Clinical Case

Autosomal dominant transmission reframes reproductive counseling in Myhre syndrome: A novel family and literature review

Maggie R. Brand,[1*] Eva Vanbelleghem,[2,3*] Alison C. Kay,[4,5,6] Anne Goriely,[4,5] Senol Demir,[7] Peter J. Hulick,[8] Breanne Prindeville,[8] Ashley W. Wong,[1] Bert Callewaert,[2,3#] Angela E. Lin[1#]

[1]Medical Genetics, Department of Pediatrics, Mass General for Children, Boston, Massachusetts, USA

[2]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

[3]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium

[4]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

[5]NIHR Biomedical Research Centre, Oxford, UK

[6]The Center for Personalised Medicine, University of Oxford, Oxford, UK

[7]Department of Medical Genetics, School of Medicine, Marmara University, Istanbul, Turkey

[8]Mark R. Neaman Center for Personalized Medicine, Endeavor Health, Evanston, Illinois, USA

* These authors contributed equally to this work.

# These authors contributed equally to this work.

Correspondence

Angela E. Lin, Medical Genetics, Department of Pediatrics, 175 Cambridge St., Mass General for Children, Boston, MA 02114, USA.

Email: lin.angela@mgh.harvard.edu

Running Title: MYHRE SYNDROME COUNSELING

CONFLICT OF INTEREST

Dr. Lin received support as an educational consultant from Myhre Syndrome Foundation (MSF)

AUTHOR CONTRIBUTIONS

Conceptualization: A.E.L., M.R.B., E.V., B.C.; Methodology and Data curation: A.E.L., M.R.B., E.V., B.C., B.P., P.J.H., S.D. Statistical analysis: E.V.; Writing-original draft: A.E.L., M.R.B, E.V., A.G., A.C.K, A.W.W. All listed authors meet authorship criteria, certifying active participation and individual contributions. Collectively, they take public responsibility for content, manuscript submission, and approval of the final draft.

FUNDING

The Clinical Research Coordinator position for the Myhre Syndrome Clinic is supported in part by the Myhre Syndrome Foundation and donors to the MGH Myhre Syndrome Clinic Sundry Fund. This study is partly supported by a grant of the EADV PPRC-2024-0022 to Bert Callewaert.

INSTITUTIONAL REVIEW BOARD

Research was approved by the Mass General Brigham Institutional Review Board (IRB) under protocols #2015P001173 and #2000P001531.

ABSTRACT

Myhre syndrome is a rare disorder that typically results from a *de novo SMAD4* variant. *De novo SMAD4* variants have recently been shown to be associated with 'selfish selection' in the male germline, explaining their exclusive paternal origin and the paternal age-effect reported for Myhre syndrome. Over recent years, there has been a steady increase in the number of families reported with an affected parent and child. We expand the literature of families with Myhre syndrome reporting a mildly affected 38-year-old mother and her 4-year-old son who carry the *SMAD4* p.(Arg496Cys) variant, consistent with all other reports of inherited Myhre syndrome. To better delineate the phenotypic spectrum, we developed a clinical severity score and compared familial cases to sporadic cases, revealing a milder phenotype in familial cases. Affected mothers with Myhre syndrome may be at increased risk of infertility and pregnancy loss. Since identification of the mode of transmission is essential for accurate reproductive counseling and appropriate clinical surveillance, we propose a nuanced reproductive and genetic counseling strategy that emphasizes awareness of potential autosomal dominant transmission, paternal age-related risk, and obstetric complications.

KEY WORDS

De novo mutations, Myhre syndrome, paternal age-effect, recurrence risk, selfish spermatogonial selection, reproductive genetic counseling

1 | INTRODUCTION

Myhre syndrome is a rare distinctive multiple congenital anomaly syndrome which has been increasingly diagnosed because of enhanced access to genetic testing (Lin et al., 2024; Vanbelleghem et al., 2024). Since the first report over 40 years ago of two affected males (Myhre et al., 1981), the phenotypic spectrum of Myhre syndrome, originally defined by short stature, distinctive facial appearance, hearing loss, thick skin, stiff joints, cardiovascular and airway abnormalities, and neurodevelopmental disabilities , has been expanded to include individuals with milder clinical manifestations. Modest genotype-phenotype differences have emerged showing that the recurrent pathogenic missense variation in *SMAD4* at codon 496 (p.Arg496Cys) is more likely to be associated with normal stature and less frequent aortic disease than individuals with codon 500 variants (p.Ile500Val); the rarity of the p.Ile500Thr, p.Ile500Met and p.Ile500Leu variants prevents robust comparison (Lin et al., 2024). The true prevalence of this genetic condition is unknown because people with mild features or those who lack access to testing may remain undiagnosed. Consistent with this, the Genome Aggregation Database (GnomAD v4.0, currently v4.1.0) reports seven individuals carrying the p.(Arg496Cys) variant and one individual carrying the p.(Ile500Val) variant in a cohort of approximately 800,000 individuals, indicating that the prevalence of Myhre syndrome may be as high as 1 in 100,000, surpassing earlier estimates of 1 in 1,000,000 (Vanbelleghem et al., 2024). Since 2022, three studies have expanded the emerging natural history in both pediatric and adult populations, adding a total of 66 new cases to the literature (Yang et al., 2022; Lin et al., 2024; Vanbelleghem et al., 2024). There has been a steady increase in familial cases, (Meerschaut at al., 2019, Demir et al., 2023, Vanbelleghem et al., 2024, Spineli-Silva at el., 2025) all associated with the *SMAD4* c.1486C>T pathogenic variant encoding p.(Arg496Cys).

A recent study has shown that Myhre Syndrome meets the criteria of a classic paternal age-effect (PAE) disorder (Wood et al., 2024). While PAE or 'selfish' *de novo* variants occur spontaneously during spermatogenesis, unusually they confer a selective advantage to mutant spermatogonial stem cells, which form clonal expansions in the testes as men age (Goriely & Wilkie, 2012; Wood &Goriely2022). This distinctive mechanism is observed in Myhre syndrome and is supported by (1) exclusive paternal origin of the causative *de novo* variants, (2) an epidemiological PAE where fathers are older than the population average, and (3) the increase in mutant sperm over time, which is anticipated to translate in an increased birth prevalence of the disorder (Wood et al 2024). The study estimated that men who had fathered a child with a *de novo SMAD4* variant were 6.3 years older than the average age of fatherhood in the general population. As a PAE disorder, sporadic cases of Myhre syndrome are anticipated to have a lower risk of recurrence due to parental gonadal mosaicism compared to most disorders caused *de novo* germline variants, which is typically estimated to be ~1-2% (Wilkie & Goriely, 2017). These new findings are not addressed in current recommendations on reproductive genetic counseling of families following the birth of a child with Myhre syndrome.

We expand the current literature by reporting on a new familial case of Myhre syndrome and comparing the phenotypic features of reported familial and sporadic cases using a newly developed clinical severity score.

2 | METHODS

2.1 | Editorial policies and ethical considerations

This research was approved by the MGH Institutional Review Board under protocol #2015P001173.

2.2 | Diagnosis and Genetic Nomenclature

Individuals with Myhre syndrome were confirmed to have a heterozygous *SMAD4* pathogenic variant through clinical diagnostic DNA sequencing tests. For the sake of brevity, the text of this article will refer to the affected protein residue rather than the cDNA nomenclature (Table 1).

Table 1. Complete nomenclature of *SMAD4* pathogenic variants (sequence variant nomenclature https://varnomen.hgvs.org/) (adapted from Table 1, Lin et al., 2024)

***SMAD4* variant (short form)**	**Complete name**	**Transcript: NM**	**mRNA change**	**Predicted protein change**
Arg496Cys	NM_005359.6(SMAD4):c.1486C>T (p.Arg496Cys)	NM_005359.6	c.1486C>T	p.Arg496Cys
Ile500Val	NM_005359.6(SMAD4):c.1498A>G (p.Ile500Val)	NM_005359.6	c.1498A>G	p.Ile500Val
Ile500Thr	NM_005359.6(SMAD4):c.1499T >C (p.Ile500Thr)	NM_005359.6	c.1499T>C	p.Ile500Thr
Ile500Met	NM_005359.6(SMAD4):c.1500A>G (p.Ile500Met)	NM_005359.6	c.1500A>G	p.Ile500Met
Ile500Leu	NM.005359.6(SMAD4):c.1498A>C (p.Ile500Leu)	NM_005359.6	c.1498A>C	p.Ile500Leu

2.3 | Phenotypic **Severity Analysis**

We compare the phenotypic features of a newly identified family with those of previously reported families and develop a clinical severity scoring system to classify the degree of phenotypic involvement (Table 2). This pragmatic severity score was derived from six key criteria: intrinsic pathology, disease progression, management options, prevalence in the general population, specificity for Myhre syndrome, and impact on quality of life. Each feature was assigned a score from 0 to 4, where 0 is “not severe” and 4 refers to the most severe manifestation according to our classification framework (Table 2). An overall severity score was calculated for all reported individuals listed in Table 4, except for those reported by Yang et al.

(2022) and Spineli-Silva et al. (2025), which were excluded due to insufficient data, and the two individuals from the newly reported family (Patients 1 and 2), who were excluded because the co-occurring variants could alter phenotyping. When the same individual was reported in multiple studies, only the most recent score was included to avoid duplication. For one individual previously reported by Vanbelleghem et al. (individual 11), updated molecular information became available indicating a *SMAD4* p.Arg496Cys variant. This information was incorporated into the current data analysis.

Table 2. Clinical severity score for the different presentation features associated with Myhre Syndrome

Phenotypic Features	Clinical Score 0-4
Restrictive cardiomyopathy	4
Multilevel airway stenosis	4
Tetralogy of Fallot, aortic valve stenosis, mitral valve stenosis, coarctation, large PDA (requiring palliation or surgery)	4
Hearing loss, moderate-severe	4
Short stature (more than -3 SD)	4
Intellectual disability, moderate-severe*	4
Aortic narrowing, thoracic and abdominal aorta, “mid-aortic syndrome” with or without branch stenosis	4
Pericarditis/pericardial restriction or pleuritis/pleural thickening or peritoneal adhesions	3
Diffusely hypoplastic aorta, moderate (Z-score of more than -2.0)	3
Restrictive lung disease	3
Endometrial cancer	3
Severe contractures	3
Behavioral changes with an impact on daily life, includes autism spectrum disorder	3
Severe expressive speech disability	3
Short stature (-2 SD to -3 SD)	2
Pericardial effusion	2
Pleural effusion	2

ASD, VSD	2
Hypoplastic aorta, mild (Z-score of -1.5 to -2.0)	2
Hypertension[1]	2
Scoliosis	2
Intellectual disability, borderline-mild*	2
Chronic, severe constipation	2
Obesity and/or overweight	2
Limited joint range of motion (extremities, spine, thorax, jaw)	1
Precocious puberty, menometrorrhagia	1

Abbreviations: ASD, atrial septal defect; PDA, patent ductus arteriosus; SD, standard deviation; VSD, ventricular septal defect

[1]Hypertension defined according to supplemental table 1, Methods, Lin et al., 2024

*The classification of borderline-mild and moderate-severe intellectual disability was based on the description in clinical reports and does not rely solely on IQ scores, which were not available for a substantial subset of individuals.

2.4 | Statistical analysis

Statistical analyses of the severity scores were performed using GraphPad Prism 10.2.3. Normality was assessed via histograms, QQ plots, and the Kruskal-Wallis test. Student's t-test was used for normally distributed data, and the Mann-Whitney U test for non-normal data. P-values of <0.05 were considered statistically significant.

3 | CLINICAL CASES

3.1 Patient 1.

The 3 years, 9-month-old male proband and his mother were referred for a multi-specialty evaluation at the Massachusetts General Hospital (MGH) Myhre syndrome clinic. Prenatal ultrasound at 20 weeks' gestation had revealed intrauterine growth restriction (IUGR). Cell-free fetal DNA screening performed early in the pregnancy was low risk for common aneuploidies. The proband was the fourth pregnancy born prematurely at 32 weeks via cesarean section to his 35-year-old mother who had 3 prior miscarriages and 37-year-old father (parental ages at conception). Growth parameters included weight of 1.45 kg (19th centile), length of 40 cm (22nd centile) and a head circumference of 28 cm (18th centile). In the postnatal period, no dysmorphic features were noted but he exhibited poor feeding, necessitating short-term tube feeding. At 9 months old, he underwent surgical repair for hypospadias.

Global developmental delays were noted at 6 months of age and a referral to early intervention services (EI) was initiated. Through EI, the proband received speech therapy, occupational therapy, and physical therapy. A developmental psychologist evaluation reported a global developmental delay, mixed receptive-expressive language disorder and a moderate risk for autism spectrum disorder. Due to developmental delays, the proband was referred to a neurologist who performed first tier genetic testing. Fragile X testing and chromosomal microarray were negative. A brain MRI showed very mild periventricular leukomalacia and white matter involvement.

The referring medical geneticist (PH) requested exome sequencing (ES) trio analysis when the proband was 2 years, 7 months. Testing revealed the presence of three (likely) pathogenic variants including a variant in *SMAD4* (c.1486C>T (p.Arg496Cys)), which molecularly confirmed the diagnosis of Myhre syndrome. The contributions to the Myhre syndrome phenotype from the two additional variants in *SMARCC2* (c.2925_2926del

(p.Ala977Cysfs*34), NM_003075.3) and *MED12L* (c.4754C>A (p.Ser1585*), NM_053002.4) will require follow up. Trio analysis identified maternal inheritance of both *SMAD4* and *MED12L* variants, while the variant in *SMARCC2* was *de novo* in the proband. Both the *MED12L* and *SMARCC2* variants are likely pathogenic although the proband's current phenotype is that of mild Myhre syndrome.

Following the molecular confirmation of the Myhre syndrome diagnosis, specialty consults and related testing were completed and a referral to the MGH Myhre syndrome clinic was initiated. At time of evaluation, the 3-year 9-month-old proband weighed 13.8 kg (15^{th} centile), and measured 95.5 cm in height (15^{th} centile). His facial appearance was mild but recognizable as Myhre syndrome, characterized by midfacial hypoplasia, a pointed chin, small mouth, short philtrum, and subjective hypertelorism. Minor joint anomalies included brachydactyly, clinodactyly and mild syndactyly of toes 2-3. He was also slightly hypotonic with stiff joints and a stiff gait. According to audiology reports, his hearing was normal, but he has had recurrent otitis media leading to placement of pressure-equalizing (PE) tubes at two years of age. His ophthalmologic exam noted "pseudostrabismus", intermittent esotropia, and hyperopia. A cardiology workup was performed, with the electrocardiogram and echocardiogram showing a normal structure, function, and sinus rhythm. The pulmonology workup was also unremarkable with no features of airway stenosis or obstructive sleep apnea.

3.2 Patient 2.

The mother of Patient 1 presented to the MGH Myhre Syndrome clinic at 38 years of age with mild Myhre syndrome features. Her weight was 51.1 kg, height was 159.9 cm, and she exhibited a mild but recognizable Myhre facial appearance, characterized by a prominent chin, small mouth with a restricted opening, short philtrum, and midfacial hypoplasia. Minor joint anomalies

included brachydactyly and clinodactyly, but no syndactyly. Her joints were mildly stiff, and contractures were absent except for a mildly decreased range of motion in the elbows.

Genetic testing confirmed that she carried the same *SMAD4* (c.1486C>T (p.Arg496Cys)) and *MED12L* (c.4754C>A (p.Ser1585*)) variants identified in her son. Her history revealed some unspecified developmental delays in childhood, and learning challenges that became more apparent at middle school and high school age. She struggled with mathematics and reading but successfully graduated from college with a bachelor's degree. She has worn eyeglasses since elementary school for myopia and astigmatism. At 19-20 years of age, she required a blood transfusion to treat severe anemia following heavy menstrual bleeding. Since then, she continued to have menometrorrhagia which has been effectively regulated by oral contraceptives have regulated her menstrual cycle. She had a history of fertility challenges including three miscarriages before conceiving her son via in vitro fertilization (IVF). A cardiology evaluation including an ECG and echocardiography was unremarkable, showing normal structure and function. However, a cardiac MRI demonstrated a trivial pericardial effusion and borderline thickened pericardium (4 mm). A likely hepatic cyst was also noted for which follow-up hepatic ultrasound has been planned.

4 | LITERATURE

To date, five families with Myhre syndrome have been reported in the literature (Demir et al., 2023; Meerschaut et al., 2019; Spineli-Silva et al., 2025; Vanbelleghem et al., 2024). Table 3 summarizes the clinical features of the previously reported families in addition to the new family reported here (pedigrees shown in Figure 1). Table 4 compares the phenotypic charactistics

features of the six familial cases with those of sporadic Myhre syndrome cases reported in natural history studies.

5 |RESULTS

5.1 Clinical features of inherited Myhre syndrome due to vertical transmission (all confirmed *SMAD4* Arg496Cys variants) (adapted from Table 1, Vanbelleghem et al., 2024)

Of the total of 18 familial cases (6 families; 8 females, 10 males), all carrying the Arg496Cys variant, median age at diagnosis was 30 years (range: 2 - 57 years). All individuals exhibited characteristic facial features with short stature in 10/18 (56%), and overweight in 5/11 (45%). Brachydactyly was observed in 7/15 (47%), limited range of motion in 8/11 (73%), and scoliosis, mostly mild, in 4/14 (29%). Congenital heart disease was found in 4/15 (27%) of cases, while arterial stenosis was present in 2/13 (15%) and hypertension in 5/11 (45%). No patient had pericarditis or restrictive cardiomyopathy, and a single patient had airway stenosis and restrictive lung disease. Intellectual disability was noted in 5/14 (36%) of cases, developmental delay in 9/14 (64%), and autism spectrum disorder in 3/9 (33%). Pseudostrabismus was present in 3/10 (30%), firm skin in 12/15 (80%), and abnormal wound healing in 3/8 (38%). 24% (4/17) of individuals had hearing loss. Constipation was reported in one individual. In three of the six families, the variant was paternally inherited, with no reported fertility issues (Patient 3, Demir et al., 2023; Patient 12, Vanbelleghem et al., 2024; Patient 4, Spineli-Silva et al., 2025). In two families, the variant was inherited from the mother, each having a history of infertility, multiple miscarriages, and who required the use of in vitro fertilization to conceive.

One of these women, Proband 1 described in Meerschaut et al. (2019), had a history of primary infertility, which led to the use of assisted reproductive treatment (ART) at 30 years of

age. She underwent three unsuccessful cycles of intrauterine insemination (IUI), followed by intracytoplasmic sperm injection (ICSI) treatment. Over nine ICSI cycles, she received 10 embryo transfers involving a total of 23 embryos. Eight of these transfers resulted in pregnancies; six ended in early miscarriage, one twin pregnancy, resulted in intrauterine demise of one fetus and the birth of her healthy child, and her second child was born following her most recent pregnancy. In the new family reported here, the mildly affected mother had a history of infertility for which intrauterine insemination was attempted. IVF resulted in three early miscarriages, followed by a successful pregnancy and the birth of her son. Prior to fertility treatments, she had normal transvaginal ultrasound, normal FSH, normal anti-Mullerian hormone, and normal testosterone. In one family, the parental origin of the transmission could not be determined (individuals 22-24 (three brothers), Vanbelleghem et al., 2024). Both parents had progressive hearing loss and passed away around age 50, with no clinical information available (Table 3).

5.2 Clinical features in reported sporadic individuals with Myhre Syndrome

In three recent natural history studies (Yang et al., 2022; Lin et al., 2024; Vanbelleghem et al., 2024), after excluding duplicates and individuals from familial cases, a total of 71 individuals with sporadic Myhre syndrome have been reported, including 47 (66%) with a codon 500 variant and 24 (34%) carrying the Arg496Cys variant. Overweight was observed in 19/70 (27%) of cases, while short stature was present in 45/58 (78%). Brachydactyly was reported in 52/70 (74%), limited range of motion in 64/67 (96%), and scoliosis in 12/59 (20%). Congenital heart disease was found in 31/64 (48%) of individuals, arterial stenosis in 10/63 (16%), and hypertension in 24/70 (34%). Airway stenosis was reported in 3/69 (4%), while restrictive lung

disease was present in 23/27 (85%). Intellectual disability was noted in 33/70 (47%) of cases, developmental delay in 67/71 (94%), and autism spectrum disorder in 50/71 (70%). Ophthalmologic findings included (pseudo)strabismus in 14/22 (64%) of cases and hyperopia in 38/69 (55%). Firm skin was present in 57/71 (80%), abnormal wound healing in 6/12 (50%), and constipation in 38/58 (66%). Hearing loss was reported in 35/71 (49%) of cases (Table 4).

5.3. Clinical severity scores

Severity scores (Table 2) were calculated for 71 individuals, including patients from Lin et al., Vanbelleghem et al., Demir et al., and Meerschaut et al. (sporadic and familial cases). Patients reported by Yang et al. and Spineli-Silva et al. were excluded because no recently updated severity scores were available. In addition, both individuals from the newly reported family (Patients 1 and 2) were excluded because the co-occurring variants could alter phenotyping. Among these, 59 (83%) were sporadic and 12 (17%) cases were familial, involving 4 different families. We first compared severity scores between different genotypes (codon 496 vs. codon 500 variants, n = 71, 35 vs. 36) which revealed significantly lower severity scores in the codon 496 group (mean: 11.7 vs. 18.8, $p<0.0001$, Figure 1A). When the scores for sporadic cases were compared to those for the familial cases, lower severity scores were observed in the familial group, with a borderline non-significant difference (mean: 16.1 vs. 11.5, $p=0.0517$, Figure 1B). When all sporadic cases were compared to only the probands of the familial cases (n=4 probands), a trend towards lower severity scores in the familial cases was observed, though the difference was not statistically significant (mean: 16.1 for all sporadic cases vs. 14.0 for probands of familial cases, $p=0.5973$, Figure 1C). Given the observed difference in severity scores between genotypes, we excluded sporadic cases with a codon 500 variant (since all

familial cases carry the Arg496Cys variant) and repeated the analysis. No significant difference in severity scores was found (mean: 11.8 for Arg496Cys sporadic cases vs. 11.5 for familial cases, p=0.8441, Figure 1D). Finally, when we restricted the analysis to probands only with the Arg496Cys variants, no significant difference was observed (mean: 11.8 for Arg496Cys sporadic cases vs. 14.0 for probands of familial cases, p=0.3941, Figure 1E).

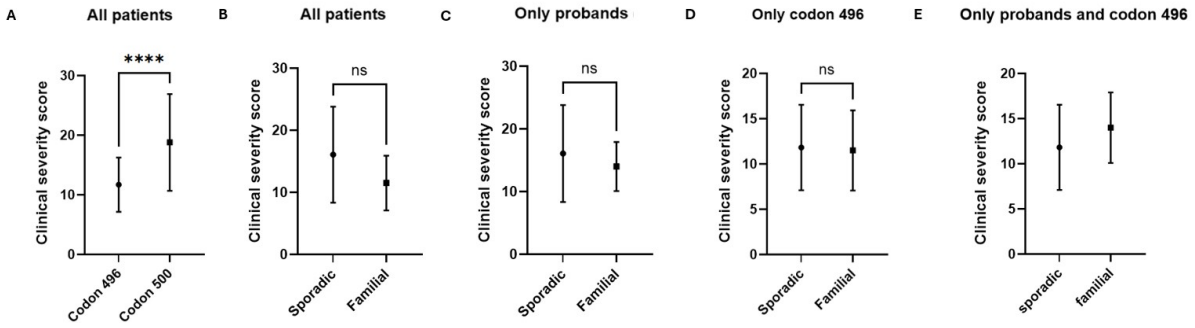

Figure 2. Comparison of severity scores across different patient groups. (A) Codon 496 (n=35) vs. codon 500 (n=36) variants, 11.7 vs. 18.8, p<0.0001. (B) Sporadic (n=59) vs. familial (n=12) cases, 16.1 vs. 11.5, p=0.0517. (C) Sporadic (n=59) vs. probands of familial cases (n=4), 16.1 vs. 14.0, p=0.5973. (D) Sporadic cases with Arg496Cys variant (n=23) vs. familial cases (n=12), 11.8 vs. 11.5, p=0.8441. (E) Sporadic cases with Arg496Cys variant (n=23) vs. probands of familial cases (n=4), 11.8 vs. 14.0, p=0.3941.

6 | DISCUSSION

6.1 | Fertility issues in Myhre syndrome

Possible fertility challenges and pregnancy loss for women diagnosed with Myhre syndrome are important topics to discuss during reproductive counseling. All reported familial cases of Myhre syndrome have been associated with the *SMAD4* Arg496Cys variant, however, a history of female infertility and early fetal loss remains a concern for these individuals. To date, no

spontaneous pregnancies have been reported in women with the Arg496Cys variant, although the sample size remains small (n = 2 confirmed affected mothers). While the mother in the newly reported family had normal laboratory results and transvaginal ultrasound findings prior to fertility treatments, it remains difficult to draw definitive conclusions regarding female fertility in Myhre syndrome. Currently, there are no detailed data on hormonal dosages and ovarian ultrasound parameters in these women. In contrast, so far fertility challenges have not been reported in men with Myhre syndrome. There have been no documented familial cases involving the Ile500 variants. Considering that the Ile500 variants are emerging as a clinically more severe, additional social or physical factors may also influence decisions around pursuing a pregnancy. Finally, an increased risk for endometrial cancer has been reported, possibly occurring more frequently in women with the Arg496Cys variant (Lin et al., 2020; Lin et al., 2024). This association may have implications for the timing and management of pregnancy, especially when IVF is required.

6.2 | Comparison of clinical features between sporadic and familial cases

Clinical features were generally more prevalent in sporadic cases compared to familial ones (Table 4). These included short stature (78% vs. 56%), brachydactyly (74% vs. 47%), congenital heart disease (48% vs. 27%), (pseudo)strabismus (64% vs. 30%), constipation (66% vs. 17%), and hearing loss (49% vs. 24%). Neurological involvement also appeared more pronounced in sporadic cases, with higher rates of intellectual disability (47% vs. 36%), developmental delay (94% vs. 64%), and autism spectrum disorder (70% vs. 33%). In contrast, certain features were more common among familial cases, such as restrictive lung disease (13% vs. 83%), overweight (27% vs. 45%), and arterial hypertension (34% vs. 45%).

Severity scores across patient groups (Table 2, Figure 1) were highest in individuals carrying a codon 500 variant when compared to those with the Arg496Cys variant. Although sporadic cases showed higher severity scores than familial cases overall (borderline non-significant, p=0.0517), this difference was no longer apparent after excluding individuals with a codon 500 variant. This suggests that the lower severity observed in familial cases is primarly due to the variant type (Arg496Cys) rather than the mode of inheritance. Milder phenotypes are likely underdiagnosed-due to ascertainment bias, whereas individuals with more severe presentations may experience reduced reproductive fitness, limiting familial transmission. Within familial cases, probands tended to have higher severity scores than other affected family members. This pattern is independent of whether the proband was a child, a parent, or another family member. Alternatively, higher mean age among familial cases may contribute to the greater prevalence of certain features due to age-related progression.

In addition to the maternally inherited *SMAD4* pathogenic variant, Patient 1 also inherited from his mother a nonsense variant in *MED12L* and was found to harbor a *de novo* frameshift variant in the *SMARCC2* gene. Loss-of-function variants in *SMARCC2* and *MED12L* are both associated with neurodevelopmental disorders, primarily characterized by developmental delay and/or intellectual disability, although severity can be variable. Given the extensive phenotypic variability of pathogenic variants in *SMARCC2* and limited literature regarding expressivity of *MED12L* pathogenic variants, it is difficult to assess the contribution of these variants on the proband's phenotype or that of his mother. This complex situation further adds to the challenge of delineating the specific phenotypic impact of the pathogenic *SMAD4* variant.

6.3 | Personalized recurrence risk counseling

The confirmation of a suspected genetic condition can have a significant impact on both the affected individual and their family, particularly regarding reproductive-decision making. In Myhre syndrome, which follows an autosomal dominant inheritance pattern, the recurrence risk in each pregnancy is 50% if one parent carries the pathogenic *SMAD4* variant in the heterozygous state. It is therefore important to perform parental segregation studies, even in apparently asymptomatic parents, to determine if the *SMAD4* variant is inherited or has occurred *de novo*. Identification of a familial *SMAD4* variant enables discussion of reproductive options, including preimplantation genetic testing (PGT) and prenatal diagnosis..

When a pathogenic heterozygous variant found in a child is absent in both parents, it most likely means that the variant occurred *de novo*, typically as a “one-off” event originating in one of the parent’s germ cells. In most cases, Myhre syndrome is caused by such *de novo* variants. However, these mutations may also occur early during gametogenesis, resulting in parental germline mosaicism, in which the variant is present in a subset of germ cells. This can theoretically confer a recurrence risk of up to 1–50% (Bernkopf et al., 2023). Recent finding that *de novo* variants associated with Myhre syndrome belong to the group of PAE disorders (Wood et al, 2024) has important implications for the genetic counseling provided to families as PAE disorders are less likely to involve parental gonadal mosaicism, and thus carry a lower recurrence risk than other sporadic disorders (Wilkie & Goriely, 2017). For parents of a child with sporadic Myhre syndrome considering fuure pregnancies, if the variant is proven to be *de novo*, it should be discussed that advanced paternal age is a general risk factor for the occurrence of *de novo* mutations.

While parent-of-origin discussions are not uncommon in genetic counseling, the recognition of PAE disorders introduces a new counseling challenge related to how *de novo*

results are communicated and understood. The finding that *de novo* variants in PAE disorders are exclusively of paternal origin represents relatively new information, which may not be widely known and can be emotionally difficult for families to process and integrate into their understanding of recurrence risk. In receiving a *de novo* result, a parent can experience a sense of relief that the cause of their child's condition was most likely not inherited from them. The introduction of *de novo* parent-of-origin information could remove this relief (Kay et al. 2024). The PREcision Genetic Counseling and REproduction (ePREGCARE) study (Kay et al., 2024) investigated UK providers views and experiences with a new *de novo* pre-conception personalized assessment tool able to provide recurrence information specific to each couple (Bernkopf et al., 2023), as an alternative to the population-average estimate of 1-2% given to couples in current practice. Providers reported that couples tended to be unaware of the paternal risks in the *de novo* context and introducing this information would require careful pre-test preparation to avoid eliciting feelings of blame and guilt (Kay et al., 2024). This is an important consideration for reproductive genetic counseling in Myhre syndrome as *de novo* variants originate exclusively in the paternal germline (Wood et al., 2024).

Nevertheless, this new understanding of *de novo* variants in PAE disorders indicates that the recurrence risk is substantially lower than the iatrogenic pregnancy loss associated with invasive prenatal testing, such as chorionic villus sampling. From a clinical perspective this may therefore not justify routine prenatal diagnostic testing in subsequent pregnancies. In the future, this may change as some countries, including the UK and Belgium, already have pilot projects aiming to identify *de novo* variants through non-invasive prenatal testing which would bypass the risk of a chorion villi biopsy (Zhang et al., 2019). Although there are currently no reports of parental somatic and germline mosaicism for a Myhre syndrome-related variants, such cases

could occur and deep sequencing of the variant across multiple tissues could help clarify this possibility (Bernkopf et al., 2023). However, we acknowledge that this approach is not routinely feasible in most clinical diagnostic settings.

7 | STUDY STRENGTHS AND WEAKNESSES

The strength of this study is the in-depth analysis and comparison of reported familial cases of Myhre syndrome using a newly developed clinical severity score, which can be generalized in future research. Comparisons between all cases, familial and sporadic, and between the Arg496 and Ile500 variants, allow the researchers to find genotype-phenotype correlations. The analysis between the two codons also allows for a more accurate determination in terms of clinical severity. Likewise, by comparing familial to sporadic cases it was possible to show that sporadic cases present a more severe phenotype than affected individuals within families, although ascertainment bias may exist.

The main weakness of this research is the small numbers of individuals, which is unavoidable given the rarity of the disorder. For example, we only had 2 families where the mutant allele had been inherited maternally and therefore it is not possible to formally show that Myhre syndrome is associated with female subfertility, which requires ART. As additional families are reported, the predominance of Arg496 in inherited cases may be further substantiated.

8 | CONCLUSION

Our research illustrates an updated approach to counseling that includes the importance of trio-based molecular testing, understanding the impact of advanced paternal age and disclosure of

parental origin for *de novo* cases, that likely will require a nuanced counseling approach, even though it likely signals a lower risk of recurrence due to gonadal mosaicism. Based on an increased number or reports of familial cases of Myhre syndrome, trio-based genetic testing is strongly endorsed. If not possible at the time of the initial proband testing, parents should be evaluated for the specific variant to determine inheritance and recurrence risk.

If the variant is found to be inherited, the recurrence risk in future pregnancies is 50% in the context of autosomal dominant disorder. Notably, we developed a severity score showing that familial cases tend to present with a generally milder phenotype than sporadic cases, potentially leading to underdiagnosis of the condition on clinical assessment.

For classical disorders associated with *de novo* variants, the recurrence risk is generally estimated at 1-2%, reflecting a theoretical risk of gonadal mosaicism. However, the recognition that Myhre syndrome is a PAE disorder implies that the contribution of mosaicism is likely to be lower (Wilkie & Goriely 2017) and therefore the risk of recurrence in a subsequent pregnancy for proven sporadic cases should be negligible. Hence, the implication of PAE in *de novo* cases with Myhre syndrome should be reassuring for the couple's future family planning but comes with the potentially difficult disclosure of the paternal origin of the genetic variant. Furthermore, the PAE likely contributes to an elevated prevalence of the disorder at the population level.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the new family. We extend our gratitude to the MGH Myhre syndrome clinical team and support staff.

REFERENCES

Bernkopf, M., Abdullah, U.B., Bush, S.J. et al. Personalized recurrence risk assessment following the birth of a child with a pathogenic *de novo* mutation. Nat Commun 14, 853 (2023). https://doi.org/10.1038/s41467-023-36606-w

Demir, S., Alavanda, C., Yesil, G., Aslanger, A. D., & Ates, E. A. (2023). A Second Family with Myhre Syndrome Caused by the Same Recurrent SMAD4 Pathogenic Variation (p.Arg496Cys). Mol Syndromol, 14(2), 175-180. https://doi.org/10.1159/000527149

Goriely, A., & Wilkie, A. O. (2012). Paternal age effect mutations and selfish spermatogonial selection: causes and consequences for human disease. *American journal of human genetics*, *90*(2), 175–200. https://doi.org/10.1016/j.ajhg.2011.12.017

Kay, A. C., Wells, J., Goriely, A., & Hallowell, N. (2024). Professionals' views on providing personalized recurrence risks for *de novo* mutations: Implications for genetic counseling. Journal of Genetic Counseling, 00, 1–13. https://doi.org/10.1002/jgc4.1910

Lin, A. E., Alali, A., Starr, L. J., Shah, N., Beavis, A., Pereira, E. M., Lindsay, M. E., & Klugman, S. (2020). Gain-of-function pathogenic variants in SMAD4 are associated with neoplasia in Myhre syndrome. American Journal of Medical Genetics. Part A, 182(2), 328–337. https://doi.org/10.1002/ajmg.a.61430

Lin, A. E., Alali, A., Starr, L. J., Shah, N., Beavis, A., Pereira, E. M., Lindsay, M. E., & Klugman, S. (2024). Corrigendum to “Gain-of function pathogenic variants in SMAD4 are associated with neoplasia in Myhre syndrome”. American Journal of Medical Genetics. Part A, 194 (6), e63497. https://doi.org/10.1002/ajmg.a.63497

Lin, A. E., Scimone, E. R., Thom, R. P., Balaguru, D., Kinane, T. B., Moschovis, P. P., Cohen, M. S., Tan, W., Hague, C. D., Dannheim, K., Levitsky, L. L., Lilly, E., DiGiacomo, D. V.,

Masse, K. M., Kadzielski, S. M., Zar-Kessler, C. A., Ginns, L. C., Neumeyer, A. M., Colvin, M. K., Elder, J. S., … MGH Myhre Syndrome Study Group (2024). Emergence of the natural history of Myhre syndrome: 47 patients evaluated in the Massachusetts General Hospital Myhre Syndrome Clinic (2016-2023). *American journal of medical genetics. Part A*, *194*(10), e63638. https://doi.org/10.1002/ajmg.a.63638

Meerschaut, I., Beyens, A., Steyaert, W., De Rycke, R., Bonte, K., De Backer, T., Janssens, S., Panzer, J., Plasschaert, F., De Wolf, D., & Callewaert, B. (2019). Myhre syndrome: A first familial recurrence and broadening of the phenotypic spectrum. American Journal of Medical Genetics. Part A, 179(12), 2494–2499. https://doi.org/10.1002/ajmg. a.61377

Myhre, S. A., Ruvalcaba, R. H., & Graham, C. B. (1981). A new growth deficiency syndrome. *Clinical Genetics*, **20**, 1–5. https://doi.org/10.1111/j.1399-0004.1981.tb01798.x

Spineli-Silva, S., Pontes, L. B., de Leeuw, N., Correia-Costa, G. R., de Wallau, M. B., Versiani, B. R., Mazzeu, J. F., Boy, R., Gil-da-Silva-Lopes, V. L., & Vieira, T. P. (2025). SMAD4 Pathogenic Variants in Seven New Brazilian Individuals With Myhre Syndrome Including a New Family. *American journal of medical genetics. Part A*, e63984. Advance online publication. https://doi.org/10.1002/ajmg.a.63984

Vanbelleghem, E., Van Damme, T., Beyens, A. et al. Myhre syndrome in adulthood: clinical variability and emerging genotype-phenotype correlations. Eur J Hum Genet 32, 1086–1094 (2024). https://doi.org/10.1038/s41431-024-01664-

Wilkie, A. O. M., & Goriely, A. (2017). Gonadal mosaicism and non-invasive prenatal diagnosis for 'reassurance' in sporadic paternal age effect (PAE) disorders. *Prenatal diagnosis*, *37*(9), 946–948. https://doi.org/10.1002/pd.5108

Wood, K. A., & Goriely, A. (2022). The impact of paternal age on new mutations and disease in the next generation. *Fertil Steril.*118(6):1001-1012. https://doi.org/10.1016/j.fertnstert.2022.10.017

Wood, K. A., Tong, R. S., Motta, M., Cordeddu, V., Scimone, E. R., Bush, S. J., Maxwell, D. W., Giannoulatou, E., Caputo, V., Traversa, A., Mancini, C., Ferrero, G. B., Benedicenti, F., Grammatico, P., Melis, D., Steindl, K., Brunetti-Pierri, N., Trevisson, E., Wilkie, A. O., Lin, A. E., … Goriely, A. (2024). SMAD4 mutations causing Myhre syndrome are under positive selection in the male germline. *American journal of human genetics*, *111*(9), 1953–1969. https://doi.org/10.1016/j.ajhg.2024.07.006

Yang, D. D., Rio, M., Michot, C., Boddaert, N., Yacoub, W., Garcelon, N., Thierry, B., Bonnet, D., Rondeau, S., Herve, D., Guey, S., Angoulvant, F., & Cormier-Daire, V. (2022). Natural history of Myhre syndrome. Orphanet Journal of Rare Diseases, 17, 304. https://doi.org/10.1186/s13023-022-02447-x

Zhang, J., Li, J., Saucier, J. B., Feng, Y., Jiang, Y., Sinson, J., McCombs, A. K., Schmitt, E. S., Peacock, S., Chen, S., Dai, H., Ge, X., Wang, G., Shaw, C. A., Mei, H., Breman, A., Xia, F., Yang, Y., Purgason, A., Pourpak, A., ... Eng, C. M. (2019). Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA. *Nature Medicine*, *25*(3), 439–447. https://doi.org/10.1038/s41591-018-0334-x

URL WEB RESOURCES

GeneReviews. https://www.ncbi.nlm.nih.gov/books/NBK425723/

MGH Myhre Syndrome Clinic https://www.massgeneral.org/children/myhre-syndrome

Myhre Syndrome Foundation https://www.myhresyndrome.org/

DATA AVAILABILITY

Additional clinical data about the two new individuals (mother and son) is not available.

ORCID numbers

Bert Callewaert https://orcid.org/0000-0002-9743-4205

Senol Demir https://orcid.org/0000-0002-8374-0359

Anne Goriely https://orcid.org/0000-0001-9229-7216

Peter Hulick https://orcid.org/0000-0001-8397-4078

Alison Kay https://orcid.org/0000-0002-1432-2516

Angela E. Lin https://orcid.org/0000-0002-1145-4572

Eva Vanbelleghem https://orcid.org/0000-0001-6380-2471

Table 3. Clinical features of families with Myhre syndrome (all *SMAD4* Arg496Cys) (adapted from Table 1, Vanbelleghem et al., 2024)

Clinical Features	This report **Patient 2**[1]	This report **Patient 1***[1]	Meerschaut et al., 2019 **Proband 1***	Meerschaut et al., 2019 **Child 1**	Meerschaut et al., 2019 **Child 2**	Demir et al., 2023 **Patient 1***	Demir et al., 2023 **Patient 2**	Demir et al., 2023 **Patient 3**	Vanbelleghem et al., 2024 **Patient 12**	Vanbelleghem et al., 2024 **Patient 13***	Vanbelleghem et al., 2024 **Patient 14**
Relationship in family	Mother of pt. 1	Son of pt. 2	Mother of child 1,2	Dau of pro. 1	Son of pro. 1	Son of pt. 3	Dau of pt. 2	Father of pts. 1,2	Bro of pt. 13, fa of pt. 14	Bro of pt. 12, uncle of pt. 14	Dau of pt. 12, niece of pt. 13
Sex	F	M	F	F	M	M	F	M	M	M	F
Age at diagnosis (years)	37	3	50	16	12	12	9	36	58	55	24
Paternal age (conception)(yrs)	NS	37	NS	NS	NS	NS	NS	28	NS	NS	33
Typical Myhre syndrome facial features	+	+	+	+	+	+	+	+	+	+	+
Severity score	6	4	18	16	9	13	11	7	12	16	4
Growth											
Weight (kg)	51.1	13.8	76	12.3	3.9	74	63	130	Unk	80.8	67.8
Overweight	-	-	NS	NS	NS	+	NS	+	NS	+	+
Height (cm)	159.9	95.5	159	96	53	161	137	172	159.5	163	158.7
Short stature	+	+	-	+	+	-	-	-	+	+	-
MSK Features											
Brachydactyly	+	+	NS	NS	NS	+	+	+	+	+	-
Limited joint ROM	-	-	+	+	NS	NS	NS	+	+	+	-
Pseudohypertrophy	-	-	NS	NS	NS	NS	NS	NS	+	+	-
MSK pain	-	-	NS	NS	NS	NS	NS	NS	-	-	+
Scoliosis	-	-	+, mild	+, severe	+, mild	-	-	-	-	-	+, mild
Cardiovascular											

Heart disease	-	-	NS	NS	NS	NS	NS	NS	-	-	-
CHD	-	-	+	+	+	NS	+	NS	-	-	-
Aorta stenosis	-	-	NS	NS	NS	-	-	NS	+	+	-
Pericarditis**	-	-	NS	NS	NS	-	-	NS	-	-	-
RCM**	-	-	NS	NS	NS	-	-	NS	-	-	-
Hypertension	-	-	NS	NS	NS	NS	NS	NS	+	+	-
Respiratory											
Airway stenosis	-	-	+	-	-	NS	NS	NS	-	NS	-
Restrictive lung disease	-	-	+	-	-	NS	NS	NS	-	NS	NS
Neurodevelopment											
ID	-	-	NS	NS	NS	+	NS	-	-	-	-
DD	+	+	NS	NS	NS	+	+	-	-	-	-
ASD/autistic behavior	-	-	NS	NS	NS	+	NS	NS	+	+	-
Ophthalmology											
Strabismus/ pseudostrabismus	-	+	NS	NS	NS	+	+	-	-	NS	-
Hyperopia	-	+	NS	NS	NS	NS	NS	-	-	NS	-
Myopia	+	-	NS	NS	NS	NS	NS	-	-	NS	-
Astigmatism	+	-	NS	NS	NS	NS	NS	-	NS	NS	NS
Hearing Loss	-	-	-	-	+	-	-	-	-	+	-
Skin											
Firm skin	+	+	+	+	+	+	+	+	+	+	+
Hyperkeratosis pilaris	+	+	NS	NS	NS	NS	NS	NS	-	NS	-
Abn. wound healing	+	+	NS	NS	NS	NS	NS	+	-	NS	-
Gastrointestinal											
Constipation	-	+	NS	NS	NS	NS	NS	NS	NS	NS	-
Abdominal pain	-	-	NS	NS	NS	NS	NS	NS	NS	NS	-

Table 3 (Continued)

Clinical Features	Vanbelleghem et al., 2024 **Patient 22**	Vanbelleghem et al., 2024 **Patient 23***	Vanbelleghem et al., 2024 **Patient 24**	Spineli-Silva et al., 2025 **Patient 1***	Spineli-Silva et al., 2025 **Patient 2**	Spineli-Silva et al., 2025 **Patient 3**	Spineli-Silva et al., 2025 **Patient 4**
Relationship in family	Bro of pt. 23 and 24	MZ bro of pt. 24, bro of pt. 22	MZ bro of pt. 23, bro of pt. 22	Dau of pt. 4, sister of pts. 2,3	Dau of pt. 4, sister of pts. 1,3	Dau of pt. 4, sister of pts. 1,2	Father of pts. 1,2,3
Sex	M	M	M	F	F	F	M
Age at diagnosis (years)	48	45	45	8	2	5	57
Paternal age (conception)(yrs)	36	34	34	NS	NS	NS	NS
Typical Myhre syndrome facial features	+	+	+	+	+	+	+
Severity score	16	9	7				
Growth							
Weight (kg)	88	Unk	Unk	34.4	44.6	35.4	70
Overweight	+	NS	NS	-	-	-	-
Height (cm)	160.2	157.5	152.4	155	161	152	153
Short stature	+	+	+	-	-	-	+
MSK Features							
Brachydactyly	-	-	-	-	-	-	-
Limited joint ROM	+	+	+	NS	NS	NS	NS
Pseudohypertrophy	NS	NS	NS	NS	NS	NS	NS
MSK pain	+	+	+	NS	NS	NS	NS
Scoliosis	-	-	-	NS	NS	NS	NS
Cardiovascular							
Heart disease	-	-	-	-	-	-	NS
CHD	-	-	-	-	-	-	NS
Aorta stenosis	-	-	-	-	-	-	NS
Pericarditis**	-	-	-	-	-	-	NS
RCM**	-	-	-	-	-	-	NS
Hypertension	+	+	+	-	-	-	NS
Respiratory							

Airway stenosis	-	-	-	NS	NS	NS	NS
Restrictive lung disease	NS	-	-	NS	NS	NS	NS
Neurodevelopment							
ID	+	-	-	+^	+	+^	-
DD	+	+	+	+	+	NS	-
ASD/autistic behavior	-	-	-	NS	NS	NS	NS
Ophthalmology							
Strabismus	-	-	-	NS	NS	NS	NS
Hyperopia	+	-	-	NS	NS	NS	NS
Myopia	-	-	-	NS	NS	NS	NS
Astigmatism	NS	NS	NS	NS	NS	NS	NS
Hearing Loss	+	+	-	-	-	-	NS
Skin							
Firm skin	+	NS	NS	-	-	-	NS
Hyperkeratosis pilaris	+	NS	+	NS	NS	NS	NS
Abn. wound healing	-	-	-	NS	NS	NS	NS
Gastrointestinal							
Constipation	-	-	-	NS	NS	NS	NS
Abdominal pain	NS	NS	NS	NS	NS	NS	NS

[1]Severity analysis excluded patients 1 and 2

*First family member diagnosed with Myhre syndrome (proband)

**The following features were not present in any of these individuals: RCM, pericarditis

^These individuals have clinical signs of intellectual disability, but have had no formal testing

Abbreviations: Abn, abnormal; ASD, autism spectrum disorder; bro, brother; CHD, congenital heart disease; dau, daughter; DD, developmental delays; F, female; Fa, father; ID, intellectual disability; M, male; Mo, mother; MSK, musculoskeletal; MZ bro, monozygotic twin brother; NS, not stated; pro, proband; pt(s), patient(s); RCM, restrictive cardiomyopathy; ROM, range of motion; Unk, unknown

Table 4. Clinical features of families with Myhre Syndrome compared to features from sporadic cases in natural history studies

Author, year, patient no.	This report	Meerschaut et al., 2019	Demir et al., 2023	Vanbelleghem et al., 2024 **Family 1**	Vanbelleghem et al., 2024 **Family 2**	Spineli-Silva et al., 2025	Yang et al., 2022	Lin et al., 2024	Vanbelleghem et al., 2024
Total no. of individuals	2	3	3	3	3	4	12 (6 new)	47 (43 new)	12* (11 new)
SMAD4 variant	Arg496Cys	Arg496Cys	Arg496Cys	Arg496Cys	Arg496Cys	Arg496Cys	Ile500: 11/12 Arg496: 1/12	Ile500 :29/47 Arg496: 18/47	Ile500: 7/12 Arg496: 5/12
Dysmorphic facial features	2/2	3/3	3/3	3/3	3/3	4/4	5-11/12	46/47	NS
Growth									
Overweight or obese	0/2	NS	2+/1 NS	2+/1 NS	1+/2 NS	NS	4/12	6/47	9/11
Short stature	2/2	2/3	0/3	2/3	3/3	1/4	NS	34/47	11/11
MSK Features									
Brachydactyly	2/2	NS	3/3	2/3	0/3	0/4	11/11	30/47	11/12
Limited joint ROM	0/2	2+/1 NS	1+/2 NS	2/3	3/3	NS	8/9	44/47	12/12
Pseudohypertrophy	0/2	NS	NS	2/3	NS	NS	9/12	27/47	9/11
MSK pain	0/2	NS	NS	1/3	3/3	NS	NS	12/47	4/12
Scoliosis	0/2	3/3	0/3	1/3	0/3	NS	NS	10/47	2/12
Cardiovascular									
Heart disease	0/2	NS	NS	0/3	0/3	3-/1 NS	NS	6/47	5/12
Congenital	0/2	3/3	1+/2NS	0/3	0/3	3-/1 NS	7/12	22/47	2/5
Arterial stenosis	0/2	NS	2-/1NS	2/3	0/3	3-/1 NS	3/7	6/47	1/9
Hypertension	0/2	NS	NS	2/3	3/3	3-/1 NS	3/11	18/47	3/12
Respiratory									
Airway stenosis	0/2	1/3	NS	2-/1 NS	0/3	NS	2/12	1/47	0/10
Restrictive lung disease	0/2	1/3	NS	1-/2 NS	2-/1 NS	NS	NS	17/17	6/10
Neurodevelopmental									
Intellectual disability	0/2	NS	1+/1-/1 NS	0/3	1/3	3/4**	9/12	15/47	9/11
Developmental delays	2/2	NS	2/3	0/3	3/3	2+/1-/1 NS	9/12	47/47	11/12
Autism spectrum disorder/autistic behavior	0/2	NS	1+/2 NS	2/3	0/3	NS	1/12	42/47	7/12
Ophthalmology									
Strabismus/ pseudostrabismus	1/2	NS	2/3	2-/1 NS	0/3	NS	5/11	NS	9/11
Hyperopia	1/2	NS	1-/2 NS	2-/1 NS	1/3	NS	6/12	26/47	6/10

Myopia	1/2	NS	1-/2NS	2-/1 NS	0/3	NS	NS	6/47	5/11
Hearing Loss	0/2	1/3	0/3	1/3	2/3	3-/1 NS	7/12	18/47	10/12
Skin									
Firm or thick skin	2/2	3/3	3/3	3/3	1+/2 NS	3-/1 NS	8/12	38/47	11/12
Hyperkeratosis pilaris	2/2	NS	NS	2-/1 NS	2+/1 NS	NS	NS	NS	2/12
Abnormal/poor wound healing	2/2	NS	1+/2 NS	2-/1 NS	0/3	NS	NS	NS	6/12
Skin									
Constipation	1/2	NS	NS	1-/2 NS	0/3	NS	NS	32/47	6/11
Abdominal pain	0/2	NS	NS	1-/2 NS	NS	NS	NS	19/47	6/9

Abbreviations: + present; - absent; MSK, musculoskeletal; NS, not stated; ROM, range of motion

*Familial cases and duplicate individuals (including four overlapping cases with Lin et al., 2024) were excluded.

**2 out of the 4 individuals in this family had clinical signs of intellectual disability, but no formal neuropsychologic testing.