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## Prenatal diagnostic conundrum involving a novel *ATP7A* duplication

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### *To the Editor:*

Whole-genome data in medical contexts have become increasingly available because of technological advances and improved sequencing efficiency. As a consequence, a rise in ambiguous situations involving novel molecular alterations and their potential association with human illness can be anticipated (1). The inability to predict clinical phenotypes based on mutations and copy number variants (2) can especially complicate prenatal decision making. We encountered a compelling instance of this problem with a couple that had lost one child to a sporadic gene mutation and serendipitously faced the prospect of a second infant with a separate rare genetic disorder.

In January 2012, a 27-year-old G<sub>2</sub>P<sub>1</sub> healthy female presented for prenatal care for her second pregnancy. Her first child was affected with Cornelia de Lange syndrome and died at 6 weeks of age because of complications from premature birth and intraventricular hemorrhage. With informed parental consent, chorionic villus sampling (CVS) at 12 weeks of the second pregnancy was performed to test the *Nipped-B-like (NIPBL)* gene, which was mutated in the deceased child. Fetal DNA testing for *NIPBL* mutations was negative. Subsequently, the remaining CVS specimen was used as a quality assurance control in prenatal evaluation of an unrelated male fetus at risk for Menkes disease, a lethal X-linked recessive neurometabolic disease caused by mutation of the *ATP7A* gene (3). The molecular diagnostic assay used, multiplex ligation-dependent probe amplification (SALSA MLPA P104, MRC-Holland, Amsterdam, The Netherlands), is most reliable when the at-risk test specimen is matched with a same-tissue control, e.g. chorionic villus. In this case, although the primary test specimen proved negative for the familial *ATP7A* alteration, we identified a duplication of exons 1–7 in the control specimen. Because Menkes disease is an X-linked

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### **Ethics approval**

Informed consent for DNA and neurochemical diagnostic testing, and approvals from Institutional Review Boards were obtained from the University of Minnesota Medical Center-Fairview, the University of Chicago, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

recessive condition and the control DNA was from a male fetus, this unexpected result contained implications for the pregnancy, and the family was recontacted.

There was no past family history of Menkes disease. One *ATP7A* allele with the exon 1–7 duplication was detected in DNA from the mother and maternal grandmother whose clinical examinations were unremarkable. The duplication was not present in the mother's healthy adult brother's X-chromosome. We had not otherwise identified this abnormality in approximately 150 assays performed with this kit in our molecular diagnostics center. On the basis of the new genomic information, and the prospect of a second infant with a potentially lethal disorder, the parents weighed carefully whether to terminate the pregnancy.

*ATP7A* duplications are estimated to represent the molecular cause of Menkes disease in 4–10% of affected patients (4, 5). All prior reported examples ( $n=24$ ) involved intragenic *ATP7A* tandem duplications, predicted to disrupt the normal translational reading frame and produce nonfunctional *ATP7A* proteins. In contrast, the exon 1–7 duplication we detected had not been reported in the literature and occurred at the 5' end of the *ATP7A* gene rather than within the gene. There are 76 copy number variants involving *ATP7A* listed in the dbVar/Gene database. Four (nsv869485, nsv869350, esv147665, and nsv491743) have a duplication breakpoint in the 5' portion of *ATP7A*. However, none of these encompass the first 100 kb of the *ATP7A* gene extending to exon 7 that is involved in this duplication. We hypothesized that, if the exon 1–7 duplication was in tandem, three *ATP7A* molecules could be generated depending on promoter selection, mRNA splicing, and position of the 5' breakpoint (Fig. 1). We reasoned that one or two of these would be functional and, based on potential molecular mechanisms of altered gene expression under various duplication scenarios (6), would avert Menkes disease.

After provided with the information and counseled concerning the potential risks and benefits, they ultimately chose to continue the pregnancy. In July 2012, at 36 weeks gestation, a 2.8 kg male infant was delivered with Apgar scores of 8 and 9. Plasma neurochemical analysis, a sensitive and specific diagnostic test for Menkes disease (7), was performed on day 1 of life, and was unequivocally normal. No other medical problems were evident, and the mother and infant were discharged home on postnatal day 3. The infant remains in good health. Further detailed characterization of the gene duplication including the final *ATP7A* transcript(s) and chromosomal localization is planned.

This case raised several questions relevant to the sometimes-competing requirements for informed consent in genetic testing and laboratory quality assurance practices. While the written consent document obtained at the time of this mother's CVS procedure indicated 'one or more genetic tests', only evaluation of the previously affected child's *NIBPL* mutation was cited specifically, and consent for *ATP7A* testing was obtained later. No formal guidelines exist on the nature of samples for routine use as quality assurance controls in molecular diagnostic testing. Institutions offering such testing typically derive their own individual policies. The use of anonymous DNA specimens, a mixture of samples, or references provided by kit manufacturers all represent practical alternative considerations, especially when tissue-specific controls for DNA extraction are unnecessary. Despite the

anxiety of this particular situation, the family remained grateful for the molecular diagnostic information and associated genetic counseling.

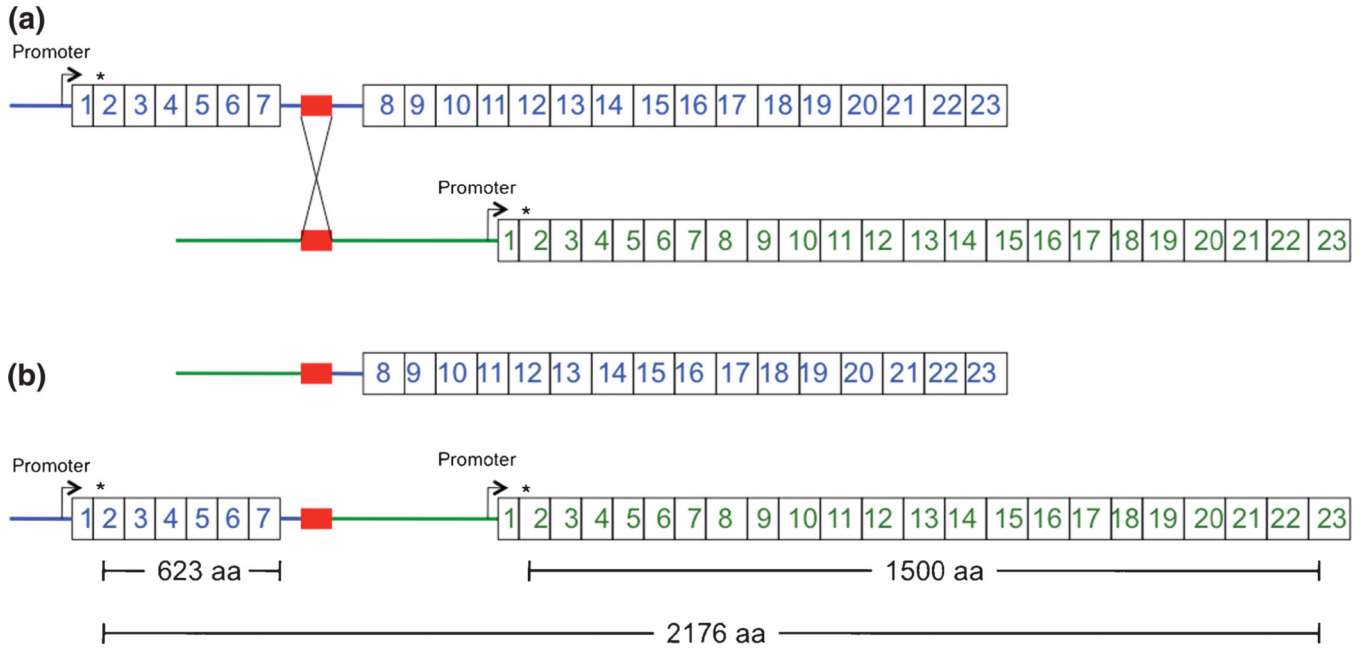
In summary, we present a case in which prospective parents, their health care providers, laboratory personnel, and consultants all wrestled with the interpretation of genomic information considered perfectly accurate, but imperfectly predictive. More detailed understanding of human genomic variation should eventually converge with advances in DNA sequencing technology to enrich both molecular medicine and prenatal diagnosis of genetic disease. At present, however, we operate in an awkward interval when our capacity to capture information often surpasses our ability to fully understand it, as illustrated by this example.

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**Fig. 1.** Proposed molecular mechanism resulting in tandem duplication of exons 1–7 of *ATP7A*. **(a)** Chromosomal misalignment of similar DNA sequences (red blocks) in intron 7 (enlarged) and the region upstream of *ATP7A* during meiosis results in an unequal crossing-over event. **(b)** The consequence of this event would be a deletion of *ATP7A* exons 1–7 on one X chromosome (top) and a tandem duplication on the other (bottom). The latter rearrangement, inherited by the male infant in this report, is predicted to generate three possible protein products, of which at least one (the 1500 amino acid normal length) should be functional. This interpretation assumes that the crossing-over event did not involve the *ATP7A* gene promoter. Asterisks denote the translation initiation site in *ATP7A* exon 2. aa, amino acids.