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Report

Rare Etiology of Autosomal Recessive Disease in a Child with Noncarrier Parents

Roger V. Lebo,[1] Lawrence R. Shapiro,[2,3] Elif Yosunkaya Fenerci,[1] J. M. Hoover,[3] Jacinta L. Chuang,[4] David T. Chuang,[4] and David F. Kronn[2,3]

[1]Center for Human Genetics, Department of Pediatrics, Boston University School of Medicine, Boston; [2]Division of Medical Genetics, New York Medical College, and [3]Westchester Medical Center, Valhalla, NY; and [4]Department of Biochemistry, Southwest Medical Center, University of Texas, Dallas

A child with maple syrup urine disease type 2 (MSUD2) was found to be homozygous for a 10-bp MSUD2-gene deletion on chromosome 1. Both purported parents were tested, and neither carries the gene deletion. Polymorphic simple-sequence repeat analyses at 15 loci on chromosome 1 and at 16 loci on other chromosomes confirmed parentage and revealed that a de novo mutation prior to maternal meiosis I, followed by nondisjunction in maternal meiosis II, resulted in an oocyte with two copies of the de novo mutant allele. Fertilization by a sperm that did not carry a paternal chromosome 1 or subsequent mitotic loss of the paternal chromosome 1 resulted in the propositus inheriting two mutant MSUD2 alleles on two maternal number 1 chromosomes.

Maple syrup urine disease is caused by homozygous mutations, at one of four gene loci, that result in accumulation of keto acids in the urine; it occurs at a frequency of ~1/224,000 newborns (Naylor and Guthrie 1982). Each of these four genes encodes one of the subunits of branched-chain keto acid dehydrogenase (Heffelfinger et al. 1983). Mutations of both alleles at the same locus result in MSUD1A (MIM 248600), MSUD1B (MIM 248611), MSUD2 (MIM 248610), or MSUD3.

In exceptional cases, autosomal recessive genetic disease results when an embryo inherits two otherwise normal chromosomes carrying a mutant gene from the only carrier parent and inherits no chromosome of this type from the parent with normal genes. Rare affected individuals have been reported with (*a*) one molecularly characterized parent who is a carrier of either cystic fibrosis or abetalipoproteinemia (Beaudet et al. 1991; Yang et al. 1999), (*b*) uniparental disomy (UPD) and biochemically characterized cystic fibrosis and cystinosis (Smith et al. 1991), or (*c*) UPD and a phenotype of Bloom syndrome or Chediak-Higashi syndrome (Woodage et al. 1994; Dufourcq-Lagelouse et al. 1999).

UPD of imprinted gene loci from the same parent results in genetic disease much more frequently than does UPD with a mutant gene. Fertilization between a disomic gamete and a gamete monosomic for the same chromosome, followed by loss of the normally inherited chromosome (trisomic rescue), is considered to be the more frequent mechanism of UPD formation, with a 3:1 preponderance of maternal UPD (Antonarakis et al. 1992). Imprinted genes have been reported to result in human genetic disease when either both chromosomes 2, 7, 14, 15, or 16 are inherited from the mother or both chromosomes 6, 11, 14, 15, or 20 are inherited from the father (Falls et al. 1999).

DNA was extracted, according to the Puregene DNA Isolation Kit protocol (Gentra Systems), from 6 ml of whole blood, by doubling all recommended volumes and excluding RNase treatment. The 20-pmol PCR-amplification primers uniquely amplified target sequences in 25–50 ng of genomic DNA in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.56 mM $MgCl_2$, 0.208 mM of each dNTP, 1 mCi of [^{32}P]-dCTP, and 1 U of *Taq* polymerase (Gibco BRL), in 12 μl. PCR proceeded by denaturing at 94°C for 5 min, then denaturing at 94°C for 30 s, annealing at 55°C for 30 s, and elongating at 72°C for 30 s, for each of 30 cycles. The last cycle was completed by a 5-min extension at 72°C. Amplification products

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Address for reprints and correspondence: Dr. Roger V. Lebo, FACMG, Center for Human Genetics, Boston University School of Medicine, 715 Albany Street, Suite 408, Boston, MA 02118. E-mail: rlebo@bu.edu

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Table 1

Parentage Testing Results

	Alleles of		
	Mother (I-1)	Propositus (II-1)	Father (I-2)
D1S1612	114, 98	114, 114	130, 102
D2S1326	248, 240	240, 236	252, 236
D3S1286	147, 143	143, 133	143, 133
D3S2427	233, 225	235, 225	235, 225
D5S112	114, 98	106, 98	106, 98
D5S346	114, 106	114, 114	126, 114
D6S1056	240, 252	268, 240	268, 256
D8S1132	144, 140	148, 140	148, 144
D9S15	208, 200	200, 200	208, 200
D10S1237	412, 404	424, 404	424, 408
D12S391	229, 218	241, 229	241, 222
D13S137[a]	122, 113	128, 122	128, 113
D13S128[a]	170, 160	166, 160	166, 160
D15S643	211, 205	211, 205	215, 211
D16S539	162, 162	162, 158	162, 158
D20S470	310, 298	310, 298	310, 302
Selected UPD results:			
D1S1612[b] (42.7 cM[c])	114, 98	114, 114	130, 102
D1S1597[b] (51.2 cM[c])	172, 164	164, 164	176, 172
D1S1598[c] (89.4 cM[c])	119, 111	119, 111	119, 115
D1S1643[c] (124.1 cM[c])	251, 251	251, 251	270, 266
MSUD2[b]	Normal/Normal	Δ10 bp/Δ10 bp	Normal/Normal
D1S1655[b] (229.1 cM[c])	196, 188	188, 188	205, 185
D1S1642[b] (262.7 cM[c])	264, 260	264, 264	270, 257
D1S1617[b] (323.9 cM[c])	135, 133	133, 133	123, 123
D1S1594[b] (338.4 cM[c])	125, 121	125, 125	121, 117

[a] Both D13S137 and D13S128 were at Wilson disease gene locus; D13S137 was used in calculation.

[b] Maternal UPD for one chromosome.

[c] Cooperative Human Linkage Center linkage-map distance.

[d] Maternal UPD for both chromosomes.

[e] Confirms maternal UPD for one or both chromosomes.

were resolved by 6% polyacrylamide gel electrophoresis using an M13 sequencing ladder as a size marker. Electrophoresed gels were dried on Whatman 3MM filter paper and were autoradiographed at −80°C.

Both E2 genes sequenced from a male child with MSUD had a 10-bp deletion IVS10del(−9;+1), which results in a 21-bp deletion in the processed mutant E2 mRNA (Chuang et al. 1999). Homozygous MSUD2 alleles on chromosome band 1p31 explained this child's clinical phenotype and suggested that the parents share a common ancestor carrying the same rare allele. In addition to an affected son, this couple also had a normal daughter and a third pregnancy that ended in spontaneous abortion. They were seen by us during the fourth pregnancy because they already had borne a child with MSUD2 and because they were of advanced age. Prenatal testing did not find the previously characterized MSUD2 mutation either in the current fetal DNA sample or previously stored maternal and paternal lymphocyte DNAs. Analysis of additional blood samples from both parents and the propositus confirmed that the propositus is homozygous for the MSUD2-gene deletion and that neither purported parent is a carrier. As expected, the mother delivered another normal baby.

Because these results could be explained by either nonparentage or a rare genetic mechanism, a parentage test was ordered for the propositus. The propositus shares alleles with the purported mother, at all of 16 independently segregating simple sequence repeat (SSR) polymorphic loci initially tested (table 1). With the allele frequencies derived either from public databases or from our accumulated database, the likelihood that I-1 is the mother of II-1 was calculated to be 1,373:1 (data not shown). At the same time, the purported father of II-1 shares with the propositus paternally derived alleles at 15 of 16 tested loci but does not share an allele at the D1S1612 locus (fig. 1*A* and table 1). Although the propositus is homozygous for a maternally derived 114-bp D1S1612 allele, the father's 102- and 130-bp alleles differ from the child's 114-bp allele, by three and four tetranucleotide repeats, respectively. The likelihood of an allele changing a single repeat during meiosis is ~1/

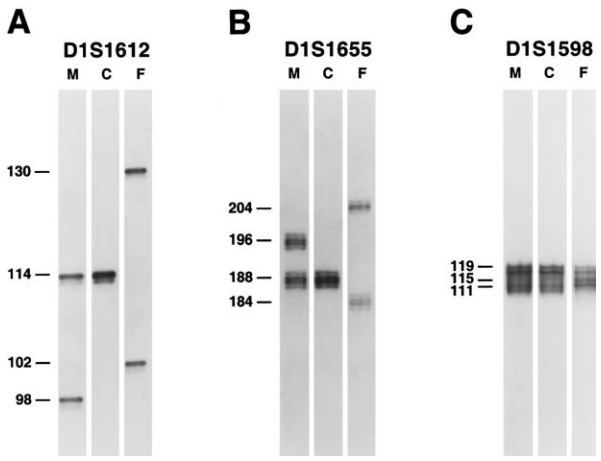

Figure 1 Polymorphic results at three chromosome 1 loci. *A* and *B,* Propositus, homozygous for one of the two maternal alleles at the D1S1612 and D1S1655 loci. *C,* Results at the D1S1598 locus, the single tested chromosome 1 locus in the propositus that is heterozygous for two different length maternal alleles (111 and 119 bp).

1,000 (Weber and Wong 1993); the likelihood of changing two SSR repeats is on the order of 1/10,000; and we have not found a three-tetranucleotide-repeat change reported. Nevertheless, the purported father II-1 shared paternally derived alleles at all 15 of 15 other tested loci.

The possibility of UPD for chromosome 1 was tested because the D1S1612 locus is in the same region of the chromosome-1 short arm as the MSUD2 locus and the propositus is homozygous for a previously unreported MSUD2 allele with a 10-bp deletion. SSR polymorphic sites were analyzed in the propositus and his purported parents at 15 loci spanning chromosome 1 from 32.7 to 338.3 cM along the Cooperative Human Linkage Center chromosome-1 linkage map. All of the propositus's chromosome-1 polymorphic fragments could have been inherited from his mother. The results at each locus were considered to be independent events, because each is sufficiently distant from the next locus. On the basis of only the allele frequencies available in the CHLC database at 11 of the 15 additional tested chromosome-1 loci, the calculated likelihood that the purported mother is the mother is 4,750,605:1 (tables 1 and 2). When multiplied by the likelihood of 1373:1 that this woman is the mother, based on the other autosomal loci tested, the combined posterior likelihood the propositus II-1 is the child of purported mother I-2 is $>6 \times 10^9$:1.

In contrast, the propositus did not share paternally derived alleles at 7 of the 16 tested chromosome-1 loci (table 1). However, the propositus II-1 is homozygous at 15 of the 16 loci tested on chromosome 1, and all alleles at all 16 loci of the child are carried by the mother (table 1). Taken together, these results demonstrate maternal UPD of chromosome 1. This conclusion is further supported by the fact that the MSUD2 locus is on chromosome 1 and that the propositus has the same unique mutation on both alleles (fig. 2 and table 1). Under the assumption that maternity and maternal UPD of chromosome 1 have been demonstrated, the likelihood that the purported father is the father, based on the 15 autosomal loci tested that are not on chromosome 1, exceeds 2×10^9:1. We conclude that the purported parents are the parents of the propositus.

The genetic mechanism resulting in maternal chromosome-1 UPD can be derived from the chromosome-1 SSR results (table 1). The propositus, II-1, is homozygous for maternally derived alleles at 15 of 16 tested chromosome-1 loci, consistent with maternal UPD from a single grandparental chromosome 1. In contrast, the fetus carried two different-length maternal alleles (118 and 112 bp) at locus D1S1598, derived from both grandparental chromosomes (fig. 1*C* and table 1). These results indicate that nondisjunction occurred in maternal meiosis II, so that the centromeres of the same two grandparental chromatids that had duplicated in the previous S-phase segregated into the oocyte prior to fertilization. The single exception, at the D1S1598 locus distal to the MSUD2 locus, carries two different maternally derived alleles resulting from one of two mechanisms: (1) a double recombination in a single chromatid with the breakpoints on either side of the D1S1598 locus prior to meiosis I (fig. 3*A*) or (2) two single recombinations, one proximal to D1S1598 in the first chromatid and the second distal to D1S1598 in the second chromatid (fig. 3*B*). Thus, the propositus carries both maternal D1S1598 alleles (fig. 1*C* and table 1). Since the MSUD2 locus is proximal to the heterozygous locus D1S1598 and the homozygous loci D1S1661 and D1S1643 on chromosome 1p (fig. 2 and table 1), the MSUD2 locus is also homozygous and carries two copies of the same mutant allele.

Because the MSUD2 mutation event occurred prior to

Table 2

Probability of Maternity with Chromosome-1 Results

Locus	Probability of Being Mother	Probability of Random Woman	Posterior Probability
D1S551	.5	[*f*(180)] = .46	1.09:1
D1S540	1.0	[*f*(156)] = .056	17.9:1
D1S1623	1.0	[*f*(268)] = .071	14.1:1
D1S1642	.5	[*f*(264)] = .402	1.24:1
D1S1667	1.0	[*f*(218)] = .031	32.3:1
D1S1617	1.0	[*f*(133)] = .142	7.04:1
D1S1594	.5	[*f*(125)] = .069	7.25:1
D1S1597	.5	[*f*(164)] = .098	5.10:1
D1S1598	.5	[*f*(118 + 112)] = .32 + .54	.58:1
D1S1661	1.0	[*f*(95)] = .233	4.29:1
D1S1648	.5	[*f*(186)] = .75	.67:1
Overall			4,750,605:1

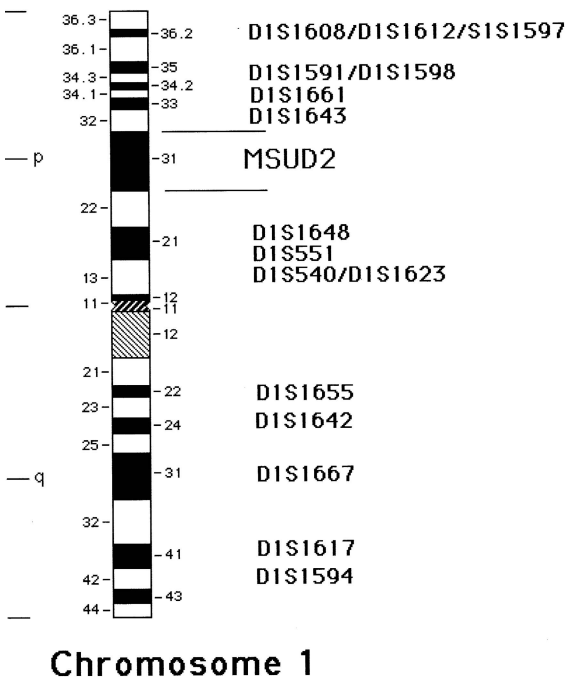

Figure 2 Chromosome 1 ideogram, with PCR-amplified locations. The approximate locations of the PCR-amplified chromosome 1 sites are based on locations and recombination distances in The Genome Database.

maternal meiosis, this cell may have given rise to a large proportion of gametes in one maternal gonad. The proportion of carrier germline cells contributes little to this couple's risk for a child with MSUD2, because nondisjunction of the mutation-carrying chromosome 1 in maternal meiosis II, selection for the abnormal genes in the subsequent oocyte, and loss of the paternal chromosome shortly after fertilization would all be required in order to produce another viable fetus with the same genotype. Therefore, this couple has a very low risk for a second fetus with homozygous MSUD2. In contrast, if all germline cells carry the MSUD2 mutation in one ovary of I-2, the likelihood that another fetus of II-1 being a carrier of the MSUD2 mutation is 25%. Given the typical frequency of germline cells carrying new mutations in studies of germinal mosaic male mice, the likelihood of a germinal mosaic transmitting another MSUD2 mutation would be in the range of 0.5%–6%. Nevertheless, this couple has been offered MSUD2 testing for all future pregnancies.

These results indicate that MSUD2 in the propositus arose from two extremely rare events: (1) a de novo mutation prior to S-phase of meiosis I in oogenesis and (2) maternal UPD resulting from maternal nondisjunction in meiosis II. The likelihood of a deleterious mutation occurring in a diploid human genotype has been estimated to be 1.6 per generation (Eyre-Walker and Keightley 1999). Although the frequency of UPD varies for different chromosomes, the frequency of UPD for chromosome 15 has been reported for both Prader-Willi syndrome (25% of 1/10,000 newborns) and Angelman syndrome (2% of 1/10,000 newborns; Amos-Landgraf et al. 1999). Therefore, the frequency of chromosome 15 UPD is anticipated to be 1/37,000 newborns. When the same frequency of UPD is used for chromosome 1, the frequency of de novo MSUD at any one of the four MSUD gene loci would be in the range of (1.6 mutations/diploid genome/generation) × (1 diploid genome/200,000 genes) × (4 MSUD genes) × (1 UPD chromosome 1/37,000 newborns) = (1 newborn with MSUD from de novo new mutation and UPD/1,156,250,000 newborns).

Given that the frequency of all four forms of MSUD is 1/224,000 newborns (Naylor and Guthrie 1982) and that the frequency is the same for all types, the MSUD carrier frequency can be calculated to be ~1/118—that is, [(1/473)2 + (1/473)2(1/473)2 + (1/473)2](1/4) = 1/224,000. When a UPD frequency of 1/37,000 newborns is assumed, the predicted frequency of MSUD from chromosome 1 UPD transmitted by a carrier parent would be 1/118 × 1/37,000 = 1/4,366,000 newborns. These frequencies predict that de novo mutation and UPD resulting in a genetic disease occurs much more rarely than the previously reported cases of UPD and genetic disease from a carrier parent.

In the case reported here, the three- and four-tetranucleotide-repeat difference between paternal and propositus allelic lengths at the D1S1612 locus suggest that

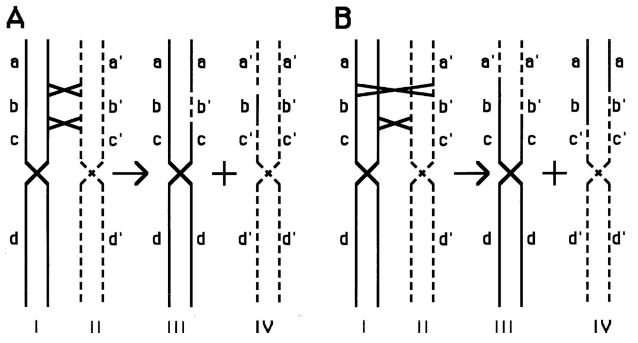

Figure 3 Meiotic recombination between homologous number 1 chromosomes. *A*, Double recombination on a single short arm of both homologous chromatids, resulting in a recombinant chromosome that is homozygous at three locations (aa, cc, and dd) and heterozygous at one location (bb′). Maternal nondisjunction in meiosis II would transmit both A-III chromatids to the oocyte. *B*, Single recombination on each chromatid, also resulting in a recombinant chromosome that is homozygous at three locations (a′a′, cc, and d,d) and heterozygous at one location (bb′). The homozygous "a" locus differs, depending on the recombination sites. These two possibilities cannot be distinguished without testing the grandparental DNAs, which were unavailable.

the likelihood of paternity is much less than that which would be expected on the basis of a single meiotic SSR change. At the same time, paternity would have been proved readily had only autosomal loci that were not syntenic to the MSUD2 locus on chromosome 1 been tested initially. This suggests that the optimal strategy in testing parentage of a propositus with an autosomal recessive genetic disease not carried by the purported parents is to test only loci on chromosomes that do not carry genes that, when mutated, would result in the propositus's genetic disease. Once parentage has been proved, subsequent UPD testing of the pertinent chromosome carrying a gene in which mutations result in the propositus's genetic disease is likely to be positive. This would be important in localizing the mutant-gene search, such as in the case of MSUD resulting from any one of four gene loci.

A decrease in recombination has been reported in demonstrated cases of UPD (Warren et al. 1987). In our case, the propositus carries two different maternal polymorphic alleles at the D1S1598 locus, as a result of two meiotic recombinations in a 295.7-cM genetic region between the D1S1612 and D1S1594 loci, where approximately six recombinations are predicted. This concomitant decrease in recombination may be related to the nondisjunction resulting in UPD.

In cases with rare genetic events, initial molecular genetic–testing results may inaccurately suggest nonparentage or reveal different relationships among individuals in the pedigree. After unusual molecular test results have been confirmed, most diagnoses, along with the reliability and recurrence risks, can be reported without addressing a newly discovered relationship. Occasionally, one individual in the pedigree needs to be consulted prior to communicating the molecular diagnosis. In these cases, effective counseling needs to proceed with care, judgment, understanding, and prudence.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, The, http://gdbwww.gdb.org/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MSUD1A [MIM 248600], MSUD1B [MIM 248611], and MSUD2 [MIM 248610])

References

Amos-Landgraf JM, Ji Y, Gottlieb W, Depinet T, Wandstrat AE, Cassidy SB, Driscoll DJ, Rogan PK, Schwartz S, Nicholls RD (1999) Chromosome breakage in the Prader-Willi and Angelman syndromes involves recombination between large, transcribed repeats at proximal and distal breakpoints. Am J Hum Genet 65:370–386

Antonarakis SE, Petersen MB, McInnis MG, Adelsberger PA, Schinzel AA, Binkert F, Pangalos C, Raoul O, Slaugenhaupt SA, Hafez M, Cohen MM, Roulson D, Schwartz S, Mikkelsen M, Tranebjaerg L. Greenberg F, Hoar DI, Rudd NL, Warren AC, Metaxotou C, Bartsocas C, Chakravarti A (1992) The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. Am J Hum Genet 50:544–550

Beaudet AL, Perciaccante RG, Cutting GR (1991) Homozygous nonsense mutation causing cystic fibrosis with uniparental disomy. Am J Hum Genet 48(6):1213

Chuang JL, Cox RP, Chuang DT (1997) Transacylase-deficient (Type II) maple syrup urine disease aberrant splicing of E2 mRNA caused by internal intronic deletions and association with thiamine-responsive phenotype. J Clin Invest 100: 736–744

Dufourcq-Lagelouse R, Lambert N, Duval M, Viot G, Vilmer E, Fischer A, Prieur M, de Saint Basile G (1999) Chediak-Higashi syndrome associated with maternal uniparental isodisomy of chromosome 1. Eur J Hum Genet 7:633–637

Eyre-Walker A, Keightley PD (1999) High genomic deleterious mutation rates in hominids. Nature 397:344–347

Falls JG, Pulford DJ, Wylie AA, Jirtle RL (1999) Genomic Imprinting: Implications for Human Disease. Am J Pathol 154:635–647

Heffelfinger SC, Sewell ET, Danner DJ (1983) Identification of specific subunits of highly purified bovine liver branched chain ketoacid dehydrogenase. Biochemistry 22:5519–5522

Naylor EW, Guthrie R (1982) Newborn screening for maple syrup urine disease (branched-chain ketoaciduria). South Med J 75:529–532

Smith ML, Pellett OL, Cahill TC, David DN, Kaskel FJ, Smolin LA, Greene AA, Weissbecker K, Dean M, Schneider JA (1991) Biochemical and genetic analysis of a child with cystic fibrosis and cystinosis. Am J Med Genet 39:84–90

Warren AC, Chakravarti A, Wong C, Slaugenhaupt SA, Halloran SL, Watkins PC, Metaxotou C, Antonarakis SE (1987) Evidence for reduced recombination on the nondisjoined chromosomes 21 in Down syndrome. Science 237:652–654

Weber JL, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2:1123–1128

Woodage T, Prasad M, Dixon JW, et al (1994) Bloom syndrome and maternal uniparental disomy for chromosome 15. Am J Hum Genet 55:74–80

Yang XP, Inazu A, Yagi K, Kajinami K, Koizumi J, Mabuchi H (1999) Abetalipoproteinemia caused by maternal isodisomy of chromosome 4q containing an intron 9 splice acceptor mutation in the microsomal triglyceride transfer protein gene. Arterioscler Thromb Vasc Biol 19:1950–1955