

## ORIGINAL ARTICLE

# Mitochondrial DNA insert into CD40 ligand gene-associated X-linked hyper-IgM syndrome

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**Abstract**

**Background:** X-linked hyper-IgM (X-HIGM), which results from mutations in the *CD40LG* gene located on chromosome Xq26.3, is the most common form of HIGM. To date, more than 130 variants of the *CD40L* gene have been reported. We described a patient with novel de novo nuclear mitochondrial DNA sequences (NUMTs) in the *CD40LG* gene that have resulted in X-HIGM.

**Methods:** Whole-exome sequencing (WES) analysis was used to screen for causal variants in the genome, and the candidate breakpoint was confirmed by Sanger sequencing.

**Results:** A new mutation of *CD40LG*, which deletes A at position 17 followed by a 147-nucleotide from mitochondrial DNA copies insertion in exon 1, was detected in a 20-month-old boy harbouring an X-HIGM combined with immunodeficiency syndrome.

**Conclusion:** This is one of the few cases of a human genetic disease caused by nuclear mitochondrial DNA sequences (NUMTs). The presented data serve to demonstrate that de novo NUMT transfer of nucleic acid is a novel mechanism of X-HIGM.

**KEYWORDS**

*CD40LG*, insertional mutation, nuclear mitochondrial DNA sequences, X-linked hyper-IgM syndrome

## 1 | INTRODUCTION

Hyper-IgM (HIGM) syndromes are a heterogeneous group of genetic disorders characterized by low or absent serum levels of IgG, IgA and IgE with normal or elevated serum IgM (Bhushan & Covey, 2001). The defect caused by HIGM results in a failure of immunoglobulin class switch recombination, with or without somatic hypermutation defects (Davies & Thrasher, 2010). X-linked HIGM (X-HIGM), which results from mutations in the *CD40LG* gene located on chromosome Xq26.3 (Allen et al., 1993; Aruffo et al., 1993;

DiSanto et al., 1993; Korthauer et al., 1993; Ramesh et al., 1993), is the most common form of HIGM. The membrane glycoprotein CD40L, also known as CD154 or gp39, which is encoded by *CD40LG* and belongs to the TNF $\alpha$  superfamily, is mainly expressed on the surface of activated CD4<sup>+</sup> T cells (van Kooten & Banchereau, 2000). To date, more than 130 variants of the *CD40L* gene have been reported. Most affect the extracellular domain, resulting in defective geometrical folding of CD40L or the prevention of CD40L/CD40 binding (Lee et al., 2005; Lopez-Saucedo et al., 2015; Wang et al., 2014). Most X-HIGM patients become symptomatic during

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the first or second year of life, during which they exhibit severe recurrent bacterial upper and lower respiratory tract infections, opportunistic infections, autoimmune disorders, tumours and failure-to-thrive (de la Morena et al., 2017).

Numerous fragments of mitochondrial DNA are present throughout the human nuclear genome (Mourier et al., 2001; Tourmen et al., 2002; Wallace et al., 1997; Woischnik & Moraes, 2002). One possible by-product of these migrations is de novo disruption of nuclear genes. The occurrence of these events has been unequivocally demonstrated to be a cause of human genetic disease. Although reports of diseases caused by mitochondrial DNA insertion in humans are rare (Goldin et al., 2004; Shay & Werbin, 1992; Srinivasainagendra et al., 2017; Turner et al., 2003), they do occur sporadically. In the present study, we describe a patient with novel de novo nuclear mitochondrial DNA sequences (NUMTs) in the *CD40LG* gene that have resulted in X-HIGM. To date, there has been no report of human X-HIGM caused by NUMTs. Therefore, the presented data not only enrich the library of human genetic diseases caused by NUMTs but also demonstrate that NUMTs are a novel mechanism of X-HIGM.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethical compliance and patient

The study was approved by the Medical Ethics Committee of the Children's Hospital of Zhejiang University (IRB No.2020-IRB-185), School of Medicine, and was performed in accordance with the Declaration of Helsinki. Clinical information about the patient was obtained at the Children's Hospital of Zhejiang University, School of Medicine. The participants in this study include the patient, his sister and his parents. Written informed consent was obtained from all participants.

### 2.2 | Sample collection

Fresh whole venous blood (5 ml) samples were collected from the patient in sodium heparin anti-coagulated syringes and delivered to the laboratory within 4 hr. About 2 ml of blood was used for genetic sequencing, and the remainder was used for isolation of peripheral blood mononuclear cells (PBMCs). Additionally, 2 ml blood samples were collected from the participating family members for genetic analysis.

### 2.3 | Genetic analysis and bioinformatics analysis

DNA was isolated from leucocytes using the QIAamp DNA Mini Kit (Qiagen Inc.) according to the manufacturer's

instructions. For exome sequencing, 1–3 µg of genomic DNA was fragmented and extracted from the sample (at an average size of 180 bp) with a Bioruptor sonicator (Diagenode). Amplified DNA was captured with the GenCap Medical exon capture kit (Li & Durbin, 2009). The DNA probes were designed to tile along the exon regions and exon–intron boundaries of the target genes. The Polymerase Chain Reaction product was purified using Solid Phase Reversible Immobilization beads (Beckman Coulter) according to the manufacturer's protocol. The enrichment libraries were applied to a Illumina HiSeq X ten sequencer for paired-read 150 bp sequencing. The bioinformatics analysis was used to identify potential pathogenic mutations.

The sequence variants detected in the *CD40LG* gene were described according to the NCBI entry NM\_000074.3. The filtered candidate breakpoint was confirmed by Sanger sequencing. The sequence that contains the breakpoint in exon 1 was amplified with Ex Taq DNA polymerase (Takara). Purified PCR samples were sequenced on an ABI 3730 Genetic Analyzer (Applied Biosystems). Sequence traces were analysed using the Mutation Surveyor (Softgenetics). The position of the breakpoint was confirmed in the patient's mother and sister using the same procedure. The obtained variant was first analysed with regard to existing databases (dbSNP, ExAC, HGMD, ESP6500, Genome Mutation, and 1000 Genomes Project) to confirm whether it had been reported before. The tertiary structures of the proteins were generated by homology modelling with SWISS-MODEL (<http://swissmodel.expasy.org/>), according to its amino acid sequence(NP\_000065.1).

## 3 | RESULTS

### 3.1 | Clinical features of the patient

A 20-month-old boy was admitted to hospital with a 40.0°C fever on July 18, 2018. He was considered to have a bacterial infection due to an increase in the presence of C-reactive protein (CRP). Although his fever was relieved after antibiotics and symptomatic treatment, it recurred several days later. The patient was the second child of his parents and was a full-term baby with a birth weight of 3250 g. He was hospitalized for 7 days due to herpangina when he was 1 year old. Apart from that, he had no other serious infections or diseases, nor did he have adverse reactions to vaccines. The parents were non-consanguineous and healthy.

Physical examination at the time of admission revealed a body temperature of 38.3°C, pulse of 124/min, respiration of 30/min and blood pressure of 96/60 mmHg. Some white material was attached to the oral mucosa and was difficult to wipe off. There were no other changes upon physical examination. A complete blood count conducted as part of

laboratory tests showed that the white blood cell count was  $9.33 \times 10^9/L$  (reference value:  $5-12 \times 10^9/L$ ) with an absolute neutrophil count of  $2.5 \times 10^9/L$  and an absolute lymphocyte count of  $5.5 \times 10^9/L$ . C-reactive protein (CRP) was up to 98.69 mg/L. A chest computed tomography (CT) indicated pneumonia. Further immunologic results are shown in Table 1. The lymphocyte subpopulations were as follows: CD3<sup>+</sup> cells, 67.89%; CD4<sup>+</sup> cells, 44.82%; CD8<sup>+</sup> cells, 48.3%; CD16<sup>+</sup> cells, 6.42%; CD19<sup>+</sup> cells, 21.48%; transitional B cells (CD19+CD38+CD24+), 15.82%; naive B cells (CD19+CD27-IgD+), 79.18%; and switched memory B cells (CD19+CD27+IgD-), 1.26%. Based on the clinical and laboratory findings, the preliminary diagnoses were HIGM, pneumonia and thrush. He was treated with intravenous cefoperazone-sulbactam to overcome infection and intravenous immunoglobulin (IVIG) for supportive therapy.

During the 1-year follow-up, although the patient was prescribed human IVIG replacement therapy at 500 mg/kg/month, he presented with recurrent fevers and serious infections, including pneumonia (which he presented with twice) and tonsillitis. During one hospitalization in March 2019, the patient was admitted to the ICU due to severe adenovirus pneumonia.

### 3.2 | Mutation in the patient's exon 1 of *CD40LG*

Whole-exome sequencing (WES) analysis identified a breakpoint on exon 1 of *CD40LG*. Furthermore, Sanger sequencing revealed a novel de novo mutation consisting of deleted A at position 17 followed by a 147-nucleotide insertion in exon 1 of *CD40LG* (c.17delAins147) (Figure 1b), which introduced a premature stop codon (TGA) (p.N61fs\*). The patient's mutation was not found in any of the databases that were searched (dbSNP, ExAC, HGMD, ESP6500, Genome Mutation, and 1000 Genomes Project), confirming its novelty. Because the mutation was located on the X chromosome, genetic analysis was also performed for the patient's mother to investigate the origin of the mutation. The results revealed that she also carried the mutation. Unfortunately, the mother was adopted, and her biological parents could not be found. We failed to further investigate the origin of the mutation. Genetic analysis was also performed on the

patient's elder sister, and she was found to not carry the mutation (Figure 1b). The family pedigree is presented in Figure 1a. The inserted sequence was submitted to the Basic Local Alignment Search Tool (BLAST) database on the National Center for Biotechnology Information (NCBI) website. It achieved the highest score (100% identity) when aligned with a fragment of mitochondrial DNA (mtDNA) encoding 12S rRNA (GenBank NC\_012920).

### 3.3 | Structure prediction of the mutant protein

Protein structure prediction by SWISS-MODEL expected an obvious change in the local structure. Through sequence alignment, we found that the mutation resulted in a translational frameshift, and the premature stop codon appeared in codon 70, which produced a truncated protein product (Figure 2).

### 3.4 | Treatment

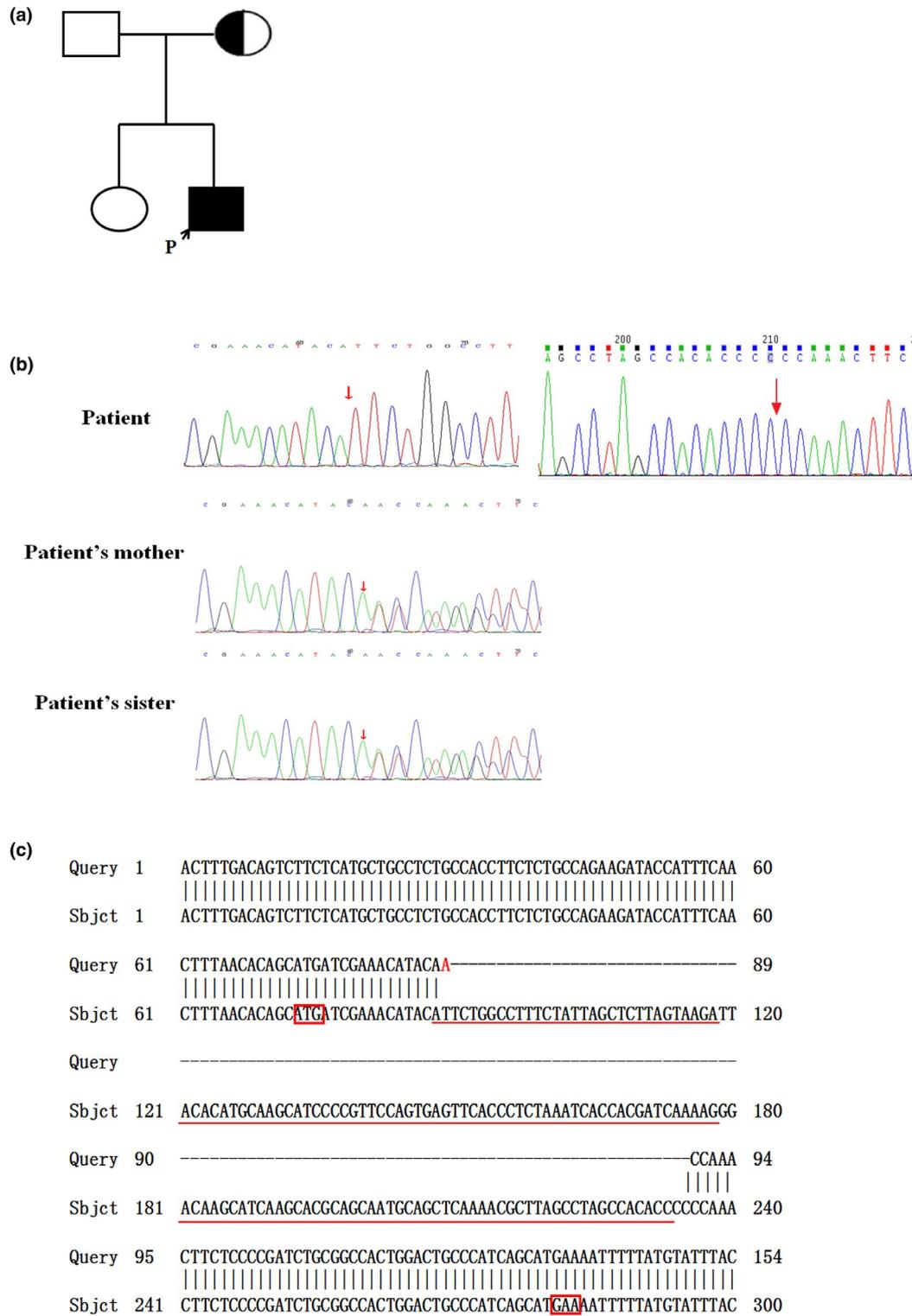
The child exhibited a poor response to IVIG therapy; severe or persistent infection still occurred. Finally, he received allogeneic peripheral blood hematopoietic stem cell transplantation (HSCT) therapy in November 2019. During the course of the 1-year follow-up, the patient presented with sporadic infections, which were easily treated, and good results were obtained with oral antibiotics.

## 4 | DISCUSSION

We reported a 20-month-old male child with X-HIGM, a primary immunodeficiency (PID) characterized by decreased serum levels of IgG and IgA and elevated IgM levels. *CD40LG* is the only gene known to cause X-HIGM, and more than 130 sense mutations, including deletions and insertions. Primarily, X-HIGM patients have exhibited missense mutations, nonmutations and splice site mutations (<http://structure.bmc.lu.se/idbase/CD40Lbase>). CD40L is a type II transmembrane glycoprotein formed by four distinct structural domains: a cytoplasmic domain, a

**TABLE 1** Patient's serum immunoglobulin levels at various periods

Immunoglobulin isotype	2018-7-16	2019-3-19	2019-5-30	Normal value
IgM [g/l]	1.23	2.26	1.58	0.4–1.28
IgG [g/l]	0.18	1.20	6.1	3.82–10.58
IgA [g/l]	0.01	0.02	0.01	0.04–1.14
IgE [IU/ml]	<18.9	<18.9	<18.9	0–100

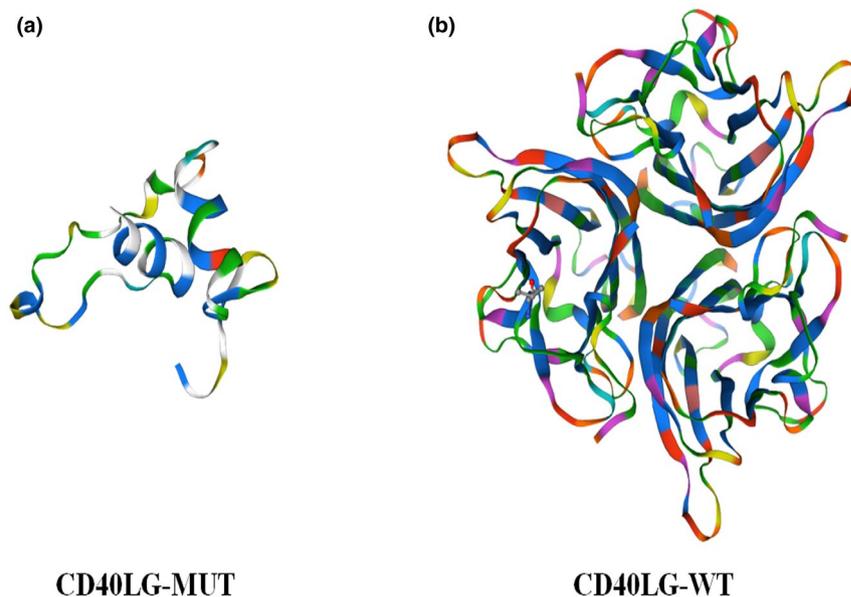


**FIGURE 1** Family pedigree and genetic analysis of *CD40LG*. Genetic analysis was performed on the patient and the patient's mother and elder sister with Sanger sequencing. (a) Family pedigree (the arrow indicates the patient). (b) Chromatogram showing the deletion of A and insertion of nucleotides in exon 1 in the patient and the heterozygous genotype of the patient's mother, in contrast to the patient's sister. (c) 147-nucleotide insertion shown by BLAST analysis of the mutation sequence and wild sequence

short transmembrane region, a unique extracellular domain and an extracellular C-terminal TNF-homologous domain (Iwata et al., 1995; Thusberg & Vihinen, 2007). *CD40LG*

contains four introns and five exons. Exon 1 encodes the intracellular and transmembrane regions and a small portion of the extracellular region, while exons 2–5 encode

**FIGURE 2** Prediction of the structure of the mutant protein. (A) The mutant protein structure of *CD40LG* (*CD40LG*-Mut) was predicted to produce a truncated protein product by the online software SWISS-MODEL. (B) The wild-type *CD40LG* protein structure (*CD40LG*-WT)



the rest of the extracellular domain (Lopez-Saucedo et al., 2015; Winkelstein et al., 2003). Interaction between CD40 and CD40L can stimulate the activation of B cells, class conversion and high-frequency mutation of somatic cells. In addition, it can induce the formation of memory B cells, with the antibody produced by B cells switching from IgM to IgG, IgA or IgE. Finally, it produces a high-affinity antibody against T-cell-dependent antigens (Xu et al., 2012). These effects influence the interaction between CD4<sup>+</sup> T cells, dendritic cells and macrophages, and they damage cellular immunity (Chatzigeorgiou et al., 2009). A murine *CD40LG* ‘knockout’ lacks expression of IgG, IgA and IgE, similar to HIGMS in humans (Renshaw et al., 1994; Xu et al., 1994). The number of mature B cells in X-HIGM patients is normal, but they cannot be converted into memory B cells, so the number of memory B cells is reduced (Revy et al., 2000; Wang et al., 2014).

In this study, Sanger sequencing data identified the mutation as c.17delAins147 in the *CD40LG*. Interestingly, the 147-bp insertion was identified in sequence to the normal human mitochondrial genome sequence and did not match any known human nuclear genomic sequence. BLAST analysis revealed that the inserted sequence had 98% identity with the mitochondrial 12S rRNA between nucleotides 664 and 805 of the human mtDNA sequence. This mutation results in p.N6Ifs\*65, a frame-shift mutation producing a premature stop codon. Furthermore, protein structure prediction with SWISS-MODEL expected a truncated protein product causing an obvious change in the local structure. The patient's protein structure suggests that severe HIGM mutation changes the protein structure much more than most single-site mutations, which mostly affect the folding and stability of proteins (Karpusas et al., 1995; Yazdani et al., 2019). Taken together, the data are consistent with

the view that this patient possesses a genomic DNA mutation caused by de novo mitochondrial–nuclear migration.

The transferred mitochondrial DNA (mtDNA) fragments in the nuclear genome are called nuclear mtDNA or NUMTs. Studies suggest that the rate of NUMTs in humans is about  $5.1\text{--}5.6 \times 10^6$  per germ cell per generation; every two human haploid genomes should be polymorphic for at least two NUMT loci (Bensasson et al., 2003; Ricchetti et al., 2004). There is increasing evidence of the involvement of NUMTs in human biology and pathology. For example, it can link a maternally inherited mitochondrial genotype with nuclear DNA polymorphism. The importance of NUMTs in human diseases is underscored by the fact that numtogenesis is an ongoing and frequent biological phenomenon. Integration of NUMTs results in not only neutral polymorphism but, more rarely, human diseases (Hazkani-Covo et al., 2010). An abundance of NUMTs is associated with cancer-related cases, such as HeLa cell (Shay & Werbin, 1992), gastric cancer (Yao et al., 2004), low-grade brain tumour (Liang, 1996) and colorectal adenocarcinoma (Srinivasainagendra et al., 2017). Five genetic cases have been reported. One involved a 41-bp mtDNA insertion at the breakpoint junction of a translocation (Willett-Brozick et al., 2001). Four others involved insertions in genes. One case study described a patient with Pallister-Hall syndrome, with a 72-bp NUMT insertion into exon 14 of the *GLI3* gene serving as the causative mutation. The NUMT insertion created a premature stop codon and predicted a truncated protein product (Turner et al., 2003). In another case, a 36-bp NUMT insertion in exon 9 of the *USH1C* gene was associated with Usher syndrome type IC (Ahmed et al., 2002). Furthermore, a 251-bp insertion of mitochondrial origin resulted in severe plasma factor VII deficiency and bleeding disease (Borensztajn et al., 2002). Transfer of a mitochondrial DNA fragment to *MCOLN1*

was reported to cause an inherited case of mucopolipidosis IV (Goldin et al., 2004).

The way in which mitochondrial nucleotide fragments escape from the mitochondria and enter the nucleus during gametogenesis, as well as the steps of DNA insertion, are poorly understood. For NUMTs to persist in nuclear genomes, mitochondrial DNA must first be exported from the mitochondria, physically reach the nucleus, and then must integrate into the nuclear chromosome, with subsequent intragenomic amplification, mutation or deletion. There are three main ways for mitochondrial genetic fragments to escape from mitochondria and travel to the nucleus (Singh et al., 2017): (a) the degradation of abnormal mitochondria (Campbell & Thorsness, 1998); (b) fusion of the mitochondria and nuclear membranes (Thorsness & Weber, 1996); and (c) engulfment of mitochondria by the nucleus (Jensen et al., 1976). In the laboratory and in nature, NUMTs enter nuclear DNA via non-homologous end joining at double-strand breaks (Hazkani-Covo & Covo, 2008; Ricchetti et al., 2004).

The appearance of NUMTs is an ongoing and frequent biological phenomenon that plays an important role in evolution and human diseases. NUMTs are one of the keys to revealing the mysterious biological implications of genomic analysis. To more clearly understand the effect of NUMT insertion, more population-level genomic data and more accurate genome sequences may be required.

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## CONFLICT OF INTERESTS

The authors declare that there are no financial or other conflicts of interest.

## AUTHOR CONTRIBUTIONS

Xuejing Li and Dan Xu gathered clinical information from the family, performed literature review, and drafted the manuscript. Beilei Chen and Yunlian Zhou performed molecular genetic analysis. Zhimin Chen and Yingshuo Wang designed the study. All authors revised the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## REFERENCES

- Ahmed, Z. M., Smith, T. N., Riazuddin, S., Makishima, T., Ghosh, M., Bokhari, S., Menon, P. S., Deshmukh, D., Griffith, A. J., Riazuddin, S., Friedman, T. B., & Wilcox, E. R. (2002). Nonsyndromic recessive deafness DFNB18 and Usher syndrome type IC are allelic mutations of USHC. *Human Genetics*, *110*, 527–531. <https://doi.org/10.1007/s00439-002-0732-4>
- Allen, R., Armitage, R., Conley, M., Rosenblatt, H., Jenkins, N., Copeland, N., Bedell, M., Edelhoff, S., Distèche, C., & Simoneaux, D. (1993). CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science*, *259*, 990–993. <https://doi.org/10.1126/science.7679801>
- Aruffo, A., Farrington, M., Hollenbaugh, D., Li, X. U., Milatovich, A., Nonoyama, S., Bajorath, J., Grosmaire, L. S., Stenkamp, R., Neubauer, M., Roberts, R. L., Noelle, R. J., Ledbetter, J. A., Francke, U., & Ochs, H. D. (1993). The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell*, *72*, 291–300. [https://doi.org/10.1016/0092-8674\(93\)90668-G](https://doi.org/10.1016/0092-8674(93)90668-G)
- Bensasson, D., Feldman, M. W., & Petrov, D. A. (2003). Rates of DNA duplication and mitochondrial DNA insertion in the human genome. *Journal of Molecular Evolution*, *57*, 343–354. <https://doi.org/10.1007/s00239-003-2485-7>
- Bhushan, A., & Covey, L. R. (2001). CD40:CD40L interactions in X-linked and non-X-linked hyper-IgM syndromes. *Immunologic Research*, *24*(3), 311–324. <https://doi.org/10.1385/IR:24:3:311>
- Borensztajn, K., Chafa, O., Alhenc-Gelas, M., Salha, S., Reghis, A., Fischer, A. M., & Tapon-Bretaudiere, J. (2002). Characterization of two novel splice site mutations in human factor VII gene causing severe plasma factor VII deficiency and bleeding diathesis. *British Journal of Haematology*, *117*, 168–171. <https://doi.org/10.1046/j.1365-2141.2002.03397.x>
- Campbell, C. L., & Thorsness, P. E. (1998). Escape of mitochondrial DNA to the nucleus in yme1 yeast is mediated by vacuolar-dependent turnover of abnormal mitochondrial compartments. *Journal of Cell Science*, *111*(Pt 16), 2455–2464.
- Chatzigeorgiou, A., Lyberi, M., Chatzilymperis, G., Nezos, A., & Kamper, E. (2009). CD40/CD40L signaling and its implication in health and disease. *BioFactors*, *35*, 474–483. <https://doi.org/10.1002/biof.62>
- Davies, E. G., & Thrasher, A. J. (2010). Update on the hyper immunoglobulin M syndromes. *British Journal of Haematology*, *149*, 167–180. <https://doi.org/10.1111/j.1365-2141.2010.08077.x>
- de la Morena, M. T., Leonard, D., Torgerson, T. R., Cabral-Marques, O., Slatter, M., Aghamohammadi, A., Chandra, S., Murguia-Favela, L., Bonilla, F. A., Kanariou, M., Damrongwatanasuk, R., Kuo, C. Y., Dvorak, C. C., Meyts, I., Chen, K., Kobrynski, L., Kapoor, N., Richter, D., DiGiovanni, D., ... Roifman, C. M. (2017). Long-term outcomes of 176 patients with X-linked hyper-IgM syndrome treated with or without hematopoietic cell transplantation. *The Journal of Allergy and Clinical Immunology*, *139*, 1282–1292. <https://doi.org/10.1016/j.jaci.2016.07.039>
- DiSanto, J. P., Bonnefoy, J. Y., Gauchat, J. F., Fischer, A., & de Saint Basile, G. (1993). CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. *Nature*, *361*, 541–543. <https://doi.org/10.1038/361541a0>
- Goldin, E., Stahl, S., Cooney, A. M., Kaneski, C. R., Gupta, S., Brady, R. O., Ellis, J. R., & Schiffmann, R. (2004). Transfer of a mitochondrial DNA fragment to MCOLN1 causes an inherited case of mucopolipidosis IV. *Human Mutation*, *24*, 460–465. <https://doi.org/10.1002/humu.20094>
- Hazkani-Covo, E., & Covo, S. (2008). Numt-mediated double-strand break repair mitigates deletions during primate genome

- evolution. *PLoS Genetics*, 4, e1000237. <https://doi.org/10.1371/journal.pgen.1000237>
- Hazkani-Covo, E., Zeller, R. M., & Martin, W. (2010). Molecular poltergeists: Mitochondrial DNA copies (numts) in sequenced nuclear genomes. *PLoS Genetics*, 6, e1000834. <https://doi.org/10.1371/journal.pgen.1000834>
- Iwata, M., Nunoi, H., Nonoyama, S., Shimadzu, M., Higuchi, S., Yanabe, Y., Migita, M., Adachi, N., & Matsuda, I. (1995). Neutropenia in patient with X-linked hyper-IgM syndrome. *Rinsho Ketsueki*, 36, 1223–1229.
- Jensen, H., Engedal, H., & Saetersdal, T. S. (1976). Ultrastructure of mitochondria-containing nuclei in human myocardial cells. *Virchows Archiv B Cell Pathology*, 21, 1–12. <https://doi.org/10.1007/BF02899139>
- Karpusas, M., Hsu, Y. M., Wang, J. H., Thompson, J., Lederman, S., Chess, L., & Thomas, D. (1995). 2 A crystal structure of an extracellular fragment of human CD40 ligand. *Structure*, 3, 1031–1039. [https://doi.org/10.1016/s0969-2126\(01\)00239-8](https://doi.org/10.1016/s0969-2126(01)00239-8)
- Korthäuer, U., Graf, D., Mages, H. W., Brière, F., Padayachee, M., Malcolm, S., Ugazio, A. G., Notarangelo, L. D., Levinsky, R. J., & Kroczeck, R. A. (1993). Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature*, 361, 539–541. <https://doi.org/10.1038/361539a0>
- Lee, W. I., Torgerson, T. R., Schumacher, M. J., Yel, L., Zhu, Q., & Ochs, H. D. (2005). Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. *Blood*, 105, 1881–1890. <https://doi.org/10.1182/blood-2003-12-4420>
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25, 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Liang, B. C. (1996). Evidence for association of mitochondrial DNA sequence amplification and nuclear localization in human low-grade gliomas. *Mutation Research*, 354, 27–33. [https://doi.org/10.1016/0027-5107\(96\)00004-8](https://doi.org/10.1016/0027-5107(96)00004-8)
- Lopez-Saucedo, C., Bernal-Reynaga, R., Zayas-Jahuey, J., Galindo-Gomez, S., Shibayama, M., Garcia-Galvez, C., Estrada-Parra, S., & Estrada-Garcia, T. (2015). CD40 ligand deficient C57BL/6 mouse is a potential surrogate model of human X-linked hyper IgM (X-HIGM) syndrome for characterizing immune responses against pathogens. *BioMed Research International*, 2015, 1–11. <https://doi.org/10.1155/2015/679850>
- Mourier, T., Hansen, A. J., Willerslev, E., & Arctander, P. (2001). The Human Genome Project reveals a continuous transfer of large mitochondrial fragments to the nucleus. *Molecular Biology and Evolution*, 18, 1833–1837. <https://doi.org/10.1093/oxfordjournals.molbev.a003971>
- Ramesh, N., Fuleihan, R., Ramesh, V., Lederman, S., Yellin, M. J., Sharma, S., Chess, L., Rosen, F. S., & Geha, R. S. (1993). Deletions in the ligand for CD40 in X-linked immunoglobulin deficiency with normal or elevated IgM (HIGM-X1). *International Immunology*, 5, 769–773. <https://doi.org/10.1093/intimm/5.7.769>
- Renshaw, B. R., Fanslow, W. C., Armitage, R. J., Campbell, K. A., Liggitt, D., Wright, B., Davison, B. L., & Maliszewski, C. R. (1994). Humoral immune responses in CD40 ligand-deficient mice. *Journal of Experimental Medicine*, 180, 1889–1900. <https://doi.org/10.1084/jem.180.5.1889>
- Revy, P., Muto, T., Levy, Y., Geissmann, F., Plebani, A., Sanal, O., Catalan, N., Forveille, M., Dufourcq-Lagelouse, R., Gennery, A., Tezcan, I., Ersoy, F., Kayserili, H., Ugazio, A. G., Brousse, N., Muramatsu, M., Notarangelo, L. D., Kinoshita, K., Honjo, T., ... Durandy, A. (2000). Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell*, 102, 565–575. [https://doi.org/10.1016/S0092-8674\(00\)00079-9](https://doi.org/10.1016/S0092-8674(00)00079-9)
- Ricchetti, M., Tekaiia, F., & Dujon, B. (2004). Continued colonization of the human genome by mitochondrial DNA. *PLoS Biology*, 2, e273. <https://doi.org/10.1371/journal.pbio.0020273>
- Shay, J. W., & Werbin, H. (1992). New evidence for the insertion of mitochondrial DNA into the human genome: Significance for cancer and aging. *Mutation Research*, 275, 227–235. [https://doi.org/10.1016/0921-8734\(92\)90026-L](https://doi.org/10.1016/0921-8734(92)90026-L)
- Singh, K. K., Choudhury, A. R., & Tiwari, H. K. (2017). Numtogenesis as a mechanism for development of cancer. *Seminars in Cancer Biology*, 47, 101–109. <https://doi.org/10.1016/j.semcancer.2017.05.003>
- Srinivasainagendra, V., Sandel, M. W., Singh, B., Sundaresan, A., Mooga, V. P., Bajpai, P., Tiwari, H. K., & Singh, K. K. (2017). Migration of mitochondrial DNA in the nuclear genome of colorectal adenocarcinoma. *Genome Medicine*, 9, 31. <https://doi.org/10.1186/s13073-017-0420-6>
- Thorsness, P. E., & Weber, E. R. (1996). Escape and migration of nucleic acids between chloroplasts, mitochondria, and the nucleus. *International Review of Cytology*, 165, 207–234. [https://doi.org/10.1016/s0074-7696\(08\)62223-8](https://doi.org/10.1016/s0074-7696(08)62223-8)
- Thusberg, J., & Vihinen, M. (2007). The structural basis of hyper IgM deficiency - CD40L mutations. *Protein Engineering, Design & Selection*, 20, 133–141. <https://doi.org/10.1093/protein/gzm004>
- Tourmen, Y., Baris, O., Dessen, P., Jacques, C., Malthiery, Y., & Reynier, P. (2002). Structure and chromosomal distribution of human mitochondrial pseudogenes. *Genomics*, 80, 71–77. <https://doi.org/10.1006/geno.2002.6798>
- Turner, C., Killoran, C., Thomas, N. S. T., Rosenberg, M., Chuzhanova, N. A., Johnston, J., Kemel, Y., Cooper, D. N., & Biesecker, L. G. (2003). Human genetic disease caused by de novo mitochondrial-nuclear DNA transfer. *Human Genetics*, 112, 303–309. <https://doi.org/10.1007/s00439-002-0892-2>
- van Kooten, C., & Banchereau, J. (2000). CD40-CD40 ligand. *Journal of Leukocyte Biology*, 67, 2–17. <https://doi.org/10.1002/jlb.67.1.2>
- Wallace, D. C., Stugard, C., Murdock, D., Schurr, T., & Brown, M. D. (1997). Ancient mtDNA sequences in the human nuclear genome: A potential source of errors in identifying pathogenic mutations. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 14900–14905. <https://doi.org/10.1073/pnas.94.26.14900>
- Wang, L. L., Zhou, W., Zhao, W., Tian, Z. Q., Wang, W. F., Wang, X. F., & Chen, T. X. (2014). Clinical features and genetic analysis of 20 Chinese patients with X-linked hyper-IgM syndrome. *Journal of Immunology Research*, 2014, 1–12. <https://doi.org/10.1155/2014/683160>
- Willett-Brozick, J. E., Savul, S. A., Richey, L. E., & Baysal, B. E. (2001). Germ line insertion of mtDNA at the breakpoint junction of a reciprocal constitutional translocation. *Human Genetics*, 109, 216–223. <https://doi.org/10.1007/s004390100564>
- Winkelstein, J. A., Marino, M. C., Ochs, H., Fuleihan, R., Scholl, P. R., Geha, R., Stiehm, E. R., & Conley, M. E. (2003). The X-linked hyper-IgM syndrome: Clinical and immunologic features of 79 patients. *Medicine (Baltimore)*, 82, 373–384. <https://doi.org/10.1097/01.md.0000100046.06009.b0>
- Woischnik, M., & Moraes, C. T. (2002). Pattern of organization of human mitochondrial pseudogenes in the nuclear genome. *Genome Research*, 12, 885–893. <https://doi.org/10.1101/gr.227202>

- Xu, J., Foy, T. M., Laman, J. D., Elliott, E. A., Dunn, J. J., Waldschmidt, T. J., Elsemore, J., Noelle, R. J., & Flavell, R. A. (1994). Mice deficient for the CD40 ligand. *Immunity*, *1*, 423–431. [https://doi.org/10.1016/1074-7613\(94\)90073-6](https://doi.org/10.1016/1074-7613(94)90073-6)
- Xu, Z., Zan, H., Pone, E. J., Mai, T., & Casali, P. (2012). Immunoglobulin class-switch DNA recombination: Induction, targeting and beyond. *Nature Reviews Immunology*, *12*, 517–531. <https://doi.org/10.1038/nri3216>
- Yao, Y. G., Bravi, C. M., & Bandelt, H. J. (2004). A call for mtDNA data quality control in forensic science. *Forensic Science International*, *141*, 1–6. <https://doi.org/10.1016/j.forsciint.2003.12.004>
- Yazdani, R., Fekrvand, S., Shahkarami, S., Azizi, G., Moazzami, B., Abolhassani, H., & Aghamohammadi, A. (2019). The hyper IgM

syndromes: Epidemiology, pathogenesis, clinical manifestations, diagnosis and management. *Clinical Immunology*, *198*, 19–30. <https://doi.org/10.1016/j.clim.2018.11.007>

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