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Molecular diagnostic challenges and complex management of consecutive twin pregnancies in a family with CD40 ligand deficiency

Running head: Molecular diagnostics of CD40L deficiency

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Abstract

X-linked hyper-IgM syndrome (XHIGM) is a primary immunodeficiency disorder caused by mutation in the gene encoding the CD40 ligand (CD40L) expressed on activated T cells. Prenatal genotyping in carriers with twin pregnancies is more challenging than in women with single pregnancies. In addition, women with twin pregnancies may decide on selective termination for which the risk of loss of the healthy fetus may exceed 7%. We report here on a family affected by XHIGM. Diagnosis of the disease was made in a male patient as late as 33 years of age. After family screening the sister of the proband conceived male twins in 2 consecutive pregnancies. In the first pregnancy, one of the male fetuses was hemizygous for the c.521A>G (Q174R) mutation in the *CD40L* gene. In the second pregnancy, ultrasound scan showed one fetus to have exencephaly and karyotyping revealed this fetus to have trisomy 18. Several options were discussed, but the parents decided on selective termination in both pregnancies. The interventions were successful in both cases and the mother now has two healthy sons. This report demonstrates the way in which advanced technologies in molecular medicine and obstetric interventions may assist families with decisions about possible selective termination in cases of life-threatening molecular or chromosomal disorders. The diagnosis of CD40L deficiency at the age of 33 year in the proband was striking and indicated that PIDs are still neglected as disease entities in the evaluation of patients with recurrent severe infectious diseases.

Keywords

CD40 ligand deficiency; chorionic villus sampling; twin pregnancies

Introduction

Studies of different types of inherited hyper-IgM (HIGM) syndromes characterized by defective antibody diversification and impaired class switch recombination revealed that intrinsic genetic defects in both T cells and B cells may lead to these immunological disorders [1-5]. In particular, defects of genes encoding for CD40 ligand (CD40L), CD40, nuclear factor-κB essential modulator, activation-induced cytidine deaminase, and uracil-DNA glycosylase have been described as the different molecular forms of the multi-faced HIGM syndrome [6-10].

X-linked HIGM syndrome (XHIGM) is a rare primary immunodeficiency disorder characterized by recurrent sinopulmonary and gastrointestinal tract infections, persistent or cyclic neutropenia, stomatitis, *Pneumocystis jirovecii* pneumonia, sclerosing cholangitis and, in rare cases, hepatocellular, bile duct carcinoma and neuroendocrine carcinoma [4, 9, 10-12]. XHIGM is caused by a mutation of *CD40L,* which maps to the long arm of the X chromosome and encodes the CD40 ligand (CD40L), an activation marker expressed on the surface of T cells [8]. Thus, in the absence of an HLA-compatible donor long-term survival rate is poor, highlighting the importance of prenatal diagnosis in affected families. The identification of the causal gene has opened up possibilities for genetic testing by DNA sequencing for affected families. We report here that diagnosis of XHIGM, a severe combined primary immunodeficiency may present clinically as a mild disease for decades indicating the lack of appropriate awareness of PID. We also present a unique and challenging clinical case of subsequent twin pregnancies in a woman with heterozygous mutation in the CD40L gene. This report demonstrates how advancement in molecular medicine and obstetric interventions may assist families with life-threatening genetic disorders.

Subjects and Methods

Subjects. All the studies described here were approved by the institutional review board and informed consent was obtained from family members. The 33-year-old proband in the family (Fig. 1) was referred to us, because he had had low concentrations of serum immunoglobulin G and A isotypes. He had suffered from recurrent respiratory tract infections, including several episodes of pneumonia and pleuropneumonia since early childhood. He also had intestinal tract infections and recurrent episodes of neutropenia consistent with XHIGM. Immunoglobulin substitution was unreasonably withheld because of the lack of serum IgA and a feared risk of anaphylaxis. No case of immunodeficiency or unexplained death was identified in the family, his parents, and 28-year-old sister were all clinically healthy. Immunophenotyping and DNA sequencing showed the proband to have XHIGM and identified his mother and sister as carriers (Fig. 1). After the diagnosis was made, the patient was put on regular intravenous immunoglobulin (IVIG) replacement therapy with 400 mg/kg dose monthly. Until now the patient refused hematopoietic stem cell transplantation. Following the detection of the CD40L gene mutation in the family, the proband's sister decided to undergo prenatal testing when she became pregnant.

Methods. Serum immunoglobulin isotype levels and lymphocyte subpopulations were determined by standard immunological assays. Lymphocyte subsets and cell-surface expression of CD40L on activated T lymphocytes was analyzed with a Coulter 500 flow cytometer.

gDNA was isolated from blood cells or chorionic villus samples (CVS) with the QIAamp DNA Blood Mini kit (QIAGEN GmbH, Hilden, Germany).

PCR amplification and sequencing of exons 1 to 5 and the flanking intron regions of the CD40L gene were used to analyze the mutations present [13]. Primer sequences are available on request. Amplicons were sequenced with the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), and analyzed on an ABI 3130 capillary sequencer (Applied Biosystems) [14]. Sequence variations are described with respect to the reference sequence, GenCard accession no. GC0XP123307 for *CD40L* cDNA, in which the c.1 position corresponds to the A of the ATG translation initiation codon.

Results

As shown in Table 1, serum IgM concentrations in the XHIGM patient was normal in contrast to the markedly decreased serum IgG and IgA levels. The number of peripheral blood lymphocytes and $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+/CD3^-$ lymphocyte subsets were in the normal range in all family members tested.

CD40L expression by activated T cells using PE-conjugated monoclonal antibodies was studied in normal controls and in members of the affected family (Table 1). Surface expression of CD40L by activated T cells was negligible in the proband with XHIGM and intermediate expression was detected in the heterozygous sister and mother (Table 1). Positive control experiments showed that the majority of T lymphocytes from all family members tested expressed $CD69^+$ suggesting that these cells were activated by PMA/ionomycin treatment (Table 1).

Sequence analysis of the CD40L gene in the proband revealed a c.521A>G missense mutation in exon 5 predicting a Q174R amino acid replacement in the TNF domain of the protein. The mother and the sister of the hemizygous male patient were carriers for the mutated CD40L allele, and both of them were clinically and immunologically healthy.

In the first pregnancy, chromosome analysis showed both fetuses to be male, and DNA sequencing showed one fetus to be hemizygous for the Q174R mutation in *CDL40L* (Fig. 1). The parents attended several detailed counseling sessions; specifically, they were offered genetic HLA typing of the two fetuses to determine whether the unaffected twin could act as a stem cell donor for his brother. However, they declined this option and eventually opted for selective termination which was performed during the 12^{th} week of gestation. DNA isolated from a blood sample drawn from the fetal heart before selective termination was sequenced and confirmed hemizygosity for the *CDL40L* mutation. The rest of the pregnancy was uneventful and a healthy male newborn was born at 37 weeks of gestation by cesarean section.

One year later, this woman again conceived male twins, this time without the use of fertility treatment (Fig 1). Ultrasound scan in the ninth week of gestation showed exencephaly in one of the twins. This malformation is invariably fatal, but chorionic villus sampling from both placentas (fused in this case) was nonetheless performed during the 11^{th} week of gestation. Molecular genetic analysis showed both fetuses to be wild type for the *CD40L* mutation and karyotyping revealed trisomy 18 in the exencephalic fetus. The parents again decided on selective termination, which was performed in the 12^{th} week of gestation, without complications. The mother gave birth of a male newborn at 38 weeks of pregnancy without further complications.

Discussion

Advanced technologies in molecular genetics and interventional medicine have greatly contributed to the complex management and to a better quality of life of families with deadly diseases caused by mutations in PID genes. Identification of the genes responsible for severe PIDs has made direct mutational analysis of both affected patients and carriers possible. Genetic counseling and prenatal testing can now be offered to families at risk. We report here the case of a patient with Q174R mutation who was diagnosed with XHIGM as late as 33 years of age. Hemizygous patients with XHIGM typically developed disease manifestations during the first 2 years of life when they present mostly with recurrent respiratory tract infections. In addition to defects in T cell mediated immunity and low immunoglobulin G and A isotypes, transient neutropenia in XHIGM patients may also predisposes them to infectious complications. The male patient described this report had had recurrent and severe respiratory tract infections since early childhood and he was diagnosed with selective IgA deficiency. The misconception that patients with low or absent serum IgA concentration should not receive IVIG has also contributed to the delay of appropriate management of this patient. This case provides an example that PIDs are still strikingly neglected diseases in the management of patients with recurrent infectious diseases [15]. The patient has been receiving IVIG infusions monthly and he has had only mild respiratory tract infections. He refused bone marrow transplantation as a primary attempt to cure his disease. Such a refusal attitude of XHIGM patients to undergoing BMT may be a more general problem as only a small number of patients registered in the ESID registry were transplanted (B. Gathmann, personal communication). A possible reason for not using BMT may be the wide heterogeneity of the severity of the disease. Also, transplants are mostly done from matched related or unrelated donors who are not always available.

We report here an intriguing and unique case of a woman heterozygous for a *CD40L* mutation who conceived two sets of male twins in consecutive pregnancies. As XHIGM remains a life-threatening PID with severe infectious complications and malignancies, poor quality of life and a poor prognosis for patients with no HLA-matched sibling, carriers of *CD40L* mutations need genetic testing for family planning. Knowledge that the women is a carrier of the disease has implications for the decision to start a pregnancy and may encourage families to make use of prenatal testing and other measures, including selective termination, if necessary. Women with one wild-type and one hemizygous fetus may decide to continue the pregnancy and analysis of HLA markers may help to determine whether the wild-type fetus may be a potential HLA-matched donor, from whom cord blood stem cells could be obtained after delivery to treat the affected twin [16]. If the fetuses present HLA mismatches, the family may consider stem cell transplantation with cells from an unrelated donor. The woman studied here decided to undergo selective termination, for which the risk of loss of the healthy fetus is 2.4 to 7.1% [17, 18]. Exencephaly, as diagnosed in one of the fetuses in the second pregnancy, is invariably fatal very shortly after birth. This malformation is often associated with severe polyhydramnios, increasing the risk of preterm or very preterm delivery of both twins. This potential complication justifies the decision of the mother to undergo selective termination.

Families choosing to undergo prenatal testing may request the information provided by these tests to help them to adjust emotionally and to make specific plans and decisions as soon as possible. In both pregnancies described here, karyotyping results were available within 24 hours, with direct sequencing of the DNA region concerned requiring a further 48 hours. In the second pregnancy the severe malformation of one of the fetuses was detected as early as the 9^{th} week of gestation.

This report demonstrates the complexity of prenatal diagnosis by ultrasound, karyotyping and molecular genetics and also the availability of selective termination in the management of discordant twin pregnancies. We also report here that diagnosis of CD40L

deficiency may be delayed by decades indicating the lack of awareness of PIDs in the general management of patients with infectious diseases.

Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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Table 2. Serum immunoglobulins and lymphocyte subsets

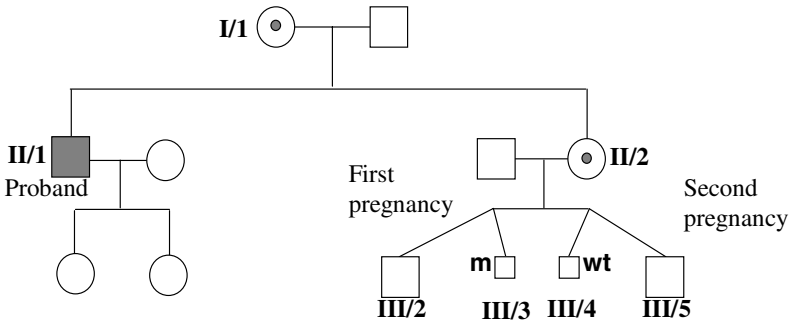
Subjects	Immunoglobulins (g/L)			Lymphocyte number (cells/mm^3)	Lymphocyte subsets (%)						
	IgG	IgA	IgM		$CD3^+$	$CD4^+$	$CD8^+$	$CD19^+$	$CD56^+/CD3^-$	CD40L[a]	CD69[a]
Proband[b]	2,10	0,40	0,9	4200	73	36	34	17	8	0.02	85
Sister	13,2	0,90	0,9	2780	77	51	23	13	9	33.7	91
Mother	13,3	4,20	0,7	1800	72	53	20	15	13	42.0	95
Father	10,5	2,90	1,1	3490	78	49	27	10	24	75.3	89
Normals	7,0-16,0	0,7-4,0	0,4-2,3	>1500	52-78	25-48	9-35	8-24	5-15	>50	>85

[a]Percentage of $CD3^+CD8^-$ lymphocytes. [b]hemizygous patient with Q174R mutation in the CD40L gene.

Figure legend

Figure 1. **CD40 ligand (CD40L) mutation and clinical phenotypes.** Pedigree of the family shows the Q174R amino acid substitution in *CD40L* which was first detected in the proband. His heterozygous sister (II/2) conceived two sets of male twins in consecutive pregnancies. In the first pregnancy, one of the fetuses (III/3) was hemizygous for the mutation, whereas, in the second pregnancy, both fetuses were wild type (III/4 and III/5), but one fetus had exencephaly. m, mutant; wt, wild type.

Figure 1


I/1
II/1
Proband
II/2
First
pregnancy
Second
pregnancy
m
wt
III/2
III/3
III/4
III/5