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Disertační práce

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Překrývání neurodegenerativních chorob: Neuropatologické, molekulárně-genetické a klinické korelace

DISERTAČNÍ PRÁCE

Overlapping neurodegenerative diseases: Neuropathological, molecular-genetic and clinical correlations

DISSERTATION

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SEZNAM POUŽITÝCH ZKRATEK

α-syn	α-synuklein
Aβ	amyloid-beta protein
AGD	nemoc argyrofilních zrn
ALS	amyotrofická laterální skleróza
FALS	familiární varianta amyotrofické laterální sklerózy
SALS	sporadická varianta amyotrofické laterální sklerózy
ALS-FTSD	amyotrophic lateral sclerosis-frontotemporal spectrum disorder
AN	Alzheimerova nemoc
ARTAG	na věk vázaná tau-astrogliopatie
BSE	bovinní spongiformní encefalopatie
bvFTD	behaviorální varianta frontotemporální lobární degenerace
CBD	kortikobazální degenerace
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CJN	Creutzfeldtova–Jakobova nemoc
gCJN	genetická Creutzfeldtova–Jakobova nemoc
iCJN	iatrogenní Creutzfeldtova–Jakobova nemoc
sCJN	sporadická Creutzfeldtova–Jakobova nemoc
vCJN	variantní Creutzfeldtova–Jakobova nemoc
CSF	mozkomíšní mok
CTE	chronická traumatická encefalopatie
DLB	demence s Lewyho tělísky
DLG2	discs large homolog of 2
EEG	elektroencefalografie
FFI	fatální familiární insomnie
FTLD-FUS	frontotemporální lobární degenerace s FUS-pozitivní patologií
FTLD-MND	frontotemporální lobární degenerace s onemocněním motorického neuronu
FTLD-tau	frontotemporální lobární degenerace s tau patologií
FTLD-TDP	frontotemporální lobární degenerace s TDP-43 pozitivními inkluzemi
FTLD-UPS	u frontotemporální lobární degenerace s inkluzemi pozitivními pro markery ubikvitin-proteazomového systému
FUS	fused in sarcoma

FXN	Frataxin
GBA	β-glukocerebrosidáza
GSS	Gerstmannův–Sträusslerův–Scheinkerův syndrom
h-tau	hyperfosforylovaný protein tau
LRRK2	leucine-rich repeat kinase 2
MM	methionin/methionin
MRI	magnetická rezonance
MSA	multisystémová atrofie
MV	methionin/valin
NFT	neurofibrilární klubka
NIA-AA	National Institute on Aging–Alzheimer's Association
OPRI	oktapeptidové opakované inzerce
PARK2	Parkin 2
PART	primární na věk vázaná tauopatie
PGRN	progranulinu
PiD	Pickova choroba
PLS	primární laterální skleróza
PN	Parkinsonova nemoc
PPA	primární progresivní afázie
nfvPPA	nonfluentní/agramatická varianta PPA
svPPA	sémantická varianta PPA
PrP^{C}	celulární prionový protein
PrP^{Sc}	scrapie-izoforma prionového proteinu
PSP	progresivní supranukleární obrna
RT-QuIC	real-time quaking-induced conversion
SD	sémantická demence
STK39	serine/Theonine kinase 39
VPSPr	variabilně k proteáze senzitivní prionopatie
VV	valin/valin

1 ÚVOD

Neurodegenerativní onemocnění jsou charakterizována ukládáním specifických, patologicky konformovaných proteinových agregátů v centrálním nervovém systému, a to jak intracelulárně, tak extracelulárně.

Nejdůležitější primárně intracelulární depozita představují:

(1) hyperfosforylovaný protein tau (h-tau) vyskytující se ve formě neurofibrilárních klubek (NFT) u Alzheimerovy nemoci (AN), [1] u tauopatií včetně frontotemporální lobární degenerace s tau patologií (FTLD-tau) [2] a tzv. nových tauopatií v podobě neuronálních, astrogliálních i oligodendrogliálních pozitivit;

(2) α-synuklein (α-syn) formující Lewyho tělíska u Parkinsonovy nemoci (PN) a demence s Lewyho tělísky (DLB), nebo oligodendrogliové inkluze u multisystémové atrofie (MSA);

(3) fosforylovaný TDP-43 typický pro frontotemporální lobární degeneraci s TDP-43 pozitivními inkluzemi (FTLD-TDP); [3]

(4) ubikvitin nacházený u frontotemporální lobární degenerace s inkluzemi pozitivními pro markery ubikvitin-proteazomového systému (FTLD-UPS); [3, 4]

(5) „fused in sarcoma“ (FUS) u frontotemporální lobární degenerace s FUS-pozitivní patologií (FTDL-FUS). [5]

Majoritně kortikálně lokalizované, primárně extracelulární agregáty ve formě tridimenzionálních plak či difuzních depozit charakterizují AN a lidská prionová onemocnění, mezi něž řadíme Creutzfeldtovu–Jakobovu nemoc (CJN; dle etiologie dále členěnou na variantu sporadickou, genetickou a získanou s podtypy iatrogenní a variantní CJN), Gerstmannův–Sträusslerův–Scheinkerův syndrom (GSS), kuru, fatální familiární insomnii (FFI) [6] a variabilně k proteáze senzitivní prionopatii (VPSPr). [7] U AN jsou extracelulární depozita tvořena amyloid-beta proteinem (Aβ) s predominancí sekundární struktury β-skládaného listu, u prionóz tzv. scrapie-izoformou prionového proteinu (PrP^{Sc}) s identickou sekundární konformací.

V extracelulárním prostoru se tedy můžeme setkat se dvěma typy patologicky konformovaných proteinů a to:

(1) Aβ u AN typicky v podobě plak;

(2) PrP^{Sc} u prionóz – u CJN častěji v podobě difuzních síťovitých agregátů, u GSS v podobě multicentrických plak.

1.1 Lidské transmisivní spongiformní encefalopatie

1.1.1 Creutzfeldtova–Jakobova choroba

Creutzfeldtova–Jakobova choroba (CJN) je nejčastějším lidským prionovým onemocněním, [8] neuropatologicky charakterizovaným spongiformní encefalopatií mozkové a/nebo mozečkové kůry a/nebo subkortikální šedé hmoty. Může být také definována jako encefalopatie s PrP-imunoreaktivitou v podobě plak a/nebo difúzních synaptických a/nebo perivakuolárních depozit. [9] Podle etiologie CJN členíme na nejčastější formu sporadickou (sCJN), genetickou (gCJN) a získanou, která je dále dělena na iatrogenní (iCJN) a variantní (vCJN). [10]

Vzhledem k nevyhnutelnému riziku iatrogenního přenosu je biopsie mozku indikována pouze ve specifických situacích s vysokou pravděpodobností léčitelné etiologie v diferenciální diagnóze. Argumenty proti biopsiím mozku v případech podezření na CJN zahrnují vysoké riziko neprůkazného výsledku [11] a nemožnost léčby (tedy neovlivnění prognózy pacienta) ani při potvrzení prionového onemocnění. [12] Případná další specifika vyšetřovacího procesu *ante mortem* budou probrána u konkrétních diagnostických jednotek.

1.1.1.1 Sporadická forma Creutzfeldtovy–Jakobovy choroby

Sporadická CJN (sCJN) začíná náhodnou konverzí fyziologického celulárního prionového proteinu (PrP^{C}) do podoby patologicky konformovaného PrP^{Sc} s převahou β-skládaného listu. Tímto mechanismem započne přibližně 85 % případů CJN. [13] Výskyt sporadické formy je celosvětově hlášen jako 1-2 případy na milion obyvatel. [14]

1.1.1.1.1 Neuropatologická charakteristika sCJN

Neuropatologicky sCJN charakterizuje spongiformní přeměna šedé hmoty, glióza, výrazný úbytek neuropilu, ztráta neuronů a agregáty PrP^{Sc} lokalizované především extracelulárně, s tím, že intracelulární lokalizace je možná. Nejnápadnějším nálezem pozorovatelným už při barvení hematoxylinem-eosinem je spongiformní degenerace mozkové kůry. Začíná několik měsíců před klinickým nástupem příznaků, následuje ji glióza, úbytek neuropilu a ztráta neuronů. [15] Jemná spongiformní přeměna doprovází synaptická depozita PrP^{Sc} typu 1, zatímco oblasti vykazující velké konfluentní spongiformní vakuoly jsou spojeny s perivakuolární přítomností PrP^{Sc} typu 2. Platí, že mezi sporadickými případy nacházíme 95 % methionin/methionin (MM) homozygotů s PrP^{Sc} typu 1 a 86 % valin/valin (VV) homozygotů či methionin/valin (MV) heterozygotů s PrP^{Sc} typu 2. [15]

Zároveň polymorfismy kodonu 129 na *PRNP* genu korelují s charakterem a lokalizací depozit PrP^{Sc} následovně:

(1) MM1: synaptická a perivakuolární depozita; [16]

(2) MM2:

- kortikální subtyp: perivakuolární pozitivita ve všech kortikálních vrstvách;
- thalamický subtyp: méně plak – bývají popisovány jako hrubé; [17]

(3) MV1: synaptická a perivakuolární depozita;

(4) MV2: „kuru-like" plaky v mozečku a perineuronální pozitivity v mozkové kůře;

(5) VV1: tečkovité synaptické pozitivity v mozkové kůře;

(6) VV2: perineuronální pozitivity, s četnými „plaque-like" formacemi a synaptickou pozitivitou PrP^{Sc} v mozkové kůře. [18]

1.1.1.1.2 Genetický podklad sCJN

Většina pacientů s sCJN jsou homozygoté pro methionin v pozici kodonu 129 na genu *PRNP*.

1.1.1.1.3 Klinický obraz sCJN

Klinika onemocnění zahrnuje rychle progredující kognitivní dysfunkci, pyramidové a/nebo extrapyramidové příznaky, cerebelární ataxii, vizuospaciální dysfunkci, myoklonus (celkový nebo postihující některé svalové skupiny) a akinetický mutismus. [19] Ke klinické diagnóze, krom přítomnosti demence a minimálně dvou dalších klinických znaků, přispívá:

(1) pozitivita 14-3-3 proteinu v mozkomíšním moku (CSF),

(2) generalizované periodické vlny zastižené pomocí elektroencefalografie (EEG),

(3) hyperintenzity ve FLAIR/DWI sekvencích magnetické rezonance (MRI) v bazálních gangliích (putamen a nucleus caudatus) nebo kortikálních oblastech (tzv. cortical ribboning),

(4) pozitivní výsledky analýzy CSF metodou real-time quaking-induced conversion (RT-QuIC). [19]

Nedávno vydaná diagnostická kritéria dle Watsona et. Al [19] rozlišují pojmy:

(1) možná CJN: klinické příznaky odpovídající CJN a vyloučení ostatních jednotek spadajících do diferenciální diagnostiky – tj. nádorové postižení, cerebrovaskulární léze, autoimunitní poruchy, neuroinfekce;

(2) pravděpodobná CJN: odpovídající symptomatologie spolu s pozitivními biomarkery: protein 14-3-3, MRI, [20, 21] EEG [22] a RT-QUIC;

(3) definitivní CJN: po neuropatologickém potvrzení diagnózy (viz Tab. 1).

Onemocnění obvykle trvá od několika měsíců do jednoho roku. Trvání delší než dva roky je vylučujícím klinickým kritériem pro možnou sCJN. [23] Zajímavostí je, že na rozdíl od vCJN

se klinické příznaky a neuropatologické nálezy liší případ od případu, což je s největší pravděpodobností dáno různými molekulárními fenotypy u jednotlivých pacientů. [24]

Progresivní neurologický syndrom a buď neuropatologické, imunohistochemické nebo biochemické potvrzení diagnózy.

Tab. 1 – Kritéria pro definitivní diagnózu sCJN.

1.1.1.2 Genetická forma Creutzfeldtovy–Jakobovy choroby

1.1.1.2.1 Neuropatologická charakteristika gCJN

Neuropatologicky je onemocnění neodlišitelné od sCJN.

1.1.1.2.2 Genetický podklad gCJN

Genetickou (gCJN), či méně přesně familiární formu CJN, podmiňuje přítomnost dědičné mutace v genu *PRNP* (kritéria v Tab. 2), vyskytující se v 10–15 % případů CJN. [25] Preference termínu „genetická CJN“ je opodstatněná ne vždy pozitivní rodinnou anamnézou. V *PRNP* je známo více než 50 mutací, [24] přičemž v České republice převládá mutace E200K, která je zároveň nejčastější mutací v Evropě, [26] následovaná mutacemi V210I a D178N – druhá zmíněná je častá v Nizozemsku, Francii, Spojeném království, Finsku a Maďarsku. [27] Penetrance onemocnění se pohybuje mezi 60 a 100 % v závislosti na dané populaci. [28]

1.1.1.2.3 Klinický obraz gCJN

Klinicky se pacienti prezentují demencí a dalšími psychiatrickými změnami spolu s ataxií a myoklonem, neuropatie jsou oproti tomu vzácné. [29]

Definitivní CJN s rozpoznanou patogenní mutací v genu *PRNP* a definitivní nebo pravděpodobnou transmisivní spongiformní encefalopatií u příbuzného prvního stupně.

Tab. 2 – Kritéria pro definitivní diagnózu gCJN.

1.1.2 Získaná forma Creutzfeldtovy–Jakobovy choroby

1.1.2.1 Iatrogenní forma Creutzfeldtovy–Jakobovy choroby

Iatrogenní forma CJN (iCJN) vzniká přenosem PrP^{Sc} během lékařských nebo chirurgických zákroků (kritéria viz Tab. 3). [30] iCJN představuje méně než 1 % všech případů CJN, [31] nicméně bylo popsáno široké spektrum procedur, při nichž k přenosu došlo. Zaznamenány jsou případy přenosu při transplantaci tvrdé pleny mozkové, rohovky, transmise z chirurgických nástrojů, zavedením hlubokých elektrod EEG, po léčbě lidským růstovým hormonem či lidským gonadotropinem i sekundární infekce variantní CJN přenesené transfuzí krevních derivátů. [28, 32, 33] Celkem je známo 492 případů [33] s rozmanitou délkou inkubační doby závislou na formě inokulace. Inkubační doba pacientů nakažených přes intracerebrálně zavedenou elektrodu se pohybovala v rozmezí 16-28 měsíců, zatímco u pacientů infikovaných

injekcemi lidského růstového hormonu došlo ke klinickému propuknutí onemocnění s latencí 5 až 30 let. [24]

1.1.2.1.1 Neuropatologická charakteristika iCJN

Neuropatologicky bývá přítomna pokročilá kortikální spongiformní přeměna s četnými vakuolami, difuzní astrogliózou a ztrátou neuronů. Kromě toho některé popsané případy vykazovaly početné „kuru-like" plaky rozptýlené v kůře mozkové, subkortikální bílé hmotě a kůře mozečku. [34]

1.1.2.1.2 Klinický obraz iCJN

Charakteristickými klinickými projevy iCJN je demence a cerebelární příznaky – především abnormality chůze a ataxie, [29] dále se objevují vizuální, psychiatrické a senzorické obtíže. [33]

Definitivní CJN (neuropatologicky potvrzená) s iatrogenním rizikem v anamnéze.

Tab. 3 – Kritéria pro definitivní diagnózu iCJN.

1.1.2.2 Variantní forma Creutzfeldtovy–Jakobovy choroby

Variantní CJN (vCJN) souvisí s nákazou produkty kontaminovanými bovinní spongiformní encefalopatií (BSE). Jako zajímavost uvádíme tři pravděpodobné případy přenosu vCJN krevní transfuzí, [32, 33] kvůli kterým platí zákaz dárcovství krve u všech osob, které žily ve Spojeném království během epidemie BSE. [35] Kromě toho byly v poslední době zaznamenány tři případy nákazy laboratorních pracovníků pracujících s BSE-kontaminovaným materiálem (v roce 2016 v Itálii, 2018 a 2021 ve Francii, přičemž poslední případ je zatím předmětem vyšetřování). [36, 37]

1.1.2.2.1 Neuropatologická charakteristika vCJN

Neuropatologicky vCJN vykazuje morfologické a imunohistochemické odlišnosti od všech ostatních typů lidských prionových onemocnění (Tab. 4). vCJN je charakterizována hojnými floridními „daisy-like" plakami [38] v mozku a mozečku, mnohdy formujícími klastry, a povšechnou akumulací PrP^{Sc} prokazatelnou imunohistochemicky. Spongiformní dystrofie bývá nejvíce vyjádřena v nucleus caudatus a putamen. Thalamus vykazuje těžkou ztrátu neuronů a gliózu, která bývá nejvýraznější v zadních jádrech a koreluje s oblastmi hyperintenzivního signálu patrného v zadním thalamu při MRI vyšetření mozku. [39] Hyperintenzity na MRI bývají patrné i v oblasti periaqueduktální šedé hmoty, pozoruhodná je absence mozkové atrofie. [40] Na rozdíl od sCJN a gCJN byla ve 100 % případů vCJN prokázána přítomnost PrP^{Sc} jak imunohistochemicky, tak western blotem, ve všech typech lymfatické tkáně (tonzily, lymfatické uzliny, slezina). [41] To vysvětluje možnost využití

biopsie tonzil při klinickém podezření na vCJN při chybění bilaterálních hyperintenzit pulvinaru na MRI. [18]

1.1.2.2.2 Genetický podklad vCJN

Až na jednu výjimku (jeden pacient s genotypem MV), [42] byli všichni pacienti s vCJN MM homozygoté. [43]

1.1.2.2.3 Klinický obraz vCJN

Klinický projev zpočátku zahrnuje psychiatrické a behaviorální příznaky s bolestivou parestézií nebo dysestézií; [29] ataxie a demence se vyvíjejí později. [24] Na rozdíl od případů sCJN a gCJN EEG obvykle postrádá periodický vzorec [44] a trvání onemocnění je delší (v průměru 13-14 měsíců).

Neuropatologické znaky zahrnují:
• početné plaky obklopené vakuolami jak v mozku, tak v mozečku („daisy-like“ plaky),
• spongiformní přeměnu a rozsáhlé ukládání PrP^{Sc} depozit v mozku a mozečku prokázané imunohistochemicky.

Tab. 4 – Kritéria pro definitivní diagnózu vCJN.

1.1.3 Gerstmannův–Sträusslerův–Scheinkerův syndrom

Gerstmannův–Sträusslerův–Scheinkerův syndrom (GSS) je definován jako pomalu progredující autozomálně dominantně dědičné neurodegenerativní onemocnění, [45] nebo jako encefalo(myelo)patie s multicentrickými PrP plakami [8] v mozkové a mozečkové kůře a bazálních gangliích. [46]

1.1.3.1 Neuropatologická charakteristika GSS

Charakteristickým neuropatologickým znakem GSS jsou multicentrické plaky, patrné bývají i hypertrofické astrocyty, aktivovaná mikroglie a ztráta neuronů. [45]

1.1.3.2 Genetický podklad GSS

GSS je prvním lidským prionovým onemocněním, u kterého byla prokázána mutace v genu *PRNP* [45] – dodnes byly popsány bodové mutace v kodonech 102, 105, 117, 131, 145, 187, 198, 202, 212, 217 a 232, [45] oktapeptidové opakované inzerce (OPRI) čítající 1–9 24násobků párů bazí, [47] nicméně v České republice je jednoznačně nejčastější mutace P102L.

1.1.3.3 Klinický obraz GSS

Klinicky se GSS manifestuje cerebelární ataxií a pomalu progredující demencí [48] s extrapyramidovými příznaky, zhoršením zraku, sluchu, myoklonem, spastickou paraparézou a hyporeflexií až areflexií dolních končetin. [48]

Mezi případy GSS s mutací P102L lze rozlišit čtyři klinické podtypy. První a početnější skupina (84 %) čítá tři podskupiny pacientů s pozdním nástupem demence (více než 36 měsíců od klinického propuknutí onemocnění), časnou ataxií a delším trváním nemoci (medián 48 měsíců). Patří sem:

(1) typický GSS,

(2) GSS s areflexií a parestézií,

(3) GSS s čistou demencí,

Druhou skupinu definuje časný nástup demence, ataxie a překotný průběh onemocnění (medián přežití 7 měsíců):

(4) GSS podobná Creutzfeldtově–Jakobově chorobě. [49]

1.1.4 Fatální familiární insomnie

Fatální familiární insomnie (FFI) je autozomálně dominantně dědičné onemocnění způsobené mutací D178N v genu *PRNP* za přítomnosti MM polymorfismu na kodonu 129. [50] V České republice FFI nebyla dosud zaznamenána.

1.1.4.1 Neuropatologická charakteristika FFI

Nejvíce postiženými oblastmi jsou mediodorzální a ventrální thalamická jádra, pulvinar a olivy. Nápadná ztráta neuronů a astrocytární glióza jsou hlavními neuropatologickými nálezy, zatímco spongiformní degenerace chybí. [51]

1.1.4.2 Klinický obraz FFI

Klinicky je FFI charakterizována farmakorezistentní nespavostí, fragmentací spánku, poruchami autonomního nervového systému, motorickými poruchami a progresivní kognitivní poruchou. [52]

1.1.5 Kuru

Kuru je definováno jako neurodegenerativní nezánětlivé infekční onemocnění. [53] Začalo se objevovat kolem roku 1900 v Papui-Nové Guineji mezi kanibalskými kmeny, přičemž epidemie kuru následně eskalovala mezi lety 1940–1950. [53, 54] Dnes je toto onemocnění považováno za vymizelé.

1.1.5.1 Neuropatologická charakteristika kuru

Neuropatologické nálezy se i přes velmi podobné klinické příznaky lišily [55] – popisována je myelinová a neuronální degenerace (s maximem postižení pontinních jader, mozečku a bazálních ganglií), mikrogliální a astrogliová proliferace, [55] mononukleární perivaskulární infiltrát, „cuffing“, [56] spongiformní transformace, [57] atrofie neuronů, disperze Nisslovy

substance a vakuolizace cerebelární Purkyňových buněk a striatálních neuronů. [58] PrP-reaktivní „kuru“ plaky byly zaznamenány u 50-75 % případů. [57, 58]

1.1.5.2 Klinický obraz kuru

Typickými klinickými známkami kuru byla cerebelární ataxie, třes a extrapyramidové příznaky jako je chorea a atetóza [53, 54] s absencí kognitivního deficitu. [29]

1.1.6 Variabilně k proteáze senzitivní prionopatie

Variabilně k proteáze senzitivní prionopatie (VPSPr) je relativně nedávno popsané prionové onemocnění poprvé zmíněné v roce 2008. [7]

1.1.6.1 Neuropatologická charakteristika VPSPr

Neuropatologicky je VPSPr charakterizována mírnou spongiformní degenerací [7] postrádající oblasti konfluentní spongiformní transformace, [53] typické jsou PrP-imunoreaktivní „mikroplaky“ a „plaque-like“ depozita. [7]

1.1.6.2 Genetický podklad VPSPr

VPSPr je považována za sporadickou formu lidského prionového onemocnění, přičemž byli zaznamenáni pacienti se všemi typy polymorfismů na kodonu 129, avšak s převahou VV homozygotů. [59] Současně bývá udáváno, že VV homozygoté vykazují rozvinutější neuropatologické postižení ve formě plak než MM homozygoté nebo MV heterozygoté.

1.1.6.3 Klinický obraz VPSPr

Medián trvání onemocnění je 2 roky s klinickou převahou psychiatrických příznaků, [7] afázie, ataxie, parkinsonského syndromu [59] a kognitivního deficitu. [7]

1.2 Alzheimerova choroba

Alzheimerova nemoc (AN) je progresivní neurodegenerativní onemocnění a vůbec nejčastější forma demence. [60] Prevalence u osob starších 65 let čítá 3 %, u osob starších 85 let 32 %, [61] což vzhledem ke stárnutí populace nevyhnutelně povede k nárustu celkového počtu případů.

1.2.1 Neuropatologická charakteristika AN

Diagnostické znaky nutné pro vyslovení neuropatologické diagnózy AN jsou:

(1) extracelulární plaky složené majoritně z Aβ,

(2) intracelulární NFT tvořená hyperfosforylovaným tau proteinem.

Kritéria jsou zapsaná v konsensuálním schématu National Institute on Aging–Alzheimer’s Association (NIA-AA). [62, 63] Extracelulární depozita Aβ jsou hodnocena podle Thalových kritérií posuzujících distribuci a lokalizaci Aβ agregátů (viz Tab. 5 a 6); Braakovo stádium

hodnotí rozsah postižení NFT (Tab. 7) a semikvantitativně je stanovována hustota neokortikálních neuritických plak Aβ podle Consortium to Establish a Registry for Alzheimer's Disease (CERAD; Tab. 8). [64] Vyhodnocením změn je vyčíslena pravděpodobnost (nízká, střední či vysoká) podílu neuropatologických změn na klinických obtížích pacienta. [63]

Ze všech druhů Aβ jsou za nejvíce toxické považovány oligomery a s největší pravděpodobností vedou k neuronální dysfunkci a degeneraci. Fibrily Aβ mají schopnost tzv. „prion-like" šíření, kdy podněcují patologické změny konformace okolních proteinů. [65] Proto oligomery Aβ ovlivňují pokles kognice více než koncentrace monomerů Aβ nebo plak samotných. [66] Nicméně je nutno dodat, že u AN je pokles kognitivních funkcí majoritně přičítán tau patologii. [67]

1.2.2 Genetický podklad AN

Asi na 5 % případů AN se podílí významná genetická predispozice – jsou známé tři kauzální geny pro autozomálně dominantně děděnou familiární AN (*APP*, *PSEN1* a *PSEN2*) a jeden genetický rizikový faktor (alela *APOEε4*) – asi 25 % osob nese jednu alelu *APOEε4*, 2-3 % alely dvě. [68]

1.2.3 Klinický obraz AN

Podle věku při začátku onemocnění se AN člení na formu s časným a pozdním začátkem s hranicí 65 let. Většina případů spadá do kategorie s pozdním začátkem, mezi případy s časným začátkem nacházíme asi 60 % pacientů s genetickou zátěží. [69, 70]

Klinicky se jak časná, tak pozdní AN v důsledku převažujícího postižení transtentoriální oblasti, parahipokampálního závitu a hipokampu projevuje amnézií a alterací epizodické paměti. Postupem neuropatologických změn do temporální, parietální a frontální oblasti klinické obtíže progredují, přidružují se poruchy řeči, exekutivních a zrakově-konstruktivních funkcí. [71]

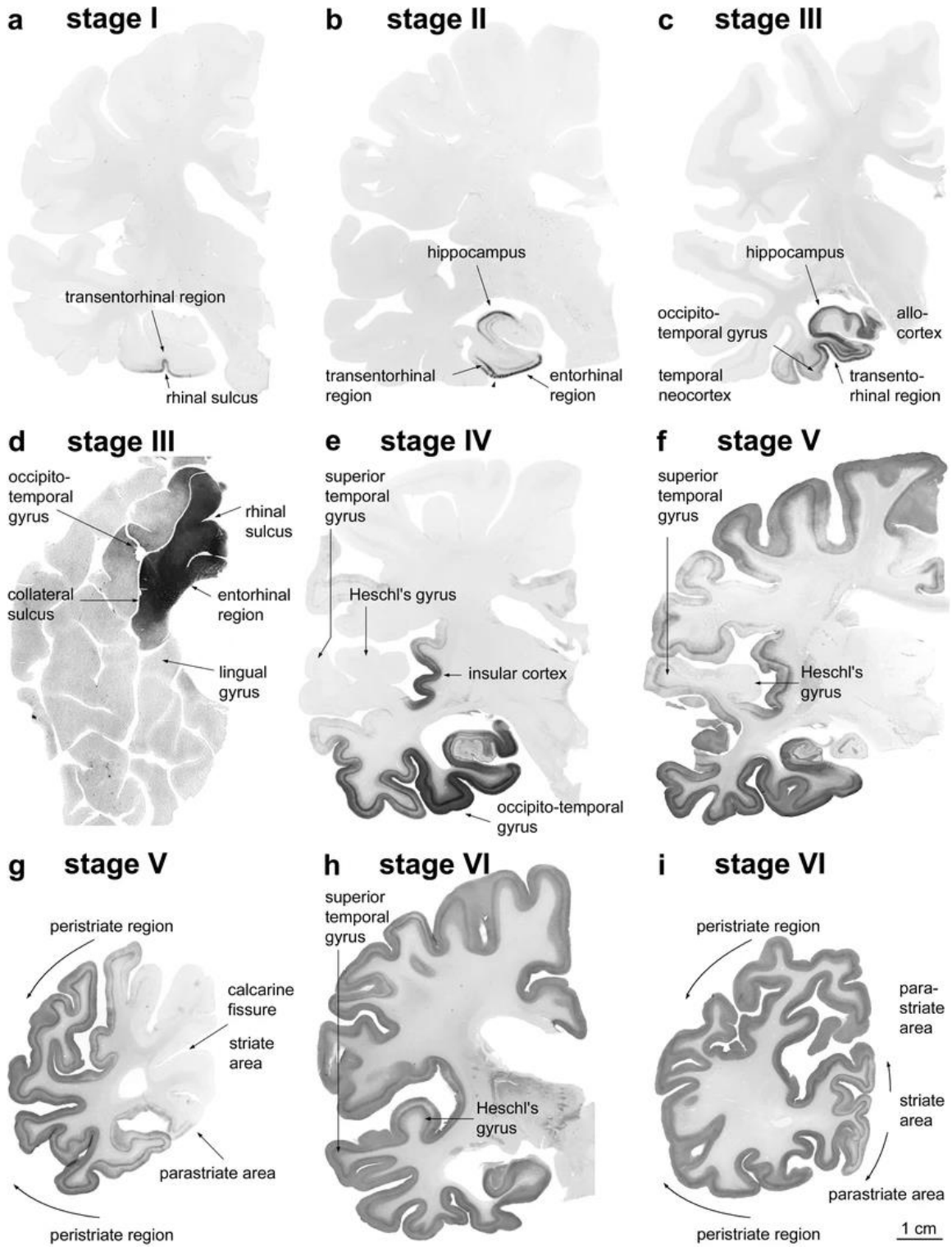
Léčba AN inhibitory cholinesterázy a memantinem může vést ke zpomalení kognitivního poklesu u pacientů s mírnou až středně těžkou demencí, ale průběh onemocnění změnit nedokáže. [72, 73]

Blok	Oblast	Fáze Aβ agregace				
		1	2	3	4	5
Frontální kortex	Šedá/bílá hmota	Jedna nebo více oblastí s Aβ	Jedna nebo více oblastí s Aβ	+	+	+
Temporální kortex				+	+	+
Parietální kortex				+	+	+
Okcipitální kortex				+	+	+
Hipokampus	Šedá/bílá hmota Sousedící temporální kortex			+	+	+
	Molekulární vrstva gyrus dentatus	-	Jedna nebo více oblastí s Aβ	+/-	+	+
	CA4	-		+/-	+/-	+
	CA1	-		+	+	+
	Zbytky entorhinální oblasti	-		+	+	+
Gyrus cinguli	Šedá/bílá hmota	-		+	+	+
Bazální přední mozek	Hypotalamus	-	-	Jedna nebo více oblastí s Aβ	+	+
	Jádra amygdaly	-	-		+	+
	Nukleus basalis Meynerti	-	-		+	+
	Putamen	-	-		+	+
	Nucleus caudatus	-	-		+	+
	Insulární kortex Šedá/bílá hmota	-	+/-	+	+	+
Střední mozek	Centrální šeď	-	-	-	Jedna nebo více oblastí s Aβ	Jedna nebo více oblastí s Aβ
	Substantia nigra	-	-	-		
Mozeček		-	-	-	-	Jedna nebo více oblastí s Aβ

Tab. 5 – Thalova stádia anatomických lokalizací Aβ agregátů. Šestistupňový skórovací systém je následně převeden na čtyřstupňové „A skóre“.

A=0	Thalovo stádium 0
A=1	Thalovo stádium 1 nebo 2
A=2	Thalovo stádium 3
A=3	Thalovo stádium 4 nebo 5

Tab. 6 – Převod Thalova stádia na čtyřstupňové „A skóre“.



Obr. 1 – Braakovo stádium neurofibrilární degenerace. Přejato z [1].

Stádium 1	B0
Stádium 2	B1
Stádium 3	B1
Stádium 4	B2
Stádium 5	B2
Stádium 6	B3
Stádium 7	B3

Tab. 7 – Braakovo stádium neurofibrilární degenerace a následný převod na B skóre.

Bez neuritických plak	C0
Řídké neuritické plaky	C1
Středně početné neuritické plaky	C2
Početné neuritické plaky	C3

Tab. 8 – CERAD skóre hustoty neokortikálních plak a následný převod na C skóre.

1.3 Tauopatie

Tau protein ve své fyziologické podobě interaguje s mikrotubuly a hraje zásadní roli v intracelulárním transportu. Tauopatie jsou skupinou onemocnění vycházející z patologické akumulace hyperfosforylovaného tau proteinu, přičemž podle počtu opakování vazebných míst pro mikrotubuly se rozlišují tři základní kategorie tauopatií – 4R-tauopatie s převahou čtyř opakování vazebného místa, 3R tauopatie s převahou tří opakování vazebného místa a smíšené 3R/4R tauopatie s kombinací obou vzorců. [71]

1.3.1 4R-tauopatie

1.3.1.1 Progresivní supranukleární obrna

Progresivní supranukleární obrna (PSP) neboli Steelův-Richardsonův-Olszewskiho syndrom, je vůbec nejčastější primární tauopatií a spadá mezi 4R-tauopatie (s akumulací tau izoformy se čtyřmi opakováními v mikrotubuly-vázající doméně) s celkem 8 různými klinickými fenotypy, [74] přičemž asociace mezi klinickými projevy a neuropatologickým nálezem není pevná. [75]

1.3.1.1.1 Neuropatologická charakteristika PSP

Neuropatologická diagnostika stojí na přítomnosti NFT a neuropilových vláken v pontu, substantii nigře, subthalamickém jádru a pallidu (nejméně ve třech z uvedených lokalizací) a nízké až vysoké hustotě NFT nebo neuropilových vláken v dalších oblastech mozku. [76]

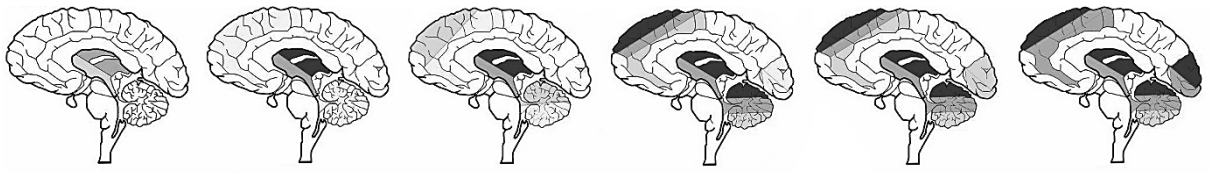
Kromě NFT a neuropilových vláken zahrnují mikroskopické rysy „tufted“ astrocyty, oligodendrogliální „coiled bodies“, ztrátu neuronů a gliózu (viz Tab. 9). [76]

1.3.1.1.2 Genetický podklad PSP

Mutace v genu *MAPT* jsou nejvíce rizikovým faktorem jak sporadických případů PSP, tak nejčastějším podkladem familiárního výskytu onemocnění. Mutace *leucine-rich repeat kinase 2* (*LRRK2*) je vzácnou monogenní příčinou PSP. [77]

1.3.1.1.3 Klinický obraz PSP

Klinické spektrum PSP je široké a zahrnuje Richardsonův syndrom, parkinsonismus, kortikobazální syndrom, nonfluentní variantu primární progresivní afázie s řečovou apraxií, frontální symptomatiku klinicky identickou s behaviorální variantou frontotemporální lobární degenerace (bvFTD) a vzácně predominantně cerebelární ataxii. [78] Nejběžnějším je Richardsonův syndrom vyznačující se pády, nestabilitou při chůzi, bradykinezí, mírnými změnami osobnosti (apatie, disinhibice), zpomalením kognitivních funkcí, pomalou, hypofonickou řečí a znesnadněním očních pohybů (tj. zpomalením vertikálních sakád, potížemi se čtením a apraxií otevírání očních víček). [79]



REGION	GP		STN		STR		FR		DE/CB		OC
Cell Stage	N/O		N		A		A		N/O		A
1	+/++	AND/OR	+/++	AND/OR	+/++	AND	-	AND	-	AND	-
2	++/+++	AND	++/+++	AND	++/+++	AND	-/+	AND/OR	-/+	AND	-
3	++/+++	AND	++/+++	AND	++/+++	AND	-/+ + +	AND	+ + -/+	AND	-
4	++/+++	AND	++/+++	AND	++/+++	AND	+ ++/+++ ++/+++	AND	++/+++ ++/+++ +	AND	- -/+
5	++/+++	AND	++/+++	AND	++/+++	AND	+ ++/+++ ++/+++	AND	++/+++ ++/+++ +	AND	+
6	++/+++	AND	++/+++	AND	++/+++	AND	++/+++	AND	++/+++ ++/+++ +	AND	++/+++

Tab. 9 – Stagingové schéma PSP dle Kovacse. Adaptováno. [80]

− Značí nepřítomnost tau inkluzí, −/+ postižení jednotlivých buněk; + mírné; + + středně závažné; + + + závažné postižení. GP = globus pallidus, STN = subthalamické jádro, STR = striatum, FR = frontální kortex, DE/CB = nucleus dentatus a bílá hmota mozečku,

OC = okcipitální kortex. U GP a DE/CB by hodnocení mělo být zaměřeno na neuronální (N) nebo oligodendrogliální (O) pozitivity; v STN neuronální pozitivity; v STR, FR a OC kortexu astrogliální (A) pozitivity.

1.3.1.2 Kortikobazální degenerace

Kortikobazální degenerace (CBD) je vzácné neurodegenerativní onemocnění spadající do skupiny 4R-tauopatií, [81] mající majoritně podobu sporadického onemocnění.

1.3.1.2.1 Neuropatologická charakteristika CBD

Neuropatologicky je onemocnění definováno kortikálními a striatálními tau-pozitivitami jak v neuronech, tak v glii, zejména v podobě astrocytárních plak a „thread-like" inkluzí v bílé i šedé hmotě, dále je přítomna fokální ztráta neuronů v kortexu a substantii nigra, balónové neurony a spongióza. [82] Imunoblotting mozkové tkáně naznačuje, že u PSP a CBD jsou přítomny biochemické rozdíly – u oněmocnění jsou nacházeny různé fragmenty tau proteinu, což znamená, že se vzájemně liší proteolytickými drahami zapojenými do patologického procesu. [83]

1.3.1.2.2 Genetický podklad CBD

Jsou reportovány nečasté genetické případy s mutací N296N v genu *MAPT*.

1.3.1.2.3 Klinický obraz CBD

Nejčastěji se CBD manifestuje asymetrickým parkinsonismem a kognitivním deficitem, ale může mít řadu jiných klinických fenotypů [84] – non-fluentní primární progresivní afázii, dysexekutivní a vizuospaciální syndrom, behaviorální symptomatiku či PSP-like syndrom. [85]

1.3.1.3 Nemoc argyrofilních zrn

Nemoc argyrofilních zrn (AGD) je často opomíjenou sporadickou 4R-tauopatií, [86] která by však měla být po Alzheimerově chorobě druhou nejčastější příčinou demence. [87] Uvádí se, že AGD je nalezeno u 5-9 % pitvaných osob dospělého věku. [88, 89] Věková distribuce onemocnění je: 10 % u osob mladších 60 let, 17 % v kategorii 61-70 let, 30 % u pacientů ve věkovém rozmezí 71-80 let a 43 % pitvaných osob nad 80 let s tím, že muži i ženy jsou postiženi stejně často. [90]

1.3.1.3.1 Neuropatologická charakteristika AGD

Typickým neuropatologickým nálezem jsou tau-pozitivní argyrofilní zrna v limbických strukturách spolu s oligodendrogliálními „coiled bodies" a neuronálními intracytoplazmatickými tau-pozitivními granulárními inkluzemi zvanými pretangly. [91] Dnes jsou u AGD neuropatologicky rozlišována čtyři stádia onemocnění (Tab. 10). Zatímco postižení anteriorní části hipokampální CA1 bývá zastiženo i u klinicky zdravých osob, zasažení

posteriorního regionu CA1 typicky doprovází klinické projevy demence. [92] Poškození kognice je klinicky zřejmé u pacientů s těžkým postižením gyrus ambiens – spoje amygdaly s temporálním lalokem. [93] Zároveň platí korelace mezi antero-posteriorním gradientem neuropatologického postižení a klinickou progresí onemocnění. [93]

1.3.1.3.2 Genetický podklad AGD

AGD je obecně považováno za neurodegenerativní onemocnění sporadického charakteru, ale byl zaznamenán případ AGD s mutací S305I genu *MAPT*. [94] Navíc se uvažuje o vlivu polymorfismů genů LDL-receptoru a α2-makroglobulinu. [95]

1.3.1.3.3 Klinický obraz AGD

Klinicky se nejčastěji projevuje pomalu progredujícím kognitivním deficitem mírného až středního stupně s častými neuropsychiatrickými obtížemi, [91] popisovány jsou abnormality chování, změny osobnosti a nálad, [88] ve většině případů dochází ke ztrátě epizodické paměti. [96] Amnézie, podrážděnost a agitovanost, následované bludy, dysforií a apatií jsou poměrně běžným klinickým obrazem. [97]

Stádium I	Přední entorhinální kortex, mírné postižení kortexu a bazolaterálních jader amygdaly, mírné postižení hypotalamického laterálního tuberálního jádra
Stádium II	Entorhinální kortex, přední oblast CA1, kortex a bazolaterální jádra amygdaly, presubikulum, hypotalamické laterální tuberální jádro, gyrus dentatus
Stádium III	Entorhinální kortex, CA1, perirhinální kortex, presubikulum, amygdala, gyrus dentatus, hypothalamické laterální tuberální jádro, mírné postižení CA2 a CA3, lehké postižení subikula, lehké postižení dalších corpora mammilaria, lehké postižení předního temporálního kortexu, insuly, přední části gyrus cinguli, orbitofrontálního kortexu, nucleus accumbens, septálních jader, ojedinělá zrna ve středním mozku
Stádium IV	Středně těžké až těžké postižení dalších kortikálních oblastí a mozkového kmene

Tab. 10 – Neuropatologická stádia AGD. [90]

1.3.1.4 Na věk vázaná tau-astrogliopatie

Pojem na věk vázaná tau-astrogliopatie (ARTAG) zastřešuje morfologické spektrum astrogliální patologie detekovatelné imunohistochemickými metodami – zejména protilátkami proti hyperfosforylované formě tau a 4R izoformě.

1.3.1.4.1 Neuropatologická charakteristika ARTAG

Tau-imunoreaktivní astrocyty představují „thorn-shaped“ astrocyty v glia limitans a bílé hmotě, stejně jako solitární astrocyty nebo jejich klastry s perinukleární cytoplazmatickou imunoreaktivitou, která se rozšiřuje do astrogliálních výběžků jako jemná fibrilární nebo granulární imunopozitivita lokalizovaná v šedé hmotě.

Při neuropatologickém hodnocení jednotky ARTAG by mělo dojít k:

1) popsání lokalizace astrogliálních tau pozitivit z celkem pěti možných:

- subpiálně,
- subependymálně,
- perivaskulárně,
- v bílé hmotě,
- v šedé hmotě,

(2) dokumentaci regionálního postižení:

- mediální temporální lalok,
- frontální,
- parietální,
- okcipitální,
- laterální temporální lalok,
- subkortikální postižení,
- zasažení mozkového kmene,

(3) dokumentaci tíže/intenzity tau astrogliopatie,

(4) popisu subregionálního zapojení.

1.3.1.4.2 Klinický obraz ARTAG

Klinickopatologické studie naznačují, že ARTAG se projevuje spíše fokálními symptomy jako je afázie, [98] v případech s pokročilou a mohutně rozšířenou astrogliopatologií se může objevit demence s parkinsonismem či bez něj. [99, 100] Velmi často se ARTAG vyskytuje jako komorbidní neuropatologie, poměrně typická je kombinace AN/ARTAG. Přítomností kopatologie ARTAG se u pacientů s AN vysvětluje např. atypický začátek s výrazným jazykovým, behaviorálním nebo vizuospaciálním deficitem, který není možné interpretovat jako důsledek AN patologie. [101] ARTAG jako relativně běžnou komorbiditu jsme zaznamenali i v retrospektivní studii případů CJN zachycených v České republice v průběhu 10 let, kdy se nejčastěji vyskytovala v kombinaci CJN/AN/ARTAG. [102]

1.3.2 Smíšené 3R/4R tauopatie

1.3.2.1 Primární na věk vázaná tauopatie

Primární na věk vázanou tauopatii (PART) neuropatologicky definuje přítomnost neurofibrilárních klubek nerozeznatelných od těch u AN a zároveň nepřítomnost extracelulárních Aβ plak. Zatímco dříve se na tyto neuropatologické změny nahlíželo jako na „změny spojené se stárnutím“ nebo „tangle-predominantní variantu AN“, dnes je PART řazena mezi FTLD-tau demence. Zůstává však čistě neuropatologickou diagnózou separovanou od jednoznačné klinické symptomatologie.

1.3.2.1.1 Neuropatologická charakteristika PART

Vzhledem k absenci Aβ plak nejsou naplněna neuropatologická kritéria pro AN a NFT jsou většinou omezena na struktury mediálního temporálního laloku, bazálního předního mozku, mozkového kmene a čichového mozku (kortexu i olfaktorických bulbů) a povětšinou korespondují s Braakovými stádii I-III hodnocenými u AN. [103] V mozkovém kmeni, včetně substantia nigra, locus coeruleus, dorzálního raphe jádra a medully oblongaty se NFT vyvíjejí v mladším věku – někdy bývají zaznamenány i u pacientů před dovršením dospělosti. [104]

1.3.2.1.2 Klinický obraz PART

Klinická symptomatologie není u řady pacientů přítomna, u jiných se vyskytují poruchy paměti, ale obecně platí, že jen minimum pacientů vykazuje závažnější klinické příznaky. [103]

1.3.2.2 Chronická traumatická encefalopatie

Chonická traumatická encefalopatie (CTE) je obecně popisována jako neurodegenerativní porucha charakteru tauopatie spojená s opakovanými traumaty hlavy, [105] přesto se má za to, že i jediné středně těžké až těžké poranění mozku může vyvolat identické progresivní neuropatologické změny. [106] Incidence CTE není známa, klinická diagnostická kritéria dosud nebyla stanovena a současná neuropatologická charakterizace CTE je považována za předběžnou. [106]

1.3.2.2.1 Neuropatologická charakteristika CTE

Neuropatologický nález se pohybuje v rozmezí od fokálních perivaskulárních epicenter NFT ve frontálním neokortexu, po těžkou 3R i 4R tauopatii postihující rozsáhlé oblasti mozku včetně mediálního temporálního laloku, což umožňuje staging do stádií I-IV (viz Tab. 11). Pozitivity 4R isoformy jsou velmi hojné, 3R isoformy spíše rozptýlené a méně početné. [107] Multifokální axonální rozšíření a ztráta axonálních výběžků byly nalezeny v hlubokých vrstvách kortexu i subkortikální bílé hmotě ve všech čtyřech stádiích CTE. Zajímavostí je, že v 85 % případů byly nalezeny také inkluze a neurity pozitivní v reakci s protilátkou proti TAR DNA-binding

protein 43 – od fokální patologie ve stádiích I-III po mnohotné pozitivity ve stádiu IV. Častá asociace chronické traumatické encefalopatie s jinými neurodegenerativními poruchami (MND, AN, DLB či FTLD) naznačuje, že opakované mozkové trauma a depozita hyperfosforylovaného tau proteinu nejspíše podporují akumulaci dalších abnormálně agregovaných proteinů včetně TAR DNA-vazebného proteinu 43, Aβ a α-syn. [108]

Stadium I	Superiorní, dorzolaterální a laterální frontální kortex, perivaskulárně v hloubce sulků
Stadium II	Mnohočetná epicentra v hloubce mozkových sulků, lokalizované šíření neurofibrilární patologie z epicenter do povrchových vrstev přilehlého kortexu, mediální temporální lalok zůstává nepostižen
Stadium III	Difuzní rozšíření NFT na frontální, insulární, temporální a parietální kortex, (nejintenzivněji postižen frontální a temporální lalok v hloubce sulků), zasažení hipokampu, amygdaly a neorhinálního kortexu. Také ve stadiu III CTE vykazuje amygdala, hipokampus a entorinální kůra neurofibrilární patologii
Stadium IV	Závažné postižení většiny oblastí mozkové kůry včetně mediálního temporálního laloku, šetřící kortex sulcus calcarinus kromě nejzávažnějších případů

Tab.11 – Neuropatologická stadia CTE. [108]

1.3.2.2.2 Klinický obraz CTE

Příznaky chronické traumatické encefalopatie se objevují většinou roky po traumatu, v I. stadiu zahrnují bolesti hlavy a poruchy koncentrace, ve II. stadiu se přidávají deprese, zvýšená výbušnost a poruchy krátkodobé paměti, III. stadium charakterizují kognitivní a exekutivní dysfunkce, IV. stadium demence, obtíže při hledání správných slov a agresivita. [109]

1.3.3 3R-tauopatie

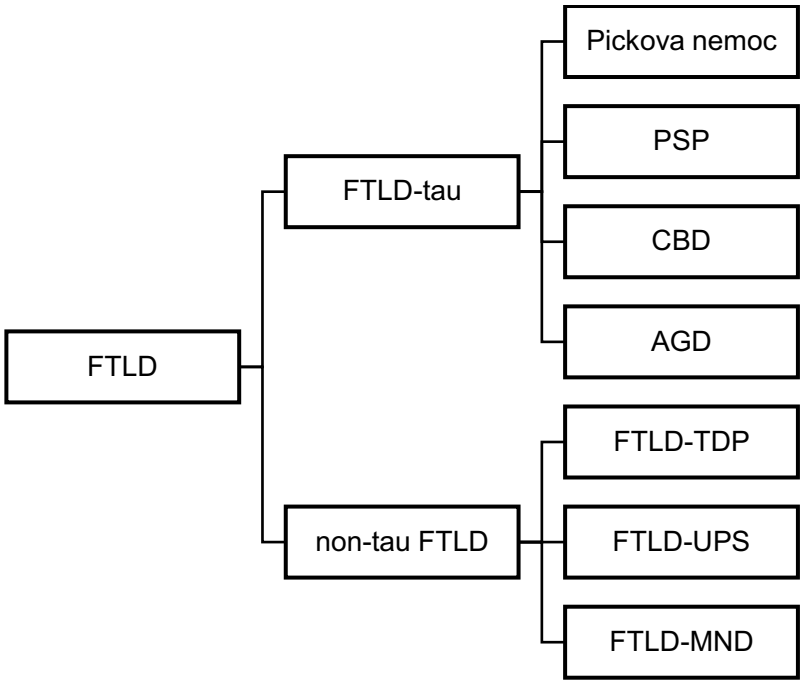
1.3.3.1 Pickova choroba

Původně splývaly termíny Pickova choroba (PiD) a frontotemporální demence – jako PiD se označovaly všechny případy demence spojené s makroskopicky zřejmou „knife blade“ atrofií frontálních a temporálních laloků, [110] dnes je termín vyhrazen pro případy demencí s tau-pozitivními Pickovými tělísky. [111] Řazena je mezi tzv. 3R-tauopatie a vyskytuje se vzácně jak v podobě sporadické, tak familiární. [112]

Problém, který vyvstal s možností imunohistochemického vyšetřování je, že ne všichni pacienti s „Pickovou chorobou“ v jejím původním pojetí měli prokazatelná Pickova tělíska. Proto vznikl koncept frontotemporálních lobárních degenerací.

1.4 Frontotemporální lobární degenerace

Frontotemporální lobární degenerace (FTLD) jsou skupinou chorob, mezi které řadíme jak některé tauopatie, tak non-tau případy. Jejich neuropatologické dělení s klinickou korelací je následující:



Obr. 1 – Rozdělení FTLD.

Vysvětlivky: FTLD-tau = frontotemporální lobární degenerace s tau patologií; PSP = progresivní supranukleární obrna; CBD = kortikobazální degenerace; AGD = nemoc argyrofilních zrn; non-tau FTLD = frontotemporální lobární degenerace s jinou než tau patologií; FTLD-TDP = frontotemporální lobární degeneracie s TDP-43 pozitivními inkluzemi; FTLD-UPS = frontotemporální lobární degenerace s inkluzemi pozitivními pro markery ubikvitin-proteazomového systému; FTLD-MND = frontotemporální lobární degenerace s onemocněním motorického neuronu.

1.4.1 Neuropatologická charakteristika FTLD a klinický obraz

Klinické fenotypy FTLD zahrnují nejčastější behaviorální variantu frontotemporální demence (bvFTD), primární progresivní afázii (PPA), kortikobazální syndrom [113] a nejméně častou sémantickou demenci (SD). [114] Klinický nález koreluje spíše s lokalizací patologických

inkluzí než s jejich typem, ale jak z názvů jednotlivých kategorií FTLD vyplývá, setkat se můžeme s inkluzemi tau, TDP-43 a ubikvitinu.

bvFTD charakterizuje postižení dorsálního laterálního prefrontálního kortexu, [115] což interaguje s oběma parietálními laloky [116] a vede ke ztrátě exekutivních funkcí. Disinhibice je spojena s degenerací pravého orbitofrontálního kortexu [117] a vede ke společensky nevhodnému chování jako je narušování osobního prostoru, nepatřičným dotekům a přílišné familiárnosti vůči cizím osobám. Pacienti mohou být impulzivní, vyskytují se potíže s gamblingem či nově vzniklým kriminálním chováním, které bývá zaznamenáno u 37-54 % bvFTD. Atrofie striata bývá spojena se stereotypy (klepání, pohupování, vyplazování jazyka) [118] a asymetrická atrofie temporálního laloku s komplexními kompulzivními projevy. [119] Obsedantně kompulzivní chování u bvFTD koreluje se ztrátou šedé hmoty globus pallidus bilaterálně, levého putamen a levého středního a dolního temporálního gyru. [120]

PPA se primárně projevuje jazykovými obtížemi. Nejnovější klinická kritéria rozlišují sémantickou variantu PPA (svPPA), nonfluentní/agramatickou variantu PPA (nfvPPA) a logopenickou variantu PPA, která však převážně koreluje s neuropatologickým nálezem AN. [121] Pokud je u svPPA majoritně zasažen levý temporální lalok, dominují obtíže jazykového charakteru s pomalou ztrátou sémantických znalostí, pokud je majoritně zasažen pravý temporální lalok, převažují behaviorální příznaky. Postupem onemocnění a bilaterální progresí neuropatologických změn dochází k překrytí klinických příznaků, přičemž progrese je pomalejší při primárním levostranném postižení. [122] nfvPPA neuroanatomicky koreluje s postižením Brocova centra (Brodmanovy arey 44 a 45) levého dolního frontálního gyru a přední části insuly. [123] S postupem onemocnění dochází ke snížení verbálních schopností až jejich vymizení – mutismus u pacientů s nfvPPA koreluje s rozšířením postižení mimo dolní frontální a insulární oblast. [124]

Kortikobazální syndrom je klinickým fenotypem charakterizovaným kortikálními a extrapyramidovými příznaky. Apraxie, kortikální senzorický deficit a fenomén „cizí končetiny“ jsou nejčastějšími kortikálními příznaky, zatímco asymetrický parkinsonismus, dystonie a myoklonus představují typické příznaky extrapyramidové. Parkinsonismus u kortikobazálního syndromu je charakteristicky asymetrický až jednostranný. [125]

Nejméně častý klinický fenotyp – sémantická demence, se projevuje příznaky poškození řečových funkcí a paměti. Typické jsou potíže s nacházením vhodných slov nebo jejich zapamatováním. Podstatná jména jsou pro pacienty obvykle nejobtížnější, což může mít

za následek užívání popisů nebo nespecifických slov. Deficitu si pacienti často nejsou vědomi. [126]

1.4.2 Genetický podklad FTLD

S FTLD je spojena vysoká genetická zátěž a až v 50 % případů se setkáváme s pozitivní rodinnou anamnézou – nalézány bývají nejčastěji mutace v genu *MAPT,* dále *progranulinu (PGRN), C9orf72, TARDBP, VCP* a *CHMP2B*. [127]

1.5 Synukleonopatie

1.5.1 Parkinsonova nemoc

Parkinsonova nemoc (PN) je forma multisystémové α-synukleinopatie. Vede k rigiditě, bradykinezi, posturální instabilitě a klidovému tremoru [128] vyplývajících ze selektivní ztráty a/nebo degenerace dopaminergních neuronů v substantia nigra, pars compacta.

1.5.1.1 Neuropatologická charakteristika PN

Neuropatologicky onemocnění charakterizuje přítomnost intracelulárních Lewyho tělísek a početných Lewyho neuritů pozitivních v reakci s protilátkou proti α-syn. [129, 130] Histopatologická diagnóza PN vyžaduje dva klíčové rysy: ztrátu neuronů a přítomnost Lewyho tělísek v substantia nigra, [131] s poměrně běžným vedlejším nálezem extraneuronálního neuromelaninu a gliózy.

1.5.1.2 Genetický podklad PN

PN může mít sporadický i genetický charakter. Existují autozomálně dominantní i autozomálně recesivní varianty, mezi něž se řadí mutace v genu pro α-synuklein (*SNCA*), *Parkinu 2 (PARK2), LRRK2, PINK1, PARK7, BST1* a v již několikrát skloňovaném *s mikrotubuly asociovaném proteinu tau (MAPT)*. [132-135] Mendelovské varianty s vysokou penetrací (např. geny *SNCA, PINK1, PARK7, LRRK2*) však stojí za méně než 10 % genetických PN. [136] Replikační studie v posledních letech odhalily 24 jednonukleotidových polymorfismů na čtyřech lokusech – *β-glukocerebrosidáza (GBA); diacylglycerolkináza θ, 110kD; SNCA* a *lidský leukocytární antigen (HLA)*, které se na zvýšeném výskytu v některých rodinách rovněž podílí. [137] Genetické riziko podle studií u kavkazské populace souvisí s alelickou heterogenitou na *LRRK2* a šesti dalších lokusech včetně *MAPT* a *GBA-SYT11*, u asijské populace spíše s *PARK16*, *Serine/Theonine kinase 39 (STK39)* a *Discs large homolog of 2 (DLG2)*. [138]

Řada jiných neuropatologií má některé, nebo dokonce všechny výše jmenované klinické znaky – klinický syndrom označovány jako „parkinsonismus“ či „parkinsonský syndrom“ zahrnuje vaskulární, traumatickou, toxickou či poinfekční etiologii. [128] *Post mortem*

neuropatologickým vyšetřením 132 pacientů s klinickou diagnózou PN bez přítomnosti demence bylo prokázáno, že 77 % těchto pacientů trpělo demencí s Lewyho tělísky (DLB), 8 % DLB + multisystémovou atrofií (MSA), 10 % tauopatií (PSP + CBD) a 4 % případů vznikla na podkladě vaskulární etiologie. [128]

Relativně vzácně se jde setkat s genetickými formami parkinsonismu spojených spíše s přítomností TDP-43 inkluzí než α-synukleinu (α-syn) – příkladem je Perryho syndrom vyvolaný mutací v *DCTN1*. [139]

1.5.1.3 Klinický obraz PN a symtpomatická terapie

Konvenční farmakologickou léčbu PN představují prekurzory dopaminu (levodopa) a symptomatická léčba včetně agonistů dopaminu, inhibitorů monoaminooxidázy a inhibitorů katechol-O-methyltransferázy. U všech pacientů s PN je léčba zaměřená na zlepšení motorických (př. třes, rigidita, bradykineze) a nemotorických příznaků (př. konstipace, kognice, nálada, spánek). [140] Medikamenty modifikující průběh onemocnění nejsou k dispozici a dlouhodobé podávání aktuálně užívaných antiparkinsonických léků po čase vyvolává „wear-off fenomén/fenomén opotřebení“ vedoucí k dalším psychomotorickým a autonomním komplikacím. [140]

1.5.2 Demence s Lewyho tělísky a Parkinsonova choroba s demencí

1.5.2.1 Neuropatologická charakteristika DLB

Demence s Lewyho tělísky (DLB) je neuropatologicky charakterizována depozity α-syn v Lewyho tělíscích a Lewyho neuritech, sekundární ztrátou populací tegmentálních dopaminových buněk a cholinergních populací bazálního předního mozku. Poměrně často je DLB doprovázena různým stupněm koexistující alzheimerovské patologie. [141]

Současná neuropatologická kritéria demence s Lewyho tělísky uvažují podíl α-syn patologie a NFT pro odhad pravděpodobnosti, že právě DLB u daného pacienta vedlo ke klinickým obtížím. [142]

Stejný neuropatologický nález provází demenci při PN, rozhodující je v těchto případech doba nástupu jednotlivých klinických příznaků (demence vs. parkinsonismu) [143] – pokud demence nastupuje více než rok po stanoveni diagnózy PN, jde o demenci při PN. [141]

Oblast mozku	Medulla		Pons		Střední mozek	Bazální přední mozek		Hipokampus		Gyrus cinguli	Temporální kortex	Frontální kortex	Parietální kortex
Anatomická oblast	dmV	irx	LC	R	SN	nbM	AC	CA2	T-O	šedá hmota	šedá hmota	šedá hmota	šedá hmota
Braakovo stádium	1	1	2	2	3	3	4	3	4	5	5	6	6
McKeith	mozkový kmen					limbické struktury						neokortex	
Amygdala predominantní							ano						
Typ depozit	LB a/nebo LN						LB	LN		LB			

Tab. 10 – Protokol BrainNet Europe, tj. přiřazení Braakova stadia a McKeithova typu a-syn inkluzí u DLB.

Vysvětlivky: dmV = dorzální motorické jádro n. vagus; irx = intermediální retikulární zóna; LC = locus coeruleus; R = raphe; SN = substantia nigra; nbM = nucleus basalis Meynerti; AC = amygdala; CA2 = cornu Ammonis hipokampu; T-O co temporo-okcipitální kortex; LB = Lewyho tělíska; LN = Lewyho neurity.

1.5.2.2 Genetický podklad DLB

Geneticky zvyšují riziko některé polymorfismy *GBA*, zmíněné již mezi geneticky podmíněnými rizikovými faktory PN. [144]

1.5.2.3 Klinický obraz DLB

Klinicky se DLB projevuje progresivní kognitivní poruchou spojenou s parkinsonismem, vizuálními halucinacemi a kolísáním pozornosti i bdělosti. [141] Mezi základní klinická kritéria patří:

(1) opakující se vizuální halucinace,

(2) kolísání pozornosti a bdělosti,

(3) parkinsonské motorické příznaky.

Známky podporující diagnózu DLB zahrnují poruchy spánkového chování v REM fázi, neuroleptické obtíže nebo nízkou absorpci dopaminového transportéru (DAT) v bazálních gangliích na single photon emission computed tomography (SPECT) nebo positron emission tomography (PET). [141]

1.5.3 Multisystémová atrofie

Multisystémová atrofie (MSA) je sporadická progresivní neurodegenerativní porucha dospělého věku projevující se v různé míře kombinací parkinsonismu, cerebelární, autonomní a motorické dysfunkce, [145] začínající nejčastěji kolem 55-60 let věku s průměrnou délkou přežití 8-9 let. [146]

1.5.3.1 Neuropatologická charakteristika MSA

Zatímco u pacientů s PN nacházíme inkluze α-syn v neuronech v podobě Lewyho tělísek či Lewyho neuritů, neuropatologickým znakem MSA jsou inkluze α-syn v glii – majoritně oligodendroglii, a neuronech, [147] a to jak v centrálním, tak periferním nervovém systému. [148] Selektivní atrofie a ztráta neuronů ve striatonigralní nebo olivopontocerebelární oblasti je základem rozdělení na dva hlavní motorické fenotypy: MSA-parkinsonského typu (= striatonigrální) a MSA-cerebelárního typu (= olivopontocerebelární). [149] Doprovodnými neuropatologickými znaky jsou aktivace glie a povšechná demyelinizace. [150]

1.5.3.2 Klinický obraz MSA

Klinicky je MSA charakterizovaná kombinací autonomních obtíží, parkinsonismu a ataxie, [149] avšak může činit velké diagnostické obtíže. Čistě autonomní symptomatika může být k nerozeznání od autonomního selhání, pacienti s parkinsonismem mohou být mylně označeni za pacienty s PN a u pacientů s cerebelární symptomatikou přichází do úvahy ataxie na genetickém podkladu, autoimunitní, paraneoplastická, vyvolaná alkoholem, chemoterapeutiky nebo např. toluenem. V řadě případů se lze u pacientů setkat s hyperreflexií, genitourinární dysfunkcí, ortostatickou hypotenzí, stridorem, poruchami REM části spánku či spánkovou apnoí. [148] Součástí klinického vyšetřování je strukturální a funkční zobrazení mozku, srdečního sympatiku, testování kardiovaskulárních autonomních funkcí, čichové testy, studie spánku, urologické vyšetření, hodnocení dysfagie a kognice. [148]

1.6 Onemocnění motorického neuronu

1.6.1 Amyotrofická laterální skleróza

Amyotrofická laterální skleróza (ALS) byla historicky považována za onemocnění výhradně motorického systému, což ji zařadilo do skupiny onemocnění motorického neuronu (MND) zahrnující spektrum poruch postihujících (1) horní motorický neuron (kortikospinální trakt), (2) dolní motorický neuron (v předních rozích míšních nebo jádrech motorických kraniálních nervů v mozkovém kmeni), nebo (3) horní i dolní motorický neuron. [151] Do dnešního dne však byla popsána řada případů zahrnujících demenci a poruchy chování, proto v současné době rozdělujeme ALS nejen podle případné existence genetické predispozice na nejčastější sporadickou (SALS; 90 %) a familiární variantu (FALS; 10 %), ale také podle přítomnosti kognitivní a/nebo behaviorální symptomatologie.

1.6.1.1 Neuropatologická charakteristika ALS

Degenerativní změny jsou většinou pozorovatelné v motorické oblasti. Nápadný je počet atrofovaných α-motorických neuronů lokalizovaných v přední šedé hmotě míchy a motorických

neuronů jader hlavových nervů v mozkovém kmeni. [152] Postiženy jsou i Betzovy buňky v primární motorické kůře. Ztráta motorických neuronů vede k chronické denervaci s neurogenní atrofií a tukovou pseudohypertrofií v příčně pruhovaných svalech končetin a svalech dýchacích, přičemž svalová vlákna 2. typu jsou zranitelnější. [153]

Imunohistochemicky je možné prokázat různé inkluze. U SALS to bývají SOD1, TDP-43, ubikvitin, p62 a OPTN (u non-SOD1 případů), u FALS oproti předchozím jmenovaným navíc FUS. [153] Inkluze mohou být „klubkovité“, tzv. „skein-like“, [154] s protáhlým nebo vláknitým tvarem, tvořící bizarní kulovité struktury v perikaryonu neuronů, nebo eozinofilní kulaté hyalinní inkluze.

1.6.1.2 Genetický podklad ALS

Přibližně 10 % případů SALS bývá spojováno s mutacemi v *SOD1, C9orf71, TARDBP* a *hnRNP A1 + hnRNP A2B1*. U FALS bývají nacházeny mutace přibližně ve 20 % případů, nejčastější je autozomálně dominantní vzorec dědičnosti, přičemž se ale lze setkat i s případy autozomálně recesivní nebo X-vázané dědičnosti. Na případech FALS se krom výše jmenovaných podílí mutace *FUS, UBQLN2, p62, ALSIN, SETX, SPG, OPTN* a *VAPB*. Mutace v genech *ALSIN* a *SETX* vedou k juvenilnímu nástupu klinických obtíží. [153]

1.6.1.3 Klinický obraz ALS

ALS je dnes klinicky členěno do pěti podskupin na:

(1) ALS s kognitivní poruchou C definovaná přítomností apatie, nebo dvou či více behaviorálních/ kognitivních změn spojených s bvFTD,

(2) ALS s poruchou chování – s exekutivní a/nebo jazykovou dysfunkcí,

(3) ALS s kombinovanou kognitivní a behaviorální poruchou – tj. kombinací obou předchozích,

(4) plně vyvinutou behaviorální variantu frontotemporální demence v kombinaci s ALS – definovanou postupným zhoršováním chování a/nebo poznávání a přítomností nejméně tří behaviorálních/kognitivních změn spojených s bvFTD, nebo nejméně dvou behaviorálních/kognitivních změn spojených s bvFTD spolu se ztrátou náhledu a/nebo psychotickými příznaky, nebo splněním kritérií pro primární progresivní afázii,

(5) komorbidní ALS a AN – tj. ALS s plně vyvinutou AN. [155]

Obecně jsou tyto případy označovány jako „amyotrophic lateral sclerosis-frontotemporal spectrum disorder“ (ALS-FTSD). [156] Obdobné klinické chování a přítomnost stejných patognomických depozit v centrálním nervovém cystému naznačují, že FTLD a ALS mohou být nikoliv dvě samostatné jednotky, ale jedno kontinuum. Počet nových případů je v Evropě přibližně 1–2,6 na 100 000 obyvatel ročně. [157] Typicky se onemocnění vyskytuje ve věku okolo 60 let, [157] vzácně před dosažením 40 let a o něco častěji u mužů (poměr M:Ž

je přibližně 1,5:1); [158] byť nástup bulbární formy je častější u žen. [159, 160] Medián doby přežití je 20–48 měsíců, nicméně skupina 10–20 % pacientů přežívá více než deset let. [161] MND také zahrnují „omezené“ fenotypy, včetně primární laterální sklerózy (PLS), progresivní svalové atrofie a progresivní bulbární obrny. [162] PLS je charakterizována izolovanou progresivní dysfunkcí horního motorického neuronu bez symptomatologie dolního motorického neuronu, která začíná v páté až šesté dekádě s postupně se rozvíjející spasticitou, hyperreflexií a mírnou slabostí. [163]

1.7 Onemocnění s opakováním tripletů

1.7.1 Huntingtonova chorea

Huntingtonova chorea je autosomálně dominantně dědičná progresivní neurodegenerativní porucha s rozličným klinickým fenotypem čítajícím choreu, dystonii, poruchy chování a kognice, [164] s typickým klinickým počátkem ve středním věku. [165]

1.7.1.1 Neuropatologická charakteristika Huntingtonovy chorey

Neuropatologicky nemoc charakterizuje selektivní ztráta GABAergních neuronů ve striatu, později progredující ve ztrátu neuronálních populací globus palidus, nucleus subthalamicus, hipokampu, substrancie nigry, hypothalamu, thalamu, mozečku a kůry mozkové. Úbytek neuronů provází zmnožení glie. Mutovaný huntingtin se majoritně hromadí v jádrech neuronů. [71]

1.7.1.2 Genetický podklad Huntingtonovy chorey

Příčinnou genetickou mutací je zmnožená cytosin-adenin-guanin (CAG) trinukleotidová repetice v genu kódujícím protein huntingtin, což vede k prodloužení polyglutaminového úseku na N-konci proteinu. [165] Při 40 a více repeticích CAG pacient s jistotou onemocní Huntingtonovou chorobou, pravděpodobně v její klasické formě se začátkem ve středním věku. Pacienti mající 60 a více repetic jsou postiženi tzv. juvenilní formou s propuknutím před začátkem adolescence. [71]

1.7.1.3 Klinický obraz Huntingtonovy chorey

Prvním klinickým projevem bývají poruchy chování – buď iritabilita s agresivitou, nezdrženlivostí a hypersexualitou, nebo apatií a depresí. [71] Často tyto příznaky doplňuje ztráta volní hybnosti, po letech se přidává chorea, dystonie, dysartrie a dysfagie. [71] Chorea, nejcharakterističtější symptom, reaguje na medikaci snižující dopaminergní neurotransmisi, psychiatrické poruchy (deprese a úzkost) mohou být řešeny symptomatickou terapií, přesto je onemocnění dodnes neléčitelné. [166]

1.7.2 Friedreichova ataxie

Friedreichova ataxie je nejčastější dědičnou ataxií s autozomálně recesivním způsobem dědičnosti a počátkem onemocnění obvykle před obdobím puberty. [167]

1.7.2.1 Neuropatologická charakteristika Friedreichovy ataxie

Nejnápadnějším neuropatologickým nálezem je atrofie především hrudní míchy, zadních kořenů míšních a nucleus dentatus mozečku, což lze zvýraznit reakcí s protilátkou proti neuron specifické enoláze. [71] Postižení Schwannových buněk a neurofilament pomáhají odhalit další imunohistochemické markery – konkrétně protilátky proti fosforylovaným neurofilamentům, S100 proteinu a myelinovému bazickému proteinu, který napomáhá odhalit patologické rozložení axonálních struktur. [71]

1.7.2.2 Genetický podklad Friedreichovy ataxie

96 % pacientů má autozomálně recesivně děděnou homozygotní expanzi trinukleotidové repetice guanin-adenin-adenin (GAA) na chromozomu 9q13, která způsobuje transkripční defekt genu *Frataxin* (*FXN*). [168] Expanze GAA v prvním intronu *FXN* vede ke snížení hladiny proteinu frataxin hojně obsaženého v mitochondriích, [168] což má za následek mitochondriální dysfunkci způsobenou poklesem klastrů železa-síry, zvýšením hladiny mitochondriálního železa a oxidativního stresu. [169]

1.7.2.3 Klinický obraz Friedreichovy ataxie

Klinická manifestace odráží léze v dorzálních kořenových gangliích, senzorických periferních nervech, kortikospinálních traktech a nucleus dentatus. Charakteristické klinické rysy zahrnují progresivní ataxii, dysartrii, zrátu vibračního čití a sekundární změny kloubního postavení. [167] Časté jsou deformace chodidel, skolióza, výrazná kardiomyopatie a postižení β-buněk pankreatu vedoucí k projevům diabetu mellitu. [170]

1.8 Komorbidní neurodegenerativní onemocnění

Rychlejší progrese onemocnění, atypický klinický průběh či snížení schopnosti odpovědi na podávanou medikaci, jsou typickými důsledky existence komorbidních případů neurodegenerativních onemocnění. Podle typu překryvu neurodegenerativních onemocnění lze vyčlenit tři podskupiny:

(1) Souběžná neuropatologická onemocnění, která definuje přítomnost dvou a více rozvinutých neurodegenerativních onemocnění, přičemž všechny jednotky splňují příslušná neuropatologická diagnostická kritéria, tj. podmínku přítomnosti specifických proteinových depozit i jejich adekvátní distribuci. Častá je kombinace AN se synukleinopatiemi či CJN s tauopatiemi.

(2) Konkomitantní neurodegenerativní patologie v ohraničených oblastech mozku, kdy je přítomno jedno primární neurodegenerativní onemocnění splňující neuropatologická diagnostická kritéria spolu se specifickými proteinovými agregáty charakteristickými pro jinou neurodegenerativní jednotku, ovšem svým omezeným rozsahem a distribucí nenaplňující kritéria finální neuropatologické diagnózy.
(3) Smíšená neuropatologie, neboli smíšená demence, je dána přítomností jednoho neurodegenerativního onemocnění naplňujícího svá diagnostická kritéria, spolu s vaskulární patologií. Nejčastěji do této kategorie spadají pacienti s AN či DLB. Vaskulární postižení má charakter rozšíření perivaskulárních prostor v oblasti bazálních ganglií a centrum semiovale. [71]
Mezi komorbidními neurodegenerativními onemocněními existují určité kombinace, které nacházíme častěji než ostatní.
U prionových onemocnění může kvůli jejich významnému vlivu na klinický průběh zůstat koexistující neurodegenerativní onemocnění *ante mortem* skryto. Analýza případů Creutzfeldtovy–Jakobovy choroby diagnostikovaných za posledních 10 let v ČR však odhalila překvapivé procento čistých vs. komorbidních případů. Paradoxně vůbec nejméně zastoupenou skupinou byla ta bez komorbidit (11,16 %), významně převýšená případy komorbidní CJN s tauopatií (62,79 %) či AN (20,47 %). Vzácně se objevily případy CJN v komorbiditě s FTLD (3,26 %) nebo synukleinopatiemi (2,33 %). [102] Zajímavostí je, že komorbidní CJN/tauopatie se na MRI projevily putaminální hyperintenzitou a CJN/AN byla spojena s pozdějším věkem nástupu onemocnění, stupněm hipokampální atrofie pozorované na MRI (nízké skóre atrofie mediálního temporálního laloku = MTA skóre) a nízkými hladinami beta-amyloidu v mozkomíšním moku. [102]
Jiným příkladem může být AN v komorbiditě s α-synukleinopatiemi, a to buď ve formě rozvinuté demence s kortikálními Lewyho tělísky nebo v podobě tzv. amygdala Lewy bodies, kdy jsou inkluze α-syn omezeny na limbické struktury, především amygdalu (ALB). [71] Náš pilotní výzkum kohorty pacientů s čistou AN, komorbidní AN/DLB a AN/ALB ukázal, že existují prominentní rozdíly ve tvaru a složení neokortikálních a archikortikálních plak, kdy bulbózní změny neuritů charakteristické pro archikortikální oblasti jsou nejvíce vyjádřeny u případů AN/DLB a následně u AN/ALB. Spekulací je, že tyto změny jsou nejvíce patrné v počátečních stádiích onemocnění, které odkrývá rychlejší progrese komorbidních případů. [171] AN a AGD jsou další častou komorbiditou, jejíž incidence roste s věkem.

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2 CÍLE PRACÍ

2.1 Mikromorfologická charakterizace archikortikálních a neokortikálních neuritických plak v případech tzv. „čisté“ Alzheimerovy nemoci a Alzheimerovy nemoci s komorbidní synukleinopatií.

Hypotéza H1: Archikortikální a neokortikální neuritické plaky se liší svou mikromorfologickou stavbou, bulbózní dystrofické neuritické změny jsou více zřetelné u komordbidních případů.

2.2 Mikromorfologická charakterizace extracelulárních proteinových depozit u komorbidních případů Creutzfeldtovy–Jakobovy choroby a Alzheimerovy nemoci, popis dystrofických neuritických změn v plakách PrP^{Sc}.

Hypotéza H2: Extracelulární depozita tvořená Aβ a PrP^{Sc} kolokalizují. Dystrofické změny v PrP^{Sc} plakách jsou identické s dystrofickými neuritickými změnami patrnými u Alzheimerovy choroby.

2.3 Mikromorfologická charakterizace extracelulárních proteinových depozit u případů Gerstmannova–Sträusslerova–Scheinkerova syndromu a jejich vztah k dystrofickým neuritickým změnám.

Hypotéza H3: Extracelulární plaky PrP^{Sc} u Gerstmannova–Sträusslerova–Scheinkerova syndromu hojně kolokalizují s dystrofickými neurity.

2.4 Charakterizace případů Creutzfeldtovy–Jakobovy choroby po neuropatologické, genetické, imunologické a klinické stránce a z pohledu radiodiagnostiky.

Hypotéza H4: Mezi případy Creutzfeldtovy–Jakobovy choroby je jen malé procento případů bez komorbidit. Komorbidity mohou mít vliv na klinické projevy, hladiny biomarkerů a výsledky zobrazovacích metod.

2.5 Utřídění názvosloví jednotlivých podtypů extracelulárních plak u Alzheimerovy choroby a prionóz, jejich vzájemné srovnání.

2.6 Shrnutí dosavadních neuropatologických poznatků o amyotrofické laterální skleróze.

2.7 Shrnutí 20 let zkušeností národní laboratoře pro prionová onemocnění s užívanými diagnostickými postupy a množstvím zachycených případů. Porovnání s dalšími státy a vysvětlení odlišností v diagnostickém procesu.

3 VÝSLEDKY PRACÍ

3.1 Mikromorfologická charakterizace archikortikálních a neokortikálních neuritických plak v případech tzv. „čisté“ Alzheimerovy nemoci a Alzheimerovy nemoci s komorbidní synukleinopatií.

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RESEARCH ARTICLE

Different Morphology of Neuritic Plaques in the Archicortex of Alzheimer's Disease with Comorbid Synucleinopathy: A Pilot Study

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Abstract: ***Background:*** Bulbous neuritic changes in neuritic plaques have already been described, and their possible effect on the clinical course of the disease has been discussed. OBJECTIVE: In our study, we focused on the location and density of these structures in patients with only Alzheimer's disease (AD) and patients with AD in comorbidity with synucleinopathies.

Methods: Utilizing immunohistochemistry and confocal microscopy, we evaluated differences of neocortical and archicortical neuritic plaques and the frequency of bulbous changes in the archicortex of 14 subjects with Alzheimer's disease (AD), 10 subjects with the Lewy body variant of Alzheimer's disease (AD/DLB), and 4 subjects with Alzheimer's disease with amygdala Lewy bodies (AD/ALB). Also, the progression and density of neuritic changes over the time course of the disease were evaluated.

Results: We found structural differences in bulbous dystrophic neurites more often in AD/DLB and AD/ALB than in pure AD cases. The bulbous neuritic changes were more prominent in the initial and progressive phases and were reduced in cases with a long clinical course.

Conclusion: Our results indicate that there is a prominent difference in the shape and composition of neocortical and archicortical neuritic plaques and, moreover, that bulbous neuritic changes can be observed at a higher rate in AD/DLB and AD/ALB subjects compared to pure AD subjects. This observation probably reflects that these subacute changes are more easily seen in the faster clinical course of AD patients with comorbidities.

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1. INTRODUCTION

"Neuritic" plaques are extracellular amyloid-beta aggregates surrounded by dystrophic neurites, reactive astrocytes, and activated microglia. Dystrophic neurites can contain aggregates of a tau protein similar to the tau in neurofibrillary tangles [1]. The pathology of tau neuritic plaque varies from simple hyperphosphorylated protein tau-positive neurites to extremely dilated dystrophic neurites called bulbous neurites [2]. Loss of neurons and an association with cognitive impairment [3, 4] make this type of plaques essential for an Alzheimer's disease (AD) diagnosis [5]. In addition, some studies have reported that the CERAD score for neuritic plaques correlates better with clinical progression than the Braak NFTs stage and no correlation has been reported for amyloid-beta aggregates [6]. A different composition of "senile" plaques between AD patients, clinically non-AD morphological comparators, and transgenic murine AD models has already been described [7]. Protein substance changes in the human hippocampus during aging may be related to age-related loss of hippocampal function [8]; dystrophic and tau-positive neurites in hippocampal plaques have also been reported [9]. The composition of "senile" plaques in Alzheimer's related pathology partly varies not only depending on the clinical stage of the neurodegeneration [10] but also on differences in neuritic plaques between the cortical and hippocampal regions (archicortex), which correlate with the stage of Alzheimer's disease [11]. It is also known that different neuritic changes are found in AD versus in Lewy body dementia (DLB) [12, 13], which is characterized by aggregates of α-synuclein in the forms of Lewy bodies and Lewy neurites [14]. In some DLB cases, Alzheimer's changes are not sufficiently expressed, while in others, they are abundant [15], in which case they are called the Lewy body variant of Alzheimer's disease (AD/DLB) [16]. Ubiquitin-

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positive neurites in the CA2 region of the hippocampus are considered to be almost DLB-specific. However, these neurites also appear in small amounts in Parkinson's disease [17]. In addition, Lippa *et al.*, reported more apparent ubiquitin-positive, AT8-negative neurites in the hippocampal CA2 and CA3 regions in DLB patients, while fewer of these neurites have been observed in AD/DLB brains and none in pure AD [18].

According to some case reports, the cognitive decline in patients with AD/DLB compared to pure AD is much faster [19]. Hypokinetic extrapyramidal and neuropsychiatric symptoms such as depression, anxiety, and hallucinations are considered to be fairly typical for AD/DLB patients [20]. At the same time, clinical data on depression have been associated with an increased presence of plaques and tau-related pathology within the archicortex [21]. Patients with AD/DLB are also reported to have shorter times to institutionalization [22] and accelerated mortality [23, 24]. Alzheimer's disease with amygdala Lewy bodies (AD/ALB) was recently described as a distinct form of α-synucleinopathy [20].

In certain cases of AD/DLB and AD/ALB, we have observed the presence of prominent swollen bulbous dystrophic neurites in archicortical plaques containing hyperphosphorylated tau protein (AT8) and ubiquitin as the leading neuropathological findings. There is evidence that hyperphosphorylated tau protein plays a major role in neurodegeneration and that neurofibrillary tangles are not a necessary condition [25]. Therefore, we must consider whether the prominent dystrophic changes in neuritic processes of archicortical plaques could be related to Lewy bodies pathology in AD/DLB and/or in AD/ALB and whether these bulbous neuritic changes contribute to the speed of cognitive decline.

The aim of this study was to evaluate the possible differences of dystrophic, hyperphosphorylated tau- and ubiquitin-positive neurites in plaques in the neocortex and archicortex within cohorts of pure AD, AD/DLB, and AD/ALB patients.

2. MATERIALS AND METHODS

2.1. Patients

A total of 28 patients, divided into three cohorts, were enrolled in the study. The first cohort included patients with "pure" Alzheimer's disease without an alpha-synuclein pathology (14 cases, age range: 62–95 years, average age: 77.29 years, average age at disease onset: 71.00, average disease duration: 75.14 months, average post-mortem interval (PMI): 42.93 hours), which had been neuropathologically defined following the NIA-AA "ABC" consensus scheme [10, 26]. The second group included patients suffering from a combination of evolved AD and evolved diffuse Lewy body disease (10 cases, age range: 68–97 years, average age: 76.30 years, average age at disease onset: 72.10, average disease duration: 50.60 months, average PMI: 54.40 hours) with the neuropathology of alpha-synuclein deposits defined by Braak's [27] as well as McKeith's [28] staging system. The third cohort comprised patients with fully developed AD and alpha-synuclein pathology of Lewy type, predominantly in the limbic system, called Alzheimer's disease with amygdala Lewy bodies (4 cases, age range: 61–96 years, average age: 74.50 years, average age at disease onset: 63.00, average disease duration: 90.00 months, average PMI: 50.00 hours), which fulfilled previously published criteria [20]. The average values for the monitored groups of patients are recorded in Table **1**. Characteristics of individual patients are summarized in Supp. 1 with other possible vascular and/or age-related co-pathologies; however, the selection of patients was mainly focused on similar ages, disease durations, and severity of the AD-related pathologies. To monitor dystrophic changes over time, patients were divided into 2 subsets based on disease duration, i.e., a short duration group (2–36 months) and a long duration group (60–168 months). The data were analyzed with respect to patient privacy and with approval by the Ethics Committee of the Institute of Clinical and Experimental Medicine in Prague and Thomayer Memorial Hospital, No G-19-18. Summary of average values for the monitored groups of patients (Table **1**).

2.2. Tissue Samples

Brains and cervical spinal cords were fixed for 3-4 weeks in buffered 10% formalin and then selected tissue blocks, following a standardized protocol, were embedded in paraffin. Five μm-thick sections were prepared and stained with hematoxylin-eosin, Klüver-Barrera, and silver impregnation methods.

2.3. Immunofluorescence and Immunohistochemistry

Briefly, 5 μm thick sections of formalin-fixed and paraffin-embedded tissue samples were deparaffinized and then incubated with the primary antibody for 20 minutes at room temperature. In β-amyloid antibody staining, 96% formic acid was applied prior to the primary antibody. As a second layer of labeling for light microscopy visualization, a secondary horseradish peroxidase-conjugated antibody (EnVision FLEX HRP, Dako M822, DK) was applied for 20 minutes at room temperature. Then the samples were incubated with DAB (Substrate – Chromogen Solution, Dako DM827, DK) for 10 minutes to visualize the reaction. At the end of the procedure, Mayer's Hematoxylin Solution was applied.

For confocal microscopy, secondary antibodies conjugated to Alexa Fluor® (see below) were used. The paraffin sections were also treated with 20X TrueBlack® (Biotium 23007, USA) diluted in 1X 70% alcohol to quench lipofuscin autofluorescence.

2.3.1. Primary Antibodies

For immunohistochemistry, 5 μm thick sections of formalin-fixed and paraffin-embedded tissue were selected from the hippocampal region and the entorhinal and transentorhinal cortex. The sections were incubated with primary antibodies against the following antigens: (1) β-amyloid (1:1000, mouse monoclonal – clone 6F/3D; Dako M0872, DK), (2) Phospho-Tau (Ser202, Thr205) Monoclonal Antibody (1:500, mouse monoclonal – clone AT8; Thermo Fisher Scientific MN1020, USA), (3) Ubiquitin (1:500, rabbit polyclonal; Dako Z0458, UK).

Table 1. Summary of average values for the monitored groups of patients.

Diagnose (No. of Cases)	Sex (M/F)	Average Age at Disease Onset (Years)	Average Age of Death (Years)	Average Disease Duration (Months)	Average Post-Mortem Interval (Hours)	ABC Score
AD (14)	8/6	71.00	77.29	75.14	42.93	14x A3B3C3
AD/DLB (10)	7/3	72.10	76.30	50.60	54.40	8x A3B3C3 2x A3B2C3
AD/ALB (4)	2/2	63.00	74.50	90.00	50.00	4x A3B3C3

2.3.2. Secondary Antibodies

Detection of immunostaining was carried out using the horseradish peroxidase–diaminobenzidine system (Envision FLEX HRP, Dako, DK) for immunohistochemistry and secondary antibodies conjugated with Alexa Fluor® 488 (1:1000, donkey anti-rabbit, H+L IgG, Thermo Fischer Scientific, USA) and Alexa Fluor® 568 (1:1000, donkey anti-mouse, H+L IgG, Thermo Fischer Scientific, USA) for immunofluorescent staining. Slides incubated with only the secondary antibody were used as specificity controls. DAPI (VECTASHIELD® Mounting Medium with DAPI, Vector Laboratories Ltd., UK) was used as a nuclear counterstain.

2.4. Microscopy Evaluation

2.4.1. Light Microscopy

Samples were examined by two pathologists. The frequency of bulbous dystrophic neurites in neuritic plaques and the frequency of these plaques were semiquantitatively evaluated in different regions of the archicortex/hippocampal formation (subiculum, dentate gyrus, cornu ammonis) on a 0–4 scale.

Comparisons of density and frequency of bulbous dystrophic neurites were categorized into 5 groups:

(a) Grade 0: the absence of bulbous dystrophic neurites. In these cases, archicortical plaques contained only a few thin dystrophic neurites that were quite similar to those in the neocortex; none of the neurites showed bulbous morphology. Plaques were present in relatively small amounts.

(b) Grade 1: sporadic/individual positivity of bulbous dystrophic neurites. Single plaques with a hint of bulbous morphology of dystrophic neurites were found.

(c) Grade 2: a mild presence of bulbous dystrophic neurites. In about half of the plaques present in the archicortex, bulbous dystrophic neurites were the most obvious feature. Compared to the previous grade, there is an increase in the total number of plaques.

(d) Grade 3: moderate positivity of bulbous dystrophic neurites. The total number of plaques, as well as the number of plaques having prominent bulbous neurites, increased, although a few plaques with discreet thin neuritic changes were still present.

(e) Grade 4: abundant diffuse positivity of bulbous dystrophic neurites. Essentially only plaques with markedly noticeable bulbous dystrophic changes were found in the archicortex. Cases with a dense population of plaques having a significantly different morphology compared to plaques in the neocortex were described as Grade 4.

2.4.2. Confocal Microscopy

Co-expression of pathogenic protein aggregates was imaged using a Leica TCS SP5 confocal fluorescent laser scanning microscope (Leica Microsystems Inc., Wetzlar, Germany). The HCX PL APO objective was chosen with 40x magnification, oil immersion, and a pinhole of 1 AU. Antirabbit donkey IgG secondary antibody was conjugated with Alexa Fluor® 488 and excited at 488 nm using a 65 mW multi-line argon laser, whereas anti-mouse donkey IgG was conjugated with Alexa Fluor® 568 Donkey at 561 nm using a 20 mW DPSS laser.

2.5. Statistical Analysis

Due to the small size of the statistical samples, the AD/ALB group was not included. The differences in grades between the AD and AD/DLB groups were investigated using the two-sided, two-sample Wilcoxon-Mann-Whitney test of median equity. The correlations between the AT8 and ubiquitin grades were investigated separately for the AD and AD/DLB groups. The relationships between group memberships and grade levels were studied using Fisher's exact test with 2x2 contingency tables, two groups of grade levels, i.e., LOW (0, 1, 2) and HIGH (3, 4) were used. A more stringent approach compared only grade 4 with the rest of the grades. The MATLAB Statistical Toolbox (MathWorks, Massachusetts) was used, and a significance level of 0.05 was used in all cases.

3. RESULTS

3.1. Morphological Comparison of Archicortical and Neocortical Plaques

In neocortical plaques, the dominant amyloid structure was accompanied by "regular" dystrophic neurites visualized by antibodies against hyperphosphorylated tau (AT8) and ubiquitin. In contrast, in the archicortex (particularly the cornu ammonis), dystrophic bulbous neurites visualized using antibodies against hyperphosphorylated protein tau (AT8), and ubiquitin were the most prominent plaques features, while central amyloid deposits were only a minor component (Fig. **1**).

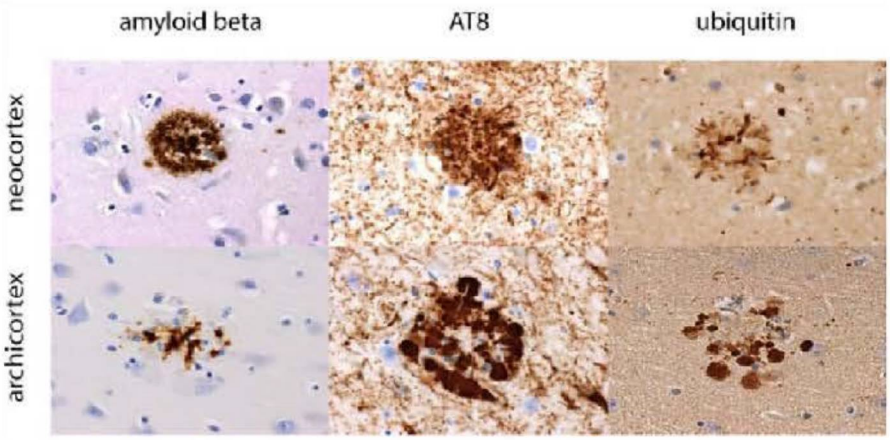

Fig. (1). Observations of prominent extremely dilated, bulbous dystrophic neuritic changes detected by AT8 and ubiquitin antibodies in the archicortex of an AD/DLB patient compared with neocortical findings. The secondary antibody was conjugated with horseradish peroxidase staining DAB. The original magnification was 400x. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.1.1. Characteristics of Neocortical Plaques

In the neocortex (i.e., the entorhinal and transentorhinal cortex), the most predominant part of the plaques was beta-amyloid, with only thin and curvilinear dystrophic neurites in the vicinity of plaque.

Neocortical plaques were either cored or non-cored diffuse plaques (green) with or without common dystrophic neurites. No bulbous changes in dystrophic neurites were observed. Our records confirm our previous immunohistochemistry observations showing that amyloid-beta was the predominant component of cortical plaques, while common dystrophic AT8 positive neurites, if present, were only a minor component (Fig. **3**).

3.1.2. Characteristics of Archicortical Plaques

In the archicortex (cornu ammonis), the co-expression of hyperphosphorylated tau protein (AT8, red) and ubiquitin (green) in the plaques with dystrophic bulbous neurites was recorded. Beta-amyloid was stained using blue-fluorescent DAPI, which is known for reacting with dense-core and diffuse amyloid plaques. Our observation showed a different level, but unequivocal positivity of hyperphosphorylated tau protein and ubiquitin in dystrophic bulbous neurites in the archicortex. However, while hyperphosphorylated tau protein was diffusely present, ubiquitin positivity was mottled (Fig. **2**).

In the archicortex (cornu ammonis), no co-expression of the hyperphosphorylated tau protein (AT8, red) and beta-amyloid (green) in the plaques with dystrophic bulbous neurites was recorded. Our current observations confirm our previous immunohistochemistry observations that dystrophic AT8 positive bulbous neurites, but not beta-amyloid, were the predominant feature of archicortical plaques (Fig. **3**).

In DLB/ALB patients, prominent bulbous dystrophic changes were recorded in the archicortex (particularly the cornu ammonis), while neocortical plaques exhibited a "common" appearance with either diffuse or cored amyloid-predominant structures (Fig. **1**). Utilizing immunofluorescence confocal microscopy, the co-expression of hyperphosphorylated tau protein (AT8) together with ubiquitin was prominently found in the bulbous dystrophic neurites of archicortical plaques (Fig. **2**), while amyloid-beta was only a minor component of these plaques (Fig. **3**). In neocortical plaques, common diffuse (or fibrillar) or cored, with predominant amyloid-beta structures with an admixture of common dystrophic neurites, were typically observed (Fig. **3**).

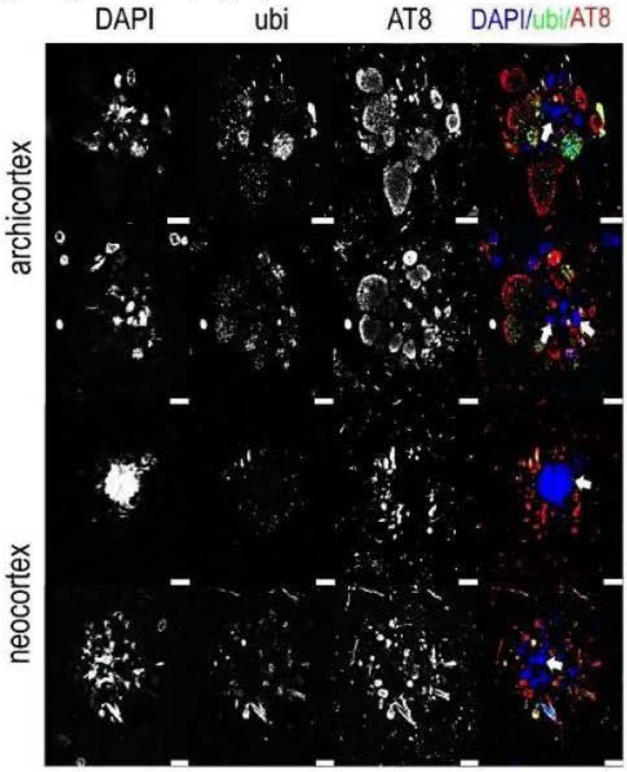

Fig. (2). Immunofluorescence visualization of different dystrophic neuritic changes in the archicortex and neocortex of an AD/DLB patient. Bulbous neuritic changes can be observed in the archicortical region. Primary antibodies: AT8 (murine antihyperphosphorylated protein tau) and anti-ubiquitin rabbit IgG. DAPI commonly counterstains nuclei but also dense fibrillar amyloid deposits (arrows).

The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (antimouse IgG, red). Scale bars indicate 10 micrometers. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

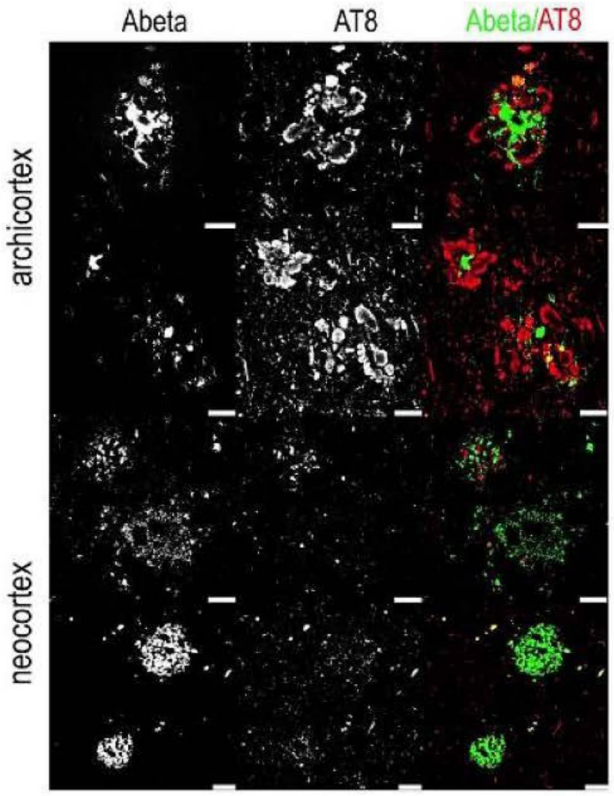

Fig. (3). Immunofluorescence visualization of common amyloid plaques in the archicortex and neocortex of an AD/DLB patient. Bulbous neuritic changes can be observed in the archicortical region. Primary antibodies: AT8 (murine anti-hyperphosphorylated protein tau) and anti-beta amyloid rabbit IgG. The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). Scale bars indicate 25 micrometers. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. Neuritic Changes in Pure AD Cases

Grade of bulbous neuritic changes in AD cases (%) (Table **2**).

3.2.1. Tau-Immunoreactive Bulbous Neurites

In a group of 14 patients with Alzheimer's disease, bulbous dystrophic neurites with a positive immunohistochemical reaction with antibodies against hyperphosphorylated tau protein (grade 4) were not present. Moderate positivity for bulbous dystrophic neurites (grade 3) was noticed in 14.29%. Mild positivity (grade 2) was observed in 28.57%. In 35.71%, sporadic individual positivity among single dystrophic neurites (grade 1) was detected. Samples with or without thin curvilinear dystrophic neurites lacking bulbous changes (grade 0) were present in 21.43% of AD cases.

3.2.2. Ubiquitin-Immunoreactive Bulbous Neurites

In the immunohistochemical reaction with anti-ubiquitin antibody, massive bulbous changes (grade 4) were observed in 7.14% of cases. Moderate changes (grade 3) were found in 28.57%. Mild changes (grade 2), the most common, were seen in 35.71%. A sporadic incidence of bulbous neurites (grade 1) was recorded in 21.43% cases. An absence of bulbous changes (grade 0) was recorded in 7.14% of AD/DLB cases.

3.3. Neuritic Changes in AD/DLB Cases

Grade of bulbous neuritic changes in AD/DLB cases (%) (Table **3**).

3.3.1. Tau-Immunoreactive Bulbous Neurites

In the Lewy body variant of Alzheimer's disease, 10 cases with a known duration were available. In the immunohistochemical reaction with an antibody against hyperphosphorylated tau protein, severe dystrophic changes with an abundance of bulbous neurites (grade 4) were observed in 20.00% cases. Moderate changes (grade 3), the most common finding, were seen in 30.00% cases. Mild changes (grade 2) were reported in 20.00% cases. The sporadic incidence of individual bulbous neurites (grade 1) was reported, the least common, in 10.00% of cases. Samples lacking bulbous dystrophic neurites were recorded in 20.00% cases.

Table 2. Grade of bulbous neuritic changes in AD cases (%).

Alzheimer's disease: 14 cases (age range: 62–95 years, average age: 77.29 years, average age at disease onset: 71.00, average disease duration: 75.14 months, average postmortem interval (PMI): 42.93 hours)				
Grade	AT8 Total Number	AT8 Percentage	Ubiquitin Total Number	Ubiquitin Percentage
0	3	21.43 %	1	7.14 %
1	5	35.71 %	3	21.43 %
2	4	28.57 %	5	35.71 %
3	2	14.29 %	4	28.57 %
4	0	0.00 %	1	7.14 %

Table 3. Grade of bulbous neuritic changes in AD/DLB cases (%).

Lewy body variant of Alzheimer's disease: 10 cases (age range: 68–97 years, average age: 76.30 years, average age at disease onset: 72.10, average disease duration: 50.60 months, average PMI: 54.40 hours)				
Grade	**AT8 Total Number**	**AT8 Percentage**	**Ubiquitin Total Number**	**Ubiquitin Percentage**
0	2	20.00 %	1	10.00 %
1	1	10.00 %	1	10.00 %
2	2	20.00 %	4	40.00 %
3	3	30.00 %	1	10.00 %
4	2	20.00 %	3	30.00 %

Table 4. Grade of bulbous neuritic changes in AD/ALB patients (%).

Alzheimer's disease with amygdala Lewy bodies: 4 cases (age range: 61–96 years, average age: 74.50 years, average age at disease onset: 63.00, average disease duration: 90.00 months, average PMI: 50.00 hours)				
Grade	**AT8 Total Number**	**AT8 Percentage**	**Ubiquitin Total Number**	**Ubiquitin Percentage**
0	1	25.00 %	1	25.00 %
1	1	25.00 %	0	0.00 %
2	1	25.00 %	1	25.00 %
3	0	0.00 %	1	25.00 %
4	1	25.00 %	1	25.0

3.3.2. Ubiquitin-Immunoreactive Bulbous Neurites

The ubiquitin-immunoreactive bulbous neurites (grade 4) were observed in the hippocampus of 30.00% of patients with Lewy body variant of Alzheimer's disease. Moderate changes (grade 3) were observed in 10.00%. Mild changes (grade 2) were found in 40.00%, while individual positivity (grade 1) and the absence of bulbous dystrophic neurites (grade 0) were seen in 10.00% of AD/DLB cases.

3.4. Neuritic Changes in AD/ALB Cases

Grade of bulbous neuritic changes in AD/ALB patients (%) (Table **4**).

3.4.1. Tau-Immunoreactive Bulbous Neurites

The third study group was 4 patients with Alzheimer's disease with amygdala predominant Lewy bodies. The group included our first AD/ALB case, where extremely prominent dilated dystrophic neurites showed the presence of both antitau and anti-ubiquitin antibodies. Abundant bulbous tau-positive changes (grade 4) were found in 25.00%. Moderate changes (grade 3) were not seen in this group. Mild changes (grade 2), sporadic changes (grade 1), and the absence of bulbous changes (grade 0) were each present in 25.00% of cases.

3.4.2. Ubiquitin-Immunoreactive Bulbous Neurites

In the analysis of ubiquitin-immunoreactive swollen neurites, in addition to 25.00% with abundant changes (grade 4),

25.00% had moderate (grade 3) changes, and 25.00% had mild (grade 2) changes. Individual bulbous neurites (grade 1) were not present in this group. In 25.00% of cases, there was no evidence of similarly altered neurites (grade 0).

3.5. Bulbous Dystrophic Changes and Disease Duration

It was evident in both AD and AD/DLB patients that the changes were more prominent in cases with shorter survival times (see Fig. **6**).

3.5.1. Tau-Immunoreactive Bulbous Neurites

For tau-immunoreactive neurites, the short duration group had an average grade of 1.80 in the AD group of patients, while in the long duration group, the average grade was 1.10. For AD/DLB cases, tendencies over time were the same. Patients in the group of patients with AD/DLB and with a short disease duration had an average grade of 2.50, while patients with a long duration had an average grade of 1.75.

3.5.2. Ubiquitin-Immunoreactive Bulbous Neurites

The average degree of ubiquitin-positive bulbous neurites was 2.60 in the group of patients with AD and short disease duration, while in those with a long duration, the number decreased to 1.78. In AD/DLB cases, the average grade was 3.17 for short duration cases and 1.75 for the long duration cases.

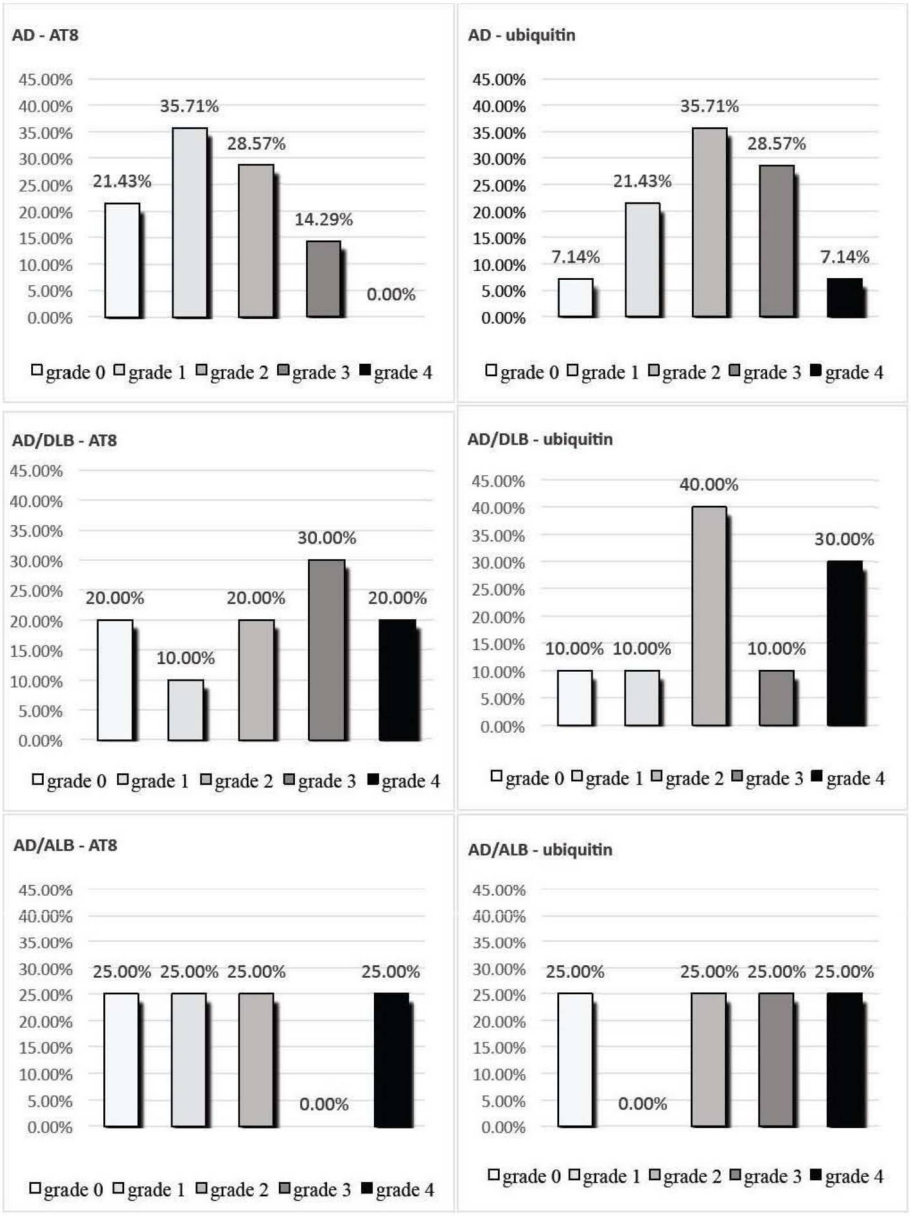

Fig. (4). Percentage comparison of bulbous dystrophic neurites in AD, AD/DLB, and AD/ALB subsets (the grading system is described in more detail in the methodology-light microscopy section). Grades of the bulbous changes are defined on the x-axis, their percentages in AD, AD/DLB, and AD/ALB cases are on the y-axis (%).

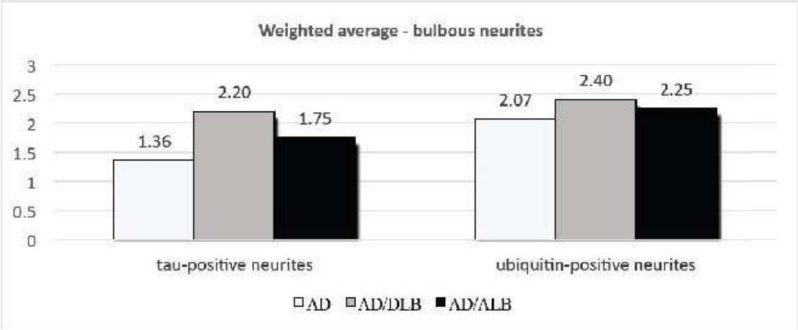

Fig. (5). Comparisons of bulbous neuritic changes were made on the basis of the calculated weighted averages for each of the monitored diseases. On the x-axis, the numbers for tau-immunoreactive bulbous neuritic changes are on the left, and the ubiquitin-reactive changes are on the right. The y-axis shows the average numerical values of bulbous changes in AD, AD/DLB, and AD/ALB.

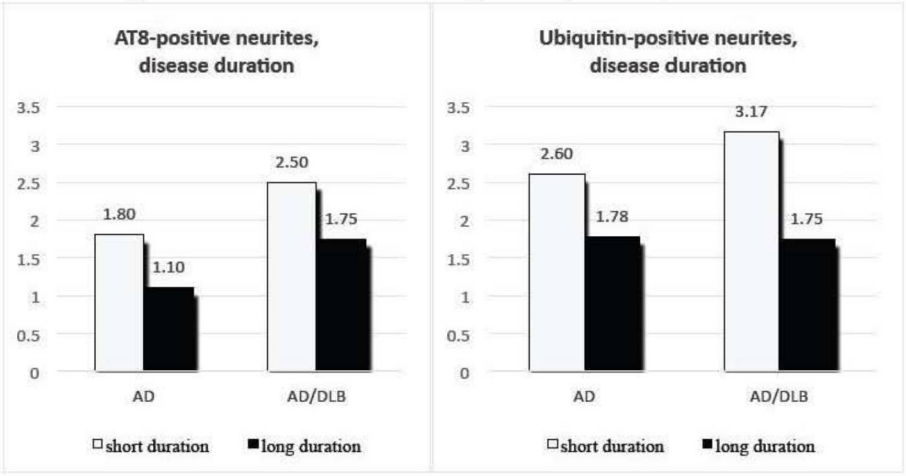

Fig. (6). Comparison of the average extent of bulbous dystrophic changes in cases with short and long disease duration. Values for AD are recorded on the left of each graph, and AD/DLB is on the right. Correlations with disease duration could not be assigned in the AD/ALB group due to the inclusion of cases without available disease duration information.

Due to the small number of AD/ALB cases, those with no duration information were included in the study; therefore, averages could not be determined.

3.6. Comparison of the Frequency of Tau- and Ubiquitin-positive Bulbous Neurites

Bulbous neurites were slightly more frequent in anti-ubiquitin than in anti-tau detection (Figs. **4** and **5**). The extent of AT8-positive vs. ubiquitin-positive neuritic changes varied at most by one grade except in 2 of the 28 cases (Supp. 1).

3.7. The Average Grade of Bulbous Neuritic Changes

The average grade was obtained by calculating weighted averages (Fig. **5**). The average for AT8-positive bulbous neuritic changes was 1.36 in AD cases, 2.20 in AD/DLB, and 1.75 in AD/ALB.

For ubiquitin-positive neurites, we observe generally higher numbers but the same tendency. The average number was 2.07 for pure AD cases, 2.40 for AD/DLB, and 2.25 for AD/ALB cases.

3.8. Statistical Analysis

The group of AD/DLB (10 patients) had a median AT8 grade that was nonsignificantly higher than the AD group (14 patients; $p = 0.1335$). The median for ubiquitin was the same, and the difference was nonsignificant (NS). There was a positive but nonsignificant correlation between AT8 and ubiquitin grades ($r = 0.4730$, $p = 0.0876$) in the AD group. But the correlation between AT8 and ubiquitin grades ($r = 0.7920$, $p = 0.0063$) was strongly statistically significant in the AD/DLB group. The contingency table for the LOW and HIGH groups of AT8 grades for AD and AD/DLB patients had a $p = 0.0850$, which was positive but had a nonsignificant dependence. In the case of ubiquitin, a $p = 1.000$ was an indicator of independence but was also NS.

4. DISCUSSION

In this study, we identified a prominent difference in the shape and composition of neocortical and archicortical plaques. For the first time, we precisely located prominent bulbous dystrophic neuritic changes in the archicortex. Additionally, we recorded different rates of these bulbous dystrophic changes between cohorts of AD and AD/DLB and AD/ALB patients, although, due to the small number of patients in the study, these were statistically nonsignificant. Our study showed that both AD/ALB and AD/DLB patients had swollen

bulbous-dystrophic neurites located in the archicortex, which were found more frequently than in patients with pure AD; however, due to the small numbers of cases in the study, it was without precise statistical significance.

The term "bulbous" change was previously used to describe dilated neurites [2] and certain levels of bulbous dilated, ubiquitin-positive neurites have been previously reported in AD subjects; however, usually without information regarding their exact location in the brain [2, 29, 30, 31]. Bulbous AT8 positive neurites were recorded in the archicortex of the transgenic 5xFAD murine model of AD, which was related to evidence that amyloid plaque in the surroundings facilitates the development of tau pathology [32]. This fact is also supported by current evidence showing that amyloid-beta plaques start to appear long before cognitive decline [30]. Generally, dystrophic neurites are common components of neuritic plaques, and their distribution in the neocortex and archicortex correlates with the Braak stage in AD [11]. Archicortical degeneration with dystrophic, ubiquitin-positive neurites differentiates AD from DLB; however, bulbous dystrophic changes were not previously reported [33].

On the other hand, a certain proportion of amyloid-beta negative plaques has been seen in the archicortex [9]. It seems that the difference between plaques in the neocortex and archicortex is primarily caused by the difference in composition and cytoarchitecture of these two developmentally distinct cortical regions. Similar findings in neocortical plaques lacking AT8-immunoreactive neurites have also been reported [34, 35], while archicortical plaques predominantly contain diffuse neuritic changes [36]. Thus, it could be speculated that differences reported in amyloid-beta plaques in the quantitative proteomics composition between non-AD, AD, and transgenic mice [7] may have been caused by location differences (i.e., hippocampus and temporal cortex). Non-AD has a predominantly neocortical location without dystrophic neurites, while in mice, the archicortex has approximately 40% of the volume of the cortical coronal sections. However, other mechanisms related to AD development, including dysregulation of lipid and glucose metabolism pathways [37], lower protein synthesis, and subsequent mitochondrial loss, must also be considered [38].

Our data suggest that the shift to the prominently bulbous form (grade 4) is more frequently recorded in AD/DLB (20% and 30% for AT8 and ubiquitin positivity, respectively) and in AD/ALB (25% and 25% for AT8 and ubiquitin positivity, respectively) compared to pure AD (0% and 7% for AT8 and ubiquitin positivity, respectively). The positive trend (lacking statistical significance) can be explained by the limited number of patients in the individual cohorts, and a much larger study should be conducted to obtain statistically more meaningful data and further evidence regarding this trend.

Our data, however, generally provide evidence that the bulbous dystrophic changes in neurites are more prominent in shorter courses of the disease (up to 36 months) rather than in longer courses of the disease (more than 60 months, see Fig. **6**). This is not surprising considering (1) that the AD/DLB pathology contributes to the development of faster cognitive loss than in AD alone [23, 39] and (2) that neuritic plaques and cognitive impairment are partly correlated [40, 41]. Some studies have even suggested that the presence or absence of morphological changes in neurites around plaques plays a critical role in the clinical course of the disease [3]. Based on the lower expression of neuritic changes in patients with long-duration AD and AD/DLB, we hypothesize that bulbous dystrophic changes are related to the initial or the progressive phase of the condition; therefore, the faster cognitive loss and faster progression to death observed in AD/DLB only help to reveal these sub-acute changes. Also, the 5xFAD murine model of AD shows that bulbous changes in the archicortex occur within one year [32]. Although there is a lack of data directly related to bulbous neuritic changes, our assumptions are supported by the studies focused on the association between dystrophic neurites and disease duration. The association is positive in the initial stages, which then becomes negative as the number of neuritic changes diminishes over the longer course of the disease [42].

CONCLUSION

Based on our pilot study, and with confirmation from our literature review, we find that (1) initially there are prominent differences in the shape and composition of neocortical and archicortical plaques, and (2) bulbous neuritic changes are expressed at higher rates in AD/DLB and AD/ALB patients compared to those with AD. We speculate that these changes appear in the early stages of the disease, and its rapid progression simply serves to make these differences visible rather than the differences themselves, causing the rapid progression. Further investigations with larger cohorts are needed for a complete statistical evaluation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The data were analyzed with respect for patients' privacy with the agreement of the Ethics Committee of the Institute of Clinical and Experimental Medicine in Prague and Thomayer Memorial Hospital, No G-19-18.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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3.2 Mikromorfologická charakterizace extracelulárních proteinových depozit u komorbidních případů Creutzfeldtovy–Jakobovy choroby a Alzheimerovy nemoci, popis dystrofických neuritických změn v plakách PrP^{Sc}.

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Extracellular Protein Aggregates Colocalization and Neuronal Dystrophy in Comorbid Alzheimer's and Creutzfeldt–Jakob Disease: A Micromorphological Pilot Study on 20 Brains

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Abstract: Alzheimer's disease (AD) and sporadic Creutzfeldt–Jakob disease (sCJD) are both characterized by extracellular pathologically conformed aggregates of amyloid proteins—amyloid β-protein (Aβ) and prion protein (PrP^{Sc}), respectively. To investigate the potential morphological colocalization of Aβ and PrP^{Sc} aggregates, we examined the hippocampal regions (archicortex and neocortex) of 20 subjects with confirmed comorbid AD and sCJD using neurohistopathological analyses, immunohistochemical methods, and confocal fluorescent microscopy. Our data showed that extracellular Aβ and PrP^{Sc} aggregates tended to be, in most cases, located separately, and "compound" plaques were relatively rare. We observed PrP^{Sc} plaque-like structures in the periphery of the non-compact parts of Aβ plaques, as well as in tau protein-positive dystrophic structures. The AD ABC score according to the NIA-Alzheimer's association guidelines, and prion protein subtype with codon 129 methionine–valine (M/V) polymorphisms in sCJD, while representing key characteristics of these diseases, did not correlate with the morphology of the Aβ/PrP^{Sc} co-aggregates. However, our data showed that PrP^{Sc} aggregation could dominate during co-aggregation with non-compact Aβ in the periphery of Aβ plaques.

Keywords: Creutzfeldt–Jakob disease; Alzheimer's disease; Aβ; prion protein; tau protein; colocalization; plaques; confocal microscopy

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1. Introduction

Deposits of extracellular protein aggregates are diagnostic findings for two separate neurodegenerative diseases, i.e., Alzheimer's (AD) and Creutzfeldt–Jakob diseases (CJD) [1,2]. Amyloid-β peptide (Aβ) is a main defining component of Aβ plaques (also called amyloid or senile plaques) observed in AD [3,4]. These extracellular deposits arise from the amyloidogenic cleavage of an integral membrane protein, called amyloid precursor protein (APP), by beta-site APP cleaving enzyme 1 (β-secretase/BACE 1), which is found on neuronal membranes [5]. In addition to APP and BACE 1, the physiological isoform of the prion protein (PrP^{C}) is also found on the outer surface of neuronal membranes; it is attached to the membrane via a glycosylphosphatidylinositol (GPI) anchor [6].

A full understanding of the physiological role of Aβ and PrP^{C} remains elusive. Briefly, Aβ plays a critical role in brain development, neuronal migration, and synaptic plasticity [7]. Additionally, Aβ interacts with Cu and Zn ions, e.g., rising copper levels increase the amount of APP on cell surfaces [8]; therefore, the increased presence of Cu ions mediates the precipitation of Aβ deposits [9]. Data from murine gene knock-outs suggest a functional

role for PrP^C in myelination maintenance in adults, neuronal plasticity in adults, and the circadian rhythm [10].

Currently, molecular interactions between Aβ and PrP, in either physiological or pathological forms, are being widely investigated, with interactions between oligomeric Aβ and physiological PrP^C receiving particular attention [11]. Other studies have focused on transfected SH-SY5Y neuroblastoma cells, cellular overexpression of PrP, decreased amyloidogenic cleavage of APP, and silencing of PrP^C genes in N2A cells, via the increased secretion of Aβ [12].

It has also been shown that the scrapie isoform of prion protein (PrP^{Sc}) could alter APP processing through stimulation of 3-phosphoinositide-dependent protein kinase 1 (PDK1 or PDPK1) and the inhibition of alpha-secretase activity, which could lead to enhanced β-secretase processing accompanied by increased Aβ production [13]. There is another connection between these two proteins; as γ-secretase cleaves the residual APP C-terminal fragment, thus creating Aβ, it leaves behind the amyloid intracellular domain (AICD) [14], which according to recent research, controls the expression of PrP^C [15].

Membrane PrP^C acts as a receptor for Aβ oligomers; this feature helps explain its involvement in AD development [16]. Nonetheless, both AD and CJD have been described as having very similar dystrophic neurites containing mostly autophagic vacuoles and autophagosomes [17].

Even though microtubule-associated protein (MAP) tau mainly forms intracellular amyloid aggregates in AD, its functional interaction with PrP^C and PrP^{Sc} has also been reported. PrP^C probably plays a critical role related to Aβ and tau protein in AD development [18], with PrP^C acting as a mediator of synaptic dysfunction induced by tau protein [19]. It is not unreasonable to expect dystrophic neurites with hyperphosphorylated tau protein in neuritic amyloid plaques. As such, dystrophic neurites in plaque-like PrP^{Sc} structures that colocalize with Aβ would also not be unexpected. There is increasing evidence that more than one neurodegeneration in the brain is possible at the same time [20]. However, the precise interactions among crucial amyloidogenic proteins in the pathophysiology of neurodegenerations remain unclear. Moreover, there is only limited information related to the morphological interactions among these brain peptides during comorbid neurodegenerations.

In our pilot study, we evaluated using immunohistochemistry and confocal microscopy, the micromorphology of PrP^{Sc} colocalized with Aβ in dystrophic neurites with compound plaques in the brains of patients with comorbid Alzheimer's and Creutzfeldt–Jakob disease [21].

2. Results

2.1. Confocal Microscopy Visualization

Using confocal fluorescent microscopy, we observed relatively rare compound plaques with either Aβ or hyperphosphorylated tau protein (h-tau) in colocalization with PrP^{Sc}. In co-aggregation with Aβ, PrP^{Sc} aggregates colocalized mainly with the non-compact (diffuse) regions of Aβ plaques, while only minor colocalization was observed in the dense regions (see Figures 1 and 2). In contrast, no colocalization between h-tau and PrP^{Sc} was observed, except for a few dot-like colocalizations (see Figure 3).

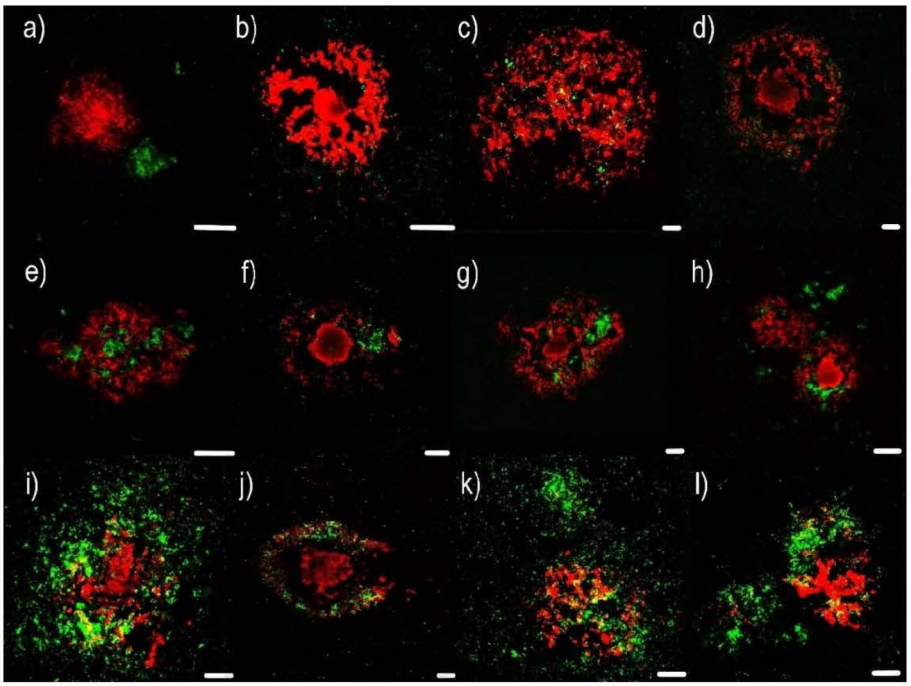

Figure 1. Immunofluorescence visualization of different types of anti-amyloid β-protein (Aβ) and prion protein (PrP^{Sc}) compound plaques in comorbid Alzheimer's (AD) and Creutzfeldt–Jakob diseases (CJD) cases. Primary antibodies: anti-PrP (rabbit recombinant monoclonal antibody) + anti-amyloid β-protein (mouse monoclonal antibody). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG; green) or Alexa Fluor® 568 (anti-mouse IgG; red). Scale bars indicate 10 micrometers. Images come from the hippocampal region (archicortical parts). (**a**,**b**) Non-compound plaques: consisting of (**a**) Aβ diffuse or (**b**) cored neuritic plaques, and lacking co-aggregating PrP structures. Images come from a 75-year-old female suffering from sCJD + AD (A1B1C1), cerebral amyloid angiopathy (CAA) 0. (**c**,**d**) Minimal compound plaques: diffuse (**c**) or cored neuritic (**d**) Aβ plaques with punctate PrP aggregates. Images come from a 69-year-old female suffering from sCJD + AD (A2B2C1), CAA 0. (**e**–**h**) Central core deposits: Neuritic non-cored (**e**) or cored (**f**–**h**) Aβ plaques with distinct PrP^{Sc} aggregates in the center of the plaque. Images come from two patients—a 79-year-old man suffering from sCJD + AD (A2B2C2), CAA 0, + ARTAG + Fahr disease, and a 71-year-old female sCJD + AD (A2B1C2), CAA. (**i**,**j**) Diffuse compound plaques: these are (**i**) neuritic non-cored or (**j**) cored Aβ plaques where co-aggregation of PrP^{Sc} at the periphery in the area of the non-compact Aβ structures is evident. The samples come from a 64-year-old male suffering from sCJD + early-onset AD (A2B2C2), CAA 0 + Wernicke's encephalopathy. (**k**,**l**) Diffuse "Yin-Yang" compound plaques: neuritic non-cored Aβ plaques having a prominent admixture of PrP^{Sc} co-aggregation predominantly localized at one pole of the plaque. The images come from a 70-year-old female patient sCJD + AD (A2B2C3) + CAA 0.

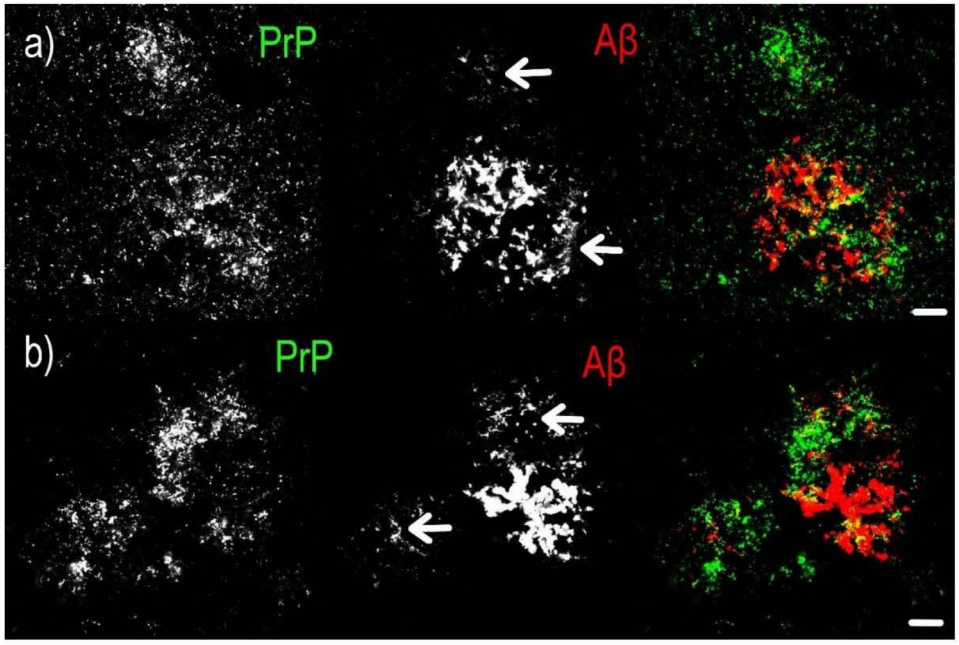

Figure 2. Immunofluorescence visualization and visualization of separated channels showing colocalization of PrP^{Sc} with Aβ in two different (**a**,**b**) non-cored plaques to demonstrate colocalization with more examples. PrP^{Sc} aggregates colocalized predominantly with non-compact Aβ of the senile plaques, while only a minor colocalization was observed in the dense parts. Both images (**a**,**b**) show diffuse "Yin-Yang" compound plaques. Arrows point to non-compact areas of Aβ plaques. Scale bars indicate 10 micrometers.

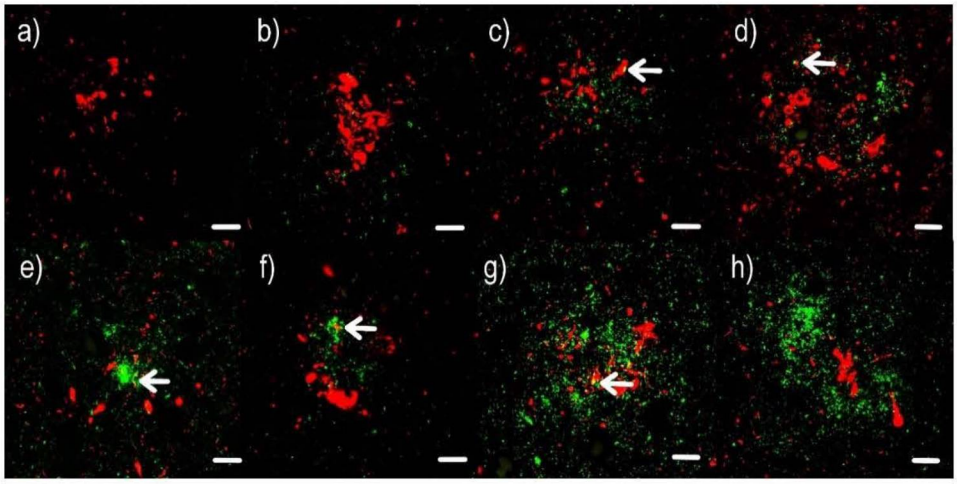

Figure 3. Immunofluorescence visualization of h-tau-positive dystrophic neurites in colocalization with PrP^{Sc} aggregates in comorbid AD and CJD cases (**a**–**h**). Primary antibodies: PrP (rabbit recombinant monoclonal antibody) + AT8 (mouse monoclonal antibody). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG, green) or Alexa Fluor® 568 (anti-mouse IgG, red). Scale bars indicate 10 micrometers. Arrows indicate minor colocalization of AT8 with PrP. Images come from the hippocampal region (archicortical parts).

Visualization of comorbid CJD and AD cases revealed that plaques varied with regard to the micromorphologies of Aβ with PrP^{Sc} between patients. In all subjects, one particular type of Aβ and PrP^{Sc} colocalization predominated. The main types of Aβ and PrP^{Sc} plaque colocalizations identified were (Figure 1):

(1) Non-compound and minimal compound plaques (10 cases out of 17):
   a. Non-compound plaques (observed in 3 cases out of 17) are without co-occurrence or colocalization of Aβ and PrPSc deposits. Pure Aβ and pure PrPSc plaque exist independently of each other (Figure 1a,b).
   b. Minimal compound plaques (Figure 1c,d) were seen most often (7 of 17 patients in whom PrPSc aggregates were present in the neocortical and archicortical parts of the hippocampal region). The most prominent feature of minimal compound plaques was Aβ (in the form of non-cored or cored plaque); however, dotted PrPSc-immunoreactivities were also present.

(2) Central core deposits—this pattern occurred in both non-cored and cored Aβ plaques (3 cases out of 17). In these cases, a rather significant PrPSc positivity was observed in central non-compact Aβ plaque structures, either with or without dense Aβ cores (Figure 1e–h).

(3) Diffuse plaques (4 cases out of 17):
   a. Diffuse compound plaques (Figure 1i,j) contain PrPSc diffusely scattered in the periphery of condensed Aβ plaques and are colocalized with surrounding non-compact Aβ (seen in a single case).
   b. Diffuse so-called "Yin Yang" compound plaques (Figure 1k,l; Figure 2) are a particular subset of asymmetric diffuse compound plaques in which PrPSc-positive structures are polarized to the sides of asymmetric plaques in colocalization with non-compact Aβ periphery (i.e., non-compact Aβ in colocalization with PrPSc, but not surrounding the entire circumference of the compact Aβ core aggregates). This type of plaques was observed in 3 cases out of 17.

Similar to colocalization with Aβ in plaques, minimal dot-like colocalization with h-tau positive dystrophic neurites in plaques was recorded (Figure 3).

We analyzed the density and type of colocalization relative to the biochemical and neuropathological properties of CJD and AD; however, no association between plaque micromorphology, type of colocalization, polymorphism at codon 129, type of PrP^{Sc}, and the AD ABC score according to the NIA-Alzheimer's association guidelines was observed (Charts 1–3).

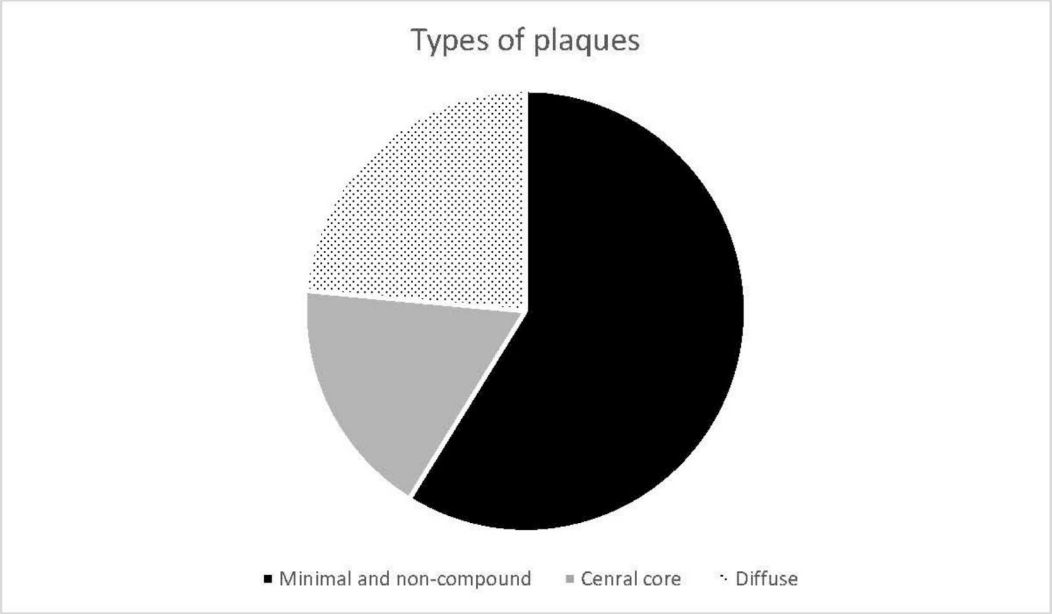

Chart 1. Relative rate of individual types of Aβ and PrP^{Sc} colocalization in plaque.

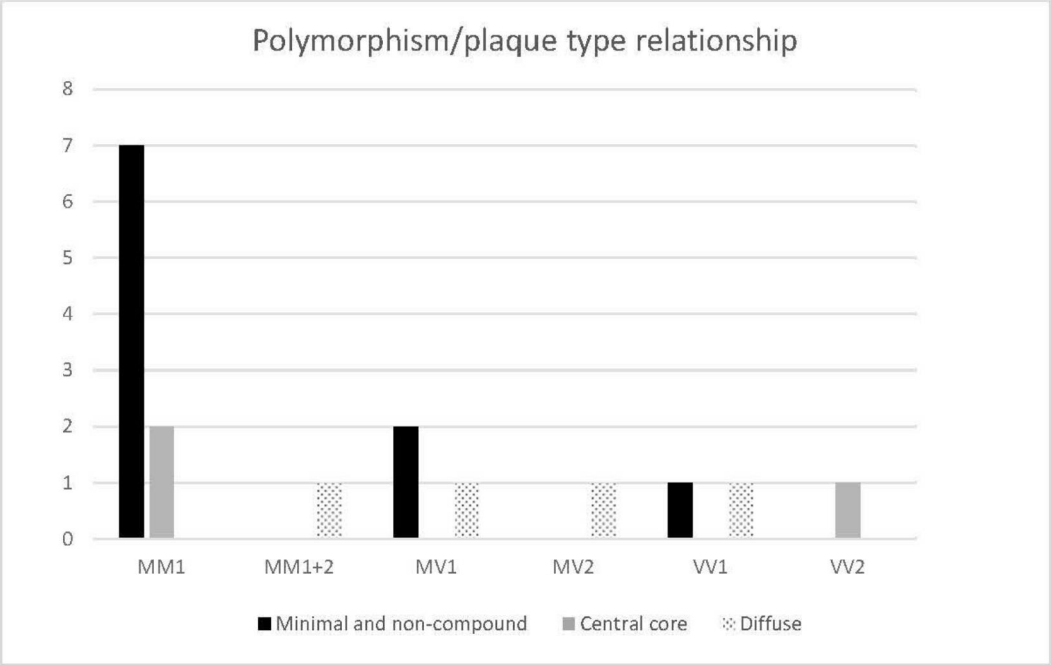

Chart 2. Relationship between methionine–valine (M/V) polymorphisms and plaque type.

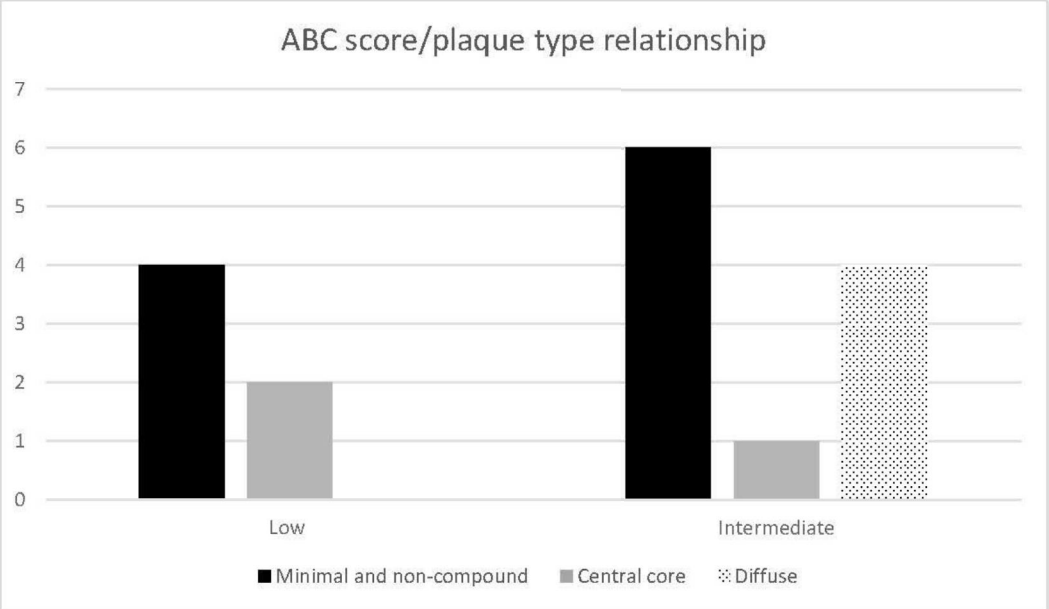

Chart 3. Dependence of plaque-type on the ABC scores.

2.2. *Immunohistochemical Examination*

In the immunohistochemical examination, parallel imaging of PrP, hyperphosphorylated tau protein (AT8), and Aβ aggregates in the same hippocampal area (Figure 4a,c) clearly showed the occurrence of PrP^{Sc}-positive aggregates in plaques with h-tau positive dystrophic neurites where Aβ was entirely missing. Thus, this observation also suggests

that PrP^{Sc} could be colocalized with h-tau-positive dystrophic neurites in Aβ plaques in the absence of Aβ structures.

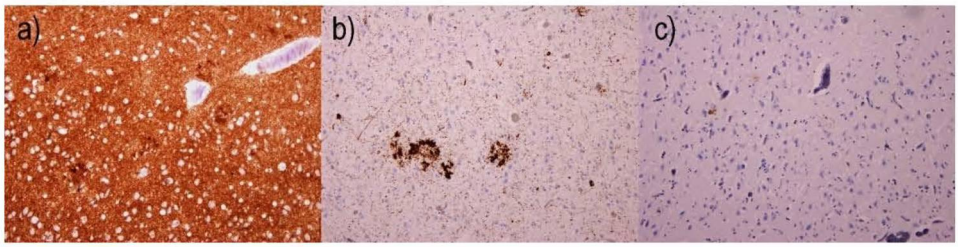

Figure 4. Immunohistochemical imaging of (**a**) PrP^{Sc}-positive plaque-like structures, (**b**) AT8-positive dystrophic neurites, and (**c**) Aβ plaques in the same hippocampal region (archicortical part) of an 80-year-old female patient. There are PrP^{Sc} "plaque-like" deposits and h-tau positive neuritic plaques in the same area lacking the equivalent of Aβ plaques. The secondary antibody was conjugated with horseradish peroxidase staining DAB. The original magnification is 100×.

3. Discussion

In the study presented, a particular affinity of PrP^{Sc} for the non-compact parts of Aβ plaques, suggesting that different subspecies of Aβ have different affinities for PrP^{Sc} aggregates, was observed. In addition, previously reported low rate of compound plaques colocalizing with Aβ or h-tau and PrP^{Sc} in comorbid AD and sCJD were considered.

The potential interaction between PrP^{Sc} and Aβ in comorbid AD and sCJD remains controversial. No significant correlation has been reported for variables influencing the development of CJD and variables determining the course of AD [22]. On the other hand, colocalization of PrP^{Sc} and Aβ in single plaques was reported in a patient with a rare prion disease called Gerstmann–Sträussler–Scheinker disease (GSS) [23]; however, this colocalization was only observed in GSS and not in sCJD [24]. On the contrary, other authors reported compound PrP^{Sc} and Aβ plaques in 11 of 12 evaluated subjects with a concomitant sCJD and AD pathology; the frequency of compound plaques ranged between 2 and 29% [25]. Others also documented the presence of PrP^{C} in senile plaques in non-sCJD AD describing dot-like Pr^{C} -immunoreactivity in diffuse plaques, isolated large coarse PrP^{C}-positive structures in neuritic plaques, and dense non-compact or amorphic aggregates in amyloid cores of senile plaques [26].

As mentioned above, the colocalization of Aβ and PrP^{Sc} in different prionopathies in comorbidity with AD was reported, and the frequency of these compound plaques was demonstrated. On the contrary, this study was focused on the detailed micromorphological relationship of PrP^{Sc}, Aβ, and AT8 in compound plaques based on confocal fluorescent microscopy.

Based on this, we recognized three types of Aβ and PrP^{Sc} colocalization, as follows: (1) no or minimum compound plaques, (2) compound plaques with a centrally dense PrP^{Sc} core only, and (3) compound plaques with or without a centrally dense PrP^{Sc} core; however, PrP^{Sc} was always present in the periphery. Our data show no or minimal colocalization in the compact region of Aβ plaques; however, in the non-compact region of Aβ plaques, which were often located in the periphery, showed a higher rate of colocalization. Non-compact Aβ plaques are mostly composed of $Aβ_{42}$, rather than $Aβ_{40}$ [27]. Similar to a study on single and double immunohistochemical stains of PrP^{C} in Aβ plaques in AD patients [26], we observed an abundance of dot-like aggregates in diffuse plaques, while in neuritic plaques, PrP deposits tended to be relatively coarse. Interestingly, there seems to be no association between plaque micromorphology, polymorphism at codon 129, type of PrP^{Sc}, and the AD ABC scores.

In addition, AT8 positive dystrophic neurites were observed in colocalization with PrP^{Sc} plaque-like structures. Dystrophic; dilated; and, in specific locations, bulbous dystrophic neurites are a common feature of Aβ plaques [21] in AD. Thus, it is not surprising

that these structures, which were detected by immunohistochemical positivity of hyperphosphorylated tau protein, are also observed in colocalization with PrP^{Sc}. However, using simple DAB immunohistochemistry, we were able to find hippocampal regions with PrP^{Sc} plaque-like positivity and h-tau-positive Aβ plaques without the expression of Aβ (Figure 4), suggesting that direct interaction between PrP^{Sc} and dystrophic neurites or directly with h-tau. Direct molecular interactions between PrP^{Sc} and tau protein have also been reported [28]. Conversely, tau pathology presented as neurofibrillary tangles was a pathognomonic finding for the Indiana Kindred variant of GSS [29]. In the P105L variant of GSS, dystrophic, tau-positive neurites and neurofibrillary tangles were observed in PrP^{Sc} plaques, even in the absence of senile Aβ amyloid plaques [30]. Associations between PrP^{Sc} plaques and tau aggregation were observed in scrapie-infected mouse brains of human tau transgenic mice [31]. Thus, direct pathological interactions between PrP^{Sc} and tau in the absence of Aβ facilitating the progression of the disease could be a plausible hypothesis for AD and sCJD comorbidity. However, our observations are based on a pilot study with a small cohort of patients, and lack a control group with separate AD and CJD cohorts. The results are sustainable for further investigation.

4. Materials and Methods

4.1. Patients

A total of 20 patients diagnosed with comorbid AD and CJD (age range 62–83 years, median age 71 years) were neuropathologically defined using the National Institute on Aging Alzheimer's Association (NIA-AA) consensus scheme [32]. Additionally, the presence of PrP^{Sc} in brain tissue was confirmed by both Western-blot and immunohistochemistry. The patient characteristics are summarized in Table 1, which includes gender, age, disease duration, the AD ABC score, [33] codon 129 methionine and/or valine polymorphisms, PrP^{Sc} [34] isoform (i.e., type 1 or 2), specification of other vascular and age-related co-pathologies, and the presence of protein 14-3-3 in the cerebrospinal fluid.

Table 1. Individual patients, including gender, age, duration of the disease, 129 codon polymorphisms with the PrP type, 14-3-3 positivity, the AD ABC score according to the NIA-Alzheimer's association guidelines, data regarding other neuropathologies, and plaque type specification.

No.	Sex	Age	Duration (Months)	Polymorph.	PrP Types Brain Tissue	Western Blot (14-3-3) CSF	AD ABC Score	Other Neuropathology	Type of Plaques
1.	M	75	2	MM	1	neg.	A2B2C2	Angiosclerotic encephalopathy, ARTAG, encephalomalacia	Non-compound
2.	F	71	1	MV	1	low pos.	A2B1C2	Angiosclerotic encephalopathy	Non-compound
3.	F	75	9	VV	1	pos.	A1B1C1	Angiosclerotic encephalopathy	Non-compound
4.	F	67	1	MM	1	pos.	A2B1C1	Angiosclerotic encephalopathy	Minimal compound
5.	F	69	2	MM	1	NA	A2B2C1	Angiosclerotic encephalopathy	Minimal compound
6.	M	62	6	MV	1	neg.	A3B2C2	Angiosclerotic encephalopathy	Minimal compound
7.	F	65	2	MM	1	pos.	A2B2C2	Angiosclerotic encephalopathy	Minimal compound
8.	M	79	1	MM	1	pos.	A1B2C1	Angiosclerotic encephalopathy	Minimal compound
9.	M	75	4	MM	1	neg.	A2B2C2	Angiosclerotic encephalopathy, ARTAG, Wernicke encephalopathy, meningioma	Minimal compound
10.	F	68	2	MM	1	pos.	A2B2C2	Angiosclerotic encephalopathy, AGD	Minimal compound + few compounds
11.	M	79	1,5	MM	1	pos.	A2B2C2	Angiosclerotic encephalopathy, ARTAG	Central core deposits

Table 1. *Cont.*

No.	Sex	Age	Duration (Months)	Polymorph.	PrP Types Brain Tissue	Western Blot (14-3-3) CSF	AD ABC Score	Other Neuropathology	Type of Plaques
12.	F	71	1	MM	1	pos.	A2B1C2	Angiosclerotic encephalopathy	Central core deposits
13.	F	80	2	VV	2	pos.	A3B1C2	Angiosclerotic encephalopathy	Central core deposits
14.	M	64	2	MV	2	low pos.	A2B2C2	Angiosclerotic encephalopathy, Wernicke encephalopathy	Diffuse compound
15.	F	70	2	VV	1	pos.	A2B2C2	Angiosclerotic encephalopathy	Yin-Yang
16.	F	70	1	MM	1 + 2	pos.	A2B2C3	Angiosclerotic encephalopathy	Yin-Yang
17.	F	80	5	MV	1	pos.	A3B2C3	Angiosclerotic encephalopathy	Yin-Yang + few compounds
18.	M	83	1	MM	1	low pos.	A2B2C2	Angiosclerotic encephalopathy, ARTAG	Lacking PrP plaques in the hippocampus
19.	F	65	5	VV	1	pos.	A1B1C1	Angiosclerotic encephalopathy	Lacking PrP plaques in the hippocampus
20.	M	85	2	MM	1	neg.	A2B2C2	Angiosclerotic encephalopathy	Lacking PrP plaques in the hippocampus

M—male; F—female; MM/MV/VV—methionine and/or valine polymorphism on codon 129; CSF—cerebrospinal fluid; ARTAG—aging-related tau astrogliopathy; AGD—argyrophilic grain disease; PrP—prion protein.

All data were analyzed with respect to patient privacy, and the study was conducted in accordance with the Ethics Committee of Thomayer University Hospital (No G-19-18) on 10 April 2019.

4.2. Tissue Samples

Brain tissue samples were fixed for 3–4 weeks in buffered 10% formalin. Then, selected tissue blocks, using a standardized protocol BrainNet Europe [35], were embedded in paraffin using an automatic tissue processor. Five-µm-thick sections were prepared and stained with hematoxylin–eosin, Klüver–Barrera, and silver impregnation methods. For analysis, representative blocks of the left hippocampal and parahippocampal areas were chosen.

4.3. Immunofluorescence and Immunohistochemistry

Briefly, 5-µm-thick sections of formalin-fixed and paraffin-embedded tissue samples were deparaffinized and then incubated with primary antibodies for 20 min at room temperature. For Aβ and PrP^{Sc} antibody staining, 96% formic acid was applied prior to the primary antibody. A second layer for light microscopy visualization, consisting of secondary horseradish peroxidase-conjugated antibody (En Vision FLEX/HRP, Dako M822, Glostrup, Denmark), was applied for 20 min at room temperature. The samples were then incubated with DAB (Substrate—Chromogen Solution, Dako K3468, Glostrup, Denmark) for 10 min to visualize the reaction. Mayer's Hematoxylin Solution was used as a counterstain.

For confocal microscopy, secondary antibodies conjugated to Alexa Fluor® (see below) were used. Paraffin sections were also treated with 20X TrueBlack® (Biotium 23007, Fremont, CA, USA) diluted in 1X 70% alcohol to quench lipofuscin autofluorescence.

4.3.1. Primary Antibodies

For immunohistochemistry, 5-µm-thick sections of formalin-fixed and paraffin-embedded tissue were selected from the hippocampal region, including the entorhinal and transentorhinal cortex. These were incubated with primary antibodies against the following

antigens: (1) Aβ (1:1000, mouse monoclonal, clone 6F/3D; Dako M0872, Glostrup, Denmark), (2) Aβ (1:5000, rabbit monoclonal, clone H31L21; Thermo Fisher Scientific 700254, Waltham, ME, USA), (3) PrP (1:8000, mouse monoclonal, clone 12F10; Bertin Pharma A03221, Bordeaux, France), (4) PrP (1:3000, mouse monoclonal, clone 6H8; Prionics 7500996, Schlieren, CH), (5) PrP (1:5000, rabbit recombinant monoclonal, clone SC57-05; Thermo Fisher Scientific MA5-32202, Waltham, ME, USA), and (6) Phospho-Tau (Ser202, Thr205) Monoclonal Antibody (1:500, mouse monoclonal, clone AT8; Thermo Fisher Scientific MN1020, Waltham, ME, USA).

4.3.2. Secondary Antibodies

Detection of immunostaining was carried out using horseradish peroxidase–diaminobenzidine (see above) for immunohistochemistry and secondary antibodies conjugated with Alexa Fluor® 488 (1:1000, donkey anti-rabbit, H + L IgG, Thermo Fischer Scientific, Waltham, MA, USA) and Alexa Fluor® 568 (1:1000, donkey anti-mouse, H + L IgG, Thermo Fischer Scientific) for immunofluorescence staining. Slides incubated with only the secondary antibody were used as specificity controls.

4.4. Microscopy Evaluation

4.4.1. Light Microscopy

The samples were examined independently by two neuropathologists focused predominantly on the archicocortical parts of hippocampal region, and the presence/absence of Aβ deposits and AT8-positive structures, in relation to PrP deposits, was evaluated. An Olympus BX51 microscope (Olympus Europa SE and Co. KG, Hamburg, Germany) was used for examination with 100× magnification. Images were captured with an Olympus DP72 camera controlled using Olympus image analysis software (Olympus Europa SE and Co. KG).

4.4.2. Confocal Microscopy

Colocalization of pathogenic protein aggregates was imaged using a Leica TCS SP5 confocal fluorescent laser scanning microscope (Leica Microsystems Inc., Wetzlar, Germany). The HCX PL APO objective with 40× magnification, oil immersion, and a pinhole of 1 AU was used. Donkey anti-Rabbit IgG secondary antibody was conjugated to Alexa Fluor® 488 and excited at 488 nm using a 65 mW multi-line argon laser, whereas Donkey anti-Mouse IgG conjugated to Alexa Fluor® 568 was excited at 561 nm using a 20 mW DPSS laser.

4.4.3. Classification of Aβ Plaques

Diffuse, neuritic non-cored and neuritic cored Aβ plaques were classified according to the literature, as previously summarized in a review article [20].

5. Conclusions

The results as presented indicate that a specific subset of Aβ, in particular the non-compact component of Aβ plaque where $A\beta_{42}$ predominates, exhibits higher levels of interaction with PrP^{Sc} and, thus, in certain circumstances, could be assumed to act as the PrPSc seeds within the brain (see Supplementary Materials). The role of PrP^{Sc} in the development of neuritic plaques, with or without the Aβ component, certainly requires further investigation.

Supplementary Materials: The following are available online at https://www.mdpi.com/1422-0067/22/4/2099/s1.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki approved in advance by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer University Hospital for the Research Project entitled: "Simultaneous histology imaging of neurodegenerative comorbidities utilizing fluorescent and multichannel confocal microscopy" on 10 April 2019.

Informed Consent Statement: No informed consent obtained as only archival tissue of dead subjects was investigated retrospectively in anonymous setting with respect to their privacy, no treatment or diagnostic intervention was performed.

Data Availability Statement: The authors confirm that all data underlying the findings are fully available without restriction. All data are included within the manuscript https://doi.org/10.3390/ijms22042099.

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3.3 Mikromorfologická charakterizace extracelulárních proteinových depozit u případů Gerstmannova–Sträusslerova–Scheinkerova syndromu a jejich vztah k dystrofickým neuritickým změnám.

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Extracellular Prion Protein Aggregates in Nine Gerstmann–Sträussler–Scheinker Syndrome Subjects with Mutation P102L: A Micromorphological Study and Comparison with Literature Data

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Abstract: Gerstmann–Sträussler–Scheinker syndrome (GSS) is a hereditary neurodegenerative disease characterized by extracellular aggregations of pathological prion protein (PrP) forming characteristic plaques. Our study aimed to evaluate the micromorphology and protein composition of these plaques in relation to age, disease duration, and co-expression of other pathogenic proteins related to other neurodegenerations. Hippocampal regions of nine clinically, neuropathologically, and genetically confirmed GSS subjects were investigated using immunohistochemistry and multichannel confocal fluorescent microscopy. Most pathognomic prion protein plaques were small (2–10 µm), condensed, globous, and did not contain any of the other investigated proteinaceous components, particularly dystrophic neurites. Equally rare (in two cases out of nine) were plaques over 50 µm having predominantly fibrillar structure and exhibit the presence of dystrophic neuritic structures; in one case, the plaques also included bulbous dystrophic neurites. Co-expression with hyperphosphorylated protein tau protein or amyloid beta-peptide (Aβ) in GSS PrP plaques is generally a rare observation, even in cases with comorbid neuropathology. The dominant picture of the GSS brain is small, condensed plaques, often multicentric, while presence of dystrophic neuritic changes accumulating hyperphosphorylated protein tau or Aβ in the PrP plaques are rare and, thus, their presence probably constitutes a trivial observation without any relationship to GSS development and progression.

Keywords: Gerstmann–Sträussler–Scheinker syndrome; PrP; plaques; co-expression

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1. Introduction

Intracellular or extracellular protein aggregates are characteristic hallmarks of neurodegenerative diseases [1]. Prion protein (PrP) and amyloid beta-peptide (Aβ) are extracellular amyloid protein deposits with a similar micromorphology that have been observed in prion diseases and Alzheimer's disease (AD) [2]. Depending on the particular type of disease, extracellular deposits in prion disorders range from diffuse synaptic positivity to patchy/perivacuolar depositions to plaque-like deposits. In these, plaques can be subdivided into "daisy" plaques in kuru and kuru-like plaques, either solitary or multicentric [2]. In GSS, PrP plaques are composed of fibrils arranged in β-sheet secondary structure [3]. In PrP plaques, a certain level of co-expression with other amyloid-forming proteins can be observed in comorbidity, particularly in Alzheimer's disease, where co-expression with Aβ plaques as well as with dystrophic neurites were previously observed [4].

Gerstmann–Sträussler–Scheinker syndrome (GSS—OMIM 137440) is a rare, slowly progressive prion disease caused by pathogenic mutations in the prion protein gene (PRNP). GSS is neuropathologically characterized by spongiform encephalopathy in different brain regions with varying severity and PrP-immunoreactive insoluble deposits mainly in the cerebral and cerebellar cortices and the basal ganglia. The most frequent mutation in GSS is in the PRNP gene (i.e., P102L); however, other mutations have been described in the literature [2]. Despite the different clinical symptomatology of the four recognized GSS P102L subtypes [5], there are no specific neuropathological changes that characterize the clinical subtypes. However, there is little data regarding the micromorphology of PrP deposits in GSS.

GSS can appear as a solitary disease; however, in some cases, comorbid neurodegenerations or comorbid neuropathology can exist, especially with hippocampal region involvement. In most cases, these are Alzheimer-related changes or so-called age-related deposits of hyperphosphorylated tau protein or protein TDP-43 [6].

Certain authors have reported co-expression of Aβ in the plaques of some GSS patients [7,8], while other studies noted the presence of hyperphosphorylated protein tau deposits [9] with three- or four-repeat tau (RD3 or RD4) proteins within PrP plaques [9,10].

Our study aimed to evaluate the micromorphology and protein composition of the hippocampal regions of archival brain material from nine confirmed GSS patients with pathogenic P102L PRNP mutations with regard to Aβ (a protein co-aggregate commonly observed in prion deposits) and hyperphosphorylated protein tau (as a marker of neuritic dystrophy). Moreover, we compared our results relative to the age of onset, disease duration, and methionine/valine (*M/V*) polymorphism at codon 129 of the PRNP gene in our cohort. We also examined data from other GSS cases published in the literature that contained information regarding micromorphology and the expression of pathological proteins.

2. Results

2.1. Immunohistochemistry

2.1.1. PrP

Either diffuse, patchy/perivacuolar, and plaque positivity, containing kuru-like plaques including multicentric plaques containing pathological PrP was confirmed (using two different antibodies (clones 12F10 and 6H4)) in the hippocampal area of all nine subjects in the cohort. In the immunohistochemical staining, the kuru-like plaques appeared small to medium-sized (approximately 2–10 μm) spheroids with central brightness and were either dispersed solitary or in aggregates called multicentric plaques (illustrated in Figure 1). In two subjects (cases 4 and 7), large plaques, up to 100 μm, were observed in hippocampal area CA1 and the subiculum (see the bottom half of Figure 2).

2.1.2. AT8 and Ubiquitin

Except for one subject, and irrespective of the expression of AD-related changes or hyperphosphorylated tau protein deposits in the form of primary age-related tauopathy (PART) and argyrophilic grain disease (AGD), there was generally no co-aggregation with hyperphosphorylated protein tau or ubiquitin in areas where PrP plaques were observed. In one subject (case 7), no co-aggregation of PrP with hyperphosphorylated protein tau was observed in areas of small solitary or multicentric plaques; a few plaques, which were slightly positive for ubiquitin, were found (top half of Figure 2). However, this subject also showed a significant co-aggregation of PrP with bulbous (extremely dilated) [11] dystrophic neurites staining for either hyperphosphorylated protein tau or ubiquitin in the same CA1 hippocampal area and the subiculum (see the bottom half of Figure 2).

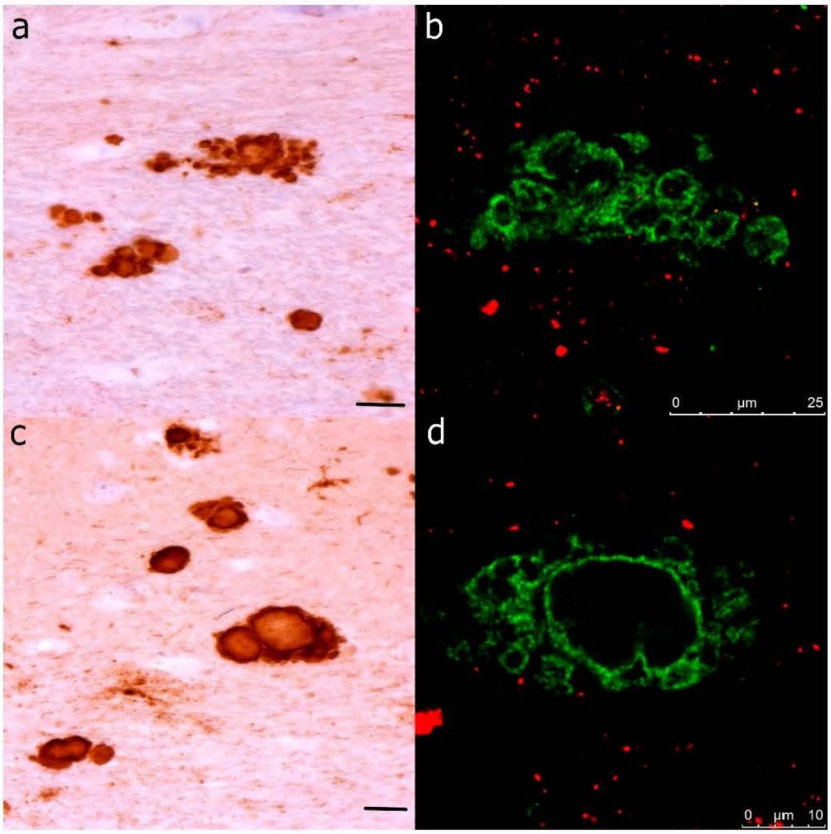

Figure 1. Illustration—Parallel observation of (**a**,**b**) multicentric and (**c**,**d**) solitary kuru-like plaques with centrally bright cores visualized using immunohistochemistry and immunofluorescence. (**a**,**c**) Primary antibody in immunohistochemical images: PrP (mouse monoclonal antibody). The secondary antibody was conjugated with horseradish peroxidase staining DAB. The original magnification was 100×. (**b**,**d**) Primary antibodies in immunofluorescent images: PrP (rabbit recombinant monoclonal antibody, green color) + AT8 (mouse monoclonal antibody, red color). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG, green) or Alexa Fluor® 568 (anti-mouse IgG, red). Scale bars indicate 25 µm in (**a**,**b**) and 10 µm in (**c**,**d**).

2.2. *Immunofluorescence*

The dominant finding from laser scanning multichannel immunofluorescence microscopy in all subjects investigated was the presence of condensed PrP aggregates, either diffuse or in the form of plaques or kuru-like plaques without any significant tau or ubiquitin co-pathology (Figure 3). Immunofluorescence confirmed that kuru-like plaques were spheroids with centrally bright cores that were either solitary or multicentric (Figure 1).

In a very few cases of plaques with diffuse deposits across the cohort of subjects investigated, dystrophic, less than more dilated neurites; however, accumulation of hyperphosphorylated protein tau or ubiquitin were observed (see Figure 4).

The only exception was subject no. 7, who exhibited bulbous changes in AT8-positive, bulbous dystrophic neurites in large diffuse PrP plaques in CA1 and the subiculum (Figure 5b,c). In the other subject with large PrP plaques located in CA1 and the subiculum (subject no. 4), only ubiquitin was recorded in dystrophic neurites (Figure 5d–f), but with no or negligible AT8 positivity (see Figure 5a).

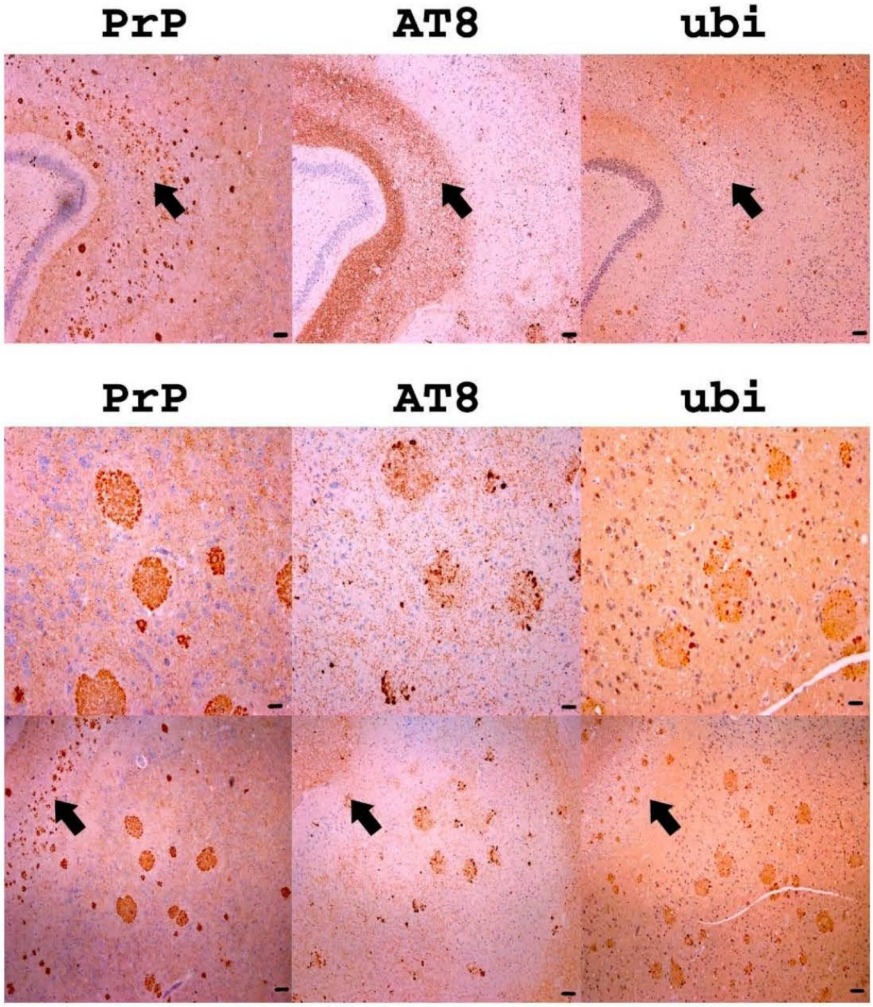

Figure 2. The top half—Immunohistochemistry observation of PrP, hyperphosphorylated protein tau, and ubiquitin in the CA1–CA2 area. No expression of hyperphosphorylated protein tau (AT8 antibody clone) and negligible ubiquitin expression was observed in areas with abundant small PrP aggregates. The magnification is 40× and the scale bars indicate 100 µm. The bottom half—Immunohistochemistry observation of PrP, hyperphosphorylated protein tau, and ubiquitin in large plaques in CA1. Bulbous changes in dystrophic neurites stained by AT8 and ubiquitin antibody were observed, but only in large PrP plaques of one particular subject; there was no co-expression in other areas (see arrows). All images come from subject no. 7. The images in the top row are zoomed details from the bottom row. The scale bars in the top row indicate 20 µm. The scale bars in the bottom row indicate 100 µm. Magnification is 100× and 40×, respectively.

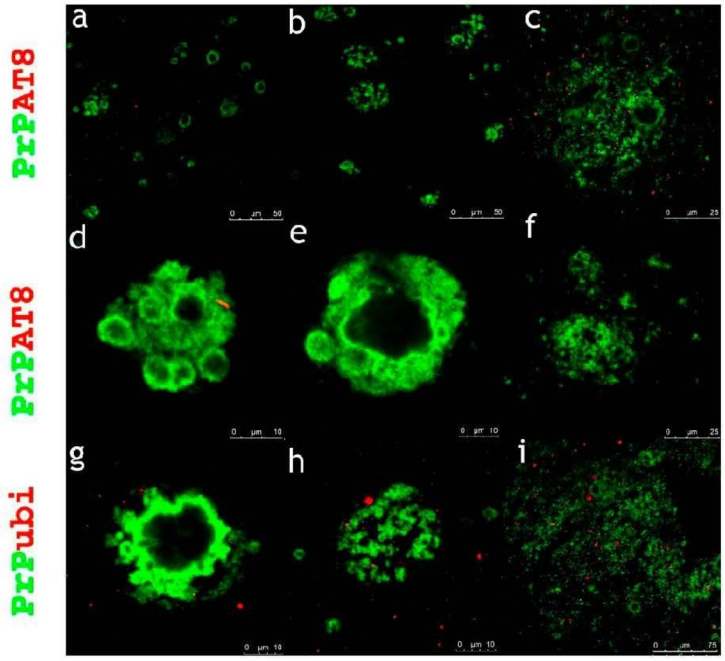

Figure 3. Condensed PrP aggregates as the dominant observation in GSS subjects. The dominant finding across the cohort was no or negligible co-aggregates with hyperphosphorylated protein tau and ubiquitin. Primary antibodies: PrP (rabbit recombinant monoclonal antibody, green color) + AT8 (mouse monoclonal antibody, red color), ubiquitin (mouse monoclonal antibody, red color). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG, green) or Alexa Fluor® 568 (anti-mouse IgG, red). Scale bars indicate 50 µm in (**a**,**b**), 25 µm in (**c**,**f**), 10 µm in (**d**,**e**,**g**,**h**), and 75 µm in (**i**).

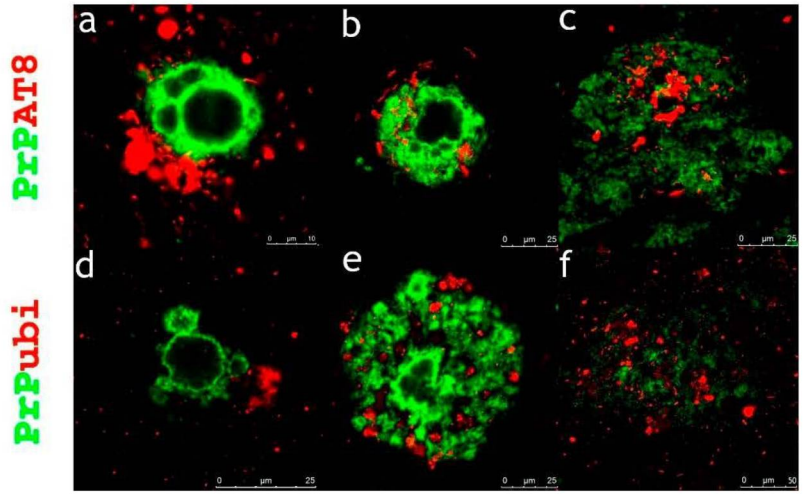

Figure 4. Few observations found condensed PrP with co-aggregates in GSS subjects. Across the cohort, few PrP aggregates either in plaques, kuru-like plaques, or diffuse exhibited a certain level of the hyperphosphorylated protein tau and ubiquitin co-pathology. Primary antibodies: PrP (rabbit recombinant monoclonal antibody, green color), AT8 (mouse monoclonal antibody, red color), ubiquitin (mouse monoclonal antibody, red color). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG, green) or Alexa Fluor® 568 (anti-mouse IgG, red). Scale bars indicate 10 µm in (**a**), 25 µm in (**b**–**e**), and 50 µm in (**f**).

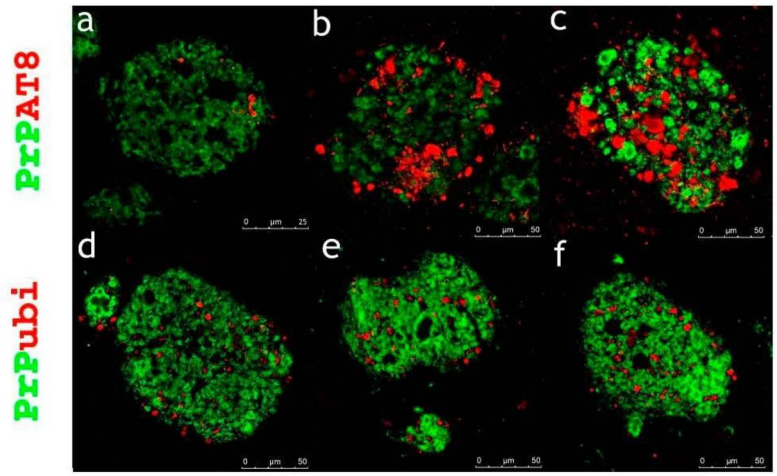

Figure 5. AT8 positive bulbous changes within huge diffuse PrP plaques—Immunofluorescence observation of co-expression PrP with hyperphosphorylated protein tau and ubiquitin in large plaques located in CA1 or the subiculum. (**a**) Large diffuse PrP with no or negligible co-pathology with hyperphosphorylated protein tau in subject no. 4; (**b**,**c**), significant co-aggregation of PrP with bulbous neurites stained for hyperphosphorylated protein tau in subject no. 7; (**d**–**f**), and significant co-aggregation of PrP with dystrophic neurites stained for ubiquitin in subject no. 4. Primary antibodies: PrP (rabbit recombinant monoclonal antibody, green color), AT8 (mouse monoclonal antibody, red color), ubiquitin (mouse monoclonal antibody, red color). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG, green) or Alexa Fluor® 568 (anti-mouse IgG, red). Scale bars indicate 25 µm in (**a**), and 50 µm in (**b**–**f**).

2.3. Statistics

Primary survival data in our cohort (Table 1) and available data from the literature (Tables 2 and 3) were analyzed relative to the presence or absence of amyloid-beta protein co-expression. None of the subjects in our cohort expressed amyloid-beta protein. The average age at death for our group was 53.78 years (±11.19 years). Data from the literature showed that in subjects with amyloid-beta protein co-expression, the average age of death was 63.92 years, which was a statistically significant difference ($p < 0.05$). Considering cases from the literature lacking Aβ-PrP co-expression, the average age of death was 52.32 years. Combining the survival data from our cohort with that from the literature, the average age of death for subjects without amyloid-beta protein co-expression was 52.74 years, which was a statistically significant difference $p < 0.005$ compared to the above-mentioned literature survival data for subjects with amyloid-beta protein co-expression.

Survival data in our study for subjects with the P102L mutation (Table 1) were also compared with data from the literature either for the P102L mutation (Table 2) or other mutations (Table 3). No statistically significant differences were found between the cohorts. The average age at death in our P102L subjects was 53.78 years compared to 52.00 years (±7.48 years) for the P102L literature group. For the P105L group from the literature, the age of death was 52.60 years and 58.67 years for the other mutations. Overall average for other than P102L mutations was 54.68 years (±10.34 years).

Statistical analysis showed a statistically significant difference of 11.18 years between the age of death in those with and without amyloid-beta protein co-expression with expectable co-expression at a later age at death. No age-related differences were observed when comparing cohorts relative to particular mutations.

Table 1. Summary of information for a Czech cohort of clinically, neuropathologically, and genetically confirmed Gerstmann–Sträussler–Scheinker syndrome, all having the P102L mutation in the PRNP gene.

Gender	Age of Onset	Duration	Age of Death	MV Polymorph	PRNP Mutation	Aβ-PrP Coloc.	AT8-PrP Coloc.	Others
1. F	42	1 year	43	MM	P102L	NO	YES	
2. M	65	3 months	65	MM	P102L	NO	YES	
3. M	37	2 years	39	MV	P102L	NO	YES	Son of subject no. 4
4. F	54	7 years	61	MM	P102L	NO	YES	PART; Mother of subject no. 3
5. M	61	5 months	61	MM	P102L	NO	YES	
6. M	56	5 years	61	MM	P102L	NO	YES	PART; Father of subject no. 7
7. F	29	10 years	39	MM	P102L	NO	YES	PART; Daughter of subject no. 6
8. F	NA	NA	69	MM	P102L	NO	YES	PART; M. Fahr
9. F	42	4 years	46	MM	P102L	NO	YES	FTLD-tau (PART, AGD), FTDL-TDP

Explanatory notes: NA—not available; coloc.—colocalization; PART—primary age-related tauopathy; M. Fahr—morbus Fahr/Fahr disease; FTLD-tau—frontotemporal lobar degeneration-tau; AGD—argyrophilic grain disease; FTLD-TDP—frontotemporal lobar degeneration with ubiquitin and TDP-43 positive neuronal inclusions.

Table 2. Summary of GSS cases having the P102L mutation in PRNP gene described in the literature with information on amyloid-beta and hyperphosphorylated tau protein colocalization with pathological prion protein including all available details. (coloc. = colocalization).

Gender	Age of Onset	Duration	Age of Death	MV Polymorph	PRNP Mutation	Aβ-PrP Coloc.	AT8-PrP Coloc.	Others, Reference
10. M	59	2 years	61	MM	P102L	NO	YES	[12]
11. F	38	7 years	55	MM	P102L	NO	YES	[12]
12. M	51	2 years	53	MM	P102L	NO	YES	[12]
13. M	59	3 years	62	MM	P102L	NO	YES	[12]
14. F	38	3 years	41	MM	P102L	NO	YES	[12]
15. M	38	6 years	44	NA	P102L	YES	YES	[13]
16. F	38	10 years	48	NA	P102L	NO	YES	[14]

Table 3. Summary of cases found in the literature having mutations other than P102L in the PRNP gene with information on amyloid-beta and hyperphosphorylated tau protein colocalization with pathological prion protein. (Explanatory note: NA—not available; coloc.—colocalization).

Gender	Age of Onset	Duration	Age of Death	MV Polymorph	PRNP Mutation	Aβ-PrP Coloc.	AT8-PrP Coloc.	Others
17. F	38	7 years	45	MV	P105L	NO	YES	Sister of subject no. 18 [1,15]
18. F	44	12 years	56	MV	P105L	YES	YES	Sister of subject no. 17, Mother of subject no. 19 [14,15]
19. M	47	2 years	49	MV	P105L	NO	YES	Son of subject no. 18 [10]
20. M	42	11 years	53	MV	P105L	NA	NO	Family with subject no. 21 [16]
21. F	50	8 years	58	MV	P105L	NA	YES	Family with subject no. 20 [17]
22. F	38	6 years	44	MV	P105L	NA	NO	Family with subject no. 23 [16]
23. F	44	12 years	46	MV	P105L	NA	YES	Family with subject no. 22 [16]
24. F	45	8 years	53	MV	P105L	NA	YES	[18]
25. F	48	21 years	69	MV	P105L	YES	NA	[7]
26. M	42	11 years	53	MV	P105L	NO	YES	[19]
27. M	50	20 years	70	MV	H187R	NO	YES	Father of subject no. 28 [20]
28. M	33	9 years	42	VV	H187R	NO	YES	Son of subject no. 27 [20]
29. F	61	6 years	67	VV	Y218NA117A	YES	YES	[21]
30. F	64	9 years	73	NA	A117V	YES	YES	Family with subject no. 31 [9]
31. M	33	6 years	39	NA	A117V	NO	YES	Family with subject no. 30 [9]
32. M	57	4 years	61	VV	D202N	NO	YES	[22]

The average disease durations were: 3.7 years for Czech cohort (all having P102L mutation), 4.7 years for P102L cases from the literature, and 9.5 years for cases having other mutations in the literature (see Scheme 1).

Scheme 1. Comparison of age of death and disease duration in the cohort of Czech cases (all having P102L mutation in *PRNP* gene), cases with P102L mutation in *PRNP* from the literature, and all other mutations recorded in the literature.

3. Discussion

Our results provide micromorphological and confocal immunofluorescence pictures of kuru-like plaques in a cohort of confirmed P102L GSS patients.

Contrary to our expectations, which arose from our literature review, see below, the dominant feature in the brains of our GSS subjects was condensed PrP plaques, and the majority of these structures did not show any Aβ- or tau-related co-pathology; in fact, Aβ was not recorded at all using two different antibodies (See Table 1). The condensed plaques were round, both small and large, with centrally bright immunofluorescent cores. The plaques were organized either as solitary or larger multicentric aggregates. The absence of expected co-pathologies can probably be explained by the lower toxicity of the primary PrP associated with the pathogenic mutations. It may also be related to the problematic transmissibility in specific GSS variants [23]. This "low toxicity" hypothesis is supported because GSS presents clinically from the fourth to the seventh decade of life with a relatively long disease course; it does not present in childhood [24] and not since childhood.

A minor feature in our GSS brains was the co-expression of PrP plaques with hyperphosphorylated protein tau (AT8) in dystrophic neurites. This finding is consistent with previously published data that unfortunately failed to describe the frequency [8,9]. In our cohort, there were few cases in which we observed dystrophic, tau-positive neurites co-expressing in the periphery of small solitary plaques as well as in multicentric aggregates. Large PrP plaques with prominent dystrophic neuritic changes were observed in the parahippocampal cortex of only one of the nine cases. This observation in archicortical areas could be explained by the different composition and structure of the archicortex compared to the developmentally distinct neocortex [11]. The greater tendency toward the presence of "bulbous" neuritic changes in archicortical plaques has already been demonstrated in cases with comorbid AD with Lewy body dementia or AD with amygdala predominant Lewy bodies [11].

Despite using two anti-Aβ antibodies, no co-expression of amyloid-beta in GSS PrP plaques was observed with either common immunohistochemistry or multichannel confocal fluorescence microscopy. Although the co-expression of pathological PrP and Aβ

protein has been previously reported, not all subjects in the investigated cohorts exhibited this feature. Ishizava et al. reported only one subject with this co-expression in a cohort of three related patients [10], Piccardo et al. reported one subject of two [25], and Risacher et al. reported no co-expression in their only subject [26]. A detailed list of available literature related to the presence or absence of amyloid-beta protein and dystrophic neuritic changes is presented in Tables 2 and 3. Although there are other reports, which used simultaneous double immunohistochemical staining methods to describe colocalization of PrP and Aβ [27], they failed to provide specifications regarding genetic mutations, making it impossible to compare their cases with our cohort.

The age at death seems to be a reasonable explanation for the difference between observations. Despite the fact that co-aggregation of Aβ and PrP has been proven, the relationship of Aβ presence with age suggests that development of "Alzheimer's" pathology develops separately and independently of the PrP aggregates. The data from literature discussed above suggest that Aβ-PrP co-aggregation occurs in older subjects. In the cited articles, the average age of death was 63.92 years in subjects co-expressing Aβ and PrP, while the average age for subjects without co-expression was 52.32 years. The statistically significant difference ($p < 0.005$) in the age of death between all subjects without co-expression (our cohort and literature data) compared to literature subjects with co-expression was 11.18 years (for detailed information, see Tables 1–3). In our cohort, the average age at death was 53.78 years, making the age hypothesis plausible. However, there are studies suggesting the possibility of neutralization of Aβ and PrP by mutual interaction. According to them, PrP is able to interact with both, Aβ oligomers as well as matured fibrils [28–30].

Even in Creutzfeldt–Jakob disease, compound PrP-Aβ plaques are not common, which agrees with our observations [4] as well as data obtained by Budka et al. [31] (only finding compound plaques in 2–29%). However, the proportion of reactive plaques in GSS that also lacked tau-positive neurites was surprisingly high, irrespective of concomitant tauopathy, namely PART and AGD, which is a frequently reported concomitant neuropathology in CJD cohorts [32].

4. Materials and Methods

4.1. Patients

A total of nine patients diagnosed with GSS (age range: 39–69 years, median age: 61 years) harboring pathogenic mutation P102L in the PRNP gene were enrolled in the study. The presence of PrP in the brain tissue was additionally confirmed using Western blot and immunohistochemistry. Patient characteristics are summarized in Table 1 and include gender, age of onset, disease duration, age of death, codon 129 methionine/valine polymorphisms, other genetic mutations, and colocalization with Aβ, and hyperphosphorylated tau protein with PrP as well as other important and/or additional information.

4.2. Tissue Samples

Brain tissue samples were fixed for 3–4 weeks in buffered 10% formalin. Then, using the BrainNet Europe standardized protocol [33], selected tissue blocks were embedded in paraffin using an automatic tissue processor. Sections 5 µm thick were prepared and stained with hematoxylin-eosin, Klüver–Barrera, and silver impregnation methods. For analysis, representative blocks of the left hippocampal and parahippocampal areas were chosen.

4.3. Immunofluorescence and Immunohistochemistry

Briefly, 5-µm-thick sections of formalin-fixed and paraffin-embedded tissue samples were deparaffinized and then incubated with primary antibodies for 20 min at room temperature. For Aβ and PrP antibody staining, 96% formic acid was applied prior to the primary antibody. A second layer for light microscopy visualization, consisting of secondary horseradish peroxidase-conjugated antibody (EnVision FLEX/HRP, Dako M822, Glostrup, Denmark), was applied for 20 min at room temperature. The samples

were then incubated with DAB (Substrate—Chromogen Solution, Dako K3468, Glostrup, Denmark) for 10 min to visualize the reaction. Mayer's Hematoxylin Solution was used as a counterstain.

For confocal microscopy, secondary antibodies conjugated to Alexa Fluor® (Thermo Fischer Scientific, Waltham, MA, USA, see below) were used. Paraffin sections were also treated with 20× TrueBlack® (Biotium 23007, Fremont, CA, USA) diluted in 1 × 70% alcohol to quench lipofuscin autofluorescence.

4.3.1. Primary Antibodies

For immunohistochemistry, 5-µm-thick sections of formalin-fixed and paraffin-embedded tissue were selected from the left hippocampal region, including the entorhinal and transentorhinal cortex. These were incubated with primary antibodies against the following antigens: (1) Aβ (1:1000, mouse monoclonal, clone 6F/3D; Dako M0872, Glostrup, Denmark), (2) Aβ (1:5000, rabbit monoclonal, clone H31L21; Thermo Fisher Scientific 700254, Waltham, MA, USA), (3) PrP (1:8000, mouse monoclonal, clone 12F10; Bertin Pharma A03221, Bordeaux, France), (4) PrP (1:3000, mouse monoclonal, clone 6H8; Prionics 7500996, Schlieren, Switzerland), (5) PrP (1:5000, rabbit recombinant monoclonal, clone SC57-05; Thermo Fisher Scientific MA5-32202, Waltham, MA, USA), (6) Phospho-Tau (Ser202, Thr205) Monoclonal Antibody (1:500, mouse monoclonal, clone AT8; Thermo Fisher Scientific MN1020, Waltham, MA, USA), and (7) Ubiquitin (1:2000, mouse monoclonal, clone Ubi-1; Millipore-Sigma MAB1510-I-25UG, Burlington, MA, USA).

4.3.2. Secondary Antibodies

Detection of immunostaining was carried out using horseradish peroxidase–diaminobenzidine (see above) for immunohistochemistry and secondary antibodies conjugated with Alexa Fluor® 488 (1:1000, donkey anti-rabbit, H + L IgG, Thermo Fischer Scientific, Waltham, MA, USA) and Alexa Fluor® 568 (1:1000, donkey anti-mouse, H + L IgG, Thermo Fischer Scientific, Waltham, MA, USA) for immunofluorescence staining. Slides incubated with only the secondary antibody were used as specificity controls.

4.4. Microscopy Evaluation

4.4.1. Light Microscopy

The samples were examined independently by two neuropathologists and focused predominantly on the archicortical parts of the hippocampal region; the presence or absence of Aβ deposits and AT8-positive structures, in relation to PrP deposits, was evaluated. An Olympus BX51 microscope (Olympus Europa SE and Co., KG, Hamburg, Germany) was used for examination with 100× magnification. Images were captured with an Olympus DP72 camera using Olympus image analysis software (Olympus Europa SE and Co., KG, Hamburg, Germany).

4.4.2. Confocal Microscopy

Colocalization of pathogenic protein aggregates was imaged using a Leica TCS SP5 confocal fluorescent laser scanning microscope (Leica Microsystems Inc., Wetzlar, Germany). An HCX PL APO objective was used with 40× magnification, oil immersion, and a 1 AU pinhole. Donkey anti-Rabbit IgG secondary antibody was conjugated to Alexa Fluor® 488 and excited at 488 nm using a 65 mW multi-line argon laser, whereas Donkey anti-Mouse IgG conjugated to Alexa Fluor® 568 was excited at 561 nm using a 20 mW DPSS laser.

4.4.3. Statistics

Student's *t*-test was used for statistical analysis.

5. Conclusions

Despite our expectations, which came from published literature, the dominant picture in the GSS brain is small, condensed plaques that are sometimes organized into more complex plaques; however, dystrophic neuritic changes that accumulate hyperphosphorylated protein tau or amyloid-beta co-expression appear to be a minor feature and may not be related to disease development. From our results, it can be concluded that co-expression with amyloid-beta can be expected when subjects die at older ages and can probably be considered a parallel and independent age-related amyloid-beta protein pathology.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki approved in advance by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer University Hospital No G-19-18, obtained 26 June 2017.

Informed Consent Statement: No informed consent obtained as only archival tissue of dead subjects was investigated retrospectively in anonymous setting with respect to their privacy, no treatment or diagnostic intervention was performed.

Data Availability Statement: The authors confirm that all data underlying the findings are fully available without restriction. All data are included within the manuscript.

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3.4 Charakterizace případů Creutzfeldtovy–Jakobovy choroby po neuropatologické, genetické, imunologické i klinické stránce a z pohledu radiodiagnostiky.

Jankovska N, Rusina R, Keller J, Kukal J, Bruzova M, Parobkova E, Olejar T, Matej R. Biomarkers Analysis and Clinical Manifestations in Comorbid Creutzfeldt-Jakob Disease: A Retrospective Study in 215 Autopsy Cases. Biomedicines. 2022 Mar 16;10(3):680. doi: 10.3390/biomedicines10030680. PMID: 35327482; PMCID: PMC8944998. **IF 4,757**

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Biomarkers Analysis and Clinical Manifestations in Comorbid Creutzfeldt–Jakob Disease: A Retrospective Study in 215 Autopsy Cases

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Abstract: Creutzfeldt–Jakob disease (CJD), the most common human prion disorder, may occur as "pure" neurodegeneration with isolated prion deposits in the brain tissue; however, comorbid cases with different concomitant neurodegenerative diseases have been reported. This retrospective study examined correlations of clinical, neuropathological, molecular-genetic, immunological, and neuroimaging biomarkers in pure and comorbid CJD. A total of 215 patients have been diagnosed with CJD during the last ten years by the Czech National Center for Prion Disorder Surveillance. Data were collected from all patients with respect to diagnostic criteria for probable CJD, including clinical description, EEG, MRI, and CSF findings. A detailed neuropathological analysis uncovered that only 11.16% were "pure" CJD, while 62.79% had comorbid tauopathy, 20.47% had Alzheimer's disease, 3.26% had frontotemporal lobar degeneration, and 2.33% had synucleinopathy. The comorbid subgroup analysis revealed that tauopathy was linked to putaminal hyperintensity on MRIs, and AD mainly impacted the age of onset, hippocampal atrophy on MRIs, and beta-amyloid levels in the CSF. The retrospective data analysis found a surprisingly high proportion of comorbid neuropathologies; only 11% of cases were verified as "pure" CJD, i.e., lacking hallmarks of other neurodegenerations. Comorbid neuropathologies can impact disease manifestation and can complicate the clinical diagnosis of CJD.

Keywords: Creutzfeldt–Jakob disease; comorbid neuropathology; Alzheimer's disease; tauopathy; MRI; beta-amyloid

1. Introduction

Creutzfeldt-Jakob disease (CJD), the most common human prion disorder [1], is neuropathologically characterized by spongiform encephalopathy involving the subcortical grey matter of the cerebral and cerebellar cortex [2]. It can also be described as encephalopathy with protease-resistant prion protein (PrP) immunoreactivity in the form of plaques, diffuse synaptic, and a patchy/perivacuolar pattern [2].

Three types of CJD are distinguished based on different etiologies [3]: in most countries, sporadic (sCJD) is the dominant form, followed by genetic (gCJD) and acquired CJD, which has two additional subtypes, i.e., iatrogenic (iCJD) [4] and variant (vCJD) [5]. A worldwide incidence of 1–2 cases of sCJD per million inhabitants is commonly reported [6]. The situation in the Czech Republic is similar, but with a slightly higher proportion of genetic cases [7].

Clinical manifestation (criteria for possible CJD) typically includes dementia with pyramidal and/or extrapyramidal signs, cerebellar ataxia, visuospatial dysfunction, myoclonus, and akinetic mutism [8]. Typical biomarkers can help establish the final clinical diagnosis: positive 14-3-3 protein in the cerebrospinal fluid (CSF), generalized periodic EEG patterns, MRI hyperintensities on FLAIR/DWI sequences in the basal ganglia (putamen and caudate) or cortical areas, i.e., cortical ribboning (criteria for probable CJD) and positive results from CSF real-time quaking-induced conversion (RT-QuIC) analysis [8]. Definite CJD is confirmed by neuropathological and immunohistochemistry examination of brain tissue [8].

CJD has long been considered a homogeneous clinical-neuropathological entity. There is, however, increasing evidence of a frequent co-occurrence of other neurodegenerative diseases in CJD cases. Kovacs et al. already published data on the relatively high incidence of tau co-pathology in CJD [9], while Rossi et al. monitored comorbid cases of CJD and Alzheimer's disease (CJD/AD) and CJD with primary age-related tauopathy (CJD/PART) [10].

The aim of our study was to retrospectively analyse neuropathological findings from autopsy specimens in a large nationwide study of definite CJD cases collected over ten years by the Czech National Center for Human Prion Disorder Surveillance and compare them to clinical, radiological, genetic, and biochemical data. We hypothesised that neuropathological comorbidities could be more frequent than previously thought and could impact disease manifestation or neuroimaging/biochemical results. To our best knowledge, this is the first comprehensive study comparing non-comorbid to comorbid CJD cases based on clinical-neuropathological correlations.

2. Materials and Methods

Postmortem confirmed CJD cases resulting from ten years of systematic prion surveillance and available clinical data, neuroimaging findings, and results of neuropathological, molecular-genetic, and immunological investigations were analysed with statistical comparison of non-comorbid versus comorbid cases.

2.1. Patients

A total of 215 patients diagnosed with definite CJD (age range 40–87 years, median 66 years) using current diagnostic criteria were neuropathologically examined, and the presence of PrP^{Sc} in brain tissue was confirmed by both western-blot and immunohistochemical methods. The genetic screening revealed 193 sporadic and 22 genetic cases that mostly had the E200K mutation in the *PRNP* gene, but the *D178* and *P102L* mutations and five octapeptide repeat insertions were also found. Clinical as well as neuroimaging data (CT or MRI) from all patients were analysed; moreover, in most cases, EEG and CSF findings were also available.

Characteristics of individual subgroups of pure or comorbid CJD are summarised in Table 1. The table contains detailed information on age, gender, codon 129 methionine and/or valine polymorphism, eventual *PRNP* mutation, specification of the type (type 1 or 2) of abnormal PrP^{Sc} isoform, protein 14-3-3 positivity, CSF neurodegenerative biomarkers (h-tau, p-tau, and Aβ), clinical data regarding dementia, pyramidal and/or extrapyramidal signs, visuospatial or cerebellar dysfunction, myoclonus, and akinetic mutism. Supplementary Material also contains EEG and MRI findings. The data were analysed with respect for patient privacy and with the consent of the local Ethics Committee

of the Institute of Clinical and Experimental Medicine in Prague and Thomayer University Hospital, No G-19-18, obtained 26 June 2017.

Table 1. Summary of available epidemiological, neuropathological, immunological and genetic data in non-comorbid vs. comorbid CJD cases. The first column shows the neuropathological diagnosis of patients, second shows the total number of cases in each group, third indicate the gender distribution (female/male). In the fourth column is age range with median age (in years) and the last two columns show methionine/valine polymorphism and presence of 14-3-3 protein in cerebrospinal fluid examined by western blot.

Diagnosis	No. of Cases	Sex	Age (Years)	Etiology	Genotype	14-3-3 Protein in CSF
CJD	24	24× F	40–78 (median 60)	21× sCJD 3× gCJD	17× MM 4× MV 3× VV	17× positive 7× negative
CJD/tau	135	73× F 62× M	49–87 (median 65)	119× sCJD 16× gCJD	85× MM 34× MV 16× VV	106× positive 29× negative
CJD/AD	44	24× F 20× M	56–85 (median 71)	41× sCJD 3× gCJD	27× MM 8× MV 9× VV	38× positive 6× negative
CJD/FTLD	7	5× F 2× M	55–78 (median 67)	7× sCJD	4× MM 3× VV	6× positive 1× negative
CJD/synuclein	5	3× F 2× M	59–76 (median 71)	5× sCJD	3× MM 2× VV	4× positive 1× negative

F—female, M—male, MM—Methionine/Methionine polymorphism, MV—Methionine/Valine polymorphism, VV—Valine/Valine polymorphism.

2.2. *Tissue Samples*

Brain tissue samples were fixed for 3–4 weeks in buffered 10% formalin. Selected tissue blocks, using a standardized protocol [11], were then embedded in paraffin using an automatic tissue processor. Five-µm-thick sections were prepared and stained with hematoxylin-eosin, Klüver-Barrera, and silver impregnation methods. Thirty-six representative blocks from standardized regions were chosen for analysis.

2.3. *Immunofluorescence and Immunohistochemistry*

Briefly, 5-µm-thick sections of formalin-fixed and paraffin-embedded tissue samples were deparaffinized and then incubated with primary antibodies for 20 min at room temperature. For Aβ and PrP^{Sc} antibody staining, 96% formic acid was applied prior to the primary antibody. A second layer for light microscopy visualization, consisting of secondary horseradish peroxidase-conjugated antibody (EnVision FLEX/HRP, Dako M822, Glostrup, Denmark), was applied for 20 min at room temperature. The samples were then incubated with DAB (Substrate-Chromogen Solution, Dako K3468, Glostrup, Denmark) for 10 min to visualize the reaction. Mayer's Hematoxylin Solution was used as a counterstain.

For confocal microscopy, secondary antibodies conjugated to Alexa Fluor® (see below) were used. Paraffin sections were also treated with 20× TrueBlack® (Biotium 23007, Fremont, CA, USA) diluted in 1× 70% alcohol to quench lipofuscin autofluorescence.

2.3.1. Primary Antibodies

For immunohistochemistry, 5-µm-thick sections of formalin-fixed and paraffin-embedded tissue were selected from the hippocampal region, including the entorhinal and transentorhinal cortex. These were incubated with primary antibodies against the following antigens: (1) PrP (1:8000, mouse monoclonal, clone 12F10; Bertin Pharma A03221, Bordeaux, France), (2) PrP (1:3000, mouse monoclonal, clone 6H8; Prionics 7500996, Schlieren, Switzerland), (3) Aβ (1:1000, mouse monoclonal, clone 6F/3D; Dako M0872, Glostrup, Denmark),

(4) Phospho-Tau (Ser202, Thr205) Monoclonal Antibody (1:500, mouse monoclonal, clone AT8; Thermo Fisher Scientific MN1020, Waltham, ME, USA), (5) Ubiquitin (1:500, rabbit polyclonal; Dako Z0458, Lakeside, UK), (6) Phospho TDP-43 (1:4000, mouse monoclonal, clone 11-9; Cosmo Bio TIP-PTD-M01, Carlsbad, CA, USA), (7) Alpha-Synuclein (1:1000, mouse monoclonal, clone 5G4; Dianova NDG-76506, Barcelona, Spain).

2.3.2. Secondary Antibodies

Detection of immunostaining was carried out using horseradish peroxidase–diaminobenzidine (see above) for immunohistochemistry and secondary antibodies conjugated with Alexa Fluor® 488 (1:1000, donkey anti-rabbit, H + L IgG, Thermo Fischer Scientific, Waltham, ME, USA) and Alexa Fluor® 568 (1:1000, donkey anti-mouse, H + L IgG, Thermo Fischer Scientific, Waltham, ME, USA) for immunofluorescence staining. Slides incubated with only the secondary antibody were used as specificity controls.

2.4. Microscopy Evaluation

2.4.1. Light Microscopy

Samples were examined, and the results of immunohistochemical methods were classified according to currently valid neuropathological criteria for individual neurodegenerative diseases. Moreover, Alzheimer's disease was subsequently scored using the National Institute on Aging–Alzheimer's Association (NIA-AA) consensus scheme [12,13], and dementia with Lewy bodies (DLB) using the DLB consensus criteria [14] with a determination of the Braak stage [15].

2.4.2. Confocal Microscopy

Co-expression of pathogenic protein aggregates was imaged using a Leica TCS SP5 confocal fluorescent laser scanning microscope (Leica Microsystems Inc., Wetzlar, Germany). The HCX PL APO objective was chosen with 60× magnification and an oil immersion pinhole of 1 AU. Anti-rabbit donkey IgG secondary antibody was conjugated to Alexa Fluor® 488 and excited at 488 nm from a 65 mW multi-line argon laser, whereas anti-mouse donkey IgG conjugated to Alexa Fluor® 568 donkey was excited at 561 nm from a 20 mW DPSS laser.

2.5. Immunological Methods

2.5.1. CSF Analysis

After a single lumbar puncture and collection, CSF samples were centrifuged at 5000 RPM for 5 min and stored in polypropylene tubes at −80 °C in aliquots to avoid thawing and refreezing until the analysis.

The presence of protein 14-3-3 beta (14-3-3β) was determined using a standardized western blot protocol (adapted from Green et al. [16]) and EURO-CJD standards, with stringent control quality. Briefly, samples in doublets were separated using sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto nitrocellulose membranes. For detection of the 14-3-3β, polyclonal antibody K-19 (1:1000, cat. #sc-629; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and after being discontinued (in 2017), monoclonal antibody B-8 (1:1000, cat. #sc-133233; Santa Cruz Biotechnology) were used. Incubation with an appropriate secondary antibody (1:1000, cat. #sc-2004; Santa Cruz Biotechnology, later with the change of primary antibody cat. #sc-516102; Santa Cruz Biotechnology) was followed by chemiluminescent detection (Pierce ECL Plus Western Blotting Substrate; cat. #32132; Thermo Scientific). A weak positive test was interpreted to mean that one sample load was positive and the other negative (the positive control was always positive). CSF levels of t-tau, p-tau, and $A\beta_{42}$ were measured during routine diagnostic testing using commercially available enzyme-linked immunoassay (ELISA) kits (INNOTEST hTAU Ag, cat. #80323/81572, INNOTEST PHOSPHO-TAU (181P), cat. #80317/81574, INNOTEST β-AMYLOID (1–42), cat. #80324/81576, all Innogenetics/FUJIREBIO); all testing was conducted according to the manufacturer's protocol. Although the individual values

of analytes in the CSF are not entirely decisive and combinations of their ratios would be more accurate [17], the values are as follows. For t-tau, the cut-off was assessed to be 1160 pg/mL with sensitivity of 90.3 % and specificity of 90.7% (AUC: 0.926, $p < 0.0001$). For p-tau and $A\beta_{42}$, the indicative normative values were determined. The values of p-tau levels > 60 pg/mL and $A\beta_{42}$ levels < 430 pg/mL were considered as abnormal. Our laboratory has extensive experience determining CSF biomarkers and successfully participates in the Alzheimer's Association's external quality control program.

2.5.2. Brain Tissue Analysis

Native brain tissue samples were frozen at −80 °C until analysis. The presence of PrPSc was determined using a standardized western blot protocol (adapted from Collinge et al. [18]). Briefly, brain homogenates were treated with Proteinase K (Proteinase K from Tritirachium album, cat. #SRE0005; Sigma-Aldrich, St. Louis, MI, USA). Samples were separated using SDS-PAGE and blotted onto nitrocellulose membranes. For detection of the PrPSc, two different monoclonal antibodies: 12F10 (1:1667, cat. #A03221.200; Bertin Bioreagent, Montigny le Bretonneux, France), and 6H4 (1:5000, cat. #01-010 and since 2017 cat. #7500996, Prionics, Zürich, Switzerland) were used. Incubation with an appropriate secondary antibody (1:2500 and 1:7500, respectively, cat. #P0447; Dako) was followed by chemiluminescent detection (Pierce ECL Plus Western Blotting Substrate, cat. #32132; Thermo Scientific, Waltham, MA, USA). After limited proteolysis, three PrPSc glycoforms were detected.

2.6. Molecular-Genetic Methods

PRNP (NC-000020.11) is a 16 kb long gene located on chromosome 20 (4686151–4701588). It contains two exons, and exon 2 carries the open reading frame, which encodes the 253 amino acids (AA) PrP protein. Exon 1 is a noncoding exon, which may serve as a transcription initiation site. Post-translational modifications result in removing the first 22 AA N-terminal fragments (NTF) and the last 23 AA C-terminal fragments (CTF).

2.6.1. Study Population

Our study was designed as a retrospective. We analysed data from 215 patients (n = 215), age range 40–87 years, median 66 years. No family history of CJD was retraced. We included patients with postmortem confirmed sCJD and then collected data regarding clinical presentation, biochemical analysis, EEG, and neuroimaging.

Gene analysis was performed at the level of genomic DNA, assuming an effect on the protein sequence. Only the coding part of the *PRNP* (NM_000311) gene and the adjacent intronic region were evaluated. Genetic analysis of genes was performed from autoptic samples of definitively confirmed cases. DNA was isolated from bone marrow (QIAamp DNA Kits).

2.6.2. Genetic Screen

Mutation analyses by *PRNP* gene sequencing were performed on genomic DNA extracted from bone marrow. The targeted gene captured all exons and the flanking intronic regions of the *PRNP* gene to cover the splice sites.

Genomic DNA was amplified using two pairs of specific PCR primers (*PRNP* 1F: 5′ TACCATTGCTATGCACTCATT 3′, *PRNP* 1R: 5′GTCACTGCCCGAAATGTATGA 3′ *PRNP*, 2F: 5′AGGTGGCACCCACAGTCAGT 3′ PRNP 2R: 5′ CCTATCCGGGACAAA-GAGAGA 3′). Primers were designed using mPCR software, and specific target regions were amplified using PCR (temperature profile in the *PRNP* 1: 95 °C/12 min, 29× (95 °C/30″, 53.1 °C/30″, 72 °C/40″), 72 °C/5′, 4 °C/∞. Temperature profile in the *PRNP* 2: 95 °C/12 min, 30× (95 °C/30″, 60.5 °C/30″, 72 °C/70″), 72 °C/5′, 4 °C/∞). PCR products were enzymatically purified using recombinant Shrimp Alkaline Phosphatase (rSAP) and Exonuclease I (Exo I). The purified products were amplified in a sequencing reaction (temperature profile: 96 °C/60″, 25× (96 °C/10″, 50 °C, 5″, 60 °C/30″), 4 °C/∞)

using BigDye Terminator v3.1 Cycle Sequencing Kit's (Applied Biosystems™). Cleanup PCR products were sequenced on an Applied Biosystems® 3130 Genetic Analyser (using the DNA sequencing Standard Operating Protocol SOPV. We use Sequencing Analysis 5.3.1 software (Applied Biosystems, Waltham, MA, USA—Life Technologies), SeqScape v.2.6 (Applied Biosystems, Waltham, MA, USA—Life Technologies) to evaluate electrophoretic sequencing data.

Results are summarised in Table 2. In our CJD samples, we present an analysis of codon 129 distribution in 215 cases. Methionine homozygotes represented 63.25%, valine homozygotes 15.35%, and methionine/valine 21.40%.

Table 2. Distribution of MV polymorphisms in pure and comorbid CJD cases.

Polymorphism	Pure CJD	CJD/tau	CJD/AD	CJD/FTLD	CJD/Synuclein
MM	17	85	27	4	3
VV	3	16	9	3	2
MV	4	34	8	0	0
TOTAL	24	135	44	7	5

VV—Valine/Valine, MM—Methionine/Methionine, MV—Methionine/Valine, total stands for total number of cases in each group.

Although the cohort of 215 patients is limited, we noticed that the ratio of valine homozygotes varied from group to group. In pure CJD, VV homozygotes form 11.00% of cases, in CJD/tau 12.50%, in CJD/AD 20.45%, in AD/FTLD 43.00%, and in CJD with comorbid synucleinopathy 40.00% of cases.

2.7. Clinical Data

This study was conceived as a retrospective data analysis. Medical records from different hospitals across the Czech Republic were assessed; in cases with insufficient data, the concerned hospitals were directly contacted to retrieve complete data. For this study, we focused on the presence/absence of key features mentioned by the current WHO diagnostic criteria for probable CJD, i.e., dementia, pyramidal or extrapyramidal signs, visuospatial or cerebellar dysfunction, myoclonus, and akinetic mutism (see Supplementary Material).

2.8. Magnetic Resonance Imaging

Available MRI scans were assessed independently by two investigators (J.K. and R.R.) to confirm typical MRI findings listed in the WHO diagnostic criteria for probable CJD, i.e., cortical hyperintensities (typically in the frontal and periinsular areas) and basal ganglia hyperintensities (putamen and caudate) in FLAIR and DWI sequences. DWI data included in all cases an acquisition with a b value equal to 1000. Hyperintensities were evaluated qualitatively, ADC maps were used only to confirm the restriction of the diffusion. No fixed windowing was used as it has been reported to have a lower area under the receiver operating characteristic curves when used by radiologists [19]. Moreover, we used semiquantitative scales for detecting focal atrophy in temporal areas (including the hippocampi) and parietal cortices—for this purpose, we used the MTA scale [20] (measuring mesial temporal atrophy) and the Koedam score [21] (assessing parieto-occipital atrophy). Furthermore, cerebrovascular lesions were coded using the Fazekas scale [22]. All available results are summarised in the Supplementary Material section.

2.9. Statistical METHODS

The data set was split into two groups using additional diagnoses; hypothesis testing was performed at $p = 0.05$. Logical explanatory variables were processed using the Fisher exact test of variable independence in 2×2 contingency tables to obtain significant Odds Ratios (OR). Real explanatory variables were processed using the two-sampled Wilcoxon–Mann–Whitney test of median equity. All the calculations were performed in the MATLAB 2019 Statistical Toolbox.

3. Results

3.1. Neuropathological Results

3.1.1. "Pure" CJD

Immunohistochemical methods revealed the presence of several comorbidities in neuropathological examinations. Of the 215 patients, only 24 cases (11.16%) had pure CJD, i.e., lacking any other pathological intra- or extracellular aggregates. The age range of the pure CJD cases was 40–78 years, and the median age was 60 years, which is statistically significantly younger than in the comorbid subgroups.

3.1.2. Comorbid CJD + Tauopathy

Based on clinical data, patients with neuropathological signs of tauopathy lacking clinical correlate were included in this group. The criteria met one hundred thirty-five patients (62.79%), of which 99 (46.05% of 215 cases) had primary age-related tauopathy (CJD/PART). Another 34 cases (15.81%) suffered from CJD/AGD; one patient had CJD/ARTAG (0.47%). Eighteen cases (8.37%) had a combination of CJD/PART and another tauopathy: 13 cases (6.04%) had argyrophilic grain disease (CJD/PART + AGD), and five cases (2.33%) had ageing-related tau astrogliopathy (CJD/PART + ARTAG). Two patients (0.93%) had a combination CJD/ARTAG+AGD, and finally, there was one case (0.47%) with CJD/PART + ARTAG + AGD. The age range of CJD/tau comorbid cases ranged from 49 to 87 years, and the median age was 65 years.

3.1.3. Comorbid CJD/AD

The cohort contained 44 comorbid cases (20.47%) of CJD/AD with possible additional co-pathology. According to the revised "ABC" classification of the National Institute on Aging–Alzheimer's Association (NIA-AA) [13], changes were classified as level "none" (1 case; 0.47%), "low" (23 cases; 10.70%), or "intermediate" (19 cases; 8.84%), no patients in the "high" category were found. The presence of tauopathies was relatively common with the Alzheimer's pathology—ARTAG was diagnosed in 10 cases (4.65%), AGD in four cases (1.86%); in one of these cases (0.47%), the criteria for ARTAG + AGD was met. The age range for this group was aged 56–85 years, and the median age was 71 years.

3.1.4. Comorbid CJD/FTLD

Considering comorbid cases of CJD and frontotemporal lobar degeneration (CJD/FTLD) with characteristic frontotemporal clinical symptomatology, seven cases (3.26%) were found, of which six cases (2.80%) had FTLD/tau, and one (0.47%) had frontotemporal lobar degeneration with positive inclusions for ubiquitin-proteasome system markers (FTLD/UPS). The age range was 55–78 years, and the median age was 67 years.

3.1.5. Comorbid CJD + Synucleinopathy

Finally, five patients (2.33%) suffered from CJD with comorbid synucleinopathy; four patients (1.86%) met the criteria for DLB, and one (0.47%) had Parkinson's disease (PD). The age ranged from 59 to 76 years, with a median age of 71 years.

3.2. CSF Analysis Results

CSF analysis was performed antemortem in about 80% of cases: 174 (80.93%) patients were tested for the presence of 14-3-3β, 168 (78.14%) patients were tested for levels of t-tau, p-tau, and $A\beta_{42}$. For CJD, the cut-off level for t-tau was set at 1200 pg/mL [17].

Results of the 14-3-3 analysis are summarised in Table 3 (full details are available in the Supplementary Material). In all groups, 14-3-3β positivity, which is one of the diagnostic criteria for probable CJD (CDC, 2018), was less common than very high t-tau levels (Table 3).

Table 3. Numbers (n) and percentage (%) of positive, low positive, negative and unanalysed results of the presence of 14-3-3β, t-tau levels and the combined presence of 14-3-3β and t-tau protein levels in CSF. Percentage is related to the whole cohort.

		14-3-3β (n)	14-3-3β (%)	t-tau (n)	t-tau (%)	14-3-3β + t-tau (n)	14-3-3β + t-tau (%)
pure CJD	pos	12	5.58	17	7.91	11	5.12
	low pos	1	0.47	N/A	N/A	8	3.72
	neg	8	3.72	3	1.40	2	0.93
	no	3	1.40	4	1.86	3	1.40
CJD/tau	pos	67	31.16	92	42.79	74	34.42
	low pos	16	7.44	N/A	N/A	27	12.56
	neg	27	12.56	13	6.05	9	4.19
	no	25	11.63	30	13.95	25	11.63
CJD/AD	pos	24	11.16	31	14.42	27	12.56
	low pos	5	2.33	N/A	N/A	6	2.79
	neg	4	1.86	2	0.93	0	0.00
	no	11	5.12	11	5.12	11	5.12
CJD/FTLD	pos	6	2.79	7	3.26	6	2.79
	low pos	0	0.00	N/A	N/A	1	0.47
	neg	1	0.47	0	0.00	0	0.00
	no	0	0.00	0	0.00	0	0.00
CJD/synuclein	pos	2	0.93	3	1.40	2	0.93
	low pos	0	0.00	N/A	N/A	1	0.47
	neg	1	0.47	0	0.00	0	0.00
	no	2	0.93	2	0.93	2	0.93

pos = positive; low pos = low positive; neg = negative; no = unanalysed; N/A = not applicable. T-tau cut-off: 1200 pg/mL; lower negative. For the combination of 14-3-3β + t-tau, "pos" means both variables are positive, "low pos" means that one of the variables is positive, and the other is negative.

When every group was tested separately, the lowest frequency of 14-3-3β positivity was found in the pure CJD subgroup (12 out of 21, 57.14%). The 14-3-3 positivity was much higher in the comorbidity subgroups (CJD/tau 67 out of 110, 60.91%; CJD/AD 24 out of 33, 72.73%; CJD/FTLD 6 out of 7, 85.71%; and CJD/others 2 out of 3; 66.67%). In pure CJD, t-tau was positive in 17 out of 20 (85.00%). T-tau positivity was higher (CJD/tau 92 out of 105, 87.62% and CJD/AD 31 out of 33, 93.94%) in the comorbidity subgroups.

3.3. Clinical Analysis Results

Clinical data were available from all patients and are summarised in Table 1. Diagnostic criteria for possible sCJD [23] were fulfilled in all 215 cases, i.e., all had dementia, and at least two of the four needed signs, i.e., (1) pyramidal or extrapyramidal signs, (2) visuospatial or cerebellar dysfunction, (3) myoclonus, and (4) akinetic mutism. The distribution was as follows: pyramidal or extrapyramidal signs 189 cases (87.90%), visuospatial signs 159 cases (73.95%), myoclonus 133 cases (61.86%), and akinetic mutism 99 cases (46.05%).

3.4. MRI Results

MRIs were available in 206 cases (95.81%), and FLAIR/DWI sequences were available in 188 of these (87.44%; for detailed information, see Table 1). Typical FLAIR and DWI findings meeting the WHO diagnostic criteria for probable CJD were found as follows: cortical hyperintensities in 146 of 188 cases (77.66%), basal ganglia (caudate and putaminal) hyperintensities in 122 of 188 cases (64.89%), and both cortical plus basal ganglia hyperintensities in 109 of 188 cases (57.98%). Manifest mesial temporal atrophy (Scheltens MTA score 2) was present in 32 of 206 cases (15.53%), severe parieto-occipital atrophy (Koedam score 2) was present in 22 of 206 cases (10.68%), and potentially clinically relevant ischemic subcortical white matter lesions (Fazekas score 2 and 3) were present in 32 of 188 cases (17.02%). For detail see Figure 1.

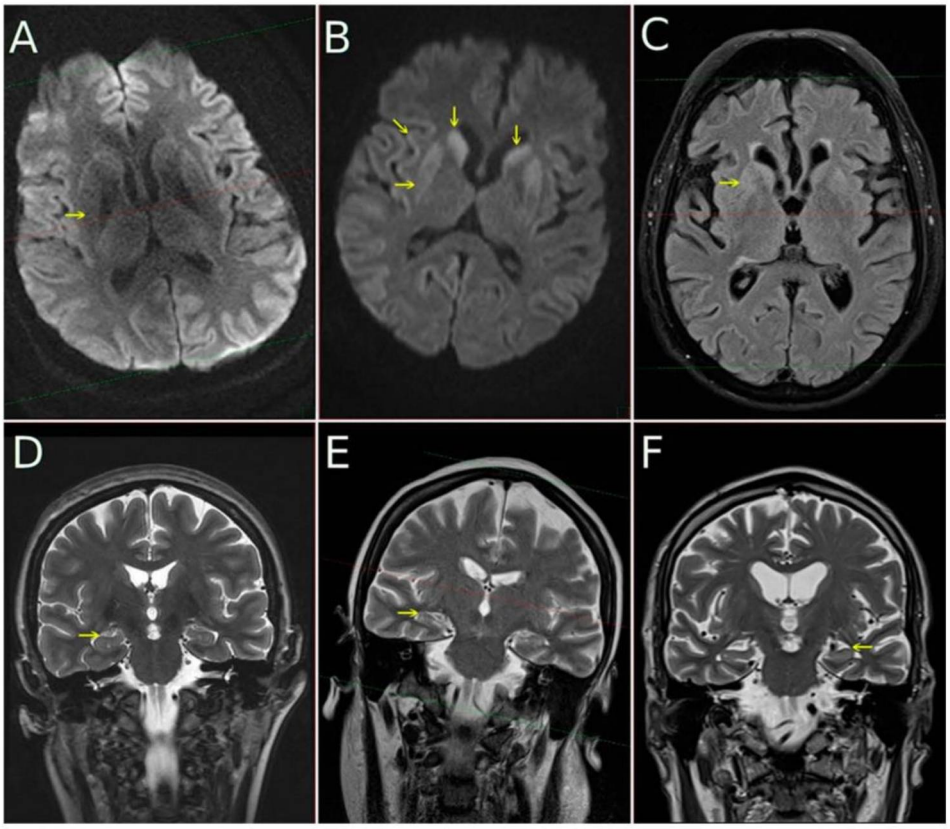

Figure 1. MRI in CJD subjects performed at 1.5T field strength: diffusion-weighed images ((**A**,**B**), DWI) with b-factor 1000, FLAIR image (**C**), coronal T2-weighted images (**D**–**F**). In the first column (**A**,**D**) data from subject with pure CJD—no DWI hyperintensity in putamina (arrow) is present and MTA is 0 (read as normal). In the second column (**B**,**E**) subject with tau comorbidity with mild hippocampal atrophy (MTA 1, arrow on (**E**)) and DWI (**B**) hyperintensity in putamina (horizontal arrow), caudates (vertical arrows) and with cortical ribboning (oblique arrow). In the third column (**C**,**F**) subject with CJD and AD comorbidity is shown. A moderate hyperintensity is visible not only on DWI (not shown), but as well on FLAIR image (**C**). Hippocampal atrophy is well pronounced, MTA 2 (arrow from right side of the (**F**), pointing to the left hippocampus which manifests clear atrophy).

3.5. Confocal Microscopy Colocalization Results

Multichannel fluorescence confocal microscopy was used in comorbid CJD case examinations to monitor the colocalization of individual pathological aggregates. We devoted our previous publication [24] to the morphology of colocalizing pathological prion protein and amyloid-beta, as well as pathological tau-positive inclusions. Compound plaques with either Aβ or hyperphosphorylated tau protein (h-tau) in colocalization with PrP^{Sc} were sparse. In contrast, PrP^{Sc} aggregates colocalized predominantly with the non-compact (diffuse) regions of Aβ plaques, and colocalization of h-tau with PrP^{Sc} had a dotted pattern. According to the NIA-Alzheimer's association guidelines, no association between the micromorphology of plaques and type of colocalization with polymorphism at codon 129, type of PrP^{Sc}, and the AD ABC score was found. See Figures 2–4.

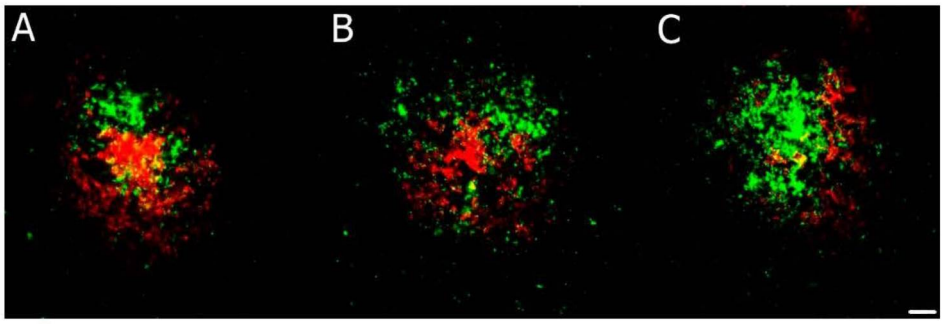

Figure 2. (**A**–**C**) Immunofluorescence illustration of different patterns of amyloid β (Aβ; red) and prion protein (PrP^{Sc}; green) colocalization in compound plaques in comorbid Alzheimer's (AD) and Creutzfeldt–Jakob diseases (CJD) cases. Primary antibodies: anti-PrP (rabbit recombinant monoclonal antibody) + anti-amyloid β-protein (mouse monoclonal antibody). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG; green) or Alexa Fluor® 568 (anti-mouse IgG; red). Scale bar indicates 10 μm. Images come from the hippocampal region (archicortical parts).

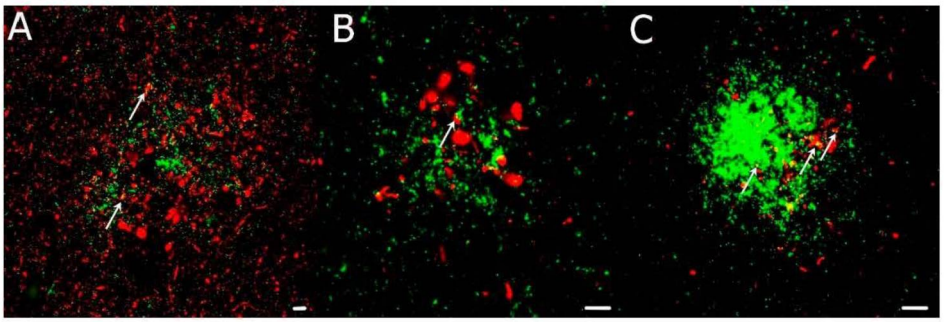

Figure 3. (**A**–**C**) Immunofluorescence illustrates h-tau-positive (red) dystrophic neurites colocalizing with PrP^{Sc} (green) extracellular deposits in comorbid CJD/AD cases. Primary antibodies: PrP (rabbit recombinant monoclonal antibody) + AT8 (mouse monoclonal antibody). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG, green) or Alexa Fluor® 568 (anti-mouse IgG, red). Scale bars indicate 10 μm. Arrows indicate minor colocalization of AT8 with PrP. Images come from the hippocampal region (archicortical parts).

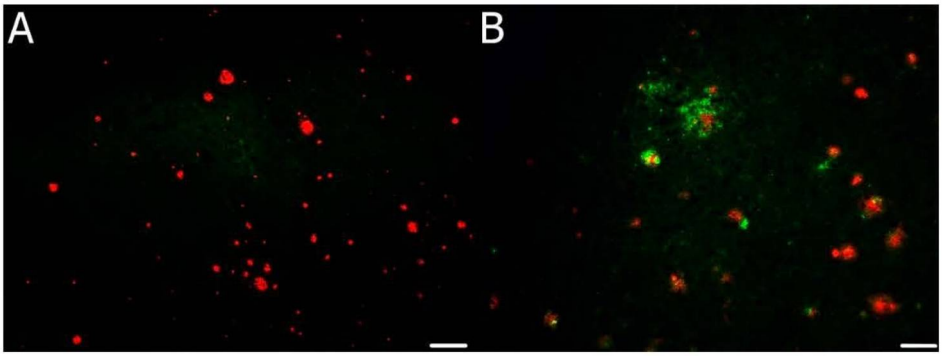

Figure 4. (**A**,**B**) Immunofluorescence illustration of the predominance of non-compound or minimal-compound plaques with minimal colocalization of Aβ (red) and PrP^{Sc} (green) in the majority of plaques. Primary antibodies: anti-PrP (rabbit recombinant monoclonal antibody) + anti-amyloid β-protein (mouse monoclonal antibody). The secondary antibody was conjugated with either Alexa Fluor® 488 (anti-rabbit IgG; green) or Alexa Fluor® 568 (anti-mouse IgG; red). Scale bars indicate 100 μm. Images come from the hippocampal region (archicortical parts).

4. Discussion

The main findings from comparing pure CJD with different comorbid subgroups were as follows: (1) pure CJD had a significantly lower age of onset; (2) tau comorbidity was associated with a higher probability of putaminal hyperintensities and had a lower MTA score on MRI; (3) AD comorbidity was associated with a higher age of onset, a lower probability for developing putaminal hyperintensities on MRI, and had significantly lower beta-amyloid levels in the CSF; (4) pure CJD compared to CJD/AD had a lower age at onset and a lower MTA score on MRI; (5) comorbid CJD/tau differed from comorbid CJD/AD by having a lower age of onset, lower MTA scores, and higher beta-amyloid CSF levels, and (6) comorbid co-pathology is not dependent on codon 129 homo/heterozygosity nor the genetic background of CJD.

First, the lower age of onset in "pure" CJD cases could be explained by the absence of age-related changes in the brain tissue. In this subgroup, early-onset prion pathology with rapid disease progression probably leads to death before the development of other comorbidities.

Second, comorbid tauopathies were more likely than in other subgroups associated with putaminal DWI hyperintensities on MRI. It is known that changes in these signals correlate roughly equally with vacuolation and the amount of PrP^{Sc} deposition (and less with astrocytic gliosis) [25]. The reason why this hypersignal is more pronounced in CJD subjects with comorbid tauopathy remains unclear since isolated tauopathies are not associated with similar MRI findings, and putaminal deposition of tau protein is not typical; however, it has been reported [26]. We can, therefore, only hypothesise that in subjects with comorbid CJD and tauopathy, the presence of tau facilitates signal increases on DWI either by tau and prion protein co-occurrence or by putaminal microstructure changes, since no micromorphological differences were visible. Lower MTA scores in the CJD/tau subgroup are even more difficult to understand, as in tau positive FTLD, pronounced frontal and temporal lobe atrophy is a frequent finding, as well as in AD, for which higher MTA scores are characteristic [27].

Third, comorbid AD was linked to a higher age of onset. Again, this is a rather surprising finding; as already discussed above, one would expect neurodegenerative comorbidities to have a significant impact relative to the destruction of brain tissue and thus lead to the earlier onset and faster disease progression. Nevertheless, CJD/AD can be viewed from different angles. Pre-existing AD development is more likely to be present at older ages, and from this point of view, it would not be unexpected that comorbid CJD/AD cases are older on average. In one of our previous publications, based on morphological findings using a multichannel fluorescent confocal microscope, we speculated that $A\beta_{42}$ might be acting as PrP^{Sc} seeds within the brain [24] since PrP-Aβ colocalization predominates in the periphery of plaques where $A\beta_{42}$ is more abundant.

Nevertheless, it is important to emphasise that compound plaques represent only a minority of plaques since PrP and Aβ plaques tended to be, in most cases, located separately or formed "minimal compound" plaques. However, even this view of CJD/AD assumes faster progression in CJD/AD comorbid cases than in "pure" CJD, although the data obtained from our 10-year surveillance did not support such a tendency. MRI findings of putaminal sparing can be hypothesised by reduced basal ganglia involvement and fewer parkinsonian features in non-comorbid AD patients compared to pure FTLD patients. The observed low beta-amyloid levels in CSF were concordant with current biomarker findings in pure AD.

A recently published Italian study [10] showed data similar to our observations: the mean age of the CJD/AD cases (71.07 years for our cohort versus 76.1 years for the Italian patients) is strikingly higher than in pure CJD cases; no link was found between the existence of comorbid AD and disease subtype, prion strain, or *PRNP* genotype; in both cohorts, there were no patients in the "high" level of AD; data from both cohorts showed low Aβ levels in the CSF. There was, however, a surprising difference in the percentage of

cases in which AD was reported: 61.5% of cases with CJD/AD reported in Italy compared to only 20.47% in our study.

Fourth, some of the previous findings became more obvious when we compared pure CJD to only comorbid CJD/AD. The difference in age of onset and MTA scores were larger between pure CJD and CJD/AD than when comparing pure CJD to all subgroups. The MTA scale is a widely used scoring system for assessing hippocampal atrophy in AD patients and may also monitor disease progression over time [28,29]. We thus suggest that AD could be present before prion disease in older patients.

Fifth, the role of AD on clinical manifestations and biomarkers in our cases was visible when comparing CJD/tau to CJD/AD subgroups. In line with previously discussed points, higher age of onset, higher MTA scores, and lower beta-amyloid CSF levels were key features of the impact of AD on our cohort.

Sixth, from the genetic point of view, we found no differences between codon 129 status of the *PRNP* gene or between genetic and sporadic CJD; in line with our pilot study, no prominent abnormalities in the deep genetic analysis of "pure" CJD cases and CJD cases in comorbidity with AD and tauopathies, respectively were seen, nor in the analysis of 15 genes related to the most important neurodegenerative diseases [30]. It has been suggested that increased expression of *Syntaxin-6* (*Stx6*) in the basal ganglia could raise the risk of prion disease, and *Stx-6* deposits have been identified as a risk factor for a 4R-tauopathy and progressive supranuclear palsy. The same study also suggests a possible role for *GAL3ST1* [31] in encoding galactose-3-O-sulfotransferase 1, which affects myelin maintenance. This would be consistent with the finding that sphingolipid metabolism is disrupted early in the pathogenesis of prion disorders in mouse models [32].

Finally, compared to a study by Kovacs et al. [9] that focused on tau pathology in CJD and identified 69.3% of cases with prominent tau co-pathology and an additional 16.0% of cases with discrete non-significant tau-immunoreactive neurites, our percentage (11.16%) of "pure" cases was surprisingly low. A possible explanation for the difference in the percentage of "pure" cases by Kovacs et al. is that they did not discuss other pathological deposits. By contrast, our study revealed cases of rare comorbidities such as CJD/FTLD or CJD/synucleinopathies; some correlations between imaging findings and co-pathologies were also found.

The main limitations of our study were the single-center expertise and the very uneven extent of neuroimaging and clinical data from patients examined in different hospitals. These factors made a more robust analysis of the data and clinical correlations difficult.

5. Conclusions

We present results from a large cohort of postmortem confirmed CJD patients (with pure CJD and neurodegenerative comorbidities) with clinical, MRI, CSF, neuropathological, and immunohistochemical data. Our retrospective data analysis found a surprisingly high proportion of comorbid neuropathologies, with only 11% of our cases being pure non-comorbid CJD. These patients were found to have the lowest age of disease onset.

The most interesting findings from our comorbid subgroup analysis were that tauopathy is linked to putaminal hyperintensity one MRIs and that AD is associated with the age of disease onset, the degree of hippocampal atrophy seen on MRIs (low MTA scores), and the low beta-amyloid levels in the CSF. However, further investigation on a broader spectrum of comorbid neuropathologies is needed before evidence of their impact on the clinical presentation can enter routine practice, especially when new biological and potentially targeted therapies become available for treating specific proteinopathies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines10030680/s1.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki approved in advance by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer University Hospital No G-19-18, obtained 26 June 2017. Cerebrospinal fluid was processed in accordance with F-IS-TN-2302012, Version No. 4. No informed consent obtained as only archival tissue of dead subjects was investigated retrospectively in anonymous setting with respect to their privacy, no treatment or diagnostic intervention was performed.

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3.5 Utřídění názvosloví jednotlivých podtypů extracelulárních plak u Alzheimerovy choroby a prionóz, jejich vzájemné srovnání.

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Review

Extracellular Amyloid Deposits in Alzheimer's and Creutzfeldt–Jakob Disease: Similar Behavior of Different Proteins?

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Abstract: Neurodegenerative diseases are characterized by the deposition of specific protein aggregates, both intracellularly and/or extracellularly, depending on the type of disease. The extracellular occurrence of tridimensional structures formed by amyloidogenic proteins defines Alzheimer's disease, in which plaques are composed of amyloid β-protein, while in prionoses, the same term "amyloid" refers to the amyloid prion protein. In this review, we focused on providing a detailed didactic description and differentiation of diffuse, neuritic, and burnt-out plaques found in Alzheimer's disease and kuru-like, florid, multicentric, and neuritic plaques in human transmissible spongiform encephalopathies, followed by a systematic classification of the morphological similarities and differences between the extracellular amyloid deposits in these disorders. Both conditions are accompanied by the extracellular deposits that share certain signs, including neuritic degeneration, suggesting a particular role for amyloid protein toxicity.

Keywords: Alzheimer's disease; Creutzfeldt–Jakob disease; Gerstmann–Sträussler–Scheinker syndrome; amyloid; senile plaques; PrP plaques; plaque subtypes

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1. Introduction

Deposits of aggregates of particular proteins are specific hallmarks of a wide range of neurodegenerative diseases [1]. Aggregates of misfolded proteins with altered degradation can be located intracellularly and/or extracellularly. The most important primary intracellular proteins include:

1. Hyperphosphorylated protein tau in Alzheimer's disease (AD) [2], tauopathies including frontotemporal lobar degenerations with tau pathology (FTLD-tau) [3];
2. Alpha-synuclein in Lewy bodies in Parkinson disease (PD) and dementia with cortical Lewy bodies (DLB) or in oligodendroglial inclusions in multiple systemic atrophy (MSA);
3. Phosphorylated TDP-43 in frontotemporal lobar degeneration with TDP-43-positive inclusions (FTLD-TDP) [4];
4. Ubiquitin in frontotemporal lobar degeneration with inclusions positive for ubiquitin-proteasome system markers (FTLD-UPS) [4,5];
5. Fused in sarcoma (FUS) inclusions in FTDL-FUS [6].

Primary extracellular protein aggregates, in optical microscopy called "plaques," can be observed in cortical locations in:

1. AD [7];
2. Prion diseases (Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI), and kuru) [8].

In all of these diseases, the term cerebral amyloidosis is widely used referring to insoluble fibrillar structures with a predominant beta-sheet conformation detectable by Congo red and thioflavin S binding [9]. These pathologic units are known to form from insoluble fibrils, giving rise to tridimensional aggregates called plaques that may exhibit different features depending on subtype.

The aim of our review is to compare and highlight similarities and differences between the two types of extracellular deposits, i.e., Aβ in AD and amyloid prion protein in prionoses, while simultaneously synthesizing the available information for didactic purposes.

2. Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common form of dementia [10]. The prevalence in those over 65 years is reported to be 3%, and in those over 85 years, it is about 32% [11]; therefore, as the human population ages, the total number of AD patients will increase. The neuropathological diagnostic hallmarks fundamental to an AD are extracellular Aβ plaques and intracellular neurofibrillary tangles (NFTs), both of which are neuropathologically defined using the National Institute on Aging–Alzheimer's Association (NIA-AA) consensus scheme [12,13]. Extracellular amyloid deposits are evaluated according to Thal's criteria, in which the phase is based on the brain areas manifesting Aβ plaques, the extent of intracellular neurofibrillary tangles, according to Braak staging [10,13], and semiquantitatively estimated density of neocortical neuritic plaques as recommended by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [14]. From all Aβ species, Aβ oligomers are considered to be the most toxic and most likely to lead to neuronal dysfunction and degeneration. Moreover, Aβ fibrils share experimental properties of transmissibility with prion proteins, and more research is needed into the "prionoid" or "prion-like" biochemical phenomena of all amyloidogenic peptides [15]. Hence, oligomeric Aβ concentrations impact cognitive impairment more than concentrations of Aβ monomers or plaques themselves [16], although the precise role of Aβ in AD pathophysiology is still not fully understood. Nevertheless, in AD, the decline in cognitive function is most closely related to the occurrence of NFTs than of Aβ deposits [17].

2.1. *Background of Aβ Plaque Formation*

Amyloid precursor protein (APP), a transmembrane protein existing in several isoforms [18], is amply expressed in brain tissue [19], and it plays a role in neuroprotection and homeostasis [20]. Additionally, APP is able to bind heparin and metals, mainly zinc [20] and copper [21]. When added exogenously, APP protects cell cultures from Aβ toxicity [22]. Through proteolysis, using β-secretase and γ-secretase [23], it creates Aβ polypeptides that are 38–43 amino acids long [24]. The whole pathway of APP processing involves the initial cleavage, by β-secretase, to clip off the N-terminal fragment (sAPPβ). Then γ-secretase cleaves the residual APP C-terminal fragment creating Aβ, and the amyloid intracellular domain (AICD) is formed. According to studies on primary neuronal cultures, cell viability is significantly reduced when β- or γ-secretase is inhibited or during Aβ immunodepletion [25].

The 42-amino-acid-long Aβ (Aβ42) is the main component of senile plaques, whereas Aβ 40, the more abundant product of APP processing [26], and which is less prone to aggregation, is common around blood vessels [27,28]—especially leptomeningeal, and small or medium-sized cortical arteries, arterioles, and capillaries [29]. While Aβ40 is described as a "closed" tetramer that is relatively resistant to the addition of additional Aβ40 units, Aβ42 is a more "open" tetramer with a tendency to generate hexameric and subsequently more stable dodecameric structures [30,31]. As mentioned above, the Aβ42 oligomers are considered to be the most toxic and causative in the development of AD [32,33].

2.2. Theory—Amyloid Cascade Hypothesis

In 1992, Hardy and Higgins [34] articulated the theory that the deposition of Aβ protein, the main component of plaques, was the causative agent of Alzheimer's pathology and that neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition. The theory is supported by:

1. An occurrence of familial Alzheimer's disease (fAD) in patients carrying an autosomal dominant mutation in genes encoding APP.
2. A higher fAD incidence was seen in families carrying the presenilin 1 (PSEN1) and presenilin 2 (PSEN2) mutations, which are the catalytic components of γ-secretase [35]. Most mutations in APP or PSEN1/PSEN2 alter APP proteolysis and result in increased production of the longer form of Aβ (i.e., Aβ42) [36].
3. Early-onset Alzheimer disease (EOAD) is manifested in patients with Down syndrome. The trisomy of chromosome 21, on which the gene for APP is located, logically leads to a triplicate of the *APP* gene. Many patients suffering from Down syndrome develop AD at an early age. The presence of Aβ plaques in these patients is often described in childhood [37], and the formation of neurofibrillary tangles occurs at about the age of 40 [38]. Thence, Down syndrome is considered to be the most significant genetic risk factor for the development of AD [39].

Although this theory dominates the field of AD research, it is not universally accepted [40–43], although the importance of the role of tau protein in the pathogenesis of AD and severity of cognitive decline has been demonstrated [36].

It is sometimes questioned for the following reasons:

1. There are patients having numerous plaques (or even fulfilling the neuropathological criteria for AD) but have no clinical signs of cognitive impairment [44].
2. Conversely, some mouse models of AD show memory deficits before the development of Aβ plaques [45].
3. While senile plaques appear first in the frontal cortex and then spread beyond the cerebral cortex to the hippocampus and beyond, neurofibrillary tangles initially develop in the limbic system [36]. To this day, the mutual relationship between these two neuropathological hallmarks is not fully understood.

The precise role of Aβ and tau protein in the pathophysiology of AD is still waiting for an explanation.

2.3. Morphological Classification of Senile Plaques (SP)

Amyloid/senile plaques are extracellular deposits of Aβ that are abundant in the cortex of AD patients [46], which, on average, are about 50 μm in diameter [47]. They can be divided into three subcategories (see summary in Table 1):

1. Diffuse/pre-amyloid plaques (Figure 1) that are predominantly 10–20 μm [48] amorphous amyloid deposits with ill-defined contours [46] and lacking dystrophic neurites [49]. Diffuse plaques are not associated with a glial response [50] or synaptic loss; hence, they are not sufficient for a neuropathological diagnosis of AD. Moreover, diffuse plaques are commonly found in the elderly without signs of cognitive decline [51]. They are evident with silver staining, but invisible with Congo red [52] or thioflavin [53].
2. Two subtypes of neuritic plaques can be distinguished.
    a. Non-cored/primitive/immature neuritic plaques (see Figure 2) are oval or spherical structures containing Aβ and altered neurites, 20–60 μm in diameter and lacking a dense Aβ region in the central part [54]; they are also associated with astrocytic and glial responses. They are reported to occur in older AD patients [55]. Similar to diffuse plaques, they do not stain with Congo red since they do not contain Aβ in the beta-sheet conformation [56].
    b. Cored/classic/dense/mature/focal neuritic plaques (Figure 3) are 20–60 μm [53] compact cores encircled by fibrillar Aβ deposits [51]. Tau-positive dystrophic

neurites [57], reactive astrocytes, and activated microglia [58,59] are found in the vicinity. Due to its relation to neuronal loss and its association with cognitive decline [60,61], these plaques are a basis for an AD diagnosis [62]. They can be visualized with silver staining [63], Congo red [64], and thioflavin [57].

3. Compact/burnt-out plaques (Figure 4) are 5–15 µm [48] in diameter, composed of a dense core that lacks a surrounding neuritic component [65].

It is not entirely clear whether non-cored neuritic plaques progress into cored and then to burnt-out plaques. In addition, it is also not known whether diffuse plaques are a common part of aging or the initial stage of neuritic plaque maturation [66].

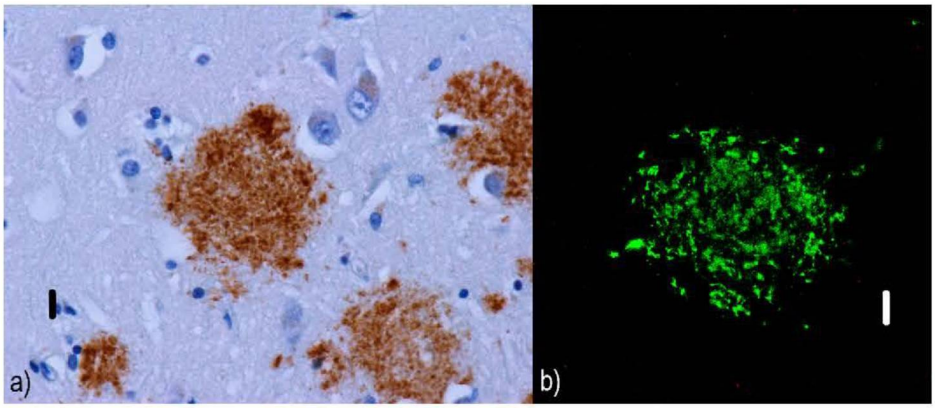

Figure 1. Diffuse plaques: (**a**) immunofluorescence visualization of diffuse Aβ plaques in an Alzheimer's disease (AD) patient. Compared to non-cored plaques, diffuse ones have less defined contours; they seem lighter and less dense. Primary antibodies: anti-beta amyloid rabbit immunoglobulin G (IgG). The original magnification was 400×. The scale bar indicates a length of 10 micrometers. (**b**) Utilizing immunofluorescence confocal microscopy, the absence of tau-positive dystrophic neurites (red) in diffuse Aβ (green) plaques is evident. Primary antibodies: Anti-beta amyloid rabbit IgG and AT8 (murine anti-hyperphosphorylated protein tau). The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). The scale bar indicates a length of 10 micrometers. The sample comes from a 92-year-old male whose neuropathological findings were a fully developed late form of Alzheimer's disease in the neocortical phase (Braak VI, Consortium to Establish a Registry for Alzheimer's Disease (CERAD) C, Thal 6) with local mild cerebral amyloid angiopathy (CAA Vonsattel grade 1). According to the revised "ABC" of the National Institute on Aging (NIA) classification, the changes associated with AD are at a "high" level (A3B3C3). This plaque was photographed in the subiculum, where diffuse and non-cored neuritic plaque were predominant.

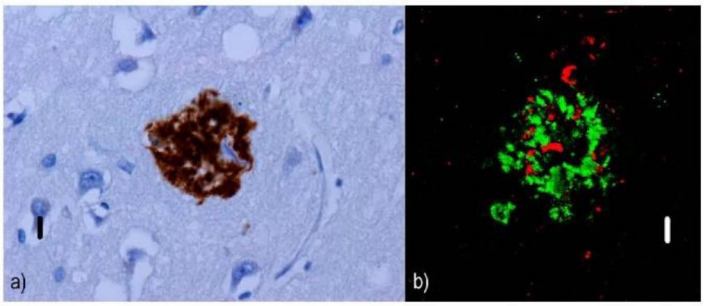

Figure 2. Non-cored neuritic plaques: (**a**) immunofluorescence visualization of non-cored Aβ plaque in an AD patient. These plaques are denser and more clearly bordered than diffuse ones. Primary antibodies: anti-beta amyloid rabbit IgG. The original magnification was 400×. The scale bar indicates a length of 10 micrometers. (**b**) Simultaneous imaging with a confocal microscope allowed us to display the presence of Aβ structures (green) as well as tau-positive dystrophic neurites (red) in the vicinity, which are a characteristic component of both types of neuritic plaques (either non-cored or cored). Note that some of the dystrophic neurites are dilated. Primary antibodies: anti-beta amyloid rabbit IgG and AT8 (murine anti-hyperphosphorylated protein tau). The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). The scale bar indicates a length of 10 micrometers. The sample comes from a 67-year-old female patient with a fully developed early form of Alzheimer's disease in the neocortical stage (Braak VI, CERAD C) with marked amyloid angiopathy (CAA Vonsattel grade 3). The changes associated with AD are at a "high" level (A3B3C3) according to the revised "ABC" classification of the NIA. This plaque comes from the amygdala region, where non-cored and cored neuritic plaques prevail in this case.

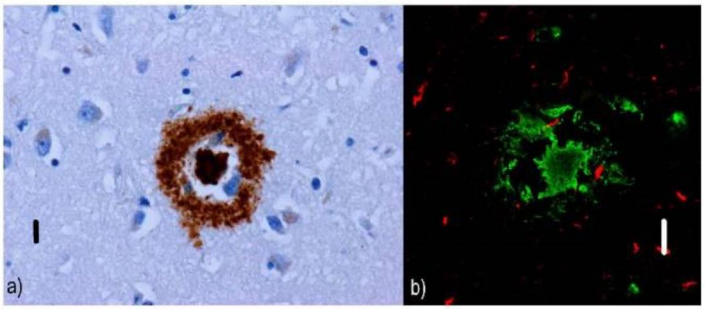

Figure 3. Cored neuritic plaques: (**a**) immunofluorescence visualization of cored Aβ plaque in an AD patient. The dense Aβ core is encircled by fibrillar Aβ deposits, which are clearly visible in cored neuritic plaques. Primary antibodies: anti-beta amyloid rabbit IgG. The original magnification was 400×. The scale bar indicates a length of 10 micrometers. (**b**) Simultaneous imaging with a confocal fluorescent laser scanning microscope shows the presence of an Aβ core with fibrillar Aβ structures (green) in the vicinity as well as a few tau-positive dystrophic neurites (red). Primary antibodies: Anti-beta amyloid rabbit IgG and AT8 (murine anti-hyperphosphorylated protein tau). The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). The scale bar indicates a length of 10 micrometers. The images are from a male 67-year-old patient with EOAD and come from the cornu ammonis, but similar findings were present in all areas of the hippocampal formation and adjacent para-hippocampal and entorhinal cortex. Neuropathological diagnosis: Fully developed early-onset form of Alzheimer's disease in the neocortical stage (Braak VI, CERAD C) with marked amyloid angiopathy (CAA Vonsattel grade 3). According to the revised "ABC" of the NIA classification, the changes associated with AD are at a "high" level (A3B3C3).

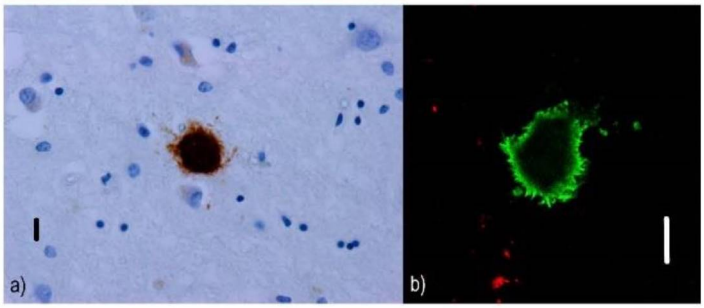

Figure 4. Burnt-out plaques: (**a**) immunofluorescence visualization of a burnt-out Aβ plaque (the dense core remains) in an AD patient. Primary antibodies: anti-beta amyloid rabbit IgG. The original magnification was 400×. The scale bar indicates a length of 10 micrometers. (**b**) Imaging of the dense Aβ nucleus (green) lacking surrounding components using a confocal microscope. Primary antibodies: Anti-beta amyloid rabbit IgG and AT8 (murine anti-hyperphosphorylated protein tau). The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). The scale bar indicates a length of 10 micrometers. This image comes from the amygdala of an 83-year-old female with a late variant of AD in the neocortical stage (Braak V, CERAD C). The changes associated with AD are at a "high" level (A3B3C3) according to the revised "ABC" classification of the NIA. Burnt-out and cored neuritic plaques were predominant in this area of the patient's brain.

Table 1. Summary of Aβ plaque types in AD.

Amyloid/Senile Plaques			
- Extracellular deposits of amyloid-β abundant in the cortex of AD patients [46] - Diameter ~50 µm [47]			
Diffuse/pre-amyloid	**Neuritic**		**Compact/burnt-out**
	Non-cored/ primitive/immature	**Cored/classic/dense/ mature/focal**	
- predominantly 10–20 µm - amorphous Aβ deposits with ill-defined contours [46] - lacking dystrophic neurites [49] - not associated with glial response [50] or synaptic loss [51] - not sufficient for the neuropathological diagnosis of AD - commonly found in brains of elderly without cognitive decline [46] - evident using silver stain - invisible with Congo red [52] or thioflavin [53]	- 20–60 µm - altered neurites lacking Aβ core in the central part [54] - invisible with Congo red [56]	- 20–60 µm - compact core surrounded by fibrillar deposits of Aβ [46] - tau-positive dystrophic neurites [57], reactive astrocytes, and activated microglia [58,59] in the vicinity - related to neuronal loss and associated with cognitive decline [60,61] - basis of Alzheimer's disease diagnosis [62] - confirmed with silver stain [63], Congo red [64], and thioflavin [57]	- 5–15 µm; - dense core without surrounding neuritic component [65] - visible using silver stain, Congo red [52], and thioflavin [53]

2.4. Dystrophic Neurites as a Component of Aβ Plaques

Dystrophic neurites in plaques may differ morphologically and immunohistochemically. Type I is described as elongated in shape, whereas type II is dilated, bulbous, or globular [67]. Certain levels of dilated, ubiquitin-positive neurites have been previously reported in AD patients, although usually without information regarding the exact brain location [68]. Based on our observations, bulbous neuritic changes are prominent mainly in archicortical structures [69].

2.5. The Molecular Composition of Aβ Plaques

1. The results of immunohistochemical examinations showed that diffuse/pre-amyloid plaques contain Aβ42 and other APP fragments lacking the C-terminus [70], apolipoprotein E [71], α1-antichymotrypsin [72], complement proteins [73,74], and heparan sulfate proteoglycan (HSPG) [75].
2. According to Armstrong [70], non-cored/primitive/immature neuritic plaques additionally contain both free and conjugated ubiquitin, paired helical filament antigen (PHF-antigen), phosphorylated tau protein, and numerous immunoreactive neurites.
3. Cored/classic/dense/mature/focal neuritic plaques consist of an Aβ42 core and a ring of alpha-synuclein. In addition to Aβ42, they contain Aβ40, complement proteins, immunoglobulins, and apolipoproteins D [76] and E. Due to the secondary binding to Aβ, zinc, copper [77], or aluminum [78] may also be part of the core, with aluminum having the lowest affinity [79]. Chromogranin, interleukine-6 [80], or catecholamine-positive neurites are constituents of the ring.

2.6. Laminar Distribution of Aβ Plaques

The internal pyramidal layer (layer V) and the external pyramidal layer (layer III) are the most affected [81]. The reason may be that APP mRNA is expressed in huge amounts by the pyramidal neurons in the internal and external pyramidal layer [82]. The degeneration of these neurons may increase APP secretion and, consequently, Aβ plaque formation [83]. Interestingly, no differences in plaque stratification were observed between patients with early-onset fAD, late-onset fAD, or sporadic AD; even the *Apo E* genotype does not appear to affect the morphology and distribution of Aβ plaques. Moreover, no differences in plaque density between the sporadic and familial AD variants have been observed [84].

3. Prion Diseases

Prion diseases are transmissible, progressive, and in all cases, fatal neurodegenerative disorders associated with an aggregation of misfolded prion protein [85]. Human transmissible spongiform encephalopathies include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), kuru, and the extremely rare fatal familial insomnia (FFI) [86]. In general, the neuropathological hallmarks of transmissible spongiform encephalopathies (TSEs) are spongiform changes, astrogliosis, and neuronal loss [87]. The toxicity of the scrapie isoform of the prion protein (PrPSc) remains controversial inasmuch as studies report different results. According to some studies, PrPSc oligomers are the most toxic form [88]; however, others state that PrPSc is not directly toxic to neurons; instead, it is the lack of the physiological cellular prion protein (PrPC) variant that leads to neuronal death [89].

Extracellular deposits and PrPSc plaques are structures visible with hematoxylin-eosin staining, while plaque-like structures can only be visualized by using immunohistochemical methods. [90] Plaques are present in 10–15% of Creutzfeldt–Jakob disease cases, 50–75% of [91,92] kuru patients, and 100% of patients suffering from Gerstmann–Sträussler–Scheinker syndrome. These amyloid plaques consist of PrP (see summary in Table 2) [93].

3.1. Molecular Background and the Composition of PrP-Amyloid Plaques

Cellular prion protein (PrPC) is a glycolipid-anchored cell membrane sialoglycoprotein localized on presynaptic membranes. PrPC appears to have neuroprotective [94] and promyelinating [95] functions; it participates in myelin maintenance, neurotransmission, zinc and copper transport, and calcium homeostasis [96–98]. It also seems to promote greater neuronal resistance after ischemic cerebral insult in laboratory rodent models [99,100]. An explanation for its numerous functions may be the ability of PrPC to interact with a variety of membrane proteins [98]. PrP is able to aggregate into amyloid [101] 8–10 nm long [102] and act as a receptor for Aβ [103,104]. According to recent research, the expression of PrPC is controlled by AICD [105], which was mentioned above as a product generated by γ-secretase cleavage in AD.

Clusterin often co-localizes in PrPSc plaques [101] and is able to bind Aβ, immunoglobulins, complement proteins, and lipids [106–113]. Moreover, prion protein also acts as a receptor for laminin, a glycoprotein mainly found in basement membranes [114].

3.2. Kuru

Kuru was the first human prionosis to be discovered and is defined as a neurodegenerative, non-inflammatory infectious disease [115,116]. Although the neurological symptoms are very similar in all patients, the neuropathological findings differ widely [117]. Shrunken neurons with dispersed Nissl bodies and intracytoplasmic vacuoles may be present, as well as vacuolated striatal neurons and cerebellar Purkinje cells [91]. A neuropathological feature may be a spongiform transformation [118] (mostly described as subtle) and neuronophagy affecting predominantly the deeper cortical layers but completely sparing hippocampal neurons. Microglial and astroglial proliferation can also be seen [117]. The most typical feature is amyloid "kuru" plaques, which are present in 50–75% [91,92] of examined brains. Immunohistochemistry has verified that the scrapie isoform of the prion protein shows synaptic and perineuronal positivity [119,120].

3.3. Creutzfeldt–Jakob Disease

Creutzfeldt–Jakob disease (CJD) is a transmissible and rapidly progressive [121] degenerative disease of the central nervous system caused by an accumulation of pathologically conformed PrP, [122] and the most common of the human prion diseases [123]. The neuropathological definition of CJD is spongiform encephalopathy in the cerebral and/or cerebellar cortex and/or the subcortical grey matter. Variations include encephalopathy with PrP immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar types) [124]. Four types, i.e., sporadic (sCJD), familial (fCJD), iatrogenic (iCJD) [125], and variant CJD (vCJD) [126], are distinguishable relative to their different etiologies [127]. The first mentioned, i.e., the sporadic type, is contingent on the accidental conversion of normal PrP to a pathological form and accounts for about 85% of CJD cases [128]. The genetic variant is conditioned by the detection of an inherited mutation in the prion protein gene (*PRNP*), which accounts for 10–15% of cases [129].

The other two types can be placed into the category of acquired CJD, i.e., the CJD variant that occurs after consumption of beef from cattle affected by bovine spongiform encephalopathy (BSE). The iatrogenic variant arises during medical or surgical procedures during which pathologically conformed prions are inadvertently transferred (e.g., during neurosurgical interventions, dura mater or corneal grafting, deep electrode insertions, or extraction of human pituitary hormones) [130]. Neuropathological changes include spongiform transformation, neuronal loss, astrocytosis, and the formation of PrP-amyloid plaques in the gray matter. The expression of neuropathological features varies significantly between individuals [131]. Importantly, amyloid plaques do not occur in all patients with sCJD, only accounting for approximately 10–15% of cases [124,132–134].

Different subtypes of sCJD are distinguishable, according to different polymorphisms at codon 129 (i.e., methionine or valine homozygosity (MM or VV, respectively) or methio-

nine and valine (MV) heterozygosity) of the *PRNP* and the type of proteinase K-resistant prion protein fragments (PrP), using a western blot examination [135].

Character and Typical Location of PrP Deposits According to the MV Polymorphism

a. MM1 subtype: synaptic and perivacuolar positivity, although cases with plaques in the white matter are so rarely encountered, we will not mention them in more detail [136].
b. MM2
   - Cortical subtype: perivacuolar positivity in all cortical layers;
   - Thalamic subtype: fewer plaques (which are usually described as coarse) [137]
c. MV1 subtype: synaptic and perivacuolar positivity;
d. MV2 subtype: distinctive "kuru-like" plaques in the cerebellum and perineuronal positivity in the cerebral cortex;
e. VV1 subtype: characterized by punctate synaptic positivity in the cerebral cortex;
f. VV2 subtype: perineuronal, with numerous plaque-like areas and some synaptic PrP positivity in the cerebral cortex [138].

As mentioned above, plaques are a neuropathological hallmark, but only for the MV2 subtype, where "kuru-like" plaques are found in the granular and molecular layers of the cerebellum [139]. Sometimes the Purkinje cell layer is also described as having an abundance of plaques [140]. They are sometimes found in the subcortical gray matter but seldom in the cerebral cortex [141]. Rarely, individuals with the MM type 1 polymorphism have plaques in the white matter. In these cases, significantly longer survivals have been reported (around 24 months) [142]. These "kuru-like" plaques are characterized by a hyaline eosinophilic core with a pale halo, both visible with hematoxylin-eosin staining.

3.4. Gerstmann–Sträussler–Scheinker Syndrome

Gerstmann–Sträussler–Scheinker syndrome (GSS) is defined as a slowly progressive hereditary autosomal dominant neurodegenerative disease [143] or encephalo(myelo)pathy with multicentric PrP plaques [124] localized in the cerebral and cerebellar cortex and the basal ganglia [144,145]. Clinically, ataxia and progressive dementia are distinctive [146]. GSS was the first human prion disease to be associated with a *PRNP* mutation. To date, point mutations at codons 102, 105, 117, 131, 145, 187, 198, 202, 212, 217, and 232 have been reported [143]. Some families carry octapeptide repeat insertions (OPRI), families having four [147], five [148], six [149,150], seven [151], eight [152], and nine [153] multiples of the 24 base pairs between codons 51 and 91 in the *PRNP* gene have been reported. In patients with 4 to 7 multiples, elongated PrP deposits are usually described, while in those having 8 or 9 OPRI, kuru-like or multicentric plaques have been found [154]. According to some studies, clinical and neuropathological variability is further affected by MV polymorphisms at codon 129; however, other researchers have failed to find any significant differences between homozygotes and heterozygotes [155].

Using silver staining methods, amyloid plaques in prion diseases can mimic burnt-out Aβ42 plaques. Nevertheless, unlike Aβ42 plaques, these PrP plaques can be clearly seen with hematoxylin-eosin staining. After proteinase pre-treatment, the presence of PrPSc can be confirmed by using specific immunohistochemistry. While PrPSc in GSS is partially sensitive to the effects of proteinase [73].

3.5. Summary of Morphological Types of PrP Plaques in TSEs

1. Unicentric/"kuru"/"kuru-like"/stellate plaques (Figure 5) are up to 30 μm [132] deposits consisting of a dense star-shaped core with thin amyloid bundles radiating into the periphery [156]. In kuru disease, the average plaque size is reported to be between 20–60 μm [117]. These plaques are surrounded by astrocytic processes that have been extensively invaded by microglia [157], although dystrophic neurites are unusual [156]. However, some studies report tau-immunoreactivity around "kuru-like" plaques [158]. "Kuru-like" plaques are present in 10–15% of sCJD patients [156],

all of whom carry the MV2 polymorphism at codon 129 [138]. In CJD cases, they occur mostly in the molecular layer of the cerebellum and the Purkinje cell layer [140]. For kuru disease, typical locations include the granular cell layer of the cerebellum, the basal ganglia, thalamus, and cerebral cortex [158]. These plaques are visible with hematoxylin-eosin staining [90], which distinguishes them from plaque-like structures.

2. Daisy/florid plaques measure up to 200 µm [132] and consist of a PrP-amyloid core surrounded by a "ring" of spongiform changes. Radiating fibrils are organized into thick structures, which stand in contrast to the thin structures seen in "kuru-like" plaques [158]. There are numerous tau-immunoreactive dystrophic neurites in the vicinity that distinguish them from "kuru-like" plaques. Moreover, Hirano bodies (in the processes around florid plaques) can sometimes also be found [158]. These plaques are characteristic [159], although not specific [160] for vCJD. They can occur anywhere in the cerebral cortex but are generally found occipitally and in the cerebellar molecular layer [161]. Florid plaques are visible when stained with hematoxylin-eosin [162].
3. Multicentric plaques (see Figure 6) are formations up to 1500 µm [132] and are composed of many cores of different sizes that have merged. Unlike "kuru-like" plaques, they are characterized by the presence of dystrophic neurites [140]. Dystrophic neurites sometimes contain paired helical filaments (PHFs) identical to those seen in the dystrophic neurites of AD patients [163]. These larger cores tend to be surrounded by smaller amyloid deposits [156]. Like the previously mentioned plaques, they can be observed with hematoxylin-eosin staining [164].
4. Pure neuritic plaques (Figure 7) are the rarest type of plaques among prion diseases. Neuritic plaques consist only of clusters of dystrophic neurites with various morphologies and lack an amyloid component. They are surrounded by astrocytic processes in the immediate vicinity [156].

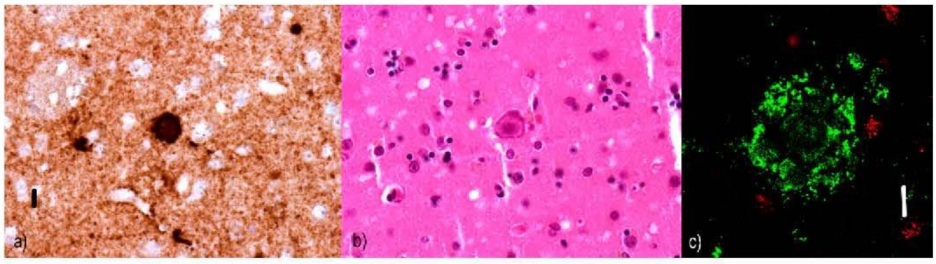

Figure 5. "Kuru-like" plaques: (**a**,**b**) Comparison of immunohistochemical images of "kuru-like" plaque with its correlate using hematoxylin-eosin staining. Primary antibodies: anti-prion protein (anti-PrP) rabbit IgG. The original magnification was 400×. The scale bar indicates a length of 10 micrometers. (**c**) The dense PrP nucleus and thin amyloid bundles in the periphery (green) of the "kuru-like" plaque were visualized using a confocal microscope. Tau-positive dystrophic neurites (red) are also included. Primary antibodies: anti-PrP rabbit IgG and AT8 (murine anti-hyperphosphorylated protein tau). The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). The scale bar indicates a length of 10 micrometers. The samples come from a 74-year-old woman suffering from CJD and come from the hippocampal formation, which contained numerous plaques; patchy synaptic and peri-vascular positivity were also present. The polymorphism at codon 129 was MV.

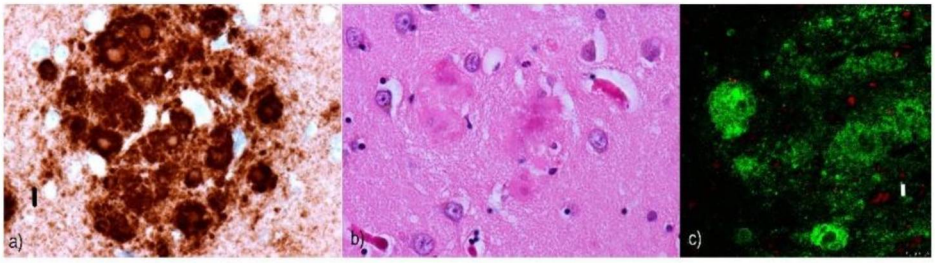

Figure 6. Multicentric plaques: (**a**,**b**) comparison of immunohistochemical staining of "multicentric" plaques distinctive for Gerstmann–Sträussler–Scheinker syndrome (GSS) with its correlate using hematoxylin-eosin staining. Primary antibodies: anti-PrP rabbit IgG. The original magnification was 400×. The scale bar indicates a length of 10 micrometers. (**c**) Numerous PrP plaques merged in a multicentric plaque (green), including tau-positive dystrophic neurites (red), visualized using confocal microscopy. Primary antibodies: Anti-PrP rabbit IgG and AT8 (murine anti-hyperphosphorylated protein tau). The secondary antibody was conjugated with either Alexa®488 (anti-rabbit IgG, green) or Alexa®568 (anti-mouse IgG, red). The scale bar indicates a length of 10 micrometers. All these images come from the occipital cortical area of a 69-year-old female with GSS in comorbidity with primary age-related tauopathy (PART). A causative point mutation in the *PRNP* gene was also detected (P102L).

Table 2. Summary of PrP plaque types in transmissible spongiform encephalopathies (TSEs).

PrP Plaques			
- **Extracellular Deposits of PrP Visible with Hematoxylin-eosin Staining**			
Unicentric/"Kuru"/"Kuru-like"/Stellate	**Daisy/Florid**	**Multicentric**	**Neuritic**
- up to 30 µm in Creutzfeldt–Jakob disease (CJD), 20–60 µm in kuru disease - consisting of a dense star-shaped core and thin amyloid bundles radiating into the periphery [156] - astrocytic processes located in the vicinity - invaded by microglia [157] - present in patients carrying the MV2 polymorphism [138] at codon 129 (total of 10–15% sCJD) [156] - visible with hematoxylin-eosin [90]	- up to 200 µm [132] - composed of PrP-amyloid core surrounded by a "ring" of spongiform changes - thick fibrils radiating into the periphery - tau-positive dystrophic neurites are present [140,159] - visible with hematoxylin-eosin [162]	- up to 1500 µm - composed of many cores of different sizes that have merged together - presence of tau-positive dystrophic neurites [140] - visible with hematoxylin-eosin staining [164]	- clusters of dystrophic neurites that do not contain amyloid structures - surrounded by astrocytic processes [156]

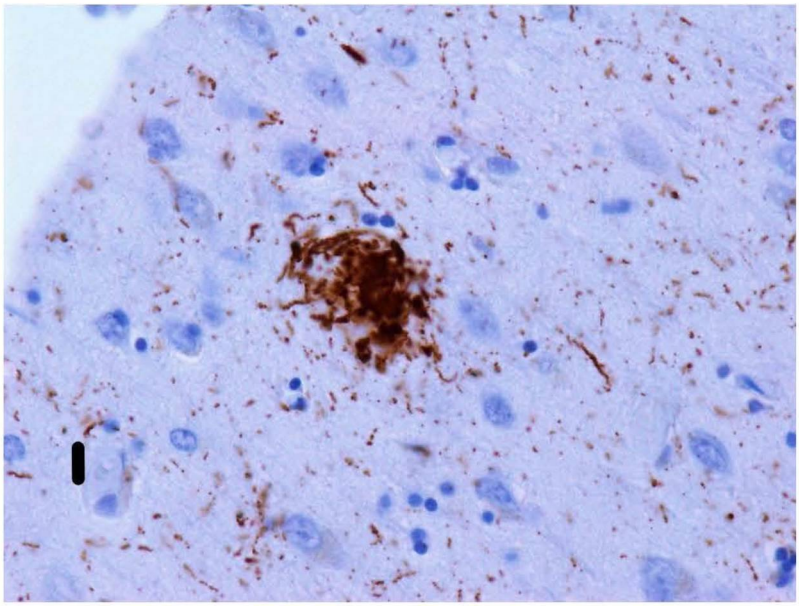

Figure 7. Neuritic plaques: purely neuritic plaque formed by only tau-positive neurites (stained immunohistochemically). These types of plaques are rarely found. In the above-mentioned 69 years old female patient (Figure 6) with GSS/PART, only a single neuritic plaque was detected. It was present in a section from the temporal cortex and found using immunohistochemical methods, but not in other sections examined using confocal microscopy. Primary antibodies: AT8 (murine anti-hyperphosphorylated protein tau). The original magnification was 400×. The scale bar indicates a length of 10 micrometers.

Both types of plaques are formed by amyloid structures—in AD by Aβ and in TSEs by prion amyloid. We tried to highlight the similarities and differences in their occurrence and behavior.

Similarities:

1. All of these diseases are based on a perturbance of proteins having physiological functions on the neuritic membrane to which they are anchored. Physiologically, they have a neuroprotective function and are able to interact with a number of other agents.
2. They are also similar to each other in the resistance of these extracellular aggregates to degradation by endogenous proteases.
3. In both AD and TSEs, extracellular aggregates may form not only compact structures such as plaques but also diffuse extracellular deposits.
4. For all mentioned diseases, extracellular deposits are mainly found in the cortical areas or in the central grey matter. Their presence in white matter is possible but exceedingly rare in TSEs and absolutely unheard of in Alzheimer's disease.
5. When forming plaques, they usually contain dystrophic neurites with similar immunohistochemical characteristics in both AD and TSEs. The neuritic morphology can vary from case to case.
6. The most toxic and neuronal death-inducing forms are oligomeric assemblies of both Aβ and PrP.

Dissimilarities:

1. While Aβ has thread-like morphology, PrP tends to be more lumpy or globular.
2. In AD, plaques probably mature, i.e., the individual types probably transform from one to the next. Nothing like "plaque maturation" has been recorded in prionoses.

3. Especially in GSS, plaque fusion and the formation of multicentric structures are distinctive. No similar trends are seen in AD.
4. For prionoses, different appearances, locations, and frequencies of extracellular aggregates are reported depending on the form and subtype. In AD, neuropathological differences between early and late-onset or sporadic and familial variants have never been described.
5. In TSEs, PrP deposits may be found intracellularly in some patients, while the occurrence of Aβ is strictly extracellular.
6. In AD and prionoses, there is a different trend relative to the spread of deposits within the brain. In AD, we distinguish five phases, with phase 1 being characterized by the presence of Aβ deposits limited to neocortical areas. During phase 2, the archicortical and paleocortical (together called allocortical) regions are affected. This is followed by a spread to the striatum and subcortical nuclei in general during phase 3. Brainstem involvement defines phase 4, and the involvement of the cerebellum defines phase 5 [165]. In prionoses, no stages are distinguishable since there is no characteristic spreading pattern over time.

4. Conclusions

To our best knowledge, this is the first systematic classification of the morphological similarities and differences between the extracellular amyloid deposits in AD and CJD. The work also clearly demonstrates the broad spectrum of these specific neuropathological entities. Better clarification of the processes of extracellular aggregate formation of different amyloidogenic proteins may be helpful for understanding the development of individual neurodegenerations and, thus, could be a useful tool for the development of effective and precise biological treatments for these progressive and fatal disorders.

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3.6 Shrnutí dosavadních neuropatologických poznatků o amyotrofické laterální skleróze. Jankovska N, Matej R. Molecular Pathology of ALS: What We Currently Know and What Important Information Is Still Missing. Diagnostics (Basel). 2021 Jul 29;11(8):1365. doi: 10.3390/diagnostics11081365. PMID: 34441299; PMCID: PMC8391180. **IF 3,992**

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Review

Molecular Pathology of ALS: What We Currently Know and What Important Information Is Still Missing

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Abstract: Despite an early understanding of amyotrophic lateral sclerosis (ALS) as a disease affecting the motor system, including motoneurons in the motor cortex, brainstem, and spinal cord, today, many cases involving dementia and behavioral disorders are reported. Therefore, we currently divide ALS not only based on genetic predisposition into the most common sporadic variant (90% of cases) and the familial variant (10%), but also based on cognitive and/or behavioral symptoms, with five specific subgroups of clinical manifestation—ALS with cognitive impairment, ALS with behavioral impairment, ALS with combined cognitive and behavioral impairment, the fully developed behavioral variant of frontotemporal dementia in combination with ALS, and comorbid ALS and Alzheimer's disease (AD). Generally, these cases are referred to as amyotrophic lateral sclerosis-frontotemporal spectrum disorder (ALS-FTSD). Clinical behaviors and the presence of the same pathognomonic deposits suggest that FTLD and ALS could be a continuum of one entity. This review was designed primarily to compare neuropathological findings in different types of ALS relative to their characteristic locations as well as the immunoreactivity of the inclusions, and thus, foster a better understanding of the immunoreactivity, distribution, and morphology of the pathological deposits in relation to genetic mutations, which can be useful in specifying the final diagnosis.

Keywords: amyotrophic lateral sclerosis; frontotemporal lobar degeneration; sporadic ALS; familial ALS; amyotrophic lateral sclerosis-frontotemporal spectrum disorder; ALS-FTSD; motor neuron disease

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1. Introduction

Amyotrophic lateral sclerosis (ALS) has classically been considered a disease exclusively affecting the motor system and, thus, it is a part of a group of disorders known as motor neuron diseases (MND), which includes a whole spectrum of disorders affecting upper motor neurons (corticospinal tract), lower motor neurons (in anterior horn or motor cranial nerve nuclei in the brain stem), or both [1]. The MNDs also includes "restricted" phenotypes, including primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), and progressive bulbar palsy (PBP) [2]. PLS is characterized by isolated progressive upper motor neuron dysfunction without lower motor neuron symptomatology that begins in the fifth to sixth decade with progressively developing spasticity, hyperreflexia, and mild weakness [3]. Unlike ALS, PLS is most often slowly progressive (survival times of 7.2–14.5 years) [3]. PMA includes lower motor neuron dysfunction; however, upper motor neuron signs may sometimes be present [4]. Even in PMA, disease survival times are longer than in ALS [5]. PBP can be divided into childhood- and adult-onset forms [6]. The childhood-onset form, also called Fazio-Londe disease, is an autosomal recessive disease associated with progressive impairment of the cranial nerves with two clinical subtypes—an early course, typically starting before the age of 6 years with mainly respiratory symptoms,

and a late course starting between 6 and 20 years with mainly motor symptoms in the upper limbs [7]. The adult-onset form attacks the bulbar region and clinically presents with swallowing, speaking, and chewing difficulties [8].

Another MND disease is spinal muscular atrophy (SMA), an inherited condition with an autosomal-recessive pattern affecting lower motor neurons that degenerate due to a lack of survival motor neuron (SMN) protein encoded by the *SMN1* gene [9]. Historically, five types of SMA, based on the age of symptoms onset and the highest physical ability achieved, are distinguished [10]. Disease severity can be modified by the *SMN2* gene, which led to the creation of the first treatment [11].

Kennedy's disease, also known as spinal and bulbar muscular atrophy, is a recessive X-linked disease affecting men (women can be carriers). It is caused by an expansion of a CAG repeat in the gene for androgen receptors [12]. Clinically, patients present with muscle weakness, proximal atrophy in the limbs, and bulbar symptomatology. Gynecomastia due to the insensitivity to androgen, heart rhythm, and urinary problems may also be present [13].

The last condition that falls under MND is post-polio syndrome (PPS), characterized by muscle weakness and/or muscle fatigability, dysphagia, dysphonia, and subsequent respiratory failure that can begin dozens of years after recovery from a poliomyelitis infection [14].

The first to describe and diagnose ALS was Jean-Martin Charcot, so ALS is sometimes referred to as Charcot's disease [15]. Charcot found and reported damage within the lateral columns of the spinal cord that leads to chronic progressive paralysis along with contractures but without muscle atrophy. On the other hand, lesions affecting the anterior horns cause paralysis and muscle atrophy but lack contractures [16]. Another common name for ALS is Lou Gehrig's disease in memory of the famous New York Yankees baseball player who died (1941) of ALS at the age of 37 [17].

In ALS, the word "amyotrophy" refers to muscle fiber atrophy, caused by selective neuronal degeneration in the anterior horns of the spinal cord, leading to weakness and fasciculations of affected muscles. The phrase "lateral sclerosis" refers to the hardening of the anterior and lateral corticospinal tracts as axons and myelin are replaced by glial cells [18]. Therefore, ALS is still often defined as a fatal neurodegenerative disease characterized by progressive muscle paralysis determined by the degeneration of motoneurons in the motor cortex, brainstem, and spinal cord [19] with the characteristic limb onset form accompanied by awkwardness, weakness, and tripping or stumbling. The bulbar onset form first appears as speech or swallowing difficulties [20]. However, today, we know that not all forms are characterized solely by motor neuron lesions—some patients also have a cognitive and behavioral impairment that is remarkably similar to the behavioral variant of frontotemporal dementia (bvFTD). Recently, these cases have become referred to as amyotrophic lateral sclerosis-frontotemporal spectrum disorder (ALS-FTSD) [21]. ALS is the most common MND in adults, with the vast majority of ALS cases being sporadic; however, about 10% have a familial history with a typical Mendelian autosomal dominant pattern of inheritance [22], although cases with autosomal recessive or X-linked inheritance are also known [23]. Regarding genetic variants, the most common cause is a hexa-nucleotide repeat expansion of GGGGCC in the first intron of chromosome 9 open reading frame 72 (*C9orf72*), which causes 30–50% of familial ALS (FALS) cases and 5% of sporadic ALS (SALS) [24,25]. These repeat expansions are also frequently found in frontotemporal dementia (FTD), which shows the molecular overlap between these two pathological units, i.e., ALS and FTD [26]. Clinically, the sporadic and familial forms are indistinguishable [27], although some studies suggest that the familial variant has an earlier onset [28].

The aim of the review is to compare the neuropathological findings in individual types of ALS in detail, including characteristic inclusions, their immunoprofiles and characteristic localizations, and thus, foster a better understanding of their relationship to genetic mutations, which can be useful in specifying the final diagnosis.

2. Incidence and Prevalence of ALS

The incidence of ALS in Europe is reported to be around 1–2.6 cases per 100,000 inhabitants per year, and the prevalence is 6 per 100,000 inhabitants per 100,000 population [22], although foci of higher frequencies occur in the Western Pacific [29]. The disease typically occurs at 58–60 years [22], rarely occurs before the age of 40, and generally, is slightly more common in men (the reported M:F ratio is about 1.5:1) [29]; however, most studies suggest that the bulbar onset form is more common in women [30,31]. The median survival time is 20–48 months, though 10–20% of patients survive more than ten years [20].

3. Etiology

The cause of ALS is not yet fully understood; however, the various genes involved in the pathogenesis of ALS share similar biological functions, which allowed the identification of some major molecular pathways that trigger selective damage in specific neurons [27]. The effect of head injury [32], viruses like herpes simplex [33], enterovirus [34], or retrovirus [35], exotoxins including excitotoxicity mediated by glutamate [36], the lysosomal-endosomal system [37], or disorders of the immune system leading to chronic inflammatory processes including autoimmune processes [38,39] have all been considered to be causal candidates, but none have been demonstrated so far. The role of epigenetics, especially defects in histone homeostasis (acetylation and deacetylation) suggested by transcriptional dysregulation indicating changes in chromatin structure, which is seen in both murine ALS models and patients, has also been assumed [40]. In addition, some ALS patients have higher physical exertion and lower body mass indexes (BMI) compared to the unaffected population, which could be related to the onset [38,41].

In the case of FALS, an autosomal dominant pattern of inheritance with the most common mutation being in *C9orf72*, superoxide dismutase 1 gene (*SOD1*), and fused of the sarcoma (*FUS*) gene are usually seen [42]. Moreover, ALS has been shown to be associated with mutations in genes with DNA/RNA regulating functions, such as TAR DNA binding protein (*TARDBP*) [43,44].

Recently published studies reported selective hypothalamic atrophy in both SALS and FALS cases, including the asymptomatic stage of FALS [45]. In FALS, atrophy correlates with body mass index (BMI), but no correlation with the severity of motor problems has been found [45].

Early motor manifestations of SALS, with the presence of TDP-43 insoluble and ubiquitinated inclusions, reflect the failure of complex adaptive motor skills leading to split hand presentation, gait disorders, split leg syndrome, and bulbar symptomatology associated with vocalization [46].

A characteristic histopathological feature of TDP-43 pathology is its limitation to the cortical region and subcortical nuclei with direct cortical projections [47]. The pathological TDP-43 protein is found in the cerebral cortex, corticofugal fibers, subcortical nuclei, and the motor neurons of the brainstem and anterior horns of the spinal cord [39]. The prion-like mechanism at the synaptic terminals of corticofugal axons is currently accepted, and theoretically explains the spread to neocortical regions and the relationship between ALS and FTD [39].

Very rarely, paraneoplastic syndrome, positive for anti-Hu antibodies, and presenting as an MND, is found. MND is considered an atypical paraneoplastic syndrome that has yet to meet the criteria set by Graus et al., [48] i.e., at least one of three conditions have to be fulfilled: (1) Post-therapeutic reduction of neurological manifestations in the absence of immune modulation; (2) the presence of onconeural antibodies; and/or (3) partially characterized onconeural antibodies and a malignancy presenting within five years of the onset of neurological abnormalities [48].

4. Clinical Manifestation

Amyotrophic lateral sclerosis is an MND [49], and the typical form is characterized by upper and lower motoneuron involvement, which is present in 65–70% of cases. Another

form is progressive bulbar paralysis with bulbar muscle involvement, which occurs in 25% of patients [50]. A rarer manifestation of the disease is progressive muscle atrophy with only lower motoneuron lesions; this occurs in 5–8% of ALS patients [39]. An extremely rare form of the disease is primary lateral sclerosis (PLS), characterized by a lesion limited to upper motoneurons and affecting 1–4% of patients [51]. In PLS, the clinical diagnosis is based on per exclusionem after excluding all other possible causes (structural, infectious, and demyelinating diseases leading to upper motor neuron syndrome or hereditary spastic paraplegias) [51]. Equally rare is the monomelic spinal amyotrophy variant with focal atrophy that usually presents as weakness in one limb [52,53].

Surprisingly often, i.e., in 55% of cases with clinically obvious dementia, 15% have been described [54] with characteristics of both ALS and cognitive impairment. In the dementia form (i.e., ALS-FTSD), five forms have been distinguished based on clinical presentation:

(1) ALS with cognitive impairment (ALSci);
(2) ALS with behavioral impairment (ALSbi);
(3) ALS with combined cognitive and behavioral impairment (ALS-cbi);
(4) Fully developed behavioral variant of frontotemporal dementia (bvFTD) in combination with ALS (ALS-FTD);
(5) Comorbid ALS and Alzheimer's disease (AD) [55].

In the fully developed disease, the clinical picture is relatively characteristic, but diagnostic difficulties may occur at the beginning of the clinical manifestation. Objective findings in the developed form are a mixed picture of central and peripheral quadriparesis, bulbar symptoms, hyperreflexia, spasticity, positive pyramidal signs, atrophy of the muscles of the upper and lower limbs and tongue, and massive fasciculations, especially of the limbs and tongue [29].

The disease most often (in two-thirds of cases) [29] begins with an asymmetric weakness and wasting of limited muscle groups, with the subsequent development of spasticity [29] typically manifesting in the upper limbs [26]. Clinical findings may imitate mononeuropathy or radiculopathy; however, weight loss, fasciculations, emotional lability, and frontal-lobe cognitive impairment [56] may also be present. Bulbar onset with articulation disorders and swallowing difficulties [26] are seen in 30% of cases; additionally, striking atrophy and fasciculations of the tongue [57] may be present. Limb weakness may begin to develop simultaneously with bulbar symptomatology or with a delay of 1–2 years [29]. As paralysis progresses, it leads to death due to respiratory failure within 2–3 years in bulbar onset and 3–5 years in the more typical limb onset variant [29].

Less often, and in cases with a focal onset, muscle weakness affects the neck muscles [26], and muscle fatigue is a common symptom [58]. Fasciculations or cramps appear in the plexus muscles of the upper and lower limbs (e.g., deltoid muscle and quadriceps femoris muscle) [39]. Atrophies of small muscles of the hand and foot (especially the interosseous muscles) leading to split-hand or split-leg signs may be clinically present [59,60]. An ALS diagnosis requires the absence of sensory signs, visual disturbances, and sphincter problems [52].

In ALS-FTSD, the degree of cognitive impairment and behavioral manifestation varies from case to case and ranges from mild cognitive deterioration bordering on a normal finding to marked changes in behavior and personality with a severe frontal syndrome. Cognitive impairment is present mainly in the bulbar form of ALS [61].

Patients with ALS may develop some form of bvFTD during the disease; conversely, some patients with bvFTD develop symptoms of motor neuron disease (from clinically insignificant fasciculations with hyperreflexia to typical ALS). The onset of motor problems and dementia usually differ, making the clinical picture of simultaneous development of ALS and bvFTD unusual [62]. ALS-FTSD has a significantly worse prognosis and shorter survival than patients with isolated forms (i.e., ALS or bvFTD independently) [63].

5. Differential Diagnosis

To standardize the diagnosis for clinical research and reduce misdiagnoses, the El Escorial criteria have been implemented [64]. Regarding diseases belonging to an ALS differential diagnosis and brain-affecting diseases, adult polyglucosan body disease (APBD) has clinical symptomatology consistent with upper and lower motor neuron lesions. However, cognitive decline, distal sensory loss, and bladder and bowel function disturbances also occur, which can clinically help differentiate the entities [52].

Among brainstem and spinal cord diseases, ALS can be mimicked by multiple sclerosis, which can cause both upper and lower motoneuron symptoms, and in some cases, may even resemble bulbar onset ALS [52]. In the predominantly spinal form, an MRI of the cervical spinal cord is performed to exclude other possibly treatable conditions; however, cervical spondylotic myelopathy or syringomyelia may include dissociated sensory loss, which is usually present in syringomyelia but starts at a younger age than most ALS cases [65]. Another disease that should be considered in the differential diagnosis is degenerative myeloradiculopathy, which can also be consistent with the clinical presentation of ALS [52].

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a hereditary X-linked lower motor neuron disease characterized by progressive muscular weakness and should also be considered in the differential diagnosis. An initial clinical manifestation usually includes muscle cramps, muscle twitching, tremor, fatigue, and slurred speech [66].

The potential for paraneoplastic encephalomyelitis, which can sometimes manifest as a motor neuron disorder, must not be overlooked. Sensory or autonomic features and ataxia may appear later in the course [67].

In dysphagia and dysarthria, which are neuromuscular transmission disorders (especially myasthenia gravis), arterial atherosclerotic disease, infiltrative tumors, and infectious or autoimmune causes should be ruled out [68]. Oculopharyngeal muscular dystrophy may also imitate ALS, although it usually involves the extraocular muscles and muscles of eyelids in contrast to ALS. In patients presenting with bulbar symptomatology lacking extraocular involvement, a muscle biopsy may be required [52].

Benign monomelic amyotrophy is a differential diagnosis for monomelic onset of ALS [52]. Generally, it is always necessary to examine the cerebrospinal fluid and perform an MRI of the brain and spinal cord. In addition to the rare variant of oculopharyngeal muscular dystrophy mentioned above, a muscle biopsy may be indicated to rule out polymyositis [69] or inclusion body myositis [70].

Furthermore, some systemic diseases can partially mimic the clinical manifestation of ALS—for example, hyperthyroidism, which can cause hyperreflexia as a corticospinal tract sign, fasciculations, weight loss, or weakness. However, many symptoms that are not typical for ALS are often present (fine tremor, tachycardia, heat intolerance, and anxiety). Weakness may also be seen in hyperparathyroidism and mimic the lower motoneuron onset form of ALS [52].

6. Types of ALS

6.1. Sporadic ALS

Sporadic ALS (SALS) is the most common form of ALS (90%)—it occurs randomly, without any known cause, with no clearly associated risk factors, and no family history of the disease, thus the etiology of most cases of SALS remains elusive.

6.1.1. Background of SALS

Only 10% of SALS cases include known disease-associated ALS mutations [71], with *C9orf72* expanded alleles being the most common [24]. Mutations in the *SOD1* gene (gene for the antioxidant enzyme protecting cells from reactive superoxide radicals, endoplasmic reticulum stress, mitochondrial dysfunction, and axonal transport disruption) [72] are found in 2–3% of SALS [73]. Another interesting gene to study appears to be *TARDBP*,

an abnormal TDP-43 fragment found in neurons and astrocytes in approximately 95% of SALS cases [74]. In less than 1% of SALS cases, mutations in hnRNP A1 and hnRNP A2B1 are detected. Oligogenic or polygenic SALS cases have an earlier age of onset [75]. The remaining cases likely represent the interplay of genetic and environmental factors.

6.1.2. Gross Findings in SALS

SALS gross findings include typically shrunken ventral/anterior roots that appear grey compared to the dorsal/posterior roots, lateral corticospinal tract, and sometimes the whole spinal cord may appear atrophic [76] (see Figure 1).

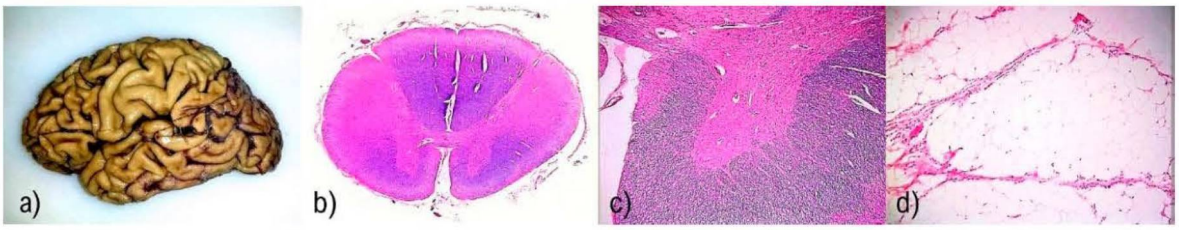

Figure 1. (**a**) Atrophy of the precentral gyrus together with atrophy of the frontal and temporal lobes may be present mainly in cases of ALS accompanied by dementia. (**b**) Marked atrophy and reactive astrogliosis of anterior horns, with atrophy of the anterior roots are the most striking findings in the standard staining method. Histochemical detection of myelin shows the most evident changes in the anterolateral cords in the forms of sclerosis. Stain: Luxol fast blue stain. (**c**) Detail of the described changes in anterior roots and horn. Stain: Luxol fast blue stain. The original magnification 200×. (**d**) Pronounced neurogenic atrophy and fatty pseudohypertrophy in the striated muscles of the respiratory muscles is a typical finding in ALS. Stain: Hematoxylin and eosin stain. The original magnification 200×.

The brain usually appears grossly normal, although there may be atrophy of the precentral gyrus. Atrophy is also evident on MRIs (on susceptibility-weighted images generated from gradient-echo pulse sequences (GRE/SWI)) as a bilateral hypointensity in the precentral gyrus, known as the "motor band sign [77]." In addition, atrophy of the frontal and temporal lobes may be seen, especially in cases clinically accompanied by dementia [78]. Brainstem atrophy, with a volume reduction of the medulla oblongata, may be noted as pontine atrophy. With neuroimaging methods, density reductions in the mesencephalic crura are apparent [79]. Moreover, severe atrophy of the muscles of the upper and lower limbs and tongue are also evident. All ALS variants share these gross findings; differences are described below in the relevant paragraphs.

6.1.3. Neuropathological Findings in SALS

Degenerative changes are mostly observable in the motor area and by the large number of atrophied α-motor neurons located in the anterior grey matter of the spinal cord and the motor neurons of the cranial nerve nuclei in the brainstem [80]; these are striking histopathological signs of the disease, together with affected Betz cells in the primary motor cortex.

The loss of motor neurons leads to chronic denervation with neurogenic atrophy and fatty pseudohypertrophy in the striated muscles of the limbs and respiratory muscles; type 2 muscle fibers are the most vulnerable (Figure 1) [81,82]. Differentiation of muscle fibers is easy using adenosine triphosphatase (ATPase) staining, in which type 1 fibers appear lightly stained, and type 2 fibers appear darkly stained—this pattern is seen at pH 9,4 but not at pH 4,3 or 4,6 where the staining is inverse (type 1 fibers are dark, while type 2 fibers are pale) [83]. Assemblages of muscle fibers associated with re-innervation via axonal sprouting from neighboring motor axons are also typical; nevertheless, this compensatory mechanism is insufficient for muscle regeneration [84]. The location of atrophy is important; in the bulbar form, atrophy of the tongue, diaphragm, and intercostal muscles predominate, while in the typical form of ALS, atrophy in the limb muscles prevails.

However, some, especially larger muscle fibers, can be paradoxically hypertrophic. In the late stages, clusters of pyknotic nuclei may be seen.

Standard staining is dominated by changes in the anterior horns, with marked atrophy, reactive astrogliosis, and atrophy of the anterior horns. In specialized histochemical detection of myelin, the most evident changes are seen in the anterolateral cords in the form of sclerosis; however, these changes can also be found in the posterior cords in longer disease courses [39]. A key diagnostic feature is the large number of atrophied large motor neurons in the anterior horns of the spinal cord, which is apparent in cervical and lumbar intumescence. The remaining neurons show signs of regressive changes, i.e., a tendency to wrinkle, the presence of lipofuscin deposits in the cytoplasm, and central chromatolysis in the nuclei [85]. A relatively characteristic feature is also phosphorylated neurofilament aggregates found as markedly swollen axons in anterior horns, generally referred to as spheroids [86].

Different types of inclusions can be found in the cytoplasm of affected neurons. Before immunohistochemical methods were routine, Bunina bodies, small eosinophilic granular inclusions, were considered diagnostically specific for ALS [87]. Now, the availability of immunohistochemical methods has broadened neuropathological examination. Anti-ubiquitin or anti-p62 [88] antibodies have revealed other types of inclusions widely present in motor neurons of the anterior spinal horns, in the brainstem, primary motor cortex, and neuronal structures in the frontal and temporal cortical areas [89]. The inclusions may be "skein-like," [90] having an elongated or fibrous shape, which form bizarre spherical structures in the perikaryon of neurons, or light eosinophilic round hyaline inclusions. The presence of ubiquitin indicates impaired proteasomal degradation (in addition to RNA metabolism disorders at multiple levels) [91] as a key mechanism [92]. Most of the affected neurons and glial cells contain cytoplasmic TDP-43-immunoreactive inclusions [93]. The physiological TDP-43 protein acts as a DNA/RNA binding protein that binds both mRNA and DNA and mediates mRNA splicing, transcription, and translation [94]. Skein-like inclusions in anterior horn neurons and their neurites in the spinal cord may also be optineurin (OPTN) immunoreactive in both SALS and non-*SOD1* FALS [95].

Oligodendrocyte dysfunction is considered by some authors to be a primary contributing factor to ALS [96] as there is prominent degeneration of oligodendrocytes in the gray matter of the spinal cord in ALS-model mice before disease onset [97]. SOD1-dependent loss of oligodendrocytes, leading to the death of motoneurons, is discussed—firstly, it is considered to happen due to defective lactate release, and secondly, by influencing cell-to-cell contact between axons and enwrapping oligodendrocytes. When SOD1 is selectively removed from oligodendrocytes, delayed disease onset as well as a longer survival period in ALS-model mice is seen. This observation could also contribute to the theory about the relationship between the impaired function of oligodendrocytes and the vulnerability of motoneurons [97].

Considering pTDP-43, Brettschneider et al. recognized and described four stages of ALS based on the specific sequential pattern of pTDP-43. Stage I is characterized by lesions in the agranular motor cortex, brainstem motor nuclei of cranial nerves XII-X, VII, V, and spinal cord α-motoneurons. For stage 2, the involvement of the prefrontal neocortex (middle frontal gyrus), brainstem reticular formation, precerebellar nuclei, and the red nucleus is characteristic. Stage 3 shows pTDP-43 pathology in the prefrontal (gyrus rectus and orbital gyri) and postcentral neocortex plus striatum. The last stage is characterized by inclusions in the anteromedial part of the temporal lobe, including the hippocampus. At all stages, oligodendroglial aggregates are also present [98].

All forms of ALS share the neuropathological signs described above. Differences between the familial form and the form combined with dementia will be described in the following paragraphs (for summary see Tables 1–3).

Table 1. Summary of characteristics genes and inclusions in SALS cases.

Disease-Associated Genes	Inclusions
✓ mutations in 10% of cases	✓ SOD1
✓ SOD1	✓ TDP-43
✓ C9orf71	✓ ubiquitin
✓ TARDBP	✓ p62
✓ hnRNP A1 + hnRNP A2B1 (< 1%)	✓ OPTN (in non-SOD1 cases)

Table 2. Summary of characteristics genes and inclusions in FALS cases.

Disease-Associated Genes	Inclusions
✓ mutations up to 20% ✓ most common—Autosomal dominant inheritance; however, cases with autosomal recessive or X-linked inheritance exist ✓ SOD1 (cognitive impairment rarely) ✓ C9orf72 ✓ TARDBP ✓ FUS ✓ UBQLN2 ✓ p62 ✓ ALSIN (juvenile-onset) ✓ SETX (juvenile onset) ✓ SPG (autosomal recessive) ✓ OPTN (autosomal recessive) ✓ VAPB ✓ VCP ✓ PGRN	✓ SOD1 ✓ FUS ✓ TDP-43 (different than ubiquitin, ubiquilin and p62 positive inclusions) ✓ ubiquitin ✓ p62 ✓ OPTN ✓ Ubiquilin—co-localize with ubiquitin itself, p62, TDP-43, FUS, and OPTN but not with SOD1

Table 3. Summary of characteristics genes and inclusions in ALS-FTSD cases.

Disease-Associated Genes	Inclusions
✓ C9orf72 (40–50% risk of development of cognitive impairment)	✓ tau ✓ FUS ✓ TDP-43 ✓ ubiquitin ✓ p62

6.2. Familial ALS

Familial ALS (FALS) is estimated to be 10% of all ALS cases [27], with the most common being autosomal dominant inheritance; however, as mentioned above, cases with autosomal recessive or X-linked inheritance have been described [23].

6.2.1. Background of FALS

To date, 16 genes associated with FALS have been identified, and up to 80 different mutations have been detected in 10–20% of familial cases. Currently, the most important genes are associated with the metabolism of the TDP-43 protein (mostly hyperphosphorylated), which plays a crucial role in the pathogenesis of the disease. Although TDP-43 is a nuclear protein, it is transferred to the cellular cytoplasm after acute neuronal injury, where it forms stress granules [99]. In addition to the *TARDBP* gene itself, the fused-in-sarcoma (*FUS*) gene is involved in physiologically similar processes as *TARDBP* (i.e., a DNA/RNA binding protein involved in brain mRNA processing and transcription) and also plays a role in the pathogenesis of ALS [100]. Mutations in *SOD1* (the first gene historically identified as an ALS causing gene) [27], ALS2 (*ALSIN*; connected to juvenile-onset ALS [101], which is ALS starting before 25 years of age) [102], and senataxin (*SETX*; also seen in the juvenile form of ALS) are also associated with FALS [23]. Considering the juvenile variants, spatacsin (*SPG*) is typically associated with autosomal recessive heritability [23]. Optineurin (*OPTN*)

is another important gene with autosomal recessive heritability [23]. Moreover, vesicle-associated membrane protein B (*VAPB*), valosin-containing peptide (*VCP*), progranulin (*PGRN*), ubiquilin 2 (*UBQLN2*; a mutation leading to ubiquitin-mediated disruption of proteasomal degradation) [103], and sequestosome 1 (*SQSTM1/p62* [104]; involved in the degradation of misfolded proteins through ubiquitin–proteasomes [105] or the autophagy-lysosome system) [106,107] are also seen in FALS. The relatively recently discovered the 72nd open reading frame on chromosome 9 (*C9orf72*) [108] has now been found in many patients with FALS, with the incidence estimated to be up to 20%. Patients with the *SOD1* mutation rarely suffer from cognitive impairment [109].

6.2.2. Neuropathological Findings in FALS

In addition to the above-mentioned neuropathological features shared with SALS, some cases of FALS have hyaline inclusions similar to Lewy bodies in the cytoplasm of neurons. They are strongly immunoreactive with anti-superoxide dismutase (SOD1) antibodies in those cases with the *SOD1* gene mutation. Inclusions immunoreactive to SOD1 are located in lower motor neurons and represent a striking pathological feature of FALS; they are due to *SOD1* mutations and are also seen in *SOD1* murine models [110]. Neuropathologically, FALS caused by the *SOD1* mutation can be distinguished from the sporadic disease by the relative sparing of the motor cortex, slight to mild corticospinal tract involvement, which is in contrast with severe atrophy of the anterior roots, and the degeneration of lower motor neurons in the sporadic form of the disease [27].

TDP-43 cytoplasmic inclusions, mainly found in the pyramidal, frontal, and temporal cortex, and hippocampal areas, are considered characteristic features of FALS with the *C9orf72* mutation; the cerebellar granule cell layer, hippocampal pyramidal neurons, and neocortex all contain ubiquilin, p62, and ubiquitin inclusions that are negative relative to the TDP-43 reaction (Figure 2) [93].

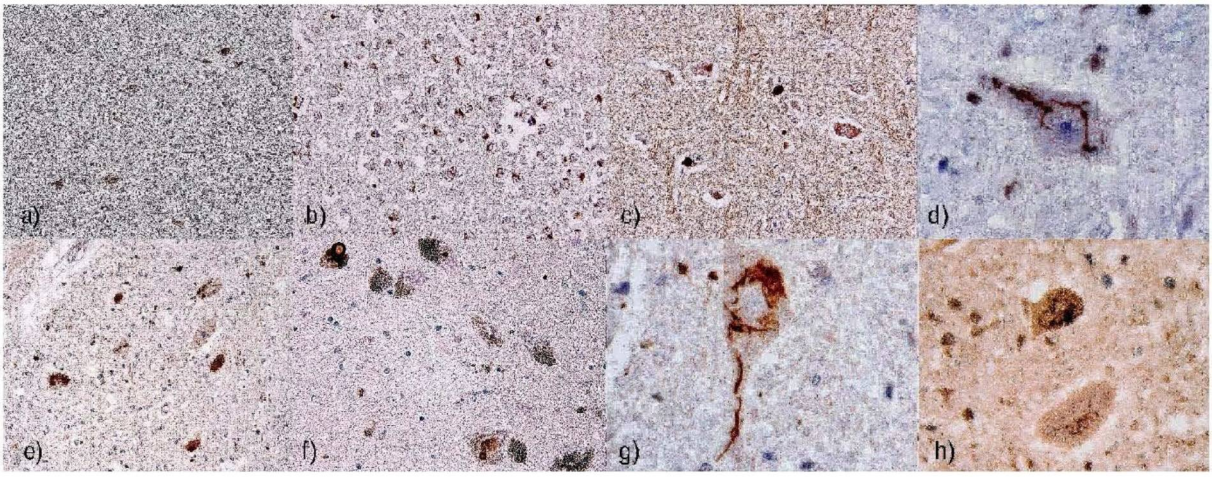

Figure 2. (**a**) Overview of inclusions positive in reaction with anti-phosphorylated TDP-43 (pTDP-43) antibody, original magnification 100×. (**b**) pTPD-43 positive cytoplasmic inclusions in the hippocampus are considered one of the characteristic features of the variant with *C9orf72* mutation, magnification 100×. (**c**) Cytoplasmic pTDP-43-positive cytoplasmic inclusions in the hypoglossal motor neurons, original magnification 200×. (**d**) Detailed shape of cytoplasmatic pTDP-43-positive inclusion in the lower motor neuron. The original magnification 400×. (**e**) Inclusions detected with anti-p62 antibodies in the mesencephalic nuclei. The original magnification was 100×. (**f**) Inclusions detected by anti-p62 antibody seen in the neurons of mesencephalic nuclei. The original magnification 200×. (**g**) Detailed morphology of p62-positive inclusions in the cytoplasma of upper motor neuron, original magnification 400×. (**h**) Morphology of ubiquitin-positive "skein-like" inclusions with characteristic elongated or fibrous shape, which form bizarre spherical structures in the perikaryon of neurons. The original magnification was 400×.

Ubiquilin-positive (ubiquitin-like-positive) inclusions have been identified in spinal cord sections of patients with X-linked FALS; the inclusions co-localize with ubiquitin itself, p62, TDP-43, FUS, and OPTN but not with SOD1. UBQLN2 immunoreactivity has also been observed in spinal cord sections of sporadic ALS, ALS with dementia, and non-*SOD1* FALS.

6.3. Amyotrophic Lateral Sclerosis-Frontotemporal Spectrum Disorder

In patients with amyotrophic lateral sclerosis-frontotemporal spectrum disorder (ALS-FTSD), inclusions positive for the anti-TDP-43 antibody reaction are usually present, so the clinical and neuropathological designation of FTLD-MND-TDP (frontotemporal lobar degeneration with motor neuron disease and TDP-43 positive inclusions) is used. However, dementia syndrome is defined as a loss of cognitive functioning and behavioral abilities to such an extent that it interferes with daily life and activities. The behavioral form (ALSbi) does not meet the condition of deterioration of self-sufficiency. Moreover, cognitive decline is often associated with a poorer prognosis [111]. Recently, the terminology was changed from "FTLD-MND" to "ALS-FTSD." At present, the term FTLD-MND-TDP is purely neuropathological, while in clinical practice, ALS-FTSD is used [21].

6.3.1. Background of ALS-FTSD

A strong genetic burden is evident in ALS-FTSD cases [112]. The pathological expansion of the "hexa-repeat" (GGGGCC) in chromosome 9 open reading frame 72 is closely related to the behavioral variant of frontotemporal dementia (bvFTD), which occurs either in direct association with ALS or as a purely cognitive impairment lacking motor neuron dysfunction. Carriers of *C9orf72* mutations have a significantly higher risk of developing cognitive impairment (40–50%) than patients lacking this mutation (8–9%) [113,114]. The spectrum of involvement in families carrying *C9orf72* gene expansions (Figure 3) differs significantly from typical ALS based on purely behavioral symptomatology (bvFTD), i.e., without demonstrable motor neuron involvement up to a combination of ALS and cognitive impairment [115]. In patients with ALS with psychosis and anosognosia, the presence of *C9orf72* mutation should be considered [21,116].

6.3.2. Neuropathological Findings in ALS-FTSD

We can distinguish three basic types of FTLD depending on the hallmark pathological protein: (1) FTLD-tau (characterized by tau-positive inclusions), (2) FTLD-TDP (with TDP-43 inclusions), and (3) FTLD-FUS (having FUS-positive inclusions); although, a small proportion of FTD cases do not express any of the above-mentioned proteins. Those that react with ubiquitin or other markers of the ubiquitin-proteasome system are called FTLD-UPS, while completely immuno-negative cases are grouped as FTLD-NOS (not otherwise specified) [117]. Widespread ubiquilin-positive inclusions are also usually observed in the hippocampal region, including patients with the *C9orf72* mutation [103].

Most ALS cases belong to the FTLD-TDP group and exhibit TDP-43 immunoreactive inclusions [118,119]; the remaining cases are in the FTLD-FUS group.

The Strong criteria [63] for the neuropathological diagnosis of ALS-FTSD remain unchanged, including the examination of the brain and spinal cord; AD must always be considered. A p62 and dipeptide repeat (DPR) pathology in the cerebellum and hippocampus is pathognomonic for *C9orf72*-linked ALS-FTSD [120]. The other neuropathological findings are identical to those cases lacking cognitive impairment [121]. It is probably not surprising that in genetic ALS-FTSD cases, SOD1- or FUS-immunoreactive inclusions can be found; moreover, alterations in microtubule-associated tau protein (tau) metabolism have been observed in ALS-FTSD, with the presence of tau deposits usually in the hyperphosphorylated form [92]. Since the primary function of tau is to provide stability to microtubules, site-specific tau phosphorylation therefore affects the interaction of tau and microtubules. Pathological hyperphosphorylation reduces the number of interactions between tau and microtubules, allowing tau to form less soluble oligomers and

subsequently leading to the formation of fibrils [122]. In addition, research on murine models shows that having coexisting pathologies in TDP-43 and tau metabolism potentiate each other [92]. Thus, it is clear that FTD and ALS can share clinical manifestations and underlying pathophysiology, which are reflected in changes in TDP-43 and tau metabolism.

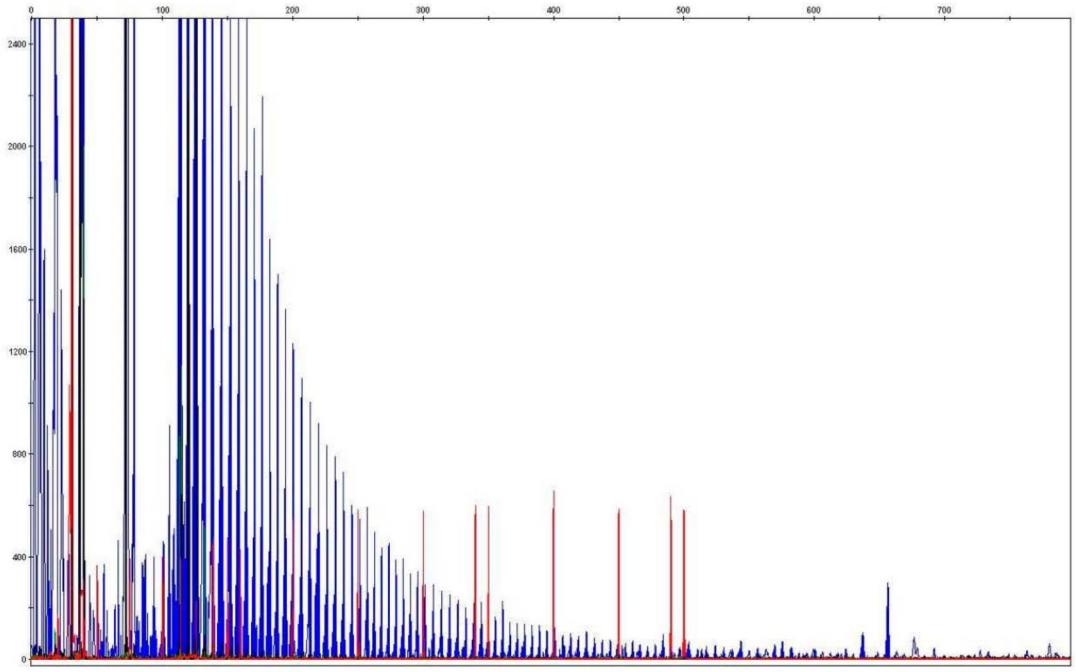

Figure 3. Determination of copy number of hexanucleotide expansion of C9orf72. Reference values: Normal allele <20 GGGGCC repeats, permuted allele 20–100 GGGGCC repeats, complete penetrance> 100 GGGGCC repeats. The blue arrow indicates the location of 20 GGGGCC repeats and the red arrow indicates the location of 30 or more repeats.

In FTLD-TDP-MND, inclusions are most common in the frontal and temporal cortex. In ALS with preserved cognition, deposits are located predominantly in the cytoplasm of motor neurons without affecting cortical structures [121].

According to the current neuropathological systemic classification, a harmonized classification system recognizes four types of FTLD-TDP pathology. Type A corresponds to Mackenzie et al. type 1 and Sampathu et al. type 3 and contains numerous short dystrophic neurites (DNs) and oval or crescent neuronal cytoplasmic inclusions (NCIs), which are located primarily in the second neocortical layer. Moderate numbers of lentiform neuronal intranuclear inclusions (NIIs) can also be present, although they are not a consistent sign of this subtype. The clinical phenotype of type A is bvFTD or progressive non-fluent aphasia and is based on mutations in the progranulin gene.

For this article, type B, equivalent to Mackenzie et al. type 3 and Sampathu et al. type 2, is the most important. Type B is characterized by moderate numbers of NCI, distributed throughout all cortical layers, with very few DNs. This condition is associated with a genetic defect in the short arm of chromosome 9, and clinically manifests as bvFTD or MND with FTD.

Type C is equivalent to Mackenzie et al. type 2 and Sampathu et al. type 1; it has a predominance of elongated DNs in the upper cortical layers and few NCIs; it leads to bvFTD or semantic dementia.

Type D is associated with the inclusion of body myopathy of Paget's disease of the bone and frontotemporal dementia caused by *VCP* mutations; there are large numbers of short DNs and numerous lentiform NIIs.

However, there are other examples of the wide-range neuropathological background of ALS/MND clinical symptomatology. The most important neurodegenerations in this field are tauopathies. Recently, we described tau protein deposits in corticospinal tract structures in patients with progressive supranuclear palsy (PSP) clinically mimicking MND. [123] Moreover, case reports of globular glial tauopathy (GGT) with the presence of tau-positive globular oligodendroglial inclusions (GOIs) with partial overlapping neuropathological features of progressive supranuclear palsy (PSP), clinically manifesting as MND and/or FTD, exist [124]. Neuronal, astrocytic, and especially oligodendroglial 4-repeat (4R) tau positive, mostly Gallyas negative, inclusions are present, whereas the GOIs in white matter correlate with the severity of neuropathologically confirmed degeneration [124]. Massive oligodendroglial involvement leading to changes in white matter (pallor, axonal loss, and gliosis) implies that these cases may represent primary oligodendrogliopathy [124]. Some authors also speculate that this entity could be an equivalent of "tau" multiple-system atrophy [125].

Considering comorbid cases and dementia in ALS, some comorbid ALS/AD cases have appeared in the literature; for example, one study reported that about 50% of ALS cases had Aβ deposits, and at least half of the cases had neuritic plaques in the neocortex [126]. Those patients with comorbid ALS/AD usually have progressive amnestic dementia, which is typical for AD and is accompanied by early distinctive impairment of episodic memory; MRIs of the hippocampus show significant atrophy [55].

Comorbid ALS/dementia with Lewy bodies (DLB) also exists. The prevalence of the DLB pathology in ALS is higher than in the general population, and it has been reported that Parkinsonian features develop in about 30% of ALS patients [127]. Levodopa-responsive parkinsonism accompanied by ALS, with symptoms that appear later in the course of the disease, is known as Brait–Fahn–Schwarz disease [128]. The incidence of this syndrome is high on the Japanese Kii Peninsula and the Micronesian island of Guam but is rare elsewhere in the world [129], although, Brait–Fahn–Schwarz syndrome was diagnosed in the Czech Republic (personal experience, not published yet).

7. Molecular Biomarkers of ALS

It seems that CFS-PGRN levels can be used to predict the type of FTD as it mirrors the FTD-subtype—the level is significantly lower in cases with TDP-43 pathology lacking a *GRN* mutation [130]. CSF-TDP-43 is also considered to be a promising CSF biomarker for ALS-FTD cases [131]. Interestingly, some researchers report higher CSF-TDP-43 levels in ALS cases than in FTLD, which might indicate more faster progression of TDP-43 pathology in ALS [132].

To predict disease development, some of the above-mentioned molecules can be used as biomarkers. Mentioned markers from the group of RNA-binding proteins in ALS are TDP-43, FUS, or hnRNPs, although some others can be used—TATA-box binding protein associated factor 15 (TAF15), which is mutated in both SALS and FALS; Ewing Sarcoma breakpoint region 1/EWS RNA binding protein 1 (EWSR1) with similar characteristics as FUS and TAF15 that they can easily aggregate leading to the toxicityn [133]; or ataxin-2 (ATXN2), which acts as a dose-sensitive modifier of TDP-43 toxic effect [134]. From the group of ALS-related genes, we could highlight *SOD1*, *C9orf72*, or spastacsin (*SPG*) [133], as well as non-coding RNA such as microRNA, circular RNA [133], or other molecules. It is worth mentioning serum uric acid, whose serum levels negatively correlate with the risk of death in patients with ALS [135]; the detection of higher levels of chitotriosidase in CSF correlates with microglial activation in the white matter of the spinal cord and may be helpful in patients with a short history of symptoms that are difficult to identify [136]. The identification of the blood neurofilament light chain (NFL) positively correlates with the progression of the disease, and a shorter survival period is indicated by increased NFL [137]. Some studies also indicate that patients with ALS have significantly higher levels of CSF total tau protein and a lower phosphorylated tau/total tau ratio than the control population [138].

8. Conclusions

Although much about the pathogenesis of ALS remains elusive, some major molecular pathways that can trigger selective damage in specific neurons are already known. Moreover, the disruption of individual ALS-causing genes can lead to the formation of pathological inclusions. Recognition of these features can help determine final diagnoses and also indicate potential links with other neurodegenerative diseases that need to be simultaneously considered.

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3.7 Shrnutí 20 let zkušeností národní laboratoře pro prionová onemocnění s užívanými diagnostickými postupy a množstvím zachycených případů. Porovnání s dalšími státy a vysvětlení odlišností v diagnostickém procesu.

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diagnostics

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Review

Human Prion Disorders: Review of the Current Literature and a Twenty-Year Experience of the National Surveillance Center in the Czech Republic

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Abstract: Human prion disorders (transmissible spongiform encephalopathies, TSEs) are unique, progressive, and fatal neurodegenerative diseases caused by aggregation of misfolded prion protein in neuronal tissue. Due to the potential transmission, human TSEs are under active surveillance in a majority of countries; in the Czech Republic data are centralized at the National surveillance center (NRL) which has a clinical and a neuropathological subdivision. The aim of our article is to review current knowledge about human TSEs and summarize the experience of active surveillance of human prion diseases in the Czech Republic during the last 20 years. Possible or probable TSEs undergo a mandatory autopsy using a standardized protocol. From 2001 to 2020, 305 cases of sporadic and genetic TSEs including 8 rare cases of Gerstmann–Sträussler–Scheinker syndrome (GSS) were confirmed. Additionally, in the Czech Republic, brain samples from all corneal donors have been tested by the NRL immunology laboratory to increase the safety of corneal transplants since January 2007. All tested 6590 corneal donor brain tissue samples were negative for prion protein deposits. Moreover, the routine use of diagnostic criteria including biomarkers are robust enough, and not even the COVID-19 pandemic has negatively impacted TSEs surveillance in the Czech Republic.

Keywords: transmissible spongiform encephalopathies; prion protein; Creutzfeldt–Jakob disease; Gerstmann–Sträussler–Scheinker syndrome; corneal donor

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1. Background—Human Prion Diseases in Review

Prion diseases are transmissible, progressive, and fatal neurodegenerative disorders associated with the aggregation of a misfolded prion protein (PrP) [1]. Human transmissible spongiform encephalopathies (TSEs) include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), kuru, and fatal familial insomnia (FFI) [2].

The cellular prion protein (PrP^C) functions as a glycolipid-anchored cell membrane sialoglycoprotein localized in presynaptic membranes that has neuroprotective [3] and pro-myelinating [4] roles. Additionally, it participates in neurotransmission, zinc and copper transport, and calcium homeostasis [5–7]. Moreover, under laboratory conditions, PrP^C promotes greater neuronal resistance after an ischemic cerebral insult [8,9].

All human prion diseases are associated with a pathological self-replicating [10] conformation of PrP, the most fundamental of which is the change of the PrP tertiary structure, by post-translational processes, into the predominant β-sheet pattern [11]. The

process results in the formation of a markedly hydrophobic form of PrP with a clear tendency toward aggregation [12], subsequent oligomerization, and formation of amyloid fibrils [13]. The pathological PrP^{Sc} (scrapie isoform of the prion protein) aggregates that arise from this process are extremely resistant to physical and chemical changes, and unlike most proteins, these molecules are not denatured by boiling [14].

Human prion diseases [15] are defined as transmissible and rapidly progressive [16] degenerative diseases of the central nervous system caused by an accumulation of pathologically conformed PrP [17].

Historically, the first mention of CJD comes from 1920 [18] and 1921 [19–21], when neurologist Hans Gerhard Creutzfeldt and neuropathologist Alfons Maria Jakob described a "nosologically very closely connected if not identical affection" of several patients. Kuru was given attention in the first half of the 20th century as it was described in Papua New Guinea among cannibalistic tribes; the disease is currently considered extinct [22]. The most recent form of TSE, i.e., Variant CJD (vCJD), was first identified in 1996 in the United Kingdom [23,24], which to this day remains the country with the highest number of cases (174) out of a total of 232 worldwide [25]. The last three known cases of vCJD came from Italy and France. An Italian patient with occupational contact with vCJD but without evidence of a laboratory incident died in 2016, and a French laboratory worker died in 2018, 7.5 years after a cutting incident with BSE transgenic mice contaminated instrument [26]. The last case that is under investigation appeared in 2021, when CJD was diagnosed in a retired French laboratory worker which led to 3-month moratorium on the study of prions in France [27].

1.1. Sporadic Human Prion Diseases

Creutzfeldt–Jakob disease (CJD) is the most common human prion disease [15]. The neuropathological characterization of CJD is spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical grey matter. It can also be described as encephalopathy with PrP immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar type) [28]. Three types, the most common being sporadic (sCJD), followed by genetic (gCJD) and acquired that can be further subdivided into iatrogenic (iCJD) [29], and Variant (vCJD) [30], are distinguished according to their different aetiologies [31]. Using Western blot, we can distinguish between PrP^{Sc} type 1 and 2 [32].

1.1.1. Sporadic Creutzfeldt-Jakob Disease

The sporadic CJD begins with an accidental conversion of physiological PrP^{C} to pathologically conformed PrP^{Sc}, which occurs in about 85% of CJD cases [33]. The worldwide incidence of sCJD is reported to be one to two cases per million [34]. Unlike vCJD, clinical signs and neuropathological findings differ from case to case, which is probably caused by different molecular phenotypes [35].

According to the diagnostic criteria recently published by Watson et al. [36] (see Table 1), three conditions can be distinguished: possible CJD (clinical presentation only and exclusion of distinct aetiologies, i.e., tumor, cerebrovascular lesions, autoimmune disorders, neuroinfection, neurodegenerative dementia, etc.), probable CJD (clinical presentation plus biomarkers: protein 14-3-3, magnetic resonance imaging (MRI) [37,38], electroencephalography (EEG) [39] with indicated sensitivity 67% and specificity 86% [40], and RT-QuIC) and definite CJD (neuropathologically confirmed).

Table 1. Diagnostic criteria for definite, probable and possible sCJD.

Possible Creutzfeldt–Jakob disease: Rapidly progressive dementia with at least two of the following symptoms: 1. myoclonus, 2. cerebellar or visuospatial dysfunctions, 3. pyramidal and/or extrapyramidal signs, 4. akinetic mutism, and duration less than 2 years.
Probable Creutzfeldt–Jakob disease: Fulfilled criteria for possible CJD with: 1. periodic sharp wave complexes at EEG, or 2. caudate/putamen hypersignal on magnetic resonance imaging (MRI) brain scan or at least two cortical regions (temporal, parietal, occipital) either on diffusion-weighted imaging (DWI) or fluid-attenuated inversion recovery (FLAIR) [41], or 3. positive cerebrospinal fluid 14-3-3 protein test, or 4. positive real-time quaking-induced conversion (RT-QuIC) in cerebrospinal fluid or other tissues.
Definite Creutzfeldt–Jakob disease: Progressive neurological syndrome and either neuropathological, immunocytochemical or biochemical confirmation.

The disease usually lasts for a few months, generally less than one year. The duration of the disease must be less than two years; longer durations are an exclusionary clinical criterion for possible sCJD [42].

Due to the risk of iatrogenic transmission, brain biopsy is only applicable in specific cases where a definitive diagnosis is critical. Arguments against brain biopsies in suspected CJD cases include the high probability of a unconclusive result [43]; additionally, it does not affect patient treatment even when the biopsy confirms clinical suspicions [44].

1.1.2. Sporadic Fatal Insomnia

Sporadic fatal insomnia (sFI) is defined as a rapidly progressive neurodegenerative disease with a clinical phenotype very similar to the fatal familial insomnia characterized by neurological and cognitive deterioration along with severe sleep impairment [45], transient diplopia [46] and cerebellar dysfunction [47], followed by dysautonomia, coma and death [45]. These patients are rare codon 129 methionine homozygotes with PrP^{Sc} type 2 and predominant thalamic involvement [48]. Microscopically, slight spongiform degeneration but severe neuronal loss with gliosis is present in thalamus and inferior olives, although immunohistochemical detection of PrP mostly shows focal or no positivity [48]. The distribution of the PrP^{Sc}-immunoreactive structures is similar in familial and sporadic form [49–51]. The features useful to distinguish sporadic versus familial form are the absence of a family history and the characteristic Asp178Asn *PRNP* mutation [49,50].

1.1.3. Variably Protease-Sensitive Prionopathy

Variably protease-sensitive prionopathy (VPSPr) is a relatively recently described prion disease identified in 2008 [52,53]. VPSPr is considered a sporadic form of human prion disease, and patients with all types of polymorphisms at codon 129, however, with a predominance of VV homozygotes, are reported [54]. At the same time, it is stated that VV homozygotes show more developed neuropathological manifestation in the form of plaques than MM homozygotes or MV heterozygotes. The median duration of the disease is 2 years with the clinical predominance of psychiatric signs [53], aphasia, ataxia, and parkinsonian syndrome [54] or cognitive decline [53]. Neuropathologically, VPSPr is

characterized by mild spongiform degeneration [53] lacking areas of confluent spongiform transformation [55], PrP-immunoreactive "microplaques", plus plaque-like deposits [53].

1.2. Acquired CJD

1.2.1. Iatrogenic, Accidentally Transmitted CJD

The iatrogenic form arises during medical or surgical procedures during which pathologically conformed prions are transferred [56]. Iatrogenic CJD is extremely rare, accounting for less than 1% of all CJD cases [57]. Transmission after dura mater grafting, from surgical instruments, after corneal transplantation, deep EEG electrode insertion, human growth hormone and gonadotropin treatment have been described [58–60] and more than 492 cases have been reported [58]. The incubation time varies widely based on the form of inoculation. Those infected via intracerebral electrodes had an incubation period of 16–28 months, whereas patients infected via peripheral growth hormone injections had a latency of between 5 and 30 years [35]. Patients with iCJD usually present with gait abnormalities and ataxia [58]. Specific diagnostic criteria also exist for iCJD (see Table 2).

Table 2. Diagnostic criteria for probable and definite iCJD.

Probable iatrogenic Creutzfeldt–Jakob disease diagnosis:	
a.	progressive cerebellar syndrome in a recipient of human cadaver-derived pituitary hormone; or
b.	probable CJD with a recognized iatrogenic risk.
Definite iatrogenic Creutzfeldt–Jakob disease diagnosis:	
a.	Definite CJD with a recognized iatrogenic risk.

1.2.2. Variant CJD

The Variant CJD (vCJD) is associated with the consumption of BSE-agent contaminated products. The clinical presentation initially includes psychiatric and behavioral symptoms, with painful paresthesia or dysesthesia [58]; ataxia and dementia develop later [35]. In contrast to sCJD and gCJD cases, EEG usually lacks the periodic pattern [61], the duration of the disease is usually longer (on average 13–14 months), and florid plaques are often present in the neuropathological findings [62]. The characteristic finding on MRI is an increased bilateral pulvinar signal (with indicated sensitivity 78% and specificity 100%). The thalamic and periaqueductal grey matter high signal, and the remarkable absence of cerebral atrophy are described [63]. In contrast to sporadic and genetic forms of CJD, the presence of PrP^{Sc} demonstrated both immunohistochemically and by Western blot was proven in all types of lymphoid tissue (tonsils, lymph nodes, and spleen) in all vCJD cases [64]. For this reason, tonsil biopsy may be used in suspected vCJD cases with corresponding clinical presentation and MRI lacking bilateral pulvinar high signal to confirm probable vCJD diagnosis [25]. With one exception (one patient with MV genotype) [65], all patients with vCJD were MM homozygotes [66], in addition, PrP^{Sc} type 2 was present in all cases [67]. Interestingly, there are three probable cases of CJD transmission via blood transfusions [59,60] from a donor suffering from vCJD; for this reason, there is a ban on donors who lived in the United Kingdom during the BSE epidemic [68]. Moreover, there are three recently known (2016, 2018, and 2021 which is under investigation) cases of CJD in former laboratory workers with occupational contact with BSE-infected brain tissue [28]. The diagnostic criteria are summarized below (Table 3).

Table 3. Diagnostic criteria for suspected and definite vCJD.

Suspected Variant CJD:	
a.	current age or age at death less than 55 years
b.	psychiatric symptoms at illness onset and/or persistent painful sensory symptoms (frank pain and/or dysesthesia)
c.	dementia, and development ≥4 months after illness onset of at least two of the following five neurologic symptoms: impairment in coordination, myoclonus, chorea, hyperreflexia, or visual signs (if persistent painful sensory symptoms exist, ≥4 months delay in the development of the neurologic signs is not required)
d.	a normal or an abnormal EEG, but not the diagnostic EEG changes seen in sporadic CJD
e.	duration of illness of more than 6 months
f.	routine investigations of the patient do not suggest an alternative, non-CJD diagnosis
g.	no history of receipt of cadaveric human pituitary growth hormone or a dura mater graft
h.	no history of CJD in a first degree relative or prion protein gene mutation in the patient
Definite Variant CJD: Neuropathologic examination of brain tissue is required to confirm a diagnosis of Variant CJD. The following confirmatory features should be present.	
a.	numerous widespread kuru-type amyloid plaques surrounded by vacuoles in both the cerebellum and cerebrum (florid plaques)
b.	spongiform change and extensive prion protein deposition shown by immunohistochemistry throughout the cerebellum and cerebrum

1.2.3. Kuru

Kuru was defined as a neurodegenerative, non-inflammatory infectious disease [69]. Kuru began to appear around 1900 in Papua New Guinea among cannibalistic tribes and the incidence subsequently escalated between 1940–1950 [69,70]. Cerebellar ataxia, tremor, and extrapyramidal symptoms such as chorea and athetosis [66,69–71] were typical symptoms of the majority of patients although neuropathological findings varied [72]. No cognitive impairment was present [58]. The neuropathological reports describe myelin and neuronal degeneration (with a maximum in pontine nuclei, cerebellum and basal ganglia), proliferation of microglia and astroglia [72], mononuclear perivascular infiltration, cuffing [73], spongiform transformation [74], shrunken neurons with dispersion of Nissl substance with intracytoplasmic vacuoles, and vacuolated cerebellar Purkinje cells and striatal neurons [75]. Amyloid "kuru" plaques were recorded in 50-75% [74,75] of the examined brains. Currently, kuru disease is considered extinct [22].

1.3. Inherited Prion Diseases

1.3.1. Genetic CJD

The genetic/familial form is conditioned by the presence of an inherited mutation in the *PRNP* gene, which occurs in 10–15% of CJD cases [76]. It is more appropriate to use the term genetic, as not every patient has known positive family history. There are more than 50 known mutations in this gene [35]. In the Czech Republic, the majority of genetic forms involve an E200K mutation, which is also the most common mutation in Europe [77,78] followed by the V210I and D178N mutations. The most common mutations in Japan are V180I, E200K, and M232R, sorted by frequency [77,79]. The D178N mutation is relatively common in certain countries in Western Europe [77,78], i.e., Netherlands, France, United Kingdom, Finland, and Hungary. Dementia is clinically described with other psychiatric changes along with ataxia and myoclonus, however, gaze palsies and neuropathies are rare [58]. The disease penetrance varies from 60 to 100%, relative to population [80]. Similar to sCJD, gCJD has its diagnostic criteria (see Table 4).

The course of the disease is usually longer; the 2-year disease duration limitation for sporadic CJD is not applicable in gCJD.

Table 4. Diagnostic criteria for probable and definite gCJD.

Probable genetic Creutzfeldt–Jakob disease diagnosis:	
a.	probable CJD and confirmed/probable CJD in a first-degree relative,
b.	a neuropsychiatric disorder plus a disease-specific prion protein gene (*PRNP*) mutation.
Definite genetic Creutzfeldt–Jakob disease diagnosis:	
a.	definite CJD with a recognized pathogenic *PRNP* mutation,
b.	and definite or probable TSE in a first-degree relative.

1.3.2. Gerstmann–Sträussler–Scheinker Syndrome

Gerstmann–Sträussler–Scheinker syndrome (GSS) is defined as a slowly progressive hereditary autosomal dominant neurodegenerative disease [81] or an encephalo(myelo)pathy with multicentric PrP plaques [28] in the cerebral and cerebellar cortex and basal ganglia [82,83]. It is the multicentric plaques that are the neuropathological hallmark of GSS, although the pattern differs among families [81]. GSS is usually manifested by cerebellar ataxia and slowly progressive dementia [84]; nevertheless, extrapyramidal symptoms, vision and hearing impairment, myoclonus, spastic paraparesis, and hyporeflexia or areflexia in the lower extremities have also been reported as common symptoms [84]. Four distinct clinical subtypes among cases with P102L mutation can be distinguished: typical GSS; GSS with areflexia and paresthesia; pure dementia GSS; and Creutzfeldt–Jakob disease-like GSS [85].

In addition, GSS was the first human TSE with a known *PRNP* mutation [81], which include point mutations at codons 102, 105, 117, 131, 145, 187, 198, 202, 212, 217, and 232 [81] or octapeptide repeat insertions (OPRI) counting 1–9 of 24 base pair multiples [86]. In the Czech Republic, the P102L mutation is the most common.

1.3.3. Fatal Familial Insomnia

Fatal familial insomnia (FFI) is an autosomal dominant inherited disease caused by a mutation D178N in the *PRNP* gene associated with the presence of the MM polymorphism at codon 129 [87]. FFI is characterized by medication-resistant insomnia, sleep fragmentation, disturbances of the autonomic nervous system, motor disorders, and progressive cognitive impairment [88]. The most affected areas are the mediodorsal and anterior ventral thalamic nuclei, followed by the pulvinar and the olives. Extensive neuronal loss and astrocytic gliosis are the main neuropathological findings, whereas spongiform transformations are missing [89]. FFI has not been found in the Czech Republic.

1.4. Differential Diagnosis of Human Prion Diseases

The clinical picture in typical forms of TSEs is relatively specific, and with additional methods (MRI, cerebrospinal fluid examination, RT-QuIC), a high degree of diagnostic certainty can be achieved. Nonetheless, the differential diagnosis needs to consider several main groups (neurodegenerative—pure or comorbid, autoimmune including paraneoplastic, infectious, toxic/metabolic [88,90,91], and tumorous). Primary neurodegenerative diseases include Alzheimer's disease (AD), frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), corticobasal syndrome (CBS), multiple-system atrophy (MSA), motor neuron disease (MND), progressive supranuclear palsy (PSP), and normal-pressure hydrocephalus [89]. However, it is important to point out that "pure" forms of neurodegenerative diseases usually do not imitate CJD; however, comorbid cases can (see Table 5). The most common diagnoses possibly clinically mimicking CJD are AD, frontotemporal lobar degeneration (FTLD), DLB, and tauopathies, more often with, or without comorbid neurodegenerative disease, including less common comorbid neurodegenerations (FTLD+DLB, AD+FTD including Pick disease, AD+PSP).

Table 5. Autopsy findings of non-prion diseases from brain tissue samples described as possible/probable CJD (2001–2020).

Neurodegenerative disorders (usually comorbid)	Alzheimer's disease	88
	Frontotemporal dementia	66
	Dementia with Lewy bodies	27
	Progressive supranuclear palsy	8
	Multiple system atrophy	4
	Corticobasal degeneration	2
	Parkinson disease	1
Neuroinfection and autoimmune diseases	Encephalitis	20
	Malignant multiple sclerosis (Marburg variant)	2
Ischemic and anoxic conditions	Subcortical vascular dementia	7
	Post-anoxic encephalopathy	7
Tumors	Primary CNS lymphoma	5
	Gliomatosis cerebri	1
	Meningeal carcinomatosis	1
	Metastatic carcinoma	1
Metabolic encephalopathy	Wernicke-Korsakoff	5
Others	Subdural hematoma	1

Paraneoplastic syndromes [92] in the form of paraneoplastic cerebellar degeneration or limbic encephalitis, autoimmune encephalitis [93], vasculitis [94], but also Hashimoto thyroiditis complicated by Hashimoto encephalopathy [95] must be included in the group of autoimmune processes that can mimic TSEs. Infectious forms are mainly caused by herpes simplex encephalitis.

Hepatic [96] or renal metabolic encephalopathies, as well as Wernicke-Korsakoff syndrome [97], which usually occurs in the chronically malnourished, especially ethylic patients, should be diagnostically considered in cases of rapidly progressive dementias. Clinically, these illnesses can have highly variable presentations.

We also noted cases of rapidly progressing dementia with atypical clinical symptoms and clinical suspicion of Creutzfeldt–Jakob disease in whom the subsequent neuropathological examination revealed the presence of a tumorous infiltration of the brain (primary, secondary, or lymphomatous) or meningeal carcinomatosis [98].

Although the neuropathological diagnosis of isolated prion disease is relatively straightforward, it is necessary to investigate possible co-pathologies in the brain that could modify the clinical, biochemical, and morphological manifestations observed antemortem. The influence of these modifying factors on the profile of biomarkers in cerebrospinal fluid is crucial [99].

1.5. CSF Biomarkers in TSE

RT-QuIC and 14-3-3 protein analysis are CSF biomarkers routinely used to confirm the diagnosis of probable CJD.

1.5.1. Protein 14-3-3

The 14-3-3 proteins are highly expressed in the brain. They are located in the cytoplasmic compartment, intracellular organelles, and in the plasma membrane of neurons [100,101]. Detection of the 14-3-3 protein in the CSF is part of the WHO diagnostic criteria for probable sCJD [39,94]. There are seven isoforms of the 14-3-3 protein, only four of them have been detected in the CSF of sCJD patients, and only two (the β- and γ-isoform)

seem to be suitable biomarkers for a differential diagnosis of sCJD [100]. This biomarker is not only present in the CSF of sCJD patients; it indicates rapid ongoing neuronal destruction in a variety of progressive neurological disorders [100,102]. Therefore, 14-3-3 positivity is not specific for sCJD, but the test should be indicated when the diagnosis of sCJD is considered. Total (t)-tau can also reflect neuronal destruction and is believed to correlate with the rate of axonal degeneration [103]. Since t-tau levels are dramatically increased in patients with prion diseases [103], we compared the ROC curves of 14-3-3β, t-tau, and their combinations in prion vs. non-prion neurodegenerative diseases. The presence of 14-3-3β was determined using a standardized Western blot protocol (used by all laboratories for the diagnosis of CJD) and followed EURO-CJD standards, with stringent quality control. We performed a standardized qualitative Western blot analysis for 14-3-3β in duplicates. Levels of t-tau were measured using commercially available enzyme-linked immunoassay (ELISA) kits (INNOTEST hTAU Ag, cat. #80323, Innogenetics/FUJIREBIO and Total-Tau ELISA, cat. #EQ 6531-9601-L, EUROIMMUN) according to the manufacturers' instructions.

Comparing 14-3-3 positivity and t-tau levels in prion diseases vs. non-prion diseases using ROC curves, the AUC values were 0.738 and 0.927 (both $p < 0.0001$), respectively (Figure 1). For 14-3-3 positivity in prion diseases, the sensitivity was 63.1%, and the specificity was 81.1%. For t-tau, the cut-off was assessed to be 1200 pg/mL, with a sensitivity of 87.5% and a specificity of 91.5%. When both variables were taken together, the AUC was 0.909 ($p < 0.0001$), which gave the highest sensitivity (93.2%) but the lowest specificity (66.7%). Our results indicate that t-tau levels or t-tau levels combined with 14-3-3 positivity work better for detecting ongoing prion disease than 14-3-3 positivity alone, which we previously described [104]. Total tau can be a helpful tool in the differential diagnosis of sCJD and thus should be measured in addition to 14-3-3, especially when RT-QuIC is unavailable due to technical reasons and/or cost [105].

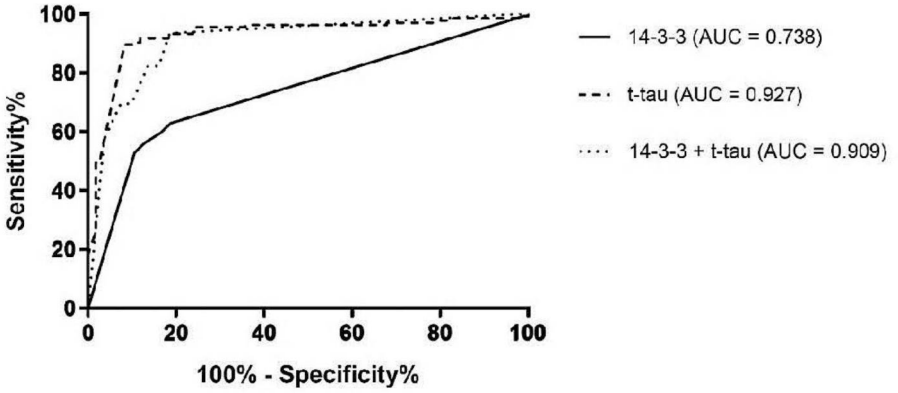

Figure 1. ROC diagrams for protein 14-3-3 positivity (solid line), t-tau values higher than 1200 pg/mL (dashed line), and the combination of protein 14-3-3 positivity and t-tau values higher than 1200 pg/mL (dotted line) in prion vs. non-prion disease cases. t-tau—total tau; AUC—area under the curve.

1.5.2. RT-QuIC

Real-time quaking-induced conversion (RT-QuIC) is used to demonstrate the ability of PrP^{Sc} (present in the CSF) to initiate the conversion of physiological PrP^{C} into a pathological conformation in which the β-sheet pattern predominates with a marked tendency toward real-time aggregation [106]. Imaging is performed by binding to thioflavin T, which emits fluorescence that can be immediately detected [106]. The method was introduced in 2010 and enabled detection of small amounts of PrP^{Sc} in approximately 90 h [106], with high levels of specificity (100% specificity, 95.8% diagnostic sensitivity), which is in contrast to other CSF biomarkers [107]. Second-generation RT-QuIC (called QuIC CSF or IQ-CSF), which uses truncated hamster PrP as a substrate, maintains a specificity of 98–100%;

however, the reaction time is reduced to 30 h [108–111]. Moreover, RT-QuIC has the ability to detect PrP^{Sc} in olfactory mucosa samples or in a skin biopsy. RT-QuIC allows an accurate and rapid diagnosis, which is significant in the differential diagnosis of treatable diseases mimicking TSEs; however, this method has not yet been introduced into clinical practice and remains available only for scientific purposes in the Czech Republic.

1.6. Definite Diagnosis of Human Prion Diseases

A definitive diagnosis of TSE is based on the detection of PrP^{Sc} in brain tissue usually from brain autopsy [94]. Because Creutzfeldt–Jakob disease is not a high priority in differential diagnostic considerations, brain biopsy should be reserved for the search of treatable causes of progressive dementia. After confirmation of prion disease by Western blot and immunohistochemical methods, molecular genetic testing is routinely performed, aimed at detecting polymorphisms of codon 129 and possible mutations in the *PRNP* gene in genetic forms. About 15% of prion diseases have a hereditary basis, and in many cases, even in the absence of a relevant family history [77].

Cases of CJD are then divided according to the polymorphism at codon 129 to the three categories: MM homozygotes, VV homozygotes, and MV heterozygotes. According to the size of the proteinase K-resistant core of PrP^{Sc} (21 and 19 kDa), type 1 and type 2 of PrP^{Sc} is recognized, however, by combination of these techniques we only achieve a division into six subcategories: MM1, MM2, MV1, MV2, VV1, and VV2. Based on histopathological criteria, the subcategory MM2 is subsequently divided into MM2-cortical (MM2-C) and MM2-thalamic (MM2-T) form with characteristic major changes in thalamus and olives [32]. Similarly, subsequent neuropathological criteria for the MV2 cases exists. Predominantly cortical (MV2C) and predominant kuru plaques form (MV2K) are recognized; moreover, a histotype (MV2K+C) sharing both characteristics is also described [112].

Each histotype has its own characteristic histological picture:

MM1/MV1:

- Spongiform degeneration: formed by fine vacuoles predominantly affecting corticostriatal-thalamic and cerebellar areas, whereas hippocampal region is relatively spared.
- PrP deposits: prevailing synaptic location.

MM2/MV2C:

- Spongiform degeneration: large confluent vacuoles.
- PrP deposits: predominant involvement of cortex and subiculum, minor involvement of brainstem with cerebellum.

MV2K:

- PrP deposits: kuru-like plaques mainly in cerebellar granular layer, plaque-like deposits in other cortical regions.

VV1:

- Spongiform degeneration: medium-sized vacuoles involving cortex, striatum, beside spared cerebellar region.
- PrP deposits: synaptic pattern.

VV2:

- Spongiform degeneration: fine or medium-sized vacuoles; more severe involvement of subcortical grey matter by comparison with cerebral neocortex, and of hippocampus plus subiculum by comparison with occipital cortex.
- PrP deposits: cerebellar plaque-like structures and perioneuronal pattern in deep cortical layers and hippocampal region.
- Others: cerebellar atrophy.

MM2T (sFI):

- Spongiform degeneration: absent in cerebellum.

- Others: moderate to severe selective atrophy of thalamus and olives.

MVK+C:

- Spongiform degeneration: extensive vacuolarization.
- PrP deposits: same as MV2K (kuru-like plaques mainly in cerebellar granular layer, plaque-like deposits in other cortical regions), in addition with perivacuolar and coarse PrP deposits in the grey matter [113].

Upon detection of PrP^{Sc}, the examined tissue is first digested with proteinase K, which degrades physiological PrP^{C}. Subsequently, monoclonal antibodies directed against PrP recognize pathological PrP^{Sc} molecules that are resistant to proteinase K digestion. Indirect immunohistochemical methods or Western blot are used as a standard, although RT-QuIC should not be omitted from the list of diagnostic methods.

1.6.1. Western Blot

Western blot (WB) is more sensitive and faster than immunohistochemical methods. The fixation time of the whole brain is about 3–4 weeks, after which a neuropathological examination can be performed.

The time from autopsy to a definitive diagnosis, including the exclusion of other possible neurodegenerative diseases, is therefore around six weeks but can take longer. WB makes it possible to demonstrate the presence of PrP^{Sc} on the second day after autopsy. For WB analysis, the frontal lobe is routinely analyzed. Nonetheless, WB analysis provides the basic information about the presence of PrP^{Sc} and can only distinguish types (1, 2, 1+2 or 3) of PrP^{Sc}. For complete information about the PrP^{Sc} strain, other methods must be performed (see below).

Western Blot in Corneal Pre-Transplantation Testing

Since the retina and optic nerve are extensions of the central nervous system, the WHO considers them to be a category I: high-infectivity tissue. In the Czech Republic, approximately 500 corneal transplants are carried out each year. Since January 2007, testing all corneal donors for PrP^{Sc} is mandatory with the goal of increasing the safety of corneal transplants. For the detection of PrP^{Sc} in brain tissue by Western blot, the frontal lobe is routinely analyzed according to WHO guidelines. Complete neuropathological work-up is not performed in corneal donors' brain tissue. Except for a few cases of CJD [114,115], no other neuropathies were determined to be transferred during the corneal transplantation to our knowledge. All tests are performed exclusively by the immunological laboratory at the NRL, which cooperates with all eye banks in the Czech Republic. During this time (2007–2020), 6590 samples were tested; all were negative, four were suspected of having PrP^{Sc}, but after further investigations, they were also found to be negative. Traceability of donors, through the National Donor Register and the National Transplant Register, for a period of 30 years is ensured by the Transplantation Act [116].

To the best of our knowledge, the Czech Republic is the only country in the world where legislation requires Western blot tests of brain tissue from every eye tissue donor. [117].

In neighboring Slovakia, where gCJD accounts for 74.2% of cases (compared to 16.28% in the Czech Republic) [118], determination of the codon 129 genotype together with detection of the E200K mutation is the method of choice for corneal donors testing [119].

1.6.2. Immunohistochemistry

An immunohistochemical examination determines the distribution of PrP^{Sc} in different parts of the brain as well as the morphology of immunoreactive structures since PrP^{Sc} may occur in the form of plaques, plaque-like structures or diffuse synaptic deposits.

According to WHO criteria, one of the above methods is enough to confirm a definitive diagnosis of prion disease, although a combination of both is recommended.

A complete definitive diagnosis of prion disease has three parts, i.e., neuropathological, immunological (Western blot), and molecular genetics. Only a combination of these approaches can provide a definitive diagnosis of sporadic or genetic prion disease.

1.6.3. Genetic Testing

Among the diagnosed cases in the Czech Republic, the distribution of polymorphisms at codon 129 is 60% methionine homozygosity (MM), 24% methionine/valine heterozygosity, and 16% valine homozygosity (VV); additionally, approximately 16% of cases are due to an inherited mutation in the *PRNP* gene.

A common coding polymorphism at codon 129 in *PRNP* between methionine and valine (c.385A>G) plays a critical role in susceptibility to prion diseases with homozygotes (i.e., MM or VV) being at higher risk. Easier dimerization and oligomerization of PrP homozygotes compared to heterozygotes is an important factor in the pathogenesis of prion diseases.

In total, approximately 50 mutations of the *PRNP* gene have been described around the world. The most common mutations in the *PRNP* gene in the Czech population are E200K, D178N, R208H, and P102L, as well as del/ins in the repetitive sequence. The general penetration of these mutations increases with age, and in some populations reaches 100% after the age of 85; in others, for example, in Slovakia or Italy, penetration is up to 60% [118].

The E200K mutation is the most widespread in the Czech Republic (30 cases), as well as in Slovakia, from where it was transferred to the Czech Republic.

The D178N mutation was found in two cases. The clinical phenotype in those cases was influenced by the polymorphism at codon 129 since the D178N mutation with valine at codon 129 causes gCJD. If methionine is present at codon 129, fatal familial insomnia develops. The genetic background of D178N could be clinically missed; for genetic counselling purposes, however, the mutation can be detected from samples of the archived tissue [120].

The P102L mutation was found in eight cases, and no clinically specific symptoms for this mutation were observed, although we described a case mimicking gCJD [121].

The R208H mutation is very rare, and one family harboring this mutation was discovered in the Czech Republic in relation to a case mimicking progressive supranuclear palsy [122].

Del/ins of two or more octapeptide repeats is considered pathogenic since octapeptide repeat insertions increase the rate of protease-resistant PrP formations. The clinical phenotype is highly variable, often blending features of both CJD and GSS disease or sometimes even lacking specific histopathological changes. Patients with insertional mutations usually display signs of illness early in life that last for several years. The molecular basis for this phenotypic heterogeneity remains elusive but seems to depend on the size of the base pair insertion and the codon 129 polymorphism.

2. Human Prion Diseases in the Czech Republic 2001–2020—Results from a Nation-Wide Survey

2.1. Numbers of Detected Cases

Autopsy verification of all suspected prion diseases is mandatory in the Czech Republic and is governed by hygienic-epidemiological surveillance. Data obtained regarding possible cases from clinical neurologists and subsequently confirmed cases are registered with the National Reference Laboratory (NRL) for Human Prion Diseases at the Department of Pathology and Molecular Medicine, Thomayer University Hospital, Prague, CZ. Autopsies are provided by the NRL [123] with clinically suspected neurodegenerations making up about one-third of performed autopsies done in the department. The NRL is the only reference center for neuropathology verification of prion disease in the Czech Republic so that the data represents the official results of prion surveillance in our country and consists of definitively neuropathologically confirmed cases.

From 2001 to 2020, a total of 305 cases of prion diseases were definitively confirmed, of which 256 cases were sCJD, 41 cases were gCJD, and 8 cases were GSS (see Figure 2), which corresponds to a total prevalence of approximately 15.25 cases per year. No cases of vCJD or FFI have ever been detected in the Czech Republic. Not all the brain samples referred to the NRL as possible/probable CJD were confirmed as definite CJD. Many other diagnostic entities were detected during autopsy, including neurodegenerations (in most cases comorbid), tumors, and autoimmune disorders (Table 5).

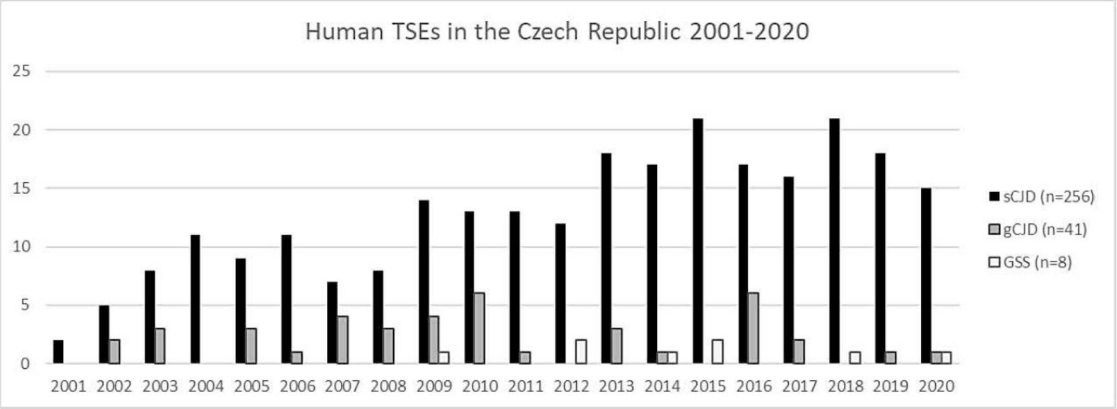

Figure 2. Number of patients who died with neuropathologically confirmed diagnose of prion disease in the Czech Republic (2001–2020). The legend shows the total number of cases for each type of prion disease.

There is an apparent trend towards an increasing prevalence of TSEs due to the optimization of diagnostic methods, and in part by the fact that the NRL is headquartered at the Department of Neurology at Thomayer University Hospital, which provides consultations intra vitam in suspected cases for the whole of the Czech Republic. The increasing prevalence is well illustrated by the fact that in the first ten years (2001–2010) of the NRL's existence, 115 cases of human prion diseases were detected, 88 of which have no hereditary background. However, over the next ten years (2011–2020), the number of confirmed TSE cases increased to 190, with 168 sporadic cases. This was an increase of 90.91% in detected sporadic cases over ten years, according to the predicted increase of diagnosed cases if surveillance was more intensive [124].

Nevertheless, during the COVID-19 pandemic in 2020, we noticed a slight decrease in the number of diagnosed patients suffering from TSEs; it was the fewest detected cases of sCJD since 2012. We speculate that this could be partly due to the greater burden on hospitals during the pandemic, which led to a reduction in TSE surveillance.

Considering incidence, in individual regions it ranges from 0.9 to 2.3 cases per million inhabitants (Figure 3), which is consistent with the worldwide numbers. The differences between individual regions are usually not significant and correlate with the existence of specialized neurological centers in the area. The age range in our cohort of patients is 39–69 years in GSS, 46–74 years in gCJD and 40–87 years in sCJD, which corresponds to a median age of 60.5 years in GSS, 56.5 years in gCJD and 67 years in sCJD. Thus, patients with the sporadic form have a higher median age than patients with genetically based TSEs (see Supplementary Material).

Figure 3. The incidence map of TSEs in individual regions of the Czech Republic. For each region, the number of cases per 100,000 population per year is indicated.

2.2. CJD in Comorbidity

There are many reports indicating that CJD very often occurs in comorbidity with other neurodegenerative diseases. Kovacs et al. [125] stated that the most common comorbidities with sCJD are tauopathies, such as primary age-related tauopathy, aging-related tau astrogliopathy, and argyrophilic grain disease (PART, ARTAG, and AGD, respectively). Our experience also supports these observations; in our cohort, approximately 62% of patients had sCJD or gCJD with a comorbid tauopathy, most often PART. The next most common comorbidity was Alzheimer's disease, of which there are twice as many as pure CJD cases. Less common combinations include CJD+FTLD and CJD+DLB, which were rarely observed. We investigated the genetic background of the various comorbidities; however, our pilot study failed to find any important relationships [126].

2.3. Brain Biopsy

During our 20 years of experience, a brain biopsy was rarely indicated. As mentioned above, brain biopsies are reserved for cases with a clinical suspicion of CJD associated with iatrogenic transmission (resulting from irreversible contamination of surgical instruments), and since CJD is incurable, a brain biopsy is the ultimum refugium in cases where the differential diagnosis includes a treatable disease.

3. Conclusions

At present, epidemiological surveillance of prion diseases in the Czech Republic is at a level comparable to other developed countries, and at the top with regard to systematic screening for PrP^{Sc} in the brain tissue of all corneal donors; the Transplantation Act, which mandates this screening, is unique in the world. The greater knowledge of clinicians and the routine use of magnetic resonance imaging and cerebrospinal fluid analysis has led to an increase in the detection of human prion diseases. In the Czech Republic, 16.28% of

cases are hereditary; therefore, subsequent genetic consultation with the deceased patients' relatives has become an important part of comprehensive approach to affected families. Although neither iatrogenic nor vCJD have been detected in the Czech Republic, the risk remains, mainly in connection with the increase in mini-invasive neurosurgical procedures and transmission via unscreened blood derivatives. The longstanding experience of our National reference center and the neuropathological feedback provided to various hospitals having referred patients with possible/probable CJD for autopsy progressively contribute to a better awareness and knowledge about the diagnostic clinical criteria and biomarkers of human prion diseases in our country.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/diagnostics11101821/s1.

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Data Availability Statement: The authors confirm that all data underlying the findings are fully available without restriction. All data are included within the manuscript.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4 ZÁVĚRY, ZHODNOCENÍ CÍLŮ A HYPOTÉZ PRÁCE

4.1 Mikromorfologická charakterizace archikortikálních a neokortikálních neuritických plak v případech tzv. „čisté“ Alzheimerovy nemoci a Alzheimerovy nemoci s komorbidní synukleinopatií.

V pilotní studii jsme se zabývali neuritickými změnami u případů komorbidní Alzheimerovy nemoci se synukleinopatií. Celkem bylo analyzováno 28 případů, 14 z nich bylo postiženo tzv. „čistou“ AN, 10 případů mělo plně vyvinutou jak AN, tak DLB a u 4 případů se jednalo o plně vyvinutou AN s Lewyho tělísky převážně v limbickém systému, majoritně v amygdale, tzv. amygdala Lewy bodies (ALB). Do kohort byli pacienti řazeni tak, aby byl u všech přibližně stejný věk v době úmrtí a tíže postižení alzheimerovskými změnami. Studie zahrnovala jak imunohistochemické metody sledované klasickou světelnou mikroskopií, tak imunofluorescenční metody zobrazované vícekanálovou konfokální fluorescenční mikroskopií – užívanými protilátkami byly protilátky proti Aβ, h-tau (AT8; Ser202, Thr205) a ubikvitinu.

Naše hypotéza vyplývající z pozorování v průběhu rutinního vyšetřování těchto případů se potvrdila. Bulbózní dystrofické změny jsou nejnápadnější v případech komorbidní AN/DLB, méně nápadné, ale přítomné v případech AN/ALB, a vzácné a málo vyjádřené v případech „čisté“ AN. Tyto změny jsou pozorovatelné především v archikortikálních plakách, u nichž jsme pomocí konfokální mikroskopie prokázali, že jsou dominantně tvořeny dystrofickými a často bulbózními neurity, zatímco Aβ extracelulární složka plak je upozaděná. Naopak v neokortikálních plakách dominuje Aβ, neuritické změny jsou méně nápadné a nemají bulbózní charakter.

Vzhledem k relativně malému počtu vzorků nejsou výsledky statisticky signifikantní, ale je jednoznačně patrný trend maximálních dystrofických změn v arichikortexu komorbidních AN/DLB případů. Domníváme se, že za rozdílnou mikromorfologií neokortikálních a archikortikálních plak stojí rozdílný vývojový původ a stavba archikortexu a neokortexu. Naší druhou domněnkou je, že dystrofické změny jsou patrné u všech případů v jejich iniciálním a progresivním stádiu, ale jelikož pacienti s čistou AN přežívají nejdéle, dochází ke známému fenoménu vyhoření neuritických změn. U komorbidních případů vzhledem k rychlejší progresi onemocnění a časnějšímu úmrtí dochází k „odkrytí“ těchto změn.

4.2 Mikromorfologická charakterizace extracelulárních proteinových depozit u komorbidních případů Creutzfeldtovy–Jakobovy choroby a Alzheimerovy nemoci, popis dystrofických neuritických změn v plakách PrP^{Sc}.

Touto studií jsme se zaměřili na mikromorfologický vzhled plak u komorbidních případů AN a sporadické CJN. Do kohorty bylo vybráno 20 komorbidních případů, které jsme vyšetřovali jak pomocí imunohistochemických, tak imunofluorescenčních metod. Předpokládali jsme, že extracelulární depozita Aβ a PrP^{Sc} budou ve většině případů kolokalizovat, a že případné rozdíly v mikromorfologickém charakteru plak by mohly být vysvětleny polymorfismy na kodonu 129 (methionin/valin) nebo neuropatologickým stádiem AN.

Zaregistrovali jsme tři základní typy plak:

(1) Plaky nesložené/samostatné a plaky s minimální kolokalizací. U první podskupiny byla depozita Aβ a PrP^{Sc} lokalizovaná zcela odděleně, u druhé podskupiny se na stavbě plak podílel především Aβ s minoritní komponentou PrP^{Sc} tečkovitého charakteru v periferních fibrilárních oblastech agregátů Aβ. Plaky s minimální kolokalizací byly zastoupeny nejčastěji.

(2) Centrálně lokalizovaná depozita PrP^{Sc} – v těchto případech kolokalizují neuritické Aβ plaky s denzním jádrem i bez něj s větším množstvím PrP^{Sc}, který se ukládá v centrálních částech Aβ plak ve fibrilární komponentně.

(3) Smíšené plaky s difuzním charakterem kolokalizace obou patologicky konformovaných proteinů. Tento obraz jsme nalezli u nejmenšího počtu případů. U jedné z podskupin byly zřejmé kolokalizace PrP^{Sc} povšechně v periferních fibrilárních oblastech Aβ plak, u druhé podskupiny se PrP^{Sc} depozita hromadila při jednom z pólů Aβ plak.

Naše původní hypotéza byla částečně potvrzena, protože jsme byli schopni nalézt případy s kolokalizujícími Aβ a PrP^{Sc} depozity, ačkoliv procentuelně jsme předpokládali větší účast případů s výraznou kolokalizací. Náš předpoklad, že charakter kolokalizací by mohl mít spojitost s polymorfismy 129. kodonu *PRNP* nebo tíží alzheimerovské patologie se nepotvrdil. Dystrofické neuritické změny jsou obdobného charakteru jako u AN a vyskytují se jak v plakách smíšených, tak v plakách tvořených čistě PrP^{Sc}. Hypotéza, že změny dystrofických neuritů budou obdobné u AN i CJN se potvrdila.

4.3 Mikromorfologická charakterizace extracelulárních proteinových depozit u případů Gerstmannova–Sträusslerova–Scheinkerova syndromu a jejich vztah k dystrofickým neuritickým změnám.

Cílem další mikromorfologické studie bylo popsat charakter PrP^{Sc} plak u Gerstmannova–Sträusslerova–Scheinkerova syndromu a jejich vztah k dystrofickým neuritickým změnám. Imunohistochemicky a imunofluorescenčně jsme vyšetřili veškeré známé případy tohoto velmi vzácného hereditárního neurodegenerativního onemocnění v České republice. Celkem se jednalo o 9 pacientů s mutací P102L v genu *PRNP*, z toho dva subjekty jsou ve vztahu otec-dcera, dva matka-syn.

Při použití dvou klonů protilátek proti prionovému proteinu (12F10 a 6H4) jsme u všech případů prokázali přítomnost charakteristických multicentrických plak, avšak v menším množství, než je v odborné literatuře uváděno. U dvou vzájemně nepříbuzných případů byly přítomny rozměrné plaky nad 100 μm lokalizované do subikula a CA1 oblasti hipokampu, u ostatních případů převládaly malé sférické plaky měřící 2–10 μm, vyskytující se často solitárně, někdy v rámci multicentrických plak bez výraznější účasti dystrofických neuritů. Pokud byly dystrofické neurity přítomny, měly povětšinou charakter nebulbózních změn. Jedinou výjimkou s nápadnými dilatovanými neurity byla pacientka č. 7 – 39letá žena s 10letým trváním onemocnění, MM polymorfismem na kodonu 129, dcera subjektu č. 6, u kterého identické neuritické změny nebyly přítomny. Podobně jako v případech AN/DLB i u této pacientky byly bulbózní změny více prominentní v archikortikálních oblastech v porovnání s vývojově a stavebně odlišným neokortexem.

Užitím dvou protilátek proti Aβ jsme v žádném z případů neprokázali kolokalizaci Aβ a PrP^{Sc}, což je nález konzistentní s poměrně nízkou kolokalizací i u případů CJN. Přesto byla překvapivá malá míra kolokalizace PrP^{Sc} a dystrofických neuritických změn patrných v reakci s protilátkou proti fosforylovasnému tau a to mj. i vzhledem k časté komorbiditě tauopatií a CJN.

4.4 Charakterizace případů Creutzfeldtovy–Jakobovy choroby po neuropatologické, genetické, imunologické i klinické stránce a z pohledu radiodiagnostiky.

Rozsáhlá retrospektivní studie probíhající ve spolupráci s imunologickou a genetickou laboratoří Národní referenční laboratoře lidských prionových chorob, Neurologickou klinikou 3. LF UK a FTN a Radiodiagnostickým oddělením Nemocnice Na Homolce si kladla za cíl podrobně charakterizovat případy CJN zachycené v České republice, popsat četnost a charakter komorbidit a případně nalézt vliv komorbidních neurodegenerativních chorob na hladiny biomarkerů, výsledky zobrazovacích metod či klinické projevy onemocnění. Všech 215 pacientů bylo podrobně neuropatologicky, geneticky a biochemicky vyšetřeno, byla získána klinická data, výsledky EEG vyšetření a MRI nálezy.

V literatuře již bylo popsáno relativně vysoké procento komorbidních případů, přesto pouhých 11,16 % „čistých“ případů CJN bylo překvapivým výsledkem. Jednoznačně nejpočetnější skupinou jsou případy komorbidní CJN s tauopatií (62,79%) – jedná se často o případy PART či AGD, ARTAG bývá zastižen častěji v komorbiditě CJN/AN/ARTAG. Dalších 20,47 % ve vyšetřené populaci tvořily případy CJN/AN, 3,26 % případů CJN/FTLD a 2,33 % CJN/synukleinopatie.

Klinicky všech 215 případů splňovalo kritéria pro možnou CJN – všichni pacienti trpěli demencí a alespoň dvěma ze čtyř nutných klinických příznaků, kterými jsou (1) pyramidální nebo extrapyramidové příznaky, (2) vizuospaciální nebo cerebelární dysfunkce, (3) myoklonus a (4) akinetický mutismus.

Ze získaných dat plyne, že:

(1) u „čisté“ CJN je významně nižší věk v době úmrtí – 60 let u „čistých“ případů vs. 65 let u CJN s taopatií, 71 let u CJN/AN či CJN se synukleinopatiemi a 67 let u CJN/FTLD;

(2) komorbidní případy CJN/tau jsou spojeny s vyšší pravděpodobností výskytu putaminálních hyperintenzit a s nižším MTA skóre na MRI;

(3) komorbidita CJN/AN je spojena s vyšším věkem nástupu, nižší pravděpodobností rozvoje putaminálních hyperintenzit na MRI a s významně nižší hladinou beta-amyloidu v CSF;

(4) čistá CJN ve srovnání s CJN/AN má nižší věk začátku onemocnění a nižší MTA skóre;

(5) komorbidní CJN s tauopatií se liší od komorbidní CJN/AN tím, že má nižší věk nástupu, nižší MTA skóre a vyšší hladiny beta-amyloidu v CSF;

(6) komorbidní patologie nezávisí na polymorfismech kodonu 129 ani genetickém pozadí CJN.

Hypotézy stanovené před zahájením retrospektivní studie se naplnily – dominují komorbidní případy, komorbidity mohou ovlivňovat jak obraz na MRI, tak hladiny biomarkerů v CSF.

4.5 Utřídění názvosloví jednotlivých podtypů extracelulárních plak u Alzheimerovy choroby a prionóz, jejich vzájemné srovnání.

V této práci jsme věnovali pozornost podrobné klasifikaci a charakterizaci extracelulárních plak u jediných dvou typů neurodegenerativních onemocnění charakterizovaných výskytem extracelulárních depozit. Informace jsme doplnili srovnáním charakteru a chování plak u obou typů onemocnění. Přestože jsou termíny pro jednotlivé typy plak užívány prakticky v každé tématické učebnici či vědecké práci, názlosloví není striktně dodržováno a často dochází k záměnám pojmů a tedy pozměnění významu informací.

U AN se držíme striktního dělení na plaky:

(1) difuzní – nedostatečné k vyslovení diagnózy AN vzhledem k chybění gliální odpovědi či ztráty neuronů,

(2) neuritické „bez jádra“ či „s jádrem“ patřící k základu diagnózy AN z důvodu vyvolání neuritické, astrocytární i mikrogliové odpovědi a ztráty neuronů,

(3) vyhořelé plaky v pozdějších stádiích onemocnění, tvořené pouze denzním jádrem bez okolní neuritické komponenty.

U prionóz mezi extracelulárními depozity rozlišujeme:

(1) unicentrické/„kuru-like“ plaky typické pro onemocnění kuru a případy sCJN s MV2 polymorfismem,

(2) floridní, „daisy-like“ plaky na periferii obklopené spongiformními změnami charakteristickými pro vCJN,

(3) multicentrické plaky vyskytující se u GSS,

(4) neuritické plaky, složené pouze z dystrofických neuritů, které se mohou vyskytovat u všech prionových chorob, ale jsou méně časté než výše zmíněné.

Jedná se o první systematickou klasifikaci morfologických podobností a rozdílů mezi extracelulárními depozity amyloidu u AN a CJN. Práce názorně demonstruje široké spektrum specifických neuropatologických změn a upozorňuje na nutnost precizního užívání názvosloví z důvodu možnosti porovnávat a doplňovat zjištěná fakta.

4.6 Shrnutí dosavadních neuropatologických poznatků o amyotrofické laterální skleróze.

V přehledovém článku byly shrnuty dosud zjištěné informace o ALS z pohledu neuropatologa ve spojitosti s genetickým podkladem a klinickým průběhem. Práce byla sestavena tak, aby porovnala neuropatologický nález u různých skupin pacientů (se sporadickou i genetickou formou a různými mutacemi), vyjádřila se k charakteristickým lokalizacím patologických depozit a imunoreaktivitě inkluzí.

Zároveň práce diskutuje ALS jako možné kontinuum s FTLD, jelikož klinický průběh u některých pacientů dokazuje spojitost mezi těmito jednotkami. K nálezu typickému pro ALS se u FTLD přidává některá z následujících variant: (1) FTLD-TDP (s TDP-43 inkluzemi), (2) FTLD-FUS (s FUS-pozitivními inkluzemi), (3) FTLD-tau (s tau-pozitivními inkluzemi), (4) FTLD-UPS (reagující s ubikvitinem nebo jinými markery ubikvitin-proteazomového systému) a (5) FTLD-NOS (neimunoreaktivní případy). Mezi případy dominují FTLD-TDP a FTLD-FUS, ale například u pacientů s mutací *C9orf72* jsou typicky nacházeny inkluze ubikvitinu či jiné součásti ubikvitin-proteazomového systému.

Ačkoli velké části patogeneze ALS stále nebylo porozuměno, některé hlavní molekulární dráhy, které mohou vyvolat selektivní poškození specifických neuronů, jsou již známy. Kromě toho může narušení konkrétních genů způsobujících ALS vést ke vzniku typických patologických inkluzí. Znalost těchto souvislostí může pomoci precizněji určit konečnou diagnózu, a odhalit potenciální souvislosti s jinými neurodegenerativními onemocněními.

4.7 Shrnutí 20 let zkušeností národní laboratoře pro prionová onemocnění s užívanými diagnostickými postupy a množstvím zachycených případů. Porovnání s dalšími státy a vysvětlení odlišností v diagnostickém procesu.

Přehledový článek shrnuje veškeré zkušenosti získané za 20 let existence Národní referenční laboratoře prionových chorob rozdělené na klinickou a laboratorní část (neuropatologickou, molekulárně genetickou a imunologickou). Vzhledem k tomu, že všechny případy tzv. možných nebo pravděpodobných prionóz podléhají povinné pitvě pomocí standardizovaného protokolu, od roku 2001 do roku 2020 bylo v našem centru vyšetřeno 305 případů sporadických a genetických prionóz včetně 8 vzácných případů GSS. V České republice navíc od ledna 2007 imunologická laboratoř NRL testuje vzorky všech dárců rohovky za účelem zvýšení bezpečnosti transplantací rohovky. V žádném z 6590 testovaných vzorků mozkové tkáně dárců rohovky nebyla prokázána depozita prionového proteinu.

V současné době hodnotíme epidemiologickou surveillance prionových onemocnění v ČR jako srovnatelnou s ostatními vyspělými zeměmi a na špičce v systematickém screeningu přítomnosti PrP^{Sc} v mozkové tkáni dárců rohovky – transplantační zákon, který tento screening nařizuje, je světovým unikátem. Dle našich statistik, které jsme publikovali v rámci této práce, vyšší povědomí lékařů o problematice prionových chorob spolu s rutinním využíváním MRI a analýzy CSF vedly k výraznému zvýšení záchytu lidských prionových onemocnění. V ČR je 16,28 % případů prionových chorob geneticky podmíněno; následná genetická konzultace s příbuznými zemřelých pacientů se stala důležitou součástí komplexního přístupu k postiženým rodinám. Přestože v ČR nebyla zachycena iatrogenní forma CJN, riziko přetrvává, a to především v souvislosti s nárůstem miniinvazivních neurochirurgických výkonů a skrze screeningově nevyšetřované krevní deriváty. Dlouholeté zkušenosti NRL a zpětná vazba poskytovaná nemocnicím, které případy s možnou/pravděpodobnou CJN odeslaly k pitvě, postupně přispívají k lepší informovanosti a znalosti o diagnostických klinických kritériích a biomarkerech lidských prionových onemocnění.

5 ZÁVĚR

Neurodegenerativní onemocnění jsou velmi častou skupinou onemocnění, o které můžeme tvrdit, že se do budoucna bude – vzhledem ke stárnutí lidské populace – celkové číslo případů zvyšovat. Přestože již mnohé bylo popsáno a pojetí se z původně ohraničených a nesouvisejících jednotek změnilo do podoby dnešního konceptu častých komorbidních případů, zůstává množství nepochopených a nevysvětlených souvislostí.

V rámci několika mikromorfologických studií korelovaných s genetickým, imunologickým a klinickým nálezem se nám podařilo odhalit odlišnost archikortikálních a neokortikálních plak. Je patrná jednoznačná tendence neokortikálních plak tvořit objemné Aβ struktury s menší účastí dystrofických neuritů pozitivních v reakci s protilátkou proti fosforylovanému tau proteinu. Naopak u archikortikálních plak je Aβ jen minoritní komponentou a převládají nápadné tau-pozitivní bulbózní dystrofické neurity. Lépe je tento trend patrný u pacientů s AN v komorbiditě se synukleinopatiemi, přičemž se domníváme, že v komorbidních případech, tj. případech s rychlejším průběhem onemocnění, dochází k odkrytí neuritických změn. Jak je totiž obecně známo, s délkou trvání onemocnění postupně dochází k „vyhořívání“ neuritické komponenty.

Druhá mikromorfologická studie popsala typy plak u pacientů s komorbidní CJN/AN. Přestože se u pacientů opakují tři základní skupiny kolokalizujících či nekolokalizujících patologických depozit tvořící extracelulární plaky, nepodařilo se nám vysledovat žádnou souvislost s polymorfismy na kodonu 129 ani tíží alzheimerovského postižení. Přesto z práce vyplývá jednoznačná tendence PrP^{Sc} kolokalizovat s fibrilárními strukturami Aβ bez tendence ke kolokalizaci v denzních partiích Aβ plak.

Prací o vzácném hereditárním neurodegenerativním onemocnění, Gerstmannově–Sträusslerově–Scheinkerově syndromu, není mnoho, proto byly podrobně neuropatologicky, molekulárně geneticky a imunologicky zpracovány veškeré případy zaznamenané v České republice, včetně čtyř pacientů v příbuzenském vztahu – všichni s mutací P102l v genu *PRNP*. V rozporu s obecnou představou GSS jako onemocnění charakterizovaného rozměrnými multicentrickými plakami jsme povětšinou nacházeli drobné sférické plaky s minimální odpovědí v okolí. Tato „nízká reaktivita“ PrP^{Sc} plak může vysvětlovat, proč v laboratorních podmínkách nikdy nedošlo k úspěšnému přenosu na jiný organismus jako je známo u případů CJN.

A nakonec, podrobnou retrospektivní studií veškerých případů CJN diagnostikovaných v České republice za posledních 10 let se nám podařilo prokázat vliv komorbidit CJN na hladiny

biomarkerů v mozkomíšním moku a obraz CNS na MRI. Podařilo se prokázat, že pacienti s „čistou“ CJN jsou v průměru významně mladší než pacienti s dalším komorbidním neurodegenerativním onemocněním, že CJN/tau mají vyšší pravděpodobnost výskytu putaminálních hyperintenzit a nižší MTA skóre na MRI; že CJN/AN je spojeno s vyšším věkem nástupu, nižší pravděpodobností rozvoje putaminálních hyperintenzit na MRI a s významně nižší hladinou beta-amyloidu v CSF, a že komorbidity nijak nesouvisí s polymorfismy na 129. kodonu *PRNP*.

Další výzkum neurodegenerativních chorob je nutný. Jedině syntéza neuropatologických, klinických, genetických a biochemických znaků může přispět k pochopení kauzálních vztahů jednotlivých neurodegenerativních chorob a jejich komorbidit, zpřesnit prognózu pacientů a případně ukázat nové alternativy terapie těchto onemocnění, s nimiž se vzhledem ke stárnutí populace setkáváme čím dál častěji.

6 SUMMARY

Neurodegenerative disorders are a common group of diseases. It is certain that total number of cases will increase in the future due to the aging of the human population. Although many facts have already been described and the outdated concept of unrelated units has changed into today's concept of frequent comorbid overlapping cases, a number of unexplained contexts remain.

Several micromorphological studies correlating genetic, immunological and clinical findings revealed a difference between archicocortical and neocortical plaques. There is a clear tendency for neocortical plaques to form bulky Aβ structures with minimal involvement of h-tau immunoreactive dystrophic neurites. In contrast, in archicocortical plaques, Aβ is only a minor component and dilated bulbous dystrophic neurites predominate. This tendency is more prominent in patients with AD in comorbidity with synucleinopathies, and we believe that in comorbid cases, i.e. cases with a faster course of the disease, the neuritic changes are just more preserved as is generally known, with the duration of the disease, the neuritic component „burns out“ gradually.

The second micromorphological study described plaque types in patients with comorbid CJD/AD. Although three basic groups of colocalizing or non-colocalizing pathological proteins forming extracellular plaques are repeatedly found in CNS, we were unable to trace any association with a polymorphism at codon 129 or stage of Alzheimer's disease. Nevertheless, the study shows a clear tendency of PrP^{Sc} to colocalize with fibrillar structures of Aβ, lacking a tendency for colocalization in dense parts of Aβ plaques.

There is minimal number of studies on a rare hereditary neurodegenerative disease Gerstmann–Sträussler–Scheinker syndrome. All cases found in the Czech Republic were processed into detailed neuropathological, molecular genetic and immunological study, including four related patients - all with the P102l mutation in the *PRNP* gene. Contrary to the general concept of GSS as a disease characterized by large multicentric plaques, we mostly found small spherical plaques with minimal response in their vicinity. This „low reactivity“ of PrP^{Sc} plaques may explain why it has never been successfully transferred to another laboratory organism, as is known from CJD cases.

Finally, a detailed retrospective study of all cases of CJD diagnosed in the Czech Republic in the last 10 years was able to demonstrate the effect of CJD comorbidities on the levels of biomarkers in cerebrospinal fluid and the CNS image on MRI. It was possible to prove

that patients with „pure“ CJD are, on average, significantly younger than patients with CJD in comorbidity; that CJD/tau cases are more likely to have putaminal hyperintensities and lower MTA score on MRI; that CJD/AD is associated with an older age of onset, a lower probability of developing putaminal hyperintensities on MRI, and a significantly lower level of beta-amyloid in the CSF; and that comorbidities have nothing to do with polymorphisms at the 129. codon of *PRNP* gene.

Further research of neurodegenerative diseases is still needed. Only the synthesis of all neuropathological, clinical, genetic and biochemical features can contribute to the understanding of the causal relationships of individual neurodegenerative diseases and their comorbidities, to refine the prognosis of patients and possibly to show new alternatives for the therapy of these diseases, which we encounter more and more often due to the population aging.

7 PŘEHLED PUBLIKAČNÍ A ODBORNÉ AKTIVITY

7.1 Publikace v recenzovaných časopisech s IF

Jankovska N, Olejar T, Kukal J, Matej R. Different Morphology of Neuritic Plaques in the Archicortex of Alzheimer's Disease with Comorbid Synucleinopathy: A Pilot Study. Curr Alzheimer Res. 2020;17(10):948-958. doi: 10.2174/1875692117999201215162043. PMID: 33327912. **IF 3,498**

Jankovska N, Olejar T, Matej R. Extracellular Amyloid Deposits in Alzheimer's and Creutzfeldt-Jakob Disease: Similar Behavior of Different Proteins? Int J Mol Sci. 2020 Dec 22;22(1):7. doi: 10.3390/ijms22010007. PMID: 33374972; PMCID: PMC7792617. **IF 5,924**

Jankovska N, Olejar T, Matej R. Extracellular Protein Aggregates Colocalization and Neuronal Dystrophy in Comorbid Alzheimer's and Creutzfeldt-Jakob Disease: A Micromorphological Pilot Study on 20 Brains. Int J Mol Sci. 2021 Feb 20;22(4):2099. doi: 10.3390/ijms22042099. PMID: 33672582; PMCID: PMC7924045. **IF 6,208**

Jankovska N, Matej R. Molecular Pathology of ALS: What We Currently Know and What Important Information Is Still Missing. Diagnostics (Basel). 2021 Jul 29;11(8):1365. doi: 10.3390/diagnostics11081365. PMID: 34441299; PMCID: PMC8391180. **IF 3,992**

Jankovska N, Rusina R, Bruzova M, Parobkova E, Olejar T, Matej R. Human Prion Disorders: Review of the Current Literature and a Twenty-Year Experience of the National Surveillance Center in the Czech Republic. Diagnostics (Basel). 2021 Oct 1;11(10):1821. doi: 10.3390/diagnostics11101821. PMID: 34679519; PMCID: PMC8534461. **IF 3,992**

Jankovska N, Matej R, Olejar T. Extracellular Prion Protein Aggregates in Nine Gerstmann-Sträussler-Scheinker Syndrome Subjects with Mutation P102L: A Micromorphological Study and Comparison with Literature Data. Int J Mol Sci. 2021 Dec 10;22(24):13303. doi: 10.3390/ijms222413303. PMID: 34948096; PMCID: PMC8704598. **IF 6,208**

Jankovska N, Rusina R, Keller J, Kukal J, Bruzova M, Parobkova E, Olejar T, Matej R. Biomarkers Analysis and Clinical Manifestations in Comorbid Creutzfeldt-Jakob Disease: A Retrospective Study in 215 Autopsy Cases. Biomedicines. 2022 Mar 16;10(3):680. doi: 10.3390/biomedicines10030680. PMID: 35327482; PMCID: PMC8944998. **IF 4,757**

7.2 Odborná sdělení na kongresech či seminářích

(1) Studentská vědecká konference 3. LF UK, listopad 2020, přednáška (10 min): Different Morphology of Neuritic Plaques in the Archicortex of Alzheimer Disease with Comorbid Synucleinopathy

(2) Studentská vědecká konference 3. LF UK, 25. 5. 2021, komentovaný poster: Extracellular Protein Aggregates Colocalization and Neuronal Dystrophy in Comorbid Alzheimer's and Creutzfeldt–Jakob Disease: A Micromorphological Pilot Study on 20 Brains.

(3) 33. evropský kongres patologie, 29. 8. 2021, komentovaný poster (15 min): Comorbid Alzheimer's and Creutzfeldt–Jakob Disease: Micromorphology of Colocalizing Extracellular Protein Aggregates Colocalization and Neuronal Dystrophy

(4) 46. sjezd českých patologů smezinárodní účastí, 25.-26. 11. 2021, 2x poster: (1) Extracellular Protein Aggregates Colocalization and Neuronal Dystrophy in Comorbid Alzheimer's and Creutzfeldt–Jakob Disease: A Micromorphological Pilot Study on 20 Brains. (2) A clinical-neuropathological retrospective study of 215 cases of Creutzfeldt–Jakobdisease found a surprisingly high number of comorbid neuropathologies. 1x přednáška: Extracelulární amyloidová depozita u Alzheimerovy a Creutzfeldtovy–Jakobovy choroby: Stejné chování různých proteinů?

7.3 Získaná ocenění

Lamblova cena za rok 2020

Cena za nejlepší původní práci v oboru patologie publikovanou v předchozím roce členem Společnosti českých patologů ČLS JEP ve věku do 35 let za práci: Jankovska N, Olejar T, Matej R. Extracellular Amyloid Deposits in Alzheimer's and Creutzfeldt-Jakob Disease: Similar Behavior of Different Proteins? Int J Mol Sci. 2020 Dec 22;22(1):7. doi: 10.3390/ijms22010007. PMID: 33374972; PMCID: PMC7792617. **IF 5,924**