Rapid Publication

Isodisomy of Chromosome 6 in a Newborn with Methylmalonic Acidemia and Agenesis of Pancreatic Beta Cells Causing Diabetes Mellitus

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Abstract

Isodisomy (ID) is a genetic anomaly defined as the inheritance of two copies of the same genetic material from one parent. ID in an offspring is a rare cause of recessive genetic diseases via inheritance of two copies of a mutated gene from one carrier parent. We studied a newborn female with a mut° form of methylmalonic acidemia and complete absence of insulin-producing β cells in otherwise normal-appearing pancreatic islets, causing insulin-dependent diabetes mellitus. The patient died 2 wk after birth. Serotyping of the HLA antigens, DNA typing of HLA-B and HLA class II loci, study of polymorphic DNA markers of chromosome 6, and cytogenetic analysis demonstrated paternal ID, involving at least a 25-centiMorgan portion of the chromosome pair that encompasses the MHC. ID probably caused methylmalonic acidemia by duplication of a mutated allele of the corresponding gene on the chromosome 6 inherited from the father. It is also very likely that ID was etiologically related to the agenesis of β cells and consequent insulin-dependent diabetes mellitus in our patient. We thus speculate on the existence of a gene on chromosome 6 involved in β cell differentiation. (*J. Clin. Invest.* 1994. 94:418–421.) Key words: congenital diabetes mellitus • human leukocyte antigen • isodisomy • methylmalonic acidemia • uniparental disomy

Introduction

Uniparental disomy (UPD)[1] is a newly recognized genetic anomaly, defined as the inheritance of both copies of genetic

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1. *Abbreviations used in this paper:* cM, centiMorgan; ID, isodisomy; IDDM, insulin-dependent diabetes mellitus; MMA, methylmalonic acidemia; MUT, methylmalonyl coenzyme A mutase; UPD, uniparental disomy.

material from one parent (1). Two subtypes of UPD can be recognized by molecular analysis: heterodisomy, defined as the inheritance of both chromosomes from one parental pair, and isodisomy (ID), defined as the inheritance of two copies of the same chromosome from one parent. UPD can cause disease if it affects a gene showing genomic imprinting, i.e., a gene whose expression depends on parental origin. ID will further result in functional reduction to hemizygosity and can thus cause a recessive disease to occur in the offspring of one carrier parent, even in the absence of genomic imprinting. Cases of UPD affecting various chromosomes have been described in association with disease (2). One case of ID of chromosome 6 has been reported in a patient with complement C4 deficiency (3).

Methylmalonic acidemia (MMA) is a rare inborn error of metabolism transmitted as an autosomal recessive trait (4). The mut° form of MMA is due to a defect in the *MUT* gene encoding the methylmalonyl coenzyme A mutase apoenzyme (5). The *MUT* locus has been mapped on the short arm of chromosome 6 (6p21) and shows genetic linkage to the MHC (5, 6). Several other disease genes are known to lie in the vicinity of the HLA complex, including the gene of one form of spinocerebellar ataxia, *SCA1* (7).

Permanent insulin-dependent diabetes mellitus (IDDM) is very rare in the neonatal period. So far, 31 well-documented cases have been published (8, 9). Its pathogenesis is unknown in two-thirds of the cases. A very early presentation of autoimmune destruction, which is a more common cause of IDDM, has never been irrefutably demonstrated. Rare causes such as aplasia of the pancreas and congenital absence of the islets of Langerhans have been reported. Isolated absence of the β cells has been shown in one case.

We reported recently on a newborn with congenital absence of insulin-producing cells and associated methylmalonyl coenzyme A mutase apoenzyme defect (9). We now show, using molecular techniques, that this patient had paternal ID for at least a 25-centiMorgan (cM) portion of chromosome 6 encompassing the *HLA* loci.

Methods

Patient. A detailed clinical description has been reported elsewhere (9). Briefly, IDDM was discovered shortly after birth in a small for gestational age female born to healthy, nonconsanguinous Caucasian parents. Because of persisting acidosis after stabilization of glycemia, an inborn error of metabolism was suspected, and MMA was diagnosed. The

child died on day 16 in spite of therapy. Enzymatic studies on cultured fibroblasts from a skin biopsy specimen were diagnostic of a mut° form of MMA. Postmortem studies showed a grossly normal pancreas. Immunohistochemistry, electron microscopy, and in situ hybridization studies using insulin mRNA probes failed to detect any insulin-producing cells in otherwise apparently normal islets. HLA typing and DNA analyses were then undertaken in the propositus and the family. The infant did not demonstrate the presence of autoantibodies to islet cells or insulin.

HLA typing. Serological HLA phenotyping was performed using the fluorochromasia cytotoxicity test (10) on peripheral blood mononuclear cells from the parents and grandparents, on cultured skin fibroblasts from the patient, and on amniotic cells from a second fetus. Before typing, skin fibroblasts and amniotic cells were incubated with recombinant γ-interferon (Boehringer Mannheim GmbH, Mannheim, Germany) and tumor necrosis factor-α (generous gift from Pr. W. Fiers, Laboratory of Molecular Biology, University of Ghent, Belgium) to enhance class I molecule expression (11).

Molecular genetics. All DNA manipulations were done using standard procedures (12). DNA was extracted from cultured skin fibroblasts of the patient, from peripheral leukocytes of the parents, and from amniotic cells of the second fetus. The B*35, B*0801, and B*51 DNA typing was performed as described (13), except that the 3' primer used for B*51 typing was B*51 5'-ggttgtagtagcggagcgcga-3'. Primers and PCR conditions for the B*44 subtyping were as follows (14): primer B*44-P1: 5'-gggccagggtctcacatca-3'; primer B*44-P4: 5'-cactgcccc-tggtacccgc-3'; the PCR reaction yielded a 298-base pair product and was performed as described in reference 13, except that the annealing temperature was 60°C and final MgCl$_2$ concentration was 1.25 mM. Hybridization was performed at 50°C with SSO-D156 probe whose sequence is 5'-ctctgtcctgctccgccac-3'. SSO-D156 recognizes the B*4401/02 subtypes and not the B*4403 subtype. *HLA* class II alleles of the DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, and DPB1 genes were defined using primers and oligonucleotide probes described (15).

The B-12 dinucleotide repeat polymorphism at the *TCTE1* locus was studied with the B-12 primers as described in reference 6. The D6S255 dinucleotide repeat polymorphic locus was studied as described in reference 16. The polymorphic trinucleotide repeat tract of the *SCA1* gene was studied as described in reference 7. The HindIII polymorphic restriction site of exon III of the *MUT* gene was studied as described in reference 5.

Chromosome analysis. Standard G-banding was performed on chromosomes from peripheral blood mononuclear cells of the patient and her parents and on fibroblasts cultured from a skin biopsy specimen of the patient. All manipulations were done according to standard procedures (17).

Results

HLA class I serotyping performed on the fibroblasts from the patient showed apparent homozygosity and lack of inheritance of maternal alleles. The patient was A11, B44 (Bw4), Cw5. The mother was A3, A11, B35 (Bw6), Cw4, DR1, DR14, DQ5, and the father was A1, A11, B8, B44 (Bw4, Bw6), Cw5, Cw7, DR4, DR15, DQ6, DQ7. Homozygosity of maternal HLA-B35 antigens was confirmed by typing the maternal grandparents. Haplotypes deduced from segregation analysis in this family are shown in Fig. 1. These results were confirmed by DNA analyses of the *HLA-B* and *HLA* class II genes (Table I).

Molecular study of the polymorphic trinucleotide repeat tract of the *SCA1* gene were as follows (the alleles are labeled arbitrarily according to sizes): mother 2, 4; father 1, 3; patient 1, 1. Study of the polymorphic HindIII restriction site in exon III of the *MUT* gene showed that the patient was homozygous for an allele shared by his parents, and study of two other
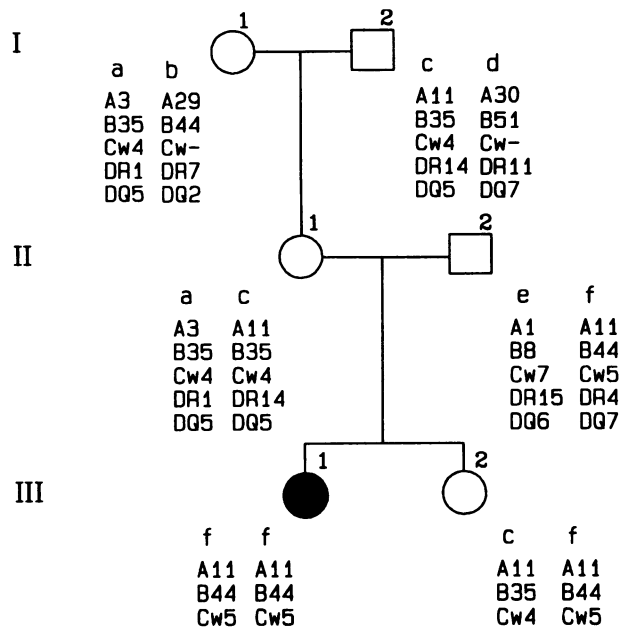

Figure 1. Serological typing of HLA antigens in the family. Squares denote male family members, and circles denote female family members. The patient (III-1) is represented by a solid symbol. Serological typing of the HLA-A, -B, -C, -DR, and -DQ antigens is indicated by upper-case letters and numbers. The haplotypes are deduced from segregation analysis and are labeled arbitrarily in lower-case letters.

polymorphic loci on chromosome 6 gave similar results, summarized in Fig. 2.

Serotyping and DNA analyses on a chorionic villus sample from a subsequent pregnancy showed normal inheritance of maternal and paternal HLA alleles, and assay of methylmalonic acid in amniotic fluid was normal. This pregnancy resulted in the birth of a healthy child (Fig. 1).

Table I. DNA Typing of HLA Genes

Haplotype	Class I	Class II
a	B*3501-08	DRB1*0101-DQA1*0101-DQB1*0501-DPB1*0402
b	B*4403	DRB1*0701/02-DRB4*0101-DQA1*0201-DQB1*0201-DPB1*1101
c	B*3501-08	DRB1*1401-DRB3*0201/02-DQA1*0101-DQB1*05031-DPB1*0601
d	B*5101-04	DRB1*1101/04-DRB3*0202-DQA1*0501-DQB1*0301-DPB1*0401
e	B*0801	DRB1*1501/03-DRB5*0101-DQA1*0101-DQB1*05031-DPB1*0401
f	B*4401-02	DRB1*0401-DRB4*0101-DQA1*0301/02-DQB1*0301-DPB1*0401

HLA alleles were typed by molecular analysis as described in Methods. Haplotypes are deduced from segregation analysis of the alleles in the family and are labeled arbitrarily in lower-case letters as in Fig. 1.
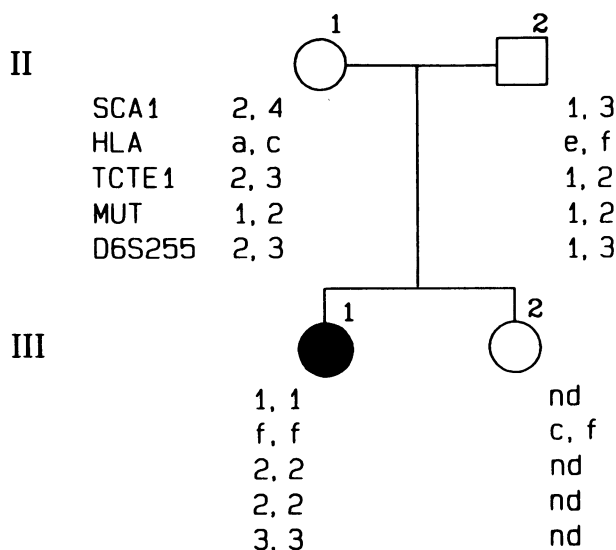
Discussion

In this newborn with MMA and IDDM related to congenital absence of insulin cells, we investigated the MHC because of its linkage to the MUT locus and its association with IDDM (18). The segregation study showed apparent absence of inheritance of maternal alleles and homozygosity for paternal alleles (Fig. 1). Such a pattern could have resulted from a vast deletion encompassing the MHC and the MUT locus on the short arm of the maternally inherited chromosome 6, with the father being a carrier for a mutated MUT allele on the chromosome he transmitted to the patient. The MUT gene is widely separated from the HLA genes on chromosome 6, with a genetic distance larger than 25 cM (6). Therefore, a deletion removing the HLA and the MUT genes would be easily detectable on chromosomes examined at the 400-band resolution level (17). Cytogenetic analysis clearly showed a normal karyotype in our patient and in her parents. This finding could still be explained by the coincidental occurrence of MMA due to inheritance of a mutated MUT allele from each parent and a complete deletion of the HLA cluster on the maternally inherited chromosome. A complete deletion of the MHC with normal high-resolution cytogenetic analysis has been reported in a case of 21-hydroxylase deficiency (19). The findings in the present case, however, were suggestive of paternal ID implicating the HLA and the MUT loci. Paternal ID was confirmed by studying the trinucleotide repeat polymorphism of the SCA1 gene, which was totally informative in this family and displayed lack of inheritance of either maternal alleles. The SCA1 gene lies ~ 20 cM telomeric to the MHC, and the MHC itself covers ~ 5 cM (20). A deletion of the SCA1 and HLA loci would thus cover 25 cM and would be easily detectable on standard cytogenetic analysis (17). There-

fore, lack of inheritance of maternal HLA and SCA1 alleles with normal cytogenetic analysis could only be explained by paternal UPD, and detection of only one paternal allele at each locus demonstrated paternal ID. Results of other polymorphic loci analyses on chromosome 6 are shown in Fig. 2. These results suggest involvement of the entire chromosome pair by paternal ID, but do not demonstrate it because of partial informativity in this family. The TCTE1 locus lies between the HLA and MUT loci at ~ 24 cM from the MHC (6); the D6S255 locus maps to the long arm of chromosome 6 (6q24-25.1) (16).

Taking these results together, MMA in our patient is best explained by reduction to hemizygosity for a mutated MUT allele on the paternally inherited chromosome 6. The absence of a reliable noninvasive test to assess the carrier status for MMA in the parents presently makes it difficult to prove this hypothesis. Nonetheless, it appears highly unlikely that in this patient homozygosity for linked loci on chromosome 6, i.e., mutated MUT alleles and paternal HLA alleles, would be unrelated chance events.

The occurrence of congenital IDDM in a child with a genetic anomaly of chromosome 6 may be merely coincidental, but this finding is intriguing. Pathologic examination showed complete absence of insulin-producing cells in otherwise normal-appearing pancreatic islets. It is also noteworthy that the HLA-DQ genotype of the patient, DQA1*0301/0302-DQB1*0301, is not associated with a significantly increased risk for autoimmune IDDM in our population (18), and no antibodies were detected against insulin nor pancreatic islets. Thus, a very early insulitis with complete destruction of the β cells in utero is a very unlikely explanation for IDDM in this case. Conversely, our observations are entirely consistent with β cell agenesis. Interestingly, the congenital absence of β cells in association with a genetic abnormality of the MHC is reminiscent of observations in transgenic mice models with abnormal expression of histocompatibility antigens in the pancreatic islet cells. In these experiments, β cell–targeted ectopic expression of class II antigens (21) or overexpression of a class I antigen (22) led to IDDM with depletion of insulin-producing cells and without signs of insulitis. However, it is hazardous to speculate that ID altered the prenatal expression of the HLA genes specifically in the developing islets as to result in β cell agenesis similar to the transgenic models. Furthermore, congenital IDDM was not observed in the patient previously described to have paternal ID of the MHC (3). Still, it should be interesting to investigate further cases of congenital IDDM for UPD of HLA genes.

Alternatively, our observation might point to a developmental gene on chromosome 6, implicated in β cell differentiation. Loss of function of such a putative gene might result from the duplication of a mutated allele carried by the father; it is tempting to speculate that a single mutational event (deletion, rearrangement), too small to be detected cytogenetically, would be responsible for both the mut° phenotype and the silencing of a gene required for β cell development. Alternatively, genomic imprinting of this putative gene could be responsible if only the maternal alleles are functionally expressed. This situation is exemplified in cases of Angelman and Beckwith-Wiedemann syndromes (2), but is unlikely in our case, as congenital IDDM was not observed in the patient previously described to have paternal ID of the HLA region (3). Future work will aim at analyzing the MUT locus region in search of the mutation responsible for MMA. Were a submicroscopic deletion or re-

arrangement detected, this approach might eventually lead to the identification of a β cell developmental gene within or at its borders.

Acknowledgments

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