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Paternal Uniparental Disomy for Chromosome 1 Revealed by Molecular Analysis of a Patient with Pycnodysostosis

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Summary

Molecular analysis of a patient affected by the autosomal recessive skeletal dysplasia, pycnodysostosis (cathepsin K deficiency; MIM 265800), revealed homozygosity for a novel missense mutation (A277V). Since the A277V mutation was carried by the patient's father but not by his mother, who had two normal cathepsin K alleles, paternal uniparental disomy was suspected. Karyotyping of the patient and of both parents was normal, and high-resolution cytogenetic analyses of chromosome 1, to which cathepsin K is mapped, revealed no abnormalities. Evaluation of polymorphic DNA markers spanning chromosome 1 demonstrated that the patient had inherited two paternal chromosome 1 homologues, whereas alleles for markers from other chromosomes were inherited in a Mendelian fashion. The patient was homoallelic for informative markers mapping near the chromosome 1 centromere, but he was heteroallelic for markers near both telomeres, establishing that the paternal uniparental disomy with partial isodisomy was caused by a meiosis II nondisjunction event. Phenotypically, the patient had normal birth height and weight, had normal psychomotor development at age 7 years, and had only the usual features of pycnodysostosis. This patient represents the first case of paternal uniparental disomy of chromosome 1 and provides conclusive evidence that paternally derived genes on human chromosome 1 are not imprinted.

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Introduction

Pycnodysostosis is a rare autosomal recessive sclerosing skeletal dysplasia that is characterized by reduced stature, osteosclerosis, acro-osteolysis of the distal phalanges, frequent fractures, clavicular dysplasia, and skull deformities with delayed suture closure (Andren et al. 1962; Maroteaux and Lamy 1962). To date, >150 patients have been reported (e.g., Sedano et al. 1968; Edelson et al. 1992), most of whom were the offspring of consanguineous parents. The pycnodysostosis locus was mapped to chromosomal band 1q21 by genetic linkage (Gelb et al. 1995; Polymeropoulos et al. 1995), and the disease gene was identified recently, by means of a positional cloning strategy, as cathepsin K, a lysosomal cysteine protease (EC 3.4.22.38) (Gelb et al. 1996). The human cathepsin K cDNA (Brömme and Okamoto 1995; Inaoka et al. 1995; Li et al. 1995; Shi et al. 1995) and genomic organization (Gelb et al. 1997; Rood et al. 1997), including the promoter and intron/exon boundary sequences, have been determined. Three mutations in the mature region of the cathepsin K prepropeptide were reported, elsewhere, in four unrelated pycnodysostosis families (Gelb et al. 1996; Johnson et al. 1996). These lesions included a missense (G146R), a nonsense (R241X), and a stop codon (X330W) mutation that altered the amino acid sequence of the mature polypeptide. Transient expression of the X330W allele resulted in normal message levels but no immunologically detectable protein (Gelb et al. 1996).

In the course of molecular analysis of a nonconsanguineous pycnodysostosis family, genotyping with polymorphic DNA markers from 1q21 revealed that the affected child had inherited a paternal haplotype but no maternal haplotype. Subsequent analyses with informative 1p and 1q telomeric markers showed inheritance of both paternal alleles. Mutation analysis detected homoallelism in the patient for a cathepsin K missense mutation that was carried by his father but not by his mother. Cytogenetic studies failed to reveal any abnormalities of chromosome 1. Therefore, it was concluded that pycnodysostosis had resulted from paternal uni-

parental disomy (UPD) for chromosome 1 with inheritance of two copies of a cathepsin K mutation carried by the father.

Aside from representing a novel mechanism for the inheritance of this rare skeletal dysplasia, this case is noteworthy for being the first example of paternal UPD for chromosome 1. Since the concept of UPD was first delineated by Engel (1980), instances of maternal and paternal UPD involving the X chromosome and the majority of the autosomes have been documented (Ledbetter and Engel 1995). For several chromosomes, UPD results in distinct phenotypes depending on the parental origin of the chromosomes. The phenomenon appears to be mediated by genomic imprinting of a specific gene or, more often, of a group of genes that are inherited from both parents but that function unequally in the offspring, depending on their parental origin. Well-established examples include the Prader-Willi and Angelman syndromes, which can result from maternal and paternal UPD for chromosome 15, respectively, and the Beckwith-Weideman syndrome, which has been associated with paternal UPD of chromosome 11 (Ledbetter and Engel 1995). For chromosomes without imprinting effect, UPD does not cause a phenotype directly, but it may be detected if two copies of a mutation for an autosomal recessive trait are inherited from one parent. For example, UPDs for chromosomes 7 and 8 were found during molecular analyses of patients with congenital chloride diarrhea and lipoprotein lipase deficiency, respectively (Hoglund et al. 1994; Benlian et al. 1996). To assemble a complete imprinting map of the human genome, it is necessary to assemble both a maternal and a paternal UPD case for all chromosomes.

Recently, Pulkkinen et al. (1997) reported the first case of maternal UPD for chromosome 1, discovered in a newborn who presented with the autosomal recessive dermatologic disorder, Herlitz junctional epidermolysis bullosa, but without dysmorphic features. Although his death at 2 months of age limited a complete assessment of the patient's psychomotor development, the authors concluded that maternal imprinting on chromosome 1 was highly unlikely. In this communication, we describe a case of paternal UPD for chromosome 1 that did not result in phenotypic abnormalities or developmental delay, suggesting that chromosome 1 can be added to the list of autosomes without imprinting.

Patient, Material, and Methods

Clinical Report

The propositus was a 3,210-g (50th percentile), 49-cm (25th–50th percentile) male, the fourth child born to healthy unrelated parents. The mother was 36 years old and of Belgian descent. The father was 38 years old and of mixed Belgian and Algerian descent. The pregnancy, labor, and delivery were uncomplicated. The propositus was first evaluated at 4.5 years of age, by a clinical geneticist (A.V.). At that time, his weight was 25 kg (95th percentile), his height was 98 cm (5th percentile), and his occipital-frontal circumference was 51 cm. Physical examination revealed a round face with prominent frontal bossing, narrow palpebral fissures, a long philtrum, micrognathia, microstomia, crowded and irregular teeth, generalized obesity, short hands (9.5 cm) with brachdactyly (third finger 3.5 cm), short feet (16 cm), and mildly dysplastic nails. Roentgenograms documented osteosclerosis with narrowed medullary spaces, proportionately short long bones, brachydactyly with acro-osteolysis, complete loss of the mandibular angle, and sclerotic cranial bones with widely open sutures. The propositus had previously sustained three pathological fractures of the lower extremities. These clinical and radiologic findings were consistent with the diagnosis of pycnodysostosis.

The patient's only other known medical problem was normocalcemic hypercalciuria with ureteral lithiasis, which was also present in his father. The propositus had normal serum parathormone and vitamin D metabolite levels. The urinary Ca/creatinine ratio was >0.2, and calciuria of 3.5–6.0 mg/kg/d was documented. No hyperoxaluria or hyperuricosuria was observed. The propositus, who is currently 7 years old, has achieved his neurodevelopmental milestones appropriately and currently performs academically at the expected grade level.

Cytogenetic and Molecular Analyses

Routine cytogenetic analysis was performed on peripheral blood lymphocyte cultures from the propositus and both parents. Genomic DNA for molecular studies was extracted from lymphocytes by standard techniques (Sambrook et al. 1989). Simple-tandem-repeat markers (STRs) from chromosome 1 were PCR-amplified with oligonucleotide primers (Research Genetics) as described elsewhere (Gelb et al. 1995). Similarly, STRs from six other chromosomes were analyzed. Mutation analysis for the cathepsin K gene was performed by PCR amplification of the seven coding exons and adjacent introns from genomic DNA of the propositus. Amplified fragments were isolated and sequenced by cycle sequencing with an ABI 377 Sequencer. These sequences were compared with wild type using the FRACTURA and AUTOASSEMBLER software packages. The identified mutation obliterated an *Aci*I site, so its presence was assayed in the propositus and his parents by PCR amplification of cathepsin K exon 7 from genomic DNA, digestion of the product with *Aci*I, and separation of the resulting fragments by horizontal electrophoresis in an

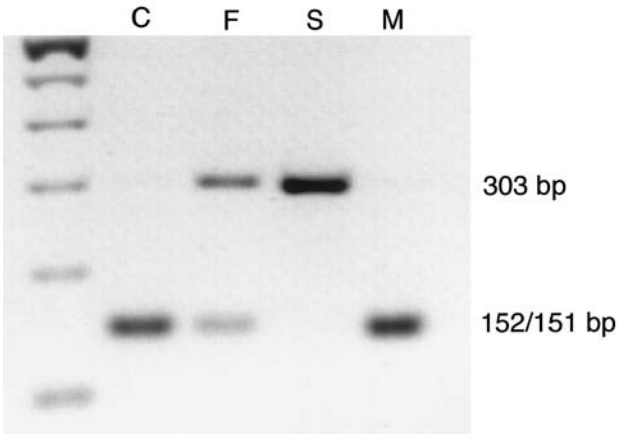


Figure 1 Restriction-fragment-length analysis for the cathepsin K A277V mutation. An ethidium bromide–stained agarose gel containing a 303-bp amplified product that was digested with *Aci*I. The digested PCR products from a normal control individual (C), the father (F), the affected son (S), and the mother (M) are shown. The normal PCR product was digested into 152-bp and 151-bp fragments, whereas the 303-bp products with the A277V defect were not digested. A 100-bp DNA ladder is shown, *left*.

agarose gel with direct visualization using ethidium bromide.

Results

Cathepsin K Mutation Analysis

Sequencing of the seven cathepsin K coding exons and their respective exon-intron boundaries successfully amplified from genomic DNA of the propositus revealed a C→T transition of nucleotide 935. This point mutation predicted the substitution of an alanine by a valine at residue 277 (A277V) in the mature cathepsin K polypeptide. Analyses of genomic DNA obtained by PCR amplification of a 303-bp fragment from the propositus and from his father identified the mutation that obliterated an *Aci*I restriction site (fig. 1). In the propositus, only an undigested 303-bp fragment was present, whereas his mother's PCR product was digested to 152-bp and 151-bp fragments (which were not resolved on the agarose gel). In contrast, digestion of the 303-bp amplimer from his father revealed two bands, the 303-bp undigested fragment and the 152-bp/151-bp digested fragments. These results were consistent with homozygosity for the A277V mutation in the propositus, heterozygosity for this mutation in his father, and absence of the mutation in his mother.

Cytogenetic Analysis

Routine G-banding of metaphase preparations of cultured lymphocytes from the propositus showed a normal 46, XY karyotype with no evidence of deletions, particularly of the chromosome 1q21 region (fig. 2). Evaluation of his parents revealed the expected 46, XY and 46, XX for his father and mother, respectively.

Polymorphic DNA Marker Analysis

Initially, several STRs from the region at 1q21 containing the cathepsin K gene were evaluated. Marker *D1S2343*, which flanked the gene by <1 cM on the telomeric side, was completely informative and revealed only one paternal allele (presumably present as two copies) and no maternal alleles in the propositus (fig. 3). Additional markers from this region, which were not fully informative, were inherited in a fashion consistent with homozygosity for the paternal allele (fig. 4). Subsequently, several STRs spread along the short and long arms of chromosome 1 were evaluated. As shown in figures 3 and 4, analysis of the propositus with several fully informative markers revealed inheritance of both paternal alleles but no maternal allele. For less-informative markers, there was no instance in which a maternal allele was unambiguously inherited by the propositus. Finally, analysis of the propositus and his parents with several STRs from chromosomes other than chromosome 1 showed typical Mendelian inheritance, with paternal and maternal alleles detected in the propositus (data not shown).

Discussion

In the course of mutation analysis of the cathepsin K gene in a patient with pycnodysostosis, the occurrence

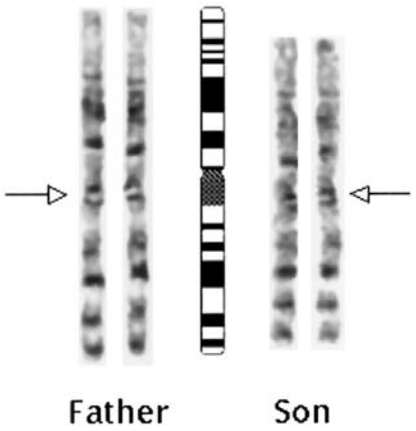


Figure 2 Chromosome 1 homologues from the proband and from his father. Cytogenetic analysis of the pycnodysostosis patient and of his father showed normal 46, XY karyotypes for both, and no cytogenetic abnormalities were observed in lymphocytes by high-resolution cytogenetic analysis (>800 bands). The chromosome 1 centromeric heteromorphism (*arrow*) was not informative on the chromosome 1 homologues in the father.

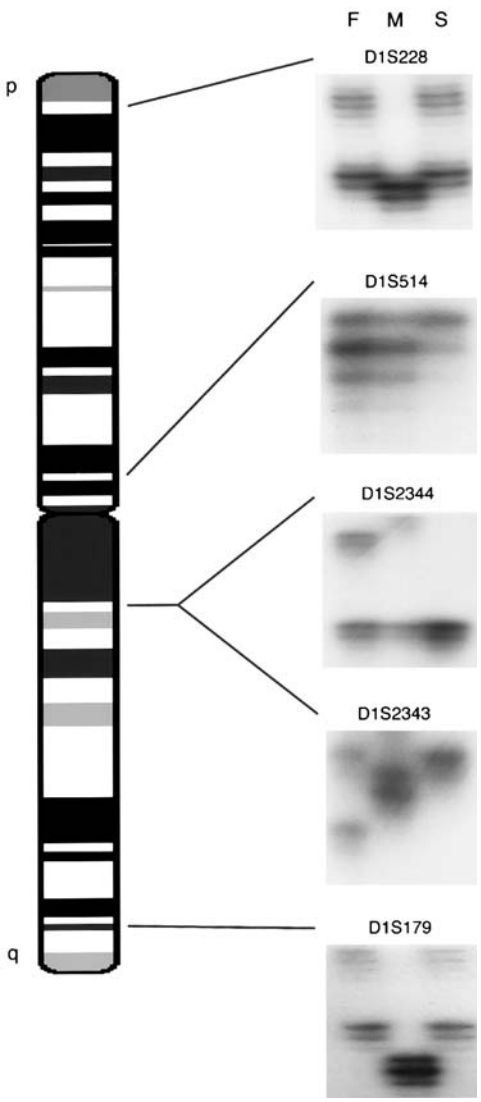


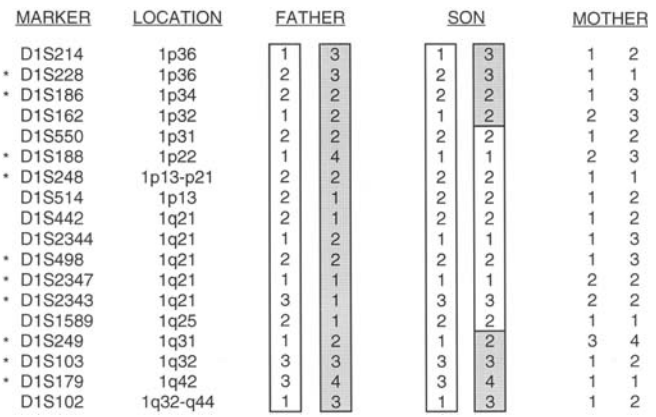
Figure 3 Polymorphic marker analyses in the proband and in his parents. Autoradiograms of several STR markers assigned to the indicated regions of chromosome 1 analyzed with DNA from the father (F), the mother (M), and their affected son (S). For fully informative markers *D1S228, D1S2343,* and *D1S179,* the affected son inherited only alleles from his father. The markers from the telomeric regions of the 1p and 1q, *D1S228* and *D1S179,* respectively, showed inheritance of both paternal alleles, whereas those in the pericentromeric region—*D1S514, D1S2344,* and *D1S2343,*—revealed inheritance of only one of the two paternal alleles. In addition, *D1S2344* and *D1S2343* closely flank the pycnodysostosis locus at 1q21, predicting inheritance of two copies of one paternal allele by the affected son. These findings are consistent with paternal uniparental disomy in the son with pycnodysostosis.

of UPD for the paternal chromosome 1 was shown to be the cause of this recessive skeletal dysplasia. This novel finding was demonstrated by analysis of the propositus with polymorphic DNA markers along chromosome 1, which revealed inheritance of both paternal alleles in some regions, indicating that, although pycnodysostosis in the proband resulted from paternal isodisomy at the relevant locus, other parts of chromosome 1 displayed paternal heterodisomy. The phenotypes observed in the propositus included pycnodysostosis resulting from the inheritance of two copies of a cathepsin K mutation from his father and familial idiopathic hypercalciuria, an autosomal dominant trait inherited from the father, and, presumably, unrelated to the UPD. Neither the gene nor the locus for familial idiopathic hypercalciuria has been determined.

Three phenotypic abnormalities that are frequently associated with UPD for chromosomes with imprinting are intrauterine growth retardation, developmental delay, and reduced stature. In this propositus, birth height and weight were normal, as was psychomotor development, but possible effects of UPD on linear growth could not be assessed, since a seminal feature of pycnodysostosis is short stature. Nonetheless, it is concluded that paternal UPD for chromosome 1 did not result in an observable phenotype, a finding that is consistent with previous predictions based on imprinting maps in the mouse. Human chromosome 1 has synteny with portions of mouse chromosomes 1, 3, 4, 8, and 13. None of those murine chromosomes has been shown to have either paternally or maternally imprinted regions (Cattanach et al. 1995).

On the basis of haplotype analyses, the mechanism responsible for UPD of chromosome 1 in this proband was paternal nondisjunction with inheritance of two chromosome 1 homologues that had recombined during meiosis I. Thereafter, either a sperm that was disomic for chromosome 1 fertilized a nullisomic egg (gamete complementation), or a trisomy 1 embryo was salvaged by the loss of the maternal copy of chromosome 1 (trisomy to disomy). These two mechanisms are indistiguishable unless mosaicism for trisomy 1 is documented. Since karyotyping of peripheral lymphocytes failed to reveal such mosaicism, and since placental tissue, which is the most likely site for such mosaicism, was unavailable in this case, gamete complementation and trisomy to disomy remain as theoretical explanations for this case of paternal UPD of chromosome 1.

It is not known what the relative probability of gamete complementation, requiring both maternal and paternal nondisjunction events, is, compared with trisomy-to-disomy salvage, which requires a paternal nondisjunction and then postfertilization chromosomal loss. Estimates of the prevalence of chromosome 1 disomy in sperm, assessed by interphase multicolor fluorescence in situ hybridization, ranged from 0.05%–0.20%, similar to the rates of disomy for other autosomes (Chevret et al. 1995; Spriggs et al. 1995). Despite the prevalence of sperm (and, presumably, eggs) that are disomic for chromosome 1, only one pregnancy with trisomy 1 in which no



	MARKER	LOCATION	FATHER		SON		MOTHER	
	D1S214	1p36	1	3	1	3	1	2
*	D1S228	1p36	2	3	2	3	1	1
*	D1S186	1p34	2	2	2	2	1	3
	D1S162	1p32	1	2	1	2	2	3
	D1S550	1p31	2	2	2	2	1	2
*	D1S188	1p22	1	4	1	1	2	3
*	D1S248	1p13-p21	2	2	2	2	1	1
	D1S514	1p13	2	1	2	2	1	2
	D1S442	1q21	2	1	2	2	1	2
	D1S2344	1q21	1	2	1	1	1	3
*	D1S498	1q21	2	2	2	2	1	3
*	D1S2347	1q21	1	1	1	1	2	2
*	D1S2343	1q21	3	1	3	3	2	2
	D1S1589	1q25	2	1	2	2	1	1
*	D1S249	1q31	1	2	1	2	3	4
*	D1S103	1q32	3	3	3	3	1	2
*	D1S179	1q42	3	4	3	4	1	1
	D1S102	1q32-q44	1	3	1	3	1	2

Figure 4 Genotype analysis of the proband and his parents with chromosome 1 markers. The polymorphic markers used for these analyses are shown on the left, with fully informative ones indicated by asterisks (*) and their cytogenetic locations noted in the second column. The genotypes of the proband and of the parents are shown with one possible phasing. The shaded areas demonstrate the alleles from the two paternal chromosome 1 homologues.

fetal development occurred has been reported, so trisomy 1 can be presumed to be lethal (Hanna et al. 1997). Interestingly, analysis of patients with Angelman syndrome caused by paternal UPD of chromosome 15 revealed that paternal isodisomy predominated, suggesting that the underlying mechanism was fertilization of a nullisomic egg by a normal sperm with subsequent salvage of a monosomy by postfertilization duplication of the paternal chromosome 15 (Mutirangura et al. 1993). This proposed mechanism was supported by a frequent incidence of advanced maternal age, increasing the likelihood that a maternal nondisjunction event had resulted in a nullisomic egg. In the present case, advanced maternal age was also noted.

Nondisjunction can occur as a result of errors in meiosis I or II. The meiotic origin of nondisjunction can be distinguished by analysis of polymorphic markers residing at or close to the centromere. Meiosis I errors result in heteroallelism near the centromere, whereas meiosis II errors cause homoallelism near the centromere. Salvage of monosomic embryos by duplication of the relevant chromosome would also result in homoallelism near the centromere, but it can often be differentiated from meiosis II errors by analysis with more telomeric markers. If any chiasmata formed during meiosis I, UPD chromosomes resulting from meiosis II errors would have heteroallelism of some markers, whereas duplicated chromosomes from postmeiotic salvage should be homoallelic at all loci. STR analysis of the propositus showed inheritance of both paternal alleles for several telomeric markers. The father was uninformative for the chromosome 1 centromeric heteromorphism, precluding its usefulness for establishing the status of the centromere. The fact that informative polymorphic markers *D1S442, D1S2344,* and *D1S2343* at 1q21 (Hoggard et al. 1995; Marenholz et al. 1996) and marker *D1S514* at 1p13 (Hoggard et al. 1995; Morissette et al. 1995) were homoallelic, as was the cathepsin K gene mutation at 1q21, provides strong evidence that the disomy resulted from a paternal meiosis II error. All of these loci reside within a few centimorgans of the centromere on the Généthon human genetic linkage map (Dib et al. 1996), and on the basis of chiasmata analyses during meiosis I in males (Hulten et al. 1982), they are unlikely to have recombined with respect to the centromere. Similar analyses of patients with the Angelman syndrome (Mutirangura et al. 1993) as well as of patients with paternal UPD for other autosomes (cited in Ledbetter and Engel 1995) have shown that isodisomy strongly predominates, presumably resulting from salvage of a monosomy caused by maternal nondisjunction. Thus, the meiosis II nondisjunction event documented here represents an unusual etiology for paternal disomy.

Pycnodysostosis was recently shown to be caused by defects in the gene for the lysosomal cysteine protease, cathepsin K, and three gene mutations have been reported. Subsequent molecular investigations of several individuals with pycnodysostosis have revealed six novel mutations, including a C→A transversion at nucleotide 935, predicting an alanine-to-glutamate substitution at residue 277 (A277E). In conjunction with the identification of the A277E mutation, >50 normal individuals were screened for the presence of the A277E lesion by means of the *Aci*I restriction assay that detected the A277V mutation in this study. No mutant alleles were found, which rules out the possibility that A277E or A277V is a polymorphism. The A277E mutation was found previously, in two unrelated patients of dissimilar ethnic backgrounds, on different haplotypes, which suggests independent mutational events. The finding in this

study of a third mutation at nucleotide 935 suggests that this is a mutational hot spot for pycnodysostosis. This is not entirely surprising, since that nucleotide occupies the middle position of a GCG codon, making it part of a CpG dinucleotide.

In summary, these studies have identified the first example of paternal UPD for chromosome 1 that was detected in a patient who had inherited two identical segments of a single paternal chromosome 1 homologue carrying a cathepsin K mutation, resulting in pycnodysostosis. The paternal UPD appears to have resulted from nondisjunction during meiosis II. The lack of an observable phenotype that can be attributed to paternal UPD for chromosome 1, combined with the previous evidence that chromosome 1 is not maternally imprinted, permits the assignment of chromosome 1 to the group of human chromosomes without imprinted genes.

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