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REVIEW

ATR-X syndrome: genetics, clinical spectrum, and management

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Abstract

ATR-X, an acronym for alpha thalassemia and mental retardation X-linked, syndrome is a congenital condition predominantly affecting males, characterized by mild to severe intellectual disability, facial, skeletal, urogenital, and hematopoietic anomalies. Less common are heart defects, eye anomalies, renal abnormalities, and gastrointestinal dysfunction. ATR-X syndrome is caused by germline variants in the *ATRX* gene. Until recently, the diagnosis of the ATR-X syndrome had been guided by the classical clinical manifestations and confirmed by molecular techniques. However, our new systematic analysis shows that the only clinical sign shared by all affected individuals is intellectual disability, with the other manifestations varying even within the same family. More than 190 different germline *ATRX* mutations in some 200 patients have been analyzed. With improved and more frequent analysis by molecular technologies, more subtle deletions and insertions have been detected recently. Moreover, emerging technologies reveal non-classic phenotypes of ATR-X syndrome as well as the description of a new clinical feature, the development of osteosarcoma which suggests an increased cancer risk in ATR-X syndrome. This review will focus on the different types of inherited *ATRX* mutations and their relation to clinical features in the ATR-X syndrome. We will provide an update of the frequency of clinical manifestations, the affected organs, and the genotype–phenotype correlations. Finally, we propose a shift in the diagnosis of ATR-X patients, from a clinical diagnosis to a molecular-based approach. This may assist clinicians in patient management, risk assessment and genetic counseling.

Background

Alpha thalassemia and mental retardation X-linked (ATR-X) syndrome has a wide clinical spectrum, affecting multiple organs and tissues. Previous descriptions have identified intellectual disability (the recommended term to replace mental retardation), facial dysmorphias, and hematological anomalies as the main clinical characteristics (Gibbons and Higgs 2000; Martinez et al. 1998; Lossi et al. 1999; Jezela-Stanek et al. 2009). Since the identification of the *ATRX* gene as causative of the ATR-X syndrome (Gibbons et al. 1995), over 200 affected individuals within more than 130 families have been described.

The ATRX protein, encoded by *ATRX* gene, is a large 2492 amino acids and widely expressed protein that has essential roles in development. It belongs to a family of DNA helicases/ATPases and has been implicated in transcriptional regulation as a chromatin remodeler, and as a transcriptional co-activator. In the N-terminal region, the ATRX protein contains a cysteine-rich region, the ATRX-DNMT3-DNMT3L (ADD) domain with a plant homeodomain (PHD), which may interact with the tail of histone H3 (Dhayalan et al. 2011; Argentaro et al. 2007) (Fig. 1B). The ADD domain is also present in the DNA methyltransferases DNMT3a and DNMT3b and in the DNMT3L protein, whose functions include imprinting. PHD domains are found in several chromatin-associated proteins involved in transcriptional regulation and protein–protein interactions. The C-terminal region of the ATRX protein contains a DNA-dependent ATPase domain of the Sucrose Non-Fermenting 2 (SNF2) family, which hydrolyze ATP to remodel nucleosomes or help repair DNA damage (Gibbons et al. 1995, Bérubé et al. 2008) (Fig. 1B). Additionally, it has been suggested that the SNF2 domain has a role in subnuclear targeting, as the C-terminal end of the ATRX protein is required for proper localization to promyelocytic leukemia (PML) nuclear bodies (NBs) (Bérubé et al. 2008). Both the ADD and SNF2 domains are highly conserved between mouse

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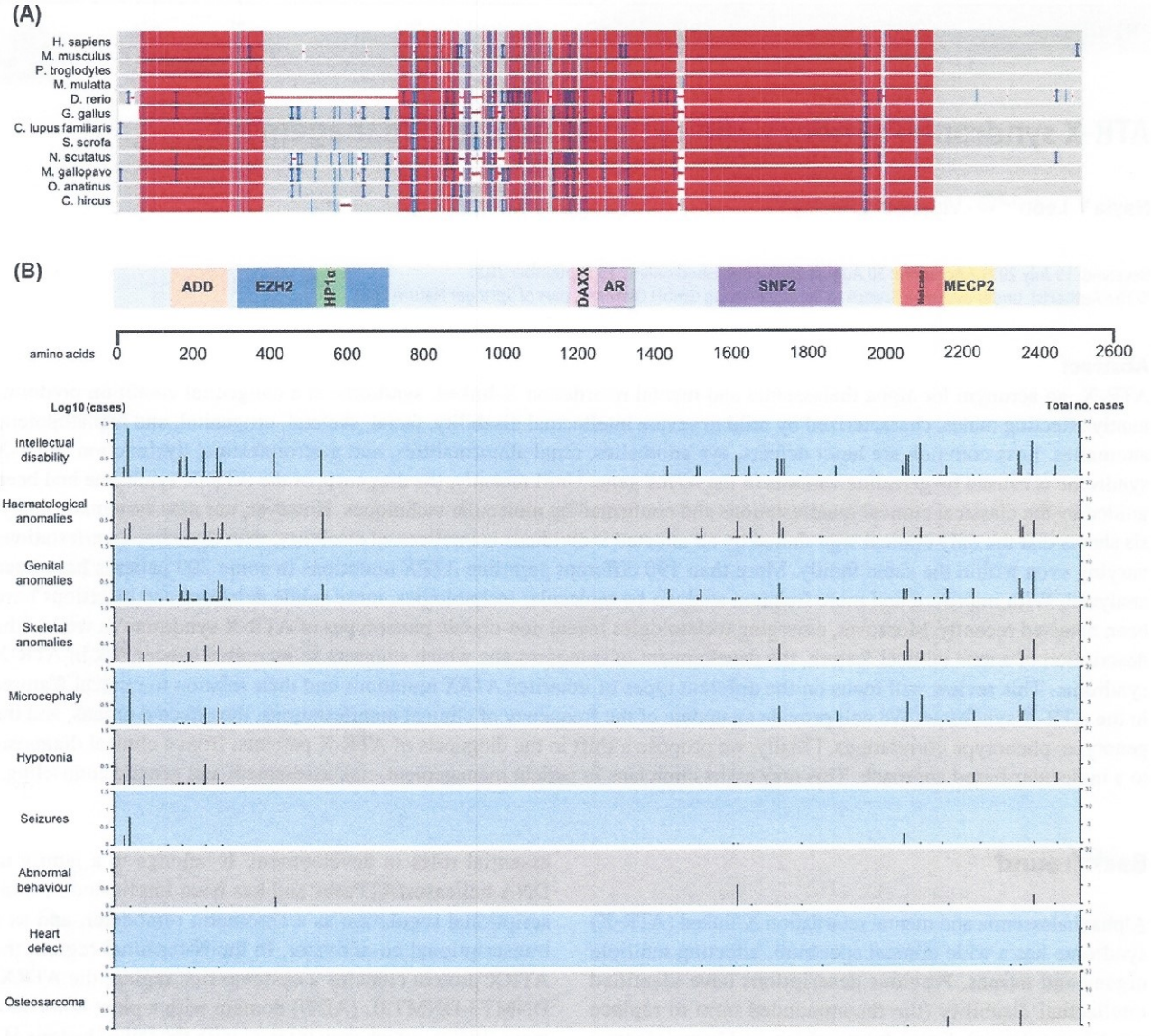

Fig. 1 ATRX protein sequence conservation and clinical mutations. **A** ATRX protein is shown in diverse species. The majority of the mutations cluster into highly conserved domains. Red color indicates highly conserved regions and blue represents less conserved ones. Image created through NCBI COBALT (Constraint-based multiple alignment tool) with 12 sequences selected through the NCBI Orthologues feature, NCBI Multiple Alignment Sequence Viewer, Version 1.19.1 (accessed on 05 May 2021). **B** Distribution of mutations in the ATRX protein that cause diverse clinical features. Note that p.R37* is the most frequent mutation (present in 28 individuals within 8 different families) and is localized outside the conserved domains

and human (Picketts et al. 1998) (Fig. 1A), which is relevant because many tissue-specific mouse knockout models have been generated (Table 1).

As a transcription factor, it is expected that ATRX regulates the expression of genes. ATRX regulates the expression of genes in a variety of ways, for example, by binding G-rich tandem repeats. ATRX loss can cause alpha thalassemia due to the down-regulation of the alpha-globin gene cluster (Law et al. 2010). Furthermore, ATRX regulates the expression of Androgen Receptor-target genes through direct AR-ATRX interaction at androgen response elements (Bagheri-Fam et al. 2011). Another mechanism by which ATRX regulates gene expression is by epigenetic changes such as chromatin remodeling and methylation. The DAXX/ATRX complex deposits the histone variant H3.3 at heterochromatic repetitive genomic regions, maintaining their condensed state in vitro (Goldberg et al. 2010; Voon et al. 2015). Additionally, individuals with *ATRX* pathogenic variants present

Table 1 Conditional *Atrx* knockout and overexpression mouse models

Organ/ system of interest	*Atrx* conditional KO/overexpression	Phenotype	Associated mechanisms	References
Muscle	Knockout Skeletal muscle	Postnatal growth delay and regeneration deficit	Mitotic defects, DNA damage and delayed progression mid-late S phase	Huh et al. (2012)
Skeletal	Knockout Forelimb mesenchyme	Brachydactyly (E15.5 and postnatal)	DNA damage and apoptosis related to p53 (E13.5-E15.5)	Solomon et al. (2013)
Eye	Knockout Retina	Loss of 2 types of neurons, amacrine and horizontal cells (E10)	–	Medina et al. (2009)
Gonad	Knockout Sertoli cells	Small testes and disruption of cords (E17.5-postnatal)	Prolonged G2-M phase and apoptosis (E15.5-E17.5)	Bagheri-Fam et al. (2011)
Brain	Knockout Forebrain	Reduction forebrain size (E11)	Apoptosis related to p53 (E11-E13.5)	Bérubé et al. (2005)
Brain	Knockout Forebrain and anterior pituitary	Reduced growth, shortened life span, lordokyphosis, cataracts, heart enlargement, and hypoglycemia	Replicative stress and DNA damage (E13.5-neonatal)	Watson et al. (2013)
Brain	Knockout Forebrain excitatory neurons	No autism-related behaviors (postnatal)	–	Martin-Kenny and Bérubé (2020)
Brain	Knockout Forebrain excitatory neurons	Impaired spatial learning and memory in males	Reduction of H3K27me3 leading to increased miR-137 expression	Tamming et al. (2020)
Brain	Overexpression	Growth retardation, neural tube defects, increased embryonic/perinatal death, seizures, abnormal behavior and craniofacial dysmorphias (E10.5-posnatal)	No increased apoptosis	Bérubé et al. (2002)

KO knockout, – not reported

differential methylation of genes involved in DNA and RNA metabolic processes that may lead to transcriptional deregulation (Schenkel et al. 2017).

In many cancers, ATRX acts as a tumor suppressor. Loss of ATRX function facilitates the alternative lengthening of telomeres (ALT) pathway in tumor cells, a critical step in establishing cellular immortality in the formation of a variety of tumors, such as gliomas (Schwartzentruber et al. 2012), neuroendocrine tumors (Jiao et al. 2011), and sarcomas (Koelsche et al. 2016; Liau et al. 2015a, b, c; Yang et al. 2015). In a zebrafish model with heterozygous deletion of *Atrx* on a p53/nf1-deficient background a wide spectrum of tumor types develop including epithelioid sarcoma, angiosarcoma, and rare types of carcinoma (Oppel et al. 2019).

As the ATRX protein is essential for embryonic development and dose-sensitive, it is likely germline complete loss of function mutations would be lethal, based on the inability to generate a conventional knockout mouse. *ATRX* germline mutations, that cause reduced protein expression, lead to ATR-X syndrome with multiple and pleiotropic congenital features, whereas loss of function somatic mutations of *ATRX* occur in a variety of tumors. While, cancer is not a general feature of the ATR-X syndrome, recently several patients with *ATRX* germline mutations have been reported with osteosarcoma. Furthermore, non-classic forms of ATR-X syndrome are now being detected (Smolle et al. 2017; Ji et al. 2017; Masliah-Planchon et al. 2018, Mirabello et al. 2020). These latest cases reveal new insights into genotype–phenotype correlation, mutation type and frequency, and a wider spectrum of clinical features. We performed a comprehensive analysis of the genetics and clinical features in the ATR-X syndrome. It is hoped that this review will help inform clinicians on the approach and management of this complex syndrome. The data collection for the analysis was obtained from databases such as Pubmed, NCBI, HGMD and COSMIC. Only reported individuals with ATR-X syndrome confirmed by molecular testing were considered. Patients reported in other cohorts were included except for unpublished individuals.

Clinical features

ATR-X syndrome comprises facial dysmorphias, intellectual disability, hypotonia, skeletal, urogenital, and hematopoietic anomalies. As ATRX is widely expressed, other tissues and organs can also be affected including heart, kidney, and intestine (Fig. 2 and Table 2). Recently, the reported range of phenotypic manifestations has expanded, predominantly towards the mild end of the spectrum, as increasing numbers of individuals have had access to diagnosis by molecular genetic testing.

The clinical manifestations in ATR-X syndrome are highly variable, even in the same family. We have undertaken a systematic analysis that reveals that five of the typical clinical manifestations (facial dysmorphias, hypotonia, skeletal, urogenital, and hematopoietic anomalies) are less frequent than reported previously (Gibbons and Higgs 2000). Notably, intellectual disability is the only clinical sign shared by all ATR-X patients. Global developmental delay is evident from birth. While ambulation and speech are accomplished late in childhood, some affected individuals never walk independently and have significant speech problems. Intellectual impairment is usually severe, compromising the autonomy of the affected individuals. Other neurologic manifestations such as hypotonia, abnormal behavior (e.g., autistic conduct, repetitive stereotypic movements, choreiform movements, aggressiveness) and seizures are highly variable. The distinct facies, previously considered an important feature for diagnosis, is only present in half of

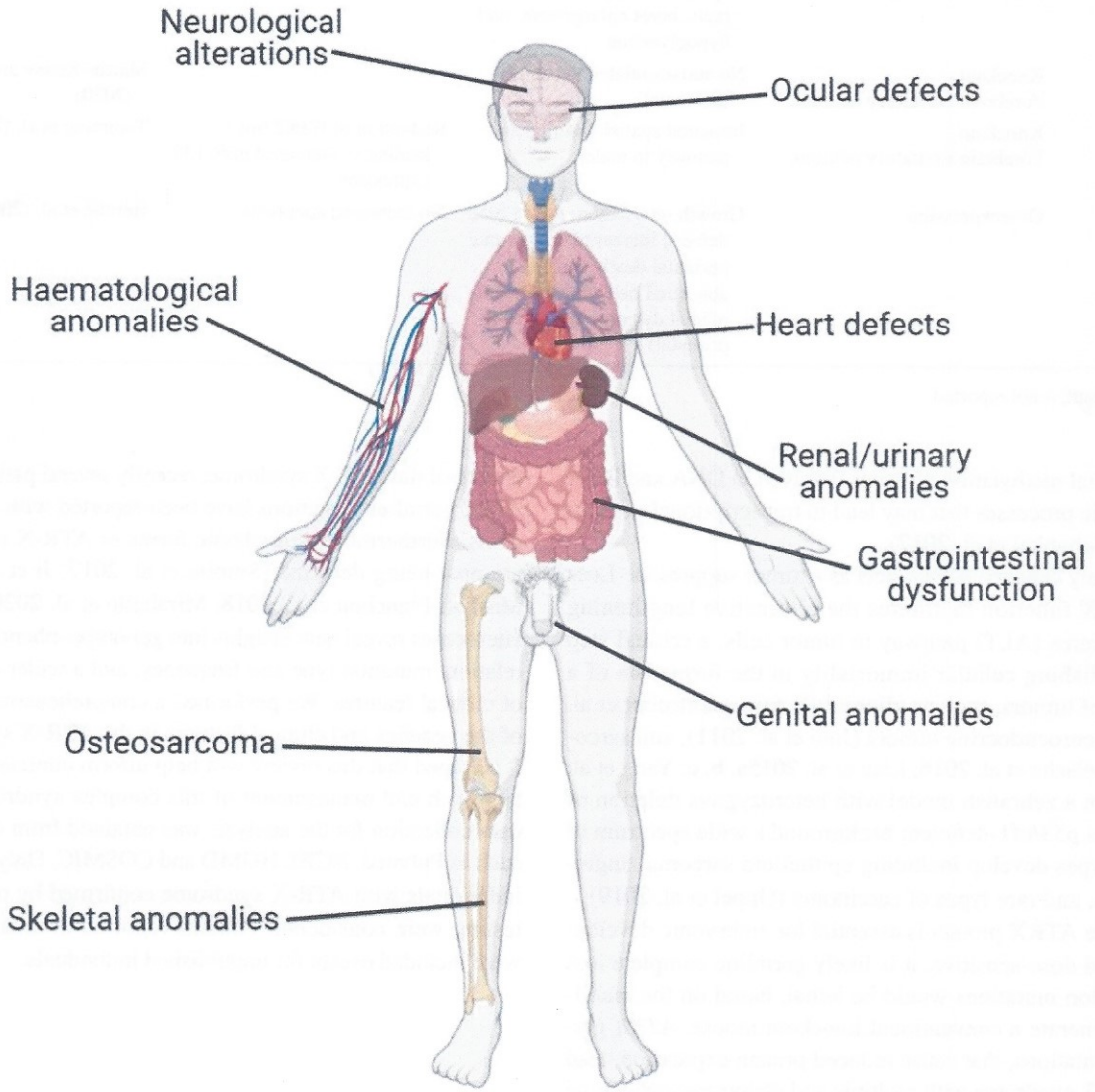

Fig. 2 Clinical features in ATR-X syndrome. Created with BioRender.com

Table 2 Frequency of clinical features in ATR-X syndrome patients

Clinical features	Total	Frequency (%)
Intellectual disability	209/209	100
Hematological anomalies[a]	92/131	70
Genital anomalies[b]	108/174	62
Characteristic face[c]	69/125	55
Skeletal anomalies[d]	51/119	43
Microcephaly	61/144	42
Hypotonia	47/140	34
Gastrointestinal problems[e]	50/167	30
Seizures	24/137	17
Abnormal behaviour[f]	16/167	10
Heart defects[g]	10/119	8
Osteosarcoma	6/209	3

Intellectual disability is the only manifestation shared by all patients with ATR-X syndrome

[a]Haematological anomalies include alpha-thalassemia, anemia and/or presence of HBH inclusions

[b]Genital anomalies include hypospadias, cryptorchidism, dysgenetic testes, micropenis, ambiguous genitalia or female external genitalia

[c]Characteristic face refers to hypertelorism, telecanthus, epicanthal folds, depressed nasal bridge, short nose, midface hypoplasia, tented upper lip, thick and everted lower lip, widely spaced frontal incisors and tongue protrusion

[d]Skeletal anomalies include short stature, pectum carinatum, kyphosis, scoliosis, brachydactyly, tapering fingers, clinodactyly, overlapping digits, bifid thumb and pes planus/varus/valgus

[e]Gastrointestinal problems include drooling, gastroesophageal reflux, constipation, required fundoplication and/or gastrostomy. Data updated and modified from Martucciello et al. (2006). Unpublished patients were not included

[f]Abnormal behavior includes autism, repetitive stereotypic movements, choreiform movements or aggressive behavior (self-injurious or aggressive against others)

[g]Heart defects include tetralogy of Fallot, septal defects, aortic regurgitation, aortic stenosis, pulmonary stenosis, aortic coarctation and bicuspid aortic valve

the reported cases. The dysmorphias are characterized by hypertelorism, telecanthus, short nose, tented upper lip, and thick lower lip. These characteristic facies are present from birth but may become less distinct with age, as the face may coarsen having an open mouth, spaced teeth, and prominent lips. While uncommon, other major clinical manifestations are ocular coloboma, cleft palate, cardiac defects, inguinal hernia, heterotaxy, and asplenia.

In a novel association with ATR-X syndrome, six patients with *ATRX* germline mutations developed osteosarcoma (Smolle et al. 2017; Ji et al. 2017; Masliah-Planchon et al. 2018; Mirabello et al. 2020), two before 10 years of age, which is uncommon. Moreover, two non-related patients with the same nonsense mutation presented two primary tumors (Ji et al. 2017; Masliah-Planchon et al. 2018), suggesting a genetic predisposition to osteosarcomas with *ATRX* germline mutations. Other patients with the same *ATRX* mutations have not shown this type of tumor. This might be due to the age when these patients were reported, with diagnosis as early as 2 years of age. Another possibility is that osteosarcoma, like most clinical manifestations of the ATR-X syndrome, has a variable expressivity. *ATRX* somatic mutations occur in 5% of all cancers (https://www.mycancergenome.org/content/gene/atrx/). Specifically, 7% of osteosarcomas have somatic mutations of *ATRX* (COSMIC: Catalogue Of Somatic Mutations In Cancer). Based on the patients analyzed to date, *ATRX* germline mutations in individuals that develop osteosarcoma are located in the C-terminal region. Whereas *ATRX* somatic mutations associated with osteosarcoma are spread throughout the ATRX protein (Fig. 3). While osteosarcoma has been recently associated to ATR-X syndrome, other clinical features have decreased their frequency compared to the previously reported (Gibbons and Higgs 2000) (Table 2). This could be explained by the detection of milder ATRX phenotypes through testing

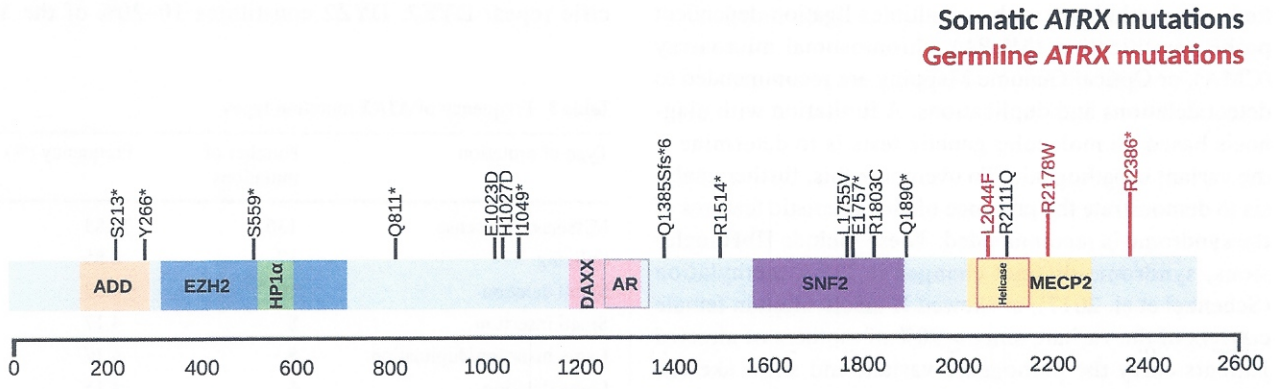

Fig. 3 *ATRX* somatic and germline mutations associated to osteosarcoma. The *ATRX* somatic mutations are spread throughout the protein, whereas the germline mutations are localized in the C-terminal region. The size of the vertical lines represents the number of samples or patients reported. Six patients with *ATRX* germline mutations have developed osteosarcoma. While 15 *ATRX* somatic mutations have been detected from 207 osteosarcoma samples. A somatic *ATRX* deletion is absent from the figure as it has not been well characterized. Horizontal bar designates amino acids

patients with few or only one ATR-X syndrome features, and the use of diagnostic molecular technologies such as whole exome sequencing (WES).

Being X-linked, ATR-X syndrome males present with clinical manifestations, while females carrying the heterozygous mutant allele are usually asymptomatic. Most female carriers have marked skewing of X chromosome inactivation (> 90:10) with favored inactivation of the X chromosome containing the pathogenic *ATRX* variant (Wada et al. 2005). Two females showed a mild ATR-X phenotype that included craniofacial features, growth retardation, and intellectual impairment (Badens et al. 2006b; Wada et al. 2005). This could be explained by the X chromosome inactivation pattern, which in the female reported by Badens et al. (2006a, b) had a marked skewed inactivation of the normal X chromosome.

Diagnosis

Historically, the diagnosis of ATR-X syndrome was based on clinical manifestations and evidence of HbH inclusions in fresh blood smears. With advancing molecular genetic technologies (e.g., gene panels, whole exome sequencing, whole genome sequencing) for diagnosis, non-classic phenotypes of ATR-X syndrome have emerged, such as the presence of only intellectual disability or osteosarcoma (Mirabello et al. 2020). Correlating *ATRX* gene mutations with phenotypes indicates that the single common clinical manifestation in ATR-X syndrome is intellectual disability. We therefore suggest including the *ATRX* gene in future intellectual disability gene panels to diagnose non-classic syndromic forms. Targeted sequencing of the *ATRX* gene as a first approach should be considered in patients with more than one hallmark clinical feature characteristic of ATR-X syndrome. Where no pathogenic variant is identified, other techniques such as multiplex ligation-dependent probe amplification (MLPA), chromosomal microarray (CMA), or Optical Genome Mapping are recommended to detect deletions and duplications. A limitation with diagnosis based on molecular genetic tests is to determine if the variant is pathogenic. To overcome this, further analysis to demonstrate the presence of characteristic features of the syndrome is recommended. These include HbH inclusions, syndrome-distinct changes in DNA methylation (Schenkel et al. 2017), or skewed X inactivation in female carriers of the variant, since > 90% of mothers of affected patients carry the pathogenic variant and show skewed X chromosome inactivation. However, it must be considered that female carriers may present a balanced inactivation pattern and that 8–27% of healthy females in the general population have skewed X-inactivation (Gibbons et al. 1992; Amos-Landgraf et al. 2006; Shvetsova et al. 2019). The shift in the diagnosis of ATR-X patients, from a clinical diagnosis to a molecular-based approach should reveal a wider spectrum of phenotypes in this syndrome and hence better characterize it.

Germline *ATRX* mutations

ATRX germline mutations cause ATR-X syndrome. 192 different germline mutations have been described in the *ATRX* gene (Table 3) (HGMD database). Most are point mutations, specifically missense, leading to reduced protein expression (Villard and Fontes 2002; Badens et al. 2006a). Deletions and duplications have also been reported in 6% of the cases (Thienpont et al. 2007; Gibbons et al. 2008; Lugtenberg et al. 2009, Cohn et al. 2009). Small deletions and insertions are more frequent than previously reported (Gibbons et al. 2008), probably due to the improvement of diagnostic molecular techniques (Table 3). The majority of germline *ATRX* mutations cluster within the helicase, SNF2 and ADD domains, pointing to essential functions of these specific domains (Fig. 1B). Germline mutations are rarely located in protein binding regions. Although no clear phenotype-genotype association has been reported for the most frequent clinical manifestations, all ATR-X syndrome patients with osteosarcoma reported to date carry mutations in the C-terminal region (Fig. 1B). Contrary to the data published by Badens et al. 2006a, b, our appraisal of published data suggests there is no difference in the severity of intellectual disability and severity of genital anomalies between mutations in the ADD and helicase domains (Badens et al. 2006a).

Germline mutations in *ATRX* have been associated with changes in the pattern of methylation of different regions in the genome. For example, presence of changes of methylation of repetitive elements, such as the ribosomal DNA (rDNA) and a region on the Y chromosome, the Y-specific repeat DYZ2. DYZ2 constitutes 10–20% of the Y

Table 3 Frequency of *ATRX* mutation types

Type of mutation	Number of mutations	Frequency (%)
Missense/Nonsense	136	70.83
Splicing	17	8.85
Small deletion	16	8.33
Small insertion	8	4.17
Large insertion/duplication	8	4.17
Large deletion	6	3.13
Small insertion/deletion	1	0.52
Total	192	

HGMD database, Aug 2021

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chromosome, distributed along the entire heterochromatic band Yq12 (Cooke et al. 1982). Mutations in *ATRX* cause some repeats (rDNA) to become hypomethylated, whereas others (DYZ2) are hypermethylated (Gibbons et al. 2000). Likewise, differential methylation pattern in promoters and CpG islands have been observed in patients with pathogenic *ATRX* variants. From the 16 genetic regions with significant differences in methylation, 13 regions were hypermethylated while normally are unmethylated (Schenkel et al. 2017).

Somatic *ATRX* mutations

Somatic *ATRX* mutations can produce alpha thalassaemia myelodysplasia syndrome, a neoplastic disorder of the myeloid lineage of haematopoiesis, or a variety of tumors, including gliomas, neuroblastomas, melanoma, osteosarcomas and neuroendocrine tumors. Similar to germline mutations, point mutations are the most frequent type of *ATRX* somatic mutations, with missense mutations alone representing 37% of all somatic *ATRX* mutations (COSMIC). However, in contrast to the germline mutations which cluster in specific domains, somatic mutations reported are evenly distributed throughout the *ATRX* gene.

Both the germline and somatic *ATRX* mutations produce hematological anomalies and increased risk of developing osteosarcoma. Around 7% of the patients with osteosarcoma harbor somatic mutations in *ATRX*. Conversely, some ATR-X syndrome patients carrying C-terminal germline mutations develop osteosarcoma (Fig. 3).

Models of ATR-X syndrome

The ATRX protein is widely expressed throughout embryogenesis and postnatal life. During mouse development, ATRX is expressed as early as when the egg is fertilized (Fulka nd Langerova 2014). ATRX is essential for development, as conditional *Atrx* knockout mice (*GATA1*-cre) starting at the 8- to 16-cell morula stage die by E9.5 due to defective formation of the extraembryonic trophoblast (Garrick et al. 2006). Therefore, mouse models with conditional knockout of *Atrx* in diverse cell types/tissues have been generated (Table 1) to understand the role of ATRX in development and diseases such as hypotonia, skeletal anomalies, ocular defects, intellectual disability, and gonadal anomalies. Some models replicate the clinical features in patients with ATR-X syndrome, leading to a better understanding of the underlying mechanisms.

ATRX interacts with a number of chromatin-associated proteins and transcription factors which may lead to functional specificity (Fig. 1B). Hence, ATRX protein has a variety of tissue and cell-specific functions. For example, it seems to have an important role in the survival of cells in forebrain, testes, and forelimb, as ATRX loss leads to apoptosis (Solomon et al. 2013; Bagheri-Fam et al. 2011; Bérubé et al. 2005) (Table 1). Mice lacking ATRX in the brain cortex (Seah et al. 2008) and forelimb mesenchyme (Solomon et al. 2013) show p53-dependent apoptosis, though the specific mechanism in gonads has not been described yet. Other studies in forebrain excitatory neurons showed that loss of ATRX leads to impaired spatial learning and memory in male mice, nevertheless no autistic behavior was observed (Tamming et al. 2020; Martin-Kenny and Bérubé 2020) (Table 1). Moreover, overexpression of ATRX in mice has also been an approach to elucidate its role. Berube et al. overexpressed ATRX in the brain, giving rise to neurodevelopmental abnormalities and craniofacial dysmorphias (Bérubé et al. 2002) (Table 1). Interestingly, no increase in cell death was observed in this ATRX transgenic mouse. In an in vitro assay, HeLa cells treated with ATRX siRNA show increased cell anomalies, such as bi-nucleation, intranuclear bridges, and lobulated nuclei (Ritchie et al. 2008). These cells had difficulty progressing through mitosis and have an increased susceptibility to death. Similarly, in the testis, programmed cell death of Sertoli cells in *Atrx* knockout mice might be a consequence of a cell cycle defect at G2/M phase (Bagheri-Fam et al. 2011). Overall, loss of ATRX causes defects in chromosome dynamics during mitosis, triggering p53-dependent apoptotic cell death. It is plausible that the defects seen in mitosis are secondary to problems in DNA replication, as shown in diverse cellular and mouse models. ATRX deficiency leading to replicative stress as shown by increased DNA damage of mouse embryonic stem cell telomeres (Wong et al. 2010). Furthermore, ATRX ablation delayed cell cycle progression in S phase, producing replicative stress and increased DNA damage in mouse myoblasts (Huh et al. 2012), neuroprogenitor cells (Watson et al. 2013), primary mouse embryonic stem cells (Clynes et al. 2014) and a human colon cancer cell line (Leung et al. 2013).

ATRX binds widely at sites rich in DNA repeats and to CpG islands (Law et al. 2010). G-rich repetitive DNA sequences are prone to form RNA–DNA hybrids (R-loops) during transcription and enable the formation of G-quadruplex (G4) structures. These DNA secondary structures have been implicated in stalling of replication, transcriptional dysregulation, and increased DNA damage. One of the functions of ATRX is to help resolve DNA secondary structures or prevent their formation (Law et al. 2010; Wang et al. 2019; Nguyen et al. 2017). Together, these findings indicate that ATRX is required for efficient DNA replication and transcription, highlighting the functions of ATRX in the maintenance of genomic stability.

Management, genetic counseling and new recommendations

The management of ATR-X syndrome is directed toward the specific symptoms in each individual.

As ATR-X syndrome affects different organs/systems, it is important that the management and follow-up is given by a multidisciplinary team including a pediatrician, orthopedist, speech pathologist, ophthalmologist, urologist, neurologist, physiotherapist, geneticist, and other healthcare professionals. Evaluation of adequate feeding by physical examination, monitoring of growth parameters and nutrition consultation is recommended, as hypotonia may result in feeding and sucking difficulty. In extreme cases nasogastric tube or gastrostomy may be required (Gibbons et al. 2021). ATR-X patients are usually dependent on primary caregivers for daily activities, so an important goal is to achieve as much autonomy as possible with physical therapy and individualized education. A neurologist is required to treat abnormal behavior (e.g., autism, repetitive stereotypic movements, choreiform movements, aggressive behavior) and seizures when present. Gastrointestinal problems such as vomiting, gastroesophageal reflux, peptic ulceration and constipation must be treated by a specialist, since aspiration is a common early cause of death (Gibbons et al. 2021). Though individuals with ATR-X syndrome present mild anemia, it does not require medical treatment. Surgical treatment to manage genital anomalies such as cryptorchidism is needed in some patients (León et al. 2019). Furthermore, detailed radiological investigation, hearing and ophthalmologic evaluation, auscultation of cardiac area and echocardiography if considered necessary, are recommended for the thorough assessment of patients with ATR-X syndrome.

The geneticist plays an important role as we determined that 92% of the mothers of affected children are asymptomatic carriers and advocate genetic counseling as an essential service to inform the risk in future pregnancies and to detect other affected individuals in the family. If the mother is the carrier of the pathogenic *ATRX* variant, she has 50% chance of having a male with ATR-X syndrome or 50% chance of having a female carrier, typically asymptomatic, in each pregnancy.

As early tumor presentation and development of two primary tumors (Ji et al. 2017; Masliah-Planchon et al. 2018) point to a genetic predisposition to osteosarcoma in patients with ATR-X syndrome, we suggest close monitoring for osteosarcoma detection. This is of particular importance in those patients with *ATRX* C-terminal mutations associated previously with osteosarcoma (Smolle et al. 2017, Ji et al. 2017; Masliah-Planchon et al. 2018; Mirabello et al. 2020). From early childhood, parents should be made aware of this risk and pay attention to development of a mass, swelling, pain or unexplained fracture in long bones (e.g., femur, tibia, humerus), especially where close to the knee or shoulder. In these cases, further investigation by a specialist is needed to exclude osteosarcoma. Furthermore, as the only clinical feature present in all patients with ATR-X syndrome is intellectual disability, we recommend including *ATRX* gene in the gene panels for intellectual impairment.

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Declarations

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