From dysgammaglobulinemia to autosomal dominant activation-induced cytidine deaminase deficiency: unraveling the mystery behind an inherited immunodeficiency first described 56 years ago

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In memoriam: Fred Rosen

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Summary

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 over six decades how progress has been made in the characterization of primary immunodeficiencies, from immunochemistry to whole-exome sequencing.

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 ten years(1). Immuno-electrophoresis of the patient's serum highlighted elevation of the 19S- γ 1 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 three generations (3). The researchers identified immunological abnormalities in 12 relatives, although only three of these presented with recurrent infections. Ten family members had elevated serum immunoglobulin (Ig) M levels, and seven 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 three generations suggested that the antibody deficiency had an autosomal dominant (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).

Hyper-IgM syndrome 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 (XR) and autosomal recessive (AR) forms of HIGM had been reported in 1961(5) and 1994(6, 7), respectively. In 1993, the first HIGM-causing genetic defect was identified; an X-linked CD40 ligand deficiency was found to cause the most common XR form of this syndrome (8-10),(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).

Activation-induced cytidine deaminase 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 nine 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 to 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 the cause of a rare AD primary immunodeficiency (PID) first described in 1963 was determined¹. This investigation of an AD inherited HIGM neatly illustrates the remarkable progress made in the exploration of PIDs over the last 6 decades.

Methods

PATIENTS

Proband III-8 and her three 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 participation in genetic testing.

SERUM IMMUNOGLOBULIN ASSAY

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

LYMPHOCYTE SUBSET PHENOTYPING

B, T and NK cell subsets were measured using flow cytometry (FACS CANTO II, Becton Dickinson, Franklin Lakes, NJ, USA). 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). 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; see the Supplementary Appendix). 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 auto-immune 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 1), 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 pneumoniae* serotypes (post pneumococcal polysaccharide vaccine).

By combining the family's data from the 1975 study(3) with the recently updated information, we determined that 15 members had presented with recurrent respiratory infections – typically

from late childhood/early adulthood. Nevertheless, only 6 out of the 15 members had received IgG replacement therapy (Figure 1). We had the opportunity to compare serum immunoglobulin levels in four patients, 44 years apart (1975 and 2019). Three patients exhibited persistent high IgM levels while the fourth patient kept with normal IgM levels. All four patients had persistent low IgA levels. Hence, the serum Ig defects varied from one individual to another but were stable over time (Table 1).

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 was used to filter the identified genetic variants (Supplementary Figure). 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 WES and 744 WGS. 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, 16)(17).

Sanger sequencing confirmed the presence of a heterozygous R190X mutation in proband III-8 and in six symptomatic family members (Figure 1), all of whom suffered from recurrent infections. Five of the seven individuals had HIGM syndrome with various IgG and/or IgA defects, and three were receiving IgG replacement therapy (Table 1). We also genotyped four asymptomatic relatives born to carriers: neither proband III-16 nor the three 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 three generations(3) (Figure 1).

Discussion

Here, we described the largest yet 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 pathophysiological mechanism involving disruption of Ig CSR and maintenance of SHM remain elusive. AID haploinsufficiency is unlikely since entire deletion of one *AICDA* allele does not impact B-cell function. A dominant negative effect seem rather plausible either by modifying the C-terminal anchor needed for the binding of the CSR co-factors, or either 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 four 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 six decades in the investigation of PIDs. In 1963, Rosen and Bougas (1) 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". At that time, serum immunoglobulins were classified in three distinct fractions (7S- γ 2, 7S- γ 1 and 19S- γ 1, where S was the sedimentation constant) on the basis of their electrophoretic and ultracentrifugation properties. In 1964 (i.e. one year after Rosen et al.'s report), 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- γ 1 fraction and no 7S- γ 1 and γ 2 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, since they are present in the 7S- γ 2 fraction only. In contrast, anti-O antibodies (present in the 19S- γ 1 fraction) were conserved in proband II-12. Twelve years later (i.e. 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. In proband II-12, E-rosette forming lymphocytes fell within normal range (79% for a normal range of 52-86%) whereas "Immunoglobulins 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, pathophysiological investigation is complicated when several different types of mutation (e.g. 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).

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Table 1: 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	<i>140</i>	<i>12</i>	<i>420</i>
III-2	M	83	R190X/WT	Recurrent infections Erythema nodosa	1064*	< 5	554
		9			<i>300</i>	< 5	<i>125</i>
III-7	M	54	R190X/WT	Recurrent infections	2027*	< 5	135
		19			<i>740</i>	63	<i>260</i>
III-8	F	64	R190X/WT	Recurrent infections	1241	47	306
		15			<i>740</i>	70	<i>438</i>
III-15	M	60	R190X/WT	Recurrent infections	710	103	587
		65			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

AID: activation-induced cytidine deaminase; WT: wild type. Age and serum immunoglobulin values in italics are taken from Feldman et al.'s 1975 study (ref 3); * receiving immunoglobulin replacement therapy; values in bold: 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.

Figure legends

Figure 1: (A) The pedigree of the family displaying an autosomal dominant 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; * denotes 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.

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Supplementary appendix: methods

WHOLE-EXOME SEQUENCING

After genomic DNA had been extracted from leukocytes, exome libraries were generated with the Twist Bioscience kit (Human RefSeq Exome Kit, 36 Mb, Twist Bioscience). Briefly, genomic DNA (500 ng) was sheared in an ultrasonicator (Covaris). Fifty nanograms of fragmented, purified, double-strand genomic DNA were used to prepare Twist exome libraries according to the manufacturer's instructions (albeit in the absence of initial enzymatic shearing) and with the addition of unique molecular identifiers (barcode adaptors, IDT). Barcoded exome libraries were pooled and sequenced with the NovaSeq6000 system (Illumina), which generated paired-end reads (100 bases + 100 bases). After demultiplexing, sequences were mapped on the human reference genome (NCBI build 37, hg19 version) using BWA, and variants were called with the GATK, SAMtools, and Picard tools. The mean depth of coverage obtained was 100X; 98% of the targeted exonic bases were covered by at least by 15 independent reads, and 97.6% were covered by at least 30 independent reads. Single-nucleotide variants were called with GATK Unified Genotyper, whereas indel calls were made with the GATK IndelGenotyper_v2. All variants with a read coverage $\leq 2x$ and a Phred-scaled quality of ≤ 20 were filtered out. All the variants were annotated and filtered with PolyWeb (our in-house annotation software).

SANGER SEQUENCING

PCR-amplified DNA was sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit and a 3500/3500xL Genetic Analyzer (both from Applied Biosystems), according to the manufacturer's instructions. The forward primer was 5'-CCAACCTCGTTTTGAAGCCA-3' and the reverse primer was 5'-GCCAGACCTGTGTTCTTCT-3'. The forward primer was also used for the sequencing reaction.

Supplementary Table S1. Filtering of annotated variants after whole-exome sequencing with an autosomal dominant genetic model.

	Number of annotated variants
	Patient III-8
Total	141,554
Non-synonymous, essential splicing, frameshift and stop-gained variants, and indels	15,975
MAF* < .01 (1%)	1,534
MAF * < .001 (0.1%)	977
MAF * < .0001 (0.01%)	711
Removal of pipeline artefacts (in-house MAF data; in one other project only)	181
Non-synonymous variants	150
Frameshift variants	21
Essential splicing variants	6
Stop-gained variants	4
CADD score ≥ 20	83
CADD score ≥ 25	42
HGMD	1

MAF: minor allele frequency. * in the Exome Aggregation Consortium database; 1000 Genomes Project; National Heart, Lung and Blood Institute Exome Sequencing Project. CADD: Combined Annotation Dependent Depletion. HGMD: Human Gene Mutation Database.