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CASE REPORT

DETECTION OF A RARE MUTATION IN A NOONAN SYNDROME SUSPECTED PATIENT: A CASE REPORT

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Abstract: Noonan syndrome (NS) is a genetic autosomal dominant condition, caused by mutations in PTPN11 and other genes. The aim of this report is to highlight a finding of a rare mutation in the RAF1 gene in a six-year-old child evaluated for Noonan Syndrome. An Ampliseq Research Panel covering A2ML1, BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1 and SPRED1 genes was used on the Ion Torrent platform. Out of 54 variants detected, a single nucleotide missense mutation c.483T>G in the RAF1 gene was classified as likely pathogenic, based on a single previous submission to Clinvar. Further investigations may shed light on the possible role of this variant in the pathogenesis of Noonan Syndrome and other RASopathies.

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INTRODUCTION

Noonan syndrome (NS) is an autosomal dominant condition with high phenotypic variability.[1] It is a clinically and genetically heterogeneous disorder that belongs to the group of RASopathy diseases, caused by mutations in genes regulating the RAS/MAPK pathway.[2] The incidence of this syndrome is approximately 1 in 1000 to 2500 people.[1]

NS was first described as a separate entity in 1963 by Dr. Jacqueline Noonan, who reported nine patients with pulmonary valve stenosis, small stature, hypertelorism, mild intellectual disability, ptosis, undescended testes and skeletal malformations.[3] Years after her publication, the understanding of the genetic causes of Noonan syndrome has increased immensely, enabling the study of the complex pathophysiological mechanisms leading to this disorder.

The RAS/mitogen-activated protein kinase (MAPK) pathway plays a fundamental role in the regulation of the cell cycle, differentiation, growth and cell senescence. Consequently, its dysregulation has serious effects on development as seen in different RASopathies. These conditions all have similar signs and symptoms and are caused by changes in the same cell signaling pathway. In addition to Noonan syndrome, the RASopathies include cardiofaciocutaneous syndrome (CFC), Costello syndrome, neurofibromatosis type 1, Legius syndrome, and Noonan syndrome with multiple lentigines. The numerous overlapping characteristics make it challenging to distinguish between different RASopathies.[4]

NS is caused by gene mutations that result in increased signal transduction down the RAS/MAPK pathway. Suspicion for NS is often raised after birth, on the basis of distinctive facial and musculoskeletal features. However, a few prenatal findings may also be observed: polyhydramnios, lymphatic dysplasia including increased nuchal translucency and cystic hygroma, relative macrocephaly, cardiac, and renal

anomalies.[5] The facial dysmorphology is more remarkable in infancy and early childhood and becomes more subtle with age.[6] Newborns with NS have a large head with a small face, a tall forehead narrowing at the temples. The eyes are wide-spaced with epicanthal folds, ptosis and horizontal or down-slanting palpebral fissures. They are usually pale blue or blue-green in color. Telecanthus and hypertelorism is also observed. The nose is short and broad. The ears are low-set and posteriorly rotated. The upper lip is noticeable with a deeply grooved philtrum and full lips. Affected individuals may have a high arched palate and micrognathia. Commonly, patients with NS have a short neck with excess skin, called webbing, and a low posterior hairline. In toddlers, the hair is wispy, whereas it is often curly or wooly in older children.[2] Birth weight and length are usually normal, but there is a subsequent decline in height and weight with age. More than half of individuals with NS have a short stature. Abnormal levels of growth hormone may be a contributor to the slow growth.[7] Delayed puberty is also observed.[8] Most males with NS have cryptochidism.[9] A characteristic feature in NS patients are chest deformities such as pectus carinatum and pectus excavatum. Abnormal pigmentation of the skin can be noticed, including café au lait spots, pigmented naevi and lentigenes. Noonan syndrome is the second most common syndromic cause of congenital heart disease (CHD), exceeded in prevalence only by trisomy 21. Pulmonary stenosis (PS), often with dysplastic valves, is the most common finding in NS patients (50-60%), followed by hypertrophic cardiomyopathy (20%) and secundum atrial septal defect (ASD) (6-10%). Other heart abnormalities are also noted.[1] Individuals with NS quite often have a bleeding diathesis (30-65%), although the symptoms are mostly mild.[10] Knowing the importance of the RAS/MAPK pathway in cell cycle regulation, it is not surprising that individuals with NS have an eightfold greater risk of developing childhood cancer than those without NS.[11] The neurologic, cognitive and behavioral side of NS is still poorly understood, but most children diagnosed with NS have normal intelligence. Few need special education and some have intellectual disability.[2]

Noonan syndrome is diagnosed on a clinical basis by its distinctive phenotypic features, but, as mentioned before, the great number of shared characteristics makes it difficult to distinguish it from other RASopathies.[4] Advancements in the field of comprehensive molecular testing such as NGS (Next Generation Sequencing) help in the identification and confirmation of NS suspected patients.[12] Pathogenic variants in the *PTPN11* gene are found in 50% of NS individuals, variants in the SOS1 gene in approximately 13%, *RAF1* and *RIT1* are each implicated in 5% and *KRAS* in fewer than 5% of patients. Other reported genes in which pathogenic variants have been found to cause NS in less than 1% of cases, include: *BRAF*, *LZTR1*, *MAP2K1*, and *NRAS*. Several additional genes associated with a Noonan-syndrome-like phenotype in fewer than ten individuals worldwide have been identified.

CASE

We present a case of a 6 years old girl suspected for Noonan syndrome. The first encounter with the patient was 4 years ago. She was sent to the University Clinic for Pediatric Diseases in Skopje for dysmorphological assessment after a surgical procedure for ASD. After a detailed anamnesis, the following information was gathered: a suspicious prenatal heart murmur was noted via Doppler sonography in the second trimester of pregnancy, polyhydramnios was observed in late third trimester, delivery was made by a Cesarean section as a first child in the family. Examination showed distinctive dysmorphological features such as curly and wispy hair, tall and wide forehead, wide-spaced eyes with epicanthal folds, low-set and posteriorly rotated ears with anoval thick helix, deeply grooved philtrum and full lips, puffy hands and deep dermatoglyphs. Karyotyping was ordered, and it showed normal female karyotype (46,XX). The clinical findings of the child pointed to Noonan or CFC syndrome. At that time genetic testing was not done. The patient was monitored by pediatricians and a psychologist every six months for the following two years. During physical exams, chest deformity was observed (a characteristic NS feature[1]), which, combined with previous findings, resulted in obtaining NS diagnosis. During that period there were admissions to the Nephrology Clinic due to a grade 2 left kidney hydronephrosis and, most recently, to the Surgery Department for a surgical procedure for infundibular pulmonary stenosis (IPS) and ASD.

MATERIAL AND METHODS

A DNA sample was obtained using salting out method from patient's whole blood, and was sent to the genetic laboratory at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje. The DNA concentration was adjusted to 20 ng/μL using the Qubit system. The Ion Ampliseq Noonan Research Panel covering 14 genes known to be related with this disorder was used. The panel assesses A2ML1, BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1 and SPRED1 genes. An automated preparation of library and templating was done using the Ion Chef system. Panel sequencing was performed on the Ion GeneStudio S5 System. Ion Reporter Software was used for data analysis.

RESULTS

Analysis of the sample resulted in identifying 54 variants. The number of exonic and intronic variants identified per gene is displayed in Table 1.

When searching ClinVar, 11 of the intronic variants were classified as benign, 2 as likely benign and 23 have not been submitted. Evaluation of exonic variants, according to ClinVar, revealed 17 benign variants and

1 single nucleotide variant, missense mutation in RAF1 gene c.483T>G (p.Asn161Lys) classified as likely pathogenic with SCV000490761.2 as submission number.

Table 1. Number of exonic and intronic variants identified per gene

Gene	Exonic variants	Intronic variants
NRAS	1	1
RIT1	1	4
SOS1	/	8
RAF1	1	1
BRAF	/	1
SHOC2	/	1
HRAS1	1	1
CBL	/	2
A2ML1	9	8
KRAS	1	1
SPRED1	2	5
MAP2K1	/	2
MAP2K2	2	1

DISCUSSION

Out of the total 54 variants identified with the Noonan Sydrome Ampliseq panel, none of them were directly associated with this disorder. The most notable change was found in the RAF1 gene. This exonic variant c.483T>G leading to amino acid change (p.Asn161Lys) was classified as likely pathogenic in the ClinVar database based on one submission from GeneDx on 29th January 2019, after fulfilling GeneDx's Variant Classification criteria. However, no information on the phenotypic and/or clinical significance of this variant is available. The mutation results in N161K protein change. N161K was not observed in approximately 6500 individuals of European and African American ancestry in the NHLBI Exome Sequencing Project, indicating it is not a common variant in these populations. The N161K variant is a semi-conservative amino acid substitution, resulting in possible change of secondary protein structure. Several in silico prediction tests are incorporated in the Ion Torrent software, and they agree in predictions of harmfulness of the predicted variant protein structure and function. For example, a SIFT algorithm and score predicts how much an amino acid substitution affects protein function. The SIFT score ranges from 0.0 (deleterious) to 1.0 (tolerated). Variants with scores closer to 0.0 are more confidently predicted as deleterious. The calculated SIFT score for the N161K change was 0.0.[13] The PolyPhen-2 score represents the probability that a substitution is damaging. With the calculated value of 0.607, this variant is possibly damaging.[14] Lastly, the Functional Analysis through Hidden Markov Models (FATHMM) score of 0.9 for coding variants is significantly associated with pathogenic variants.[15] Having these predictions in mind, the variant is classified as likely pathogenic.

There are more than 20 defined RAF1 mutations causing Noonan Syndrome. The RAF1 gene codes the synthesis of a protein that is part of the RAS/MAPK signaling pathway, which transmits chemical signals from outside the cell to the cell's nucleus. RAS/MAPK signaling helps control the cells proliferation, differentiation, migration and apoptosis. The RAF1 gene mutations change single amino acids in the RAF1 protein. Those changes disrupt the regulation of the RAS/MAPK signaling pathway by increased protein activity. It is believed that this disruption in normal cell processes plays a role in the development of signs and symptoms of Noonan syndrome, with an accent on cardiac defects.

In conclusion, we report a rare, likely pathogenic RAF1 gene variant discovered in our patient using the Noonan AmpliSeq Panel, with unconfirmed clinical significance. Further investigation of the variant and, if possible, analysis of the parents, could help with the clarification of the impact on protein function and, hopefully, in identifying new pathogenic variants causing Noonan Syndrome and similar RASopathies.

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