

Evaluation of the X-chromosome inactivation patterns in females with Gabriele-de Vries syndrome and expansion of clinical spectrum

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Abstract

Gabriele-de Vries syndrome (GDVS) is a syndromic form of intellectual disability caused by pathogenic variants in the *YY1* gene, which encodes a ubiquitously expressed transcription factor that plays a crucial role in the X-chromosome inactivation (XCI) process. Recent studies have implicated *de novo* *YY1* pathogenic variants in skewed XCI in females with GDVS. Here, we report clinical and molecular features of 11 GDVS patients (ten females), including eight newly identified cases, two being a familial case. Patients exhibited the core phenotype of GDVS but with notable clinical heterogeneity, displaying additional features such as autism spectrum disorder, thyroid dysfunction, hearing impairment, and dysarthria. We also discuss thyroid and other endocrine alterations, autoimmune conditions, and movement disorders in GDVS. Eight *YY1* variants were analyzed *in silico*, exhibiting high pathogenicity scores and predicted structural or functional impact, with most affecting DNA binding or zinc finger domain interactions. Finally, we investigated the XCI patterns of ten female patients, and XCI skewing (moderate or extreme) was detected in blood samples from all of them. Our findings expand the clinical and molecular spectrum of GDVS, in addition to reinforcing a potential involvement of *YY1* mutations in modulating XCI patterns in females.

1. Introduction

Gabriele-de Vries Syndrome (GDVS; OMIM #617557), first described in 2017, is a neurodevelopmental autosomal dominant condition caused by *YY1* (OMIM *600013) haploinsufficiency (Gabriele et al. 2017). It is characterized by developmental delay (DD), mild to profound intellectual disability (ID), craniofacial dysmorphisms and other highly variable phenotypes, comprising low birth weight, multiple congenital anomalies, feeding difficulties, atypical behavior, among others (Gabriele et al. 2017; Nabais Sá et al. 2019).

GDVS diagnosis relies on genomic testing with the detection of heterozygous pathogenic/likely pathogenic *YY1* variants, including missense and loss-of-function (LoF) mutations, and 14q32.2 microdeletions encompassing the *YY1* gene (Nabais Sá et al. 2019). It is a rare condition, with 48 patients reported so far, 44 for whom a general clinical description is available (Gabriele et al. 2017; Morales-Rosado et al. 2018; Carminho-Rodrigues et al. 2020; Bae et al. 2021; Imafidon et al. 2021; Balakrishnan and Ranganath 2021; Malaquias et al. 2021; Tan et al. 2021; Zech et al. 2021; Zorzi et al. 2021; Alali and Vitalone 2022; Cherik et al. 2022; dos Santos et al. 2022; Ferng et al. 2022; Khamirani et al. 2022; Asato et al. 2023; Chaves et al. 2023; Chawla et al. 2023; Koruga et al. 2023; Woo et al. 2023; Luo et al. 2024; Shin et al. 2024; Srouf et al. 2024; Topa et al. 2024; Yang et al. 2024; Mudassir et al. 2025; Pal et al. 2025; Huang et al. 2025) (see the **Supplementary file – Table S1**). Gabriele et al. (2017) also reported other 13 individuals who carried deletions encompassing the *YY1* gene.

The *YY1* gene, located on chromosome 14q32.2, encodes a transcription factor involved in numerous physiological roles, such as DNA replication, cell differentiation, apoptosis, and neurogenesis (Jung et al. 2023; Verheul et al. 2020). In particular, *YY1* plays a central role in the process of X-chromosome inactivation (XCI), a mechanism that evolved in mammals to equalize X-linked gene expression between biological sexes (Jeon and Lee 2011; Makhoul et al. 2014; Jégu et al. 2017; Sun et al. 2021; Dossin and Heard 2022). It has been shown that *YY1* serves as a transcriptional activator of *XIST* in both human and mouse contexts (Chapman et al. 2014; Makhoul et al. 2014; reviewed in Dossin and Heard 2022). Moreover, studies conducted in mice proposed that *YY1* protein binding to the *Xist* locus and its transcript acts as a bridge that allows *Xist* RNA to coat the future inactive X-chromosome (Xi) (Jeon and Lee 2011; Thorvaldsen et al. 2011; reviewed in Jégu et al. 2017 and Sun et al. 2021). Due to the stochastic nature of XCI, its patterns in healthy females show a continuous distribution, with extreme skewed inactivation (< 10:90/>90:10) observed only in a small proportion of them, approximately 2% (Amos-Landgraf et al. 2006). Strikingly, female patients with pathogenic *YY1* variants for whom XCI patterns have been analysed exhibited marked skewing of XCI in peripheral blood (dos Santos et al. 2022; Chaves et al. 2023). If confirmed in a larger group of female patients, this phenomenon could have implications on our understanding of *YY1*'s role in XCI regulation and/or the broader effect of *YY1* mutations in affected females.

Herein, we report detailed clinical features and genetic findings of eleven patients with GDVS, including eight previously unreported cases, and present XCI analysis of ten females carrying causal *YY1* variants. Furthermore, we performed *in silico* analysis of the *YY1* missense variants identified in the GDVS female patients.

2. Patients and methods

Ethics approval

for this study was granted by the Institute of Biosciences - University of São Paulo Ethics Committee (CAAE 80921117.5.0000.5464), the Institutional Ethics Committee from the State University of Rio de Janeiro (CAAE 46769315.5.0000.5259), and the French Ethics Committee (French Ministry of Research and Innovation) under approval number DC-2020-4073. Written informed consent was obtained from the patients' legal guardians. All procedures adhered to the principles outlined in the Declaration of Helsinki, and its subsequent amendments. To ensure confidentiality and data protection, all participant data were anonymized using numerical identification codes, with database access restricted to authorized research team members, in compliance with data protection regulations.

We recruited female patients who had previously undergone Whole Exome Sequencing (WES), and were found to carry pathogenic/likely pathogenic *YY1* variants. Clinical characterization of the patients was obtained through analysis of previously conducted health tests or reports from healthcare professionals and parents.

Peripheral blood samples from probands, their parents and siblings (when applicable) were collected for DNA extraction using standard methods. Sanger sequencing analysis or WES of parents was conducted to perform segregation analysis. For Sanger sequencing, PCR was carried out under standard conditions, and amplicons were sequenced in both directions using BigDye (Thermo Fisher) according to the manufacturer's instructions, with capillary electrophoresis performed on an ABI 3730 DNA Analyzer (Thermo Fisher Scientific Inc.).

All variants were re-evaluated and classified according to the American College of Medical Genetics (ACMG) guidelines (Richards et al. 2015) and ClinGen updates. Causal variants that had not been previously reported were submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>).

To estimate the proportion of cells carrying an active maternal *versus* paternal X-chromosome, we employed methylation-sensitive restriction enzyme assays. The XCI patterns of patients P1–P6, P8 and P9 were determined by assessing differential methylation at different X-chromosome *loci*. One of them is located in a polymorphic region (CAG repeats) of the first exon of the *AR* gene (mapped to Xq12) (Allen et al. 1992); another is mapped to a polymorphic region (GAAA repeats) near the *RP2* gene (Xp13.3) (Machado et al. 2014); another is mapped to a polymorphic region (CCG repeats) near the *HMGB3* gene (Xq28) (Musalkova et al. 2015), and the last one is mapped to a polymorphic region (CCG repeats) near the *TMEM185A* gene (Xq28) (Musalkova et al. 2015).

Each XCI assay included one male negative control and one female control with a known extremely skewed XCI. Reproducibility of the data was based on the assay of all samples in duplicate. XCI patterns were calculated in agreement with Bittel et al. (2008), using an average of the duplicates. Ratios $\leq 70:30$ were considered random XCI, 71:29–90:10 as moderate skewing, and $> 90:10$ as extreme skewing, based on criteria reported by Tan et al. (2025).

The six missense variants linked to the YY1 protein were analyzed through AlphaMissense (Cheng et al. 2023), and structural modeling based on the AlphaFold 3 database (Varadi et al. 2024). Models incorporating each identified mutation were generated based on the wild-type configuration provided by the 1UBD PDB structure. This approach allowed the exploration of the specific structural perturbations induced by the mutations. Residue interactions with DNA and ions were examined using PDBsum for the wild-type structure (Laskowski et al. 2018). Also, the Variant Effect Predictor (VEP) from Ensembl (McLaren et al. 2016) was used to assess dosage sensitivity of the *YY1* gene. The two frameshift variants leading to premature stop codons were evaluated by LOFTEE through VEP from Ensembl.

3. Results

Clinical description

Below, the eight newly identified patients GDVS cases are described in more detail, and clinical data for Patients 9–11 are summarized. Complete clinical information can be found in the **Supplementary file – Table S2**.

Patient 1

Patient 1 (P1) was a Chilean female with a healthy sister and no family history of genetic diseases. She exhibited intrauterine growth restriction and was born prematurely at 36 weeks of gestation, with a birth length of -2.4 SD, and a birth weight of -3.2 SD. Congenital hypotonia and global DD, including delayed motor, speech and language development, were reported. Physical exams at the age of 2 years and 4 months revealed decreased body weight (-4.1 SD), craniofacial asymmetry, large forehead, malar flattening, a pointed chin, low-set and posteriorly rotated ears, periorbital fullness, downslanted palpebral fissures, eyelid ptosis, almond-shaped eyes, telecanthus, convergent strabismus, wide, concave nasal bridge, bulbous nose tip, short columella, downturned corners of mouth, micrognathia, sparse hair, hypertrichosis, marfanoid habitus, big hands and feet, and long fingers with joints hypermobility. Feeding difficulties in consuming solid foods, gastroesophageal reflux, and constipation were reported. She presented with impairment of visual pursuit, atopic dermatitis, bilateral neurosensory hearing impairment, nonverbal autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), self-injurious behavior, sleeping disturbance, unsteady gait and had nasolacrimal duct obstruction, recurrent Moro reflex, poor pincer grasp, and recurrent infections. Brain Magnetic Resonance Imaging (MRI) performed at 3 months revealed a thin *corpus callosum*. P1 passed away due to sepsis with multiple organ failure and pulmonary hemorrhage, after an infection caused by multiple pathogens, including *Streptococcus* bacteria, adenovirus and *Pseudomonas aeruginosa*.

Patient 2

Patient 2 (P2) is a Brazilian female born via cesarean section after *in vitro* fertilization. She has a healthy dizygotic twin sister and no family history of genetic diseases. Her mother developed maternal diabetes during gestation. P2 had intrauterine growth restriction and was born at term (37 weeks) small for gestational age, with a low birth weight (-2.8 SD). Large forehead, downslanted palpebral fissures, eyelid ptosis, thin and sparse hair, micrognathia, and a pointed chin were observed. The patient had congenital torticollis, recurrent Moro reflex and was treated with cranial orthosis between 7 and 10 months of age. She exhibited global DD, including motor and speech development. Hypotonia was noted between 2 to 3 years old, and modest axial ataxia was reported at 18 months, progressing to global ataxia by 3.5 years. MRI revealed mild hydrocephalus. Cranial magnetic resonance imaging at 3 years revealed faint foci of hyperintense signal on T2/FLAIR scattered in the periventricular and deep white matter, potentially related to zones of terminal myelination/gliosis (at 3 years). She is currently 5 years old and has manifested unsteady gait, dysarthria, and sleeping disturbance. She is undergoing assessment for ASD.

Patient 3

Patient 3 (P3) is a Brazilian female born from a healthy couple who has two healthy siblings (a younger paternal brother and an older maternal sister). She has a paternal cousin with a mild ID. Her mother displayed maternal hypertension during pregnancy. P3 displayed intrauterine growth restriction and was born prematurely from 35 weeks of gestation by cesarean section. The patient was small for gestational age and was born with a low birth weight (-3.1 SD). She had neonatal jaundice and was treated with phototherapy. P3 exhibited nasolacrimal duct obstruction (treated by surgical intervention), congenital hypotonia, and recurrent emesis. She manifested febrile seizures until 1 year of age. Graves' disease and Hashimoto's thyroiditis were diagnosed and treated with Methimazole (later suspended). The patient's clinical features at 16 years old also include mild ID, reading and writing difficulties, ADHD, and anxiety. Physical exams showed short stature, a large and prominent forehead, receding hairline, epicanthus and telecanthus, proptosis, bilateral ptosis, long philtrum, overfolded helix, simple ears, micrognathia, thick lower lip, pointed chin, arachnodactyly, camptodactyly, tapered phalanx of fingers of the hands,

marfanoid habitus, and big hands and feet. She displayed recurrent falls during childhood. Audiometry, echocardiogram, and abdominal ultrasound exams exhibited normal results.

Patient 4

Patient 4 (P4) is a French female, one of six siblings from a family originally from the Maghreb. She was born prematurely, had growth delay, speech delay, and academic difficulties with schooling in a medical-educational institute due to mild intellectual disability. She presents with facial dysmorphism including broad forehead, nose with bulbous tip, upslanting palpebral fissures with almond-shaped eyes, enlarged columella, prominent philtrum, pointed chin. She has obesity (1.53 m for 98 kg, BMI 42), convergent strabismus of the left eye, hypermetropia and occasional dizziness. Brain MRI showed normal brain morphology. She had maternal hypertension during her first pregnancy, which happened to be a spontaneous bichorial biamniotic twin pregnancy (mother of patient 5).

Patient 5

Patient 5 (P5) is a French male born from a twin bichorial biamniotic pregnancy (son of P4), after an emergency caesarean section at 30 weeks and 3 days, due to severe intrauterine growth restriction. His birth weight was 640 g, length was 31,7 cm and OFC was 25,5 cm. Apgar score was 7 at 5 min, pH at 7,26 and lactate at 3,1. He presented with axial hypotonia, tubulopathy and retinopathy due to prematurity. Cardiac ultrasound showed hypertrophic and hypertrabeculated left ventricle associated to a patent foramen ovale with left-right shunt. Abdominal ultrasound did not identify any visceral malformation but a thickening of the walls of the left pyelon associated with a small ectasia of the caliceal tracts upstream. Prenatal brain MRI and postnatal transfontanelar ultrasounds showed normal brain morphology. Ophthalmologic control showed normal retina but revealed hypermetropia and convergent strabismus. He presented with facial dysmorphism including brachycephaly, broad forehead, nose with bulbous tip, upslanting palpebral fissures, enlarged columella, smooth and prominent philtrum, thin upper lip, pointed chin, faded cupid's bow, low set ears with overfolded helix. His hands had a single transverse palmar crease. He held his head up at three months (adjusted age for prematurity), but is unable to sit or stand without support at 12 months and needs physiotherapy and psychomotricity follow-up. He shows good social interaction.

Patient 6

Patient 6 (P6) is a French 6 years old female born from healthy non-consanguineous parents. She has a healthy older brother. Motor delay was noted at 3 months old with hypotonia, sitting was acquired at 12 months and walking at 2 years and a half. Speech delay was noted with only a few words at 3 years. Physical examination revealed multiple hemangiomas and congenital naevus, marfanoid habitus with arachnodactyly, hyperlaxity and pectus excavatum. Broad forehead was noted. She presented some difficulties for feeding. Examination by cardiac echography and cerebral MRI were normal. Ophthalmologic examination was normal. Array-CGH testing showed normal results.

Patient 7

Patient 7 (P7) is a girl who was born in Germany after 39 + 5 gestational weeks of an uneventful pregnancy. Her parents and two older siblings are healthy, and there is no history of genetic disease in her family originating from Vietnam. She had a low birth weight (3 P, -1.91 z) and developed recurrent cyanosis after birth. The patient exhibited dystrophy due to severe feeding issues with swallowing difficulties and recurrent emesis. An inefficient motility of the esophagus and gastroesophageal reflux were diagnosed. Her motor development and socio-emotional development were delayed, while speech development was normal. She has recurrent infections intermittently treated with long-term antibiotic medication. Physical exams showed craniofacial asymmetry, periorbital fullness, malar flattening, protruding ears, and short columella. The patient's clinical features at 7 years old also include learning disability (IQ 82), attention-deficit disorder (ADD), lack of orientation, Graves' Disease, decreased body weight (< 1 P, -3.07 z), and IgA deficiency.

Patient 8

Patient 8 (P8) is a Brazilian girl born at term. Her birth weight was 2,200 g and her length was 50 cm. She was small for gestational age and did not suck effectively. The patient presented failure to thrive and underwent recurrent hospitalizations due to upper respiratory infections and acute gastroenteritis. Ultrasonography examination at 9.5 years indicated enlarged thyroid gland, with heterogeneous and diffusely hypoechoic texture, diagnosed as hyperthyroidism. At 8.18 years, an electrocardiogram revealed sinus tachycardia and left anterosuperior fascicular block. A physical examination at 11 years and 10 months showed low weight 18.6 kg (z-4), height 141.5 cm, and head circumference 46.5 cm. The observed clinical features were neuropsychomotor developmental delay, intellectual disability, hyperthyroidism, microcephaly, facial dysmorphisms (small face, closed anterior fontanelle, mild midface hypoplasia, proptosis, thick lips), goiter, small nipples, symmetrical limbs, and long, thin fingers with verrucous lesions. Karyotyping was normal.

Patients 9, 10 and 11

Patients 9 (P9), 10 (P10) and 11 (P11) had already been described in the literature by Carminho-Rodrigues et al. (2020), dos Santos et al. (2022), and Chaves et al. (2023), respectively.

P9 presented with left divergent strabismus, was diagnosed with ADHD, and showed a delay of ~ 2 years in cognitive abilities (Carminho-Rodrigues et al. 2020). There were gait and speech difficulties, without cognitive regression. Neurological exams revealed static ataxia with normal muscular tone, kinetic, resting and postural tremor and myoclonic jerks in the left arm. Physical examination at age 21 disclosed facial dysmorphisms (long and asymmetric face, broad forehead, mandibular prognathism, malar flattening and short philtrum), small ears, long nose, full nasal tip, high palate, Gingko leaf-shaped upper lip indentation and thick lower lip. All four members had reduced muscular tone and there was postural and kinetic action tremor in her body. She manifested generalized dystonia, mainly impairing the neck and left hand, and cerebellar features that included gait and stance ataxia.

P10 is a Brazilian patient born from a healthy non-consanguineous couple by caesarean delivery at full term (dos Santos et al. 2022). Intrauterine growth restriction and hypoactivity were noted. Bilateral hearing loss was detected at 1 year of age, and when she was 3 years old, she failed to thrive and had frequent bilious vomiting. Global developmental delay, hypotonia, low weight, short stature, feeding difficulties, and some pneumonia events were reported, and physical examination revealed flat and triangular face, malar hypoplasia, short palpebral fissures, triangular nose, smooth philtrum, microstomia, arched and narrow palate, thin upper lip, irregular placement of teeth, micrognathia, low-set ears, protruding and malformed auricles, pointed chin, asymmetric thoracic cage, *pectus carinatum*, small nipples, scoliosis, digital thumb, arachnodactyly, camptodactyly, chorioretinitis, hypoplasia of labia majora and breast, and walking difficulties. Skeletal abnormalities were progressive and included femur and knee dysplasia (surgically treated) and severe scoliosis with osteoporosis. When she was 22, absence of sphincter control, sporadic non-febrile seizures, and feeding issues were still present, she used a wheelchair and exhibited anxiety.

P11 is a Brazilian girl who had been described by Chaves et al. (2023). Here, we update her clinical presentation (**Supplementary file – Table S2**). Patient 11 exhibited mild ID, delayed speech and language development, ADHD, ASD, self-injurious behavior (trichotillomania), and insomnia. Convergent strabismus was treated surgically by the age of 10. She presented with Hashimoto’s thyroiditis, constipation, recurrent sinus infection (3–8 years), hypotonia, decreased body weight, and atopic dermatitis. Physical signs at 10 years included preauricular pit, periorbital fullness, epicanthus, hypertelorism, almond-shaped eyes, thick lower lip, micrognathia, arachnodactyly, marfanoid habitus, and scoliosis).

Genetic findings

Table 1 summarizes the molecular characteristics of the *YY1* variants detected in the 11 GDVS patients (ten females) described in this study: eight are previously unreported cases and three have already been reported (Carminho-Rodrigues et al. 2020; dos Santos et al. 2022; Chaves et al. 2023).

Table 1
Molecular features of YY1 variants and X-chromosome inactivation data of 11 GDVS cases

	Patient 1	Patient 2	Patient 3	Patient 4 (mother of patient 5)	Patient 5	Patient 6	Patient 7
YY1 variant (NM_003403.5)	c.1102T > C (p.Phe368Leu)	c.1147_1151dup (p.Cys385Metfs*18)	c.690dupA (p.Asp231Argfs*3)	c.690dupA (p.Asp231Argfs*3)	c.690dupA (p.Asp231Argfs*3)	c.1114A > C (p.Thr372Pro)	c.1057T > (p.Phe353)
Nationality	Chile	Brazil	Brazil	France	France	France	Germany
Age at referral	2 years	5 years	16 years	30 years	6 months	6 years	7 years
XCI data (blood)	AR 71:29 RP2 - NI	AR 73:27 RP2 67:33	AR 71:29 RP2 72:28	AR - NI TMEM185A - NI HMGB3 98:2	- (male)	AR 54:46 TMEM185A 53:47 HMGB3 24:76	AR 50:50 RP2 27:73
ACMG classification	likely pathogenic (PM1 moderate, PM2 moderate, PM6 moderate, PP2 supporting)	pathogenic (PVS1 strong, PM2 moderate, PM6 moderate, PS4 strong)	pathogenic (PVS1 strong, PM2 moderate, PS4 moderate, PM6 moderate)	pathogenic (PVS1 very strong, PM2 moderate, PS4 moderate, PM6 moderate)	pathogenic (PVS1 very strong, PS4 moderate, PM2 moderate)	pathogenic (PS4 strong, PM1 moderate, PP2 supporting, PM2 moderate, PM6 moderate, PP3 supporting)	likely pathogenic (PS4 moderate, PM2 moderate, PM1 supporting, PM6 moderate, supporting)
AlphaMissense	0.9999	0.9999	-	-	-	0.9938	0.9998
LOFTEE	-	low confidence (end_truncated)	high confidence	high confidence	high confidence	-	-
Biochemical change	Phenylalanine is a large, hydrophobic, and aromatic amino acid. Leucine is also hydrophobic but is an aliphatic amino acid due to the lack of an aromatic ring. The change maintains the hydrophobic character but may influence the local folding	Cysteine can form disulfide bridges with another cysteine, which improves the protein's stability and 3D structure. Although methionine also contains sulfur, it does not affect the formation of disulfide bridges. The substitution of cysteine by methionine may compromise the formation of disulfide bridges, affecting the structural integrity of the protein	Aspartic acid is a polar amino acid characterized by its negative charge, which enables it to engage in electrostatic interactions and contribute to the formation of salt bridge formation. Arginine is a basic amino acid with a larger, positive charge and the ability to form multiple hydrogen bonds. The substitution represents a charge reversal. It may alter local electrostatic interactions, possibly by disrupting or creating salt bridges			Threonine is a polar amino acid with a hydroxyl group (-OH) in its side chain, allowing it to participate in hydrogen bonding and contribute to the stabilization of secondary structures. Proline is unique among amino acids due to its cyclic structure, where the side chain forms a covalent bond with the backbone nitrogen, restricting backbone flexibility and disrupting regular secondary structures. The substitution of threonine by proline may disrupt local secondary structure	Phenylalanine is a large, hydrophobic and aromatic amino acid. Leucine is also hydrophobic but it is an aliphatic amino acid due to the lack of an aromatic ring. The change maintains hydrophobic character but may influence the local folding
NI: not informative; NA: not available; AlphaMissense scores: 0-0.34: likely benign; 0.34-0.564: VUS; 0.564-1: likely pathogenic							

All patients, except for P5, were found to carry *de novo* mutations. P5 inherited a *YY1* frameshift variant from P4, his affected mother. Variants were reclassified regarding their pathogenicity, following ACMG/ClinGen criteria (Table 1) and Clingen updates. Three novel *YY1* variants were identified in the present study in P1, P2 and P8.

A pathogenic heterozygous *YY1* missense variant [chr14:100.277.457 T > C (hg38); *YY1*(NM_003403.5):c.1102T > C(p.Phe368Leu)] was identified in P1. A pathogenic heterozygous *YY1* frameshift variant was identified in P2 [chr14:100.277.501 C>CTATGT (hg38); *YY1*(NM_003403.5):c.1147_1151dup(p.Cys385Metfs*18)]. In P3, P4 and P5, a pathogenic heterozygous *YY1* frameshift variant [chr14:100.262.306 G > GA (hg38); *YY1*(NM_003403.5):c.690dup(p.Asp231Argfs*3)] was detected. This variant was previously reported in ClinVar (ID 1331550, classified as likely pathogenic) and in the literature (Cherik et al. 2022). A heterozygous missense *YY1* variant was detected in P6 [chr14:100.277.469 A > C (hg38); *YY1*(NM_003403.5):c.1114A > C(p.Thr372Pro)], already described in ClinVar (ID 1703497, as pathogenic). A missense *YY1* variant was found in P7 [chr14:100.276.643 T > C (hg38); *YY1*(NM_003403.5):c.1057T > C(p.Phe353Leu)] in heterozygosis, which has been previously reported as likely pathogenic in ClinVar (ID 2413123) and in the literature (Topa et al. 2024). Lastly, in P8, another heterozygous *YY1* missense variant [chr14:100.276.629 C > T (hg38); *YY1*(NM_003403.5):c.1043C > T(p.Thr348Ile)], not previously reported, was revealed. Figure 1 shows the position of each *YY1* variant in the *YY1* protein.

Schematic diagram of *YY1* protein, based on dos Santos et al. (2022). Position and nomenclature of *YY1* pathogenic variants of patients whose XCI patterns have been evaluated are represented. Circles, triangles and stars portray missense, frameshift and splicing variants, respectively. Gray and black filled circles/triangles/stars represent moderate (ratios between 71:29 and 90:10) and extreme (> 90:10 ratios) skewing, respectively, in blood DNA samples for the marker that exhibited the highest deviation from a random pattern (*AR*, *RP2*, *TMEM185A* or *HMGB3*). Not in scale

XCI patterns in blood samples

XCI patterns were assessed for 10 patients (P1-P4 and P6-P11). The XCI analysis was performed using DNA samples extracted from peripheral blood. P1 showed moderate skewing for the *AR* locus at an average ratio of 71:29 and was homozygous for the *RP2* locus and hence not informative for this marker. In P2, the *AR* and *RP2* analysis yielded ratios of 73:27 (moderate skewing) and 67:33 (random), respectively. The XCI analysis for P3 revealed moderate skewing for *AR* and *RP2* loci (71:29 and 72:28), respectively). P4 was not informative for *AR* or *TMEM185A*, but displayed extreme skewing for *HMGB3* (98:2). P6 exhibited random XCI for the *AR* (54:46) and *TMEM185A* (53:47) markers, but moderate skewing for *HMGB3* (24:76). The XCI analysis using P7's samples showed a random inactivation ratio for *AR* (50:50), but moderate skewing for *RP2* (27:73). Figure 2 shows these results for three patients, as examples, and the XCI ratios are presented in Table 1, including those of the three already published GDVS cases (P9–P11). P9 (whose clinical presentation had been previously reported by Carminho-Rodrigues et al. (2020) exhibited moderate skewing for both the *AR* (19:81) and *RP2* (87:13) loci. P10 was not informative for the *AR* marker, and the analysis for the *RP2* locus indicated moderate skewing (86:14) (data from dos Santos et al. 2022). P11's samples demonstrated extremely skewed XCI (94:6) for the *AR* locus (data from Chaves et al. 2023).

Filled circles in the pedigree charts represent females diagnosed with GDVS. Red rectangles highlight moderate XCI skewing. u: undigested; d: digested. The graphics are not in scale. **a)** P1's pedigree chart. **b)** XCI assay using P1's blood DNA samples for the *AR* locus (71:29 average ratio). **c)** P2's pedigree chart. **d)** XCI assay using P2's blood DNA samples for the *AR* locus (73:27 average ratio). **e)** P8's pedigree chart. **f)** Graphics resulting from XCI assay using P8's blood DNA samples for the *AR* locus (91:9 average ratio)

In silico analysis of *YY1* protein 3D structure

YY1 protein contains four zinc-finger domains in its C-terminal region that are essential for DNA binding and transcriptional regulation. Variants located within this region, such as the missense here reported (p.R303Q, p.I348T, p.L353P, p.L368P, p.S369C, and p.M385V), occur within or near the zinc-finger motifs; in terms of structural interactions, the residues Phe368 and Asn369 interact directly with DNA, while Cys385 interacts with zinc. Therefore, all of them are predicted to alter zinc coordination or disrupt the interface with DNA. All the six missense variants listed in Table 1 exhibited high pathogenicity scores according to AlphaMissense, a deep learning method for predicting the pathogenicity of single amino acid substitutions in human proteins based on protein structure and evolutive conservation, with five exceeding 0.9 (AlphaMissense cutoffs: $\geq .564$, likely pathogenic; $0.565 - 0.340$, likely neutral; ≤ 0.340 , likely benign). These structural disturbances likely reduce *YY1*'s DNA-binding affinity and impair its transcriptional regulatory function, providing a plausible molecular basis for the associated developmental phenotypes. The changes in the 3D structure of the protein caused by these six missense variants (303R, 348I, 353L, 368L, 369S, and 385M) are represented in Fig. 3. Although not designed to evaluate LoF variants, Alphamissense can be applied to generated models of truncated proteins, assuming that truncated forms can occur if they escape from the NMD. We evaluated two of the LoF variants: c.690dupA(p.Asp231Argfs*3) and c.1147_1151dup(p.Cys385Metfs*18); the third one can not be evaluated because it is a splice site mutation. The c.690dupA(p.Asp231Argfs*3) variant had a high confidence classification in LOFTEE, suggesting loss of function, whereas the c.1147_1151dup(p.Cys385Metfs*18) variant had a low confidence classification with the truncation site near to the end of the gene. The model for p.Cys385Metfs*18 exhibits C-terminal region loss, extending beyond the α -helical segment, while the experimental structure remains fully resolved throughout the entire length of the molecule (Fig. 3). The c.690dupA(p.Asp231Argfs*3) variant, however, could not be depicted in the 3D model, possibly indicating an association with specific protein characteristics. The AlphaFold database shows that the structural model for the *YY1* protein (ID: P25490) could indicate that residues 1–295 are situated within a disordered region, a finding that is also supported by DisProt. While the AlphaMissense score for the p.Asp231Argfs*3 variant is lower than that of other variants, it is important to note that this variant is situated within a structurally disordered region. Considering the variants in the study, only p.Asp231Argfs*3 is located outside the zinc finger domain. However, it could play additional functional roles, especially within the Gly-rich region that is linked to HCFC1 interactions. Also, dosage sensitivity data suggest that *YY1* is dosage intolerant, as evidenced by high pHaplo and pTriple scores (0.96 and 0.99, respectively).

4. Discussion

GDVS is caused by *YY1* haploinsufficiency due to truncating alterations or missense variants, the latter often mapping to the *YY1* zinc-finger motifs (Gabriele et al. 2017). Here, we compiled detailed clinical and molecular features of eleven cases (**Supplementary file – Table S2**), three of them previously reported (Carminho-Rodrigues et al. 2020; dos Santos et al. 2022; Chaves et al. 2023).

The patients displayed clinical traits already reported in GDVS, including intrauterine growth restriction, low birth weight, global DD, hypotonia, gait disturbances and craniofacial dysmorphisms, such as broad and prominent forehead, periorbital fullness, eyelid ptosis, micrognathia, pointed chin, and downslanted palpebral fissures (Gabriele et al. 2017; Morales-Rosado et al. 2018; Carminho-Rodrigues et al. 2020; Bae et al. 2021; Balakrishnan and Ranganath 2021; Malaquias et al. 2021; Tan et al. 2021; Zorzi et al. 2021; Alali and Vitalone 2022; Cherk et al. 2022; dos Santos et al. 2022; Ferng et al. 2022; Khamirani et al. 2022; Asato et al. 2023; Chaves et al. 2023; Chawla et al. 2023; Koruga et al. 2023; Woo et al. 2023; Shin et al. 2024; Srouf et al. 2024; Topa et al. 2024; Yang et al. 2024; Mudassir et al. 2025; Pal et al. 2025; Huang et al. 2025). It is interesting to note that hearing impairment, which was recorded in our series, has been previously described in four patients (Bae et al. 2021; dos Santos et al. 2022; Cherk et al. 2022; Asato et al. 2023). Behavioral issues are relatively common in this condition, and were also presented by patients recruited for the present investigation. Attention Deficit Disorder was present in P1 (with Hyperactivity - ADHD), P3 (ADHD), P6, P7, and P11 as well as in several other individuals with GDVS (Gabriele et al. 2017; Morales-Rosado et al. 2018; Carminho-Rodrigues et al. 2020; Alali and Vitalone 2022; Cherk et al. 2022; Khamirani et al. 2022; Chaves et al. 2023; Chawla et al. 2023). P3, P9 and P10 displayed anxiety, which has also been previously reported in GDVS (Gabriele et al. 2017; Cherk et al. 2022 - supplementary material; dos Santos et al. 2022; Khamirani et al. 2022). P1 exhibited autism and self-injurious behavior, both of which have already been described (Gabriele et al. 2017; Cherk et al. 2022; Ferng et al. 2022; Khamirani et al. 2022). P2 is currently being evaluated for ASD. Sleeping disturbances were observed in P1 and P2, similarly to other cases in the literature (Gabriele et al. 2017). P1, P3, P7 and P8 had recurrent infections, also documented in individuals with GDVS (Tan et al. 2020; Cherk et al. 2022 - supplementary material; Khamirani et al. 2022).

Significant clinical heterogeneity was also observed. P2 exhibited dysarthria, which seems to be a rare feature, only reported by Ferng et al. (2022) and Chawla et al. (2023). Other uncommon clinical characteristics here described include congenital torticollis (P2), which has only been reported by Cherk et al. (2022), and abnormalities of the nasolacrimal duct (P3; obstruction), also reported once (Gabriele et al. 2017; stenosis).

We found an increased frequency of thyroid and autoimmune conditions in this group of patients. P3 and P7 were diagnosed with Graves' disease; in P3, the condition later evolved into Hashimoto's thyroiditis. P11 also exhibited Hashimoto's disease, and P8 was diagnosed with hyperthyroidism. Graves' disease and Hashimoto's thyroiditis are both autoimmune thyroid diseases; the Hashimoto's thyroiditis may produce hyper- or hypothyroidism manifestations, while Graves' disease causes hyperthyroidism (Liu et al. 2023; Davies et al. 2020). It is interesting to note that thyroid dysfunction has been previously reported in GDVS, mainly hypothyroidism and thyroid nodules (Gabriele et al. 2017; Alali and Vitalone 2022; Cherk et al. 2022; Asato et al. 2023; Pal et al. 2025; Huang et al. 2025). Remarkably, previous *in silico* analysis of protein-protein interactions of *YY1* protein disclosed enrichment of thyroid hormone signaling, among other biological pathways (dos Santos et al. 2022).

Other endocrine abnormalities in GDVS include growth hormone deficiency, although rarely described (Gabriele et al. 2017). Besides, Morales-Rosado et al. (2018) published a case of a GDVS patient with the diagnosis of autoimmune myasthenia gravis. In addition, one of the patients (P1) presented with clinical signs that have not been previously reported for GDVS patients to our knowledge, namely hypertrichosis and atopic dermatitis. A more detailed clinical reevaluation of P11 – previously described (Chaves et al. 2023) – revealed that she also exhibited atopic dermatitis, a finding not noted in the original publication. Atopic dermatitis is a condition that has been associated with an increased risk of developing autoimmune diseases, as studied and discussed by Ahn et al. (2024). However, it is a frequent manifestation in the normal population, making it hard to ascertain this clinical feature to GDVS. Taken together, these observations hint that thyroid/endocrine disorders (especially hypothyroidism) and autoimmune conditions may represent an important clinical aspect of GDVS, as also proposed by Alali and Vitalone (2022) and Huang et al. (2025). These features should be considered in the clinical evaluation and management of GDVS patients.

Among the approximately 44 individuals with GDVS clinically described in the literature to date, 20 presented with movement disorders, among whom 12 exhibited dystonia (Gabriele et al. 2017; Morales-Rosado et al. 2018; Carminho-Rodrigues et al. 2020; Balakrishnan and Ranganath 2021; Zorzi et al. 2021; Malaquias et al. 2021; Ferng et al. 2022; Khamirani et al. 2022; Cherk et al. 2022; Chawla et al. 2023; Shin et al. 2024; Srouf et al. 2024; Mudassir et al. 2025) (**Supplementary file – Table S1**). The *YY1* variant carrier reported by Zech et al. (2021 - supporting information) also presented with dystonia; however, since this was the only clinical feature documented for that individual, this case was not included among those with a comprehensive clinical description. Notably, 18 of these 20 clinically characterized cases with dystonia and/or other movement disorders also exhibited neurodevelopmental deficits, such as DD and/or ID (Gabriele et al. 2017; Morales-Rosado et al. 2018; Carminho-Rodrigues et al. 2020; Balakrishnan and Ranganath 2021; Zorzi et al. 2021; Ferng et al. 2022; Khamirani et al. 2022; Cherk et al. 2022; Shin et al. 2024; Srouf et al. 2024; Mudassir et al. 2025). The remaining two cases (Malaquias et al. 2021; Chawla et al. 2023) did not prominently manifest the core GDVS phenotype, although they did exhibit other nonspecific clinical features commonly reported in the syndrome, including low birth weight and facial dysmorphisms, such as pointed chin and bulbous nose. These observations underscore a phenotypic overlap between *YY1*-related movement disorders and neurodevelopmental deficits. Rather than representing distinct diagnostic categories, these phenotypes appear to constitute a single clinical entity associated with *YY1* disruption, with a wide and variable manifestation spectrum. This full picture of the GDVS is still evolving; therefore, describing additional cases is a key step to broaden the clinical and molecular spectrum of the condition, with implications for improvement of diagnosis and clinical management.

Figure 1 depicts the *YY1* protein and the location of the pathogenic variants carried by the affected individuals reported in this study, along with the corresponding XCI pattern observed in the assessed females. P1, P6, P7, P9 (Carminho-Rodrigues et al. 2020) and P10 (dos Santos et al. 2022) carried missense variants mapped to sequences encoding part of the zinc-finger domain (DNA-binding). The missense variant identified in P8 affects an amino acid located between two zinc finger motifs. The remaining five patients, P2, P3/P4/P5 (frameshift) and P11 (Chaves et al. 2023; splice site), carry LoF variants. Interestingly, six out of the eleven variants here reported (variants identified in P1, P2, P6, P7, P9 and P10) impact the zinc-finger DNA binding domains.

According to GnomAD, *YY1* displays intolerance to missense (z-score = 3.6) and loss-of-function variants (pLI score = 1). Also, the pHaplo (0.96) and pTriple (0.99) values indicate that the *YY1* gene is dosage-sensitive; and alterations in its expression may lead to pathological effects.

YY1 is a ubiquitously expressed transcription factor involved in the regulation of a wide range of gene promoters, acting either as a repressor or as an activator. Importantly, *YY1* is a pivotal gene in the initiation of XCI, since it activates *XIST* transcription in human and mouse contexts (Chapman et al. 2014; Makhoul et al. 2014) and might serve as a docking protein for *Xist* transcripts to the inactive X-chromosome (Jeon and Lee 2011). *YY1* interacts with numerous chromatin modifiers (Verheul et al. 2020), and is also known to directly and indirectly interact with numerous XCI key regulators (see discussion in dos Santos et al. 2022). In *Drosophila*, *YY1* has been shown to participate in the recruitment of polycomb group proteins, which are fundamental for maintaining transcriptional repression of genes (Wilkinson et al. 2006). Polycomb group protein recruitment to DNA by *YY1* leads to the methylation of histone H3 on lysine 27 (H3K27) (Wilkinson et al. 2006). The polycomb complexes PRC1 and PRC2 mediate specific histone modifications, which are involved in the XCI process (Sun et al. 2021). Also, *YY1* may be implicated in recruiting polycomb complexes to the Xi (Thorvaldsen et al. 2011). Additionally, *YY1* interacts with histone deacetylases (HDACs) engaged in gene silencing, such as HDAC3, and histone acetyltransferases (HATs) (Verheul et al. 2020). Studies point to HDAC3 involvement in XCI, deacetylating histones upon interaction with *XIST* RNA and helping gene silencing and RNA polymerase II exclusion from the Xi (McHugh et al. 2015; Żylicz et al. 2018). Moreover, a recent study in mice suggests that the *YY1* binding to X-linked genes can act as a barrier to *Xist*-mediated silencing until XCI late stages (Bowness et al. 2024).

Given *YY1*'s prominent involvement in the XCI process, we hypothesized that germline *YY1* mutations could drive or impact the XCI pattern in females, leading to skewing (dos Santos et al. 2022; Chaves et al. 2023). To date, only two published studies have assessed XCI patterns in female patients with *YY1* mutations (dos Santos et al. 2022; Chaves et al. 2023), and both reported XCI skewing. Here, a heterogeneous XCI pattern was observed among the eight cases newly evaluated (P1-P4 and P6-P9). In spite of that, it is noteworthy that in total, seven patients (P1, P2, P3, P4, P7, P9 and P10) exhibited moderate XCI skewing for at least one marker, and three individuals (P4, P8 and P11) showed extreme XCI skewing. These findings may reinforce a potential involvement of *YY1* mutations in modulating the XCI pattern *in trans*, although the mechanistic link is not clear.

It is crucial to recognize that XCI skewing can arise due to several mechanisms, such as the stochastic nature of the molecular mechanism, the presence of an X-linked variant unrelated to ID, or even the activity of escapee X-linked genes (Peeters et al. 2023). An impressive number of variants in X-linked genes are associated with ID (Migeon 2020). Plenge et al. (2002) first described that female carriers of harmful variants causing X-linked ID (XLID) disorders displayed XCI skewing, with findings suggesting a negative selection against cells harboring the mutation on the active X-chromosome. Notwithstanding the potential protective effect provided by XCI (Migeon 2020), female carriers of those XLID pathogenic variants may exhibit skewing favoring the mutant allele, and can still present ID (Vianna et al. 2020; Chaves et al. 2023).

Gabriele et al. (2017) reported that lymphoblastoid cell lines (LCLs) from two individuals carrying missense mutations [c.1138G > T (p.Asp380Tyr)] and [c.1097T > C (p.Leu366Pro)] displayed wild-type *YY1* levels, after analysis by RNA-seq and Western blot. Upregulation of *YY1* was detected in induced pluripotent stem cells (iPSCs) derived from another affected individual with a missense variant (Pereira et al. 2025). Jeon and Lee (2011) proposed that *YY1* zinc-fingers interact with both *XIST* DNA and RNA by binding to different motifs on the DNA and RNA. Therefore, it is plausible to speculate that *YY1* isoforms containing missense variants may exhibit reduced affinity to *XIST* DNA or RNA, resulting in a preferential binding of the wild-type *YY1* isoform. In the other way, LoF *YY1* mutations, which are known to lead to a strong reduction in the amount of protein in LCLs (Gabriele et al. 2017) and iPSCs (Pereira et al. 2025) derived from patients with GDVS, would result in a reduced availability of functional *YY1*. This reduction induces global decrease of *YY1* occupancy in the genome (Gabriele et al. 2017; Pereira et al. 2025); however, if this condition impacts the XCI pattern is a question not yet addressed.

Recent findings (Bertin et al. 2026) suggested that females may exhibit an altered dynamic utilization of the two X-chromosomal alleles. This would distort the *locus*- and lineage-specific reactivation of the inactive X, a mechanism proposed to serve as a critical reservoir during differentiation, thereby enhancing the resilience of female neural tissue. Taking into account that *YY1* interacts with a myriad of elements involved in XCI and its reduced levels in *YY1*-LoF mutations and/or disrupted activity of *YY1*-missense mutations, it is tempting to suggest that these harmful *YY1* variants could drive a primary XCI skewing, but no obvious explanation can be provided at this point. Alternatively, *YY1* depletion caused by germline pathogenic variants could indirectly lead to XCI skewing, by altering progenitor cell development or survival during early development, thereby reducing the available cell pool and increasing the likelihood of biased inactivation patterns. We may also speculate that females with *YY1* mutations can develop in the brain a disrupted version of the heterogeneous expression pattern of escapee X-linked genes (Peeters et al. 2023). This later hypothesis is interesting when associated with the autoimmune conditions reported here. Autoimmune diseases disproportionately affect women compared to men, with contributing factors such as estrogen, X-linked genes, and microbiota composition; there are several studies addressing how XCI features may drive the sex disparity in autoimmunity of females (reviewed in Mousavi et al. 2020).

In conclusion, our study expands the clinical and molecular understanding of GDVS. Considering the limited number of reported GDVS cases in the literature, it is of paramount importance to describe new cases along with their clinical and molecular data for improving diagnosis accuracy and exploring potential therapeutic strategies for the syndrome. Moderate XCI skewing was detected in blood samples from seven patients, and extremely skewed XCI patterns were found in three patients, reinforcing that *YY1* mutations may impact XCI patterns. Future functional studies to assess the impact of the *YY1* mutations on XCI could employ patient-derived cells to evaluate *XIST* transcription and its interaction with the X-chromosome. Addressing this gap in future research will be crucial to fully elucidate the mechanistic links between *YY1* dysfunction and XCI alterations in GDVS.

Declarations

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Human Research Ethics Committee of the Institute of Biosciences of the University of São Paulo (CAAE 80921117.5.0000.5464), Institutional Ethics Committee from the State University of Rio de Janeiro (CAAE 46769315.5.0000.5259), University of São Paulo Ethics Committee (CAAE 80921117.5.0000.5464), and the French ethics committee (French Ministry of Research and Innovation, DC-2020-4073. All data presented in this manuscript were fully anonymized, and no identifying images or personal information are included.

Consent to participate

We obtained written informed consent from the patients' legal guardians.

Consent to publish

Written informed consent for publication of clinical and genetic data was obtained from the patients' legal guardians.

Data Availability

The reported variants are available in the ClinVar database of genetic variants.

ClinVar Variation ID: 2582783, Accession: VCV002582783.1 (P1)

ClinVar Variation ID: 3236170, Accession: VCV003236170.1 (P2)

ClinVar Variation ID: 1331550, Accession: VCV001331550.4 (P3, P4 and P5; not submitted by us)

ClinVar Variation ID: 1703497, Accession: VCV001703497.1 (P6; not submitted by us)

ClinVar Variation ID: 2413123, Accession: VCV002413123.6 (P7; not submitted by us)

ClinVar Variation ID: 4530027, Accession: VCV004530027.1 (P8)

Additional data may be made available by the corresponding author upon reasonable request. Data sharing will be considered in accordance with participant privacy and ethical standards.

Author Contributions

Ana Cristina Victorino Krepischi and Cíntia Barros Santos-Rebouças conceived and designed the study. Laura Machado Lara Carvalho, Matheus Augusto Araújo Castro, Cíntia Barros Santos-Rebouças, and Ana Cristina Victorino Krepischi established collaborations, recruited patients, and coordinated the collection of clinical data and samples. Bianca Mie Sato Kurashima, Andressa Pereira Gonçalves, and Silvia Souza da Costa performed XCI pattern assays. Bianca Mie Sato Kurashima performed Sanger sequencing, wrote the initial version of the manuscript, and prepared figures 1 and 2 under the guidance of Ana Cristina Victorino Krepischi, Laura Machado Lara Carvalho, and Cíntia Barros Santos-Rebouças. Rafael Mina Piergiorgio and Cíntia Barros Santos-Rebouças performed *in silico* analyses. Rafael Mina Piergiorgio prepared figure 3. Lina Quteinheh, Maria Teresa Carminho-Rodrigues, Vincent Michaud, Fanny Morice-Picard, Caroline Rooryck, Matthias Begemann, and Larissa Mattern conducted formal analyses of patients. Francisco Cammarata-Scalisi, Mariana de Carvalho Moreira, Suely Rodrigues dos Santos, and Elis Vanessa de Lima Silva clinically evaluated patients. All authors contributed to Writing – Review & Editing and have read, revised, and approved the final version of the manuscript.

Competing Interests

The authors declare no conflicts of interest.

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Figures

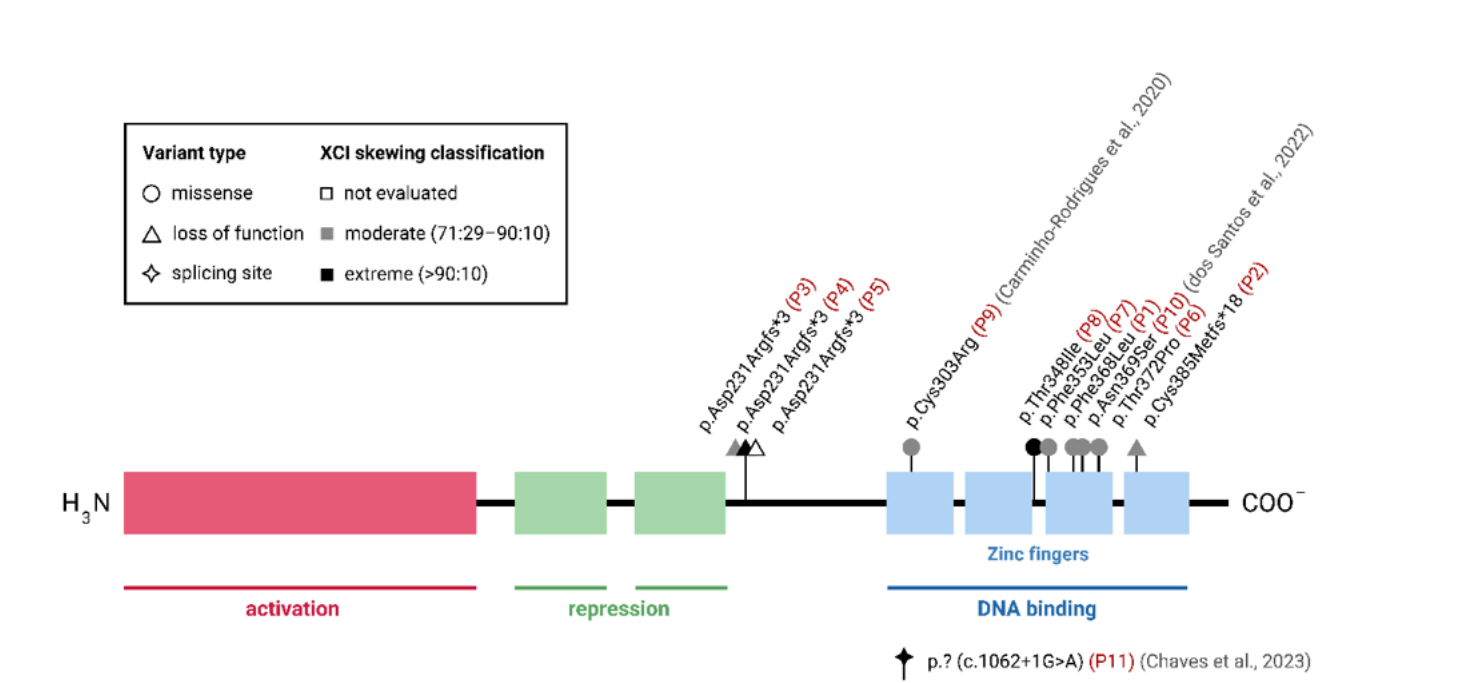


Figure 1
YY1 protein structure and position of pathogenic variants identified in the patients

Schematic diagram of YY1 protein, based on dos Santos et al. (2022). Position and nomenclature of YY1 pathogenic variants of patients whose XCI patterns have been evaluated are represented. Circles, triangles and stars portray missense, frameshift and splicing variants, respectively. Gray and black filled circles/triangles/stars represent moderate (ratios between 71:29 and 90:10) and extreme (>90:10 ratios) skewing, respectively, in blood DNA samples for the marker that exhibited the highest deviation from a random pattern (*AR*, *RP2*, *TMEM185A* or *HMGB3*). Not in scale

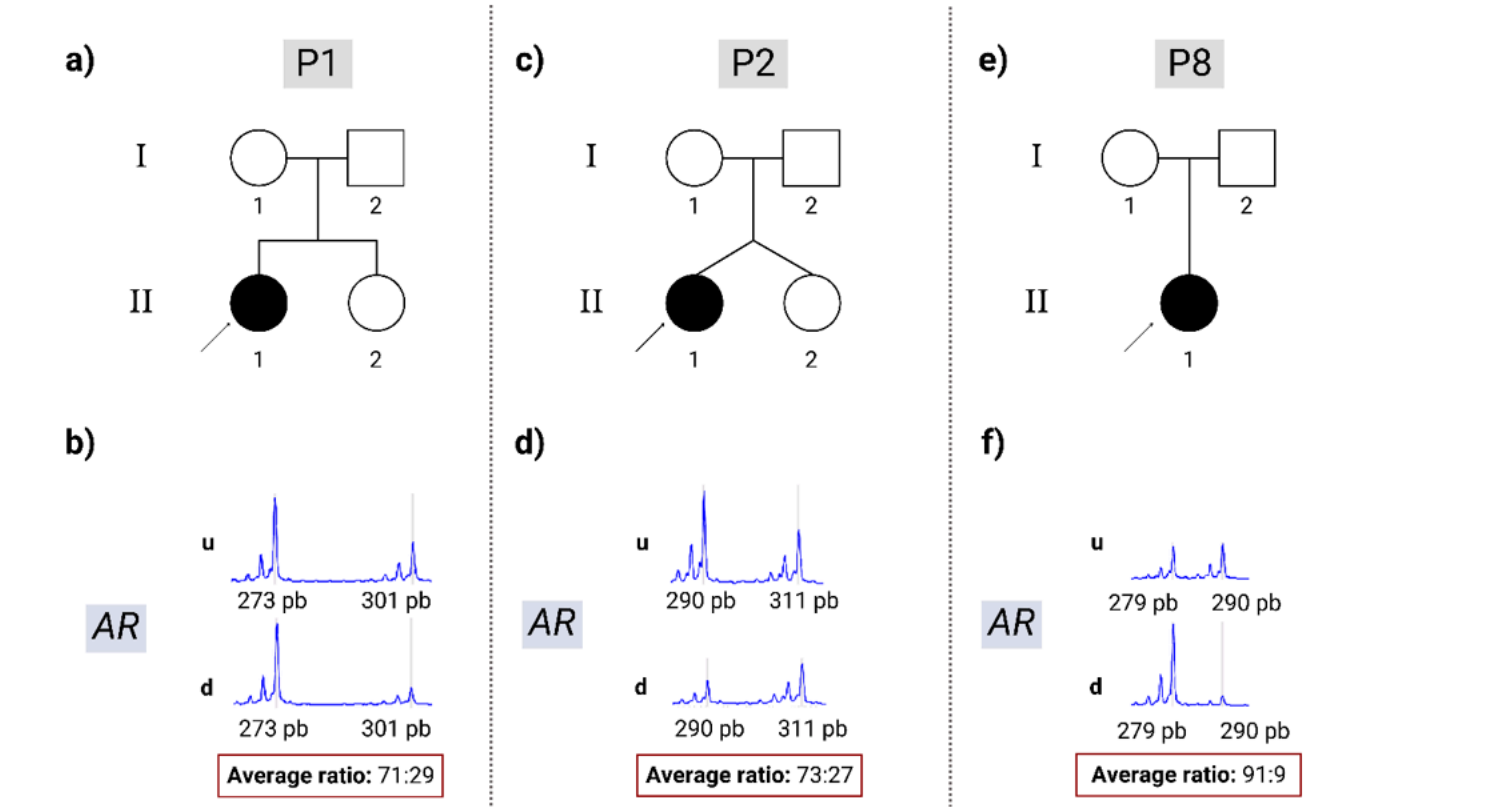


Figure 2

Pedigree charts of three female patients exemplifying the XCI study

Filled circles in the pedigree charts represent females diagnosed with GDVS. Red rectangles highlight moderate XCI skewing. u: undigested; d: digested. The graphics are not in scale. **a)** P1’s pedigree chart. **b)** XCI assay using P1’s blood DNA samples for the *AR* locus (71:29 average ratio). **c)** P2’s pedigree chart. **d)** XCI assay using P2’s blood DNA samples for the *AR* locus (73:27 average ratio). **e)** P8’s pedigree chart. **f)** Graphics resulting from XCI assay using P8’s blood DNA samples for the *AR* locus (91:9 average ratio)

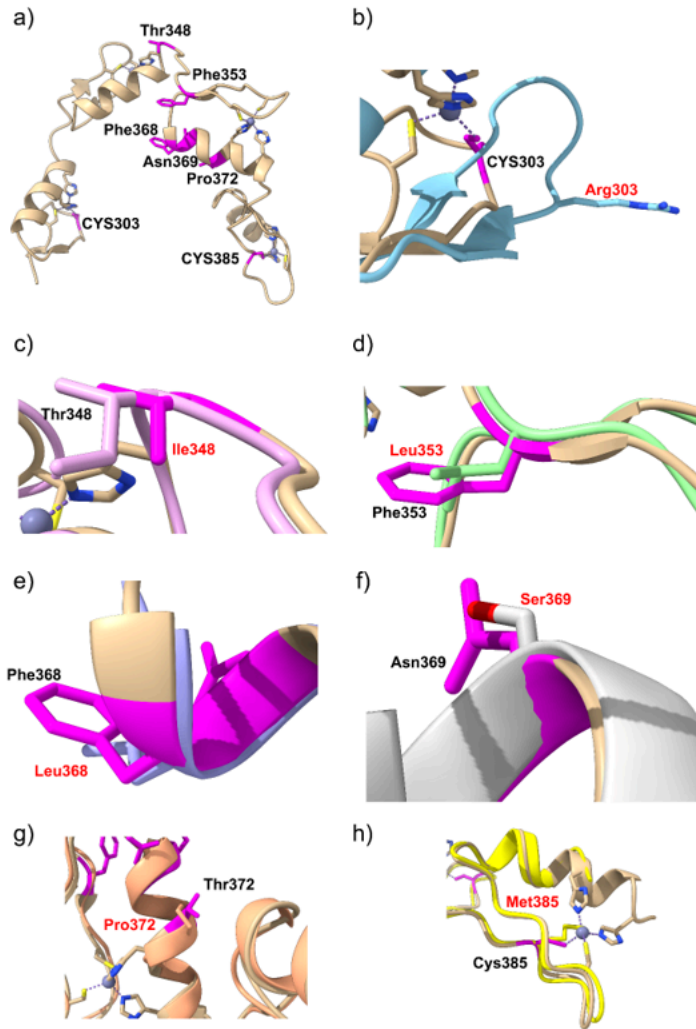


Figure 3

In silico analysis of the missense variants and p.Cys385Metfs*18 included in this study

a) Three-dimensional model of the YY1 protein complexed with DNA, obtained from the PDB database (ID: 1UBD). The regions highlighted in pink indicate the amino acid positions examined in this study. **b–h)** Structural overlap between the structural models generated by AlphaFold 3 and the crystallographic model. The altered residues are identified, and the side chains are highlighted. The variant p.Cys385Metfs*18 (h) introduces a premature stop codon and is therefore represented as a truncated protein model. The model (yellow) exhibits a loss of the C-terminal region extending beyond the α -helical segment, while the experimental structure (beige) remains fully resolved throughout the entire length of the molecule. Since the 231R mutation did not cover the crystallographic model, it was not represented in this figure. The text presented in red highlights the missense changes depicted in images b to h

Supplementary Files

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