outgrowth resulting a JMML-like MDS/MPN disease. Patient J384 died within 3 months of his initial diagnosis without undergoing transplant. Cryopreserved bone marrow was not available to define the acquisition of pathogenic sequence variants in this patient.

This case is the first description of GATA2 mutations in a patient suspected of having JMML but who, on retrospective review, did not manifest all of the classic features. In addition, this is the first report of biallelic GATA2 mutations in a hematologic malignancy. Finally, this is also the first reported case of a concurrent KRAS mutation and monosomy 7 in a patient with a presumed germline GATA2 mutation. Although GATA2 mutations are rare in JMML, this work expands the spectrum of hematologic cancers associated with germline GATA2 mutations and supports sequencing this gene in patients with atypical features of JMML, particularly those with monosomy 7.

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To the editor:

Does the haplotype Met408-Del420, which was apparently predictive for imatinib efficacy, really exist and how strongly may it affect OCT1 activity?

Recently, the Met408-Del420 haplotype in the OCT1 gene (the combination of methionine at codon 408 and a deletion of another methionine at codon 420) was associated with lack of transport activity for imatinib and tetraethylammonium (TEA⁺) and with poor clinical outcome in chronic myeloid leukemia patients.¹
To explore the relevance of the Met408-Del420 haplotype in other clinical contexts, we genotyped 371 Caucasians who were previously studied for the effects of OCT1 polymorphisms on pharmacokinetics or efficacy of metformin, tropisetron, or tramadol.2-4 To our great surprise, none of the homozygous Met408 carriers carried the Met420-deletion allele (Figure 1A). In comparison, 52% of the homozygous Val408 carriers carried the Met420 deletion (12 homozygous and 36 heterozygous carriers; 9% of the Met420 heterozygous carriers). This suggested a lack of the Met408-Del420 haplotype in our population (n = 47; Figure 1A). In comparison, the Met408Val and Met420del genotypes in the samples of the 1000 Genomes Project.5 The haplotype combinations in human embryonic kidney 293 cells and measured the effects of Met408Val substitution were similar in size both with the Met420 and Del420 background and vice versa. This suggests independent additive effects and a lack of epistatic interaction between the 2 polymorphisms.

Figure 1. Haplotype combinations of the Met408Val and Met420del polymorphisms in the OCT1 gene in humans and their functional activity. (A-B) Combined representation of the Met408Val and Met420-deletion genotypes (A) and haplotypes (B) of Caucasians from our previous clinical studies and from selected (CEU and GRB) or all populations from the 1000 Genomes Project (the individual genotype data that was used in the calculations was obtained from http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/).5 Individual haplotypes were inferred using PHASE version 2.1.6 The genotype/haplotype frequencies are given as number (and %) of all individuals in the corresponding population. CEU, population of Utah residents (CEPH) with Northern and Western European ancestry; GRB, population of British in England and Scotland. (C) Strategy (upper 2 panels) and an example of the results (lower panel) of haplotype sequencing analyses using semiconductor-based massively parallel sequencing. A 155-bp region from the chromosome 6 was amplified via PCR. The region contained both the Met408Val and Met420-deletion loci. This haplotype-specific single-molecule DNA analysis enabled us to experimentally verify the presence of different haplotype combinations in individuals heterozygous for the Met408Val and Met420-deletion polymorphisms. The PCR products were purified, pooled together, subjected to template preparation using Ion OneTouch, and sequenced using Ion PGM (both from Life Technologies, Darmstadt, Germany). We obtained on average 19 000 sequencing reads per chromosome that covered both loci. The results were visualized using Integrative Genomics Viewer version 2.3 (Broad Institute; http://www.broadinstitute.org/igv/). Each gray line represents a single sequencing read. The reads containing the Val408 allele are denoted with a G indicating a disagreement with the presence of ATG in the human reference sequence (that is given in the bottom of the figure). This is a representative example of part of the reads of a single sample demonstrating that individuals heterozygous for both Met408 and Met420 deletion did not carry the 2 alleles on the same chromosome. (D) Comparison of the uptake activity of hOCT1 carrying all theoretically possible combinations of the Met420del and Met420Val polymorphisms. The uptake was measured in human embryonic kidney 293 cells stably transfected with the hOCT1 variants or with an empty control plasmid pcDNA3. The stably transfected cells were generated using the FlpIn system for targeted chromosomal integration (Life Technologies) following a procedure described before.7 The uptake was measured as the total cellular accumulation after a 2-minute incubation at 37°C with 1 mM MPP+ 5 μM TEA, or 5 μM ASP8. The concentrations used were below (MPP+) or close to (ASP) the known Km values for the substrates. The concentrations of TEA and MPP used in this study were identical to the one used by Giannoudis et al. and Shu et al., respectively. Two minutes of incubation were within the linear range for time dependence of the OCT1-mediated uptake. Shown are means and standard error of the means of at least 3 independent experiments each performed in duplicate. Met420 deletion caused a significant 70% decrease in TEA uptake. The decrease was less strong (18.6%) when ASP was used and missing when MPP was used as a substrate. In comparison, the exchange of Met408 to Val408 caused only a 12% increase in TEA uptake. The effects of Met408Val substitution were similar in size both with the Met420 and Del420 background and vice versa. This suggests independent additive effects and a lack of epistatic interaction between the 2 polymorphisms.

polymerase chain reaction (PCR) amplicon and resequenced the products using semiconductor-based sequencing. In all double-heterozygous individuals in our population (n = 47; Figure 1A), we found only the Val408-Del420 and not the Met408-Del420 haplotype (Figure 1C). To confirm that this finding was not restricted to our population, we also analyzed the Met408Val and Met420del genotypes in the samples of the 1000 Genomes Project.4 The haplotype Met408-Del420 was not present in other European populations or worldwide (Figure 1A-B).

Secondly, we expressed all 4 possible Met408Val and Met420del combinations in human embryonic kidney 293 cells and measured transport of 1-methyl-4-phenylpyridinium+ (MPP+), TEA+, and 4-(4-dimethylamino) styryl-N-methylpyridinium+ (ASP+). We observed strong, substrate-specific effects of the Met420 deletion on OCT1 activity. However, the activity of the Met420 deletion was
only marginally higher with valine408 than with methionine408. Our results are in line with the previously reported lack of relevant differences in MPP⁺ uptake⁸ 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 questioned⁹ and recent attempts to demonstrate imatinib transport by OCT1 failed.¹⁰

Finally, careful inspection of the three-dimensional (3D) model published by Giannoudis et al¹ 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 α-helical structures 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

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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

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Does the haplotype Met408-Del420, which was apparently predictive for imatinib efficacy, really exist and how strongly may it affect OCT1 activity?

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