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Rare copy number variation in extremely impulsively violent males

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The genetic correlates of extreme impulsive violence are poorly understood, and there have been no studies that have systematically characterized a large group of affected individuals both clinically and genetically. We performed a genome-wide rare copy number variant (CNV) analysis in 281 males from four Czech prisons who met strict clinical criteria for extreme impulsive vio-lence. Inclusion criteria included age ≥ 18 years, an ICD-10 diagnosis of Dissocial Personality Disorder, and the absence of an organic brain disorder. Participants underwent a structured psy-chiatric assessment to diagnose extreme impulsive violence and then provided a blood sample for genetic analysis. DNA was genotyped and CNVs were identified using Illumina Huma-nOmni2.5 single-nucleotide polymorphism array platform. Comparing with 10851 external pop-ulation controls, we identified 828 rare CNVs (frequency ≤ 0.1% among control samples) in 264 participants. The CNVs impacted 754 genes, with 124 genes impacted more than once (2-25 times). Many of these genes are associated with autosomal dominant or X-linked disorders affecting adult behavior, cognition, learning, intelligence, specifically expressed in the brain and relevant to synapses, neurodevelopment, neurodegeneration, obesity and neuropsychiatric phe-notypes. Specifically, we identified 31 CNVs of clinical relevance in 31 individuals, 59 likely clini-cally relevant CNVs in 49 individuals, and 17 recurrent CNVs in 65 individuals. Thus, 123 of

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281 (44%) individuals had one to several rare CNVs that were indirectly or directly relevant to impulsive violence. Extreme impulsive violence is genetically heterogeneous and genomic analysis is likely required to identify, further research and specifically treat the causes in affected individuals.

KEYWORDS

antisocial personality disorder, copy number variation, dissocial personality disorder, genetics, impulsive violence, rare variants

1 | INTRODUCTION

Violent behavior is a poorly understood evolutionary, biological and sociological phenomena.[1] It is a complex trait resulting from multiple genetic and environmental factors, and their interactions. Despite the growing impact of violent behavior on both the individual and society, there is a paucity of research in this area[2–4]; partly because research in violent behavior raises serious ethical and legal concerns.[5–9] In addition, most research has focused on the attribution of specific psychiatric disorders to individuals with violent behavior,[10] rather than pursuing a basic understanding of the underlying pathophysiology.

Research in this area is critical in order to better understand the pathophysiology of these disorders, classify them and provide treatment to affected individuals. Over the last few decades developments in cognitive neuroscience and behavioral genetics have provided new insights into the nature of violent and criminal behavior by identifying genetic, gender-dependent, physiological, biochemical and neural correlates of impulsivity, violence, and related personality disorders.[2,11–13]

Twin and family studies have shown that violence, particularly irritable/impulsive violence, has substantial heritability, ranging from 44%-72%,[14–16] and these strong heritability estimates have stimulated genetic investigations.[17]

Linkage studies led to the identification of a causal mutation in the gene encoding X-linked monoamine oxidase A (MAOA),[18,19] with confirmation in other families.[20] Experimental validation of MAOA function on behavioral patterns was found in animal models[21] as well as in association studies in humans[22,23]; for review see.[24,25]

Candidate gene-based research focusing on the determination of a relationship between aggressive behavior and sex-determining and sex-specific genes and common allelic variants in genes involved in neurotransmission, hormone regulation, drug metabolism, neurodevelopment, neuroadaptation and regulation of hypothalamic-pituitary-adrenocortical axis has been mostly inconclusive.[2,24,26] Several genome-wide association studies on aggressive traits have failed to consistently identify genes associated with violent behavior.[24]

Targeted resequencing and a search for rare variants in 14 candidate genes involved in the dopamine and serotonin pathways in a cohort of 228 extremely violent Finnish prisoners showed a premature stop codon variant of serotonin receptor 2B (HTR2B). This variant was significantly over-represented in cases compared with the control Finnish population (7.5% vs 1%; odds ratio 3.1; 95% confidence interval 1.3-7.7; P = 0.01). The variant cosegregated with impulsivity in multiple families and the phenotype was recapitulated in $Htr2b^{-/-}$ mice.[27]

Aggression and impulsivity have also been reported in several individuals with large genomic rearrangements (copy number variations [CNVs]) in chromosomal regions 1q21.1.,[28,29] 15q13.3[29–32] and 16p13.11[33,34] and 16q22.2-q23.1.[29] These regions contain genes that affect neurobiological pathways, suggesting that haploinsufficiency of genes acting in these pathways may predispose to aggressive and impulsive behavior,[29,35] as well as to other neuropsychiatric disorders.[36,37]

Aggression and impulsivity may also be linked to the effect of de novo rare genetic variants. This theory is supported by a study showing that children born to men older than 60 were more likely to be convicted of violent crimes than children born to men aged 40 to 60 years.[38] The number of de novo variants increases with advanced paternal age,[39,40] and de novo variants have been shown to be a major cause of neuropsychiatric disorders.[41,42]

Research in this area has been hampered by a limited ability to holistically phenotype violent individuals, to subcategorize endophenotypes, to collect sufficient cohorts of psychiatrically and psychologically well assessed individuals and to obtain biological materials for genetic studies. Moreover, no single work has yet systematically studied the greater spectrum of allelic variation from sequence-level to copy number variation (CNV), in individuals with this phenotype.

In order to assess the association between impulsive violence and rare chromosomal abnormalities and CNV changes, we performed whole genome genotyping and exome sequencing in over 300 extremely impulsively violent males who underwent a standardized and detailed clinical characterization and met standard diagnostic criteria for dissocial personality disorder (DPD). It was our intent to enrich the population for individuals who might have a genetic rather than environmental basis for their violence.

2 | MATERIALS AND METHODS

2.1 | Subjects

The study received approval by the Ethical Committee of the First Psychiatric Clinic, Prague and General University Hospital in Prague. Subjects were recruited between June, 2011 and March, 2016 from four high security male prisons, two of which have specialized program for prisoners with personality disorders. A stringent recruitment protocol was developed. First, we selected individuals who, based upon legal proceedings, had been convicted of at least two violent attacks (ie, bodily harm, robbery, murder or attempted murder) and met standard diagnostic criteria for DPD. These violent acts were impulsive and without pre-meditation. Individuals were not included in the study if the violent acts were the result of defensive aggression (ie, response to persistent violent bullying or actions performed in order to avoid capture or bodily harm). Other inclusion criteria were age > 18 years and the absence of an organic brain disorder. The psychiatrist (J.V.) then carried out 55 prison visits to review prisoner records and determine whether the subject also met the standard diagnostic criteria for DPD. The term DPD is not used in the US Diagnostic and Statistical Manual, which uses the diagnosis of antisocial personality disorder for a similar cluster of symptoms. Individuals with DPD have high levels of impulsivity, high negative emotionality and low conscientiousness and associated behaviors, including irresponsibility and violence. The diagnosis of DPD was made only after a detailed discussion of each subject with the first author and the psychologists.

Using this procedure psychologists screened the records of 6390 individuals and invited 488 inmates to participate in the study. Thirty-four subjects declined participation, with the remaining 454 participants signing a written informed consent once the nature and aims of the study were fully explained. The investigators confirmed with the prison staff and related to the prisoners that there would be no other benefits to participation and that nonparticipation would not be associated with the loss of any benefits or standing. This was routinely reinforced and monitored by the study staff.

A board-certified psychiatrist (J.V.) assessed all 454 invited participants using the following methods: (a) the Eysenck Impulsivity Inventory for assessment of personality traits of impulsivity, venturesomeness and empathy (IVE), which is a classical trait marker that has been validated in the Czech population; (b) the Mini-International Neuropsychiatric Interview—M.I.N.I., version 5.0.0 (MINI 5.0) using the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) criteria[43]; (c) the Hare Psychopathy Checklist-Revised (PCL-R); (d) the Childhood Experience of Care and Abuse questionnaire (CECA.Q; part 5 and 6, concerning physical punishment before age 17 and unwanted sexual experiences.[44] The CECA.Q was read to all participants during interviews to improve the accuracy of the fixed category responses.[45] Individuals were considered to be impulsive if they scored ≥8 on the Eysenck IVE Impulsivity scale, which indicates increased impulsivity based on a Czech normative study.[46] The principal reason for using Eysenck's test was that it provides quantified data on impulsivity. Individuals who did not complete the entire battery of questionnaires or who did not cooperate with the Eysenck test were also included in the study if they met the following criteria: (a) after MINI interview with subjects they scored a maximal impulsivity score of 2 on the PCL-R, and (b) after extensive review of relevant personal prisoner files and psychiatric assessment by the first author of the study, the team of psychologists and psychiatrists reached agreement on the impulsive nature of the subject's repetitive violence. The review procedures were identical for all individuals. The nationality and ethnicity of the participants were determined by self-identification.

2.2 | DNA isolation

Genomic DNA was extracted from whole blood samples using the Qiagen DNA micro kit (QIAgen, Hilden, Germany). The quantity and quality of the isolated DNA was verified using the NanoDrop 2000 (Thermo Fisher Scientific, Praha, Czech Republic).

2.3 | Microarray genotyping, CNV detection and gene content annotation

Genotyping was performed using the Illumina Human Omni2.5 SNP array platform (Illumina, San Diego, California) at The Microarray Facility of The Centre of Applied Genomics of The Hospital for Sick Children in Toronto as described.[47] Relevant microarray data were deposited in the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under the accession GSE116022. Quality control procedures and CNV calling were performed as previously described.[47,48] In brief, three CNV calling algorithms were used for both cases and controls: iPattern,[49] PennCNV,[50] and QuantiSNP.[51] We then analyzed "stringent" CNVs detected by at least two methods. Detecting large CNVs particularly sex chromosomal aneuploidies is challenging.[48,52] In addition, these large CNVs are sometimes fragmented. If large CNVs were found, they were merged and their identity confirmed by examining the probe density and "B" allele frequencies in the region. The annotation pipeline at The Centre for Applied Genomics was used for annotating CNVs. The genomic coordinates used are based on Human Genome Build GRCh37/hg19.

2.4 | Controls and rare CNV detection

Comparisons were made with external population controls comprised of 10 851 samples genotyped on different microarray platforms.[52] This includes 2884 samples genotyped on Illumina Human Omni2.5 SNP

array platform (platform-matched controls) from the KORA (Cooperative Health Research in the Region of Augsburg; n = 1775) and COGEND (Collaborative Genetic Study of Nicotine Dependence; n = 1109) cohorts. Ethnically matched population controls were available from the database of genomics variants maintained by the Czech National Center for Medical Genomics (n = 468) (http://ncmg.cz/en) and individuals investigated for other nonrelated clinical phenotype; (n = 86).[53] The control Roma population (n = 200) consisted of healthy individuals who were mostly parents and biologically unrelated family members of families who were studied for rare Roma-specific genetic diseases of pediatric onset.[54,55] These individuals self-identified to be of Roma ancestry as part of an assessment by the Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University, Prague and Department of Pediatrics and Adolescent Medicine, Children's Faculty Hospital, Košice. We defined rare CNVs as having a frequency ≤ 0.1% among control samples, using a 50% reciprocal overlap strategy as previously described.[47,48,56] We additionally restricted the rare variants to: (a) those with ≤0.1% frequency among variants in cases, (b) those with no identical CNV (using 50% reciprocal overlap strategy) in ethnically matched controls, and (c) overlap with no clusters of CNVs in Database of Genomic Variants (DGV)[57] controls. We also removed those with less than 75% overlap with copy number stable regions, according to our stringent CNV map of the human genome.[58] CNVs with an overlap of >75% with a structurally unstable region including segmental duplications and centromeres of human genome were also excluded. We preferentially investigated CNVs >10 kb and covered by ≥10 consecutive probes. However, if clinically relevant, we also considered CNVs <10 kb.

2.5 | Ancestry determination

The ancestry of the cases were determined from genotype data obtained from the HumanOmni2.5 microarray and whole exome sequencing as previously described.[47,59]

2.6 | Global burden, gene-set and gene network analyses of rare CNVs and prioritization of candidate genes

We performed a global burden of rare CNVs using two metrics: (a) the total length of rare CNVs per subject, and (b) the number of genes per participant with at least one exon partially overlapped by rare CNVs using in house scripts in R[60] (Supplementary Information Appendix S1: R-scripts). We stratified CNVs to different bins based on their length: 20 to 100 kb, 100 to 500 kb, and those >500 kb. Population stratification based on principle component analysis of single nucleotide polymorphism data extracted using the open-source whole genome association analysis toolset PLINK[61] were used as covariates. We also performed burden analysis on 34 gene sets[56,62,63] representing those with function in human brain or human orthologs of mouse genes implicated in nervous system and behavioral phenotypes using the general linear model function. Gene set analysis was performed and candidate genes were prioritized through functional annotation tools available in DAVID (Database for Annotation, Visualization, and Integrated Discovery) 6.8,[64] TOPPGENE suit database,[65] Genopedia,[66] Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA)[67] and by individual expert evaluation. Gene network analysis was performed using the Cytoscape.[68]

2.7 | Validation of prioritized variants by alternative genotyping methods

Existence of selected CNVs was independently assessed either in exome sequence data that were available for each of the participants (DNA fragmentation, sequencing library preparation, library sequencing and CNV variant detection and annotation were performed as described[59]) and/or validated and genotyped using a Universal Probe Library copy number assays using the LightCycler 480 Instrument (Roche Life Science, Prague, Czech Republic).

3 | RESULTS

3.1 | Clinical characterization of subjects

In total, 313 of 454 (69%) participants were included in the study. (Figure 1).

The mean age of the participants was 31.5 years (SD = 11,6; range 18-70 years). Substance abuse was diagnosed in 268 (86%) participants (96 alcohol and 172 illegal drugs, mostly methamphetamine). Suicidal attempt was reported by 75 (24%) participants. The majority of participants (70%) completed an elementary school education, 1% did not finish elementary school, 24% graduated from a secondary technical school and 5% from a secondary general school. None of the participants had obtained a university degree. Participants had been convicted of a mean of 8.6 criminal offenses (SD = 5,1; range 2-27), with 134 participants (43%) reported a history of criminal conviction in their relatives. Fifty-two (17%) reported a history of conviction in their fathers, 2 (1%) in their mothers, 25 (8%) in a sibling, and 39 (12%) in a sibling and either in a father or mother. Sixteen participants (5%) reporting a history of criminal conviction in other relatives. Childhood maltreatment was reported by 90 (29%), physical abuse by 80 (26%), and sexual victimization by 18 (6%) of participants. Descriptive statistics of PCL and IVE are provided in Table 1.

3.2 | Quality control of CNV detection and population stratification of participants

From 313 samples, 286 were genotyped and 281 passed stringent quality control for CNV detection (Figure 1).[49] The Czech Republic has a population of 10.5 million people. The largest ethnic group comprised of individuals of North-Central European ancestry. The second largest ethnic group, with estimated 245 800 individuals (2.3% of the total population) is of Roma descent. Fifty-seven participants self-identified as having Roma ancestry at the assessment. Using principal component analysis, we identified 161 individuals (57%) as of North-Central European ancestry and 120 individuals (43%) as of Roma ancestry (Figure 2).

3.3 | The analysis identified 828 rare CNVs in 264 participants

Two participants (6998 and 7498) had duplication of chromosome X (eg, 47,XXY). At least one rare CNV fulfilling the selected criteria was

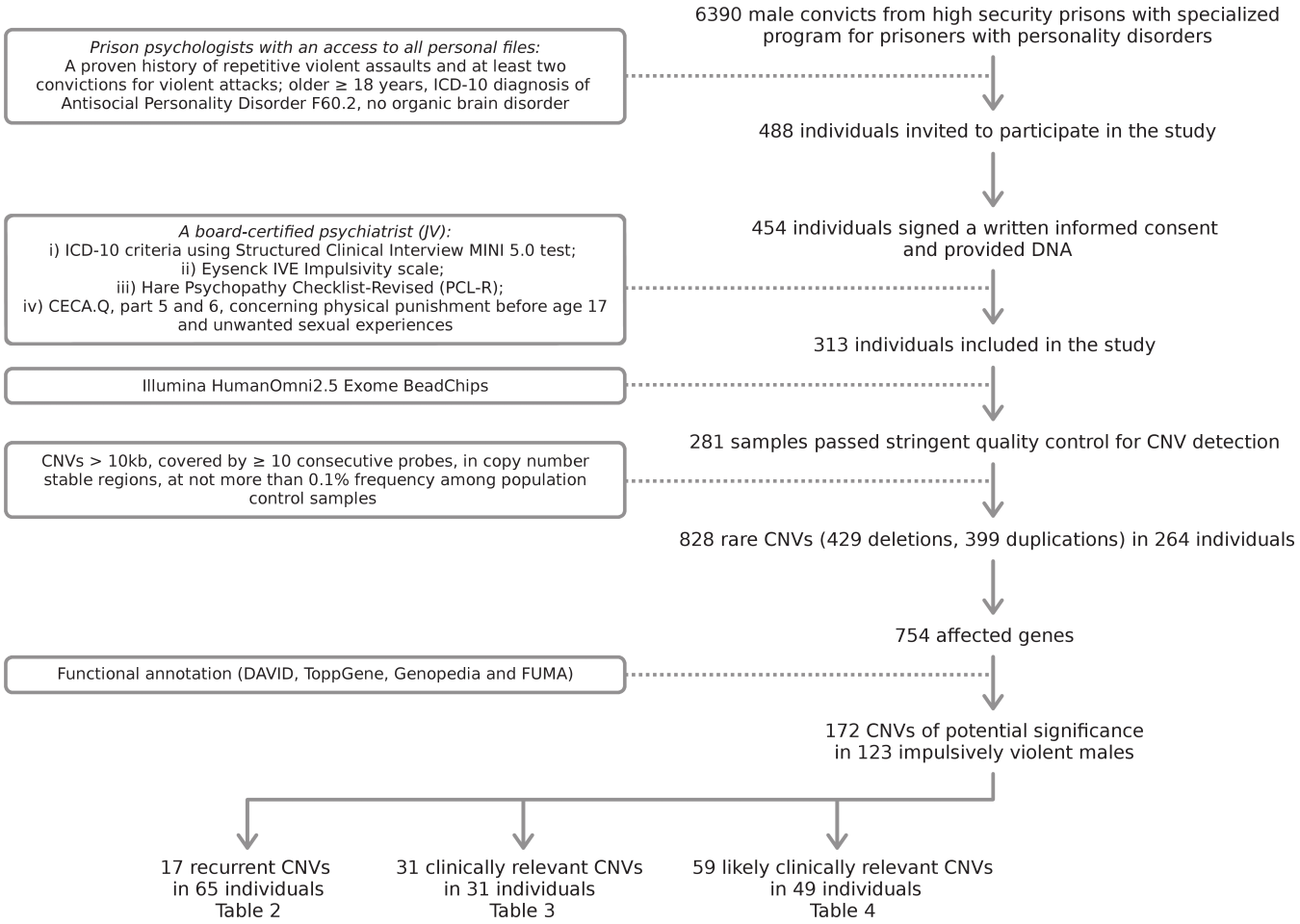

FIGURE 1 Work flow diagram and summary of results

TABLE 1 Descriptive statistics of 313 males with an ICD-10 diagnosis of DPD

	N	Mean	Minimum	Maximum	SD	Cronbach´s alpha
PCL	313	28.59	4	40	7.01	0.84
Scale 1 interpersonal	313	4.55	0	8	2.49	
Scale 2 affective	313	5.92	0	8	2.12	
Scale 3 life style	313	8.10	0	10	1.90	
Scale 4 antisocial	313	7.34	1	10	2.27	
IVE						0.71
Impulsivity	272	13.13	8	19	2.83	
Empathy	272	11.90	3	19	3.50	
Venturesomeness	272	10.80	1	16	3.33	

Abbreviations: IVE, Eysenck IVE impulsivity scale; PCL, hare psychopathy checklist.
Cronbach's alpha quantifies the internal consistencies of corresponding tests.
Cronbach's alpha ≥0.7 is considered acceptable and ≥0.8 good.

identified in 264 (94%) of 281 participants. This represents in total 828 rare CNVs (429 deletions, 399 duplications) (Supplementary Information Appendix S1: list "all CNVs"). Distribution of CNV numbers and CNV amounts per participant are shown in (Figure 3). From 828 identified CNVs, 533 were genic (eg, impacting exons, untranslated regions and introns of a gene) with 251 deletions and 282 duplications. There were 420 CNV_S impacting protein coding exons, with 159 deletions and 261 duplications. Identified C^NVs impacted 754 different genes (Supplementary Information Appendix S1: list "genes") with 124 genes impacted more than once (2-25 times), (Figure 3).

3.4 | Rare CNV burden, gene set and interactome analysis and functional annotation of rare CNVs identified in impulsively violent males showed over-representation of disrupted genes in several functionally relevant categories

We stratified CNVs into three categories: 20 to 100 kb, 100 to 500 kb and >500 kb, compared global CNV burden against platform-matched male controls (KORA + COGEND) and found no significant differences in the size and number of genes impacted by rare CNVs. For gene-set enrichment, we tested neuro-sets published before[56,62,63], but none met statistical significance. We functionally annotated the resulting set of 754 genes impacted by rare CNVs using several gene list annotation programs, including DAVID, TOPPGENE, Genopedia and FUMA (see Methods). This analysis identified 97 genes in 87 individuals with established links to Online Mendelian Inheritance in Man (OMIM) database, from which 33 genes are associated with an autosomal dominant (AD) and five genes with X-linked transmitted diseases (Supplementary Information Appendix S1: list "OMIM

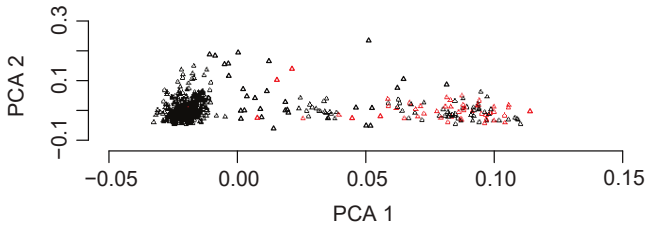

FIGURE 2 Principal component analysis of participants included in this study. Red triangles denote individuals who identified themselves to be of Roma ancestry at the assessment. Black triangles denote other participants. This analysis identified 161 individuals of North-Central European ancestry and 120 individuals as of Roma origin

genes"). The analysis further identified over-representation of genes impacted by rare CNVs in several functional categories. The most over-represented genes in the category of cellular components were found in a gene sets related to synapses (GO:0045202), neurons (GO:0097458), mitochondria (GO:0005739) and the RNA polymerase III complex (GO:0005666) and ionotrophic glutamate receptor. In the category of biological processes, the most over-represented genes were found in a gene sets related to synaptic signaling (GO:0099536), regulation of ion transport (GO:0043269), cell to cell signaling (GO:0007267), regulation of dendrite development (GO:0050773), behavior (GO:0044708) and neurogenesis (GO:0022008). In the categories of gene sets associated with human phenotypes through genome-wide association studies (GWAS), the most over-represented genes were in a category related to obesity, intelligence, schizophrenia, neuroticism, bipolar disorder, autism and cognitive decline (Figure 4A; Supplementary Information Appendix S1: "GWAS hits"). Interactome analysis showed 12 groups of interacting gene clusters that are relevant to impulse control, neurodevelopment and neurodegeneration like synaptic protein interactions, ionotrophic glutamate receptor activity, transmission of nerve impulses and synaptic vesicle cycle, vesicle-mediated transport, axon guidance and cell adhesion, clathrin-mediated endocytosis, Rab-regulation of trafficking, nervous system development, DNA replication, RNA polymerase transcription, and glycosylphosphatidylinositol-mediated membrane protein anchoring (Figure 4B). Genes impacted by CNVs were overexpressed in brain and specific brain regions compare with other tissues, with some of the genes demonstrating brain-specific expression patterns (Figure 5).

3.5 | Clinical and biological interpretation of genic CNVs defined 31 clinically relevant CNVs and 142 likely clinically relevant CNVs in 123 impulsively violent males

To assess the clinical relevance of individual genes impacted by CNVs, we considered their clinical associations, biological function, the patterns of gene expression obtained from The Genotype-Tissue Expression Project Portal (GTEx),[69] genic intolerance to deletions and duplications[70] and loss of function mutations[71] (Supplementary Information Appendix S1: list "constrains and expression"), presence in

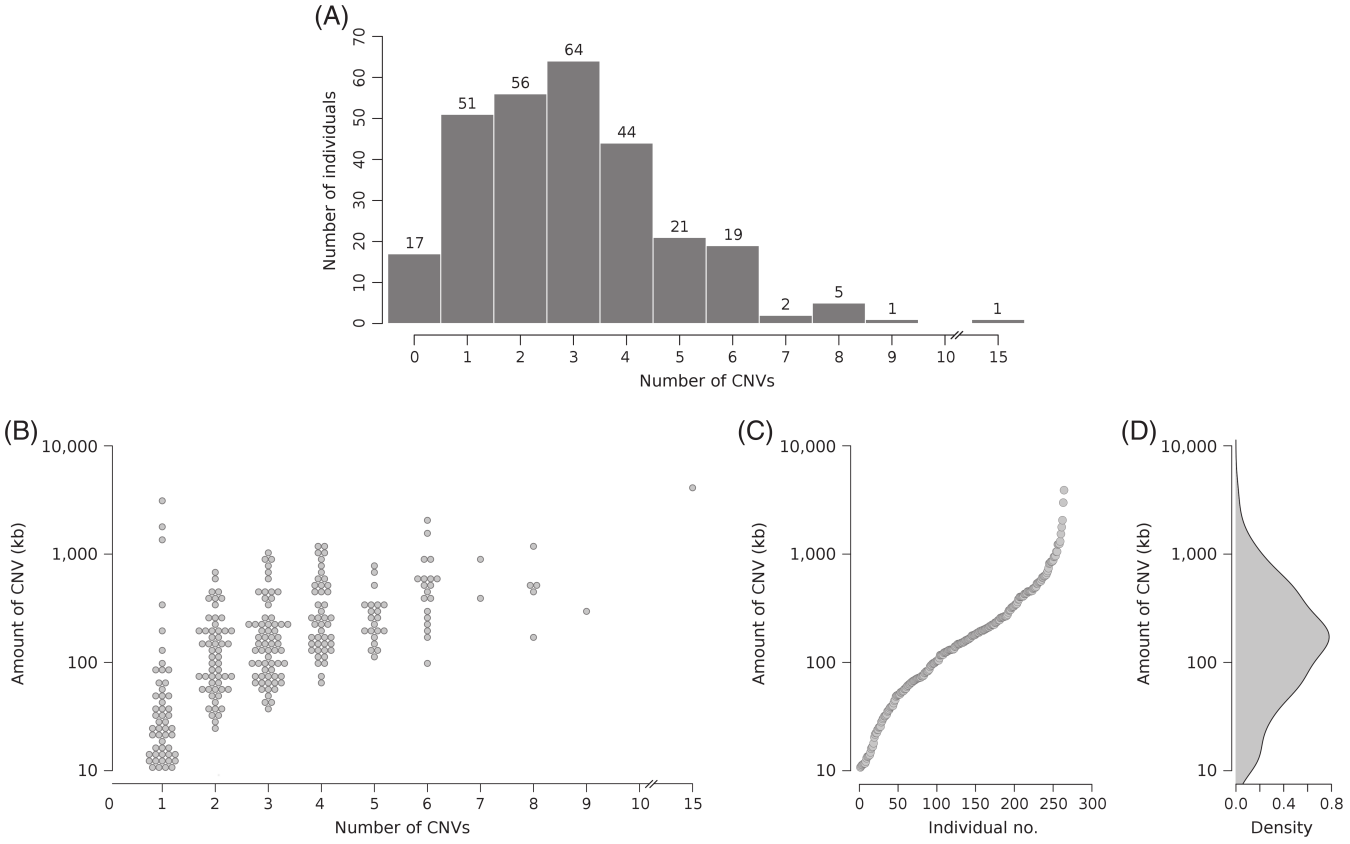

FIGURE 3 Distribution of CNV numbers and CNV amounts in 281 impulsively violent individuals. (A) Distribution of CNV numbers, (x-axis), per individual; (B) Distribution of CNV numbers (x-axis), and total CNV amounts in bp, (y-axis), per individual. (C) Distribution of total CNV amounts in bp across individuals. (D) Density plot of CNV amount per individual

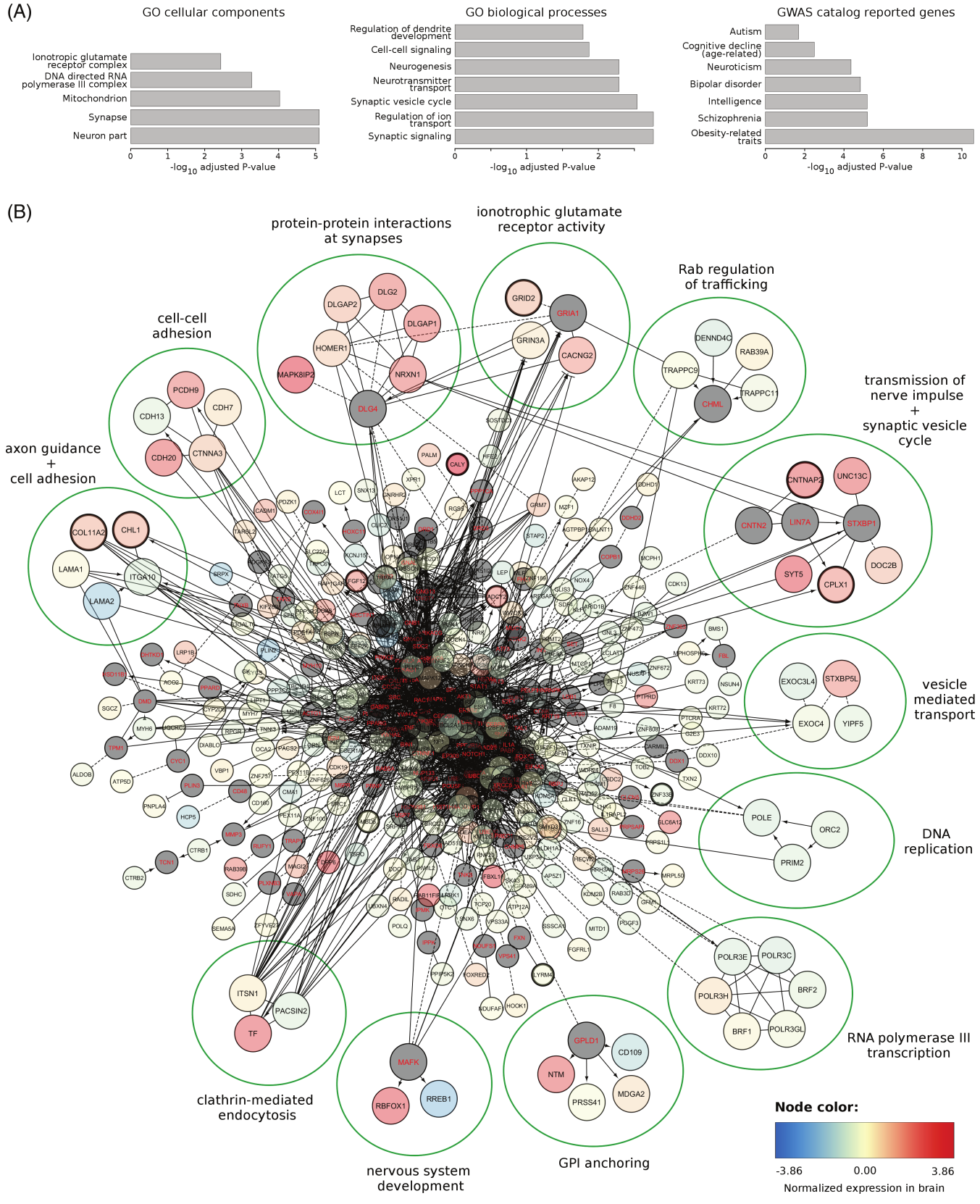

FIGURE 4 Gene set over-representation and gene network analysis of 754 genes impacted by rare CNVs identified in 264 individuals with an ICD-10 diagnosis of dissocial personality disorder. (A) Significant over-representation of impacted genes in the categories of cellular components, biological processes and those associated with human phenotypes through genome-wide association studies. Hypergeometric tests were performed to test if genes of interest are overrepresented in any of the pre-defined gene sets; multiple test correction (Benjamini-Hochberg) was performed per category in FUMA reported gene sets with adjusted P value ≤0.05. (B) Functional interaction network of genes impacted by rare CNVs showing groups of interacting gene clusters relevant to impulse control, neurodevelopment and neurodegeneration. Identified clusters (enlarged nodes in green ellipses) were annotated by Gene Onthology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome

regulatory regions defined by the Encyclopedia of DNA Elements (ENCODE)[72] and the recurrent presence of similar CNVs in individuals with neurobehavioral phenotypes reported in the Database of Genomic Variation and Phenotype in Humans (DECIPHER).[73] This analysis defined 31 CNVs as clinically relevant and 142 CNVs as likely clinically relevant (Figure 1). Eighty-four individuals had one, 31 individuals had two, 6 individuals had three and 2 individuals had four of these rare potentially clinically significant CNVs.

3.6 | Analysis of recurrent rare CNVs identified three males with a microduplication impacting MBD5 and suggested association of impulsive violence with an intragenic deletion of CHL1

Seventeen CNVs classified as clinically relevant or likely clinically relevant were recurrently detected in 65 participants (Table 2). Forty-nine individuals had one, 12 individuals had two, and 4 individuals had three of these CNVs. With the exception of a microduplication impacting CALY, all of these recurrent variants were identified in participants of Roma origin. To determine if these recurrent CNVs represented rare alterations associated with impulsive violence or common variants of Roma population, we used quantitative polymerase chain reaction (qPCR) and genotyped all these alterations in 90 to 180 Roma control samples.

In three individuals, we identified a microduplication impacting MBD5, which encodes Methyl-CpG-Binding Domain 5 (MBD5) protein. MBD5 mutations result in a syndrome (MIM 156200) characterized by AD intellectual disability and a broad range of neurodevelopmental symptoms including aggressive behavior.[159]

In 13 individuals, we identified likely clinically relevant deletions in intron 1 of CHL1, which encodes Cell adhesion molecule L1-like protein (CHL1). CHL1 is a modulator of the serotonergic system; mutations in this gene have been associated with deficits in behavior, cognition and social interactions.[74]

The MBD5 and CHL1 recurrent CNVs did not occur in participants who were directly related. The microduplication in MBD5 did not occur in controls, and the CHL1 deletion was significantly overrepresented compared with controls (Fisher's exact test (FET) two-tailed, $P = 0.038$).

The other 15 recurrent functionally relevant CNVs included: (a) a microduplication of CPLX1, a gene that modulates neurotransmitter release and is a candidate gene for Wolf-Hirschhorn syndrome[75]; (b) a microduplication of NF1, mutations of which are often associated with learning disabilities or behavioral problems[76]; (c) an intragenic deletion of CNTNAP2, a gene that may affect brain development and acquisition of higher cognitive functions[77]; (d) an intragenic deletion FGF12, mutations of which are associated with AD early-onset epileptic encephalopathy with cerebellar atrophy (MIM 617166)[78]; (e) a deletion of brain-specifically expressed TMEM235; (f) a microduplication affecting exon2 of ADCY2, a gene that is associated with bipolar disorder[79]; (g) a microduplication of exons 3-6 of CALY that encodes calcyon, a protein implicated in various neuropsychiatric disorders including schizophrenia, bipolar disorder, attention-deficit hyperactivity disorder and drug dependence[80]; (h) a duplication of exons 39-64 of COL11A2, a gene in which mutations cause the AD connective tis-sue disorder Stickler syndrome (MIM 184840), and rare de novo CNVs have been also found in Finnish families with autism spectrum disor-ders[81]; (i) a microdeletions in GRID2 encoding the glutamate receptor channel delta-2 subunit (GRID2), variations of which has been associ-ated with obsessive-compulsive disorder[107] and AD adult-onset, slowly progressive, cerebellar ataxia[82]; (j) a deletion of ZNF385D, mutations of which have been associated with reading disability (RD) and language impairment[83] and (k) a duplication of FAAH encod-ing fatty acid hydrolase (FAAH) which is known to have a role in medi-ating responses to stress, including stress-associated behavior and variations of which has been associated with susceptibility to drug addiction (MIM 602935). The qPCR genotyping, however, showed that all these CNVs were found in similar frequencies in Roma controls and thus probably represent population-specific rare polymorphisms whose clinical significance is unclear.

3.7 | Clinically relevant CNVs identified in 31 individuals

Thirty-one individuals each had one unique clinically relevant CNV. In addition to two individuals with Klinefelter syndrome (eg, duplication of chromosome X; 47,XXY) and three individuals with recurrent microduplication impacting MBD5 we identified 26 clinically relevant CNVs in 26 other individuals (Table 3).

There were five CNVs that overlapped in length with CNVs in DECIPHER and ClinGen that were identified in multiple individuals with intellectual disability and behavioral abnormalities: (a) a 600-kb deletion (chr16:21839340-22440319) encompassing 10 genes (EEF2K; CDR2; RRN3P3; POLR3E; NPIPB4; C16orf52; MFSD13B; UQCRC2; PDZD9 and VWA3A) affecting EEF2K (which encodes postsynaptic eukaryotic elongation factor 2 kinase and balance inhibitory and excitatory synaptic transmission[84];) and CDR2 (which encodes cerebellar degeneration related protein 2, a major candidate gene[85] of the 6p12.2 microdeletion syndrome [OMIM 136570]); (b) a 3.5 Mb duplication (chr22:41887922-45396440) that impacts 70 genes some of which are implicated in autism (TCF20), steroid-dependent stress and anxiety (TSPO), drug metabolism (CYP2D6), synaptic transmission (PACSIN2), neuronal homeostasis (MPPED1), with others specifically expressed in brain (LINC00634, SHISA8); (c) a 3-Mb duplication (chr11:23211700-26188592), affecting LUZ2P, a brain-specific protein of unknown function; (d) a 1.7 Mb duplication (chr19:27791257-29562474) affecting several noncoding RNAs and genes (LINC01532; LOC102724908; LOC100420587; LINC00906; LOC101927151; LOC102724958; and LINC00662) whose function is unknown; and (e) a 1.3-Mb duplication (chr12:127396986-128

pathway terms. Node colors correspond to the relative expression of a given gene in brain normalized to other tissues (see the inset). Black gene symbols represent genes impacted by CNVs, red gene symbols represent linker genes (not impacted by CNVs). The nodes with twice increased width of the border represent genes that were recurrently impacted by particular CNV (see Table 2). Edges are displayed as "- > "for activating/catalyzing, "-|" for inhibition, "-" for functional interactions (FIs) extracted from complexes or inputs, and "---" for predicted FIs

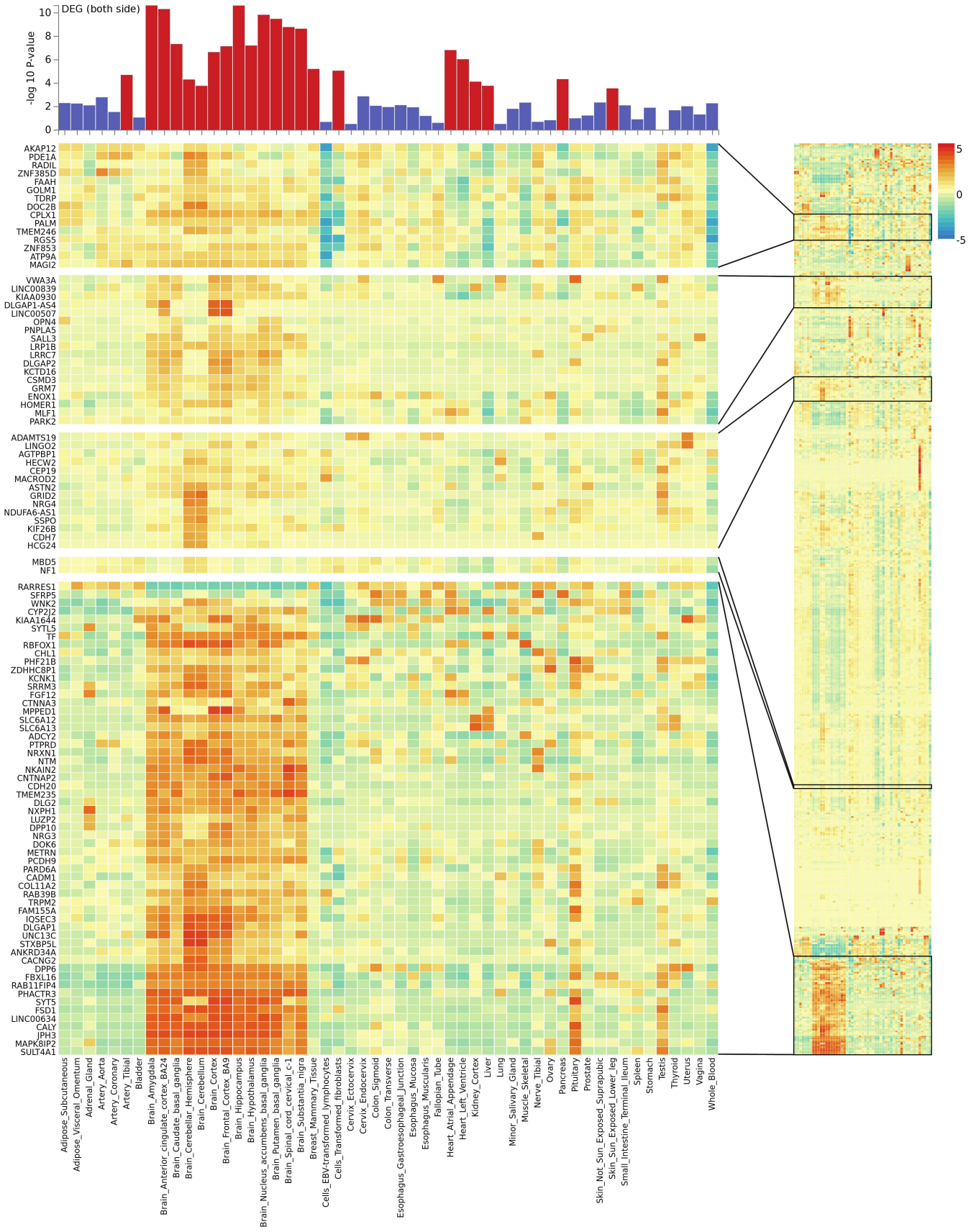

FIGURE 5 Expression patterns generated in FUMA of 754 genes impacted by CNVs identified in 264 individuals with an ICD-10 diagnosis of dissocial personality disorder. Heat map representation of expression patterns of all impacted genes across 53 tissue types based on GTEx v7 RNA-seq data is shown on the right. Genes that are specifically expressed in brain and/or specific brain regions are shown in detail on the left. Tissues significantly enriched for impacted genes (DEG; sets of genes which are more (or less) expressed in a specific tissue compared to other tissue types) at Bonferroni corrected P-value ≤0.05 are highlighted in red on the top. The −log10(P values) in the graph refer to the probability of the hypergeometric test

TABLE 2 Recurrent CNVs of potential significance identified in 65 males with an ICD-10 diagnosis of DPD and their frequencies in controls

Chr	Start	End	Size	Type	Candidate gene	Clinical associations	Ethnicity	n cases	n ctrls	P-value	% all ext. Controls	% all male ext. Controls	% PM ext. Controls	% PM male ext. Controls
chr2	148 943 206	149 091 754	148 549	Dup	MBD5	Mental retardation, autosomal dominant 1 (MIM 156200)	R	3/120	0/180	0.063	0.000	0.000	0.000	0.000
chr3	269 208	283 807	14 600	Del	CHL1	Nonspecific mental retardation, modulator of the serotonergic system	R	13/120	8/181	0.038	0.009	0.020	0.000	0.000
chr4	759 335	784 546	25 212	Dup	CPLX1	Essential for normal performance of complex behaviors	R	12/120	12/56	Ns	0.028	0.040	0.069	0.152
chr17	29 692 316	29 727 873	35 558	Dup	NF1	Learning disabilities or behavioral problems	R	8/120	7/181	Ns	0.000	0.000	0.000	0.000
chr7	146 334 587	146 371 099	36 513	Del	CNTNAP2	Susceptibility gene implicated in multiple complex neurodevelopmental disorders, including autism, intellectual disability, and schizophrenia	R	7/120	10/91	Ns	0.009	0.020	0.000	0.000
chr3	192 247 712	192 266 586	18 875	Del	FGF12	Epileptic encephalopathy, early infantile 47 autosomal dominant (MIM 617166)	R	6/120	4/90	Ns	0.000	0.000	0.000	0.000
chr11	89 959 903	89 986 667	26 765	Dup	DISC1FP1	A susceptibility gene for major psychiatric disorders	R	5/120	6/181	Ns	0.000	0.000	0.000	0.000
chr17	76 219 646	76 239 564	19 919	Del	TMEM235	Specifically expressed in brain, function unknown	R	5/120	3/181	Ns	0.000	0.000	0.000	0.000
chr5	7 412 827	7 472 191	59 365	Dup	ADCY2	Association with ADHD and bipolar disorder	R	4/120	2/182	Ns	0.055	0.100	0.069	0.152
chr3	21 680 527	21 732 347	51 821	Del	ZNF385D	Associated with reading disability and language impairment	R	4/120	4/180	Ns	0.000	0.000	0.000	0.000
chr1	46 812 747	46 961 781	149 035	Dup	FAAH	Susceptibility to drug addiction; increased levels of FAAH activity are involved in the deficits in social behavior (MIM 602935)	R	4/120	4/180	Ns	0.000	0.000	0.000	0.000
chr6	33 114 647	33 139 662	25 016	Dup	COL11A2	Otospondylomegaepiphyseal dysplasia, autosomal dominant; Stickler syndrome type III (MIM 184840)	R	3/120	1/159	Ns	0.018	0.040	0.035	0.076
chr10	135 129 012	135 141 572	12 561	Dup	CALY	Calcyon; role in neuronal development, synaptic function, and neurodegeneration	C	2/161	Nd	Ns	0.009	0.020	0.035	0.076
chr6	5 242 102	5 393 392	151 291	Del	FARS2, LYRM4	Associated with cognitive deficit in schizophrenia	R	2/120	3/90	Ns	0.018	0.020	0.035	0.000
chr4	93 233 136	93 302 533	69 398	Del	GRID2	Associated with obsessive-compulsive	R	2/120	2/90	Ns	0.000	0.000	0.000	0.000

TABLE 2 (Continued)

Chr	Start	End	Size	Type	Candidate gene	Clinical associations	Ethnicity	n cases	n ctrls	P-value	% all ext. Controls	% all male ext. Controls	% PM ext. Controls	% PM male ext. Controls
						disorder; GRID2 mutations span from congenital to mild adult-onset cerebellar ataxia.								
chr10	42 680 389	43 374 443	694 055	Dup	LINC00839, ZNF33B	Expressed in brain, molecular function is unknown	R	2/120	Nd	Na	0.028	0.040	0.035	0.076
chr16	16 363 239	16 682 080	318 842	Dup	NPIPA8, NPIPA7	Expressed in brain, 16p13.11 recurrent microduplication neurocognitive disorder susceptibility locus	R	2/120	Nd	Na	0.018	0	0	0

Abbreviations: ADHD, attention-deficit hyperactivity disorder; R, C-individuals of Roma or Caucasians origin; n cases and controls—number of individuals with particular variant/number of individuals from the corresponding population; na, not available; nd, not done; Ns, not significant; P-value, Fisher exact test statistic value for differences in CNV frequencies between cases and controls; frequencies of CNVs in all external controls (n = 10851), external control males, and platform-matched (PM) external controls (n = 2884) and male external controls are provided.

705 029) affecting several noncoding RNAs including LINC00507, which is specifically and age-dependently expressed in brain, suggesting it may be involved in the brain development of higher primates.[86]

There were 18 CNVs that affected genes definitively associated with AD neurologic phenotypes. These include: (a) a duplication impacting the open reading frame of KIF26B, which is associated with AD cerebellar ataxia[87] (OMIM 614026); (b) a contiguous deletion of XPR1 associated with AD basal ganglia calcification[88] (OMIM 605237) and LHX4 associated with AD combined pituitary hormone deficiency type 4 (MIM 262700); (c) a duplication impacting RAB39B and CLIC2 that was found in individuals with X-linked intellectual and developmental disability[89,90]; (d) a duplication impacting the open reading frame of KMT2C that has been associated with AD Kleefstra syndrome 2 (606833) and neurodevelopmental phenotypes[91,92]; (e) a duplication impacting the open reading frame of CACNG2 associated with AD mental retardation 10 (OMIM 602911) and nonsyndromic intellectual disability[93]; (f) a duplication impacting the open reading frame of MTOR associated with Smith-Kingsmore syndrome (OMIM 616638)[94]; (g) a duplication impacting the open reading frame of CDK13 associated with AD intellectual developmental disorder (OMIM 617360) and behavioral problems[95]; (h) a duplication of ZFYVE27 associated with AD spastic paraplegia 33 (MIM 610244), (i) a deletion of the regulatory region of ARID1B associated with Coffin-Siris syndrome 1 (MIM 135900) presenting with a broad range of neurodevelopmental and behavioral abnormalities including hyperactivity and aggressive behavior[96]; (j) a heterozygous multiexonic deletion of the NRXN1 that has been detected in multiple patients referred for intellectual disability, autism spectrum disorder, or seizures (MIM 614332)[97]; (k) a microduplication in LRRC7, de novo variants of which were found in patients with neurodevelopmental disorders[98]; (l) a heterozygous deletion encompassing exon 3 of DPP associated with AD mental retardation 33 (MIM 616311); (m) a microduplication in the ACMSD that encodes aminocarboxymuconate semialdehyde decarboxylase (ACMSD), which prevents the accumulation of the neuronal excitotoxin quinolinate that is associated with suicidal behavior,[99] and mutations of which leads to AD parkinsonism[100]; (n) a multiexonic deletion of CDH7 leading to AD CHARGE syndrome (MIM 214800), which is often associated with behavioral abnormalities[101]; (o) an exonic deletion of DLG2, encoding a synaptic protein that is linked to developmental disorders and intellectual disability,[102] bipolar disorder[103] and autism[104]; (p) a multiexonic deletion of PTPRD that is highly constrained against loss-of-functions in the ExAC database, reported in numerous individuals with behavioral abnormalities in the DECIPHER database, and variants of which have been associated with a range of neurobehavioral phenotypes affecting conformity,[105] and associated with mood instability,[106] obsessive-compulsive disorder[56,107] and attention-deficit hyperactivity disorder.[108] Deletion and haploinsufficiency of PTPRD alter locomotion, sleep behaviors and cocaine-conditioned place preference in mice.[109] Another microdeletion of PTPRD affecting regulatory region within intron 2 is reported later in this investigation as likely clinically relevant; (q) a multiexonic deletion of GRN leading to AD frontotemporal lobar degeneration with ubiquitin-positive inclusions (MIM 607485), which presents in early stages with impulsive socially inappropriate behavior (Hsiung, 2007); and (r) a multiexonic deletion of RBFOX1, a

TABLE 3 Clinically relevant copy number variants identified in 31 males with an ICD-10 diagnosis of dissocial personality disorder

SID/Heredity	Et	Chr	Start	End	Size	Type	Candidate genes	Clinical association
6998/0	C	chrX	1	155 270 560	155 270 560	Dup		Klinefelter syndrome
7498/1	R	chrX	1	155 270 560	155 270 560	Dup		Klinefelter syndrome
7542/0	C	chr22	41 887 922	45 396 440	3 508 519	Dup	TCF20, TSPO, CYP2D6	70 genes; overlapping CNVs found in multiple individuals with ID
499G/0	C	chr11	23 211 700	26 188 592	2 976 893	Dup	LUZP2	Brain-specific protein of unknown function; overlapping CNVs found in multiple individuals with ID
1221G/1	C	chr19	27 791 257	29 562 474	1 771 218	Dup	LINC00662	Brain expressed ncRNAs; overlapping CNVs found in multiple individuals with ID
3492G	C	chr12	127 396 986	128 705 029	1 308 044	Dup	LINC00507	Age-dependently expressed in brain; overlapping CNVs found in multiple individuals with ID
7503/0	C	chr1	245 804 664	246 651 577	846 914	Dup	KIF26B, SMYD3	AD cerebellar ataxia 53 (OMIM 614026); overlapping CNVs found in multiple individuals with ID
7567/1	R	chr16	21 839 340	22 440 319	600 980	Del	EEF2K, CDR2	10 genes, recurrent 16p12.1 microdeletion (OMIM 136570); neurodevelopmental susceptibility locus
2507G/0	R	chr1	180 089 858	180 656 059	566 202	Del	XPR1, QSOX1	Seven genes, XPR1 is associated AD basal ganglia calcification, idiopathic, 6 (OMIM 605237)
3553G	C	chr16	6 322 276	6 770 650	448 375	Del	RBFOX1	A candidate for aggressive behavior; CNVs enriched in children with developmental disorders
3486G	C	chrX	154 119 023	154 563 670	444 648	Dup	RAB39B	Eight genes, mental retardation, X-linked; RAB39B is involved also in Parkinson disease and autism
1220G/1	C	chr11	84 034 817	84 469 196	434 380	Del	DLG2	Synaptic protein linked to developmental disorders, ID, bipolar disorder and autism
7707/0	C	chr1	145 382 349	145 765 424	383 076	Del	RBM8A, GNRHR2	21 genes, mice heterozygous for Rbm8a deletion exhibit aberrant neurogenesis and microcephaly
1101G/0	R	chr18	63 504 872	63 859 664	354 793	Del	CDH7	AD CHARGE syndrome (MIM 214800), synaptic expression
7575/0	R	chr7	151 748 737	152 050 571	301 835	Dup	KMT2C	AD Kleefstra syndrome 2 (MIM 606833)
859G/0	C	chr9	10 225 428	10 512 307	286 880	Del	PTPRD	Associated with mood instability, major depressive disorder, anxiety disorder and schizophrenia; behavioral abnormalities in Ptprd−/− mice
972G/0	C	chr22	36 695 173	36 966 418	271 246	Dup	CACNG2	Five genes, CACNG2 is associated with AD mental retardation 10 (OMIM 602911) and intellectual disability
7453/1	R	chr1	11 305 316	11 530 369	225 054	Dup	MTOR	Smith-Kingsmore syndrome (OMIM 616638)
7576/1	C	chr14	47 150 378	47 315 071	164 694	Del	MDGA2	Haploinsufficiency alters cortical dynamics and cognitive function
2325G/0	C	chr7	39 919 353	40 073 434	154 082	Dup	CDK13	Associated with AD intellectual developmental disorder (OMIM 617360) and behavioral problems
1602G/0	C	chr2	51 118 081	51 266 798	148 718	Del	NRXN1	Heterozygous intragenic deletions found in patients with ID and autism (MIM 614332)
7672/1	R	chr2	148 943 206	149 091 754	148 549	Dup	MBD5	Mental retardation, autosomal dominant 1 (MIM 156200)
3482G/1	R	chr2	148 943 657	149 091 754	148 098	Dup	MBD5	Mental retardation, autosomal dominant 1 (MIM 156200)
7545/1	R	chr2	148 943 657	149 050 617	106 961	Dup	MBD5	Mental retardation, autosomal dominant 1 (MIM 156200)
7549/1	R	chr10	99 490 436	99 573 168	82 733	Dup	ZFYVE27	Associated with AD spastic paraplegia 33 (MIM 610244)

TABLE 3 (Continued)

SID/Heredity	Et	Chr	Start	End	Size	Type	Candidate genes	Clinical association
7336/0	C	chr5	78 767 898	78 819 404	51 507	Dup	HOMER1	Heterozygous knockout mice showed increased aggression in social interactions with conspecifics
1103G/1	R	chr6	157 334 519	157 372 427	37 909	Del	ARID1B	Regulatory region of the gene that is associated with AD Coffin-Siris syndrome 1 (MIM 135900)
7588/1	C	chr2	135 572 382	135 600 749	28 368	Dup	ACMSD	Associated with AD Parkinson's disease
3476G	R	chr1	70 377 391	70 399 318	21 928	Dup	LRRC7	De novo variants found in patients with neurodevelopmental disorders
7590/0	C	chr7	154 161 235	154 172 154	10 920	Del	DPP6	AD mental retardation 33 (MIM 616311)
7551/0	C	chr17	42 425 313	42 432 774	7462	Del	GRN	AD frontotemporal lobar degeneration with ubiquitin-positive inclusions (MIM 607485)

Abbreviations: Et, ethnicity; ID, intellectual disability; R, C, individuals of Roma or Caucasians origin; SID, id number/heredity (father and/or sibs) – 1 yes, 0 no.

candidate gene for aggressive behavior[110] that controls neuronal excitation in the mammalian brain,[111] regulates the expression of synaptic and autism-related genes,[112] is highly constrained against loss-of-function mutations in the ExAC database, and is reported in numerous individuals with autism,[81,113] epilepsy,[114] bipolar disorder[103] and behavioral abnormalities in the DECIPHER database; another microdeletion of RBFOX1 affecting the regulatory region within intron 1 is reported later in this investigation as likely clinically relevant.

Three CNVs were classified as clinically relevant based on relevant phenotypes observed in heterozygous knockout mice models. These include: (a) a heterozygous 400-kb deletion (chr1:145382349-145765424) impacting 21 genes including RBM8A, haploinsufficiency of which disrupts embryonic cortical development and results in microcephaly in mice[115] and intellectual disability in humans[116]; (b) a heterozygous deletion of the last exon of MDGA2, haploinsufficiency of which leads to an autism-like phenotype including stereotypy, aberrant social interactions and impaired memory in mice[117] and which is considered a candidate gene for autism in humans[118] and (c) a microduplication of a regulatory region and exon 1 of HOMER1, encoding a protein that regulates the functional assembly of postsynaptic density proteins at glutamatergic synapses, and haploinsufficiency of which in mice leads to increased aggression in social interactions.[119] A single individual (288868) with a similar microduplication demonstrating aggressive behavior and intellectual disability is reported in the DECIPHER database.

3.8 | Fifty-nine rare, likely clinically relevant CNVs were identified in 49 individuals

There were 41 individuals who had one, seven individuals with two, and one individual with four of these CNVs (Table 4). The most notable CNVs in this group included: (a) a deletion of a regulatory region in intron 2 of HECW2, mutations of which cause an AD neurodevelopmental disorder with hypotonia, seizures and absent language (MIM 617268)[120]; (b) a microduplication in CDH13, a gene that impacts GABAergic function in hippocampus and cognition,[121,122] and which has been associated with extreme violent behavior in a cohort of Finnish prisoners[123] and attention-deficit hyperactivity disorder[124]; and (c) a microduplication in neurotrimin (NTM), a gene that has been associated with aggressiveness in attention-deficit hyperactivity disorder,[35] late onset Alzheimer disease,[125] and is linked to developmental delay[126] in humans and whose loss leads to a deficit in emotional learning in Ntm $^{(-/-)}$ mice.[127]

Other CNVs affect genes that controls synaptic signaling (DLGAP2, DLGAP1, RPH3AL, GRM7, PTPRD, NRXN1, P2RX2, MAPK8IP2, JPH3, DOC2B, KCTD16, SDK1, IQSEC3, SNX6, DDC, PALM, GLT8D1, WNK2) and re-uptake of GABA (SLC6A12, SLC6A13); and/or are associated with neurobehavioral phenotypes like aggressive behavior, autistic disorders, schizophrenia, attention-deficit hyperactivity disorder, bipolar disorder or sporadic parkinsonism in human and/or mouse models (DLGAP2, NRXN1, IL1RAPL2, RBFOX1, MACROD2, SEMA5A, CSMD3, DDC, CDH13, MAPK8IP2, NXPH1, GRM7, SLC6A12, SLC6A13, DLGAP1, P2RX2, LAMA1, GRIN3A, CMYA5, DDC, IPO11, RPH3AL, TRANK1, SDK1, NINJ1, NRG3, MIDN).

Some CNVs affected genes that are specifically expressed in the brain, and/or constrained against deletions and loss of function mutations, (Figure 5 and Supplementary Information Appendix S1: list "constrains and expression"), identifying them as novel candidate genes for impulsive behavior. These genes included FBXL16, PHACTR3,[126] CDH20,[107] FAM155A, DOK6,[128] NKAIN2,[129] STXBP5L,[130] or PCDH9,[131,132] UNC13C,[133–135] and DPP10,[132] C7orf26, FSD1, GLT8D1,[136] or GNL3.[137] Other candidate genes included DNAJC15 and UBE2V2. DNAJC15 is consistently reported to be affected in some patients with cognitive impairment reported in the DECIPHER database. UBE2V2 has been found overexpressed in brains of aggressive dogs.[138]

4 | DISCUSSION

Impulsively violent behavior is a complex trait that results from multiple genetic and environmental factors,[4] but the specific genes and their variants (mutations) conferring risk are poorly characterized and largely unknown.[17,139]

With the relative failure of a genome-wide and candidate gene-centered association studies[35,140] and the development of novel genotyping and sequencing techniques, it has been postulated that

TABLE 4 Likely clinically relevant copy number variants identified in 49 males with an ICD-10 diagnosis of dissocial personality disorder

SID	Et	Chr	Start	End	Type	Size	Candidate gene	Clinical association
7542/0	C	chr7	6 647 725	6 697 851	Del	50 127	C7orf26	Overexpressed in brain, constrained against deletions and loss-of-function mutations
		chr16	736 998	766 690	Del	29 693	WDR24, METRO	WDR24 is a component of the GTPase activating proteins toward Rags complex (GATOR2) regulating mTORC1 pathway. METRO regulates glial cell differentiation and promotes the formation of axonal networks during neurogenesis.
		chr19	1226555	1251781	Del	25227	MIDN, C19orf26	MIDN is a regulator of Parkin expression, deletions associated with Parkinson's disease. C19orf26 is specifically expressed in brain, its molecular function is unknown
		chr22	51 035 885	51 048 721	Del	12 837	MAPK8IP2	IB2 is expressed in the brain and is highly enriched within postsynaptic densities; cognitive deficits in IB2 (−/−) mice due to synaptic transmission deficits
3550G	C	chr3	163 800 507	164 042 296	Dup	241 790	MIR1263	Recurrent duplications in ID
		chr18	7 080 135	7 171 561	Del	91 427	LAMA1	De novo and rare inherited CNVs in the hemiplegic form of cerebral palsy.
1121G/1	C	chr5	9 013 766	9 083 548	Del	69 783	SEMA5A	Associated with autism spectrum disorders; de novo microdeletion of SEMA5A found in a boy with autism and ID
		chr10	84 139 399	84 186 263	Del	46 865	NRG3	Specifically expressed in brain; regulates impulsive action.
502G/0	C	chr7	8 394 248	8 540 295	Dup	146 048	NXPH1	Involved in synaptic transmission and differentiation of synaptic contacts. Associated with ADHD and autism.
		chr16	87 693 428	87 706 185	Del	12 758	JPH3	Amplification of repeats within JPH3 leads Huntington disease-like 2
525G/1	C	chr18	66 982 638	67 072 149	Dup	89 512	DOK6	Promotes RET-mediated neuritis outgrowth
		chr19	4 320 022	4 372 780	Dup	52 759	FSD1	Specifically expressed in brain, molecular function unknown;
7590/0	C	chr6	124 264 221	124 352 163	Del	87 943	NKAIN2	Specifically expressed in brain; associated with extraversion and neurotism
		chr19	20 718 734	20 925 934	Dup	207 201	ZNF626, ZNF737	Overlapping amplifications of ZNF626 were found in multiple individuals with ID and behavioral abnormalities. ZNF737 has been associated with reading disability and ADHD
7539/1	C	chr5	61 885 501	61 904 389	Dup	18 889	IPO11	Rare variants are associated with ADHD in Caucasians.
		chr11	100 704 714	101 436 561	Dup	731 848	TMEM133	Constrained against duplication CNVs, biological function unknown
7468/1	C	chr7	50 530 869	50 541 135	Del	10 267	DDC	DOPE decarboxylase, association with nicotine dependence; functional common polymorphisms in the DDC gene might contribute to neural processes relevant to neuropsychiatric illness and treatment.
		chr20	58 326 432	58 342 420	Del	15 989	PHACTR3	A regulator of neuritis outgrowth and neuroplasticity in the brain.
2698G	R	chr9	10 547 083	10 595 208	Del	48 126	PTPRD	Associated with mood instability, major depressive disorder, anxiety disorder and schizophrenia; behavioral abnormalities in Ptprd−/− mice
2700G/1	R	chr15	54 888 538	54 926 493	Del	37 956	UNC13C	Constrained against mutations, overexpressed in brain; rare mutations in dementia in a Finnish cohort.
3473G	R	chr20	14 481 403	14 604 808	Del	123 406	MACROD2	Rare CNVs identified in individuals with ADHD; overlapping CNVs found in multiple individuals with ID and autism
3487G	R	chr2	116 342 244	116 362 694	Del	20 451	DPP10	

TABLE 4 (Continued)

SID	Et	Chr	Start	End	Type	Size	Candidate gene	Clinical association
								Linked to ADHD; constrained against mutations, overexpressed in brain
3491G	C	chr16	6 874 144	6 891 002	Del	16 859	RBFOX1	A candidate for aggressive behavior; CNVs enriched in children with developmental disorders
3496G	C	chr8	896 237	929 794	Del	33 558	DLGAP2	Rare CNVs in autism spectrum disorders; Dlgap2 −/− mice show exacerbated aggressive behaviors
3555G	C	chr13	108 294 075	108 358 850	Del	64 776	FAM155A	Specifically expressed in brain, constrained against duplications and loss of function mutations
1063G/1	R	chr18	3 821 489	3 848 159	Dup	26 671	DLGAP1	Association with ADHD; Dlgap1 (−/−) mice exhibit alterations of the postsynaptic density and selective reductions in sociability.
1070G/1	C	chrX	104 559 318	105 083 783	Dup	524 466	IL1RAPL2	Specifically expressed in brain. Overlapping CNVs found in multiple individuals with ID and autism. Microdeletions in females with severe ID, hypotonia and behavioral abnormalities.
1098G/0	C	chr3	6 933 866	6 983 750	Dup	49 885	GRM7	Glutamate receptor; associated with ADHD; rare CNV variants identified in patients with psychiatric disorders
1119G/0	C	chr12	133 137 528	133 253 195	Dup	115 668	POLE, P2RX2	Overlapping CNVs found in multiple individuals with ID and autism
1120G/0	C	chr11	130 821 810	131 476 469	Dup	654 660	NTM	Overlapping CNVs found in multiple individuals with ID and autism, associated with aggressiveness
1213G/0	R	chr7	4 018 597	4 476 949	Dup	458 353	SDK1	Association with ADHD; CNV variants in the brain of schizophrenia patients
1214G/1	C	chr6	57 220 778	57 632 044	Dup	411 267	PRIM2	Overlapping CNVs found in multiple individuals with ID
1220G/0	C	chr8	140 805 932	140 869 942	Del	64 011	TRAPPC9	De novo mutation in schizophrenia, mental retardation, autosomal recessive 13 (MIM 613192)
1594G/1	C	chr16	82 179 289	83 668 937	Dup	1 489 649	CDH13	Genetic background of extreme violent behavior; associated with ADHD
1616G/1	C	chr2	197 250 296	197 264 236	Del	13 941	HECW2	Neurodevelopmental disorder with hypotonia, seizures, and absent language (MIM 617268)
2562G/1	C	chr11	89 232 161	89 822 105	Dup	589 945	NOX4	Overlapping CNVs found in multiple individuals with ID and behavioral abnormalities
379G/0	C	chr8	1 117 573	1 144 799	Del	27 227	DLGAP2	Rare CNVs in autism spectrum disorders; Dlgap2 −/− mice show exacerbated aggressive behaviors
477G/0	C	chr13	67 380 739	67 397 532	Del	16 794	PCDH9	Specifically expressed in brain; CNVs in autism; Pcdh9 (+/−) mice showed long-term social recognition impairment
478G/0	C	chr19	746 206	770 528	Dup	24 323	PALM	Implicated in plasma membrane dynamics in neurons, phosphoprotein associated with brain synaptic plasma membranes. It is also abundant in several endocrine tissues
483G/0	C	chr13	43 452 859	43 792 305	Dup	339 447	ENOX1, DNAJC15, EPSTI1	Overlapping CNVs found in multiple individuals with ID and autism
486G/0	C	chr7	149 509 048	149 522 447	Dup	13 400	SSPO	Associated with structural connectivity and information processing in the brain.
530G/0	C	chr9	95 892 712	96 015 165	Dup	122 454	WNK2, NINJ1	WNK2 regulates cell volume and/or GABAergic signaling; NINJ1 plays a role in nerve regeneration; Ninj1 (−/−) mice are reminiscent of mouse models of neuropsychiatric disorders.

TABLE 4 (Continued)

SID	Et	Chr	Start	End	Type	Size	Candidate gene	Clinical association
546G/0	C	chr3	121 081 880	121 155 725	Dup	73 846	STXBP5L	Plays a role in vesicular trafficking and neurotransmitter release
7019	C	chr12	133 137 528	133 272 761	Dup	135 234	POLE, P2RX2	Overlapping CNVs found in multiple individuals with ID and autism
7412	C	chr17	2220	103 469	Del	101 250	DOC2B, RPH3AL	DOC2B act as calcium sensor to trigger spontaneous release from synaptic vesicles. Overlapping deletions of RPH3AL were found in multiple individuals with ID
7467/0	C	chr9	103 860 319	104 371 736	Del	511 418	GRIN3A, ZNF189, PPP3R2	PLPPR1 and ZNF189 are specifically expressed in brain; GRIN3 is involved in the development of synaptic elements
7469/0	R	chr1	36 626 006	36 645 615	Del	19 610	MAP7D1	Facilitate axon elongation of cortical neurons
7501/0	C	chr14	35 021 090	35 061 728	Dup	40 639	SNX6	Ablation of SNX6 leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. Interacts with HOMER1
7536/0	R	chr5	79 069 469	79 120 797	Del	51 329	CMYA5	Associated with schizophrenia in multiple populations
7577/1	C	chr18	3 981 701	3 998 627	Dup	16 927	DLGAP1	Association with ADHD; Dlgap1 (−/−) mice exhibit alterations of the postsynaptic density and selective reductions in sociability.
7578/0	R	chr8	48 964 607	48 999 011	Del	34 405	UBE2V2	Differential expression of the UBE2V2 in brains of aggressive and nonaggressive dogs.
7617/0	C	chr12	260 682	592 524	Dup	331 843	SLC6A13, SLC6A12, IQSEC3	SLC6A13 encodes major transporter for gamma-aminobutyric acid; SLC6A12 encodes a transporter of betaine; IQSEC3 is expressed specifically in the adult brain and rare CNVs were identified in patients with anxiety disorders
7618/0	C	chr2	50 952 084	50 970 602	Del	18 519	NRXN1	Microdeletions have been associated with neurodevelopmental disorders, including autism, schizophrenia, intellectual disability, speech and language delay, epilepsy and hypotonia.
7636/1	R	chr5	143 416 602	143 645 872	Dup	229 271	KCTD16	Determines the kinetics of the gamma-aminobutyric acid B (GABAB) receptor response; may contribute to hyper-reactivity to aversive stimuli in neuropsychiatric disorder
7674/1	C	chr3	36 948 417	37 017 024	Del	68 608	TRANK1	Associated with bipolar disorder.
7700/	R	chr5	61 698 663	61 977 629	Dup	278 967	IPO11	Rare variants are associated with ADHD in Caucasians.
7703/1	R	chr3	52 596 914	52 735 364	Del	138 451	GNL3, GLT8D1	Associated with bipolar disorder in individuals of European ancestry.
863G/0	C	chr8	113 219 539	113 281 792	Dup	62 254	CSMD3	Candidate gene for ID and autism
972G/0	C	chr2	50 495 613	50 513 774	Del	18 162	NRXN1	Microdeletions have been associated with neurodevelopmental disorders, including autism, schizophrenia, intellectual disability, speech and language delay, epilepsy and hypotonia.

Abbreviations: ADHD, attention-deficit hyperactivity disorder; Et, ethnicity; ID, intellectual disability; R, C, individuals of Roma or Caucasians origin; SID- id number/heredity (parents and/or sibs) – 1 yes, 0 no.

genetic components of complex phenotypes, such as impulsively violent behavior,[139] may be deciphered by detailed genetic analysis of extreme cases[141–144] and analysis of individual genetic profiles with a specific attention to rare variants of potential clinical and biological significance.[145,146]

Following this hypothesis we assessed rare CNVs in 281 individuals from a cohort of 313 male participants that were classified by strict criteria as having impulsive violence. These individuals resided in Czech high security male prisons, two of which have specialized program for prisoners with personality disorders. Of 313 offenders,

134 (43%) reported a history of criminal conviction in their relatives, suggesting that a genetic component may be enriched in this cohort.

For genotyping, we used Illumina HumanOmni2.5 Exome BeadChips that allowed sensitive detection of CNVs >10 kb and applied stringent criteria for CNVs definition. We compared the frequencies of all 1525 identified CNVs with KORA, COGEND and internal population-matched control cohorts and defined in 264 participants a set of 828 rare CNVs. Identified rare CNVs impacted 754 genes and 124 genes were impacted more than once (2-25 times).

Functional annotation and evaluation of genes impacted by CNVs provided important insights into the genetics and pathophysiology of impulsive, violent behavior.

We found that many of the identified CNVs occurred in pathways that are relevant to impulsive violence. Impacted genes were over-represented in functional categories and biological processes related to synapses, neurons, mitochondrial metabolism, human behavior and learning. We also found over-representation of impacted genes in a category of gene sets that are associated, through genome-wide association studies, with neurobehavioral phenotypes like intelligence, schizophrenia, neurotism, bipolar disorder, autism and cognitive decline. Interestingly, the impacted genes are most over-represented in a category of obesity-related traits. This finding supports the established link between obesity, impulsivity and behavioral problems in children and adults.[147,148] Impacted genes were also enriched for those that are more abundantly and specifically expressed in brain and brain-specific regions related to impulse control. The most prominent enrichment was seen in the anterior cingulate cortex, amygdala, basal ganglia and frontal cortex, key structures in the circuitry underlying emotion regulation.[149] Most importantly, 97 genes (12%) of the impacted genes are known to cause monogenic diseases reported in the OMIM database. From these, 33 genes (5%) are associated with an AD and five genes with X-linked transmitted diseases, a finding that is compatible with heterozygous deletions or duplications caused by identified CNVs.

The potential contribution of CNVs to the pathophysiology of the impulsive violence was extensive, with 123 (44% of all) participants having 173 CNVs of potential clinical significance.

Important finding was the identification of 17 rare recurrent CNVs of potential significance that were present in 65 participants. This finding suggested either that these variants are clinically and/or biologically significant or that their recurrence reflect the effect of population stratification that is typical for rare variants.[146] To assess population structure, we performed principal component analysis of genotype data from whole exome sequencing and microarray data and found that 161 individuals from the studied cohort were of North-Central European ancestry and 120 individuals of Roma origin. The majority of the genes recurrently impacted by CNVs were found in individuals of Roma origin. The Roma represents the largest ethnic minorities in Czech Republic, its neighboring countries Slovakia and Hungary and across Europe.[150] Roma minorities originate from several founder populations that split, underwent several extensive reductions in population size and admixture events due to pogroms and holocaust during the last 700 years and thus formed multiple genetically and socially divergent and geographically dispersed groups.[151,152]

The divergence, dispersion, endogamy and genetic admixture with majority population led to a high prevalence of deleterious genetic mutations that are rare among other populations and even private to specific Roma groups.[153] The heterogeneity of the Roma populations and limited access to DNA samples with sporadic information on health status of control individuals from Roma populations prevented rigorous association testing of recurrent likely clinically relevant CNVs identified in Roma participants. Nevertheless, rare CNVs impacting genes specifically expressed in brain and involved in adult behavior, cognition and learning (NF1, MBD5, CHL1, FGF12, ZNF385D and CNTNAP2), synaptic signaling (NF1, GRID2, CPLX1, FGF12), neuronal development and homeostasis processes (NF1, CHL1, GRID2) and drug addiction (FAAH) identified in control Roma populations deserve further investigations due to the increased prevalence of anxiety, neuropsychiatric diseases, hereditary seizure disorders, and depression in this population.[154]

In other 31 individuals (11% of all), we identified 31 rare CNVs that we classified as clinically relevant. These include two individuals with 47,XXY (Klinefelter syndrome), which is frequently associated with specific neurobehavioral features and personality traits.[155] The 47,XXY is one of the most common chromosomal disorders, occurring in approximately 1 in 500 to 800 males in the general population.[156] Our results suggest that this genetic disorder may be over-represented in this cohort. The other recurrently identified CNV was a microduplication of exon 4 and its adjacent intronic regions in MBD5. MBD5 is a dosage sensitive gene, mutations of which lead to AD or sporadic developmental delay, motor delay, language impairment, autistic-like symptoms and behavioral problems.[157–159] This CNV was identified in three individuals, all of whom reported a history of conviction for criminal activity in their families. This is compatible with an AD inheritance pattern present in the MBD5-associated neurodevelopmental disorder. Comparison of exome-derived genotypes showed that these three individuals are not closely genetically related. However, all three individuals share several rare single nucleotide variants around MBD5 locus. This is suggestive that this particular CNV has been inherited from their common ancestor and thus may represent another relatively frequent deleterious mutation that is specific to Roma population. The other 26 identified clinically relevant CNVs were private. The 19q11-19q12 duplication, 16p12.1 microdeletion, microdeletions of DLG2[102] and ARID1B[96,160] and microduplications of MTOR,[94] ACMSD,[100,161] MDGA2[117] and ZFYVE27[162] were present in individuals who also reported history of criminal conviction in their relatives, further supporting their either firmly established or highly suspected clinical relevance. For the remaining 18 clinically relevant CNVs, the criminal history of siblings was either not mentioned or denied. However, in all these cases, the impacted genes are verifiably associated with monogenic AD neuropsychiatric phenotypes.

In an additional 49 individuals (17% of all), we identified 59 rare CNVs that we classified as likely clinically relevant. Their contribution to the phenotype of impulsively violent behavior is not as clear as for the category of clinically relevant variants. However, all the impacted genes represent highly phenotypically relevant candidates and their contribution require further investigations.

In summary, the primary aim of our study was to describe rare copy number variation in a group of participants who were currently in prison, volunteered to participate in this study without coercion,

and who met strict criteria for impulsive violence. We expected that these extreme cases would be enriched for rare CNV variants with large phenotypic effects, and that the genes impacted by these CNVs would identify specific biological pathways and candidate genes contributing to impulsively violent behavior.

In this study, we identified 107 unique causative, likely causative, or possibly causative CNVs in approximately 44% (123 of 281) of individuals with impulsive violence. There were many different genes and pathways involved, without the identification of a predominant gene or pathway. This research indicates that extremely impulsively violent individuals likely do not share a common genetic condition, but rather each individual has one to several unique abnormalities that increase impulsivity that may lead to violence. Thus, an individualized approach will be important in the scientific and clinical evaluation of each individual. These findings explain the failures of prior analyses such as genome-wide and candidate gene-based association studies to identify significant findings. In addition, this study shows that attempts to classify extremely violent individuals into groups of defined psychiatric disorders are likely to be unsuccessful, due to the widely diverse genetic and biologic pathophysiology that we identified.

There were several weaknesses to the study. First, we encountered some difficulties in phenotyping violent, antisocial individuals, who often were uncooperative with structured tests, even after receiving compensation. The misclassification of individuals may have resulted in the inclusion of some individuals who did not have true impulsive violence. We believe that this would have likely diluted our findings.

Another potential weakness is that some of our genetic findings may have been associated with violent behavior in general instead of impulsive violence. In the future, studies of all individuals with violent behavior will help to further clarify these findings. Inclusion of all violent individuals was beyond the scope of our study.

In addition, the nature of the study did not allow for collection, phenotyping and genotyping of parents and siblings of study participants. Consequence of the absence of clinical and genetic information on family members was that we could not determine with certainty whether each CNV we identified cosegregated with impulsively violent behavior or arose de novo. While de novo CNVs are relatively uncommon, with one study reporting frequencies of de novo CNVs in size from 5 to 100 kb from 0.1 to 0.01 per generation,[41] they can occur and be asymptomatic. While the CNVs we identified were rarely identified in controls and genome databases, CNVs are often unique. Given the large number of CNVs found and the large number of comparisons performed, and given the relatively small sample size, there is a significant possibility that some of the CNV findings were in fact false positives. Future research will need to be performed to deter-mine the clinical significance of each CNV.

We also encountered a problem with the population stratification of the cohort. Heterogeneity of the Roma populations and limited access to DNA samples with sporadic information on health status of control individuals from the Roma populations did not allowed us to perform a more rigorous assessment of CNVs frequencies and association testing of recurrent likely clinically relevant CNVs identified in Roma participants. Another weakness of our study was the inclusion of prisoners. While rigid criteria resulted in their characterization as having impulsive violence, discrimination and selective prosecution may have resulted in a different population than if we were able to include individuals who were not in prison. Given the high rates of substance abuse and suicide attempts in the offender sample, we also do not know which finding represent specific associations to impulsive violence or to substance abuse.

Despite these weaknesses, we can conclude that the extreme forms of impulsively violent behavior have in many cases a strong genetic component. This genetic component is individually specific, and underlies the biologically diverse origin and phenotypic presentation. In many of the investigated cases the genetic findings point to specific disorders for which treatments may be present or developed. As the diagnosis of psychiatric disorders has been found to improve prisoner care, we believe that the identification of these disorders may also be helpful to prisoner care. Individuals with these disorders may benefit more from counseling. In addition, training for prison workers in the approach to individuals prone to impulsive violence may be helpful to the care of these individuals. We strongly believe that these results are preliminary and require further validation.

It is also extremely important to point out that while the presence of these CNVs in the prison population may have contributed to impulsive, violent behavior, the converse is not true: the finding of these or similar CNVs in the general population may or may not be markers of impulsive, violent behavior. Extensive, population-based studies would be required in order to obtain even a limited understanding of the effect of these CNVs in the general population.

Genotyping of extremely violent individuals may result in the diagnosis of neurologic diseases, allowing for early detection of disease symptoms and/or placement of these incarcerated individuals into yet to be developed specific correctional facilities.[163,164] The ultimate goal of this work is to identify targets and develop individually specific treatments for impulsively violent people. Several of our findings provide strong examples of how this approach could be beneficial to affected individuals. For example, FAAH inhibitors already used in human,[165] modulate impulsivity in deprived rats[166] and may be considered in individuals found here with duplications of FAAH. Abnormal cognitive and social behaviors in Arid1b-heterozygous mice were rescued by treatment with a positive allosteric $GABA_A$ receptor modulator.[160] In addition, aberrant behaviors in Ninj1 knockout mice are ameliorated by fluoxetine.[167]

Our findings thus warrant detailed genetic analysis in individuals with extremely impulsively violent behavior and provide motivation to perform further studies in a similar cohort of extremely impulsively violent women that has been collected by J.V. and in other populations.

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AUTHOR CONTRIBUTIONS

J.V. recruited and clinically assessed cohort of impulsively violent males, collected blood for DNA isolation and co-wrote the manuscript. M.Z. carried out microarray data analysis, interpret the data and co-wrote the manuscript. H.H., I.J., D.M., H.T., L.N. and K.H. were responsible for sample handling, genotyping, whole exome sequencing and qPCR CNV analysis. V.S. and A.P. carried out microarray data analysis and interpreted the data. V.J, P.O., M.P. and K.P. clinically assessed cohort of impulsively violent males. J.Š. provided control samples. J.W. and M.W.-S. carried out microarray data analysis and interpreted the data. A.J.B., S.W.S. and S.K. carried out data analysis, interpreted the data and co-wrote the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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ORIGINAL ARTICLE

Steeper Delay Discounting for Potentially Real versus Hypothetical Cigarettes (but not Money) in Czech Republic Smokers

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Abstract

A relatively large literature suggests that hypothetical and potentially real monetary rewards yield similar patterns of responses in delay (DD) and probability (PD) discounting. However, the much smaller literature concerning hypothetical versus potentially real cigarettes is more mixed and the generalizability of findings from both of these literatures outside the United States is unclear. The present study extended research comparing hypothetical and potentially real delayed and probabilistic monetary and cigarette outcomes to adult smokers (N = 59) in the Czech Republic using a within-subjects design. Comparison of hypothetical versus potentially real outcomes across tasks revealed Czech smokers discounted the value of potentially real cigarettes (but not money) more steeply than hypothetical cigarettes on the DD, but not PD, task. Findings also suggest a gender effect in which male participants discounted the value of money and cigarette outcomes more than did women for DD (but not PD). The relevance to methodological factors, cultural factors, and gender effects in discounting are discussed.

Keywords Delay discounting · Probability discounting · Hypothetical outcomes · Cigarette smoking · Gender

Introduction

Delay discounting (DD) and probability discounting (PD) are behavioral measures of choice that indicate the extent to which an individual is sensitive to delayed (in DD) and probabilistic (in PD) rewards. In humans, DD is measured by posing a series of choices between a relatively small reward available right now (e.g., $10) and a larger reward available after a delay (e.g., $100 in a day). The size of the small reward is adjusted across subsequent questions (e.g., $20, $30) and a similar series of questions is asked using several different delays (e.g., 1 week, 1 month, 1 year). Preferences for smaller–sooner and larger–delayed rewards is indicated as a series of indifference points indicating the current subjective value of the reward at the different delays. The preference for smaller–sooner rewards is consistent with the behavioral definition of impulsive choice (Rachlin et al., 1991). PD is similar, except that individuals choose between a series of smaller–certain rewards (e.g., $10 for sure) and larger, but probabilistic rewards (e.g., a 50% chance of $100). As in DD, the smaller–certain amount is adjusted and a similar series of choices is given for several probabilities (e.g., 10%, 25%). A tendency to choose the probabilistic rewards in PD indicates a pattern of risk-taking, or insensitivity to probabilistic rewards (see Green & Myerson, 2004).

Patterns of DD are associated with many problem health outcomes, including illicit drug use and abuse (MacKillop et al., 2011), cigarette smoking (Bickel et al., 1999), sexual risk-taking (e.g., Johnson & Bruner, 2012; Mahoney & Lawyer, 2018; Lawyer & Schoepflin, 2013), and obesity (Rasmussen et al., 2010), among others. The relationship between DD and health problems led DD to be described as a "transdisease" process (Bickel et al., 2012, 2019; Bickel & Mueller, 2009) that may be a fundamental mechanism for health problem behaviors. The literature on PD and health-related decisions is a bit more sparse, but suggests that patterns of PD are associated with cigarette smoking (Reynolds et al., 2004), gambling (Holt et al., 2003),

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sexual risk-taking (Mahoney & Lawyer, 2018), and body fat percentage (Rasmussen et al., 2010). Taken as a whole, the preference for smaller more immediate rewards (in DD) and larger and riskier rewards (in PD) represent important decision-making factors that underlie a range of health problem behaviors.

Given the clear relationship between discounting and human health decisions, it is important that discounting procedures yield valid data regarding decisions for health-relevant outcomes. One important concern is the predominant use of hypothetical rewards to characterize patterns of discounting. In the vast majority of discounting studies, participants make choices between hypothetical rewards that they will not receive. Hypothetical rewards offer several advantages over using real rewards (e.g., where providing real rewards is unethical, infeasible, or even illegal), but skepticism about analogousness of real and hypothetical rewards is appropriate. A number of studies directly compare patterns of discounting for hypothetical rewards to discounting for potentially real (participants receive one or more randomly selected rewards from their pool of responses) rewards, typically money. Most of this research compares real and hypothetical rewards for DD using nondrug-using samples and suggests that discounting for hypothetical monetary rewards yields data that are indistinguishable from those that are real (Johnson & Bickel, 2002; Lagorio & Madden, 2005; Lawyer et al., 2011; Madden et al., 2003, 2004; Robertson & Rasmussen, 2018; cf. Hinvest & Anderson, 2010). The results of the few studies comparing real and hypothetical monetary rewards in substance-using or -dependent participants (Baker et al., 2003; Green & Lawyer, 2014; Lawyer et al., 2011) reported the same outcomes. The vast majority of these studies used a within-subjects design in which each participant's response patterns vis-à-vis hypothetical rewards are compared to their response patterns vis-à-vis real rewards, though several studies (Green & Lawyer, 2014; Madden et al., 2004) made between-groups comparisons. Most of these studies also used a lottery system in which the participant received one or more of their choices in the real outcomes condition, though Hinvest and Anderson (2010) delivered the outcome after each DD choice.

Fewer studies have investigated PD for potentially real and hypothetical rewards and the conclusions are mixed. Green and Lawyer (2014; using a between groups design) and Lawyer et al. (2011) and Robertson and Rasmussen (2018; using within-subjects designs) found similar patterns of discounting across hypothetical or potentially real monetary rewards. It is worth noting that two other studies (Hinvest & Anderson, 2010; Jikko & Okouchi, 2007) reported on studies comparing PD for real and hypothetical monetary outcomes. In these studies, real rewards were delivered after each choice (rather than chosen at random after the task). The results were inconsistent: Jikko and Okouchi found different patterns of PD for money, but Hinvest and Anderson did not. The limited number of studies on the issue and the inconsistency in the findings for (potentially) real and hypothetical outcomes calls for more research to clarify the issue.

One issue that is relatively unaddressed in this context is the growing research literature making it clear that patterns of discounting are not uniform across commodities. For example, people tend to discount the value of consumable rewards (e.g., food, drugs) at a steeper rate than nonconsumable rewards (e.g., money) even when the value of the commodities is standardized (Estle et al., 2007; Green & Myerson, 2004; Odum & Rainaud, 2003). This is likely because monetary rewards are "fungible" (Holt et al., 2016) commodities that retain their value over time and can be exchanged for other rewards. Indeed, cigarette smokers tend to discount the value of food and cigarettes more steeply than money and health outcomes (Odum et al., 2002).

This raises the question about whether patterns of discounting for potentially real and hypothetical outcomes are similar in the context of nonmonetary rewards. Two studies compared real and hypothetical nonmonetary rewards, but yielded different findings. Green and Lawyer (2014) compared discounting patterns for hypothetical and potentially real money and cigarettes in a sample of smokers. As mentioned above, they found that smokers discounted hypothetical and potentially real money similarly, but they found also that smokers yielded steeper patterns of DD and PD for potentially real cigarettes than for hypothetical cigarettes. This raises the possibility that the equivalence of discounting for real and hypothetical rewards might not be uniform across commodities. However, Robertson and Rasmussen (2018) found that discounting for hypothetical and potentially real food rewards were statistically equivalent. It is possible that differences between these studies are tied to differences in methodological design (Robertson and Rasmussen's used of a within-subjects design; Green and Lawyer used a between groups design), commodity (food vs. cigarettes), or substance-use status (nonclinical college students vs. cigarette smokers). The divergent findings among these studies indicate a significant need for continued research.

Another methodological issue in the discounting literature that has received relatively little attention is the extent to which data gathered in one culture meaningfully generalizes to others. All available research concerning real and hypothetical discounting rewards reviewed here has come from the United States (except for Hinvest & Anderson, 2010, which was conducted in England). As such, the findings from the studies published to date on this particular methodological issue have unclear generalizability vis-à-vis other countries and cultures. There are important cultural factors (e.g., perception of and attitudes about time) that should raise concern about generalizing discounting findings across cultures, but only a couple of studies have examined discounting from a cross-cultural perspective. Du et al. (2002) compared DD and PD for money across U.S., Chinese, and Japanese samples

and found culturally specific differences in DD and PD. Wang et al. (2016) surveyed students from 45 different countries with a series of decision-making questions, some of which mimic DD and found significant variability in time discounting across countries. As such, although the process of discounting is likely universal, the generalizability of methodological comparisons should be established across cultural contexts to ensure the validity of discounting data.

The purpose of the present study was to extend the comparison of potentially real and hypothetical money and cigarettes in DD and PD in a community sample of adult smokers from the Czech Republic. This study helps address two important gaps in the discounting literature. First, it represents the first effort to determine if findings about potentially real versus hypothetical money and cigarettes in the United States generalize to smokers in the Czech Republic. Second, it extends the existing research on hypothetical versus potentially real rewards to smokers recruited from a community sample.

Method

Participants

Adult smokers (N = 59) residing mainly in the community of Prague, Czech Republic, were recruited using a snowball technique, including participants that had already participated in previous studies and were interested in this study. Further recruitment was done using public newspaper announcements, leaflets, and promotion on a social network (Facebook) advertising the study. The data collection took place at the National Institute of Mental Health in Klecany and then at the University of New York in Prague. All participants were at least 18 years of age. The sample was relatively evenly split between male (N = 26) and female (N = 33) participants. The mean age of the sample was 36.7 years of age (SD = 12.3). Participants were included based on their own perception of being a smoker and interest in the study revealing further information about their habits. The sample as a whole scored, on average, a 4.19 (SD = 2.4) on the Fagerstrom Test for Cigarette Dependence (FTCD; Fagerstrom, 2012; Heatherton et al., 1991). If the participant scored six points and higher, they were evaluated as being nicotine-dependent; however, due to the small representation of nicotine-dependent individuals, the group was not further divided, representing a smoking population as a whole.

Measures

Delay and Probability Discounting Tasks

Data for delay and PD for money and cigarettes were established using a web application that posed questions using the same algorithm used in previous research (Baker et al., 2003; Lawyer et al., 2011). The large amount for the monetary tasks was 250 Czech Koruna (Kč), which is similar in value to $10USD. The large amount for the cigarette tasks was 20 cigarettes. Indifference points for both rewards were established across five different delays (1 day, 7 days, 1 month, 6 months, and 1 year) and five different probabilities (90%, 75%, 50%, 25%, and 10%) with the smaller–sooner and certain amounts adjusted incrementally.

Participants completed four different DD tasks and four different PD tasks. Within each task, participants answered discounting questions in relation to money (two tasks) and cigarettes (two tasks). Within each commodity, participants answered discounting questions in relations to purely hypothetical and potentially real rewards. In hypothetical rewards tasks, participants were informed that they would not receive any of the rewards. In the potentially real tasks, participants were informed that one of their questions would be chosen at random and they would receive whichever choice they made (i.e., the smaller–sooner or the larger–delayed reward in the DD task or the smaller–certain or probabilistic reward in the PD task). The instructions associated with each task were drawn from previous similar studies (Lawyer et al., 2011) and were presented in the Czech language.

Procedure

Procedures were similar to those reported in Lawyer et al. (2011). All participants provided informed consent upon arriving to the laboratory, followed by a brief demographics survey and the FTCD. Participants completed all discounting tasks and self-report measures in a single session lasting approximately 45 min. In each session, participants completed discounting tasks in a counterbalanced fashion such that discounting for one commodity (money or cigarettes) was completed before moving on to the next commodity. The order of hypothetical and potentially real tasks also was counterbalanced.

Compensation

At the end of the session, one question from the potentially real DD and PD tasks for each commodity (one for money; one for cigarettes) was ostensibly chosen at random and each participant received the rewards associated with their choices on each question. In reality, only PD questions were chosen for compensation, given complications associated with the delivery of delayed rewards. After a question was selected at random, the actual reward was determined by drawing poker chips from a bag based on the probability in the randomly selected question. Participants could receive up to 250 Kč based on their responses to the potentially real rewards. The same procedure took place for potentially real cigarette

reward, which could result in one pack of 20 cigarettes. In a case in which participants received no money or cigarette rewards due to chance, they were compensated with 150 Kč (however, all participants received money, cigarettes, or both).

Statistical Methods

Characterization of Discounting

Rate of discounting was calculated by fitting the hyperbolic decay function (Mazur, 1987) to individual and group-median indifference point data using nonlinear regression in GraphPad Prism©. Due to significant skew in the distribution, the b values were $\log_{10}$-transformed for parametric analysis. Residual sum of squares (RSS) was used to characterize model fit in place of R^2, because nonlinear regression can produce uninterpretable R^2 values (Johnson & Bickel, 2008). Discounting also was characterized by estimating individual area under the curve (AUC; Myerson et al., 2001) values for all discounting tasks. AUC provides an atheoretical characterization of discounting that complements b values derived from the hyperbolic model. AUC values range from 0 to 1, with small numbers indicate more impulsive choice in DD and less risky choice in PD.

We also we characterized the frequency of nonsystematic response patterns using Johnson & Bickel (2008) atheoretical algorithms used to identify patterns of discounting that deviate significantly from generally expected patterns of decision making that may complicate interpretation of b values derived from the hyperbolic function described above (see Smith et al., 2018). Consistent with Johnson & Bickel (2008) a participant's discounting pattern was identified as "nonsystematic" if (1) any indifference point was greater than the previous one by greater than 20% and/or (2) the last indifference point was not less than the first by at least 10%. These data were used descriptively and for separate analyses. All data were included in primary analyses.

Results

Initial Data Review

Technical issues led to one participant's DD for hypothetical money task to not be recorded. Initial exploration of findings suggested that there were significant gender effects across some of the discounting tasks. To characterize potential gender effects without increasing the likelihood of Type I errors from multiple comparisons, we used a series of mixed-methods ANOVA to test for differences in discounting across outcomes (hypothetical and potentially real) across each task with gender entered as a covariate. Each mixed-model ANOVA was conducted using $\log_{10}$-transformed b values and raw AUC values in separate analyses for each discounting task.

Nonsystematic Response Patterns

Visual inspection of the frequency of nonsystematic response patterns suggests that a nontrivial number of response patterns that deviated from broad expectations about discounting patterns, especially in the DD tasks and for Johnson & Bickel's (2008) second algorithm. This suggests that indifference points for a significant number of participants did not diminish as a function of delay. A comparison of the frequency of nonsystematic response patterns for DD versus PD indicated that the rate of nonsystematic responding was significantly greater for DD (M = 1.28; SD = 1.5) than for PD (M = .57; SD = 1.07) (paired samples t (57) = 4.06, p < .001). There were no differences in rate of nonsystematic responding for gender or commodity.

Comparison of Hypothetical Versus Potentially Real Rewards

Fit of the hyperbolic decay function to hypothetical and potentially real money and cigarettes are shown in Table 1 and suggest no difference in mean model fit across tasks. Table 2 shows the frequency of nonsystematic response patterns across tasks. Figure 1 shows median indifference point values (with the hyperbolic decay function fit to median indifference point data), mean AUC estimates, and median (untransformed) b values for delay discounting tasks. Figure 2 shows the same data for probability discounting.

Delay Discounting

Money The mixed-model ANOVAs revealed no differences in rates of DD for real and hypothetical money when looking at $\log_{10}$-transformed b values [F (1, 56) = 1.07, p > .05, partial η^2 = .02] or area under the curve [F (1, 56 = 1.95, p > .05, partial η^2 = .03) (see Fig. 1). However, there was a significant effect for gender for both $\log_{10}$-transformed b values [F (1, 56)

Table 1 Median (upper, lower quartiles) residual sum of squares (RSS) values produced by the hyperbolic decay function when fit to individual choice patterns across hypothetical and potentially real rewards

	Hypothetical	Potentially Real	Z	sig
Money Discounting				
Delay	.05 (.01, .12)	.05 (.01, .14)	-.52	ns
Probability	.05 (.02, .12)	.07 (.02, .13)	-1.78	ns
Cigarette Discounting				
Delay	.04 (.01, .11)	.05 (.01, .13)	-.83	ns
Probability	.05 (.02, .12)	.04 (.01, .11)	-.499	ns

Table 2 Frequency of nonsystematic response patterns across discounting tasks (N = 59 total for all tasks*)

	Algorithm		
	1	2	Either
Delay Discounting			
Hypothetical Money	1	19	20
Potentially Real Money	2	15	17
Hypothetical Cigarettes	4	21	22
Potentially Real Cigarettes	3	14	15
Probability Discounting			
Hypothetical Money	2	4	6
Potentially Real Money	1	5	6
Hypothetical Cigarettes	2	8	9
Potentially Real Cigarettes	2	11	12

*data were missing for one DD task for hypothetical monetary outcomes

= 13.78, $p < .001$, partial $\eta^2 = .20$] and area under the curve [F (1, 56 = 13.02, $p = .001$, partial $\eta^2 = .19$). There were no interactions (see Fig. 3).

Cigarettes The mixed-model ANOVAs revealed no differences in rates of DD for real and hypothetical cigarettes when comparing $\log_{10}$-transformed b values [F (1, 57) = 3.16, $p = .08$], but there was an effect for area under the curve [F (1, 57 = 4.51, $p = .04$, partial $\eta^2 = .07$) (see Fig. 1). In addition, there was a significant effect for gender for both $\log_{10}$-transformed b values [F (1, 57) = 7.38, $p = .009$, partial $\eta^2 = .12$] and area under the curve [F (1, 57 = 6.12, $p = .016$, partial $\eta^2 = .10$) (see Fig. 3).

Probability Discounting

Money The mixed-model ANOVAs revealed a nonsignificant trend toward differences in rates of PD for real and hypothetical money when looking at $\log_{10}$-transformed b values [F (1, 57) = 4.00, $p = .05$, partial $\eta^2 = .07$] an no effect for area under the curve [F (1, 57 = .14, $p > .05$, partial $\eta^2 = .002$) (see Fig. 2). There were no gender effects or interactions (see Fig. 3).

Cigarettes The mixed-model ANOVAs revealed no differences in rates of discounting for real and hypothetical cigarettes when comparing $\log_{10}$-transformed b values [F (1, 57) = 1.44, $p > .05$] or

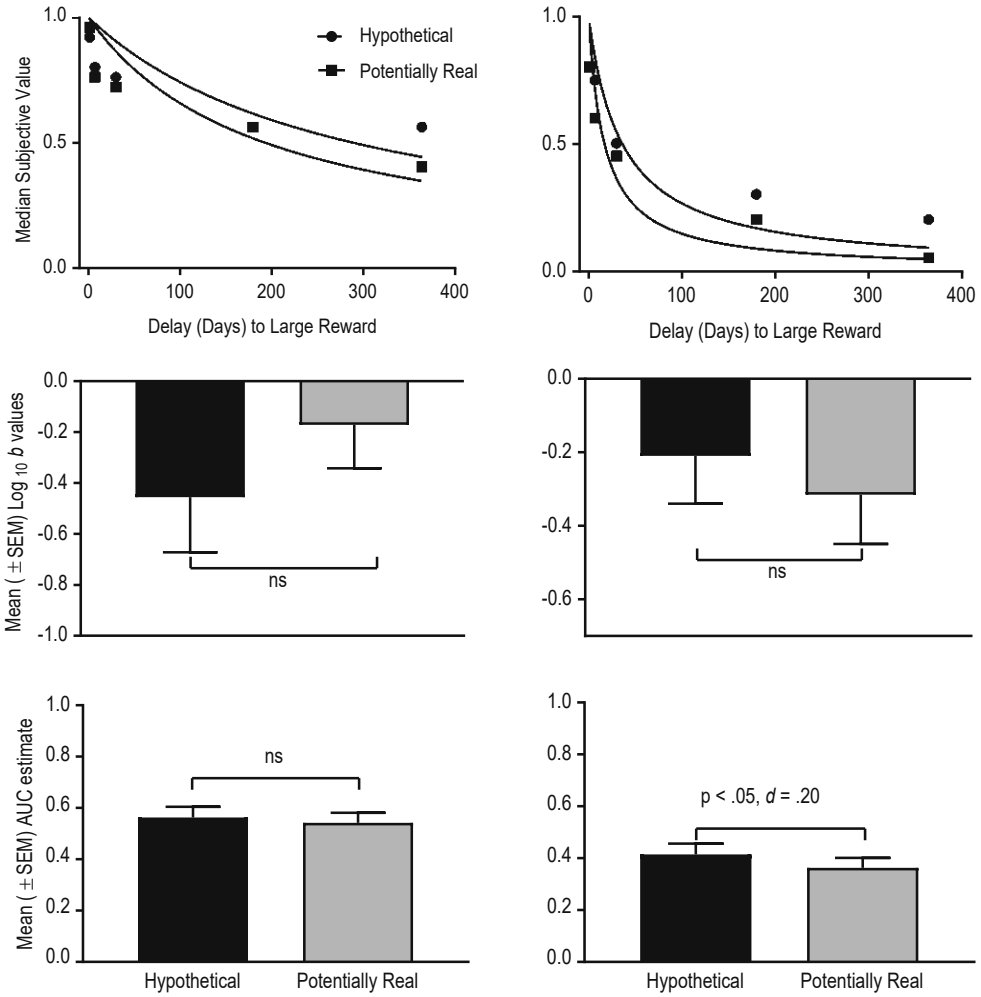

Fig. 1 Comparison of delay discounting for hypothetical and potentially real money (left) and cigarettes (right). The top panels show the median subjective value of hypothetical and real rewards. The middle panels show mean (± SEM) $\log_{10}$-transformed b values calculated using the hyperbolic decay function. The lower panels show mean (± SEM) area under the curve (AUC) values

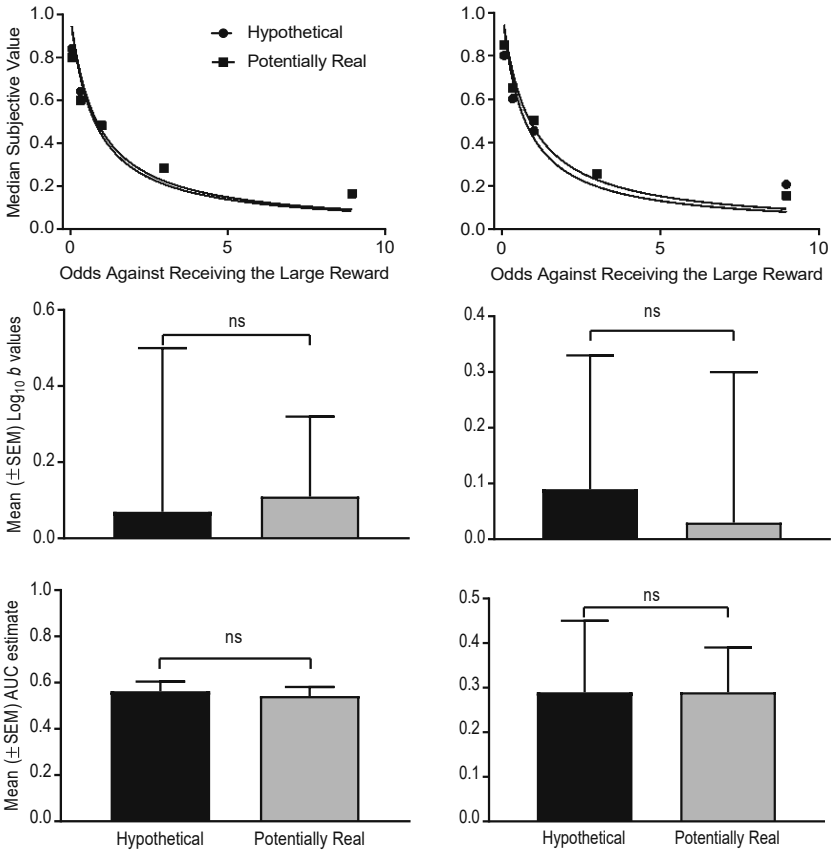

Fig. 2 Comparison of probability discounting for hypothetical and potentially real money (left) and cigarettes (right). The top panels show the median subjective value of hypothetical and real rewards. The middle panels show median (± SEM) b values calculated using the hyperbolic decay function. The lower panels show mean (± SEM) area under the curve (AUC) values

area under the curve [$F(1, 57 = 2.02, p > .05$, partial $\eta^2 = .03$) (see Fig. 2). There were no gender effects or interactions (see Fig. 3).

Discussion

The findings of the present study add to a large literature regarding important methodological aspects of the discounting paradigm, which has become one of the most commonly used behavioral measures of impulsive choice in the research literature. It also extends the relatively small number of studies on commodity-specific discounting patterns to a novel community sample of participants and raises potentially interesting questions about gender differences in discounting.

When comparing patterns of delay and PD for relatively small amounts of money (an amount approximately

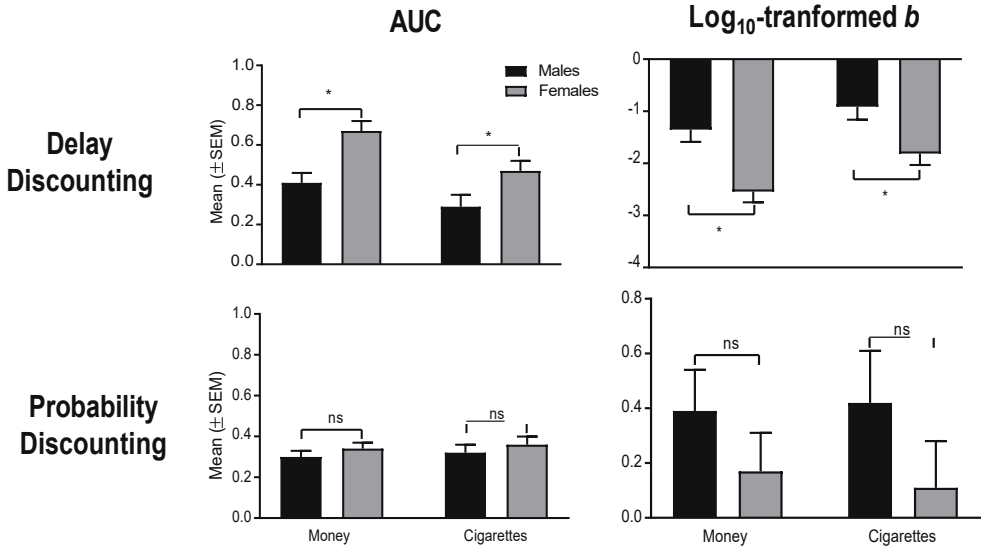

Fig. 3 Gender comparisons for delay and probability discounting for money and cigarettes (hypothetical and potentially real outcomes combined) between male (N = 26) and female (N = 33) participants

equivalent to $10USD) we found no empirical distinction between patterns of DD and PD for hypothetical and potentially real monetary rewards. This is consistent with the majority of studies comparing DD patterns for hypothetical and potentially real monetary rewards among a range of samples (Bickel et al., 2009; Johnson & Bickel, 2002; Madden et al., 2003; Lawyer et al., 2011) and, most important for this study, cigarette smokers (Lawyer et al., 2011). It is also consistent with studies published to date finding functional equivalence for PD when comparing hypothetical outcomes with both real (Hinvest & Anderson, 2010) and potentially real rewards (Lawyer et al., 2011; cf. Jikko & Okouchi, 2007).

A less clear picture emerged, though, when comparing hypothetical and potentially real cigarettes. In this study, Czech smokers exhibited more impulsive DD patterns for potentially real than for hypothetical cigarettes. It is worth noting that this difference was evident for the AUC estimates rather than the b parameter derived from the hyperbolic decay function. In this case, AUC should be considered the better metric of discounting, because the frequency of "flat" nonsystematic response patterns for DD threatens the interpretability of the b parameter of the hyperbolic decay function derived using nonlinear regression (see Johnson & Bickel, 2008). Regardless, these findings suggest that Czech smokers discount the value of potentially real cigarettes more steeply than hypothetical cigarettes, at least for DD.

These findings are consistent with Green and Lawyer's (2014) findings among U.S. smokers and suggest similar patterns among Czech smokers. This may be relevant to discounting researchers studying discounting for cigarettes across cultural contexts, but the place of these findings in the larger commodity-specific discounting literature is less clear. Our findings are inconsistent with Robertson and Rasmussen's (2018) findings that DD for potentially real versus hypothetical food is statistically equivalent. This may have some bearing on the growing literature on commodity-specific DD in which a growing number of studies examine DD in relation to an ever-increasing number of nonmonetary commodities. Commodity-specific discounting is important in light of studies suggesting that discounting for health-related commodities (e.g., sex, food) predict some human health problem behaviors better than does discounting for money (e.g., Lawyer & Schoepflin, 2013; Rasmussen et al., 2010). Taken together, these findings suggest that continued focus on methodological aspects of DD across and within cultural contexts is warranted.

Unlike DD, we found no difference between potentially real and hypothetical cigarettes on the PD task. It is not clear why we did not replicate Green and Lawyer's (2014) findings that smokers exhibited steeper PD for potentially real versus hypothetical outcomes. It is possible that the difference in findings indicates that Green and Lawyer's findings simply do not extend to Czech samples, that they represent Type I error, or that any effect for potentially real outcomes on PD for cigarettes is quite small. Given Robertson and Rasmussen's (2018) findings indicating statistical equivalence of PD for food, it would be reasonable to assert that PD for nonmonetary outcomes do not differ when they are potentially real or hypothetical, but more research on this issue would enhance confidence in such assertions.

One potentially interesting and unexpected set of findings in this study is the gender differences in the DD measures. Our findings that men discounted the value of delayed (but not probabilistic) money and cigarettes more than did women should be considered provisional because the study was not designed to test for gender differences and the relatively small sample size makes broad generalizations problematic. However, these findings might be relevant to the small and mixed literature that yields divergent findings that men are steeper discounters than women (Kirby & Marakovic, 1996; Wilson & Daly, 2004), women were steeper discounters than men (Beck & Triplett, 2009; Mahoney & Lawyer, 2018), and that there are no gender differences in discounting (e.g., Epstein et al., 2003; Mahoney & Lawyer, 2018), at least for money. An even smaller literature on gender differences in discounting for commodities other than money suggests that men tend to discount steeper than do women (Johnson & Bruner, 2012; Lawyer & Schoepflin, 2013), at least in the context of sexual rewards. Gender differences for sexual outcomes correspond well to evolutionary perspectives on gender and the value of immediate sexual opportunities (Haselton & Buss, 2000) and to data regarding gender differences in sex drive (e.g., Baumeister et al., 2001), but it is not clear why men and women might discount the value of cigarettes differently, as our data suggest. These differences cannot be explained via gender differences in dependence, because there were no gender differences on the FTCD. At any rate, conclusions about gender differences in discounting should await theory-driven research with sufficient sample sizes (or perhaps a meta-analysis) to better determine the role of gender, perhaps as a moderator of discounting patterns.

This is the first study to date to examine patterns of discounting for hypothetical versus potentially real monetary and nonmonetary rewards in a population outside the United States. The extension of findings from one culture into another represents an important step in determining the extent to which cultural factors may influence findings from decision-making studies. The small sample size in this study precludes broad assertions about how the findings speak to culturally specific patterns of discounting, but this research represents a small step toward determining the extent to which findings drawn from one nation (the United States) extend to another (the Czech Republic). Levinson and Peng (2007) argue that behavioral economics research has largely ignored the role of cultural factors in economic decision making and demonstrated that data from behavioral paradigms that might

appear to represent relatively universal processes can yield different choice patterns across different cultures. Their culturally oriented lamentation from years has gone largely unaddressed in discounting research, though Du et al. (2002) first compared discounting patterns across cultures and Kim et al. (2012) reported significant differences in discounting patterns across U.S. and Korean students. Although our article did not directly compare U.S. and Czech patterns of responding, the differences seen in Czech participants compared to similar studies suggests that the discounting literature would benefit from drawing cultural connections between behavioral economic patterns of decision making.

Availability of Data and Materials Data supporting the findings reported in this manuscript can be acquired by contacting the corresponding author.

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Declaration

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest. This research involved human subjects participants and was approved by the Czech National Institute for Mental Health Institutional Review Board prior to study commencement. All participants provided informed consent prior to their participation.

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Potentially real and hypothetical food and monetary outcomes in delay and probability discounting are similar in a Czech sample

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ABSTRACT

Most of the studies that compare potentially real (PR) to hypothetical outcomes with delay discounting (DD) and probability discounting (PD) compare monetary outcomes in American college samples and shows that hypothetical and PR monetary outcomes are discounted at similar rates. Fewer, if any, studies have examined discounting for PR vs hypothetical outcomes in non-American samples with outcomes other than money. Using a choice-questionnaire format and a within-subjects design, the relation between PR and hypothetical outcomes was examined in a Czech Republic sample. Importantly, food-related and monetary outcomes for both delay and probability discounting were examined. Sixty participants were recruited from the greater Prague area and completed four discounting tasks: the Food Choice Questionnaire, Monetary Choice Questionnaire, Probability Monetary Choice Questionnaire, and Probability Food Questionnaire in randomized order for both PR and hypothetical outcomes. Each of these measures has three magnitudes of outcomes embedded in the choices. For food-related outcomes, PR and hypothetical food outcomes were discounted similarly and significantly correlated across two of the three magnitudes of the Food Choice Questionnaire and across all three magnitudes of the Probability Food Choice Questionnaire. For monetary outcomes, PR outcomes and hypothetical outcomes were discounted similarly and were significantly correlated across all magnitudes of the Monetary Choice Questionnaire and Probability Monetary Choice Questionnaire. Magnitude effects were found across all four measures. These findings suggest that hypothetical and PR food and money outcomes are discounted similarly for both DD and PD and extends the discounting literature on similarity between real and hypothetical discounting to food-related outcomes to European community samples and discounting choice questionnaires.

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Czech republic; delay discounting; food discounting; food choice questionnaire; impulsivity; monetary choice questionnaire; potentially real outcome

Delay discounting (DD), a facet of impulsivity, is the devaluing of a commodity or outcome as the delay to its receipt increases (Ainslie, 1975; Madden & Johnson, 2010;

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Rachlin, 1995). The tendency to strongly value immediate outcomes over those that are delayed is evident in health problems such as substance use disorder and obesity, in which the immediately reinforcing properties of drugs outweigh the long-term health benefits of abstinence. Indeed, higher levels of delay discounting (i.e., impulsivity) have been found in cigarette smokers (e.g., Bickel et al., 1999), cocaine-dependent (e.g., Heil et al., 2006), heroin-dependent (e.g., Kirby et al., 1999), and obese individuals (e.g., Fields et al., 2011; Hendrickson & Rasmussen, 2013, 2017; Jarmolowicz et al., 2014; Rasmussen et al., 2010; Weller et al., 2008) relative to controls. Because discounting is associated with a number of health-related problems, it has been referred to as a trans-disease process (Bickel et al., 2012; Bickel & Mueller, 2009).

Delay discounting (DD) is measured by presenting a series of choices between a smaller, more immediate outcome vs. a larger outcome available after a delay. A typical example might be: "Would you prefer 5 USD now or 10 USD in a day?". Preferences for smaller, sooner outcomes are considered impulsive and preferences for larger, later outcomes are considered self-controlled (Bickel et al., 2012; Bickel & Mueller, 2009). Delay discounting for food has also been examined by presenting choices between smaller, sooner versus larger, delayed amounts of food (e.g., Hendrickson et al., 2015; Rasmussen et al., 2010).

Some researchers have pointed out that uncertainty is also an inherent though independent, property of delayed outcomes (Green et al., 1999; Holt et al., 2003; Myerson et al., 2003). For example, if a person chooses 1000 USD after 5 years (the longer delayed outcome) over 500 USD now, there is some doubt of whether this outcome will still be available after the 5 years elapses. Probability discounting (PD) assesses sensitivity to uncertainty or risk. With PD, individuals make choices between a series of smaller, certain outcomes vs. larger, less certain outcomes. An example might be: "Would you prefer 5 USD for certain or 10 USD with a 50% chance of receiving that outcome?". Preferences for more likely or certain outcomes (i.e., the former) are called risk averse and preferences for less certain, larger outcomes are called risky (i.e., the latter; see Estle et al., 2007; Green et al., 1999; Johnson & Bickel, 2002). Risky choice can also be a problematic behavioral pattern. For example, a person who is prone to risky choices may be more likely to gamble (i.e., keeping the sure amount of money in your pocket vs. risking more for a lower likelihood; see meta-analysis by Kyonka & Schutte, 2018) or make risky sexual choices (e.g., prefers to not use a condom because it certainly feels good over the risk of getting an STD; Berry et al., 2019)

In studies using both DD and PD with humans, hypothetical outcomes, as opposed to real outcomes, often are used for assessing choices (Jikko & Okouchi, 2007; Madden et al., 1997; Odum et al., 2006; Rasmussen et al., 2010; Robertson & Rasmussen, 2018), as giving real outcomes, especially money, to participants can be cost-prohibitive. In addition, using real items that are immediately consumable, such as food, may alter the motivating operations of the outcome (MO; see Laraway et al., 2003; Tapper, 2005) within session. For example, the MOs that are present during the first choice of a discounitng task involving food will likely not be similar to those involved in the last choice, as within-session satiation or habituation (see McSweeney, 2004) may play a role. Therefore, the use of some types of real outcomes may potentially create confounding variables.

To ensure that hypothetical and real outcomes are discounted similarly, researchers have compared delay discounting for hypothetical outcomes to those that are potentially real (PR). With PR outcomes, participants are instructed to make choices as though they will actually receive the outcome. After the task, one choice is randomly sampled and the real outcome is given to the participant. For example, of 20 delay discounting choices, one that could be sampled might be: "Would you rather have 5 USD now or 10 USD tomorrow?". If the participant chose 5 USD now, then they would receive five dollars before they left the laboratory; if they chose 10 USD tomorrow, they would receive ten dollars the next day.

The comparison of PR and hypothetical outcomes, however, has focused primarily on *delay discounting for monetary outcomes*. Moreover, participants have also almost exclusively been drawn from American college samples (e.g., Lagorio & Madden, 2005; Madden et al., 2003, 2004; Robertson & Rasmussen, 2018) or younger adult community samples that do not have clinical problems such as substance abuse disorders (Johnson & Bickel, 2002; Matusiewicz et al., 2013) – situations in which discounting may be more homogeneous among the sample. These studies all show that hypothetical and PR monetary outcomes are discounted at similar rates.

There are also some studies that have tested differences between PR and hypothetical monetary outcomes in smokers (Baker et al., 2003; Lawyer et al.; Lawyer et al.,) – a population that is more likely to show steeper discounting than non-smokers (Baker et al., 2003; Bickel et al., 1999). Like the other studies, these studies show no differences in discounting with PR vs hypothetical outcomes when monetary outcomes were used.

There are three additional studies to our knowledge that have investigated hypothetical vs. PR outcomes with commodities other than money. Robertson and Rasmussen (2018) examined the differences in PR and hypothetical food outcomes with DD and PD using a computerized adjusting amount task (Richards et al., 1999) and found PR and hypothetical outcomes were statistically equivalent. This was in a college sample, however. In two other studies, Green and Lawyer (2014) and Lawyer et al. () reported on PR vs. hypothetical comparisons with cigarettes in a sample of smokers in an American and Czech sample, respectively. Both studies replicated that discounting rates did not differ between hypothetical vs. PR monetary outcomes. However, both studies found that PR cigarettes were discounted *more strongly* for both delay and probability discounting than hypothetical cigarettes. A number of reasons for this finding is possible. One, both studies used a non-college sample of smokers, which may have increased the heterogeneity of variance of the sample, thereby allowing for larger differences in conditions. Two, the outcomes that were used were specifically related to the population of interest (i.e., cigarettes with a sample of cigarette smokers). Three, the finding may have to do with the immediately consumable nature of these outcomes (i.e., cigarettes are more immediately consumable than money). However, this latter possibility may not be the reason, as the Robertson and Rasmussen (2018) study with the immediately consumable outcomes of food did not find differences in DD or probability with PR vs hypothetical food.

Given that differences in PR and hypothetical cigarettes were found in the Green and Lawyer (2014) and the Lawyer et al. () study in community-sample of smokers across two continents and the differences were found with cigarettes, it is important to examine the robustness of similarity between PR and hypothetical outcomes in community samples with outcomes other than money. This is especially important given that especially steep

discounting has been found with the outcome of choice that is relevant to the demographic features of the sample (see Estle et al., 2007; Friedel et al., 2014; Hendrickson et al., 2015; Odum et al., 2006; Odum & Rainaud, 2003). For example, individuals with substance abuse disorders show steeper discounting for their preferred commodity (e.g., cocaine, heroin) compared to money (Heil et al., 2006; Madden et al., 1997; Petry, 2001). Further, obese individuals exhibit steeper discounting for food, but not lower-magnitude amounts of money (e.g., Hendrickson & Rasmussen, 2013, 2017; Hendrickson et al., 2015; Rasmussen et al., 2010). These studies, however, have all used hypothetical outcomes. It is possible that consumable items that are relevant to the population of interest, such as food to obese individuals, may be discounted more steeply when outcomes are PR, rather than hypothetical. Therefore, the extent to which differences between PR vs hypothetical outcomes exist should be tested on community samples, and this testing should include non-monetary outcomes.

The current study had two aims that involved replication and extension of findings from comparing PR and hypothetical outcomes with discounting. First, we tested the extent to which DD and PD for food differed as a function of hypothetical or PR outcome type in a community sample from the Czech Republic. Using a community sample from a European nation extends the hypothetical-PR similarity trend beyond the studies that are published with American college samples. We included also included PD in our study, as few studies test differences between PR and hypothetical outcomes using this process that is related to DD. Second, we attempted to replicate the extent to which DD and PD for money differed as a function of hypothetical or PR outcome type in this same sample. Importantly, to extend the literature, we tested both aims using choice ques-tionnaires (Hendrickson et al., 2015; Kirby & Maraković, 1996). To date, there are no published studies that compare PR vs hypothetical outcomes using choice question-naires. Using different measures of discounting to determine the extent to which PR and hypothetical outcomes are similar builds confidence in the ability to use them inter-changeably in studies. We hypothesized that DD and PD would be similar for real and hypothetical food and monetary outcomes.

Method

Participants

A power analysis assuming a 2 × 3 ANOVA with repeated measures, an effect size of 0.4, and power of 0.95 (alpha = 0.05), yielded a suggested *n* of 52. Sixty participants (80% female) were recruited from the community of Prague, Czech Republic via daily news-paper advertisements, fliers placed at subway stations, and ads through social media such as Facebook. Once the participant was contacted, they scheduled a time to come to the laboratory for one session. The data were collected at the National Institute of Mental Health in Klecany. Table 1 shows demographic characteristics of the sample. Participants were predominantly female and the average age of 42.9 (*SEM* = *2.23*) years old.

Table 1. Demographic Characteristics of Sample.

	Total (N = 60)
	Mean (S.E.)
Age	42.86 (2.23)
Gender (% female)	80%
Nationality (Czech)	98%
Income	1,200,000 Kč (19,993)
Currently employed	71.6%
%Married#	45%
Graduated High School or above	98%
Religious Preference (%None)	70%
Weight (kg)	72.65 (1.59)
BMI	25.32 (0.49)
% Body Fat	28.41 (0.95)
Subjective hunger (0–100)	34.17 (3.66)
Alcohol abuse (AUDIT)	7.25 (0.85)
Drug abuse (DAST)	0.95 (0.29)
Hours since last meal	3.14 (0.69)
Hours since last snack	2.81 (0.29)

Measures

Food Choice Questionnaire (FCQ). The FCQ (α = 0.92; Hendrickson et al., 2015) is a 27-item measure of delay discounting for food-related outcomes across small (8–13 bites), medium (25–35 bites), and large (40–50 bites) magnitudes. There are nine choices for each magnitude. Before administering the FCQ, a 5/8-in white cube is placed in front of the participant and they are asked to imagine it is a bite of her favorite food. Within each magnitude, individuals are instructed to make choices between two hypothetical food outcomes in which one of the food outcomes is available immediately (e.g., 4 bites now) and the other is available after a delay (e.g., 8 bites in 1 hour). The range of delays for the choices is 1/2 to 24 hours. Impulsivity values are calculated for each of the three magnitudes. See Hendrickson et al. (2015) for scoring of DD values.

Money Choice Questionnaire (MCQ)

The MCQ (α = 0.92; Kirby & Maraković, 1996; Kirby et al., 1999) is a 27-item of delay discounting for monetary outcomes across small (USD$25-$35; CK equivalent: 528-740 USD), medium (USD$50-$60; CK$1058-1270), and large (USD$75-$85; CK$1587-1799) magnitudes. There are nine choices for each magnitude. Individuals are presented with choices between a smaller, immediately available amount of money vs. a larger, delay amount of money though the money values and delay range differ (1–360 days). See Kirby and Maraković (1996) for scoring of DD values.

Probability Choice Questionnaires for Money

The Probabilistic Money Choice Questionnaire (PMCQ) (α = 0.94; Madden et al., 2009) is a 30-item measure of probability discounting (risk aversion) in which an individual

makes choices between smaller, certain amounts of money versus larger, less certain amounts of money. It estimates discounting rates across small (USD$20 vs. 80 USD; CK $423 vs 1693 USD), medium (USD$40 vs. 100 USD; CK$846 vs. 2116 USD), and large (USD$40 vs. 60 USD; CK$846 vs. 1270 USD) magnitudes (ten choice questions for each magnitude). See Madden et al. (2009) for scoring.

Probabilistic Food Choice Questionnaire

The Probabilistic Food Choice Questionnaire (PFCQ; α = 0.93; Rodriguez et al., 2018) is a 39-item measure of probability discounting for food outcomes that was adapted from the FCQ and PMCQ. The measure estimates food discounting across small (8–14 bites), medium (26–36 bites), and large (40–50 bites) magnitudes (13 choices for each magnitude). For each magnitude, individuals select between smaller, certain amounts of food (e.g., 15 bites for sure) vs. larger, less certain amounts (e.g., 75% chance of receiving 30 bites). See Rodriguez et al. (2018) for scoring.

Physical Measurements

Researchers collected participants' heights using a two-meter portable ruler. Weight and percent body fat (PBF), and body mass index (BMI) were gathered and calculated using a Tanita 2204® body scale. PBF was calculated via the scale through bioelectric impedance. BMI was calculated by dividing weight in kgs by height in meters squared (kg/m^2).

Drug Abuse Screening Test (DAST-10)

The DAST measures problematic drug use (excludes alcohol). Ten response items are coded as "yes" or "no" to produce a total score from 0 to 10. This measure was used to control for substance use, which has been shown to be associated with steeper discounting (see e.g., W. K. Bickel et al., 2014).

Alcohol Use Disorders Identification Test-C (AUDIT-C)

This 3-item self-report measures screens for problematic alcohol use (Bush et al., 1998). Each question has five response options and a total score ranging from 0 to 12. The higher the score, the more functional impairment the drinking behavior has. The AUDIT-C is a condensed version of the AUDIT and has similar accuracy rates (Reinert & Allen, 2007). This measure was used to control for problematic drinking, which is correlated with steeper discounting (e.g., Kollins, 2003; Petry, 2001).

Weschler Adult Intelligence Scale-III (Information and Similarities) interview (WAIS-III). A clinical psychologist conducted this interview with each participant as a quick assessment of cognitive ability. Scoring higher than an 8 on at least one of two subtests ensured there were no intellectual disabilities as intellectual function has been shown to be inversely related to discounting (e.g., De Wit et al., 2007).

Procedure

Participants were asked not to eat for at least 2 hours prior to the session. Upon arrival to the experiment, they were seated individually in a quiet room with the researcher. Participants then provided informed consent. After consent, participants were asked how long since their last meal and snack, and to rate how hungry they felt on a scale of 0–100 (0 = no hunger, 100 = extremely hungry).

Participants were then administered the FCQ, MCQ, PMCQ and PFCQ twice – one with potentially real outcomes and one with hypothetical outcomes. The order of measures and real vs hypothetical outcomes was counterbalanced across participants to eliminate order effects.

Under the hypothetical outcome type measures, participants were instructed to answer as if they were going to actually receive the rewards, but no rewards would be given to them. Under the potentially real (PR) condition, participants were given the same instructions, but told that at the end of the task they would randomly actually receive the actual outcome of one of their choices and they were given the actual outcome.

After the second discounting task, participants also were asked to complete a series of questionnaires via paper and pencil about demographics (e.g., gender, income, religion, marital status, etc.), as well as information regarding health practices (i.e., DAST-10 and AUDIT). Participants also completed the WAIS-III subscale interview. Researchers then measured the participant's height, weight, and percent body fat.

Finally, the outcome for the PR condition was determined. Participants drew a slip of paper from a bag indicating a specific trial from either the PD or DD task. If they drew the probability discounting alternative, the specific trial from the probability discounting questions was determined by drawing a slip of paper from a second bag. If, for that trial, the participant chose a certain outcome, they receive that outcome. If instead the participant chose a probabilistic outcome for that trial, they were presented a bag containing poker chips. The bag contained two different colors of poker chips with the distribution of each color corresponding to the probability. For instance, if the probability was 50%, the bag contained 5 poker chips that were green and 5 poker chips that were red. Selecting a green poker chip resulted in obtaining the outcome and selecting the red poker chip resulted in receiving no outcome. In reality, everyone received the same outcome (100 CZK) immediately due to the time and dificulty of delivering the rewards after the delay. For the FCQ/PFCQ, participants were given a choice between salty snacks including crackers, nuts or grits (a Czech salty crunchy snack) and sweets including chocolates, gummy bears or cookies. Each participant also received a chocolate bar for their participation.

Analysis

For each participant, delay discounting and probability discounting values for money and food were determined from the scoring of the MCQ and the FCQ described in Kirby and Marakovíc (1996) and Hendrickson et al. (2015), respectively. Briefly, each choice item on the measures has a predetermined *k* value that corresponds to indifference. If the participant chooses the smaller, sooner reward, their discounting rate is greater than the

predetermined *k* value. For the subsequent choice(s), if the participant chooses the larger, later value (preference reversal), the *k* value for that participant would be the geometric mean of the pre-determined *k* values on the current and last choice. If a participant had >1 preference reversal, a geometric mean of the *k* values that correspond to those preference reversals is calculated. A higher delay discounting value represents higher impulsivity for the delay discounting measures. Lower probability discounting values represent higher risk aversion (lower risky choice). Values for probability discounting for money and food were determined similarly based on analyses described by Madden et al. (2009) and Rodriguez et al. (2018), respectively.

The degree to which PR and hypothetical outcomes were related was tested in two ways. First, discounting data were heavily skewed, so values were log-transformed before analyses were conducted – a common practice in discounting research. A two-way repeated measures ANOVA with magnitude (three levels) and outcome type (PR vs. hypothetical) were used to determine statistical significance separately for four dependent variables: food DD, food PD, money DD, and money PD. It was hypothesized that a main effect of magnitude would be found, but there would be no differences between PR and hypothetical outcomes or an interaction.

Second, because rejecting the null hypothesis does not mean that two variables are related, another set of analyses was conducted. Similar to other studies that compare real and PR outcomes (Johnson & Bickel, 2002; Lagorio & Madden, 2005; Lawyer et al., 2011; Madden et al., 2003, 2004), we conducted Pearson product-moment correlations on the PR and hypothetical discounting data to determine the extent to which there were related. We hypothesized that the correlation between real and hypothetical outcomes would be positive and significant. Finally, we examined correlations of demographic and health variables, such as BMI, substance use, etc. with discounting. Any significant correlations would be statistically controlled analyses using an ANCOVA.

Results

Delay Discounting for Hypothetical versus Potential Real Monetary Outcomes

Figure 1 shows log-transformed delay discounting rates for hypothetical and PR monetary outcome types across three magnitudes of values from the MCQ. A two-way ANOVA with repeated measures revealed main effects of magnitude, F (2, 218) = 61.02, $p < 0.01$, $\eta\rho^2 = 0.36$). Post hoc contrasts on magnitude showed that small magnitude money was discounted more steeply than medium ($p < 0.01$) and medium was discounted more steeply than large ($p < 0.01$). There were no main effects of outcome type (PR vs. hypothetical outcomes; $p = 0.34$; $\eta\rho^2 = 0.008$) or a magnitude X type interaction ($p = 0.97$). None of the demographic variables correlated significantly (see Table 2) to any of the magnitudes of PR or hypothetical monetary outcomes, so they were not controlled in this analysis.

To show the relation between PR and hypothetical monetary values, Pearson correlations were conducted on discounting values. There was a significant correlation between outcome type for small magnitude money ($r = 0.47$, $p < 0.01$). In addition, outcome types

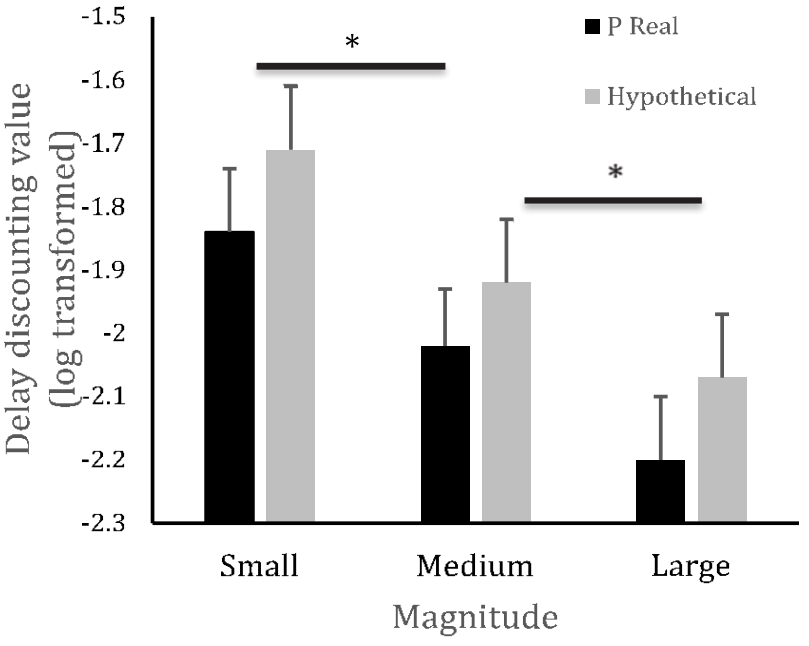

Figure 1. Mean delay discounting rates (log transformed) for potentially real and hypothetical money as a function of magnitude. Error bars = 1 SEM; *$p < 0.01$.

Table 2. Correlation Matrix for Magnitudes for Delay Discounting of Real and Hypothetical Money Outcomes.

	1	2	3	4	5	6
1. Small Hyp	–	0.85**	0.80**	0.47**	0.55**	0.54**
2. Med Hyp	0.85**	–	0.94**	0.42**	0.51**	0.52**
3. Large Hyp	0.81**	0.94**	–	0.55**	0.60**	0.64**
4. Small Real	0.47**	0.42**	0.55**	–	0.73**	0.64**
5. Med Real	0.55**	0.51**	0.60**	0.73**	–	0.93
6. Large Real	0.54**	0.52**	0.64**	0.64**	0.93**	–

$^{**}p < 0.01$

were strongly correlated for medium ($r = 0.51$; $p < 0.01$), and large ($r = 0.64$, $p < 0.01$) magnitudes of money (see Table 2).

Delay Discounting for Hypothetical versus Potential Real Food Outcomes

Due to administration error, only 52 participants' data were useable for the food discounting portion of the study. Since 52 participants were necessary for study based on the power analysis; however, the analysis was still powered adequately.

Figure 2 shows mean discounting values (log-transformed) for PR and hypothetical food outcomes across all three food magnitudes from the FCQ. A two-way repeated measures ANOVA revealed main effects of magnitude, $F(2, 196) = 8.01$, $p < 0.01$, $\eta\rho^2 = 0.08$), with post-hoc contrasts showing small magnitude food was discounted more steeply than medium ($p < 0.01$) and large ($p < 0.01$) outcomes; there were no

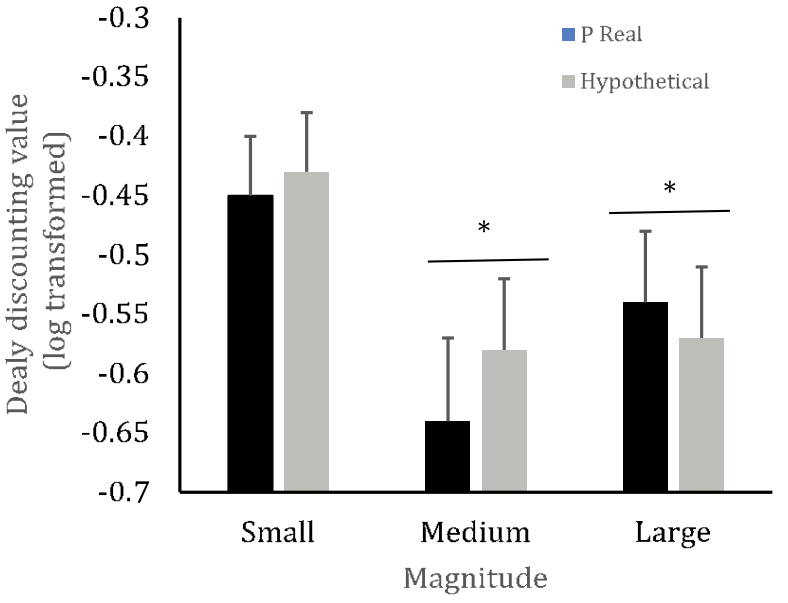

Figure 2. Mean rates of delay discounting (log transformed) for potentially real and hypothetical food as a function of magnitude. Error bars = 1 SEM; *$p < 0.01$.

differences between medium and large food outcomes. There were no main effects of outcome type ($p = 0.79$) and no interaction ($p = 0.52$). To identify potential confounding variables, food discounting was correlated with various demographic and health measures, such that significant relations would be statistically controlled in additional analyses (see Table 3). None of the measures of subjective hunger, BMI, drug use, or alcohol use correlated with any of the food discounting measures for small or large magnitude FCQ. However, for the medium magnitude with real outcomes, the drug use self-report measure was positively correlated to food discounting ($r = 0.30$, $p = 0.03$) and self-report measure for alcohol use was negatively correlated with the medium magnitude for hypothetical outcomes on the FCQ ($r = -0.33$, $p = 0.02$). Therefore, for the medium magnitude analyses, we statistically controlled for these variables; this, however, did not change the statistical outcomes.

To examine the strength of the relation between PR and hypothetical food, Pearson correlations were conducted on each food discounting magnitude (see Table 3). Values for PR and hypothetical food were significantly correlated for small ($r = 0.46$, $p < 0.01$)

Table 3. Correlation Matrix for Magnitudes of Delay Discounting for Real and Hypothetical Food Outcomes.

	1	2	3	4	5	6
1. Small Hyp	–	0.53**	0.45**	0.46**	0.59**	0.26
2. Med Hyp	0.53**	–	0.83**	0.25	0.14	0.44**
3. Large Hyp	0.45**	0.83**	–	0.17	0.06	0.59**
4. Small Real	0.46**	0.25	0.17	–	0.01	0.36*
5. Med Real	0.06	0.14	0.06	0.01	–	0.0
6. Large Real	0.26	0.44**	0.59**	0.36*	0.0	–

*$p < 0.05$
**$p < 0.01$

and large (r = 0.59, p < 0.01) magnitudes. However, for the medium magnitude, the correlation between outcome types was not significant.

Correlations for delay discounting, outcome type, and magnitude

To replicate previous research with discounting magnitude, Table 2 also shows correlations for differing magnitudes of monetary outcomes with both PR and hypothetical outcome type. Every magnitude of hypothetical money correlated significantly and strongly with one another (rs = 0.80–0.94) and every magnitude of PR money correlated significantly with one another (rs = 0.64–0.93). Importantly, all hypothetical monetary outcomes correlated significantly with every magnitude of potentially real outcomes (rs = 0.42–0.64).

Table 3 shows similar correlations for food. For food, all hypothetical outcome magnitudes correlated significantly with one another (rs = 0.45–0.83). Only small and large PR outcomes were significantly correlated (r = 0.36). Finally, small hypothetical outcomes were significantly correlated with medium potentially real outcomes (r = 0.59) and medium hypothetical outcomes were significantly correlated with large real outcomes (r = 0.44).

Probability Discounting for Hypothetical versus Potential Real Monetary and Food Outcomes

The top of Figure 3 shows probability discounting values for PR and hypothetical monetary outcomes. There was a main effect of magnitude; small magnitude money had steeper values than medium and large, F (2, 202) = 53.70, p < 0.01, $\eta\rho^2$ = 0.35. Post-hoc contrasts revealed that smaller outcomes were discounted significantly more than medium (p < 0.01) and medium more than large (p < 0.01). There was no main effect of magnitude type or an interaction (p > 0.15). None of the demographic variables were correlated with monetary or food PD, so were not statistically controlled in the analyses (Table 4).

Table 4 also shows there were significant correlations between real and hypothetical outcomes for small (r = 0.35, p < 0.05), medium (r = 0.56, p < 0.01), and large (r = 0.58, p < 0.01) probability monetary outcomes. Table 4 also shows all magnitudes of PR and hypothetical monetary outcomes. All magnitudes of hypothetical monetary outcomes strongly and significantly correlated with one another (rs = 0.53–0.80) and all magnitudes of PR monetary outcomes strongly and significantly correlated with one another (rs = -0.54–0.88). All magnitudes of real and hypothetical monetary outcomes correlated significantly with one another as well (rs = 0.34–0.58).

The bottom of Figure 3 shows probability discounting for real and hypothetical food outcomes. There was a main effect of magnitude, F (2, 204) = 16.03, p < 0.01, $\eta\rho^2$ = 0.14, but no main effect of outcome type (p = 0.84), or an interaction (p = 0.71). No demographic or health variables were correlated with probability discounting for food (Table 5), so none were controlled in these analyses.

Table 5 shows there were significant correlations between real and hypothetical food outcomes for small (r = 0.46, p < 0.01), medium (r = 0.54, p < 0.01), and large (r = 0.58, p < 0.01) magnitude probability discounting. In addition, all magnitudes of PR food

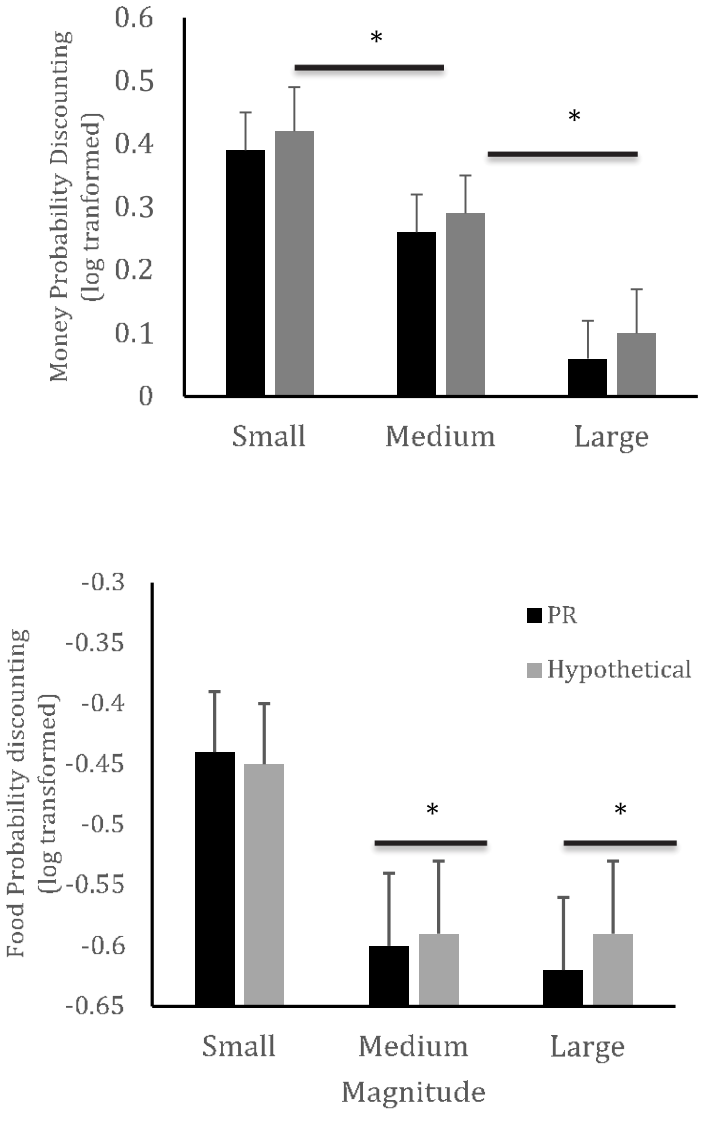

Figure 3. Mean rates of probability discounting for potentially real and hypothetical money (top) and food (bottom) as a function of magnitude. Error bars = 1 SEM; *$p < 0.01$ magnitude differences.

outcomes strongly and significantly correlated with one another (*rs* = 0.53–0.85) and all magnitudes of hypothetical food outcomes strongly and significantly correlated with one another (*rs* = 0.61–0.93). All magnitudes of real and hypothetical food outcomes correlated significantly with one another as well (*rs* = 0.37–0.58).

Discussion

This study compared delay and probability discounting for PR vs. hypothetical monetary outcomes and PR vs. hypothetical food-related outcomes in a community sample of participants in the Czech Republic. There were no significant differences between

Table 4. Correlation Matrix for Magnitudes of Probability Discounting for Real and Hypothetical Monetary Outcomes.

	1	2	3	4	5	6
1. Small Hyp	–	0.88**	0.67**	0.35*	0.48**	0.40**
2. Med Hyp	0.88**	–	0.80**	0.34*	0.56**	0.47**
3. Large Hyp	0.67**	0.80**	–	0.36*	0.51**	0.58**
4. Small Real	0.35*	0.34*	0.36*	–	0.80**	0.54**
5. Med Real	0.48**	0.56**	0.51**	0.80**	–	0.76**
6. Large Real	0.40**	0.47**	0.58**	0.54**	0.76**	–

*$p < 0.05$
**$p < 0.01$

Table 5. Correlation Matrix for Magnitudes of Probability Discounting for Real and Hypothetical Food Outcomes.

	1	2	3	4	5	6
1. Small Hyp	–	0.63**	0.61**	0.46**	0.48**	0.40**
2. Med Hyp	0.63**	–	0.93**	0.39**	0.54**	0.53**
3. Large Hyp	0.61**	0.93**	–	0.37**	0.54**	0.58**
4. Small Real	0.46**	0.39**	0.37**	–	0.72**	0.53**
5. Med Real		0.54**	0.54	0.72**	–	0.85**
6. Large Real	0.40**	0.53**	0.58**	0.53**	0.85**	–

*$p < 0.05$
**$p < 0.01$

monetary DD for PR vs. hypothetical monetary outcomes using the MCQ at any of the three magnitudes. In addition, correlational data showed that PR and hypothetical money outcomes were significantly and strongly related across all three magnitudes. These findings replicate previous research (Baker et al., 2003; Johnson & Bickel, 2002; Lawyer et al., 2011; Madden et al., 2003, 2004; Robertson & Rasmussen, 2018) that discounting for hypothetical and potentially real monetary outcomes are directionally related; in other words, individuals who are impulsive for real monetary outcomes tend to also be impulsive for hypothetical monetary outcomes and that the values are strongly related.

The results also replicate and extend the similarity of real and hypothetical effects in several important ways. First, the replication occurred with a European sample. To date, most of the data on this topic are from American college samples. Most (98%) of the sample in the current study was Czech. Importantly, the current sample was middle aged (mean = 42 years old). Most graduated high school (98%) and 45% had graduated college. The sample was primarily female, and of middle-class income (1,200,000 koruna = ~ USD$53,700). Most were employed (70%). The sample was also of normal BMI on average. This sample differed somewhat from American samples and college samples used in other PR vs hypothetical studies in that the sample was older, employed, and had lower BMI (mean BMI in America is 26.5 or overweight; Center for Disease Control, 2020).

The replication of similarity of PR vs hypothetical monetary outcomes in samples from more than one continent, culture, and nation increases confidence in the use of PR vs. hypothetical outcomes across a broader range of individuals. The racial, ethnic, and socioeconomic diversity of the sample, however, was quite homogeneous. In addition, the vast majority of the sample was female, which also limits generalization. Future studies should consider using samples beyond those of European descent and with

community samples that have a broader range of socioeconomic diversity to gain a fuller characterization of racial, ethnic, cultural, gender-based, and socioeconomic characteristics in discounting. For example, food insecurity – an indicator of poverty – has been shown to be associated with steeper discounting for food and money (Rodriguez et al., 2020).

Second, in the present study the similarity between PR vs. hypothetical outcomes in discounting was also extended to choice questionnaires. Since the original MCQ was developed by Kirby and Maraković (1996), a number of choice questionnaires have been developed for measuring discounting with other commodities such as food and cigarettes (e.g., Hendrickson et al., 2015; McKillop, et al, 2011). Choice questionnaires have the advantage of quickly generating discounting values in less time than other discounting measures and with a wider range of subjects that include younger participants (Hendrickson & Rasmussen, 2017; Hendrickson et al., 2015). Moreover, discounting values generated from the MCQ and FCQ correlate well with computerized versions of monetary and food delay discounting, respectively (Hendrickson et al., 2015). It is important to determine the extent to which the results generated by choice questionnaires generalize to more behavioral discounting procedures by demonstrating phenomena such as magnitude effects and similarity between PR vs hypothetical outcomes, etc. This study is the first to show that real vs hypothetical outcomes are similar across magnitudes with the MCQ, FCQ, PMCQ, and PFCQ.

Food delay discounting

There were also no statistical differences between PR vs. hypothetical food outcomes at any of the three magnitudes of the FCQ. Importantly, however, hypothetical food outcomes were significantly related to PR outcomes for both small and large magnitudes though not medium outcomes. For small magnitude (8–13 bites), these results replicate and extend findings from Robertson and Rasmussen (2018), who reported bites of food in the small magnitude range (10 or fewer) are discounted similarly (i.e., they are statistically equivalent) whether they are PR or hypothetical. Though the discounting procedures were different (computerized adjusting amount in Robertson & Rasmussen; choice questionnaires in the present study), the delay range for the Robertson and Rasmussen (2018) paper was similar (1–20 hours) to the small magnitude range of delays in the current paper (1/2 hr to 24 hrs). The results, then, extend the similarity of PR and hypothetical food from smaller bites to larger magnitude bites (40–50) and to discounting that is quantified by choice questionnaires.

Though there was no statistical difference between PR and hypothetical food outcome for the medium magnitude, there was no significant correlation between them, meaning that the relation was not robust. It may be the case that the amount used in the discounting task is indeed important for hypothetical vs real food outcome similarity. Moreover, it is noteworthy to point out the relations between the small, medium and large magnitudes across real and hypothetical food outcomes was nuanced compared to those of the MCQ which were consistent and robust. This may not be surprising, however, given that money is a more generalizable reinforcer compared to food (a primary reinforcer). Indeed, the reinforcing value of money is less sensitive to establishing operations such as deprivation and properties related to food such as perishability

and the ability to be immediately consumed are less relevant. Money is also more fungible than food. Indeed, perishability, and low fungibility have been shown to affect discounting rates (e.g., Holt et al., 2016). This study indeed supports why money is a useful outcome to use in discounting studies. However, other outcomes that may be more sensitive to other variables (such as deprivation, perishability, etc.), such as food, drug, or sex, also show utility in discounting because they are often related to reinforcer pathologies, such as obesity or drug abuse (see W. K. Bickel et al., 2014; MacKillop et al., 2011). More research is needed to understand factors that affect non-monetary discounting, such as food.

Probability discounting for money and food

The PMCQ produced PD data that did not differ for PR vs. hypothetical outcomes across three magnitudes of money. Moreover, PR and hypothetical outcomes correlated significantly with one another across all magnitudes. These data replicate and extend research on PD for monetary outcomes that compares PR vs. hypothetical outcomes (e.g., Hinvest & Anderson, 2010; Jikko & Okouchi, 2007; Matusiewicz, et al., 2013) by extending similarity of PR vs hypothetical monetary outcomes to probability choice questionnaires.

For the PFCQ, values for PR vs hypothetical outcomes were significantly and strongly correlated across all three magnitudes. This is the first study to compare real vs hypothetical food outcomes with PD using a choice questionnaire; the results support those of Robertson and Rasmussen (2018), which showed similarity of PR and hypothetical food using a computerized PD task; therefore the present study replicates and extends the results of Robertson and Rasmussen (2018).

Magnitude

Importantly, magnitude effects were found with both money and food delay discounting. For money, small magnitude money was more steeply discounted than medium magnitude and medium magnitude was discounted more steeply than large magnitude money. This pattern replicates other research (e.g., Kirby & Maraković, 1996, 1996). In addition, small magnitude food was discounted more steeply than medium and large amounts. This magnitude effect also replicates other research with the FCQ (Hendrickson & Rasmussen, 2017; Hendrickson et al., 2015) and further validates the use of the FCQ to measure food discounting.

A magnitude effect was also found for PD for money and food, but in a direction that is inverse to what has been published with PD. Typically with PD, as magnitude of the outcome increases, rates of discounting increase (e.g., Green et al., 1999; Rodriguez et al., 2018; Weatherly & Terrell, 2014), though in the original study with the PMCQ (Madden et al., 2009), there were no magnitude differences. In the present study, smaller amounts of PR and hypothetical money and food were discounted more steeply than medium and large amounts. It is unclear why an inverse relation in magnitude and discounting was found with the PMCQ and PFCQ in this study, but may have to do with the nature of parameters of the choice questionnaire. One major difference of the PMCQ compared to other probability discounting tasks is that the probabilities on the PMCQ are more

constricted. For example, participants choose differing amounts of money across three magnitudes of specific probabilities. This compares to other typical PD studies which use a full range of probabilities (0–1.0) (e.g., Holt et al., 2003; Myerson et al., 2003; Rasmussen et al., 2010; Robertson & Rasmussen, 2018; Weatherly & Terrell, 2014). More research may be required to understand why a reverse magnitude effect was found for these measures.

In this study, some of the demographic variables that have previously been associated with discounting were not strong predictors of discounting. Alcohol and drug use (e.g., Heil et al., 2006; W. K. Bickel et al., 2014; Petry, 2001) were not robust predictors of monetary discounting in this sample, but the base rate of these variables (especially clinical-level alcohol and drug use) was quite low in the sample. Indeed, the relation between non-clinical drinking and discounting is inconsistent. MacKillop et al. (2007), for example, found no difference in hazardous drinkers and controls while a meta-analysis by MacKillop et al. (2011) found only a small effect size (0.26) in the relation between drinking and discounting. Moreover, obesity effects reported previously (Hendrickson & Rasmussen, 2013; Hendrickson et al., 2015; Jarmolowicz et al., 2014; Rasmussen et al., 2010; Weller et al., 2008) were not seen with money or food discounting in the current sample. However, the distribution of the sample skewed strongly toward thinness. Therefore, it is not surprising that these variables did not predict discounting processes in this sample.

In sum, this study replicates and extends the research on PR vs. hypothetical money and food outcomes in several important ways. Similarity of outcome type was replicated for DD and PD with a non-American sample using choice questionnaires with monetary and non-monetary outcomes. The effects were replicated across three magnitudes for DD and PD for money, for two of three magnitudes for food DD, and across all three magnitudes for food PD. Future research should focus on examining the nuances of non-monetary outcomes in discounting and the use of participant samples that vary on different cultural and socioeconomic dimensions.

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