New heritable ATRX mutation identified by whole exome sequencing and review

Zahra Shahbazi
Pasteur Institute of Iran
Golale Rostami
Pasteur Institute of Iran
Mohammad Hamid (hamid143@yahoo.com)
Pasteur Institute of Iran https://orcid.org/0000-0002-4625-0713

Research

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Abstract

Background ATRX gene encodes a member of the SWI2/SNF2 family of proteins that may act as a transcriptional factor and plays a significant role in the epigenetic regulation of gene expression. The mutations in the ATRX gene have been shown to cause two types of disorders: inherited mutations lead to alpha thalassemia X-linked mental retardation (ATR-X) syndrome and acquired somatic mutations cause alpha thalassemia myelodysplastic syndrome (ATMDS). Here we report a case of ATRX gene mutation without completely features of ATR-X or ATMDS syndromes. Moreover we review previous reports of ATRX gene mutations in both ATR-X syndrome and ATMDS. Methods Patient was a 29-year-old male of Arab ancestry with normocytic anemia, low mean corpuscular hemoglobin (MCH), haematocrit percent and red blood cell (RBC) count. He had no sign of mental retardation and facial dysmorphism. The whole exome sequencing method was used to find the disease-causing variants. Moreover we searched HGMD, Ensembl, OMIM and COSMIC databanks for all mutation reported in ATRX gene so far, and Pubmed, WOS, Science direct and springer link for articles that reported ATRX gene mutations. Results We identified a hemizygous missense ATRX gene mutation (ATRX, c.2388A>C, p. K796N) as a new disease-causing variant in the patient. Conclusion According to previous findings, inherited ATRX mutations are associated with a broad spectrum of clinical presentations. Therefore a person with a mild α-thalassemia phenotype may also have mutation in ATRX gene. Accordingly, it is critical for geneticist and physicians to increase awareness in molecular diagnosis of α-thalassemia patients.

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematopoietic stem cell disorders characterized by peripheral blood cytopenias and microcytic red blood cell (RBC) indices in the absence of iron deficiency as a result of ineffectual erythropoiesis [1, 2]. In contrast to the generic macrocytic anemia in MDS [3], the anemia of ATMDS (α-thalassemia myelodysplastic syndrome; OMIM catalog #300448) is often specify as microcytic and hypochromic [2]. About 25% of patients with alpha thalassemia myelodysplastic syndrome (ATMDS) will progress to acute myelogenous leukemia (AML), the risk of this progression appears to be similar to that of the MDS patient population [4]. A high ratio of MDS patients have acquired somatic mutations in the chromatin remodeling factor ATRX [1]. The ATRX gene encodes a member of the SWI2/SNF2(Switching defective/Sucrose nonfermenting) family of proteins [5] that may act as a transcriptional factor and plays a significant role in the epigenetic regulation of gene expression [6]. The unusual α-thalassemia and microcytosis in the ground of MDS is due to a decreased synthesis of α-globin related to a dramatic down regulation of α-globin gene expression by inactivating mutation in the ATRX gene [7]. On the other hand, inherited mutations in the ATRX gene, have been shown to cause an alpha thalassemia X-linked mental retardation (ATR-X) syndrome, (α thalassemia with retardation encoded on the X chromosome; OMIM catalog #301040). In this disease mild α- thalassemia have been found in boys with severe mental retardation, facial dysmorphism, and urogenital abnormalities [8]. Here we report a new hereditary missense mutation in

exon 9 of the ATRX gene in a young MDS patient with mild α-thalassemia. We also reviewed reports of ATRX gene mutations in databases and previous accessible publications.

Materials And Methods

Sample collection and DNA extraction

Relevant informed consent was obtained from the patient. Two milliliters of peripheral blood were collected and DNA was extracted from patient's peripheral blood leukocytes by salting out method. High quality DNA samples, with the OD260/280 ratio of 1.8–2.0, at the concentration of 100 ng/µl were used for further analysis. This study was also reviewed and approved by the Ethics Committee of Pasteur Institute of Iran.

Molecular Investigation

In order to investigate causing gene, exome sequencing was performed using Illumina HiSeq PE150 (Novagen, Beijing, China) by Agilent SureSelect Human All Exon V6 Kit with an average coverage of 100x-fold and 150 bp read-length.

Sanger Sequencing

In order to approve founded variation, we performed Polymerase chain reaction (PCR) and Sanger sequencing method on the patient's DNA sample and his relatives (The primers sequences are available in Table 1). PCR amplification was carried out in a final volume of 30 µl PCR master mix (Ampliqon) containing 100–200 ng genomic DNA, and 10 pmol of each primer.

Table 1
PCR primers used for the PCR-sequencing of exon 9 of ATRX gene

Primer name	Primer sequence 5'>3'
ATRX-F	GATGAGTCACAGTTCTTCTTCAG
ATRX-R	TCTTTTTTTGGTGGTTCTGGC

Evaluation of PCR products was done by electrophoresis on 1% agarose gel. Finally, the PCR products were sequenced at Macrogene Company (South Korea). The sequencing results were analyzed by Chromas (V2.6) and CLC workbench(V5.5 CLC bio) software and NM_000489.3 was used as reference sequence for ATRX (Fig. 1). The pathogenicity of the variant was re-evaluated using the updated guideline for interpretation of molecular sequencing by the American College of Medical Genetics and

Genomics (ACMG) considering the allele frequency in the population database, immunological/functional data, familial segregation and parental genotype (https://www.acmg.net/).

Data Collection

Information on previous reports of pathogenic variants in the ATRX gene was obtained by examining databases such as Web of science(www.webofknowledge.com), Science direct(www.sciencedirect.com), Springer link(https://link.springer.com/) and Pubmed(www.ncbi.nlm.nih.gov › pubmed). We also used bioinformatics databases like NCBI(www.ncbi.nlm.nih.gov), Ensembl(www.ensembl.org), HGMD (www.hgmd.cf.ac.uk), COSMIC(https://cancer.sanger.ac.uk/cosmic), UniProt(www.uniprot.org) and OMIM(https://www.omim.org/) in writing this review article.

Results

A 29-year-old male of Iraq-Arab ancestry was found to have normocytic anemia (low hemoglobin (Hgb) (5.6 g/dL), low mean corpuscular hemoglobin (MCH) (26.8 pg) and haematocrit percent (18.3%). He was normal for some quantities including mean corpuscular volume (MCV) (96.4fL), white blood cell (WBC) count ($4.3 \times 10^3/mm^3$), platelet count (PLT) (199×10^3 $/mm^{3)}$ and lymphocyte, monocyte, eosinophil, basophil percent (36.9%, 4.5%, 0.8%, 0.2 respectively). Interestingly his RBC count was low $2.34 \times 10^6/mm^3$, as one of the hallmarks of MDS. He had no family history of thalassemia and no evidence of iron deficiency or other hemoglobinopathy. On physical examination, there was no sign of mental retardation, facial dysmorphism, urogenital abnormalities and palpable lymphadenopathy or splenomegaly.

Whole Exome Sequencing analysis identified A > C transition at c.2388 position in codon 796 of exon 9 of the ATRX gene leading to the putative missense p.K796N mutation. To the best of our knowledge, the mutation has never been described before in MDS in the Single Nucleotide Polymorphism Database (https://www.ncbi.nlm.nih.gov), Ensemble (http://m.ensemble.org) and HGMD (http://www.hgmd.cf.ac.uk). This variation is likely pathogenic variant based on ACMG. The mutation is predicted to be damaging by PredictSNP, Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping-2 (PolyPhen-2) softwares (Table 2). Verification Sanger sequencing results showed hemizygous alteration in patient, heterozygous situation for his mother (a wild type allele and an allele with a single mutation p.K796N) and his father was hemizygous for wild type allele (Fig. 1).

Table 2
Interpretation scores for the pathogenicity of p.K796N variant in ATRX gene

Software	PredictSNP	Polyphen-1	Polyphen-2	SIFT
Score	51% Deleterious	59% Deleterious	43% Deleterious	53% Deleterious

Literature review for ATR-X syndrome showed Non-homogeneous distribution of mutations in different domains of ATRX gene. According to the review of this study, most of the previously reported mutations which lead to clinical consequences of ATR-X syndrome were occur in conserved domain of the ATRX gene including ADD, ATPase and C-terminal domains (Fig. 2). Moreover there was more variety of mutation types reported in ATRX gene for ATR-X syndrome (Fig. 3). About ATMDS, there was a homogeneous distribution of mutations in different domains of ATRX gene. In other words, there was no significant difference in the mutation distribution in different domains of ATRX gene in ATMDS reported cases. Moreover there were multiple reports of mutations in spacer region between EZH2 and DAXX domains in some ATMDS patients, the region that we found our patient's mutation, while in the ATR-X syndrome no mutation was reported in this region. Also there was less variety of mutation types reported in ATRX gene for ATMDS (Fig. 3).

Anyway in both diseases in the terms of variant types, missense variant was the most common, afterwards splicing, small deletion and nonsense variants were commonest types respectively (Fig. 3). According to the review of this study, most of mutations which lead to clinical consequences occurred in these conserved domains.

Discussion

MDS is a heterogeneous group of clonal hematopoietic stem cell disorders characterized by peripheral blood microcytic and cytopenias RBC indices as a result of ineffectual erythropoiesis in the absence of iron deficiency [1, 2]. Here we report a case of MDS without usual presentation of ATR-X syndrome or ATMDS. The patient was a male case with ATRX hereditary mutation manifested mild α-thalassemia and no sign of mental retardation, facial dysmorphism, and urogenital abnormalities as the signs of ATR-X syndrome. His mother was carrier and has a normal appearance and intellect, and no signs of α-thalassemia. Our patient has shown neither the severe hematologic signs expected in ATMDS nor the widespread manifestations of the ATR-X syndrome, this may be due to the fact that the mutation in this patient occurs outside of highly conserved domains of ATRX gene. Another possibility is that this mutation is hypomorphic or modifier genetic and epigenetic factors contributing to modulating the effects of this mutation.

Literature Review

Somatic ATRX mutations: ATMDS

Although aberrant templates of hemoglobin synthesis are almost always inherited, sporadically individuals with previously normal hematology may develop abnormal hemoglobin synthesis as an acquired abnormality [9]. There are a number of reports describing α thalassemia as newly acquired traits in the background of hematologic malignancy [10]. The first cases were described in 1960 [11]. Similar patients were early found that have reduced α/β globin chain synthesis ratios [12, 13]. This syndrome

was characterized by a marked hypochromic and microcytic anemia with the presence of HbH (β4 tetramers) and named α-thalassemia myelodysplastic syndrome or ATMDS [14]. In the light of these findings, an acquired α thalassemia patients registry (www.imm.ox.ac.uk/groups/mrc_molhaem/home_pages/Higgs/index.html) was established in the early 1980s [15]. ATMDS predominantly occurs in male (male-to-female ratio greater than 6:1) within the 7th decade of life. Although the term ATMDS is correct in most cases, acquired α thalassemia is not uniquely limited to MDS and has been reported in the context of other myeloid malignancy [14]. Acquired α thalassemia is not limited to the geographical regions in which the inherited forms of α thalassemia are common (e.g., the Mediterranean basin, Southeast Asia, Africa, and Melanesia). Most of ATMDS patients have been of Northern European descent and few Mediterranean and Asian patient have been reported to date. It is feasible that this distribution represents ascertainment bias. If microcytic, hypochromic red cell indices or other signs of thalassemia were apperceived in a patient with hematologic malignancy originating from an area in which inherited forms of thalassemia are common, it is likely that these findings would be related to a previously unrecognized inherited thalassemia, and such a hypothesis would usually be correct. However, rare cases of acquired α thalassemia in patients with these areas of the world origin may have been missed. On the other hand, thalassemia red cell indices in an individual with hematologic malignancy who originate from outside of the malaria belt (e.g., from Northern Europe) are unanticipated and should operate further evaluations [4, 14, 15].

At least 2 molecular mechanisms for acquired α-thalassemia are presented today: cis-acting defects including acquired deletion of the α-globin gene cluster limited to the neoplastic clone and, more commonly, inactivating somatic mutations of the trans-acting regulator of globin gene expression ATRX, which cause significant down regulation of α-globin gene expression [4, 10].

A major clue to identifying the trans-acting molecular defect resulting ATMDS came from a cDNA microarray study of RNA extracted from peripheral blood neutrophil from a man newly diagnosed with ATMDS, and comparing the results with pooled granulocyte RNA from several normal individuals. The findings demonstrated that the ATRX gene was one of the genes with the lowest expression in the ATMDS patient compared to control group, a result that was confirmed by RT-PCR. An ATRX point mutation (G > A) in the canonical splice donor site of intron one was then found in this ATMDS male patient. This variant likely resulted in nonsense-mediated decay (NMD) of the ATRX transcript. It was perhaps fortunate that the first ATMDS patient analyzed in this way had a null mutation in ATRX, as many of the other point mutations described so far would not be expected to affect the level of ATRX mRNA [5].

The patient’s ATRX somatic mutation in ATMDS often restricted to myeloid cell line, and other cells including buccal cells and a lymphoblastoid cell line may have normal genotypes [4]. It is now demonstrated that most patients with ATMDS have an acquired somatic splicing abnormality or point mutation involving ATRX [10].

Atr-x Syndrome

The rare association of α-thalassemia and mental retardation (MR) was presented over 36 years ago in northern European origin patients by Weatherall and colleagues [16]. It is now distinct that this association may occur as a result of two quite distinguished mechanisms; one resulting from large deletions in telomeric region of chromosome 16 (α thalassemia with retardation on chromosome 16, ATR-16 syndrome OMIM catalog #141750); the other, caused by mutations in ATRX gene (ATR-X syndrome) [17]. The main clinical features of ATR-X syndrome include severe psychomotor delay, abnormal facial appearance, microcephaly, urogenital abnormalities and a variable degree of α thalassemia, a condition caused by deficient α-globin expression [18, 19]. Patients are characterized during early childhood [18]. ATR-X syndrome is predominant in males, and almost all female carriers have a normal appearance and intellectual ability, although approximately one in four carriers has subtle signs of a-thalassemia, which show an skewed pattern of X-inactivation that lead to the expression of the mutant allele [20, 21]. The molecular cause of ATR-X is mainly from point mutations in the ATRX gene [14]. More over in some cases the disease appears to raise de novo. In fact, a number of families have been reported in which some or all of the affected members with mutations of ATRX, and the characteristic manifestations described previously, have no signs of α-thalassemia [5, 20, 22, 23]. In ATR-X patients which have abnormal development of the genitalia (e.g., male pseudohermaphroditism), testis characteristics occurs but the cellular components maturation is failed. It is possible that many of the genes whose regulation is perturbed by ATRX mutations have critical rule in terminal differentiation. It is noteworthy that ATR-X patients do not have an increased incidence of cancer [19, 24].

ATRX gene and protein

The ATRX gene is located on the Xq13.3; more precisely it is located from base pair 77,504,878 to base pair 77,786,269 on chromosome X and contains 35 exons (Reference sequence NG_008838.3; https://www.ncbi.nlm.nih.gov/). ATRX is a relatively large protein consists of 2492 amino acids (283 kDa). It is a chromatin- associated protein with ATRX-DNMT3-DNMT3L (ADD) domain (encoded by exons 8–10) containing GATA-like zinc finger at the N-terminus, and a long C-terminal that pack together to form a single globular domain containing a helicase/ATP domain (encoded by exons 18–31) [25]. The previous domain formed by seven conserved “helicase” motifs found in DNA-stimulated ATPases and DNA helicases of the SNF2/SWI2 (Switching defective/Sucrose nonfermenting) protein family. The SWI/SNF complexes act as global gene regulators, changing the chromatin structure and altering the accessibility of transcriptin factor to DNA in a subset of specific genes [26]. The ADD and helicase/ATPase are extremely conserved domains. Studies over the recent ~ 15 years have discovered numerous roles for ATRX, some of which may appear to be inconsistent. For example, the fact that ATRX mutations consequence is the loss of α-globin gene expression and that ATRX physically binds to the α-globin gene cluster, proposes a transcriptionally activating role for ATRX [6]. However, its localization to telomeres, pericentric heterochromatin and the inactive X chromosome signify a role in the establishment and/or maintenance of transcriptionally silent chromatin. These proteins frequently exist in

multicomponent complexes that remodel chromatin and thereby influence multiple epigenetic nuclear processes (e.g., DNA replication, DNA repair, DNA methylation, recombination, transcription). The recent study on endogenous ATRX has confirmed that it is an ATPase and like other members of this family has translocase activity, therefore can move along double stranded DNA in the presence of ATP in vitro. ATRX is widely expressed throughout development [15, 27]. Many evidence support the critical role of ATRX protein in the cerebral development and the survival of neurons in developing cortex and hippocampus [28]. The protein partners that are in interaction with ATRX, including HP1α, EZH2, MeCP2 and macroH2A, also implicate its role in heterochromatin structure and function [29]. The other names of ATRX gene are RAD54 (alpha thalassemia/mental retardation syndrome X-linked), JMS (Juberg-Marsidi syndrome), MRX52 (mental retardation, X-linked 52) (https://ghr.nlm.nih.gov/gene/ATRX).

ATRX mutations

The CpG methylation at heterochromatic loci is disturbed in patients with inherited mutations in ATRX gene. The reality that ATRX mutations affect α but apparently not β globin expression may be informative. These two gene clusters are embedded in exactly different chromosomal environments, which supports the vision that ATRX influences gene expression via one or more of the epigenetic aspects that distinguish these different regions[15]. Disturbance of ATRX function in model organisms leads to defective sister chromatid adherence and congression, telomere dysfunction due to ALT-pathway (Alternative Lengthening of Telomeres) perturbation, and aberrant patterns of methylation [14].

Various types of mutations including missense, nonsense, in/del, duplication and splice site have now been reported in ATRX gene in related to ATR-X syndrome (Fig. 2,3). It has been hypothesized that in ATR-X syndrome, both copies of gene are inactivated in the patient, one by mutation and the other by X inactivation [30]. Considering the monogenic nature of ATR-X syndrome, it is important to recognize the type of mutations involved and their clinical severity. For example, the ADD domain amino acids mutations lead to more severe psychomotor phenotypes than the helicase domain mutations. Interestingly, a nonsense mutation at residue 37 of ATRX is associated with a milder phenotype than the phenotype created by missense mutations in the ADD and helicase domains [17]. The nonsense mutation at residue 37 is spliced out of a proper subset of transcripts, which partially compensate ATRX protein function. Interestingly, mutations in genes that encode proteins cooperate with ATRX, such as DAXX, have not been identified in patients with ATR-X syndrome [30].

The hematologic phenotype in patients with ATMDS is in general, more severe than that seen in boys with congenital ATR-X syndrome. For example, α/β globin synthesis ratios are usually very low (< 0.2 in 52% of patients) and the amounts of HbH are large (median 30%) in a patients with ATMDS, compared to boys with inherited ATR-X syndrome, who commonly have only mildly reduction in α/β synthesis ratios with low and sometimes undetectable amounts of HbH [15]. The suggestion is that the different in hematologic effects of ATRX mutations in erythroid cells depends on mutation occurrence time during development or, more likely, on the cellular context in which the mutation occurs [4, 15].

The ATRX gene mutations (http://www.hgmd.cf.ac.uk/) are clustered in highly conserved domains including ADD and helicase (Fig. 2), suggesting functionally importance of these domains [4, 10, 31, 32]. It appears that none of the mutations leads to a true null allele but rather are hypomorphics caused by protein destabilizing effects of the mutations [19, 33, 34]. Also the patients with the same mutations may have very different degrees of α-thalassemia, suggesting that the effect of the ATRX protein on α-globin expression may be modified by other genetic and epigenetic factors. This is most clearly illustrated by comparing the hematology phenotype of cases with identical mutations[6]

The ATRX gene mutations were first discovered in oncology in pancreatic neuroendocrine tumors (PanNETs)[35], where ATRX and DAXX mutations are associated with a better prognosis. Further studies have identified mutations in SWI/SNF family proteins in nearly 19.6% of human cancers, including gliomas, demonstrating that SWI/SNF is the most frequently mutated chromatin-regulatory complex with a potential “driver” role in human cancer [36].

Here we report a male case with ATRX hereditary mutation manifested mild α-thalassemia and no sign of mental retardation, facial dysmorphism, and urogenital abnormalities. His mother was carrier and has a normal appearance and intellect, and no signs of α-thalassemia. Our patient has shown neither the severe hematologic signs expected in ATMDS nor the widespread manifestations of the ATR-X syndrome, this may be due to the fact that the mutation in this patient occurs outside of highly conserved domains including ADD and helicase. The other possibility is that this mutation is hypomorphic or modifier genetic and epigenetic factors contributing to modulating the effects of this mutation. Moreover the symptoms of the disease did not occur at birth, as expected in the ATR-X syndrome, and he showed disease symptoms earlier than the age expected foe ATMDS disease.

Conclusion

According to previous report, inherited ATRX mutations leas to alterations in expression of the α-globin genes and are associated with a broad spectrum of clinical presentations, from an asymptomatic α-thalassemia trait that is only of reproductive consequences, to the sever α-thalassemia mental retardation, facial dysmorphism, and urogenital abnormalities. Therefore a person with a mild α-thalassemia phenotype may also have mutation in ATRX gene. As conclusion, it is critical for geneticist and physicians to increase awareness in molecular diagnosis of α-thalassemia patients.

Abbreviations

AML
acute myeloid leukemia
ATMDS
Alpha-Thalassemia Myelodysplastic syndromes
ATRX gene
Alpha-Thalassemia/Mental Retardation Syndrome gene

ATR-X syndrome
Alpha-Thalassemia/Mental Retardation Syndrome
COSMIC
Catalogue Of Somatic Mutations In Cancer
DNMT
DNA methyltransferase
HbH
Hemoglobin H
Hgb
Hemoglobin
HGMD
Human Gene Mutation Database
MCH
Mean Corpuscle Hemoglobin
MCV
Mean corpuscular volume
MDS
Myelodysplastic syndromes
MR
Mental Retardation
OMIM
Online Mendelian Inheritance in Man
PCR
Polymerase Chain Reaction
PLT
Platelets
RBC
Red Blood Cells
SWI/SNF
SWItch/Sucrose Non-Fermentable
WES
Whole Exome sequencing
WBC
White Blood Cells

Declarations

- Ethics approval and consent to participate. This study was also reviewed and approved by the Ethics Committee of Pasteur Institute of Iran.
- Consent for publication: Not applicable

- Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
- Competing interests: Not applicable
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- Authors' contributions: Zahra Shahbazi: Analyzed the data, read past articles, and wrote the manuscript
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Figures

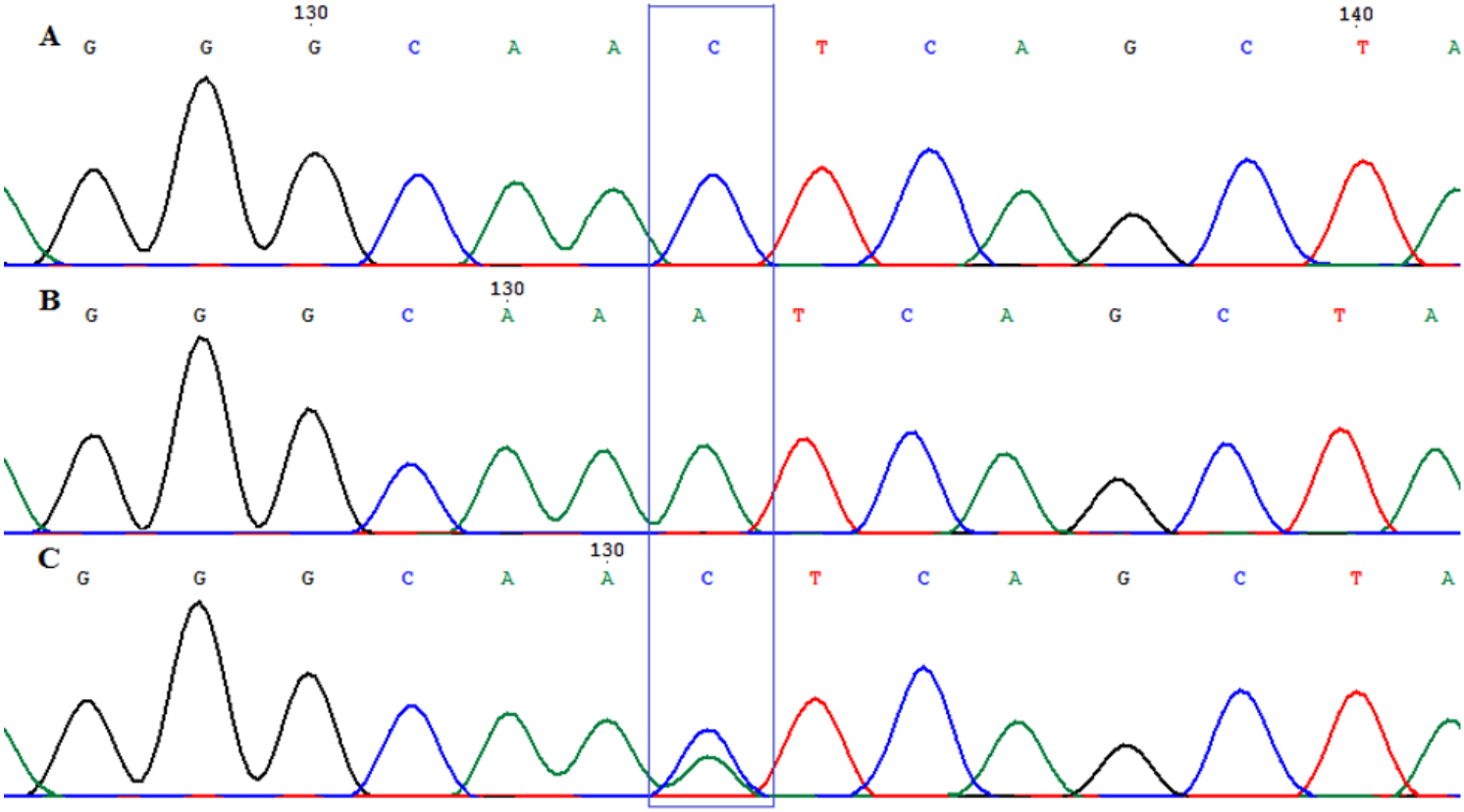

Figure 1

ATRX gene Sanger sequencing result for the proband shows hemizygous genotype for mutant C allele (a), patient's father is normal (b) and his mother is heterozygous (c).

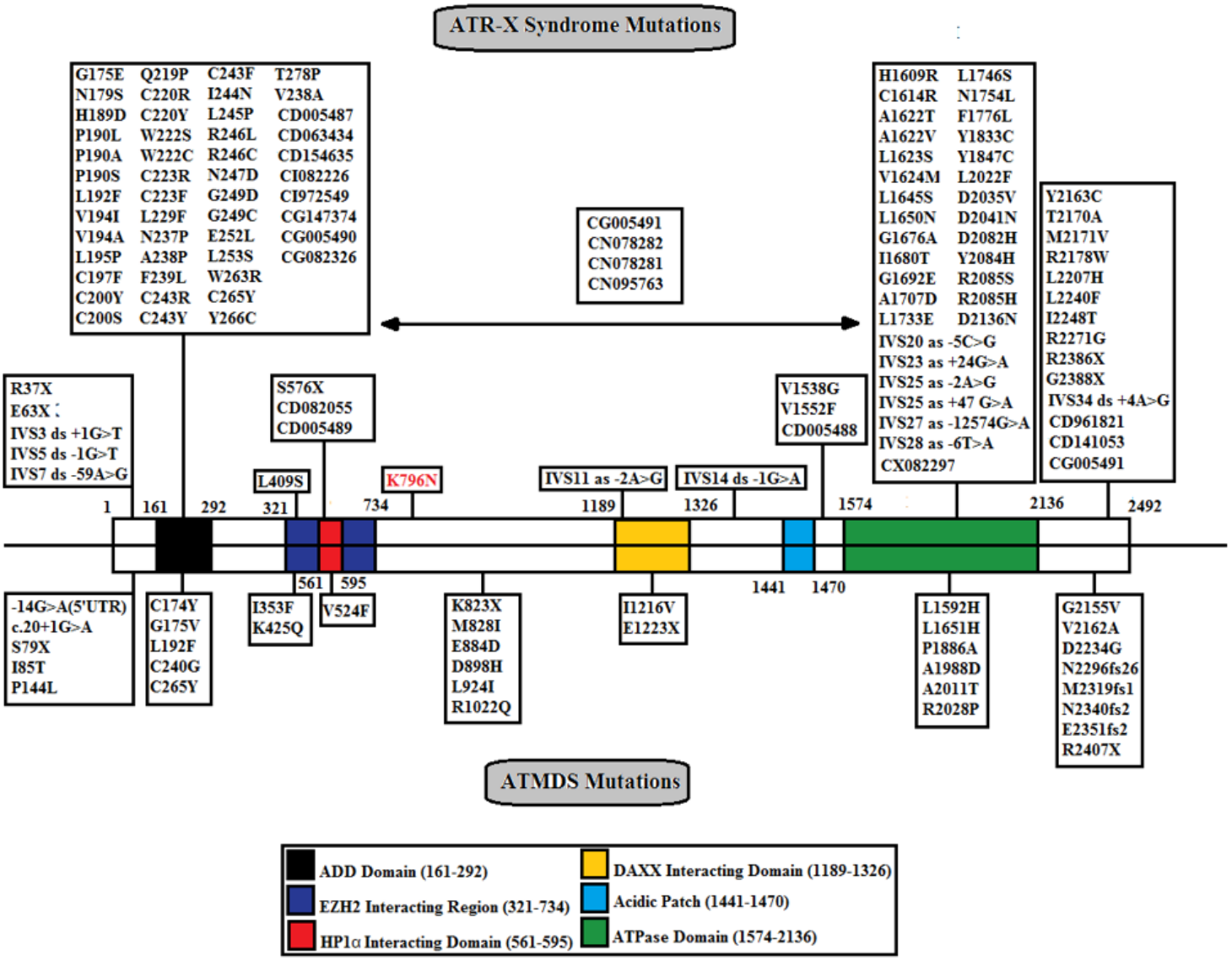

Figure 2

The diagram of ATRX protein and the distribution of mutations previously reported in its 6 domains. The mutation found in the current study is shown with red color.

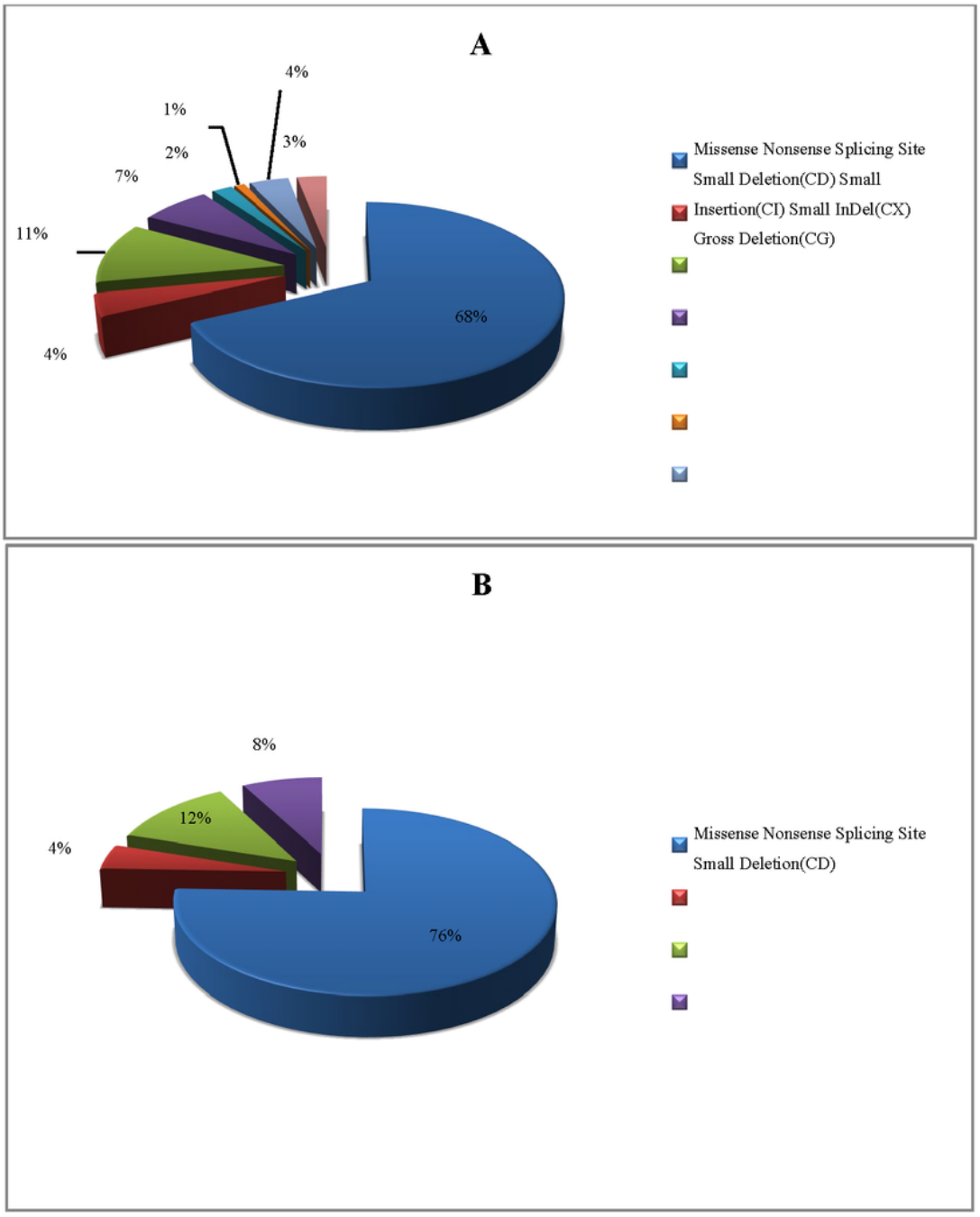

Figure 3

The ATRX gene mutation types reported to date in ATR-X syndrome (A) and ATMDS (B).