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Genetic analysis in patients with Noonan syndrome in the Republic of Macedonia

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Authors declare no conflict of interest

Introduction

Noonan syndrome is autosomal dominantly inherited disease with an incidence of 1:1000 to 1:2500 newborns. It is caused by different gene mutations involved in the RAS/MAP kinase signaling pathway in the cells. Variable phenotype including variable expression of dysmorphic features and visceral organ affection is common. Different gene mutations are found in approximately 60-70% of tested patients.

Aim

To report mutational analysis in 10 patients with clinical phenotype concordant with the Noonan syndrome.

Methods

Clinical diagnosis was based upon the presence of majority of minor and/or major abnormalities characteristic for Noonan syndrome. Genomic DNA was extracted from peripheral leukocytes. Massively parallel sequencing was done as 150bp paired-end sequencing on MiSeq (Illumina, San Diego, California, United States). A total of 37 Kbps of genomic DNA was analyzed, including 14 Rasopathy-related genes, *NRAS, RIT1, SOS1, RAF1, BRAF, SHOC2, HRAS, CBL, KRAS, PTPN11, SPRED1, MAP2K1, NF1*, and *MAP2K2.* Among the called variants, common variants were excluded using public databases, 1000 Genomes database (http://browser. 1000genomes.org), Exome Variant Server (http://evs.gs.wahsington.edu) and Exome Aggregation Consortium (http://exac.broadinstitute.org). The identified variants were annotated based on human reference genome GRCh37/hg19.c

Results

Symptoms in patients were in various combinations (Table 1, Fig 1, 2, 4). Six patients (60%) had a relevant mutation: 4 patients had PTPN11 mutation(40%), one had RAF1 and one had KRAS mutation (Table 2). No mutation was detected in 4 patients (40%) two of whom had most of the characteristics of Noonan syndrome (Fig 4). Search for mutations of some other or regulatory gene mutation in this family is warranted. Patient with the RAF1 mutation had severe myocardiopathy as previously described, however the same finding appeared in a child with PTPN11(Fig 3). Four children had pulmonary stenosis that was treated surgically in three. Short stature was detected in 7 children (70%), three carrying heterozygous mutation in PTPN11 gene and treated with growth hormone with a modest response. The child with a KRAS mutation was short and had a significant developmental delay.

Fig 1. Patient 6. Facial features, hand appearance with deep palmar creases and laxicity, one caffé au lait spot

Table 1. Phenotype and clinical findings in eight patients with Noonan syndrome

Patient	2	3	4	6	7	8	9	10
Mutation	PTPN11	PTPN11	PTPN11	PTPN11	RAF1	KRAS	none	none
Height at diagnosis (percentile)	< 3rd	10th	10th	< 3rd	< 3rd	< 3rd	< 3rd	10th
Bone age	delayed	normal	normal	normal	NA	delayed	delayed	NA
Hypertelorism	+	+	+	+	+	+	+	+
Down-slanted palp.fissures	+	+	+	+	+	+	+	+
Eye features	exophthalmos	ptosis, exophthalmos	ptosis, exophthalmos	ptosis, exophthalmos	ptosis, strabismus, exophthalmos	ptosis	ptosis	ptosis
Ophtalmological	normal	normal	normal	normal	myopia	normal	normal	normal
Low set ears	+	+	+	+	+	+	+	+
Post.rotated ears	+	+	+	-	+	+	-	-
Hearing difficulties	-	-	NA	-	-	-	-	-
High arched palate	+	NA	+	+	+	+	+	+
Prominent philtrum	+	+	+	+	+	+	+	+
Full lips	-	-	+	+	+	-	+	+
Hair	sparse	-	sparse, pluckable	sparse, curly	sparse	sparse	normal	normal
Macrocephaly	+	+	+	-	+	+	+	+
Webbed/short neck	+	+-	+	short	+	+	+	+
Low posterior hairline	+	+	+	+	+	+	+	+
Chest deformities	-	pectus carinatum	-	pectus excavatum	pectus excavatum	pectus excavatum	pectus excavatum	pectus excavatum
Wide spaced nipples	+	+	+	+	+	+	+	+
Cardiac anomalies	PS, VSD	PS	hypetrophic myocardiopathy	VSD	hypetrophic myocardiopathy	PS	PS	none
Skin changes	-	lentigines	hyperkeratose, café-au lait, lentigines	café-au lait, lentigines, lymphoedema	-	-	lentigines	-
Deep palmar creases	-	+	+	+	+	+	+	+
Genitalia	bilateral cryptorchidism	-	bilateral cryptorchidism	delayed puberty	unilateral cryptorchidism	NA	delayed puberty	NA
Developmental delay	+	+	+	-	++	++	+	+

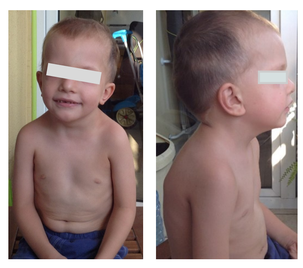

Fig 2. Patient 8. Facial features in a child born with IVF with a short stature, minor facial features. KRAS mutation. Tween sister is normal.

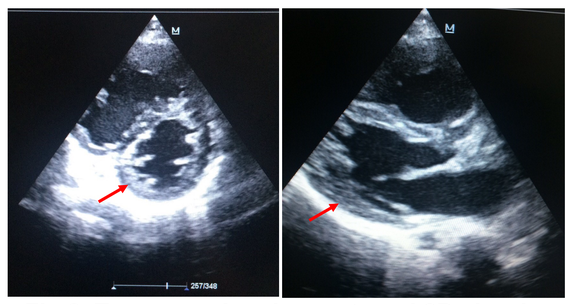

Fig 3. Transversal and parasternal 2D view in patient 4 Hypertrophic myocardiopathy, RAF 1 mutation

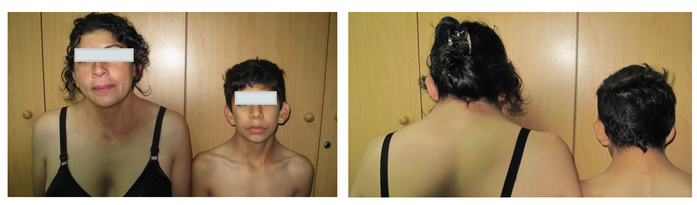

Fig 4. Patients 9 and 10, mother and son. Typical phenotype; no mutation detected.

Table 2. Molecular analysis

No	GENE/CHRomos.	DNA	AA	Zygosity	Exon	Information
S1						
S2	PTPN11/12q24	NM_002834.3:c.417G>C	p.Glu139Asp	Het	4	Known mutation (PMID 11992261)
S3	PTPN11/12q24	NM_002834.3:c.922A>G	p.Asn308Asp	Het	8	Known mutation (PMID 11704759)
S4	RAF1/3p25	NM_002880.3:c.770C>T	p.Ser257Leu	Het	7	Known mutation (PMID 17603482)
S5						
S6	PTPN11/12q24	NM_002834.3:c.1403C>T	p.Thr468Met	Het	12	Known mutation (PMID 12058348)
S7	PTPN11/12q24	NM_002834.3:c.1403C>T	p.Thr468Met	Het	12	Known mutation (PMID 12058348)
S8	KRAS/12p12	NM_004985.3:c.458A>T	p.Asp153Val	Het	5	Known mutation (PMID 16474405)
S9						
S10						

Conclusions

- This is the first report of the molecular analysis of Noonan syndrome in a country from Balkan region.
- Clinical recognition of the syndrome was successful.
- Two rare mutations detected in a small number of patients require further genetic analysis in this region.

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Syndromes : Mechanisms and Management

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