Mutational screening of the CD40 ligand (CD40L) gene in patients with X linked hyper-IgM syndrome (XHIM) and determination of carrier status in female relatives

M L Prasad, M Velickovic, S A Weston, E M Benson

X linked hyper-IgM syndrome (XHIM; MIM 308230) is a severe primary immunodeficiency caused by mutations in the gene encoding the CD40 ligand (CD40L, also called CD154).1 CD40L is a surface molecule present on activated T cells, which interacts with CD40 on the surface of B cells to provide an essential signal for B cell proliferation and immunoglobulin class switching.2 As a consequence of this interaction with CD40 on the surface of B cells''

The mutations are heterogeneous, with functional deficiency ranging from a complete lack of CD40L protein expression to missense mutations that interfere with its interaction with CD40 on the surface of B cells. They are recorded in a CD40L mutation database (found at http://bioinf.uta.fi/base_root/).3

In our study, we report the identification of mutations in the CD40L gene in 11 Australian patients from 10 unrelated families with XHIM phenotype.

MATERIAL AND METHODS

Clinical samples
Eleven patients with a clinical XHIM phenotype and greatly reduced serum concentrations of IgG and IgA, but normal or increased serum concentrations of IgM, and with a history of an increased susceptibility to infections, were referred to our laboratory by clinicians around Australia for the confirmation of diagnosis. The patients were all male, aged between 1 and 27 years, and were unrelated, with the exception of two individuals who were first cousins (patients 8 and 9; table 1). Table 1 summarises the patients’ data. First degree female relatives of a proband were also assessed as requested. Thirty healthy individuals were investigated as controls. Informed consent for the CD40L mutational analysis was obtained from the patients or their respective guardians.

Molecular analysis
Genomic DNA was obtained from whole blood. All five exons of the CD40L gene, including the exon–intron boundaries, were individually amplified with primers described previously.4 11 Sequencing reactions were performed using the dye deoxyterminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA) with an automated ABI373 DNA sequencer. Detected mutations were confirmed by sequencing in the opposite direction from an independent PCR product.

RESULTS

Using direct sequence analysis of exon specific PCR products, we were able to detect 10 mutations in 11 patients from 10 unrelated families. Mutations were observed in both coding and non-coding regions of the entire CD40L gene. Seven have been described previously,2 7–9 11 and three were new mutations, which were not on the CD40L database or in the literature. Table 1 summarises the mutations for individual patients.

No correlation was seen between the clinical phenotype and the site of the mutation, which is in agreement with the previously published data.12

We also tested the XHIM carrier status in female members of seven families, after identifying mutations in the patients (table 1). Healthy female carriers were identified by direct

Abbreviations: XHIM, X linked hyper-IgM; CD40L, CD40 ligand; TNFα, tumour necrosis factor α; PCR, polymerase chain reaction
sequencing of the exon specific PCR products, a simple and reliable method that precludes the presence of false positives. Ten of 15 female family members revealed both a mutated allele and a normal allele, indicating that they were XHIM carriers (table 1).

**DISCUSSION**

Eleven patients across Australia with a clinical XHIM phenotype were analysed using direct sequencing and were found to carry mutations in the CD40L gene. It is notable that six of 10 mutations, two of which are new, were found in exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that six of 10 mutations, two of which are new, were found in exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11). It has been found previously that most of the CD40L gene mutations are located within exon 5 (patients 8, 9, and 11).

The mutation nomenclature follows the guidelines of den Dunnen and Antonarakis and HUGO (www.hgvs.org/mutnomen/). The numbering of nucleotide and amino acid positions refers to the cDNA sequence (GeneBank accession number L07414.1), where the A of the ATG translation initiation start site represents nucleotide +1. The reported age is the age of the patient when flow cytometry and molecular analysis were performed.

New mutation.

A, aunt; AA, amino acid; FC, flow cytometry; GM, grandmother; M, mother; ND, not done; S, sister.

**Table 1 CD40L mutations in 11 Australian patients**

<table>
<thead>
<tr>
<th>Patient no/age at diagnosis</th>
<th>CD40L expression (FC)</th>
<th>Exon/intron</th>
<th>Nucleotide change</th>
<th>AA change</th>
<th>Effect</th>
<th>CD40L database</th>
<th>Carrier status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1 year Absent</td>
<td>Exon 3</td>
<td>c.322G&gt;T*</td>
<td>E108X</td>
<td>Not reported</td>
<td>M+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/1 year Absent</td>
<td>Intron 3</td>
<td>c.346+2T&gt;C</td>
<td>A123E</td>
<td>Reported</td>
<td>M+, GM+, S, S-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/2 years Absent</td>
<td>Exon 4</td>
<td>c.368C&gt;A</td>
<td>AA change in TNF-α domain</td>
<td>Reported</td>
<td>M+, GM+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/12 years Absent</td>
<td>Intron 4</td>
<td>c.409+1G&gt;A</td>
<td>AA change in TNF-α domain</td>
<td>Reported</td>
<td>M+, GM+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/6 years Absent</td>
<td>Exon 5</td>
<td>c.420G&gt;T</td>
<td>W140C</td>
<td>Reported</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/20 years Present</td>
<td>Exon 5</td>
<td>c.431G&gt;A</td>
<td>AA change in TNF-α domain</td>
<td>Reported</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/27 years Absent</td>
<td>Exon 5</td>
<td>c.464T&gt;C</td>
<td>L155P</td>
<td>Reported</td>
<td>M-, S-, S-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/13 years, cousin of 9</td>
<td>Exon 5</td>
<td>c.499G&gt;T*</td>
<td>G167X</td>
<td>Not reported</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/14 years, cousin of 8</td>
<td>Exon 5</td>
<td>c.499G&gt;T*</td>
<td>G167X</td>
<td>Not reported</td>
<td>M+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/1 year Absent</td>
<td>Exon 5</td>
<td>c.521A&gt;G</td>
<td>Q174R</td>
<td>Reported</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/5 years Absent</td>
<td>Exon 5</td>
<td>c.654C&gt;A</td>
<td>W140C</td>
<td>Reported</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Take home messages**

- We identified 10 mutations (including three new ones) in the CD40L gene in 11 patients with X linked hyper-IgM syndrome (XHIM), reflecting the heterogeneity of this gene.
- Accurate and reliable molecular testing of patients with suspected XHIM is required.
- Such testing also provides valuable information for counselling female family members at risk for being XHIM carriers, in addition to offering the possibility of prenatal testing.

**Authors’ affiliations**

M L Prasad, M Velickovic, S A Weston, E M Benson, Department of Immunopathology, ICPMR, Westmead Hospital, Westmead 2145, Sydney, Australia

Correspondence to: Dr E Benson, Department of Immunopathology, ICPMR, Westmead Hospital, Westmead 2145, Sydney, Australia; elizab@westgate.wh.usyd.edu.au

Accepted for publication 12 July 2004

**REFERENCES**
