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Case Report

PTPN11 and *FLNA* variants in a boy with ambiguous genitalia, short stature, and non-specific dysmorphic features

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Highlights

- Digenic variants of *PTPN11* and *FLNA* are possibly associated with ambiguous genitalia in boys with Noonan syndrome.
- Whole-exome sequencing is a powerful tool for the diagnosis of patients with atypical disease manifestations.

Abstract. Noonan syndrome is a congenital disorder characterized by distinctive facial appearance, congenital heart defects, short stature, and skeletal dysplasia. Although boys with Noonan syndrome frequently exhibit cryptorchidism, a mild form of 46,XY disorders of sex development (DSD), they barely manifest more severe genital abnormalities. Here, we report a boy with ambiguous genitalia, short stature, and non-specific dysmorphic features. He had no cardiac abnormalities or skeletal dysplasia. His score in the Noonan syndrome diagnostic criteria (36 of 157 points, 23%) was lower than the cutoff for diagnosis (50%). Whole-exome sequencing identified a *de novo* heterozygous variant (c.922A>G: p.Asn308Asp) in *PTPN11* and a maternally inherited hemizygous variant (c.1439C>T: p.Pro480Leu) in *FLNA*. The *PTPN11* variant was a known causative mutation for Noonan syndrome. *FLNA* is a causative gene for neurodevelopmental and skeletal abnormalities and has also been implicated in 46,XY DSD. The p.Pro480Leu variant of *FLNA* was assessed as deleterious by *in silico* analyses. These results provide evidence that whole-exome sequencing is a powerful tool for diagnosing patients with atypical disease manifestations. Furthermore, our data suggest a possible role of digenic mutations as phenotypic modifiers of Noonan syndrome.

Key words: DSD, hypospadias, oligogenic, mutation screening, WES

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Introduction

Noonan syndrome is a congenital disorder that typically arises from monoallelic pathogenic variants in the RAS/MAPK signaling genes including *PTPN11* (1). Patients with Noonan syndrome characteristically present with facial dysmorphologies, congenital heart defects, short stature, and skeletal dysplasia to varying degrees (2). In addition, boys with Noonan syndrome frequently exhibit cryptorchidism, a mild form of 46,XY disorders of sex development (DSD) (3), although these individuals rarely manifest more severe genital abnormalities such as hypospadias (4).

46,XY DSD is a multifactorial disorder caused by genetic and environmental factors. To date, more than 300 human genes have been implicated in this condition (5). These genes include *FLNA*, an X chromosomal gene that encodes a multifunctional protein (NM_001110556). *FLNA* is known as a causative gene for neurodevelopmental and skeletal abnormalities (6). Rare variants in *FLNA* have been identified in a few patients with 46,XY DSD without neuronal or skeletal anomalies (7, 8).

Here, we report a boy who exhibited ambiguous external genitalia, short stature, developmental delay, and non-specific dysmorphologies. Whole-exome sequencing (WES) identified a pathogenic *PTPN11* variant that explains most of his clinical features and a rare variant in *FLNA* that may be associated with the 46,XY DSD phenotype.

Case Presentation and Molecular Analysis

This study was approved by the Institutional Ethics Committee of the National Center for Child Health and Development. Written informed consent was obtained from the patient's parents. The boy was born at 35 wk and six days of gestation after an uncomplicated pregnancy. He was the first child of a non-consanguineous Japanese couple. His body size at birth was within the normal range [(height, 45 cm (–1.9 SD); weight 2,172 g (–1.6 SD); and head circumference, 31 cm (–1.3 SD)]. He had no family history of Noonan syndrome or DSD. At birth, he showed severe micropenis (14 mm, reference range: 25–39 mm) and hypospadias with a urethral opening at the perineal part of the penis, together with chordee, bifid scrotum, and cryptorchidism (**Fig. 1a**). Magnetic resonance imaging detected testes of 1–2 mL at the bilateral inguinal region (**Fig. 1a**). A human chorionic gonadotropin stimulation test on the 10th postnatal day yielded a mild increase in testosterone levels (**Table 1**). Endocrine evaluation at two months of age showed an increased level of follicle-stimulating hormone and a normal level of anti-Müllerian hormone (AMH), together with significant gonadotropin responses to gonadotropin-releasing hormone stimulation comparable to that in pubertal children (**Table 1**). G-banding showed a normal 46,XY karyotype, and fluorescence *in situ* hybridization detected a signal of *SRY*. Therefore, the patient was diagnosed with syndromic 46,XY DSD of unknown etiology and assigned to the male sex. He manifested

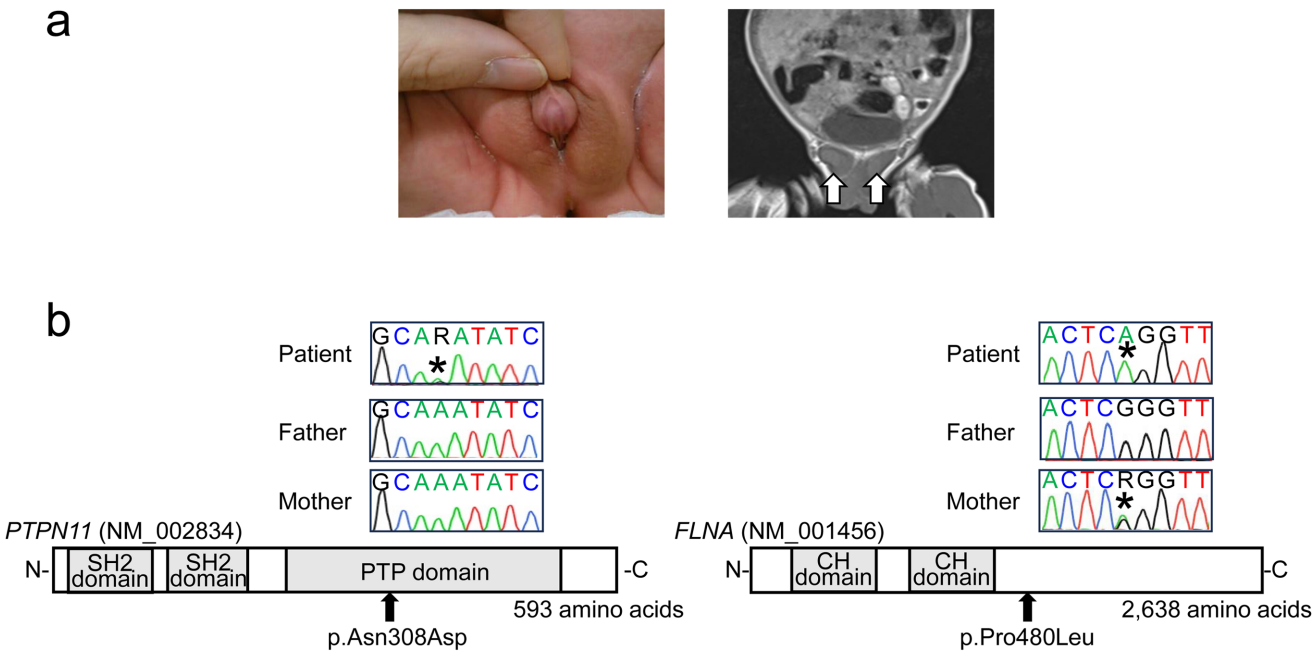

Fig. 1. (a) Genital findings of the patient. The left panel shows ambiguous external genitalia with micropenis, hypospadias, cryptorchidism, and bifid scrotum. The right panel is the result of magnetic resonance imaging showing bilateral undescended testes at the inguinal regions. White arrows indicate the testes. (b) *PTPN11* and *FLNA* variants identified in the patient. The mutated nucleotides are indicated by asterisks. The positions of the variants in the PTPN11 and FLNA proteins are indicated by thick arrows. PTP domain, protein tyrosine phosphatase domain; SH2 domain, src-homology 2 domain; CH domain, calponin homology domain.

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growth failure from one month of age and thereafter grew along the –3.0 or –3.5 SDS growth curves. During the first year of life, his developmental milestones were appropriate; he attained head control at three months of corrected age and could walk alone at 1.2 yr of corrected age. However, at 2 yr of age, he was noted to have mild language delay. He underwent orchidopexy at 1.4 yr of age and urethroplasty and chordee repair at 2.5 yr of age.

At 2.6 yr of age, the patient underwent systematic clinical and genetic evaluations. His height was 79.7 cm (–3.2 SD), and his weight was 9.8 kg (–2.5 SD). His motor development was almost equivalent to his age, whereas he had a mild speech delay. Several physical characteristics, such as sparse hair, wide-set eyes, epicanthic folds, thin lips, and pectus carinatum, were noted. Genomic DNA samples were obtained from the patient and his parents. Microarray-based comparative genomic hybridization ruled out pathogenic copy number variations in the patient's genome. WES using trio samples was performed as described previously (9). We searched for *de novo* variants, as well as rare (< 1% in the general population) damaging variants in 336 causative/candidate genes for 46,XY DSD (5) and 165 causative/candidate genes for growth failure (10). As a result, we identified two *de novo* variants and one damaging variant in 46,XY DSD causative genes (**Table 2**). First, a *de novo* heterozygous missense substitution (c.922A>G: p.Asn308Asp) was detected in *PTPN11* (**Fig. 1b**). This variant has previously been identified in multiple patients with Noonan syndrome (11). The variant was classified as "pathogenic" according to the ACMG/AMP guidelines (PS1 + PS2 + PM1 + PM5 + PP1 + PP2 + PP3 + PP4). Second, a *de novo* heterozygous missense substitution (c.3706C>T, p.Arg1236Trp) was identified in *TAF1L*, a testis-specific gene of unknown function. This variant was assessed as "likely pathogenic" (PS2 + PM2 + PP3). Lastly, a hemizygous missense substitution (c.1439C>T, p.Pro480Leu) was identified in *FLNA* (**Fig. 1b**). This variant was assessed as a "variant of unknown significance (VUS)" (PM5 + PP1 + PP3) according to the ACMG/AMP guidelines, and has been submitted as a variant with "conflicting interpretations of pathogenicity" in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). The variant was assessed as deleterious by *in silico* assays and had a low frequency in the general population. The *FLNA* variant was shared by the patient's mother.

Since the patient was found to carry a pathogenic

Table 1. Endocrine data of the patient

Age at examination	Hormone value of the patient			Reference value		
				Prepubertal (mini-puberty)	Prepubertal	Pubertal (Tanner 3)
10 d	Testosterone (nmol/L)[a]	Stimulated	6.4	No reference	5.1–6.9	17.4–24.7
2 mo	LH (IU/L)[b]	Baseline	3.2	0.6–4.1	0–0.4	0.8–4.2
		Peak	37.7	No reference	0.4–6	18.2–38.0
	FSH (IU/L)[b]	Baseline	6.5	0.4–3.0	0.6–3.0	2.9–10.8
		Peak	30.6	No reference	6.3–15.6	5.8–22.3
	Testosterone (nmol/L)					
	Baseline (ELISA)[b]	Baseline	5.1	0.7–7.6	0.5–0.8	2.4–4.2
	AMH (pmol/L)	Baseline	606	425–1,810	529–1,057	No reference

AMH, anti-Müllerian hormone. [a]A human chorionic gonadotropin stimulation test (3000 IU/m^2/dose [max. 5000 IU] i.m. for three consecutive days; blood sampling on days one and four). [b]A GnRH stimulation test (100 μg/m^2 [max. 100 μg] bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 min).

Table 2. Rare variants identified in the patient

Gene		Variant								
Name	Mode of inheritance[a]	cDNA	Protein	Zygosity	CADD_phred[b]	1000G_all[c]	ToMMo[d]	gnomAD[e]	Inheritance	Amino acid conservation[f]
PTPN11	Autosomal dominant	c.922A>G	p.Asn308Asp	Heterozygote	22.3	–	–	1.2×10^{-5}	*De novo*	High
TAF1L	Unknown	c.3706C>T	p.Arg1236Trp	Heterozygote	25.7	–	–	–	*De novo*	High
FLNA	X-linked	c.1439C>T	p.Pro480Leu	Hemizygote	26.6	–	–	7.5×10^{-5}	Mother	High

[a]Mode of inheritance of the associated disease. [b]Functional prediction using combined annotation-dependent depletion (CADD, https://cadd.gs.washington.edu/). Scores above 20 are indicative of damaging effects. [c]Allele frequency in the 1000 Genomes Project (https://www.genome.gov/27528684/1000-genomes-project). [d]Allele frequency in the Tohoku Medical Megabank Organization Database (ToMMo, https://www.megabank.tohoku.ac.jp/). [e]Allele frequency in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/). [f]Conservation of the amino acid at the variant site across species: high, conserved from fish to humans; medium, conserved from mice to humans; and low, conserved only among primates.

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variant in *PTPN11*, we re-examined his phenotype focusing on the clinical features associated with Noonan syndrome. He had some facial dysmorphologies, but no Noonan syndrome-specific facial appearance (Supplementary Table 1). Echocardiography and electrocardiography ruled out congenital heart defects. Consequently, his score on the diagnostic criteria of Noonan syndrome (36 of 157 points, 23%) was lower than the cutoff value for diagnosis (50%) (Supplementary Table 1) (12).

Discussion

We performed WES for a boy with ambiguous external genitalia, short stature, and non-specific dysmorphic features. We identified a *de novo* p.Asn308Asp variant of *PTPN11*, a known causative mutation for Noonan syndrome (1). Notably, the patient lacked Noonan syndrome-specific features, such as characteristic facial appearance, skeletal dysplasia, and congenital heart defects (1). Consequently, the patient had a low score in the diagnostic criteria of Noonan syndrome (Supplementary Table 1) (12). Hence, he was diagnosed with Noonan syndrome only after the detection of the *PTPN11* variant, although he exhibited several non-specific dysmorphic features, such as short stature, moderate hypertelorism, epicanthic folds, and cryptorchidism, which are consistent with Noonan syndrome (1). These results highlight the phenotypic diversity of Noonan syndrome and provide evidence that WES is useful for diagnosing patients with mild or atypical phenotypes of congenital syndromes. On the other hand, WES frequently identifies pathogenicity-unknown variants. For example, our patient carried a VUS in *TAF1L* (described below). Hence, WES results need to be interpreted carefully during genetic counseling.

The patient exhibited ambiguous genitalia with cryptorchidism and hypospadias. Of these, cryptorchidism is a common feature of Noonan syndrome observed in 40–80% of the cases (1, 4). Cryptorchidism in Noonan syndrome is assumed to result from partial Sertoli cell dysfunction due to the perturbed RAS/ERK signaling (13) or reduced expression of *INSL* and other genes involved in the testicular descent (14). The mildly elevated gonadotropin levels of our patient are consistent with partial gonadal dysfunction in Noonan syndrome (13). However, hypospadias has rarely been documented in boys with Noonan syndrome. For example, Athota *et al*. reported that none of 221 male patients with Noonan syndrome exhibited hypospadias, while 97 had cryptorchidism (4). Ea *et al*. performed sequence analysis for 293 male patients with isolated hypospadias and identified no pathogenic mutations in *PTPN11*, except for one VUS (5). Thus, the severe 46,XY DSD phenotype of our patient including hypospadias cannot be explained by the *PTPN11* variant alone. In this context, the patient carried no additional *de novo* variants except for the p.Arg1236Trp variant in *TAF1L*. The significance of this *TAF1L* variant was unknown because this gene has not been linked to human disorders. In addition, the patient had no pathogenic variants in known causative genes for 46,XY DSD, with the exception of the p.Pro480Leu variant in *FLNA*. Loss-of-function variants of *FLNA* have been causally associated with neurodevelopmental and skeletal disorders (15, 16). Although 46,XY DSD is not a common symptom of patients with *FLNA* mutations, hypospadias and cryptorchidism have been reported in a few cases (7, 8, 17). Importantly, Carrera-Garcia *et al*. proposed that FLNA is involved in the nuclear translocation and activation of the androgen receptor (17). Hence, the p.Pro480Leu variant in our patient may have caused his 46,XY DSD phenotype by affecting the androgen signaling pathway. Indeed, severe hypomasculinization of the patient under the presence of normal levels of testosterone and AMH is consistent with partial androgen insensitivity (18). While he exhibited no neuronal or skeletal features indicative of *FLNA* abnormalities, this may reflect the broad phenotypic variations of *FLNA* mutations (17). Notably, Tsai *et al*. reported a male patient with rare variants in both *PTPN11* and *FLNA* (p.Asp61Gly and p.Glu622Lys, respectively) (19). The patient manifested a typical phenotype of Noonan syndrome together with cryptorchidism and hypospadias. Tsai *et al*. confirmed that the patient carried no pathogenic variants in other DSD-associated genes. These results are consistent with our findings and indicate that *FLNA* variants can facilitate the development of 46,XY DSD in boys with Noonan syndrome. However, since no *in vitro* functional assays have been performed for p.Pro480Leu and p.Glu622Lys, it remains uncertain whether these variants affect the protein function of FLNA. Considering that cryptorchidism and hypospadias are relatively common multifactorial disorders, we cannot exclude the possibility that these abnormalities in the patients reported by us and Tsai *et al*. were caused by hitherto unrecognized genetic or environmental factors. The significance of *FLNA* variants in the etiology of 46,XY DSD needs to be confirmed in future studies.

In summary, we report a boy with 46,XY DSD and non-specific dysmorphic features, who carried rare variants in *PTPN11* and *FLNA*. The results of this study provide evidence that WES is a powerful tool for diagnosis of patients with atypical disease manifestations. Furthermore, our data suggest a possible role of digenic mutations as phenotypic modifiers of Noonan syndrome.

Conflict of interests: The authors declare no conflicts of interest.

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References

1. Roberts AE. Noonan syndrome. [updated 2022 Feb 17]. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW *et al*. editors. GeneReviews®[Internet] 2001. Seattle (WA): University of Washington, Seattle; 1993–2023.
2. Romano AA, Allanson JE, Dahlgren J, Gelb BD, Hall B, Pierpont ME, *et al*. Noonan syndrome: clinical features, diagnosis, and management guidelines. Pediatrics 2010;126: 746–59. [Medline] [CrossRef]
3. Domenice S, Batista RL, Arnhold IJP, Sircili MH, Costa EMF, Mendonca BB. 46,XY differences of sexual development. 2022. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, *et al*. editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000–.
4. Athota JP, Bhat M, Nampoothiri S, Gowrishankar K, Narayanachar SG, Puttamallesh V, *et al*. Molecular and clinical studies in 107 Noonan syndrome affected individuals with PTPN11 mutations. BMC Med Genet 2020;21: 50. [Medline] [CrossRef]
5. Ea V, Bergougnoux A, Philibert P, Servant-Fauconnet N, Faure A, Breaud J, *et al*. How far should we explore hypospadias? next-generation sequencing applied to a large cohort of hypospadiac patients. Eur Urol 2021;79: 507–15. [Medline] [CrossRef]
6. Robertson S. X-linked otopalatodigital spectrum disorders. [updated 2019 Oct 3]. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, *et al*. editors. GeneReviews® [Internet] 2005. Seattle (WA): University of Washington, Seattle; 1993–2023.
7. Zidoune H, Ladjouze A, Chellat-Rezgoune D, Boukri A, Dib SA, Nouri N, *et al*. Novel genomic variants, atypical phenotypes and evidence of a digenic/oligogenic contribution to disorders/differences of sex development in a large North African cohort. Front Genet 2022;13: 900574. [Medline] [CrossRef]
8. Rjiba K, Mougou-Zerelli S, Hamida IH, Saad G, Khadija B, Jelloul A, *et al*. Additional evidence for the role of chromosomal imbalances and SOX8, ZNRF3 and HHAT gene variants in early human testis development. Reprod Biol Endocrinol 2023;21: 2. [Medline] [CrossRef]
9. Igarashi M, Masunaga Y, Hasegawa Y, Kinjo K, Miyado M, Saitsu H, *et al*. Nonsense-associated altered splicing of MAP3K1 in two siblings with 46,XY disorders of sex development. Sci Rep 2020;10: 17375. [Medline] [CrossRef]
10. Yang L, Zhang C, Wang W, Wang J, Xiao Y, Lu W, *et al*. Pathogenic gene screening in 91 Chinese patients with short stature of unknown etiology with a targeted next-generation sequencing panel. BMC Med Genet 2018;19: 212. [Medline] [CrossRef]
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al*. ACMG Laboratory Quality Assurance Committee Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17: 405–24. [Medline] [CrossRef]
12. Duncan WJ, Fowler RS, Farkas LG, Ross RB, Wright AW, Bloom KR, *et al*. A comprehensive scoring system for evaluating Noonan syndrome. Am J Med Genet 1981;10: 37–50. [Medline] [CrossRef]
13. Moniez S, Pienkowski C, Lepage B, Hamdi S, Daudin M, Oliver I, *et al*. Noonan syndrome males display Sertoli cell-specific primary testicular insufficiency. Eur J Endocrinol 2018;179: 409–18. [Medline] [CrossRef]
14. Hutson JM. Cryptorchidism and Hypospadias. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, *et al*. editors. Endotext [Internet] 2022. South Dartmouth (MA): MDText.com, Inc.; 2000–.
15. Jamuar SS, Lam AT, Kircher M, D'Gama AM, Wang J, Barry BJ, *et al*. Somatic mutations in cerebral cortical malformations. N Engl J Med 2014;371: 733–43. [Medline] [CrossRef]
16. Parrini E, Mei D, Pisanti MA, Catarzi S, Pucatti D, Bianchini C, *et al*. Familial periventricular nodular heterotopia, epilepsy and Melnick-Needles Syndrome caused by a single FLNA mutation with combined gain-of-function and loss-of-function effects. J Med Genet 2015;52: 405–12. [Medline] [CrossRef]
17. Carrera-García L, Rivas-Crespo MF, Fernández García MS. Androgen receptor dysfunction as a prevalent manifestation in young male carriers of a FLNA gene mutation. Am J Med Genet A 2017;173: 1710–3. [Medline] [CrossRef]
18. Chen Z, Li P, Lyu Y, Wang Y, Gao K, Wang J, *et al*. Molecular genetics and general management of androgen insensitivity syndrome. Intractable Rare Dis Res 2023;12: 71–7. [Medline] [CrossRef]
19. Tsai MC, Weng YH, Lin YF, Wang YC, Yu HW, Chou YY, *et al*. Whole-exome sequencing identified rare genetic variants associated with undervirilized genitalia in Taiwanese pediatric patients. Biomedicines 2023;11: 242. [Medline] [CrossRef]

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