From Dysgammaglobulinemia to Autosomal-Dominant Activation-Induced Cytidine Deaminase Deficiency: Unraveling an Inherited Immunodeficiency after 50 Years

Jehane Fadlallah, MD, PhD[1], Loic Chentout, MS[2], Bertrand Boisson, PhD[3,4], Aurore Pouliet, BS[5], Cecile Masson, MS[6], Florence Morin, PharmD[7], Anne Durandy, MD[2], Jean-Laurent Casanova, MD, PhD[3,4,8,9,10], Eric Oksenhendler, MD[1,*], and Sven Kracker, PhD[2,*]

The genetic investigation of a family presenting with a dominant form of hyper IgM syndrome published in 1963 and 1975 revealed a R190X nonsense mutation in activation-induced cytidine deaminase. This report illustrates the progress made over 6 decades in the characterization of primary immunodeficiencies, from immunochemistry to whole-exome sequencing. *(J Pediatr 2020;223:207–11).*

In 1963, Fred Rosen and James Bougas reported on a 37-year-old woman (proband II-12) having suffered from recurrent infections and severe bronchiectasis over the previous 10 years.[1] Immunoelectrophoresis of the patient's serum highlighted elevation of the 19S-γ1 globulin fraction and a profoundly low 7S-γ globulin fraction. The features of the 1963 case were reminiscent of Dr Rosen's description of two boys in 1961.[2]

In 1975, Feldman et al studied one of the proband II-12's nephews (III-2) and then described in *The Journal of Pediatrics* the clinical and biological features of 37 family members over 3 generations.[3] The researchers identified immunological abnormalities in 12 relatives, although only 3 of these presented with recurrent infections. Ten family members had elevated serum IgM levels, and 7 had low serum levels of IgG, IgA, or both. In most family members, the lymphocyte count (assessed by the percentage of E-rosette-forming lymphocytes and the percentage of immunoglobulin-bearing cells) was within the normal range. Analysis of the family's pedigree over 3 generations suggested that the antibody deficiency had an (AD) mode of inheritance. This kindred was the first to be diagnosed with an AD form of an immunological and clinical condition that was designated hyper IgM syndrome (HIGM) in 1979.[4]

HIGM encompasses a number of genetic defects that impair class switch recombination (CSR) and somatic hypermutation (SHM) and result in normal or elevated serum IgM levels and abnormally low serum levels of other Igs. The first kindreds with X-linked recessive and autosomal recessive (AR) forms of HIGM had been reported in 1961 and 1994, respectively.[5-7] In 1993, the first HIGM-causing genetic defect was identified; an X-linked CD40 ligand deficiency was found to cause the most common X-linked recessive form of this syndrome.[8-11] Mutations in the *AICDA* gene (encoding activation-induced cytidine deaminase [AID]) responsible for the most frequent AR form of HIGM were described in 2000.[12]

AID initiates the B-cell-specific CSR and SHM processes.[13] It is now known that a range of biallelic mutations, premature stop codons, and deletions in *AICDA* affect both CSR and SHM.[12,14] Along with the AR form of HIGM, *AICDA* is also involved in a rare AD form of HIGM that was genetically characterized for the first time in 2003 in a Japanese woman with a mild clinical phenotype.[15] The causative heterozygous R190X mutation in *AICDA* generates a premature stop codon in exon 5 and results in the loss of the last 9 C-terminal amino acids in the AID protein (part of the nuclear export signal domain). In 2005, Imai et al demonstrated that the R190X mutation was associated with an impairment in CSR but—in contrast with AR HIGM—not in SHM.[16]

In 2018, one of the initial patient's nieces (III-8) underwent genetic testing in our center. Here, we report on how

AD	Autosomal dominant
AID	Activation-induced cytidine deaminase
AR	Autosomal recessive
CSR	Class switch recombination
HIGM	Hyper IgM syndrome
PID	Primary immunodeficiency
SHM	Somatic hypermutation

From the [1]Department of Clinical Immunology, Saint-Louis Hospital, Assistance Publique–Hôpitaux de Paris, and Paris Diderot University, Paris, France; [2]Human Lymphohematopoiesis Laboratory, Institut Imagine, Inserm U1163, Paris Descartes Sorbonne, Paris Cite University, Paris, France; [3]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Inserm U1163, Paris, France; [4]St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, New York, NY; [5]Genomics Core Facility, Institut Imagine, Inserm U1163, Paris Descartes Sorbonne, Paris Cite University, Paris, France; [6]Bioinformatics Facility, Institut Imagine, Inserm U1163, Paris Descartes Sorbonne, Paris Cite University, Paris, France; [7]Immunology and Histocompatibility Laboratory, Saint-Louis Hospital, Assistance Publique–Hôpitaux de Paris, Paris, France; [8]Howard Hughes Medical Institute; [9]Rockefeller University and Rockefeller University Hospital, New York, NY; and [10]Pediatric Immunology and Hematology Unit, Necker Hospital for Sick Children, Assistance Publique-Hôpitaux de Paris, Paris, France

*Contributed equally.

Funded by Inserm, the Agence Nationale de la Recherche, as part of the Investment for the Future Program (ANR-10-IAHU-01) and by ANR-19-CE17-0012-01 (ANR-AID), ANR-14-CE15-0009, the Ligue Contre le Cancer–Comité de Paris, the Fondation ARC pour la Recherche sur le Cancer, and the Centre de Référence des Déficits Immunitaires Héréditaires (CEREDIH), the Rockefeller University, Paris Descartes University, and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (P01AI061093). The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2020.03.024

the cause of a rare AD primary immunodeficiency (PID) first described in 1963 was determined.[1] This investigation of an AD inherited HIGM neatly illustrates the remarkable progress made in the exploration of PIDs over the last 6 decades.

Methods

Proband III-8 and her 3 children underwent a physical examination. New or updated clinical information was obtained for all family members. A blood sample was obtained from proband III-8 and 10 of her relatives. All assessed family members had given their written, informed consent to participate in genetic testing.

Serum Immunoglobulin Assay

Serum IgG, IgA, and IgM levels were measured in a nephelometric assay.

Lymphocyte Subset Phenotyping

B, T, and natural killer cell subsets were measured using flow cytometry (FACS CANTO II, Becton Dickinson, Franklin Lakes, New Jersey). Naive and memory T-cell subsets and naive, transitional, marginal zone, and memory B-cell subsets were determined in proband III-8.

Whole-Exome Sequencing and Sanger Sequencing

Genomic DNA was extracted from leukocytes provided by 11 family members. For proband III-8, barcoded exome libraries were prepared and sequenced with the NovaSeq6000 system (Illumina, San Diego, California). Sequences were then mapped on the human reference genome (NCBI build 37, hg19 version). Variants were called with the Genome Analysis Toolkit (GATK), SAMtools, and Picard tools. All the variants were annotated and filtered with PolyWeb (our in-house annotation software; **Appendix and Table I** [available at www.jpeds.com]). Sanger sequencing was used to detect the R190X mutation in all tested relatives.

Results

Proband III-8 (a 60-year-old woman) had suffered from recurrent bronchitis since childhood. There was no evidence of an autoimmune or lymphoproliferative disorder. The proband had never received IgG replacement therapy. Blood tests confirmed the diagnosis of HIGM, with an elevated level of IgM and normal levels of IgG and IgA (**Table II**), and lymphocyte immunophenotyping show that neither the switched memory B-cell ($CD19^+CD27^+IgD^-$) population nor the T-cell compartment was defective (data not shown). The proband exhibited normal IgG titers against tetanus toxin and protective titers against 6 out of 7 *Streptococcus pneumonia* serotypes (postpneumococcal polysaccharide vaccine).

By combining the family's data from the 1975 study with the recently updated information, we determined that 15 members had presented with recurrent respiratory infections—typically from late childhood/early adulthood.[3] Nevertheless, only 6 of the 15 members had received IgG replacement therapy (**Figure**). We had the opportunity to compare serum immunoglobulin levels in 4 patients, 44 years apart (1975 and 2019). Three patients exhibited persistent high IgM levels and the fourth patient kept with normal IgM levels. All 4 patients had persistent low IgA levels. Hence, the serum Ig defects varied from one individual to another but were stable over time (**Table II**).

To identify the genetic cause of the disease, genomic DNA from proband III-8 was whole-exome sequenced. In view of the mode of inheritance, an AD genetic model

Table II. Clinical and immunologic characteristics of the 11 family members having undergone genetic testing

Family member	Sex	Age (years)	AID	Clinical phenotype	Serum immunoglobulins IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)
II-2	F	*38*	R190X/WT	Recurrent infections Mouth ulcers	***140***	***12***	***420***
		83			1064*	**<5**	**554**
III-2	M	*9*	R190X/WT	Recurrent infections Erythema nodosa	***300***	***<5***	*125*
		54			2027*	**<5**	135
III-7	M	*19*	R190X/WT	Recurrent infections	*740*	***63***	***260***
		64			1241	**47**	**306**
III-8	F	*15*	R190X/WT	Recurrent infections	*740*	***70***	***438***
		60			710	**103**	**587**
III-15	M	65	R190X/WT	Recurrent infections	1047*	**<5**	**466**
III-16	M	63	WT/WT	Non-Hodgkin lymphoma	1300*	304	73
IV-1	F	22	WT/WT	Asymptomatic	1075	225	113
IV-2	F	19	WT/WT	Asymptomatic	1318	137	**221**
IV-3	M	18	WT/WT	Asymptomatic	978	122	97
IV-4	F	34	R190X/WT	Recurrent infections	792	**58**	**224**
IV-5	M	28	R190X/WT	Recurrent infections	**68**	**<5**	145

WT, wild type.
Age and serum immunoglobulin values in italics are taken from the 1975 study by Feldman et al.[3]
Values in bold are outside the normal range. Normal ranges were defined as follows: IgG: 675–1253 mg/dL; IgA: 104–337 mg/dL; IgM: 52–146 mg/dL.
*Receiving immunoglobulin replacement therapy.

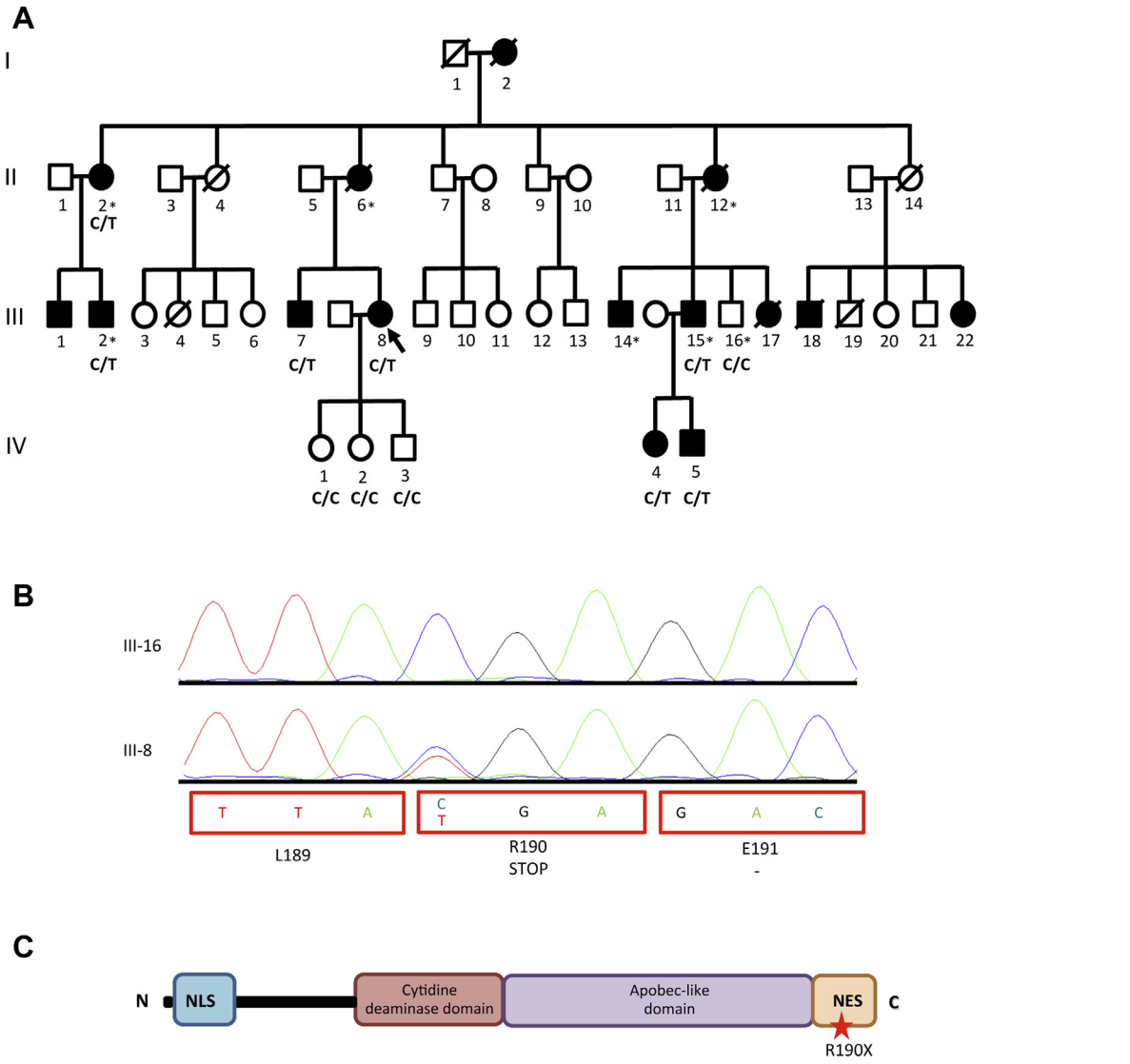

Figure. **A**, The pedigree of the family displaying an AD inherited trait. Members having undergone genetic testing are either C/C (wild type) or C/T (carriers of the R190X mutation). Black filled symbols represent affected members with recurrent infections and abnormal serum Ig levels. *Ig replacement therapy. The *arrow* denotes the proband in the present study. **B,** Electropherogram showing the c.568 C>T mutation (generating a STOP codon) in proband III-8, compared with proband III-16. **C,** Graphical representation of the AID protein, showing the R190X mutation in the C-terminal domain.

was used to filter the identified genetic variants. A heterozygous C-to-T nonsense mutation at position g.8756909 (GRCH37; NM_020661.2) on chromosome 12 (c.568 C>T in exon 5 of the AICDA gene) was detected. This variant was absent from the gnomAD database (https://gnomad.broadinstitute.org) and in Polyweb, an in-house database containing 16060 whole exome sequencing and 744 whole genome sequencing. The R190X heterozygous nonsense mutation in AID has been linked to an AD HIGM syndrome with a mild clinical phenotype, hypogammaglobulinemia of variable intensity, conserved SHM, and a variable disruption of CSR.[15-17]

Sanger sequencing confirmed the presence of a heterozygous R190X mutation in proband III-8 and in 6 symptomatic family members (**Figure**), all of whom suffered from recurrent infections. Five of the 7 individuals had HIGM syndrome with various IgG and/or IgA defects, and 3 were receiving IgG replacement therapy (**Table II**). We also genotyped 4 asymptomatic relatives born to carriers: neither proband III-16 nor the 3 asymptomatic children of proband III-8 carried the R190X mutation. It is noteworthy that proband III-16 had been considered to be asymptomatic in 1975 but had since developed non-Hodgkin lymphoma and secondary immunodeficiency requiring IgG replacement therapy.

An analysis of the family's pedigree highlighted the presence of the heterozygous R190X mutation with Mendelian segregation and variable phenotypic expression and in males and females over 3 generations (**Figure**).[3]

Discussion

Herein we describe a kindred with AD HIGM caused by AID R190X mutation. As in the few previously reported families, the clinical and biological phenotype was variable yet mild in most investigated patients. This rare PID has only been included in the latest International Classification of Human Inborn Errors of Immunity.[18]

The underlying molecular pathophysiologic mechanisms involving disruption of Ig CSR and maintenance of SHM remain elusive. AID haploinsufficiency is unlikely because entire deletion of one *AICDA* allele does not impact B-cell function. A dominant negative effect seems rather plausible either by modifying the C-terminal anchor needed for the binding of the CSR co-factors, or by the nuclear accumulation of the mutated protein, altering the native protein function.[16,17] Functional investigation provided evidence that the domain encoded by exon 5 of the *AICDA* gene provides a link between DNA damage and repair during CSR.[19,20]

In 4 tested patients, a pair of serum Ig measurements 44 years apart revealed the persistence of abnormal Ig levels. The kindred's medical history nicely illustrates the technical progress achieved over the last 6 decades in the investigation of PIDs. In 1963, Rosen and Bougas first investigated proband II-12 for recurrent infections and bronchiectasis; the researchers used the available immunochemical methods to characterize what was referred to as dysgammaglobulinemia.[1] At that time, serum immunoglobulins were classified in 3 distinct fractions (7S-γ2, 7S-γ1 and 19S-γ1, where S was the sedimentation constant) on the basis of their electrophoretic and ultracentrifugation properties. In 1964, the World Health Organization designated the 19S γ-globulin as "IgM."[21] Immunoelectrophoresis (based on goat anti-19S gammaglobulin, horse anti-gammaglobulin and horse anti-human serum) was used to distinguish more precisely between the different immunoglobulins.[22] Thus, proband II-12 was found to have an elevated 19S-γ1 fraction and no 7S-γ1 and γ2 fractions. Furthermore, vaccine responses and isohemagglutination tests for Gram-negative bacteria bearing O and H antigens were used as functional assays. No anti-H antibodies were found, because they are present in the 7S-γ2 fraction only. In contrast, anti-O antibodies (present in the 19S-γ1 fraction) were conserved in proband II-12. In 1975, progress in immunologic techniques had enabled scientists to distinguish between T and B lymphocytes. T-cell counts were assessed by determining the percentage of lymphocytes that formed rosettes with sheep red blood cells. B-cell counts were evaluated in an immunofluorescence assay after the lymphocytes had been staining with a fluorescein-conjugated specific goat anti-human heavy chain antibody. Immunoglobulin-bearing cells (defined by the presence of a uniform rim or patchy surface fluorescence) were considered to be B-cells. Thus, in proband II-12, E-rosette forming lymphocytes fell within normal range (79% for a normal range of 52%-86%), whereas "Ig-bearing cells" were decreased for IgG and IgA (1% and 2%, respectively).[3]

In the following years, the advent of fluorescence-activated cell sorting considerably improved the process of lymphocyte phenotyping. In the last decade, the investigation of PIDs has been dominated by genetics. Although Sanger sequencing of candidate genes involved in the immune system has been used since 1980, whole exome sequencing is now the reference technique for uncovering the involvement of novel genes and revealing new modes of transmission. To date, more than 400 genes have been causally implicated in PIDs, and the number is increasing exponentially year after year.[23] Nevertheless, pathophysiologic investigation is complicated when several different types of mutations (eg, gain-of-function and loss-of-function mutations) in a single gene are involved or (as in the present case) when the PID features differ in the mode of inheritance (either recessive or dominant). ■

We thank the participating family for their cooperation and Dr Fred Rosen (deceased) for his contributions to the field.

Submitted for publication Nov 26, 2019; last revision received Feb 17, 2020; accepted Mar 12, 2020.

Reprint requests: Jehane Fadlallah, MD, PhD, Département d'Immunologie Clinique, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010, Paris, France. E-mail: jehane.fadlallah@aphp.fr

References

1. Rosen FS, Bougas JA. Acquired dysgammaglobulinemia: elevation of the 19s Gamma globulin and deficiency of the 7s gamma globulin in a woman with chronic progressive bronchiectasis. N Engl J Med 1963;269:1336-40.
2. Rosen FS, Kevy SV, Merler E, Janeway CA, Gitlin D. Recurrent bacterial infections and dysgamma-globulinemia: deficiency of 7S gamma-globulins in the presence of elevated 19S gamma-globulins. Report of two cases. Pediatrics 1961;28:182-95.
3. Feldman G, Koziner B, Talamo R, Bloch KJ. Familial variable immunodeficiency: autosomal dominant pattern of inheritance with variable expression of the defect(s). J Pediatr 1975;87:534-9.
4. Immunodeficiency. Report of a WHO scientific group. Clin Immunol Immunopathol 1979;13:296-359.
5. Jamieson WM, Kerr MR. A family with several cases of hypogammaglobulinaemia. Arch Dis Child 1962;37:330-6.
6. Conley ME, Larche M, Bonagura VR, Lawton AR 3rd, Buckley RH, Fu SM, et al. Hyper IgM syndrome associated with defective CD40-mediated B cell activation. J Clin Invest 1994;94:1404-9.
7. Callard RE, Smith SH, Herbert J, Morgan G, Padayachee M, Lederman S, et al. CD40 ligand (CD40L) expression and B cell function in agammaglobulinemia with normal or elevated levels of IgM (HIM). Comparison of X-linked, autosomal recessive, and non-X-linked forms of the disease, and obligate carriers. J Immunol 1994;153:3295-306.
8. Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. Nature 1993;361:539-41.
9. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G. CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. Nature 1993;361:541-3.
10. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell 1993;72:291-300.
11. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science 1993;259:990-3.

12. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). Cell 2000;102:565-75.
13. Petersen-Mahrt SK, Harris RS, Neuberger MS. AID mutates E. coli suggesting a DNA deamination mechanism for antibody diversification. Nature 2002;418:99-103.
14. Minegishi Y, Lavoie A, Cunningham-Rundles C, Bedard PM, Hebert J, Cote L, et al. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. Clin Immunol 2000;97:203-10.
15. Kasahara Y, Kaneko H, Fukao T, Terada T, Asano T, Kasahara K, et al. Hyper-IgM syndrome with putative dominant negative mutation in activation-induced cytidine deaminase. J Allergy Clin Immunol 2003;112:755-60.
16. Imai K, Zhu Y, Revy P, Morio T, Mizutani S, Fischer A, et al. Analysis of class switch recombination and somatic hypermutation in patients affected with autosomal dominant hyper-IgM syndrome type 2. Clin Immunol 2005;115:277-85.
17. Ta VT, Nagaoka H, Catalan N, Durandy A, Fischer A, Imai K, et al. AID mutant analyses indicate requirement for class-switch-specific cofactors. Nat Immunol 2003;4:843-8.
18. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2020;40:24-64.
19. Zahn A, Eranki AK, Patenaude AM, Methot SP, Fifield H, Cortizas EM, et al. Activation induced deaminase C-terminal domain links DNA breaks to end protection and repair during class switch recombination. Proc Natl Acad Sci U S A 2014;111:E988-97.
20. Kracker S, Imai K, Gardes P, Ochs HD, Fischer A, Durandy AH. Impaired induction of DNA lesions during immunoglobulin class-switch recombination in humans influences end-joining repair. Proc Natl Acad Sci U S A 2010;107:22225-30.
21. Cooper MD, Faulk WP, Fudenberg HH, Good RA, Hitzig W, Kunkel H, et al. Classification of primary immunodeficiencies. N Engl J Med 1973;288:966-7.
22. Scheidegger JJ. A micro-method of immuno-electrophoresis. Intl Arch Allergy Appl Immunol 1955;7:103-10.
23. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol 2018;38:96-128.

Table I. Filtering of annotated variants after whole-exome sequencing with an AD genetic model

	No. of annotated variants
Variant analysis	**Patient III-8**
Total	141 554
Nonsynonymous, essential splicing, frameshift and stop-gained variants, and indels	15 975
MAF* <0.01 (1%)	1534
MAF* <0.001 (0.1%)	977
MAF* <0.0001 (0.01%)	711
Removal of pipeline artefacts (in-house MAF data; in one other project only)	181
Nonsynonymous variants	150
Frameshift variants	21
Essential splicing variants	6
Stop-gained variants	4
CADD score ≥20	83
CADD score ≥25	42
HGMD	1

1000 Genomes Project, National Heart, Lung and Blood Institute Exome Sequencing Project; *CADD*, combined annotation-dependent depletion; *HGMD*, Human Gene Mutation Database; *MAF*, minor allele frequency.
*In the Exome Aggregation Consortium database.