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ARTICLE

Chediak-Higashi syndrome associated with maternal uniparental isodisomy of chromosome 1

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Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder (incidence around 1 in 10^6 births), characterised by a complex immunologic defects, reduced pigmentation, and presence of giant granules in many different cell types. It most likely results from defective organellar trafficking or protein sorting. The causative gene (*LYST*) has recently been identified and shown to be homologous to the beige locus in the mouse. CHS has always been reported associated with premature-termination-codon mutations in both alleles of *LYST*. We report a unique patient with CHS, who was homozygous for a stop codon in the *LYST* gene on chromosome 1 and who had a normal 46,XY karyotype. The mother was found to be a carrier of the mutation, whereas the father had two normal *LYST* alleles. Non-paternity was excluded by the analysis of microsatellite markers from different chromosomes. The results of 13 informative microsatellite markers spanning the entire chromosome 1 revealed that the proband had a maternal isodisomy of chromosome 1 encompassing the *LYST* mutation. The proband's clinical presentation also confirms the absence of imprinted genes on chromosome 1.

Keywords: Chediak-Higashi syndrome; maternal isodisomy; chromosome 1; LYST mutation

Introduction

Chediak-Higashi syndrome (CHS) (MIM: 214500) is a rare autosomal recessive immune disease, characterised by a partial oculocutaneous albinism, a predisposition to pyogenic infections and the presence of large granules in many cell types.[1,2] Unless treated by bone marrow transplantation, death usually occurs in childhood from a lymphohistiocytic syndrome, the so-called 'accelerated phase' of the disorder, though some patients have a relatively milder clinical course.[3] The hallmark of CHS is the presence of giant organelles-lysosomes, melanosomes and giant inclusion bodies seen in virtually all granulated cells.[2] The *CHS* gene product is likely to be involved in the biogenesis, structure, or function of these different organelles, possibly in organelle trafficking or protein sorting to organelles.[4] A similar disorder occurs in a wide variety of mammalian species, most importantly, the *beige* mouse which shares many features with human CHS.[5]

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The *CHS* locus was mapped to chromosomal band 1q42–43[6,7] and the disease gene (*LYST*) was identified by its homology to the mouse beige gene.[8,9] This gene encodes a large cytosolic as well as microtubule associated protein.[4,9,10] The precise function of *LYST*, however, remains unknown. Mutation analyses of the *CHS* gene have been somewhat hindered by the large size of the CHS cDNA (13.5 kb), and thus far, only eight pathologic mutations have been identified, all of which leading to a truncated CHS protein.[9,11,12]

We herein report the case of a cytogenetically normal male affected with CHS as a result of homozygosity for mutation of the *LYST* gene. Homozygosity for the *LYST* gene was the result of maternal disomy of the entire chromosome 1. The proband's clinical presentation is consistent with the absence of imprinted genes on maternal chromosome 1.

Subjects and Methods

Case Report

The proband (VA) was a 3200 g male, the eldest child of healthy unrelated parents. The mother was 24 and the father 27 years old at the patient's birth. The proband had one younger, clinically unaffected sister. The pregnancy, labour, and delivery were uncomplicated. Diagnosis of Chediak-Higashi syndrome was performed at the age of $5\frac{1}{2}$ years in the proband when he developed features characteristic of an accelerated phase of the disease. These included fever, oedema, hepatosplenomegaly, lymphadenopathy, pancytopenia, coagulation disorder and infiltration of most organs by lymphocytes and histiocytes. In addition, the patient presented with a partial oculocutaneous albinism, whilst giant granulations were detected in his leukocytes. Postnatal physical and mental development were totally normal. At this age, height was 108.5 cm, and weight was 20 kg. The child died at 6 years of age from veino-occlusive disease following a bone marrow transplantation attempt.

Conventional and Molecular Cytogenetics

Routine chromosome analysis of the proband was performed on RHG and GTG banded metaphases from B-EBV lymphoblastoid cell line. RBG banding was also performed on prometaphases from a FdU synchronised lymphoblastoid cell line by using a terminal 5-bromo-2'-deoxyuridine (BrDU) pulse for at least 7 hours of culture. Twelve metaphases were analysed. Fluorescence *in situ* hybridisation (FISH) analysis was performed with a chromosome 1q42–q43 specific probe (YAC 906H7, locus D1S235), closely linked to the *CHS* locus. This probe was amplified by *Alu*-PCR using the *Alu* specific primers, *Alu*278:5'-GGCCTCCCAAAGTGCTGGGATTACAGGC-3' and *Alu*IV: 5'-CGACAGAGCAGACTCCGTCTCA-3', and biotin-16-dUTP labelled by nick translation according to the Boehringer Mannheim protocol. The detection of the probe was performed with three amplification steps (Avidin-Texas red; Avidin-Biotin; Avidin-Texas red). Chromosomes were counterstained with DAPI.

Molecular Investigations

After appropriate informed consent, genomic DNAs from the proband and his parents, isolated from peripheral blood, were analysed with the polymorphic markers depicted in Figure 1 and Table 1 as previously described.[6] Non-paternity was tested by the use of nine microsatellite markers from five different chromosomes. The results of the informative markers are reported in Table 1. For mutation analysis of the *LYST* gene, cDNA was prepared from blood cell proband, amplified by PCR in nine overlapping fragments covering the entire sequence. These fragments were subject to a protein truncation test (PTT) analysis (manuscript in preparation). PCR product leading to a transcribed fragment of abnormal size was then directly sequenced. In the parents, sequence analysis was performed on a 150 bp PCR fragment DNA encompassing the mutation region.

Results

The proband was a 6-year-old male from a non consanguineous family with a clinical presentation of Chediak-Higashi syndrome. He was referred during an accelerated phase of the disease. Several polymorphic DNA markers from 1q42–q43 region containing the *Chediak-Higashi* gene were evaluated. Markers D1S2649, D1S235 and D1S2680 flanking the gene in a 2 cM region, were completely informative and revealed only one maternal allele (presumably present in two copies) and no paternal alleles in the propositus (Figure 1). In contrast, both maternal and paternal alleles were detected in his healthy sister. Ten additional microsatellite markers spanning the length of chromosome 1 which were partly or fully informative, were analysed in the parents and the proband. The proband was homozygous for all these markers. In each case, inheritance of one maternal allele was detected. Analysis of the proband and his parents with several polymorphic markers from other chromosomes showed typical Mendelian inheritance, with paternal and maternal alleles detected in the proband (Table 1).

Cytogenetic analysis of the patient using RHG, GTG, and RBG banding of metaphases and prophases from a lymphoblastoid cell line showed a normal 46,XY karyotype. FISH study with YAC 906H7 showed a fluorescent signal on each chromosome 1, confirming the absence of deletion of D1S235 locus (data not shown).

A Protein Truncated Test[13] was established to analyse the *LYST* gene in several patients with Chediak-Higashi syndrome (manuscript in preparation). This test enabled us to identify in the proband, a shorter protein than the one observed in controls *in vitro* translation assay of a PCR fragment. The latter

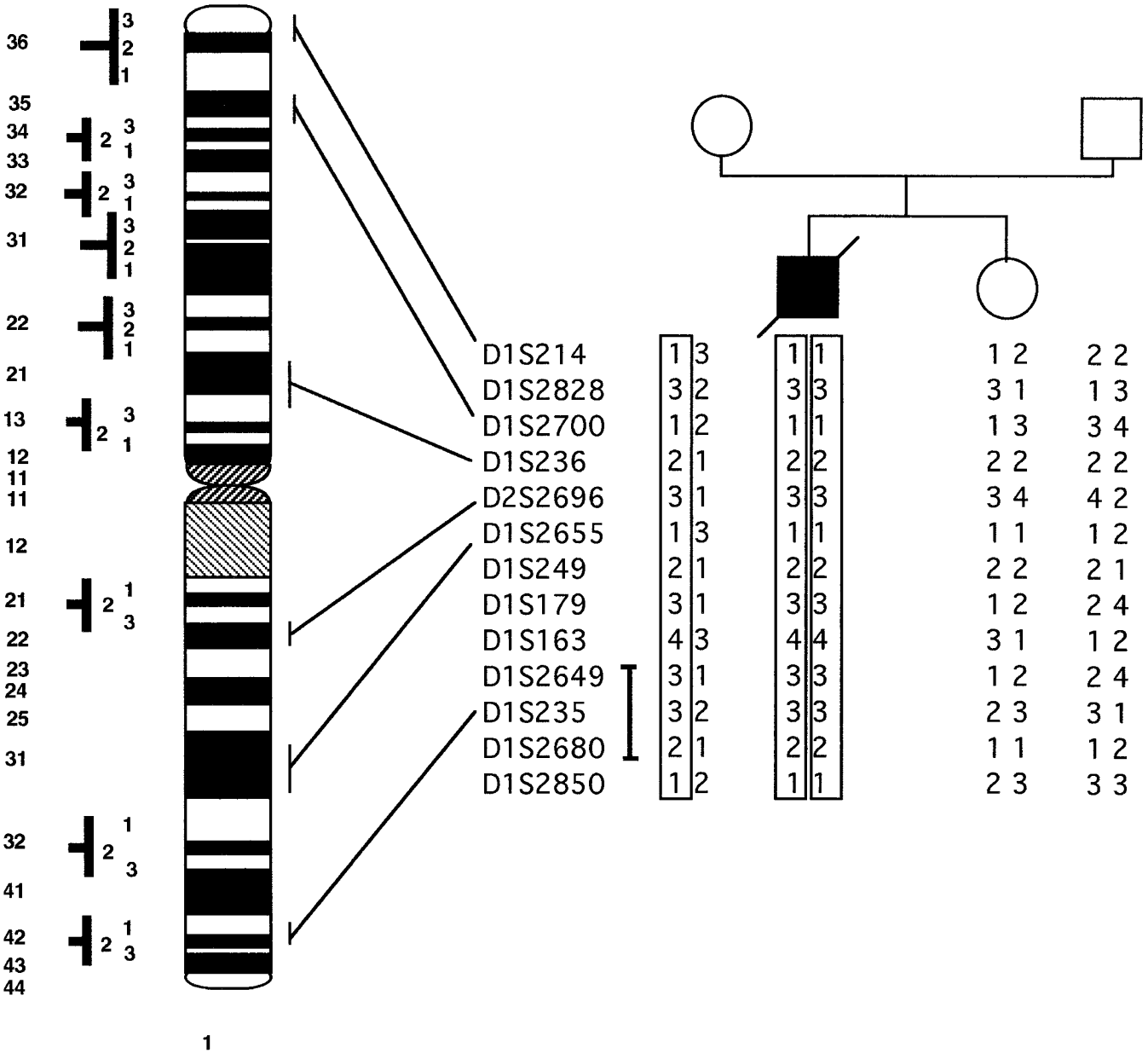

Figure 1 *Idiogram of chromosome 1. Result of the allelic distribution of 13 informative polymorphic markers spanning the chromosome 1 in the different members of the CHS family. Localisation of the markers relative to G bands are shown on the left.*

Table 1 Microsatellite marker segregation in the proband and his parents

Locus	*Proband*	*Father*	*Mother*
D10S1645	34	32	14
D10S195	32	33	12
D15S962	23	23	13

corresponded to nucleotides 2452 to 3886 of the *LYST* sequence[9] (data not shown). Direct sequencing of this fragment revealed an homozygous T deletion between nucleotide position 2620–2623 (Figure 2), leading to a termination codon at amino acid position 898. Sequencing of this region from the parents' DNA confirmed that the mother was heterozygous for this mutation, whereas no mutation was detected in the father's DNA (Figure 2).

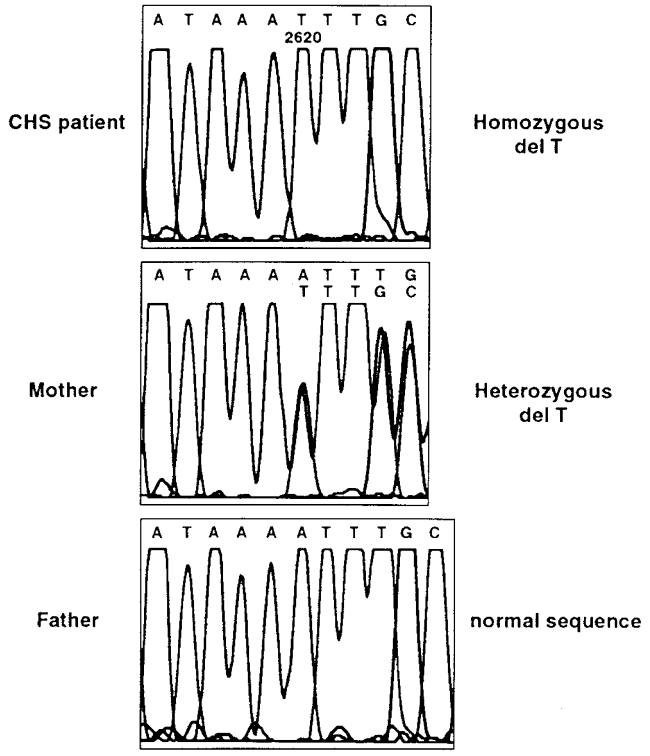

Figure 2 *Results of direct automated nucleotide sequencing using a reverse primer. The patient's PCR product was homozygous for a T deletion (identified as* 'A' *in the complementary sequence shown here) at nucleotide position 2620, resulting in a termination codon at position 898. The mother's PCR product was heterozygous for the same deletion with visualisation of one base pair shifting of the corresponding allele sequence following the deletion. Normal sequence was detected in the father's PCR product.*

Discussion

In the course of the molecular analysis of a non-consanguineous CHS family, genotyping with polymorphic DNA markers from 1q43 revealed that the affected child had inherited a maternal haplotype but no paternal haplotype. The same results were obtained for all the tested markers belonging to 1p and more centromeric 1q region, whereas both parental haplotypes were identified for the other chromosomes. Cytogenetic study as well as *in situ* hybridisation analysis failed to reveal any abnormalities of chromosome 1, especially in the q42–q43 region. Mutation analysis detected an homozygous base pair deletion in the patient *LYST* gene leading to a frameshift and subsequent stop codon which was present as an heterozygous mutation in the mother's DNA and absent in the father's. Therefore, it was concluded that CHS in this patient resulted from maternal uniparental isodisomy from chromosome 1 with inheritance of two copies of a LYST mutation carried by the mother.

Patient chromosome 1 displayed homozygosity for all the marker loci examined. Although several mechanisms may lead to the occurrence of uniparental disomy (UPD), maternal isodisomy detected in this patient most probably results from the fertilisation by a nullisomic paternal gamete of a monosomic maternal gamete mutated in the *LYST* gene, followed by a postzygotic duplication of the maternal chromosome 1. Other UPD mechanisms, ie non-disjunction events during meiosis I or II in one parent, followed by gametic complementation, or trisomy rescue[14,15] will lead to heterodisomy. This is the third case of chromosome 1 maternal UPD,[16,17] whilst only one chromosome 1 paternal UPD has been reported so far.[18] Although this represent a small sample size, this difference might alternatively be related to the high frequency of chromosome 1 paternal nullisomy (0.25%) observed in the population.[19] Single chromosome duplication increases the risk of homozygosity for deleterious recessive genes. It provides a unique genetic mechanism for inheritance of autosomal recessive disease from a single carrier parent. Such a phenomenon has previously been described in several conditions[20] including cartilage hair hypoplasia on chromosome 9,[21] familial Mediterranean fever on chromosome 16[22] or Duchenne muscular dystrophy on chromosome X.[23] Chediak-Higashi syndrome is a rare autosomal recessive disorder whose frequency is estimated to be $1/10^6$ birth. The majority of the cases were born in consanguineous families. Occurrence of this syndrome in non-consanguineous families, as the case reported here, should lead to considering UPD as a possible mechanism of a rare disease expression. Recognition of such a mechanism would have important implication in further genetic counselling in these families. In this family, Mendelian inheritance would have predicted a risk of 1/4, whereas the actual risk of recurrence is almost zero.

Apart from the Chediak-Higashi syndrome, the patient has no other unusual conditions. He had a full-term birth weight of 3200 g, without indications of either intrauterine growth retardation, dysmorphic features, or developmental abnormalities. This strengthens the observation made in two other settings which suggests that there are no imprinted gene(s) in maternally derived chromosome 1.[16,17] In the first reported case, absence of imprinting was observed in a 2-month-old infant[17] and in a young adult in the second

case.[16] Pericentromeric heterodisomy with partial isodisomy characterised these two latter cases, whereas the paternal chromosome 1 UPD reported has pericentromeric isodisomy and distal heterodisomy. The patient described here has isodisomy of the entire chromosome 1, but no apparent additional phenotypic consequence seems to result from this genetic difference.

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