Retinal Dystrophy Due to Paternal Isodisomy for Chromosome 1 or Chromosome 2, with Homoallelism for Mutations in RPE65 or MERTK, Respectively

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Uniparental disomy (UPD) is a rare condition in which a diploid offspring carries a chromosomal pair from a single parent. We now report the first two cases of UPD resulting in retinal degeneration. We identified an apparently homozygous loss-of-function mutation of RPE65 (1p31) in one retinal dystrophy patient and an apparently homozygous loss-of-function mutation of MERTK (2q14.1) in a second retinal dystrophy patient. In both families, the gene defect was present in the patient’s heterozygous father but not in the patient’s mother. Analysis of haplotypes in each nuclear kindred, by use of DNA polymorphisms distributed along both chromosomal arms, indicated the absence of the maternal allele for all informative markers tested on chromosome 1 in the first patient and on chromosome 2 in the second patient. Our results suggest that retinal degeneration in these individuals is due to apparently complete paternal isodisomy involving reduction to homoallelism for RPE65 or MERTK loss-of-function alleles. Our findings provide evidence for the first time, in the case of chromosome 2, and confirm previous observations, in the case of chromosome 1, that there are no paternally imprinted genes on chromosomes 1 and 2 that have a major effect on phenotype.

Uniparental disomy (UPD) arises when a diploid individual carries both homologs of a chromosomal pair from a single parent (uniparental heterodisomy) or two copies of a single parental chromosome (uniparental isodisomy) (Engel 1980). Uniparental heterodisomy occurs most frequently in cases in which both homologs are maternally derived (maternal UPD), and uniparental isodisomy occurs most frequently in cases in which both homologs are paternally derived (paternal UPD), with the overall prevalence of maternal UPD being three times greater than that of paternal UPD (Kotzot 1999). Possible mechanisms of UPD include gamete complementation, trisomic zygote rescue (reduction to disomy), postzygotic monosomy duplication, and somatic crossing-over (Engel and DeLozier-Blanchet 1991; Robinson 2000). Disease phenotypes associated with UPD can be due to trisomy mosaicism, unmasking of autosomal recessive gene defects by reduction to homoallelism, or imprinting (Engel 1998; Kotzot 1999). Studies of UPD in humans and mice played an important part in establishment of the role that imprinting plays in parent-of-origin–specific gene expression and in identification of imprinted regions of the genome that have profound effects on fetal growth, development, and viability (Preece and Moore 2000). Studies of autosomal recessive disorders identified a number of cases of UPD resulting in reduction to homoallelism of disease-associated mutations (Engel 1998).

Inherited retinal dystrophies are a genetically heterogeneous group of diseases responsible for visual deficits that vary in severity and age at onset and include some
forms of congenital blindness. Mutations in a number of genes expressed in the photoreceptor cells or the adjacent retinal pigment epithelium (RPE) have been shown to cause autosomal dominant and recessive forms of retinal degeneration (Phelan and Bok 2000). Our previous studies identified two disease genes, RPE65 and MERTK, involved in essential aspects of RPE physiology necessary for photoreceptor-cell function and viability (Gu et al. 1997; Gal et al. 2000). RPE65 (MIM 180069) encodes a unique protein localized in the RPE smooth endoplasmic reticulum and is required for the conversion of vitamin A to 11-cis retinal, the chromophore of the visual pigments (Hamel et al. 1993; Nicotelli et al. 1995; Redmond et al. 1998). Mutations in RPE65 cause early-onset severe retinal degeneration that is often diagnosed as Leber congenital amaurosis (Gu et al. 1997; Arnhens et al. 1997). Over 50 different disease-associated mutations have been reported, with current estimates indicating that RPE65 mutations are responsible for ~10% of cases of autosomal recessive retinal dystrophy with early onset and severe phenotype (Thompson et al. 2000). MERTK (MIM 604705) encodes a tyrosine kinase receptor that is the human ortholog of the Royal College of Surgeons (RCS) rat retinal-dystrophy gene (D’Cruz et al. 2000). In the RCS rat, mutations in Mertk cause retinal degeneration as a result of defective phagocytosis of shed photoreceptor outer segments by the RPE (Mullen and Lavail 1976; Edwards and Szamier 1977). Disease-associated mutations of MERTK have been reported in patients with autosomal recessive retinitis pigmentosa (Gal et al. 2000).

We identified two families in which a mutation of RPE65 or MERTK, present in apparently homozygous form in the affected individual, was carried in heterozygous form by only one parent (Gal et al. 2000; Thompson et al. 2000). In the first case (patient 1723), a 51-year-old man diagnosed with Leber congenital amaurosis in childhood had an apparently homozygous mutation of RPE65, IVS1+5g→a, that is the most frequently occurring mutation in this gene (comprising ~19% of all reported RPE65 disease alleles) (Thompson et al. 2000). The mutation identified in patient 1723 was present in heterozygous form in his unaffected father and brother but was not present in his mother. Evidence suggesting that no maternal RPE65 allele was transmitted to the patient was obtained by analysis of two intragenic DNA polymorphisms; a microsatellite repeat (D1S2803) in the promoter region (Nicotelli et al. 1998) and an intronic single-nucleotide polymorphism (IVS6+22c→t). The patient appeared to be homozygous for both markers, and only the paternal allele was detected (fig. 1). Further analysis of 19 informative microsatellite markers, spread out along both arms of chromosome 1, suggested complete absence of the maternal homolog, since the patient appeared homozygous for the paternal allele in each case. Genotypes obtained for 12 other partially informative DNA polymorphisms on chromosome 1, including an intragenic 2-bp insertion/deletion-type polymorphism (2383ins/del) located in the 3’ UTR of RPE65 (Nicotelli et al. 1995), were also consistent with the assumption of paternal isodisomy of chromosome 1. The assumed biological relationship of the patient to his parents was confirmed by analysis of a number of microsatellite markers on chromosomes 2, 3, 6, and X (data not shown). Routine cytogenetic analysis of metaphase spreads prepared from peripheral blood showed a normal male karyotype, 46,XY.

Patient 1723 has no obvious physical abnormalities and is currently engaged in a high-level professional career. He was born after a full-term pregnancy, when his mother and father were 22 and 25 years of age, respectively. The patient has hyperbetalipoproteinemia, which is also present in his father and in his father’s siblings, as well as benign hyperbilirubinemia (Gilbert syndrome). Neither condition can be ascribed to the patient’s UPD status. Onset of his retinal disease occurred at age ~5 years, with severe visual loss and nystagmus. At age 8 years, his visual acuity was 20/200, with a refraction of +4.00. Signals on rod and cone electroretinograms were not detectable. At age 50 years, he had only small islands of vision remaining. The course and severity of the patient’s visual disorder fit well within the range of clinical phenotypes exhibited by other reported patients with retinal dystrophy who had mutations of RPE65 (Gu et al. 1997; Arnhens et al. 1997; Morimura et al. 1998; Perrault et al. 1999; Lorenz et al. 2000; Lotery et al. 2000; Thompson et al. 2000).

In the second case (that of patient arRP185), a woman diagnosed with retinitis pigmentosa had an apparently homozygous MERTK mutation, IVS10–2a→g, that was present in heterozygous form in her unaffected father but was not present in her mother (Gal et al. 2000). Analysis of a number of exonic and intronic, partially informative DNA polymorphisms was consistent with the assumption of a paternal isodisomy, since the patient appeared to be homozygous for one of the paternal alleles (data not shown). Further analysis of 17 informative microsatellite markers spread out along both arms of chromosome 2 suggested complete absence of the maternal homolog (fig. 2). Data obtained for eight other partially informative DNA polymorphisms on chromosome 2 were also consistent with the assumption of paternal isodisomy of chromosome 2. The assumed biological relationship of the patient to her parents was confirmed by analysis of a number of microsatellite markers on chromosomes 3–6, 9, 11, and 14–19 (data not shown). Routine cytogenetic analysis of metaphase
Figure 1  Genotype analysis of chromosome 1 in the family of patient 1723, who carries an RPE65 mutation. The disease-relevant RPE65 mutation is shown in boldface italics; marker order is as in the NCBI draft of the human genome (build 25), with cytogenetic locations derived from NCBI Locus Link and Genome Database Map View. Data from a total of 12 additional chromosome 1 polymorphisms were only partially informative, because the parents shared at least one allele. All data were consistent with paternal uniparental disomy of chromosome 1.

chromosome spreads prepared from peripheral blood showed a normal female karyotype (46,XX).

Patient arRP185 (currently 34 years of age), the mother of two healthy children, appears phenotypically normal in all aspects except for her retinal disease and a general connective-tissue weakness. She was born after a full-term pregnancy, when her mother and father were 36 and 33 years of age, respectively, and was of normal birth weight. She was noted to be night blind in pre-school, and, in primary school, she had poor vision that was uncorrectable by refractive lenses. At 12 years of age, she was diagnosed with retinitis pigmentosa. At 18 years of age, her peripheral vision was largely reduced (10° visual fields). At her current age, she has 5-degree visual fields and has difficulty reading. Her visual handicap is similar to that of the other retinal dystrophy patients reported to have mutations of MERTK (Gal et al. 2000).

Our studies have identified the first known cases of UPD resulting in retinal degeneration. In both cases, the patient's ocular phenotype appears to result from reduction to homoallelicism of a disease-causing mutation of paternal origin, since marker analysis suggests that each patient carries two complete and identical homologs of a single paternal chromosome. Such cases of uniparental disomy can be thought of as resulting from compensatory UPD—that is, from replacement of a missing chromosome by early somatic duplication in cases of postzygotic monosomy (Robinson 2000). The mutations involved, IVS1+5g→a in RPE65 and IVS10−2a→g in MERTK, are predicted to interfere with correct splicing and to generate loss-of-function alleles of known disease genes that are expressed in the RPE and are necessary for photoreceptor cell function. The associated ocular phenotypes appear to be very similar to those of other patients who carried mutations in the same genes in homozygous or compound heterozygous form, without additional severe abnormalities in our patients that would indicate multiple autosomal recessive defects, major effects of imprinting, or both.

Previous studies have identified UPD involving various chromosomes in patients affected with different auto-
somal recessive disorders (Engel 1998). Reported cases of UPD of chromosome 1 (UPD 1) involving full or partial isodisomy include a number of cases of maternal UPD 1 (Pulkkinen et al. 1997; Field et al. 1998; Dufourcq-Lagelouse et al. 1999), as well as cases of paternal UPD 1 (Gelb et al. 1998; Chen et al. 1999; Miura et al. 2000; Takizawa et al. 2000). Of the latter, two are cases of likely complete paternal isodisomy associated with either (a) congenital insensitivity to pain, with anhidrosis due to mutation in TRKA (MIM 191315) (M iura et al. 2000), or (b) Herlitz junctional epidermolysis bullosa due to mutation in LAMC2 (M IM 150292) (Takizawa et al. 2000), with both patients having phenotypes characteristic of autosomal recessive disease but being too young (ages 8 mo and 2 years) to exclude with certainty any additional abnormalities suggestive of an effect of imprinting. Cases of UPD of chromosome 2 (UPD 2) reported elsewhere include a case of paternal i(2p) with maternal i(2q) in a phenotypically normal female with two isochromosomes (Albrecht et al. 2001). All other cases were of maternal UPD 2, with four reported cases apparently due to trisomy rescue resulting in uniparental heterodisomy (Harrison et al. 1995; Webb et al. 1996; Hansen et al. 1997; Heide 2000) and with two reported cases of maternally derived isochromosomes i(2p),i(2q) (Bernasconi et al. 1996; Shaffer et al. 1997).

The case of UPD 2 described in the present study represents the first example of complete paternal isodisomy of chromosome 2, providing evidence, for the first time, that there are no paternally imprinted genes on chromosome 2 that have a major effect on growth or development. The case of UPD 1 described in the present study represents the first example of complete paternal isodisomy of chromosome 1 in an adult individual, establishing that there are no paternally imprinted genes on chromosome 1 that have a major effect on phenotype. In addition, our findings suggest that, in general, the total number of recessive mutations per chromosome in any one individual may be low. It is tempting to speculate that future genetic studies may find that UPD of chromosomes or chromosomal regions unaffected by imprinting may be a more common cause of autosomal recessive disease than has been appreciated previously.
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Electronic-Database Information

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- Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for RPE65 [MIM 180069], MERTK [MIM 604705], LAMC2 [MIM 150292])

References


