only marginally higher with valine408 than with methionine408. Our results are in line with the previously reported lack of relevant differences in MPP\(^+\) uptake\(^5\) and do not confirm the strong effects of the Met408Val polymorphisms on TEA\(^+\) uptake reported by Giannoudis et al.\(^1\) We did not use imatinib in this study because the validity of the Giannoudis et al data that suggest OCT1-mediated transport of imatinib has been questioned\(^9\) and recent attempts to demonstrate imatinib transport by OCT1 failed.\(^10\)

Finally, careful inspection of the three-dimensional (3D) model published by Giannoudis et al\(^1\) used to explain the interactions between Met408Val and Met420del reveals that the model comprises only of 11 instead of 12 transmembrane helices (helix 1 is missing). Using the same approach and software, we obtained a 3D model with a helical cytoplasmic-loop domain between helices 6 and 7 that did not contain a β-sheet structure as described by Giannoudis et al. The long cytoplasmic loop is conserved in structurally homologous transporters like the glucose/H\(^+\) symporter (Protein Data Bank entry 4LDS) and Xyle (Protein Data Bank entries 4GC0 and 4JA4). In all these structures, the intracellular loop forms a compact arrangement of 3 helices without β sheets. Consistently, secondary structure predictions using the software Jpred also propose a helical structure for the loop segment. Therefore, the conclusion of Giannoudis et al that the mutations alter the intracellular loop conformation seems very speculative given the fact that the applied modeling procedure seems to highly influence the predicted 3D structure.

In conclusion, our study demonstrates that the Met420 deletion exists exclusively together with the Val408 allele in humans and suggests that the Met420 deletion leads to substrate-specific loss of activity that is only marginally affected by the Met408Val polymorphism.

References


To the editor:

Heckathorn disease revisited

In 1975, Ratnoff and Lewis described a family with an X-linked, moderately severe, inherited bleeding disorder and named it Heckathorn disease after the 34-year-old index patient (patient V-14, Figure 1).\(^1\) The patient and his maternal uncle (IV-3), also a bleeder, underwent extensive investigations and were found to have factor VIII deficiency. The functional antihemophilic factor (AHF) level varied from 0.10 to 0.75 U/mL over a 6-year period in patient V-14 and from 0.10 and 1.20 U/mL over a 3-month period in patient IV-3. Four women (IV-5, V-12, VI-5, and VI-8) were identified as obligate carriers. The authors concluded that Heckathorn disease represents
as eparaté for mo of A HFd e
ciency and its chief distinguishing feature
was the fluctuation of AHF in affected men. They believed that, in
time, other families with Heckathorn disease would be identifi
ced. The index patient’s daughter (patient VI-8), who was born in
1964 and had no children, was seen in August 2013 for clearance
prior to her spinal surgery. She bruised easily but did not bleed
excessively. Her physical examination was unremarkable. A com-
plete blood count, prothrombin time, partial thromboplastin time,
factor VIII activity (which was 80% and 121% on 2 occasions),
von Willebrand factor studies, and platelet aggregation were
normal. Factor VIII gene sequencing showed 1 mild pathogenic
mutation (1 copy) with nucleotide change c.908C>A. The amino
acid alteration was p. ALA303GLU (traditional nomenclature:
ALA284GLU). This finding established her as a carrier of hemo-
philia A.

Factor VIII is a single-chain peptide with multiple domains
known as A1-A2-B-A3-C1-C2.2-4 During clot formation, cleavage
of factor VIII increases its procoagulant activity followed by first-
order decay in the procoagulant activity and generation of a hetero-
dimer consisting of the A1 and A2 subunits. The A2 subunit
dissociates from the A1/A3-C1-C2 in a pH-dependent manner
resulting in loss of procoagulant activity.5 Normally, ARG531 lies at
the interface of the A1 and A2 subunits. Mutations that directly
contact ARG531 (ALA284GLU, ALA284PRO) or are adjacent to it
(SER289LEU) are associated with lower clotting activity, especially
in assays that use incubation, as is done in 2-stage assays. The A2
subunit can also dissociate spontaneously.5,6

There are 3 hemophilia mutations involving the ARG531 or
adjacent region that show increased dissociation of A2 subunit.
These are ALA284GLU, ALA284PRO, and SER289LEU. In vitro
studies of these missense mutations show a twofold lower activity
when the factor VIII assay is done with incubation. When cleaved
by thrombin, A2 dissociation is threefold faster for SER289LEU and
ALA284GLU.6

Fluctuations in factor VIII levels seen by Ratnoff and Lewis5 may
have resulted from their methods, which involved incubation of
plasma7,8 and measurement of AHF activity at 2 different institutions
at different times. Also, factor VIII levels are influenced by age,
glucose, and insulin levels, fibrinogen level, pregnancy, surgery,
hemolytic anemia, and hyperthyroidism.9

In summary, we have shown that Heckathorn disease is hemophilia
A due to a missense mutation resulting in nucleotide change c.908C>A
and amino acid alteration p. ALA303GLU (traditional nomenclature:
ALA284GLU).

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Contribution: S.N. and A.K. designed the study. All authors interpreted the data
and wrote and revised the manuscript.

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