Complete paternal uniparental isodisomy for chromosome 1 revealed by mutation analyses of the *TRKA (NTRK1)* gene encoding a receptor tyrosine kinase for nerve growth factor in a patient with congenital insensitivity to pain with anhidrosis.

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Abstract

Uniparental disomy (UPD) is defined as the presence of a chromosome pair that derives from only one parent in a diploid individual. The human *TRKA* gene on chromosome 1q21-q22 encodes a receptor tyrosine kinase for nerve growth factor and is responsible for an autosomal recessive genetic disorder: congenital insensitivity to pain with anhidrosis (CIPA). We report here the second case of paternal UPD for chromosome 1 in a male patient with CIPA who developed normally at term and did not show overt dysmorphisms or malformations. He had only the usual features of CIPA with a homozygous mutation at the *TRKA* locus and a normal karyotype with no visible deletions or evidence of monosomy 1. Haplotype analysis of the *TRKA* locus and allelotype analyses of whole chromosome 1 revealed that the chromosome pair was exclusively derived from his father. Non-maternity was excluded by analyses of autosomes other than chromosome 1. Thus, we have identified a complete paternal isodisomy for chromosome 1 as the cause of reduction to homozygosity of the *TRKA* gene mutation, leading to CIPA. Our findings further support the idea that there are no paternally imprinted genes on chromosome 1 with a major effect on phenotype. UPD must be considered as a rare but possible cause of autosomal recessive disorders when conducting genetic testing.

Introduction

Uniparental disomy (UPD) is defined as the presence of a chromosome pair that derives from only one parent in a diploid individual (Engel 1980). Heterodisomy is the inheritance of a pair of chromosome homologs from a parent, whereas isodisomy implies the inheritance of two copies of a single parental homolog. This unusual non-Mendelian transmission of parental genes may cause recessive disorders with reduction to homozygosity or developmental disturbances attributable to aberrant effects in the zygote (Ledbetter and Engel 1995). UPD was first demonstrated in diploid cell lines derived from four cases of mosaic Down syndrome (Niikawa and Kajii 1984). Subsequently, maternal UPD for chromosome 7 in a patient with short stature and cystic fibrosis was described as the first clinical case of UPD in human, presumably resulting from a monosomy 7 duplication (Spence et al. 1988).

Recently, two cases of UPD for chromosome 1 were reported: the first case of maternal UPD (Pulkkinen et al. 1997) and the first case of paternal UPD (Gelb et al. 1998) for this chromosome. Both cases were ascertained through a rare recessive condition and did not show overt additional phenotypes. The third case displayed maternal heterodisomy for chromosome 1, associated with no apparent phenotypic effects (Field et al. 1998), whereas the fourth case involved maternal isodisomy for chromosome 1 associated with Chediak-Higashi syndrome (Dufourcq-Lagelouse et al. 1999).

Congenital insensitivity to pain with anhidrosis (CIPA; MIM 256800) is an autosomal recessive genetic disorder characterized by anhidrosis (inability to sweat), the absence of reaction to noxious stimuli, and mental retardation (Dyck 1984; Swanson 1963). The human *TRKA (NTRK1)* gene, located on chromosome 1q21-q22 (Weier et al. 1995), encodes a receptor tyrosine kinase for nerve growth factor (Kaplan et al. 1991; Klein et al. 1991). We have identified the genetic basis for this disorder by detecting mutations in the *TRKA* gene derived from patients (Indo et al. 1996). Recently, we have developed a comprehensive strategy to screen for *TRKA* mutations, on the basis of the structure and organization of the gene (Indo et al. 1997; Mardy et al. 1999) and characterized eight intragenic polymorphic sites (Miura et al. 2000). These should facilitate the detection of CIPA mutations and aid in the diagnosis and genetic counseling of this painless but severe disorder with devastating complications. We report here the second case of paternal UPD for chromosome 1 that has been revealed by molecular analyses of a patient with CIPA.

Materials and methods

Clinical history and findings

The proband KI-24 was a 2-year-old boy, the first child of healthy parents of Japanese ancestry. Family history was unremarkable, and there was no known consanguinity. The patient had developed normally at term and did not show overt dysmorphisms or malformations. Intrauterine growth retardation was not observed. He was born at 39-week's gestation after an uncomplicated pregnancy when his father and mother were 23 years and 19 years old, respectively. His birth weight was 2550 g and was appropriate for his gestational age in Japanese (2480-3680 g). The newborn physical examination was unremarkable. However, unexplained fever was first noted at 2 weeks of age. Diagnosis of CIPA was clinically given at 3 months of age. The patient showed no response to noxious stimuli, such as injections, nor to a sweat-test. Later, self-mutilating behaviors, such as biting of his tongue, and mental retardation became evident. His body-weight gain was slow around 1 year of age, because of intercurrent febrile episodes attributable to CIPA, but it recovered to the normal range at 2 years of age. His body-height was always within the normal range. He had only the usual features of CIPA.

Mutation analysis

DNA samples from peripheral bloods were obtained from the proband and family members: parents, paternal grandmother, paternal aunt, and maternal grandparents. Informed consent was obtained from all subjects tested. *TRKA* exons were amplified from genomic DNA by polymerase chain reaction (PCR) with primers designed for exons 1-17. The PCR products were sequenced as described elsewhere (Mardy et al. 1999).

Haplotype and allelotype analyses

Haplotypes of the proband and his parents were characterized by using seven single nucleotide polymorphic markers and one microsatellite marker (D1S506) within the *TRKA* locus, as described elsewhere (Miura et al. 2000). Microsatellite markers outside the *TRKA* locus spanning the entire chromosome 1, and those of chromosomes 2-22, were characterized. Each marker was amplified from genomic DNA by AmpliTaq Gold and primer pairs of the Linkage Mapping Set Version 2 (PE Applied Biosystems), according to the manufacturer's

protocol. PCR products were resolved on an ABI PRISM 310 Genetic Analyzer, and the results were analyzed with ABI PRISM GeneScan and Genotyper softwares (PE Applied Biosystems). For chromosome 1, all 31 primer pairs included in the Linkage Mapping Set were used.

Results

Cytogenetic analysis of peripheral lymphocytes revealed a normal male karyotype with no visible deletions or evidence of monosomy 1 (data not shown). Direct sequencing from the patient revealed a homozygous C deletion at nucleotide 1726 in exon 14; this causes a frameshift and premature termination codon after amino acid Arg 548 (R548 fs). His father was heterozygous for the R548 fs mutation, but his mother was homozygous for the wild-type allele (data not shown). This mutation abolished the restriction enzyme site for *Bsp*1286I. Restriction enzyme analysis confirmed that the patient was homozygous for the mutation, the father was heterozygous for the mutation, and the mother was homozygous for the wild-type allele (Fig 1). The mutation was derived from the paternal grandmother but was not detected in the maternal grandparents (data not shown). No putative mutations were detected throughout the coding regions or at the exon-intron junctions of the *TRKA* gene in the mother.

To define the mode of inheritance in this family further, we characterized haplotypes of the proband and his parents, by using seven single nucleotide polymorphic markers and one microsatellite marker (D1S506) within the *TRKA* locus, as described elsewhere (Miura et al. 2000). The R548 fs mutation is relatively common in Japanese CIPA families and shows linkage disequilibrium with a rare haplotype in normal chromosomes, strongly suggesting that it is a common founder mutation (Miura et al. 2000). In the patient, the two chromosomes with the R548 fs mutation were linked to a rare haplotype CIPA1, which had previously been found to be associated with more than 90% of the R548 fs chromosomes. The father had two haplotypes, the CIPA1 and a haplotype A, the most common in normal Japanese controls. In contrast, the mother had two separate haplotypes, A and B, which can be distinguished on the basis of three intragenic polymorphic sites encompassing nucleotide 1726. Thus, we excluded the possibility that the mother had a large deletion in the *TRKA* gene. The father only transmitted the mutant allele to the patient. These analyses indicate the non-Mendelian inheritance of the *TRKA* locus haplotypes in this family.

In order to determine whether this non-Mendelian inheritance involved all or part of chromosome 1 and to exclude non-maternity in this family, we performed allelotype analyses for the patient and his parents. As shown in Fig. 2, the patient was homozygous at all 31 loci, 18 of them being exclusively derived from the father. The patient inherited two copies of one paternal allele, although some markers were not fully informative. To ascertain that maternity was correct, microsatellite markers from the other chromosomes were tested. The primer pairs for autosomal loci were tested, and 34 informative loci are shown in Table 1. Allelotype

analysis of the loci other than chromosome 1 in the patient and his parents demonstrated typical Mendelian inheritance, with paternal and maternal alleles being detected in the patient, indicating a high likelihood of maternity. Thus, we identified complete paternal isodisomy for chromosome 1 as the cause of reduction to homozygosity of the R548fs mutation, leading to CIPA.

Discussion

Several mechanisms for UPD have been proposed that are based on a meiotic nondisjunction event (Engel 1993). These include gamete complementation, postzygotic monosomy duplication, and trisomy rescue (reduction to disomy of a trisomic conception). In gamete complementation, a nullisomic gamete and a disomic gamete are fertilized. In monosomy duplication, a nullisomic gamete and a normal gamete are fertilized, followed by post-zygotic duplication of the monosomic chromosome. In trisomy rescue, a disomic gamete and a normal gamete are fertilized, followed by post-zygotic loss of the extra chromosome. Two explanations can be considered in the present case. One is paternal monosomy duplication associated with a nullisomic maternal gamete, as follows: a nullisomic ovum resulting from maternal meiotic nondisjunction was fertilized by a monosomic sperm giving a monosomic zygote. Subsequently, the single chromosome was duplicated, leading to the complete isodisomy. The other is paternal meiosis II nondisjunction without crossing over (recombination) of two homologous paternal chromosomes, resulting in a trisomic conceptus, with subsequent reduction to disomy through loss of the maternal chromosome. A failure to detect mosaicism in the karyotype of peripheral lymphocytes leads us to favor the former as the explanation for the findings in this patient. However, the latter is also tenable, since placental tissues of the patient were not available for analysis.

Recent reports of UPD for chromosome 1 have suggested that there are no imprinted genes on chromosome 1. At least four cases of UPD for chromosome 1, including three maternal and one paternal, have been reported. Three maternal UPDs for chromosome 1 were described in a male newborn with Herlitz junctional epidermolysis bullosa (Pulkkinen et al. 1997), in a female with insulin dependent diabetes mellitus (Field et al. 1998), and a male with Chediak-Higashi syndrome (Dufourcq-Lagelouse et al. 1999). Allelotypes of the first two cases indicated pericentromeric heterodisomy with partial isodisomy, whereas that of the third suggested complete isodisomy. In contrast, one paternal UPD for chromosome 1 was described in a male with pycnodysostosis (Gelb et al. 1998). The allelotype of this case indicated centromeric isodisomy and telomeric heterodisomy. None of these four cases shows an abnormal karyotype or additional phenotypes other than those associated with each disorder. These results indicate that UPD for chromosome 1 with a normal karyotype does not show an apparent phenotype in human. Recently, paternal UPD for chromosome 1 has been reported in a female with short stature, ptosis, micro/retrognathia, myopathy, deafness and sterility (Chen et al. 1999). Analysis of her karyotype indicates the presence of two isochromosomes, i(1p) and i(1q). The adverse phenotypes of this patient have been ascribed

to one or more recessive mutations, genomic imprinting, or a combination of both. In the present study, we have described a male case of complete paternal uniparental isodisomy for chromosome 1 with a normal karyotype. His physical growth remains normal. His mental retardation is probably attributable to one of the features associated with CIPA. The disorder in this child is caused by the paternal contribution of two copies of a defective *TRKA* gene, with no evidence for additional abnormalities. Thus, our findings further support the idea that there are no paternally imprinted genes on chromosome 1 with a major effect on phenotype.

UPD has been recognized as a genetic mechanism for the non-Mendelian inheritance of autosomal recessive disease from a single carrier patient (Engel 1998). The recurrent risk of CIPA is usually 0.25, since its mode of inheritance is autosomal recessive. However, the recurrent risk of CIPA in the present family would be almost zero, because the recurrent risks for UPD is expected to be extremely low. Generally, adverse phenotypes associated with UPD for chromosome 1 will be rare when it happens to be detected in clinical situations, such as a routine prenatal diagnosis for chromosome aberrations or other genetic disorders. However, it should be kept in mind that autosomal recessive disorder(s) mapped on chromosome 1 could occur with reduction to homozygosity in any case of UPD. The risk of a recessive disorder for any individual case of UPD is probably quite low, although a number of UPD cases have been ascertained on the basis of an autosomal recessive disorder. The highest risk would be associated with complete isodisomy, in which the risk for an average-sized human chromosome (~5% of the genome) would be approximately similar to that of children of first cousin mating (approximately 6% homozygosity by descent; (Ledbetter and Engel 1995). The risk for the lengthy chromosome 1 might be higher compared with an average- sized human chromosome. UPD must be considered as a rare but possible cause of autosomal recessive disorders when conducting genetic testing.

Acknowledgments

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Figures

Fig. 1. Restriction digestion analysis of a single base C deletion at nucleotide 1726 in the *TRKA* gene in family KI-24 with CIPA.

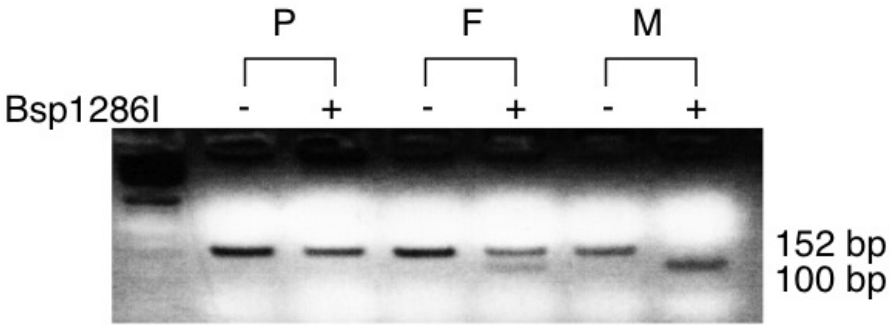

Fig. 1. Restriction digestion analysis of a single base C deletion at nucleotide 1726 in the *TRKA* gene in family KI-24 with CIPA. *TRKA* exon 14 was amplified from the genomic DNA of the proband and family members by PCR. PCR products of 152 bp were digested with *Bsp* 1286I and electrophoresed on an agarose gel, as described by Indo et al. (1996). A *Bsp* 1286I site is present in the normal control sequence, providing fragments of 102 and 50 bp (the latter is not visible). The single base deletion disrupts this site, preventing digestion.
+, - Presence and absence of the enzyme for incubation, respectively. The digested PCR products are shown from the patient (*P*), his father (*F*), and mother (*M*).

Fig. 2. Allelotype analysis of the proband and his parents with chromosome 1 markers.

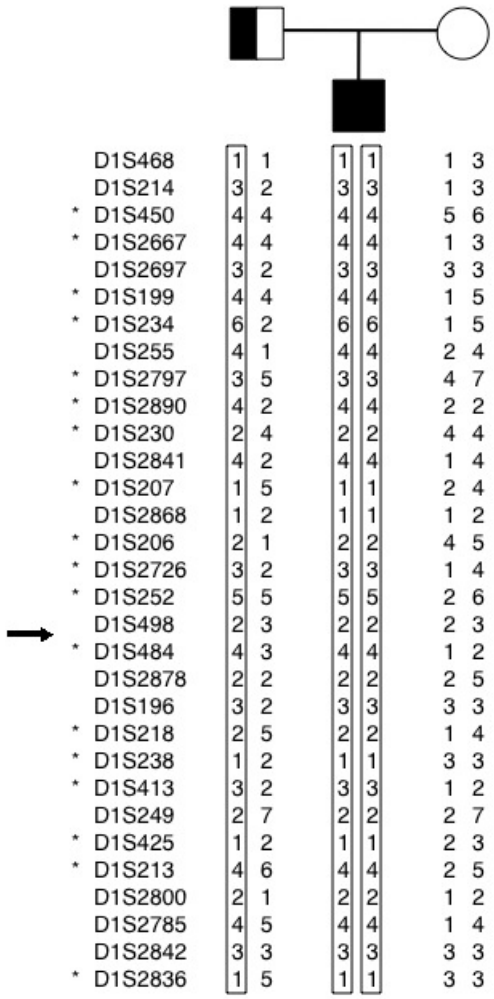

Fig. 2. Allelotype analysis of the proband and his parents with chromosome 1 markers. The polymorphic markers used for these analyses are shown *left*, with fully informative ones being indicated by *asterisks*. Thirty-one polymorphic markers were located on chromosome 1 in an average distance of 10 cM from the 1p terminal to the 1q terminal regions. Alleles are labeled arbitrarily according to sizes. The allelotypes of the proband and parents are shown with one possible phasing. *Arrow* Locus of the *TRKA* gene

Table 1 Allelotype analysis of the loci on autosomes beside chromosome 1

	Alleles					
Marker	Father		Patient		Mother	
D2S367	3	2	3	5	5	4
D3S1292	2	6	2	1	1	3
D3S1569	5	2	5	5	5	3
D4S1592	3	3	3	2	2	4
D4S415	7	7	7	5	5	6
D5S419	5	1	5	6	6	6
D5S424	5	3	5	2	2	2
D6S287	2	2	2	2	2	3
D6S292	7	6	7	6	6	7
D7S507	2	1	2	4	4	5
D7S519	4	2	4	3	3	3
D8S277	3	5	3	3	3	6
D8S550	3	4	3	1	1	3
D9S164	2	2	2	3	3	1
D9S286	4	2	4	6	6	4
D10S192	5	1	5	3	3	1
D10S249	5	3	5	5	5	6
D11S4175	5	5	5	3	3	2
D11S4191	7	6	7	3	3	6
D12S1617	7	2	7	4	4	5
D12S99	5	5	5	6	6	4
D13S217	2	5	2	4	4	1
D13S285	6	6	6	2	2	1
D15S1007	4	5	4	7	7	5
D15S205	1	8	1	4	4	7
D17S787	2	1	2	5	5	5
D18S1161	1	7	1	4	4	5
D18S59	3	4	3	5	5	5
D19S902	3	3	3	2	2	1
D20S195	2	1	2	4	4	3
D20S196	1	7	1	7	7	7
D21S266	3	6	3	5	5	5
D22S280	6	3	6	2	2	4
D22S283	6	5	6	4	4	2