

## SHORT REPORT

# 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

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**Aims:** To analyse the gene encoding the CD40 ligand (CD40L) in 11 Australian patients from 10 unrelated families with the X linked hyper-IgM (XHIM) phenotype.

**Methods:** The CD40L gene was screened for mutations using direct sequencing of exon specific polymerase chain reaction (PCR) products.

**Results:** Ten mutations were identified. Seven of these mutations have been described previously, whereas three new nonsense mutations were identified, namely: E108X (c.322G>T), G167X (c.499G>T), and C218X (c.654C>A). Ten of 15 female family members revealed both a mutated allele and a normal allele, indicating that they were XHIM carriers.

**Conclusion:** The 10 mutations (including the three new ones) identified in this study reflect the heterogeneity of the CD40L gene, and indicate the need for accurate and reliable molecular testing of those patients suspected of XHIM.

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/](http://bioinf.uta.fi/base_root/)).<sup>5</sup>

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.<sup>4 11</sup> 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,<sup>2 7–9 11</sup> 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.<sup>12</sup>

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

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).<sup>1</sup> 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.<sup>2</sup> As a consequence of CD40L deficiency, patients with XHIM are characterised by very low amounts or the absence of IgG, IgA, and IgE, with normal to raised serum IgM concentrations, although, more recently, panhypoglobulinaemia of IgA, IgG, and IgM was seen in 26% of these patients.<sup>1 3</sup> This defective production of most isotypes of immunoglobulin renders affected individuals extremely susceptible to recurrent bacterial infections, starting in the 1st year of life.<sup>1</sup>

“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”

The gene that encodes CD40L has been mapped to Xq26.3–27.1 and is a member of the tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) superfamily.<sup>2</sup> It consists of four introns and five exons, encoding a type II membrane glycoprotein of 261 amino acids, with a short intracytoplasmic tail (22 amino acids), a transmembrane region (24 amino acids), and an extracellular TNF $\alpha$  homologous domain (215 amino acids).<sup>4</sup>

To date, over 130 unique CD40L mutations have been identified in patients with XHIM, which are spread throughout the entire length of the gene. The mutations are

**Abbreviations:** XHIM, X linked hyper-IgM; CD40L, CD40 ligand; TNF $\alpha$ , tumour necrosis factor  $\alpha$ ; PCR, polymerase chain reaction

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

Patient no/age at diagnosis	CD40L expression (FC)	Exon/intron	Nucleotide change	AA change	Effect	CD40L database	Carrier status
1/1 year	Absent	Exon 3	c.322G>T*	E108X		Not reported	M+
2/1 year	Absent	Intron 3	c.346+2T>C		Skipping of exon	Reported <sup>6</sup>	M+, GM+, S+, S-
3/2 years	Absent	Exon 4	c.368C.>A	A123E	AA change in TNF $\alpha$ domain	Reported <sup>2</sup>	M+, GM+
4/12 years	Absent	Intron 4	c.409+1G>A		AA change in TNF $\alpha$ domain	Reported <sup>6</sup>	ND
5/6 years	Absent	Exon 5	c.420G>T	W140C	AA change in TNF $\alpha$ domain	Reported <sup>6</sup>	ND
6/20 years	Present	Exon 5	c.431G>A	G144E	AA change in TNF $\alpha$ domain	Reported <sup>7</sup>	M+, GM+, A+
7/27 years	Absent	Exon 5	c.464T>C	L155P	AA change in TNF $\alpha$ domain	Reported <sup>8</sup>	M -, S -, S -
8/13 years, cousin of 9	Absent	Exon 5	c.499G>T*	G167X		Not reported	ND
9/14 years, cousin of 8	Absent	Exon 5	c.499G>T*	G167X		Not reported	M+
10/1 year	Absent	Exon 5	c.521A>G	Q174R	AA change in TNF $\alpha$ domain	Reported <sup>9</sup>	ND
11/5 years	Absent	Exon 5	c.654C>A*	C218X		Not reported	S-

The mutation nomenclature follows the guidelines of den Dunnen and Antonarakis<sup>10</sup> and HUGO ([www.hgvs.org/mutnomen/](http://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.

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, a region with the greatest homology to TNF $\alpha$ .<sup>12</sup> In addition, three of our mutations—A123E (c.368C>A), W140C (c.420G>T), and L155P (c.464T>C)—which are found in exons 4 and 5, were previously implicated in a functional defect of the CD40L molecule. This defect is caused by the failure of the assembled proteins to interact with the receptor molecule.<sup>13</sup> Codon W140 was also described as a hotspot for CD40L mutations.<sup>5</sup> To date, the CD40 database lists eight families with mutations occurring at this codon. Four families in the CD40L database have a W140X substitution—two families have the W140R substitution and one family each has the W140G or W140C substitution; the W140C substitution was also found in our present study (table 1, patient 5).

"It is notable that six of 10 mutations, two of which are new, were found in exon 5"

The accurate diagnosis of XHIM is facilitated by reliable molecular testing, not only in patients with the typical phenotype or a positive family history, but also in previously undefined hypogammaglobulinaemia. It has been reported that men previously diagnosed with common variable immunodeficiency were subsequently diagnosed with XHIM. Although these patients may not develop the life threatening complications of XHIM, other affected family members could have a more severe phenotype.<sup>14</sup> Furthermore, testing provides valuable information for counselling female family members at risk for being XHIM

## 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

carriers, in addition to offering the possibility of prenatal testing.

This is the first Australian study to describe mutations in the CD40L gene in patients with XHIM. Three mutations identified in our study were new, not being present in the CD40L database or having been reported in the literature, and three had previously been described as playing an important role in CD40L and CD40 interactions.

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## REFERENCES

- 1 Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiency. *Clin Immunol* 1999;**93**:190-7.
- 2 Hollenbaugh D, Grosmaire LS, Kullas CD, et al. The human T cell antigen gp39, a member of the TNF family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. *EMBO J* 1992;**11**:4313-21.

- 3 **Gilmour KC**, Walshe D, Heath S, *et al*. Immunological and genetic analysis of 65 patients with a clinical suspicion of X linked hyper-IgM. *J Clin Pathol* 2003;**56**:256–62.
- 4 **Shimadzu M**, Nunoi H, Terasaki H, *et al*. Structural organization of the gene for CD40 ligand: molecular analysis for diagnosis of X-linked hyper-IgM syndrome. *Biochim Biophys Acta* 1995;**1260**:67–72.
- 5 **Notarangelo LD**, Peitsch MC. CD40lbase: a database of CD40L gene mutations causing X-linked hyper-IgM syndrome. *Immunol Today* 1996;**17**:511–16.
- 6 **Seyama K**, Nonoyama S, Gangsaas I, *et al*. Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome. *Blood* 1998;**92**:2421–34.
- 7 **Macchi P**, Villa A, Strina D, *et al*. Characterization of nine novel mutations in the CD40 ligand gene in patients with X-linked hyper IgM syndrome. *Am J Hum Genet* 1995;**56**:898–906.
- 8 **Lin Q**, Rohrer J, Allen C, *et al*. A single strand conformation polymorphism study of CD40 ligand. Efficient mutation analysis and carrier detection for X-linked hyper IgM syndrome. *J Clin Invest* 1996;**97**:196–201.
- 9 **Katz F**, Hinshelwood S, Rutland P, *et al*. Mutation analysis in CD40 ligand deficiency leading to X-linked hypogammaglobulinemia with hyper IgM syndrome. *Hum Mutat* 1996;**8**:223–8.
- 10 **den Dunnen JT**, Antonarakis SE. Mutations nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000;**15**:7–12.
- 11 **Seyama K**, Kira S, Ishidoh K, *et al*. Genomic structure and PCR-SSCP analysis of the human CD40 ligand gene: its application to prenatal screening for X-linked hyper-IgM syndrome. *Hum Genet* 1996;**97**:180–5.
- 12 **Notarangelo LD**, Hayward AR. X-linked immunodeficiency with hyper-IgM (XHIM). *Clin Exp Immunol* 2000;**120**:399–405.
- 13 **Garber E**, Su L, Ehrenfels B, *et al*. CD154 variants associated with hyper IgM syndrome can form oligomers and trigger CD40-mediated signals. *J Biol Chem* 1999;**274**:33545–50.
- 14 **Jones AM**, Gaspar HB. Immunogenetics: changing the face of immunodeficiency. *J Clin Pathol* 2000;**53**:60–3.



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