

Case Report

Clinical and molecular analysis of Noonan syndrome in Indonesia: a case report

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Noonan syndrome (NS; OMIM#163950) is a relatively common autosomal dominant disorder with a worldwide prevalence of approximately 1:1,000 to 1:2,500. The syndrome is characterized by distinctive facial features, congenital heart defects (CHD), and short stature. Distinctive facial features consist of a broad and high forehead, hypertelorism, downslanting palpebral fissures, a high arched palate, low set and posteriorly rotated ears with a thick helix, and a short neck with excess nuchal skin and low posterior hairline. Additional relatively frequent features include chest deformities, cryptorchidism in males, mild intellectual disability, and bleeding diathesis.^{1,2}

In 2001, missense mutations in the *PTPN11* gene were reported in about 50% of NS cases. This gene encodes the tyrosine protein phosphatase non-receptor SHP2, which is involved in ERK activation via RAS-MAPK pathway.³ Later, several other genes involved in the RAS-MAPK pathway were found to be mutated in NS individuals without *PTPN11* mutations, including *KRAS*, *SOS1*, *RAF1*, *NRAS*, *BRAF*, *MAP2K1*, and *RIT1*, as well as mutations in *SHOC2* and *CBL* causing an NS-like disorder (Noonan syndrome-like disorder with loose anagen hair/NSLH and Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia/NSLL, respectively).^{2,4,5} The involvement of two other genes, *A2ML1* and *RRAS* is still unclear.^{6,7}

Despite the relatively high frequency of NS in the general population, no Indonesian patients have been reported. Here, we report the first Indonesian case with a confirmed molecular diagnosis of NS.

Keywords: Noonan syndrome, Turner syndrome, *PTPN11* gene, molecular analysis

The Case

A fifteen-year-old girl was suspected to have Turner syndrome and was referred by her pediatric endocrinologist to the Center for Biomedical Research (CEBIOR), Semarang, Central Java, Indonesia, for a cytogenetic analysis. She was short compared to the average height of her peers. On physical examination, her height was 137 cm (<3rd

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Figure 1. The posterior view of an Indonesian patient with Noonan syndrome shows a low posterior hairline and a webbed neck.

centile) and her weight was 39 kg (<5th centile). She had a normal head circumference (54 cm). Facial dysmorphisms included a high forehead and depressed nasal bridge. In addition, she had a webbed neck, a low posterior hairline, and clinodactyly of both 5th toes. The parents consented only to showing a picture of the back of the patient (**Figure 1**). The result of growth hormone (GH) testing was within the normal range, as were thyroid function tests. Chromosomal analysis showed a normal female karyotype (46, XX), even after reculture and counting more cells in order to exclude

mosaicism for Turner syndrome. Further history-taking by the pediatrician and medical geneticists revealed that she had a congenital heart defect. At the age of 10 years, echocardiography showed mild pulmonary regurgitation, tricuspid regurgitation, and trivial mitral regurgitation. Follow-up echocardiography showed normal heart valves, an intact interatrial and interventricular septum, and good systolic function. Bone age radiography at the age of ten years was consistent with that of a 7-year-old girl. Moreover, she had normal menstrual cycles and breast development. No other family members had the same phenotype. Based on the clinical and additional investigations, NS was suspected.

Molecular analysis of the *PTPN11* gene was performed in the Genome Diagnostics Laboratory of the Radboud University Medical Center in Nijmegen, The Netherlands. The DNA was extracted from peripheral blood lymphocytes using a salting-out method. All exons and flanking splice sites of the *PTPN11* gene were sequenced on a 3730 automated sequencer (*Applied Biosystems*, Foster City, CA). Primer sequences and PCR conditions are available upon request. A missense mutation in exon 3 was identified: c.317A>C, p.(Asp106Ala) (based on GenBank Accession Number NM_002834.3, nomenclature according to the Human Genome Variation Society (HGVS) guidelines.⁸ Segregation analysis in both parents revealed that the mutation was *de novo* in the child (**Figure 2**).

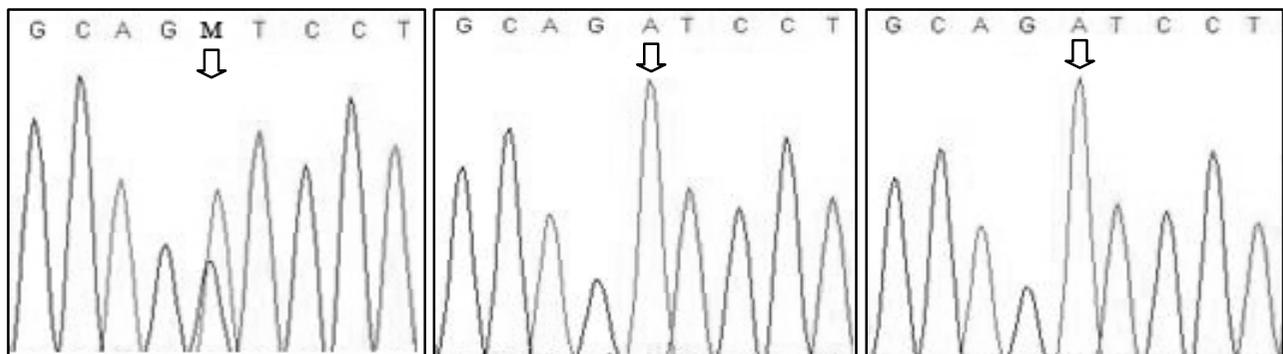


Figure 2. Electropherograms of the patient-parents trio. (A): patient, (B): father, and (C): mother. The mutation was absent in both parents, confirming *de novo* occurrence. The position of the c.317A>C mutation is indicated by the arrow. The “M” represents the heterozygous A/C.

Discussion

In our patient with a clinical suspicion of NS, a *de novo* missense mutation in the *PTPN11* gene was detected. This mutation [c.317A>C; p(Asp106Ala)] was first reported in 2002 by Tartaglia *et al.* in two sporadic cases and in one family.⁹ Our patient had a webbed neck, low posterior hairline, a congenital heart defect, and short stature, all of which are consistent with the NS features. The patient also showed the typical dysmorphic facial features, as previously described.¹⁰

In the worldwide population, NS is relatively common. The incidence was estimated to be approximately 1:1,000 to 1:2,500.¹ In 2013, the Indonesian population was estimated to consist of almost 250 million people.¹¹ Therefore, between 100,000-250,000 NS cases should be present in Indonesia. Nevertheless, to our knowledge, no NS cases from Indonesia have been reported in either national or international literature. We would not expect NS to be less prevalent in Indonesia than in other parts of the world, since most mutations occur *de novo*. Furthermore, the heart defect is generally not lethal in NS, so it is also unlikely that most NS patients in Indonesia die at a young age, before they are recognized as NS patients. The most likely explanation for the lack of reporting on NS in Indonesia is that the clinical features are not observed to be peculiar so NS cases are often not recognized. A clinical and molecular diagnosis of NS is important, however, because parents should be informed as to the etiology of the condition in their child, the risk of recurrence in future pregnancies, and treatment options.

Noonan syndrome shares many phenotypic similarities with Turner syndrome (TS), the most well known chromosomal aberration in females. However, differentiation between NS and TS can be determined by chromosomal analysis. Turner syndrome is the most prevalence complete or mosaic monosomy X in females, while NS is a single gene mutation affecting both females and males.¹² Furthermore, to aid the diagnostic process, several scoring systems have been developed. The most commonly used method was developed by van der Burgt *et al.* in 1994 (Table 1). A clinical diagnosis of NS can be made when the typical facial dysmorphism plus another major sign are present, or two minor signs or suggestive facial dysmorphism plus two major signs are present, or

Table 1. Clinical features of our patient compared to those in published cases of NS

Clinical features*	Our patient
Facial	
A: Typical dysmorphism	+
B: Suggestive dysmorphism	
Cardiac	
A: PVS, HCM, and/or ECG typical of NS	
B: Other defects	+
Height	
A: <3 rd percentile	+
B: <10 th percentile	
Chest wall	
A: Pectus carinatum/excavatum	NA
B: Broad thorax	
Family history	
A: First-degree relative with definite NS	-
B: First-degree relative with suggestive NS	
Other	
A: ID, cryptorchidism, and lymphatic dysplasia	+
B: One of ID, cryptorchidism, or lymphatic dysplasia	

* Adapted from van der Burgt (2007),¹ A: major sign, B: minor sign, NA: not available, PVS: pulmonary valve stenosis, HCM: hypertrophic cardiomyopathy, ECG: electrocardiogram, ID: intellectual disability.

three minor signs are present.¹

Facial features change with age and become less noticeable in adulthood. Thus, some adults may be recognized as having NS after their child has been diagnosed with NS. While birth length and weight are usually normal in NS patients, feeding difficulties in the neonatal period often occur and may cause failure to thrive. This condition can resolve spontaneously later in infancy. During childhood, the mean height is within the lower end of the normal range. With delayed puberty and reduced or absent pubertal growth spurt, the mean height falls below the normal range. A growth catch-up may present in the late teen years.^{2,13}

More than 70% of NS patients have a broad spectrum of CHDs. The two most common defects are pulmonary valve stenosis (PVS), with or without dysplastic valve, and hypertrophic cardiomyopathy (HCM). The presence of PVS is most commonly associated with a mutation in *PTPN11*, whereas HCM is associated with a mutation in *RAF1* and *RIT1*.^{2,4} Other relatively frequent features are unilateral or bilateral cryptorchidism in males, skeletal abnormalities particularly superior pectus carinatum and inferior pectus excavatum, as

well as bleeding diathesis.^{1,2} Management guidelines for NS, including an NS growth chart were released in 2010.¹⁴ Administration of GH showed short-term growth acceleration, but outcomes of long-term treatment remain inconclusive.¹⁵ Therefore, the policy to offer GH treatment to NS patients differs among countries.¹⁶ Regular cardiac evaluations are recommended during GH treatment.¹⁷ Furthermore, as NS is a genetic and multisystem disorder, a multidisciplinary team approach to patient care is essential for better quality of life in NS individuals.

In conclusion, we report the first Indonesian NS case with molecular confirmation. Establishing a molecular diagnosis is not only important for confirmation of the diagnosis, but also for genetic counseling to patients and their families. Since most of the mutations occur *de novo*, the recurrence risk for future offspring is very low, less than 1%. However, when one of the parents appears to have NS, the recurrence risk is 50%.¹⁸ The publication of this Indonesian NS case is important to raise awareness for this relatively common disease, as it has likely been underestimated in the Indonesian population. Establishing a clinical diagnosis, and when possible a molecular confirmation, is important for patients and their families.

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Conflict of interest

None declared.

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