Molecular characterization using exome sequencing of two probands with the undiagnosed developmental delay from Central Brazil

Caracterização molecular usando sequenciamento de exoma de dois probandos com atraso desenvolvimento não diagnosticados do Brasil Central

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ABSTRACT

Noonan syndrome (NS) is a heterogeneous autosomal dominant disorder caused by germline mutations in genes belonging RAS-MAPK pathway. Herein, we described two patients with developmental delay and syndromic features from Central Brazil diagnosed with NS using an exome sequencing target gene panel. Germline mutation in genes that participates in the RAS-MAPK signaling pathway are associated with developmental disorders that share particular clinical features such as craniofacial dysmorphisms, congenital heart defects, musculoskeletal and ocular abnormalities, and neurocognitive impairment. The exome sequencing through the intellectual disability gene panel was an effective approach to identify de novo pathogenic mutations in SOS1 and PTPN11 genes that are responsible for Noonan syndrome and was an efficient method to direct the adequate clinical management and better follow-up of the probands and their families.

Keywords: SOS1; PTPN11; WES; Noonan Syndrome; Developmental delay

RESUMO

A síndrome de Noonan (SN) é uma doença autossômica dominante heterogênea causada por mutações na linha germinativa em genes pertencentes à via RAS-MAPK. O objetivo deste estudo foi relatar dois casos de pacientes com atraso de desenvolvimento e características sindrômicas do Brasil Central com diagnóstico de SN, por meio do sequenciamento de exoma usando um painel de genes. A mutação da linha germinativa em genes que participam da via de sinalização RAS-MAPK está associada a distúrbios de desenvolvimento que compartilham características clínicas particulares, como dismorfinos craniofaciais, defeitos cardíacos congênitos, anormalidades musculoesqueléticas e oculares e comprometimento neurocognitivo. O sequenciamento do exoma através do painel de genes da deficiência intelectual foi uma abordagem eficaz para identificar mutações patogênicas de novo nos genes SOS1 e PTPN11 responsáveis pela síndrome de Noonan e foi um método eficiente para direcionar o manejo clínico adequado e melhor acompanhamento dos probandos e suas famílias.

Palavras-chave: SOS1; PTPN11; WES; Síndrome de Noonan; Atraso no desenvolvimento

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INTRODUCTION

Noonan syndrome (NS) is a heterogeneous autosomal dominant disorder caused by germline mutations in genes belonging to the rat sarcoma viral oncogene (RAS)/mitogen-activated protein kinase (MAPK) pathway. In general, those genes encode proteins to interact with extracellular growth factors to regulate cellular proliferation, differentiation, survival, and metabolism (KOH et al., 2019; LEE; YOO, 2019; WANG; SHI; JIAO, 2020). Both downstream and upstream mutations in the RAS-MAPK genes are related to a wide range of developmental alterations in NS (TAFAZOLI et al., 2017).

The estimated global incidence of Noonan Syndrome is around 1 in 1,000-2,500 live births. Affected individuals present multiple congenital malformations, including dysmorphic craniofacial features, short stature, congenital heart diseases, skeletal abnormalities, and developmental delay (KOH et al., 2019; SHOJI et al., 2019; TEKENDO-NGONGANG et al., 2019). Cognitive decreases such as attention deficit, impaired social cognition, developmental delay, learning and intellectual disabilities and various degrees of behavioral problems are frequently observed among children with NS (PIERPONT; TWOROG-DUBE; ROBERTS, 2015; JOHNSON et al., 2019).

Nowadays, germline gain-of-function mutations in 30 RAS-MAPK genes have been implicated in six different syndromes, leading to the classification of those conditions in a group called the RASopathies (LEE; YOO, 2019; KIM; BAEK, 2019; ATHOTA et al., 2020). In this context, NS is the most common RASopathy and PTPN11 on chromosome 12q24.1 was the first gene described as responsible for its etiology (TARTAGLIA et al., 2001). Since then, there are fourteen different genes associated with NS (BABAN et al., 2019; ATHOTA et al., 2020). Missense mutations in PTPN11 gene account for 40-50% of cases. Additionally, mutations in SOS1, RAF1, and KRAS genes account for 10–20%, 3–17%, and <5% of cases, respectively (PIERPONT et al., 2009; VAN TRIER et al., 2018; LEE; YOO, 2019; TEKENDO-NGONGANG et al., 2019).

In Brazil, since 2014 the Comprehensive Care for People with Rare Diseases National Policy, where rare diseases affect 1.3 in 2,000 individuals. This regulation was instituted with guidelines for comprehensive care of patients from the Brazilian Unified Health System (Sistema Único de Saúde – SUS), aimed at improving the quality of life of people, through actions of promotion, prevention, early detection, timely treatment,
reduction of disability, and palliative care. However, after more than six years since publication, little progress has been made in the implementation process, one of which is the lack of effective availability of molecular and genetic diagnostic tests in SUS.

As a consequence, patients do not receive an adequate diagnosis and medical follow-up. In this sense, is urgently the strengthening and effective application of the rare disease policy with the incorporation and offering by the public health system of effective molecular and genetic diagnostic tests, especially for those rare syndromes that are associated with the ID.

Herein, we describe two isolated cases with developmental delay and syndromic features from Central Brazil that were diagnosed with NS after using an exome sequencing target gene panel. This report contributes to improving the phenotypic interpretation of a rare genetic disease and highlights the diagnostic effectiveness of an exome sequencing in a developing country.

**MATERIALS AND METHODS**

**Case presentation**

*Proband 1 (WES006)*

A female proband born at 36 weeks’ gestation to a consanguineous 35-year-old mother and 43-year-old father by cesarean section. At birth her weight was 2,800g (<15th) and her crown-heel length was 46cm (<15th). At birth, she was unable to suck, presented gastroesophageal reflux, pulmonary stenosis, and a heart murmur. She underwent surgery to correct the cardiac malformation. At the age of 11 months, she had a weight of 5,270 g (<3rd) and a height of 63cm (<3rd). Physical examination revealed her developmental delay and tracheomalacia. Her craniofacial dysmorphisms included epicanthal folds, hypertelorism, depressed nasal bridge, low-set posteriorly rotated ears, large philtrum, thin upper lip, and micrognathism. At the age of 6 years, she weighed 21kg (50th) and was 1.13m tall (<50th). Her head circumference was 50 cm. Physical examination revealed high anterior hairline, curly hair, short-webbed neck, pectus excavatum, and widely spaced nipples. Her craniofacial dysmorphisms included low-set ears without tags, ptosis, epicanthal folds, hypertelorism, sparse eyebrows, down-slanting palpebral fissures, large philtrum, thin upper lip, high arched palate, dental malocclusion, micrognathism, bulbous nose. Despite surgical correction for cardiac malformation, she still presents pulmonary stenosis, and heart murmur. She was also diagnosed with
myopia, astigmatism, and attention-deficit/hyperactivity disorder. No previous history of birth defects in the family was reported (Figure 1; Table 1).

**Figure 1** – Pedigree and phenotype features of the girl with Noonan Syndrome. (A) Pedigree of the proband 1 family exhibiting consanguineous parents. (B) Pictures of proband 1 showing dysmorphic features characteristic of Noonan Syndrome.

A male proband born by natural birth at 38 weeks’ gestation to non-consanguineous progenitors aged 31- and 37-year-old for mother and father, respectively. At birth, he weighed 3,500g (50th) and his crown-heel length was 49cm (<50th). He also presented respiratory difficulty and remained at an intensive care unit for 5 days after birth. Additionally, it was observed bilateral cryptorchidism later corrected with a surgical procedure. He also presented pulmonary stenosis, atrial septal defect, ventricular septal defect, and patent ductus arteriosus. He underwent surgery to correct the cardiac malformation. At 15 years old, he weighed 25.5kg (<3rd) and was 1.31m tall (<3rd). His head circumference was 52 cm. Physical examination revealed short-webbed neck, pectus excavatum, and widely spaced nipples. His craniofacial dysmorphisms included low-set ears without tags, ptosis, epicanthal folds, hypertelorism, down-slanting palpebral
fissures, large philtrum, high arched palate, dental malocclusion, and micrognathism. He also had myopia and astigmatism, developmental delay, and attention-deficit/hyperactivity disorder. No previous history of birth defects in the family was reported (Figure 2; Table 1).

**Figure 2** – Pedigree and phenotype features of the boy with Noonan Syndrome. (A) Pedigree of the proband 2 family exhibiting non-consanguineous parents. (B) Pictures of proband 2 showing dysmorphic features characteristic of Noonan Syndrome.

Both patients’ parents signed informed consent forms approved by the Ethics Committee on Human Research from the Pontifical Catholic University of Goiás (CAAE: 04114917.0.0000.0037) that also include parental authorization to release children’s images for scientific purposes. The study was performed in accordance with the Declaration Helsinki.
Table 1 Clinical features of two probands with Noonan Syndrome.

<table>
<thead>
<tr>
<th>Case</th>
<th>WES006</th>
<th>WES010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital heart defects</td>
<td>PS</td>
<td>PS, ASD, VSD, PDA</td>
</tr>
<tr>
<td>Short stature</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Low-set ears</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Curly hair</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ptosis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Epicanthal folds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sparse eyebrows</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Down-slanting palpebral fissures</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bulbous nose</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Large philtrum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thin upper lip</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dental malocclusion</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>High arched palate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Micrognathism</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Short-webbed neck</td>
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<td>+</td>
</tr>
<tr>
<td>Pectus deformity</td>
<td>PE</td>
<td>PE</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Astigmatism</td>
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<td>+</td>
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<tr>
<td>Myopia</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Developmental delay</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Attention deficit/hyperactivity disorder</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

PS=pulmonary stenosis; ASD=atrial septal defect; VSD=ventricular septal defect; PDA=patent ductus arteriosus; PE=pectus excavatum.

Whole Exome Sequencing (WES) followed by target gene panel analysis

Genomic DNA was isolated from whole blood samples using Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare Life Sciences, Piscataway, New Jersey, USA), following the manufacturer’s instructions. Exome sequencing (WES) was performed on each patient and both parents using the intellectual disability gene panel version DG-2.16, customized by Genome Diagnostics Nijmegen from Radboud
University Medical Center, and based on last genome build available (hg19/GRCh37). Exome enrichment (Agilent SureSelectXT Human All Exon 50Mb) and exome sequencing (Illumina HiSeq) were done at BGI-Europe (Denmark).

RESULTS

Both patients were admitted to Replicon Research Group at Pontifical Catholic University of Goiás, Brazil because of developmental delay in combination with diverse dysmorphisms. All genetic tests were carried in the family trios. Initially, we screened for genetic rearrangements using G-banding karyotypes and Chromosomal Microarray Analysis. However, both approaches showed no chromosomal alterations. Following, we ran an intellectual disability gene panel by exome sequencing. In the patient 1 (WES006) was identified a de novo pathogenic missense variant in SOS1 Chr2(GRCh37):g.39249914C>T; NM_005633.3:c.1655G>A (p.Arg552Lys) and in the patient 2 (WES010) was detected a de novo pathogenic missense variant in PTPN11 Chr12(GRCh37):g.112915455T>C; NM_002834.4; c.854T>C; p.(Phe285Ser). Parental WES analysis confirmed that the mutations identified were de novo events (Table 2).

<table>
<thead>
<tr>
<th>Case</th>
<th>Locus</th>
<th>Transcript</th>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide substitution</th>
<th>Amino acid substitution</th>
<th>Variant</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>WES006</td>
<td>chr2:39249914</td>
<td>NM_005633.3</td>
<td>SOS1</td>
<td>11</td>
<td>c.1655G&gt;A</td>
<td>p.Arg552Lys</td>
<td>Missense</td>
<td>de novo</td>
</tr>
<tr>
<td>WES010</td>
<td>chr12:112915455</td>
<td>NM_002834.4</td>
<td>PTPN11</td>
<td>8</td>
<td>c.854T&gt;C</td>
<td>p.Phe285Ser</td>
<td>Missense</td>
<td>de novo</td>
</tr>
</tbody>
</table>

DISCUSSION

Germline mutation in genes that participate in the RAS-MAPK signaling pathway have been associated with developmental disorders that share particular clinical features such as craniofacial dysmorphisms, congenital heart defects, musculoskeletal and ocular abnormalities, and neurocognitive impairment (RAUEN, 2013). The degree of cognitive delay varies for each person, but individuals with a mutation in SOS1 or PTPN11 genes have showed mild or no cognitive impairment (KIM; BAEK, 2019).

Here, we reported two probands with the clinical diagnosis of global developmental delay, and variable phenotypic traits such as congenital heart defects, craniofacial dysmorphisms, and pectus deformities. Initial screening for genetic rearrangements in the family trios using G-banding karyotypes and chromosomal microarray analysis were uneventful. Thus, no chromosomal aberrations or genomic
gains and losses were found in the families. However, dominant de novo missense mutations in \textit{SOS1} and \textit{PTPN11} genes were revealed by exome sequencing using an intellectual disability gene panel.

In proband 1, exome sequencing showed a de novo \textit{SOS1} c.1655G>A (p.Arg552Lys) missense mutation. The mutation of residue Arg552, located in the short helical linker (HL) connecting the plekstrin homology (PH) and the RAS-exchange motif (Rem) domains, leads to the substitution of a strongly conserved nucleotide and its consequent amino acid residue change. The nucleotide variant identified in our patient and its association with Noonan Syndrome was first described by Tartaglia et al. (2007). Nowadays, \textit{SOS1} mutations have been characterized as causative for Noonan Syndrome 4 (NS4) (#OMIM610733).

The \textit{SOS1} gene is a ubiquitously expressed and its product is a guanine nucleotide exchange factor (GEF), which regulates RAS proteins by catalyzing GDP/GTP exchange. \textit{SOS1} protein participates in autoinhibition and altered amin acid residues could contribute to autoinhibition by stabilizing the interaction between histone folds and the PH-Rem linker or the interaction between DH (the Dbl homology) and the Rem domains (AOKI; MATSUBARA, 2013; ROBERTS et al., 2013). Disruption of this interaction could affect the orientation of the DH-PH unit and Rem domain, leading to the pathogenetic mechanism due to the autoinhibition delivery followed by an enhanced GEF activity, sub-adjacently increasing RAS-GTP levels (TARTAGLIA et al., 2007).

According to Tartaglia, Gelb and Zenker (2011), the most majority of the \textit{SOS1} mutations identified are missense changes and affect multiple domains, and the substitutions of residue Arg552 account for around 30% of total \textit{SOS1} mutations in NS4.

In proband 2, the exome sequencing identified a de novo \textit{PTPN11} c.854T>C p.(Phe285Ser) missense mutation. This pathogenic mutation led to the substitution of a highly conserved amino acid residue localized in the protein-tyrosine phosphatase (PTP) domain. The PTPN11 protein is a member of the protein tyrosine phosphatase family, which is expressed in most tissues and plays a regulatory role in various cell signaling, events that are important for the diversity of cell functions (KOSAKI et al., 2002).

The \textit{PTPN11} gene encodes SHP-2, a cytoplasmatic protein tyrosine phosphatase (PTP) that positively modulates RAS signaling (TARTAGLIA et al., 2002). Most missense mutations detected in the \textit{PTPN11} gene have been heterozygous base substitutions and have been clustered mainly to exon 3 or exon 8, resulting in amino acid
substitutions within the N-SH2/PTP interdomain. These substitutions influence negatively the activity of the protein as a result of a conformational change into the constitutively active form, promoting gain of function in the mutated SHP-2 (KOSAKI et al., 2002; TARTAGLIA et al., 2002; TARTAGLIA; GELB; ZENKER, 2011; AOKI; MATSUBARA, 2013).

Germline mutations in residue Phe285 contribute to the N-SH2/PTP interaction and catalytic function with a role in maintaining the overall PTP structure, and the specificity in the amino acid substitution is relevant to the functional deregulation of SHP-2 and disease pathogenesis. Pathogenic variants in the PTPN11 gene have been the first described as causative for Noonan syndrome 1 (NS1) (#OMIM163950) and account for around 50% of affected individuals (TARTAGLIA et al., 2002; TARTAGLIA et al., 2006; WANG; SHI; JIAO, 2020).

In each proband, mutations in main genes related to Noonan Syndrome were identified. Both probands had phenotypic features consistent with of Noonan Syndrome, such as hypertelorism, low-set-ears, down-slanting palpebral fissures, epicanthal folds, micrognathism, short-webbed neck, sunken chest (pectus excavatum), and developmental delay. Moreover, both cases were born with pulmonary stenosis, the most prevalent congenital cardiovascular disease observed in NS individuals with SOS1 and PTPN11 mutations (AOKI et al., 2016; PIERPONT; DIGILIO, 2018).

According to Pierpont, Tworog-Dube and Roberts (2015), who evaluated attention skills in cohort children with Noonan syndrome, patients experienced attention deficits and hyperactivity considerably more frequent than their unaffected siblings. Both our probands showed attention-deficit/hyperactivity disorder. Furthermore, up to 80% of boys diagnosed with Noonan syndrome have unilateral or bilateral cryptorchidism (ROBERTS et al., 2013), and proband 2 had bilateral cryptorchidism.

Van der Burgt (2007) criteria defined several common features in NS individuals, such as short stature that is observed in 50-80% of the NS patients. Head and neck abnormalities are often notable (95%), ear abnormalities are observed in 44-90%, high arched palate and dental malocclusion are noticed in 34-45%, and ophthalmological problems are detected in 95% (VAN DER BURGT, 2007; LEE; YOO, 2019). All these features are observed in our probands, establishing the clinical and molecular diagnosis for Noonan Syndrome.
CONCLUSION

Rare and complex disorders must always be investigated and reaching a diagnosis could be challenging. For our probands with the developmental delay where the karyotype and CMA testing has shown no alterations, the exome sequencing through intellectual disability gene panel was an effective approach to identify de novo mutations in SOS1 and PTPN11 genes that are responsible for Noonan syndrome, confirming its usefulness to increase the diagnostic yield of undiagnosed developmental delay. Notwithstanding, the exome sequencing by a customized panel was an efficient method to direct the adequate clinical management, reduce diagnostic costs and promote better follow-up of the probands and their families, and finally guiding a more suitable genetic counseling for the families.

Importantly, WES is being used more and more in clinical practice for molecular and genetic diagnostics in individuals with intellectual disabilities (LINDSTRAND et al., 2019). Our findings corroborate the importance of using a customized panel for diagnosis in these families, with still access deficit in the Brazilian Public Health System. In Brazil and other countries, WES is a high-cost test and is not covered by health insurance. Therefore, we recommended the introduction and recognition of an exome sequencing panel for ID by Brazilian Unified Health System to improve the molecular and genetic diagnostic reality for ID and neurodevelopmental disorders, increasing the number of patients with a diagnosis and providing adequate clinical management and better follow-up of the probands and their families.

REFERENCES


BABAN, A. et al. SOS1 Mutations in Noonan Syndrome: Cardiomyopathies and Not Only Congenital Heart Defects! Report of Six Patients Including Two Novel Variants


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