Simple and Effective Strategies for Detection of Allele Dropout in PCR-Based Diagnosis of Wilson Disease

To the Editor:

Drs. Lam and Mak, in a recent article in this journal (1), described the mechanisms leading to allele dropout in the PCR-based diagnosis of Wilson disease (WD) and reported potential solutions to this problem. We propose 2 strategies that would enable unequivocal and rapid identification of allele dropout in WD.

In WD, an autosomal recessive disorder, mutations in the \textit{ATP7B} gene lead to accumulation of copper in the liver and the brain (2). In PCR-based detection of WD carriers and presymptomatic individuals in affected families, the presence of numerous single-base variations in the \textit{ATP7B} gene gives rise to allele dropout, the nonamplification of one of the alleles (1). In WD compound heterozygotes, if the wild-type allele corresponding to an identified mutation fails to amplify, the PCR product shows apparent homozygosity, leading to faulty diagnosis. Screening the entire \textit{ATP7B} gene to exclude allele dropout, as recommended by Lam and Mak (1), is an arduous job because \textit{ATP7B} is \(~80\) kb long with 21 exons and a coding length of more than 6.5 kb. The alternative suggestion, to design primers for PCR from intronic sequence-lacking nucleotide variants (1), can be hampered by lack of information on single-base variations, because HAPMAP project data (http://www.hapmap.org/) do not provide complete information on all the neutral nucleotide variants in different population groups.

We suggest a much simpler and faster method for detection of allele dropout. Because WD is a simple Mendelian disorder that follows an autosomal recessive mode of inheritance, normal parents of a WD pa-

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Fig. 1. Flowchart to determine allele dropout in WD patient suspected to be homozygous for a mutation.

D13S133, D13S316, D13S314 are dinucleotide repeat markers flanking WD locus with a large number of alleles. To ensure detection of any underlying heterogeneity between 2 WD-alleles in sporadic patient, genotyping for all 3 markers is recommended. Segregations of mutant alleles (\textit{m1}, \textit{m2}) and wild-type alleles (\textit{wt1}, \textit{wt2}) are shown.
tient are obligatory carriers of one mutant chromosome each (see Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol52/issue8). Therefore, both parents of a patient with a homozygous mutation should be heterozygous for the same mutation. Screening the parents will reveal either the presence of single mutant allele in both parents, suggesting that the patient is genuinely homozygous (no allele dropout) or the absence of the mutant in one of the parents, suggesting allele dropout. In the latter case, PCR should be repeated with new pairs of primers designed to avoid the location of known single-base variations and to restore amplification of the failed allele (1). Then the new amplicon can be sequenced to freshly determine the genotype of the patient. A heterozygous genotype for the mutation should lead to a search for the second ATP7B variation. On the other hand, a homozygous genotype suggests the presence of the same mutation in both alleles, but from different haplotype backgrounds. However, in isolated inbreeding populations and in families with reported consanguinity among the parents of a WD patient, a homozygous mutation found in the patient is unlikely to result from allele dropout.

We recommend the use of PCR with fluorescent-labeled primer pairs to genotype WD patients for highly heterozygous and multiallelic dinucleotide repeat-markers (D13S133, D13S316, and D13S314) flanking the disease locus. Samples showing heterozygosity for any of the 3 markers should be reinvestigated for genotyping at the identified mutation, as shown in Fig. 1. Homozygosity found at all 3 marker loci strongly suggests homozygosity for the identified mutation.

Patients with a homozygous mutation may not have the same allele size at the selected marker locus if the 2 mutant alleles do not have a common origin (3). However, in case of a prevalent mutation, postmutation change in the allele size of repeat markers from slippage during replication could lead to different allele sizes that might suggest different origins (a de novo event) for the same mutation. Haplotypes based on multiple single-base variations could be constructed to test for a common origin (4). To genotype 10 homozygous patients born to nonconsanguineous parents, we used a D13S133 marker reported to be highly heterozygous, with 20 alleles ranging in size from 130 bp to 189 bp (4). All 10 homozygous patients had a homozygous genotype for the repeat marker. Interestingly, repeat-alleles associated with different mutations also differed in size (see Table 1 in the online Data Supplement). The rare event of recombination by the repeat markers located at a moderate distance from the WD locus can be addressed by genotyping any intragenic single-base variation informative in the study population. The study was cleared by the internal review committee on human research according to the regulations of the Indian Council of Medical Research.

In short, we suggest that genotyping the parents of a WD patient suspected to be homozygous for a mutation and/or genotyping for a marker linked to the disease locus in the patient will unequivocally address the issue of allele dropout without labor-intensive mutation screening of ATP7B.

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References

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Magnesium Contamination from Terumo Blood Collection Tubes

To the Editor:
Components of blood collection tubes have been implicated as interfering substances in various assays, especially immunoassays (1–5). Possible interfering components for blood collection tubes include the lubricants, clot activators, surfactants, and barrier gels. In 1 study, a surfactant was identified as the interfering (1). To our knowledge, no problems have been reported with general chemistry assays. Here we describe an apparent contamination of Terumo tubes with magnesium.

In an evaluation of collection tubes, we collected samples from volunteers via a Vacutainer system into 3 tubes (Greiner, BD, or Terumo). The samples were obtained with no conscious order of draw, and the 3 types of tubes were treated identically. The fill volume of drawn blood was 5–7 mL for all tubes. The samples were stored at room temperature (~21°C) for <45 min before centrifugation. Magnesium was measured by a xylyl blue method on a