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Further Evidence for the Implication of *LZTR1*, a Gene Not Associated with the Ras-Mapk Pathway, in the Pathogenesis of Noonan Syndrome

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Abstract

Background: Noonan Syndrome (NS) is a relatively common autosomal dominant condition, caused by germline mutations in different genes involved in the RAS MAP Kinase signaling pathway. Although clinically heterogeneous, characteristic findings include typical facial features, short stature, chest deformity and congenital heart diseases.

Methods: Here, we present the clinical and molecular characterization of a Tunisian patient with NS. A comprehensive mutations analysis of 29 genes belonging to the RAS pathway or encoding for interactors was performed, using targeted next generation sequencing.

Results: The results revealed a novel pathogenic substitution affecting the *LZTR1*, whose mutations have been described only in 5 cases of NS.

Conclusion: This report supports the implication of *LZTR1* in Noonan syndrome. Next Generation Sequencing seems a suitable method for mutation detection in clinically and genetically heterogeneous syndromes such as NS.

Keywords: Noonan syndrome; RASopathy; Targeted NGS; RAS-MAPK pathway; *LZTR1*

Introduction

The RAS-MAPK pathway is essential in cell growth, differentiation, senescence and in regulating cell cycle [1-3]. Germline mutations of genes involved in the RAS/MAPK signaling pathway result in a spectrum of phenotypically overlapping syndromes named RASopathies or RAS/mitogen-activated protein kinase (MAPK) syndromes [4,5]. These disorders include neurofibromatosis type 1 (NF1, OMIM 162200), Legius syndrome (NFLS, OMIM 611431), Noonan syndrome (NS, OMIM 163950), Noonan syndrome with multiple lentigines (also called LEOPARD syndrome, LS, OMIM 151100), Costello syndrome (CS, OMIM 218040), cardiofaciocutaneous syndrome (CFCS, OMIM 115100), Noonan-like syndromes, hereditary gingival fibromatosis (HGF, OMIM 135300), and capillary malformation-arteriovenous malformation (CMAVM, OMIM 608354). The most frequent RASopathy remains NS with a prevalence estimated to be between 1:1000 to 1:2500 live births [6,7].

Noonan syndrome (NS, OMIM 163950) is an autosomal dominant multisystem disorder characterized by a wide phenotypic spectrum including distinctive facial dysmorphism, postnatal growth retardation, short stature, ectodermal and skeletal defects, congenital heart anomalies, renal anomalies, lymphatic malformations, bleeding difficulties and variable cognitive deficits [8-10].

NS was already associated with *PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF,MAP2K1/2 SHOC2, CBL and RIT1* gene mutations [2,7,11]. Recently, novel gene variants affecting *RRAS, RASA2, A2ML1, SOS2* and *LZTR1* have been shown to be associated with NS and RASopathies [12].

Here we report a new case of a Tunisian patient with Noonan syndrome caused by *LZTR1* mutation.

Case Presentation

The patient, a 6-year-old Tunisian boy, was the second child of healthy unrelated parents aged 26 (mother) and 41 years (father). The family history was unremarkable. His two brothers were healthy. Pregnancy and delivery were normal, and the boy was born at term. His birth weight was 3300 g, his length 49 cm and his head circumference 33 cm. He sat alone at 2½ years, walked at 4 years 10 months and had speech delay.

The patient was referred for consultation because of psychomotor retardation and facial dysmorphism. On examination at the age of 4 years, his weight was 13 kg (-3 SD), his height 89 cm (-4,3 SD) and his head circumference 46 cm (-2,6 SDS, Standard Deviation Score). He

had facial dysmorphism suggestive of Noonan syndrome including frontal bossing, downslanting palpebral fissures, thick lips, anteverted nose, low-set and posteriorly rotated ears and short webbed neck withlow posterior hairline, dental caries, thoracic deformation with pectus excavatum, hypospadias, cryptorchidism, fingers' hyperlaxity, valgus flat feet, loose excess skin on hands and heart murmur (Figure 1).

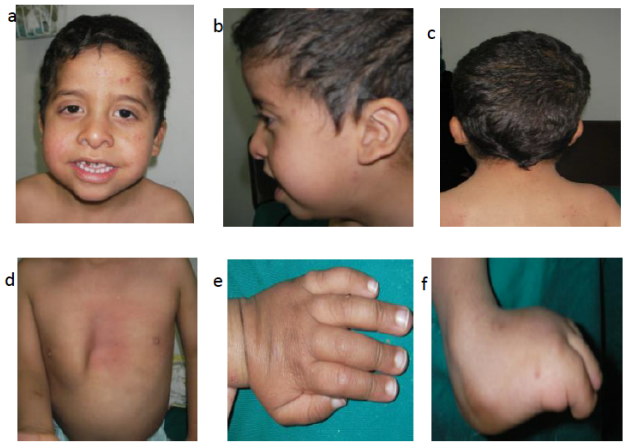

Figure 1: Photographs of the patient at 4 years. (a) downslanting palpebral fissures, thick lips, anteverted nose, dental carries; (b) low-set and posteriorly rotated ears; (c) short webbed neck with low hairline; (d) thoracic deformation with pectus excavatum; € loose excess skin on hands and (f) valgus flat foot.

The cardiac exam showed hypertrophic cardiomyopathy, ostium secundum atrial septal defect and mitral anomaly.

Ophthalmologic examination, auditory and visual evoked potentials and brain magnetic resonance imaging were normal.

Laboratory analyses showed normal levels of TSH and FT4, IGF1 and blood phenylalanine. Urinary glycosaminoglycan screening was negative. Blood count revealed normochromic and normocytic anemia. Chromosomal analysis was normal: 46, XY.

Methods

Genomic DNA of the patient was manually extracted from peripheral blood collected in EDTA tubes according to standard salting out methods and purified by *QIAmp DNA microkit* (Qiagen).

Genomic DNA of asymptomatic patient's relatives (mother, paternal uncle and brother) was extracted from buccal cells using *QIAmp DNA blood mini Kit (Qiagen*-Cat.No.51 104).

The Ion Torrent PGM system was used to sequence exons and splicing sites of 29 genes of the RAS MAPK signaling pathway involved in Noonan syndrome and other rasopathies, i.e.,: the commonly mutated *PTPN11, SOS1, RAF1, KRAS, BRAF, NRAS, HRAS, MAP2K1, MAP2K2, SHOC2, CBL, SPRED1* genes and less common ones such as *LZTR1* (more details about the complete list of genes are provided under request). The library kit was made with Agilent Haloplex technologies. The sequence analysis software was VarAFT (http://varaft.eu). Prediction of functional effects of nsSNPs was done with UMD predictor, MutationTaster and PolyPhen. Variants of interest were verified using the Integrative Genomics Viewer and validated by bidirectional Sanger Sequencing.

Results

A total of 163 variants were detected across the 29 genes analyzed. Filtering these results using *in silico* software predictors of mutation's impact revealed only one heterozygous variant as potentially pathogenic: a heterozygous missense mutation in exon 4 of the *LZTR1* gene predicted to lead to a missense amino acid change (NM_006767:c.347C>T, p.Ala116Val). This alteration was validated using Sanger sequencing in proband's and available relatives, showing that it appeared *de novo* (Figure 2).

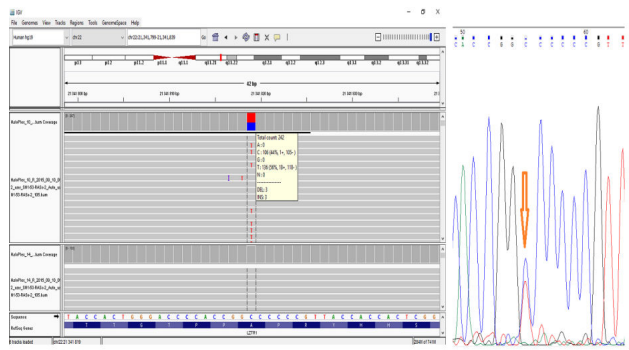

Figure 2: Sequencing results. IGV browser visualization of the targeted NGS sequencing results showing the heterozygous c. 347C>T substitution in the LZTR1 gene in the patient and the Sanger Sequencing validation.

Discussion

In this study, we report a case of NS with typical clinical findings, harboring a mutation in *LZTR1* (Leucine-Zipper-like Transcriptional Regulator-1), a gene rarely associated with NS.

LZTR1, located at 22q11.21, encodes a protein member of the BTB-Kelchsuperfamily implicated in several fundamental cell processes. The implication of *LZTR1* in human disease was first reported with the DiGeorge Syndrome, as it was deleted in the majority of DiGeorge Syndrome patients [13]. More recently, somatic mutations with loss of heterozygosity in *LZTR1* and germline loss-of-function variants in *LZTR1* were respectively associated with glioblastoma multiforme [14] and schwannomatosis [15,16].

Germline *LZTR1* variants in Noonan syndrome patients were first reported by Chen et al. in [17]. The authors performed Next Generation Sequencing in a cohort of 27 NS patients without a known NS gene mutation. Two of these patients had *LZTR1* variants (p.R237Q and p.A249P) which were not considered as responsible for the NS phenotype, since the authors considered *LZTR1* as a gene already associated with DiGeorge syndrome. In 2015, Yamamoto et al. identified rare variants of *LZTR1* using whole-exome sequencing in 6/50 Brazilian probands (p.G248R, p. R284C, p.H287Y, p.Y119C, p.I647Vand p.F447L) and one Polish family (p.S247N) with NS and lacking mutation in the known NS genes [18]. Two of these variants were considered nonpathogenic because of their presence in unaffected relatives (p.F447L) or the weak of *in silico* pathogenicity prediction

(p.I647V). The remaining five variants, p.G248R, p. R284C, p.H287Y, p.Y119C and p.S247N, were predicted to cause NS as they segregated with the NS phenotype or were *de novo* events and predicted to be deleterious by *in silico* analysis. Moreover, the *LZTR1* variants identified in Brazilian patients were found in only 1/107 control cohort supporting their implication in NS. All the reported *LZTR1* variants are localized in highly conserved kelch (KT) domain and are predicted to disrupt protein function. Kelch domain shape may also be responsible for binding to other proteins [19,20]. The missense heterozygous variant found in *LZTR1* in our patient is localized in KT1 domain. *In silico* analysis predicted pathogenicity. Analysis of a control database of WES in 50 Tunisian patients affected by other disorders showed no *LZTR1* variant. The mechanism by which mutations in *LZTR1* causes NS is still unknown. Yamamoto et al. [18] suggested that missense heterozygous variants in *LZTR1* may cause dysregulation of the RAS/MAPK pathway by increasing ERK signaling through a loss of tumor suppressor function.

The clinical findings of NS patients with *LZTR1* variants were similar to *PTPN11* positive individuals with the exception of short stature which was not frequent in the Brazilian cohort [19]. Our patient, contrary to what has been reported, had a short stature at -4,3 SD (Standard Deviation).

Table 1, updated from Yamamoto et al. [18], summarizes the clinical features seen in reported cases of NS patients with *LZTR1* variants and highlights similarities between clinical findings seen in the 5 previously reported cases and in our case.

	Brazil F3	**Brazil F4**	**Brazil F5**	**Brazil F6**	**Poland F1**	**Tunisian (this report)**
	Proband	**Proband**	**Proband**	**Proband**	**Proband**	**Proband**
Clinical findings						
Sex	Female	Female	Male	Female	Male	Male
Age	11 years 5 months	14 years	16 years 1 month	30 years	18 years	4 years 9 months
Perinatal data						
Gestational age	Term	Term	35 weeks	Term	Term	Term
BW, g	2270	2750	2130	3930	4000	
Length, cm	45		47	52	53	
Typical facial features	+	+	+	+	+	+
Current height	131.5 cm	146 cm	172.6	146	183	93
Short/webbed neck	+	-	-	-	+	+
Pectus deformity	+	+	-	-	+	+
Cardiac abnormality	PVS/ASD	PVS	PVS/ASD	LVH	MVI	CIA/HCM
Cryptorchidism	NA	NA	+	NA	-	+
Renal abnormality	-	-	-	-	-	-
Abnormal hemostatis	-	+	+	-	-	-
Factor XI deficiency		-	-	-	-	-
Ophthalmological abnormality	+		+	-	-	-
Ectodermal findings	-	-	-	-	+	+
Curly hair	-	-	-	-	-	-
Sparse eyebrows	-	-	-	-	-	+
Hyperkeratosis pilaris	-	-	-	-	-	-
Ulerythema ophriogenes	-	-	-	-	-	+
Tumours	-	-	-	-	+	-
Developmental delay	-	-	+	-	+	+
Learning ability	-	-	+	-	+	

Other findings	Lacrimal duct			Lymphedema, varicose veins		
Mutation (NM_006767,3)	**c.742G>A; p.G248R**	**c.850C>T; p..R284C**	**c.859C>T; p.H287Y**	**c.356A>G; p.Y119C**	**c.740C>A;p.5247N**	**c.347C>T;p.A116V**
ASD: Atrial Septal Defect; BW: Birth Weight; WHO: World Health Organisation; HCM: Hypertrophic Cardiomyopathy; LVH: Left Ventricular Hypertrophy; MVI: Mitral Valve Insufficiency; NA: Not Applicable; NS: Noonan Syndrome; PVS: Pulmonary Valve Stenosis; SDS: SD Score						

Table 1: Summary of clinical details of Noonan syndrome patients with *LZTR1* variants.

Our case further supports the implication of *LZTR1* in the pathogenesis of NS. Nevertheless, functional studies are required to unravel the precise implication of *LZTR1* variant of p.Ala116Val in Noonan syndrome.

Conclusion

The identification of causative mutations that underlie genetically heterogeneous syndromes such as Noonan Syndrome has been greatly facilitated by the emergence of high throughput sequencing. In this context, Targeted NGS methods can be used as a cost effective first line genetic test for confirmation of NS cases. Thus, an early and accurate genetic diagnosis and suitable management of patients will be possible. The case we report supports the involvement of *LZTR1* in the pathogenesis of typical Noonan syndrome.

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Competing Interests

The authors declare that they have no competing interests.

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