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Examiner's Report

Doctoral dissertation of **Mohammed Farrag** entitled "*Molecular Pathology of Selected Porphyrias with Skin Manifestation*".

Field of study: Biochemistry and Pathobiochemistry

Training Institution: Department of Pediatrics and Adolescent Medicine 1st Medical Faculty of the Charles University

Supervising Professor: Pavel Martasek, M.D., D.Sc.,

Nature of the work: Inherited disorders of heme biosynthesis, collectively referred to as porphyrias, can be life threatening or substantially diminish the quality of life. Although the genes involved in heme biosynthesis and mutations thereof have been known for quite some time, therapeutic intervention strategies are inadequate in large part due to the lack of genotype-phenotype correlation in these diseases. Furthermore, molecular dissection of mutation outcomes is also lacking. The PhD dissertation of Mr. Farrag attempts to rectify this paucity of data by revealing a new homozygous mutation (in Arabic patients) in uroporphyrinogen decarboxylase (UROD), causing porphyria cutanea tarda (PCT). It identifies how this mutation is likely to impact protein structure and activity. Furthermore it nicely combines molecular genetics, biochemistry, and epidemiological results under one roof to underscore how defects in UROD can be elucidated. Mr. Farrag's dissertation also highlights the discovery of a novel mutation in ferrochelatase, causing erythropoietic protoporphyria in Czech patients. The work is timely and is of great interest to the porphyria community. It is also relevant to those interested in how naturally occurring mutations impact protein structure and activity.

Organization of the dissertation: The overall makeup of the dissertation is solid and acceptable. The studies have been described in adequate detail, with careful attention paid to what is already known in the literature and how this investigation sheds new light on the topic. The dissertation is clearly hypothesis driven and the overall objectives are laid out clearly. The nature of the system and its suitability for probing is also elaborated well. The diverse methodologies and approaches are outlined in sufficient depth for independent reproduction in the two publications authored by Mr. Farrag. The

results presented in both tables and figures are satisfactory. Finally, the overall summary and discussion portions of the dissertation place in perspective the results found here with those that are already in the literature. Taken together, the dissertation is very good.

Questions: (a) Currently, the Human Gene Mutation Database lists 78 missense mutations in UroD. These do not cluster in any one area, but are distributed throughout the polypeptide chain. Needless to say, all of them cannot interfere with substrate binding! What is the general consensus regarding how these mutations cause disease? (b) Whereas F55I mutation in UROD has been shown to cause disease, would a conservative substitution of the type F55Y expected to alter activity? (c) What do molecular modeling studies reveal in this regard? (d) The 2003 article of Phillips et al describing the crystal structure of UroD only showed molecular interactions between the protein and product analogs (coproporphyrinogen I, III). It did not have any experimental data on how substrate (uroporphyrinogen) is recognized by UROD. Did the modeling in this dissertation identify how the substrate would reside in the active site pocket and whether F55I would interfere with its positioning? (e) One of the accepted manuscripts indicates that "the relatively moderate skin problems of the children correlate well with the high residual activity of UROD". As the proband is homozygous for F55I, how would this correspond to significant perturbation of the UROD active site vis-à-vis substrate binding? (f) Similarly, does the 19% activity found for heterologously expressed F55I-UROD is representative of "high residual activity" in the patient? (g) Based on what is known about PCT, how could we improve on existing therapeutic approaches for treating patients with moderate to severe disease?

Summary: The PhD dissertation of Mr. Mohammed Farrag has the potential to generate new therapeutic intervention strategies for PCT. Therefore, I strongly recommend that the "Biochemistry and Pathobiochemistry" PhD committee accept this dissertation, in its present form, as the basis for conferring Doctor of Philosophy degree to Mr. Mohammed Farrag.

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