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Somatic Hypermutation Defects in Two Adult Hyper Immunoglobulin M Patients

Hülya Yilmaz (md.hulya.yilmaz@gmail.com)
Ankara University Faculty of Medicine: Ankara Universitesi Tip Fakultesi https://orcid.org/0000-0001-5664-5893

Sinem Fırtına
Istinye University

Merve Sarıtaş
Istanbul University

Müge Sayitoğlu
Istanbul University

Muhlis Cem Ar
Istanbul University

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Abstract

Hyper immunoglobulin M (HIGM) syndrome is a rare disorder of the immune system with impaired antibody functions. The clinical picture of the patients varies according to the underlying genetic variation. In this study, we identified two novel variants in AID and UNG genes, which are associated with autosomal recessive type HIGM, by targeted next generation sequencing (NGS) panel. A biallelic 11 base pair deletion (c.278_288delATGTGGCCGAC) in the coding sequence of activation-induced cytidine deaminase (AID) gene was identified in a 36-years-old patient. Biallelic two base pair insertion in exon 7 of Uracil nucleoside glycosylase (UNG) gene (c.924_925insGG) was identified in 40-years-old patient. Both variants were confirmed by Sanger sequencing. HIGM, like many of the other primary immunodeficiencies, is a rare and difficult-to-diagnose entity with heterogeneous clinical phenotypes. It should be suspected in patients with a history of early-onset recurrent respiratory infections, enlarged lymph nodes and autoimmune disorders. There might be a delay in diagnosis until adulthood especially in subtle cases or if HIGM is not included in the differential diagnosis due lacking of awareness. In this regard, genetic testing with NGS-based diagnostic panels provide a rapid and reasonable tool for the molecular diagnosis of patients with immunodeficiencies and hence, decrease the time to diagnose and prevent infection-related complications associated with increased morbidity and mortality.

Introduction

Hyper IgM syndrome (HIGM) comprises a rare and heterogeneous group of primary immunodeficiency disorders presenting with recurrent infections and is characterized by impaired B and T-cell functions which result in increased or normal immunoglobulin M (IgM) levels along with low or undetectable immunoglobulin A (IgA) and immunoglobulin G (IgG) levels (1). The clinical picture of HIGM varies depending on the developmental stage of B cell maturation at which the genetic defect occurs and its inheritance pattern (2). Several genetic variations with X-linked *(CD40L, NEMO, BTK, SAP)*, autosomal dominant (*NFKB1A, PIK3CD, PIK3R1, NFKB1)* and autosomal recessive (*CD40, AID, UNG, PMS2, ATM, NBN, MSH etc.*) inheritance have been identified in patients with HIGM.

X-linked HIGM (HIGM1) constitutes 65 - 70% of all HIGM cases with an estimated prevalence of 2 in 1,000,000 males. The underlying molecular defect leads to impaired CD40-CD40L signalling (3). Activation-induced cytidine deaminase (AID=AICDA) deficiency (HIGM2) is the second most common type of HIGM and estimated to affect fewer than 1:1,000,000 individuals. Other types of HIGM are extremely rare. There are only a few reported cases with deficiencies of *CD40* (2, 4, 5, 6) and uracil N-glycosylase (*UNG*) (7, 8), and other class-switch recombination (CSR) defects (9, 10). CD40/CD40 ligand (*CD40L*) deficiencies lead to impaired cellular and humoral immune responses, whereas activation-induced cytidine deaminase (*AID*) and uracil N-glycosylase (*UNG*) deficiencies are usually associated with defective humoral immunity.

UNG gene product encodes an uracil DNA glycosylase which has an important function for eliminating uracil from DNA and initiates base excision repair (BER) pathway. *AID* gene product functions as a RNA

editing deaminase in B-cells involving in somatic hyper mutations (SHM) and class-switch recombination (CSR) of immunoglobulin genes. Germinal centre B-cells require the expression of these two crucial enzymes, *AID* and *UNG*, for DNA editing, and defects in these processes result in HIGM.

Patients with *AID* deficiency generally present with recurrent sinopulmonary and gastrointestinal infections, as well as with lymphoid hyperplasia (11, 12). There is an increased susceptibility to opportunistic and bacterial infections. Furthermore, 20-25 % of patients with HIGM develop autoimmune disorders including immune cytopenia, nephritis, inflammatory bowel disease, hepatitis, and arthritis (13, 14, 15, 16, 17, 18).

Although a clinical picture similar to AID deficiency is expected to occur in patients with *UNG* gene defect, their phenotype has not been clearly identified due to the low number of reported cases. In this study, we report two novel variations in autosomal recessively inherited *AID* and *UNG* genes leading to HIGM phenotype in two adult Turkish patients.

Material And Methods

Patients

One male and one female patients of 36 and 40 years of age, respectively, from consanguineous families were diagnosed with HIGM at the Haematology Outpatient Clinics of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty based on IUIS criteria described elsewhere (19). The clinical and immune phenotypic characteristics of the cases are given in Tables 1 and 2. Patient 1 (P1) had a more severe clinical picture with a history of multiple episodes of pneumonia, and upper respiratory tract infections having started at the age of seven and was diagnosed with HIGM two years later. The other patient (P2) gave a past medical history of recurrent attacks of pneumonia and upper respiratory tract infections leading to deafness at early childhood. She was diagnosed with HIGM at the age of 28 with a delay of about 20 years. Both of the patients had bronchiectatic lungs.

Table 1
Immunophenotypic features of the patients

	P1 with AID deficiency	*P2 with UNG deficiency*	*Normal range*
Serum IgM (mg/dL)	*985*	*283*	*40-230*
Serum IgA (mg/dL)	25	<24	70-400
Serum IgG (mg/dL)	180	<146	700-1600
$CD19^+$ (/mm^3)	154	330	100-500
$CD27^+$(% of $CD19^+$)	38	0.8	10-40
AID, activation-induced cytidine deaminase; UNG, uracil N-glycosylase ; IgM levels before Ig substittion therapy			

Table 2
Features of the patients

	P1	**P2**
Protein affected	AID	UNG
Inheritance	AR	AR
Clinical features		
Bacterial infections	+	+
Opportunistic infections	-	-
Bronchiectasis	+	+
Obstructive pulmonary disease	+	+
Lymphadenopathy	-	-
Autoimmunity	-	Type I DM
Tumors	-	Cervix carcinoma
Immunologic features		
AR, autosomal recessive; AID, activation-induced cytidine deaminase; UNG, uracil N-glycosylase; DM, diabetes mellitus.		

Mutation Analyses

Genomic DNA was isolated from the peripheral blood mononuclear cells of the patients and their family members by using QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Quantity and quality of DNA

were determined by using Nanodrop 2000 (Thermo Fisher Scientific, Germany). A targeted next generation sequencing panel with known 22 genes (*BTK, IGHM, IGLL1, CD79a, CD79β, BLNK, PIK3R1, TCF3, ICOS, CD19, CD81, MS4A1, CD21, TNFRSF13B, LRBA, TNFRSF13C, TWEAK, NFKB2, CD40LG, CD40, AID, UNG, TRNT1, TTC37*) in primary immunodeficiency diseases designed by SmartChip-TE technology (WaferGen, USA) was used for screening. Enrichment of target regions was performed by Seq-Ready™ TE Multisample NanoDispenser according to manufacturers' protocol. Quantification of libraries was done by Qubit dsDNA HS assay kit (Invitrogen, USA) with using Qubit 4 Fluorometer. Samples were sequenced with Illumina Miseq reagent V2 kit on Illumina Miseq (Illumina USA) sequencer. Variant validation in two patients and allelic segregation analysis in their families were performed using Sanger sequencing.

To predict the functional impact of the variant, SIFT, Polyphen and Mutationtaster prediction tools were used. The available public databases including dbSNP, The Human Gene Mutation Database (HGMD), GnomAD, and the Exome Aggregation Consortium (ExAC) were used for frequency data.

Results

In this study, we report two novel variations associated with autosomal recessive type HIGM. Both families had a consanguineous marriage were referred to our centre for further evaluation and treatment. P1 was diagnosed with HIGM, after having detected low IgG, A and high IgM levels while he was investigated for his frequently recurring upper and lower respiratory tract infections at the age of 9. He had neither a family history of primary immunodeficiency nor other related diseases. Intravenous immunoglobulin (IVIG) replacement therapy every 3 weeks was begun based on the history of frequent respiratory infections. Bilateral tonsillar hypertrophy noted during his childhood seemed to have shrinked following regular IVIG replacements. When he was referred to our adult hematology outpatient clinics at the age of 21 his serum Ig levels were as follows: IgG:180 mg/dL, IgA:25 mg/dL, IgM:985 mg/dL (Table 1). At the time of genetic analysis serum levels of immunoglobulins under replacement therapy were 1236 mg/dL, 0 mg/dL, and 1052 mg/dL, for IgG, IgA and IgM, respectively. He had no signs of enlarged lymph nodes, autoimmune diseases, cytopenia, or diarrhea during the follow up (Table 2). Most prominent clinical features included advanced bronchiectasis and frequent respiratory infections, for which he has been receiving prophylactic oral trimethoprim/sulfamethoxazole in addition to IVIG replacement therapy. His pulmonary functions were poor resulting in an oxygen saturation of 84% on room air. He has been suffering from chronic respiratory failure for the past few years of his 27 years follow-up after diagnosis. In P1, we identified a novel 11 bp deletion (c.278_288delATGTGGCCGAC) in the coding sequence of *AID* gene (Ref. ENST00000229335.6). c.278_288delATGTGGCCGAC variation, neither found in ExAC nor in 1000G databases, was suggested as "disease causing and likely pathogenic" by the prediction tools. This frameshift deletion p.H93LfsTer21 is located in exon 3 of AID gene and assumed to be leading to mRNA degradation by nonsense mediated decay mechanisms. Familial segregation analysis confirmed the parents as heterozygous for the AID gene deletion (Figure 1A).

P2 was admitted to the hospital with recurrent upper and lower respiratory tract infections when she was 28 years old. She had undergone tonsillectomy and adenoidectomy and had already hearing loss in her

20s due to chronic otitis media. Her physical examination was unremarkable with regard to organomegaly and lymphadenopathy. She gave a history of herpes virus infection and type 1 diabetes mellitus that was diagnosed when she was 5 years old. Her serum Ig levels were as follows: IgG:146 mg/dL, IgA:<24 mg/dL, IgM:283 mg/dL (Table 1). Upon detection of low IgG, A and normal IgM levels, a diagnosis of hyper IgM was made and IVIG replacement treatment was initiated with every 3 weeks intervals. Subcutaneous immunoglobulin (SCIG) treatment was started when she had recurrent infective episodes in the 3rd week of IVIG replacement and her IgG levels was found to be below 500 mg/dl. Following a infection-free 3 months period under SCIG treatment, IVIG treatment had to be reinitiated upon the request of the patient due to the discomfort which the patient was experiencing during the subcutaneous administration of the immunoglobulin. Her serum levels of IgG, IgA and IgM were 958 mg/dL, 0.1 mg/dL, and 444 mg/dL, respectively at the time of genetic testing. Recently, the patient had to undergo hysterectomy due to cervical cancer. Her 12 years of follow-up after diagnosis has been otherwise unremarkable under immunoglobulin replacement therapy. In P2, we identified two base pair insertion in exon 7 of *UNG* gene (ENST00000242576.2) which caused a novel frameshift mutation (c.924_925insGG, p.Asp309GlyfsTer15) and predicted as VUS (variant unknown significance) classification. This variant is a stop loss variation resulting in abnormal extension of catalytic domain of UNG protein. Genetic screening of the affected families revealed that the clinically unaffected parents did not carry the mutations, suggesting that the identified novel sequence alteration is a *de novo* mutation in our patient (Figure 2A).

Discussion

HIGM is a rare disorder of the immune system with impaired antibody functions. The clinical picture of the patients varies according to the underlying genetic variation. In this study, two novel variations associated with autosomal recessive type HIGM were reported in two different families who had consanguineous marriages that were referred to our centre for further evaluation and treatment. A novel 11 base pair frameshift variant in *AID* gene was identified in a 36-years-old (P1) male patient. AID gene, expressed only in germinal centre B cells, is the major regulator of antibody diversity involving in somatic hypermutation, gene conversion and class switch recombination steps of antibody production by B cells (20, 21). Our variant was located in the exon 3 of 5 in AID gene and bioinformatic analysis showed that this variant leads to absence of AID protein. AID is required for both SHM and CSR and abnormal function or absence of AID protein has been associated with HIGM phenotype.

Biallelic two base pair insertion in *UNG* gene associated with HIGM has been reported for the first time in this study. This variation, located in the catalytic domain of *UNG* gene, leads to abnormal protein sequence. The catalytic domain of UNG protein is an evolutionarily conserved domain of the protein and has a critical role in removing uracil base pairs created by AID. Abnormal and extended domain structure might have blocked the activity of catalytic domain and caused the HIGM phenotype in this patient.

Symptoms started in early childhood in both P1 and P2 while their diagnosis was delayed for 2 and 20 years, respectively. Cases in the literature have been reported to usually receive their diagnosis in the early

years of life. In the study of Minegishi et al., all patients with AID mutations had recurrent attacks of otitis and sinusitis within the first 2 years of life. However, most of the cases were not diagnosed with immunodeficiency until their second or third decades. In patients with delayed diagnosis bronchiectasis seemed to be the most prominent clinical entity which usually developed after the age of 35 (22). Unlike the literature, the symptoms of P1 started at a relatively late age. Early diagnosis and timely onset of immunoglobulin replacement therapy (within the first decade of life), however, could not prevent the development of bronchiectasis and progress of respiratory failure in that patient.

Both of our patients had a long-lasting history of recurrent infectious episodes, mainly involving upper and lower respiratory tract. Chronic otitis media resulting in permanent hearing loss had occurred in the patient with *UNG* variation (HIGM5) before HIGM diagnosis was made. Both patients had radiologic clinical features of established bronchiectasis having resulted from recurrent pneumonias and delayed diagnosis. In line with the limited data in the current literature, the clinical picture of the patient with *UNG* variation (HIGM5) was milder than that with *AID* deficiency (HIGM2) (23). Almost all individuals with *AID* or *UNG* deficiency have been reported to develop lymphoid hyperplasia (22, 24, 25), which was found to resolve in 75% of the cases following IVIG replacement therapy (25). None of the two patients reported here showed lymphoid hyperplasia at the time of their genetic diagnosis, which they received in their thirties. This may be explained by the fact that our patients had been on long-standing regular IVIG replacement therapy when the underlying genetic variations were identified.

Contrary to current data (25), no autoimmune disease was detected in the patient with *AID* deficiency, while P2 was diagnosed with early onset type I diabetes mellitus. There is limited data whether HIGM is associated with increased risk for developing cancer. We reported here a case who developed cervix cancer while on immunoglobulin replacement therapy. Similar to the literature, peripheral blood B cell counts were within normal limits in two cases (7, 11, 25). However, the percentage of CD27+ IgD- memory B cells was low in the patient with UNG deficiency, while normal in the patient with AID deficiency.

HIGM, like many of the other primary immunodeficiencies, is a rare and difficult-to-diagnose entity with heterogeneous clinical phenotypes. It should be suspected in patients with a history of early onset recurrent respiratory infections, enlarged lymph nodes and autoimmune disorders. There might be a delay in diagnosis until adulthood especially in subtle cases or if HIGM is not included in the differential diagnosis due lacking of awareness. In this regard, genetic testing with NGS-based diagnostic panels provide a rapid and reasonable tool for the molecular diagnosis of patients with immunodeficiencies and hence, decrease the time to diagnosis and prevent infection related complications associated with increased morbidity and mortality.

Declarations

Ethical Approval: All procedures performed in studies involving human participants followed the institutional research committee's ethical standards and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to Participate: The patients consent to the use of their clinical information to the redaction of this study.

Consent to Publish: The patients formally consented to the use of their clinical information to be published for this study.

Authors Contributions: Yilmaz H., Fırtına S., Ar M.C., Sayitoğlu M., and Sarıtaş M. designed research; Ar M.C., Yilmaz H., Sayitoğlu M., Fırtına S., and Sarıtaş M. conducted review and editing; Ar M.C.,and Yilmaz H., provided funding acquisition, project administration, and resources; Ar M.C., Yilmaz H., Sayitoğlu M., and Fırtına S., wrote the paper.

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Competing Interests: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
Availability of data and materials: Data available on request from the authors

Code availability: Not applicable

References

1-Schneider LC. X-linked hyper IgM syndrome. Clinical reviews in allergy & immunology. 2000;19(2): 205-215.

2-Lougaris V, Badolato R, Ferrari S, Plebani A. Hyper immunoglobulin M syndrome due to CD40 deficiency: clinical, molecular, and immunological features. Immunological reviews. 2005;203(1): 48-66.

3-Liu X, Zhou K, Yu D, Cai X, Hua Y, Zhou H, Wang C. A delayed diagnosis of X-linked hyper IgM syndrome complicated with toxoplasmic encephalitis in a child: A case report and literature review. Medicine. 2017;96(49).

4-Kutukculer N, Moratto D, Aydinok Y, Lougaris V, Aksoylar S, Plebani A, Notarangelo LD. Disseminated cryptosporidium infection in an infant with hyper-IgM syndrome caused by CD40 deficiency. The Journal of paediatrics. 2003;142(2):194-196.

5-Mazzolari E, Lanzi G, Forino C, Lanfranchi A, Aksu G, Ozturk C, Kutukculer N. First report of successful stem cell transplantation in a child with CD40 deficiency. Bone marrow transplantation. 2007;40(3):279.

6-Lanzi G, Ferrari S, Vihinen M, Caraffi S, Kutukculer N, Schiaffonati L, Giliani S. Different molecular behavior of CD40 mutants causing hyper-IgM syndrome. Blood. 2010;116(26): 5867-5874.

7-Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, Ochs HD. Human uracil–DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nature

immunology. 2003;4(10):1023.

8- Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD. Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. Blood. 2000; 105(5):1881-1890.

9- Etzioni A, Ochs HD. The hyper IgM syndrome—an evolving story. Pediatric research. 2000; 56(4):519.

10-Imai K, Catalan N, Plebani A, Maródi L, Sanal Ö, Kumaki S, Hermine O. Hyper-IgM syndrome type 4 with a B lymphocyte–intrinsic selective deficiency in Ig class-switch recombination. The Journal of clinical investigation. 2003;112(1):136-142.

11-Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, Tezcan I, Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 2000;102(5):565-575

12- Trotta L, Hautala T, Hämäläinen S, Syrjänen J, Viskari H, Almusa H, Seppänen M, Enrichment of rare variants in population isolates: single AICDA mutation responsible for hyper-IgM syndrome type 2 in Finland. European Journal of Human Genetics. 2016;1473.

13-Erdős M, Garami M, Rákóczi É, Zalatnai A, Steinbach D, Baumann U, Maródi L, Neuroendocrine carcinoma associated with X-linked hyper-immunoglobulin M syndrome: report of four cases and review of the literature. Clinical Immunology. 2008;129(3):455-461.

14-Winkelstein JA, Marino MC, Ochs H, Fuleihan R, Scholl PR, Geha R, Conley ME, The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. Medicine. 2003; 82(6):373-384.

15-Hervé M, Isnardi I, Ng YS, Bussel JB, Ochs HD, Cunningham-Rundles C, & Meffre E, et al. CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. Journal of Experimental Medicine. 2007;204(7):1583-1593.

16-Lacroix-Desmazes S, Resnick I, Stahl D, Mouthon L, Espanol T, Levy J, Ochs H, Defective self-reactive antibody repertoire of serum IgM in patients with hyper-IgM syndrome. The Journal of Immunology. 1999;162(9):5601-5608.

17-Moazzami B, Yazdani R, Azizi G, Kiaei F, Tafakori M, Modaresi M, Aghamohammadi A. Respiratory Complications in Patients with Hyper IgM Syndrome. *Journal of clinical immunology*. 2019;*39*(6):557-568.

18-Fazel A, Kashef S, Aleyasin S, Harsini S, Karamizadeh Z, Zoghi S, Rezaei N. Novel AICDA mutation in a case of autosomal recessive hyper-IgM syndrome, growth hormone deficiency and autoimmunity. Allergologia et immunopathologia. 2017;45(1):82-86.

19-Picard C, Gaspar HB, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Holland SM. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of

immunity. *Journal of clinical immunology*. 2018;*38*(1): 96-128.

20-Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson N O, Honjo T. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *Journal of Biological Chemistry*. 1999;*274*(26):18470-18476.

21-Longacre A, & Storb U. A novel cytidine deaminase affects antibody diversity. *Cell*. 2000; *102*(5):541-544.

22-Minegishi Y, Lavoie A, Cunningham-Rundles C, Bédard PM, Hébert J, Côté L, Durandy A. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. Clinical Immunology. 2000;97(3):203-210.

23-Imai K, Catalan N, Plebani A, Maródi L, Sanal Ö, Kumaki S, Hermine O. Hyper-IgM syndrome type 4 with a B lymphocyte–intrinsic selective deficiency in Ig class-switch recombination. The Journal of clinical investigation. 2003;112(1):136-142.

24-Aghamohammadi A, Parvaneh N, Rezaei N, Moazzami K, Kashef S, Abolhassani H, ... & Hammarström L, et al. Clinical and laboratory findings in hyper-IgM syndrome with novel CD40L and AICDA mutations. Journal of clinical immunology. 2009;29(6): 769.

25-Quartier P, Bustamante J, Sanal O, Plebani A, Debré M, Deville A, Horneff G. Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to activation-induced cytidine deaminase deficiency. Clinical immunology. 2004;110(1): 22-29.

Figures

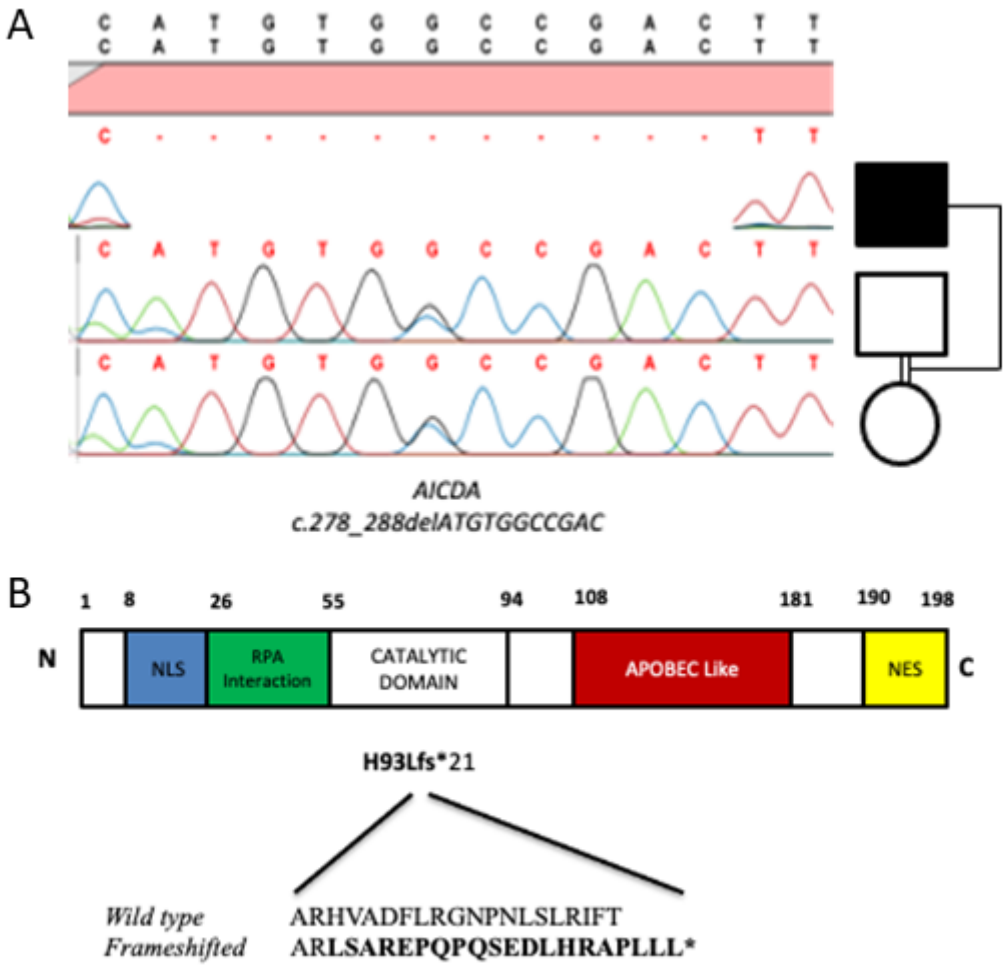

Figure 1

A) Pedigree and allelic segregation of AICDA variant . Affected index case is indicated by filled symbol, and unaffected parents are indicated by an open symbol. Sanger sequencing chromatograms identifiying homozygous c.278_288delATGTGGCCGAC mutation in the proband and his parents heterozygous. B) Graphical view of AICDA protein; NLS; nuclear localization signal; NES; nuclear export signal, RPA; replication protein A, interaction binding site, catalytic domain and APOBEC-like domain.

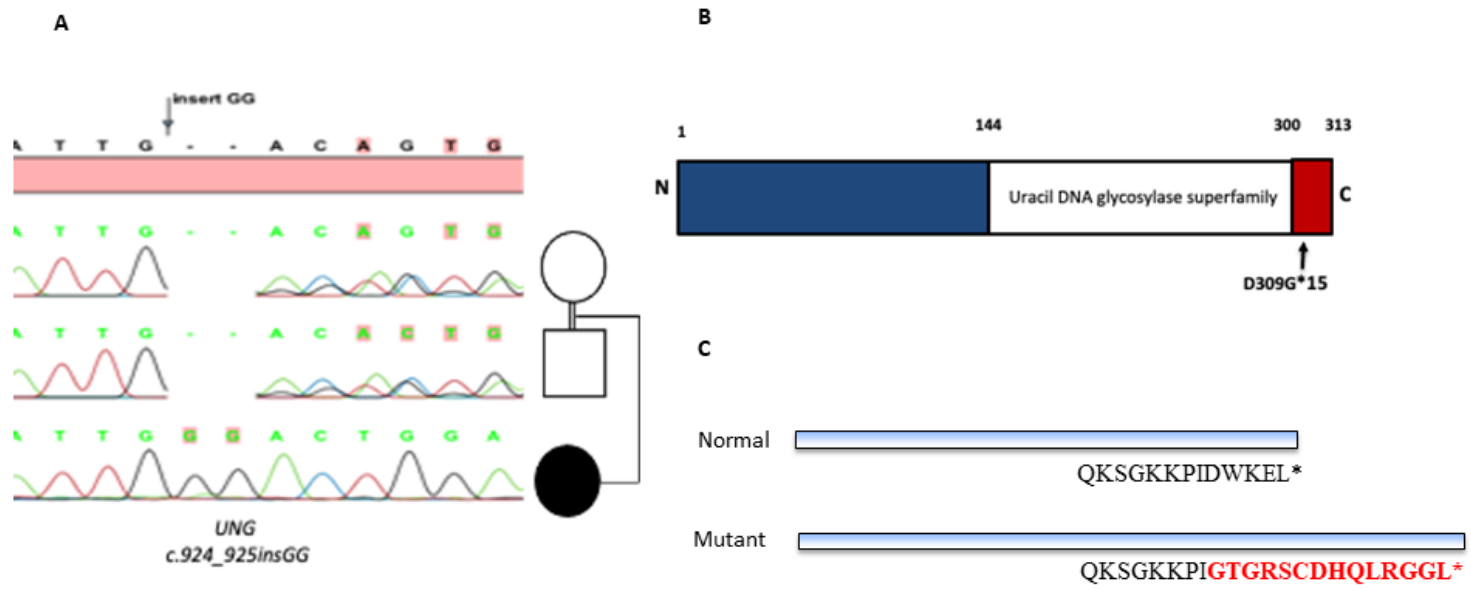

Figure 2

A) Pedigree and allelic segregation of UNG variant. Affected index case is indicated by filled symbol, and unaffected parents are indicated by an open symbol. Sanger sequencing chromatograms identifiying homozygous c.924_925insGG mutation in the proband and his parents heterozygous. B) Graphical view of GG insertion on UNG protein. C) New amino acid sequence after GG insertion.