Fernando A. Rodríguez*, Nancy Unanue, María Isabel Hernández, Karen E. Heath and Fernando Cassorla

Molecular characterization of Chilean patients with a clinical diagnosis of Noonan syndrome

Abstract

Background: Noonan syndrome (NS) is an autosomal dominant syndrome characterized by typical dysmorphic features, cardiac anomalies as well as postnatal growth retardation, and is associated with Ras-MAPK pathway gene mutations. The purpose of this study was to improve the diagnosis of Chilean patients with suspected NS through molecular analysis.

Methods: We screened 18 Chilean patients with a clinical diagnosis of NS for mutations in PTPN11 by high resolution melting (HRM) and subsequent sequencing.

Results: Three PTPN11 missense mutations were detected in 22% of analyzed patients. Of these, two (c.181G>A and c.1510A>G) were previously reported and one was the novel substitution c.328G>A (p.E110K) affecting the linker stretch between the N-SH2 and C-SH2 domains of SHP-2 protein.

Conclusion: Molecular studies confirmed the clinical diagnosis of NS in 4 of 18 patients, which provided support for therapeutic decisions and improved genetic counseling for their families.

Keywords: Noonan syndrome; PTPN11 mutations; short stature.

***Corresponding author: Dr. Fernando A. Rodríguez,** Institute of Maternal and Child Research, School of Medicine, University of Chile, Avenida Santa Rosa 1234, piso 2, PO Box 226-3, Santiago (8360160), Chile, Phone: +56 (2) 29770855, Fax: +56 (2) 24247240, E-mail: frodriguezr@med.uchile.cl

Nancy Unanue, María Isabel Hernández and Fernando Cassorla: Institute of Maternal and Child Research, School of Medicine, University of Chile, Santiago, Chile

Karen E. Heath: Institute of Medical and Molecular Genetics (INGEMM), Hospital Universitario La Paz, Universidad Autónoma de Madrid, IdiPAZ, CIBERER, ISCIII, Madrid, Spain

Introduction

In 1968, Jacqueline Noonan described nine patients with Turner-like physical features, hypertelorism, and pulmonary stenosis (1). Subsequent research described a new syndrome currently known as Noonan syndrome (NS, MIM 163950). The cardinal features of NS are distinctive dysmorphic facial features with hypertelorism, ptosis and low-set ears; congenital heart defect (most commonly pulmonary stenosis) and/or hypertrophic cardiomyopathy; and postnatal growth retardation and chest deformity (pectus carinatum or excavatum). Bleeding diathesis, ectodermal anomalies, lymphatic dysplasias, cryptorchidism, and cognitive deficits have also been reported (2, 3). The incidence for NS has been reported to be between 1 in 1000–2500 live births (4), although mild expression of the syndrome may be considerably more common.

NS is inherited as an autosomal dominant trait. Mutations in seven genes involved in the Ras-MAPK pathway (PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, and MEK1) comprise the etiology in approximately 75% of individuals with a clinical diagnosis of NS (5). PTPN11 mutations account for approximately 50% of these cases (6–9). This gene encodes the non-receptor protein tyrosine phosphatase SHP-2 (10), a positive regulator of the Ras-MAPK pathway, which is conformed by two SH2 domains in tandem (N-SH2 and C-SH2) and a C-terminal phosphatase domain (PTP) (11). The vast majority of PTPN11 mutations associated with NS result in an increased phosphatase activity of SHP-2, which upregulates signaling through the Ras-MAPK pathway (12).

Several studies have suggested that NS patients with PTPN11 mutations require a specific clinical follow-up compared with patients with no PTPN11 mutations, due to their higher prevalence of cardiac disorders such as pulmonary stenosis (7, 13, 14). Consequently, molecular confirmation of this clinical diagnosis is of utmost importance in order to make appropriate management decisions. Thus, the aim of this study was to perform a molecular analysis of all coding exons of *PTPN11* in a group of 18 Chilean patients with a clinical diagnosis of NS.

Materials and methods

Subjects

Eighteen children with a clinical diagnosis of NS were recruited at the Institute of Maternal and Child Research, University of Chile in

Santiago, Chile. Each patient was assessed by a clinical geneticist (NU) and an endocrinologist (MIH). The diagnosis of NS was established following the criteria of van der Burg et al. (15). All recruited patients were sporadic cases, and as such no familial cases were included in the cohort. This study, as well as informed consent for DNA extraction, was approved by the Ethics Committee of Hospital Clínico San Borja-Arriarán, Santiago, Chile.

DNA extraction

Peripheral blood samples were obtained from patients after signing the informed consent form, and whenever possible, from relevant family members for DNA extraction. Genomic DNA was isolated from blood lymphocytes using the Wizard Genomic DNA Purification Kit (Promega, WI, USA).

Point mutation screening

Point mutations in PTPN11 were screened by high resolution melting (HRM) analysis. Briefly, the 15 exons of PTPN11 were amplified with specific primers (Supplementary Table 1). The PCR conditions were 1× Megamix GOLD PCR Mastermix (Microozone, Southampton, UK); 0.3 μM each oligonucleotide; 1× LCGreen™ Plus + (Idaho Technologies, Salt Lake City, UT, USA); 5% DMSO and 15 ng genomic DNA in a total volume of 10 μL. The annealing temperatures for the amplification of the different exons are described in Supplementary Table 1. Amplification products were analyzed in a 96-well Lightscanner™ HR96 system (Idaho Technology Inc., Salt Lake City, UT, USA), and those exons with abnormal profiles relative to control samples (at least three) were sequenced in an ABI3130XL sequence analyzer (Applied Biosystems, Foster City, CA, USA).

Results

Three PTPN11 missense mutations were detected in 4 out of 18 patients (22% of analyzed patients). Two heterozygous missense substitutions were detected in exon 3, c.181G>A (p.D61N) and c.328G>A (p.E110K), in patients NS14 and NS15, respectively (Figure 1A–C). The two exon 3 mutations were not detected in the mothers. We were unable to contact the fathers, but no family history suggestive of the NS phenotype was reported in these individuals.

The third mutation, located in exon 13 c.1510A>G (p.M504V), was detected in patients NS12 and NS21 (Figure 1D and E). In both cases, the mutation was de novo, since it was not detected in their parents.

The clinical characteristics of the PTPN11-mutation-positive patients are indicated in Table 1. Both patients with exon 3 mutations presented typical NS facial features with cardiopathy, but with variable stature. In contrast, the two patients with the exon 13 mutation exhibited short stature and variable NS facial features, but no cardiac disease.

Six different intronic single nucleotide variations in the PTPN11 gene were detected in seven patients without PTPN11 mutations (Table 2), one of whom (c.137 +49 A/T) was not reported previously.

Discussion

The present study documented PTPN11 mutations in 4 out of 18 Chilean patients with a clinical diagnosis of NS. In this study, we found a mutation frequency of 22%. Similar frequencies have been reported by Tartaglia et al. (6), Musante et al. (16), and Papadopoulou et al. (7) at 37%, 26% and 29%, respectively. These authors studied only sporadic cases as we did. This study is the first to discover PTPN11 mutational analysis of patients with NS carried out in Chile, and the second in South America (17).

Most PTPN11 mutations reported to date are missense changes that cluster in the N-SH2/PTP interdomain binding network. Residues at this network stabilize SHP-2 in its close-catalytically inactive conformation. Consequently, these mutations destabilize SHP-2 close conformation and favor a shift towards the open-catalytically active state (18, 19). Two out of the three mutations (c.181G>A and c.1510A>G) detected in this study have been reported previously (6–8, 12, 16, 20) in NS patients. Both mutations perturb sequences of the N-SH2/PTP interdomain (6, 20). The third mutation c.328G>A (p.E110K) observed in our patient, has not been reported previously, generating a glutamic acid to lysine change at position 110 of SHP-2. Tartaglia et al. (21) reported a similar substitution (c.329G>C) affecting codon 110 (p.E110A) in a NS patient. The glutamic acid 110 localizes at the linker stretch, which connects the N-SH2 and C-SH2 domains of SHP-2. Mutations affecting this linker are predicted to alter the relative orientation or mobility of N-SH2, which may abrogate its autoinhibitory function.

The intronic SNVs not reported previously (c.137 +49 A/T) have not been observed in more than 2500 Caucasian controls (NHLBI Exome Sequencing Project, http://evs.gs.washington.edu/EVS/). This should be investigated further in the general Chilean population in order to rule out an association with NS.

In our cases without PTPN11 mutations and relative normal height, especially in those without developmental delay or mental retardation, it is also necessary to study possible *SOS1* mutations (22). Other patients presented with sensorineural hearing loss, which prompted us to search for mutations, particularly RAF1 and BRAF, which are associated with LEOPARD syndrome (MIM 151100)

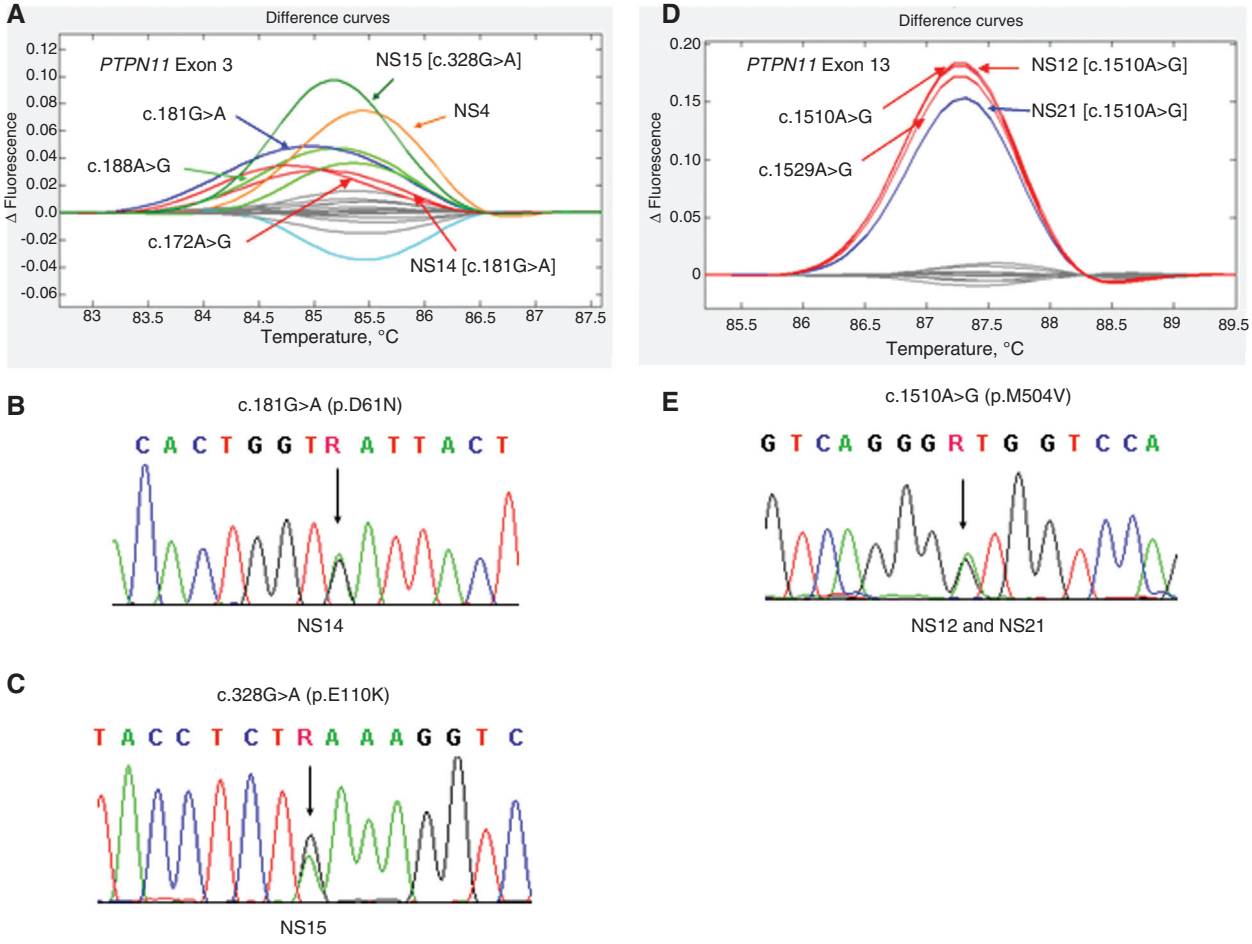

Figure 1 PTPN11 missense mutations detected in patients with NS. Results of the HRM analysis for PTPN11 exon 3 (A) and 13 (D) of patients (NS14-15 and NS12-21, respectively) and controls with known nucleotide changes. Sequence analyses of PTPN11 exon 3 shows a heterozygous substitution of guanine c.181 by adenine (R) in patient NS14 (B) and a substitution of guanine c.328 by adenine (R) in patient NS15 (C). Sequence analysis of PTPN11 exon 13 shows a heterozygous substitution of adenine c.1510 by guanine (R) in patients NS12 and 21 (E).

(23). Finally, in cases with short stature and low IGF1 levels, SHOC2 mutations may also be involved (24).

Recently, we performed screening for hotspots in the SOS1, RAF1, and KRAS genes in those patients without PTPN11 mutations. Only intronic single nucleotide variations of KRAS were detected in these patients.

In summary, this first molecular characterization of a cohort of Chilean NS patients resulted in the following: i) the detection of three pathogenic PTPN11 mutations in 4 out of 18 (22%) patients, and ii) the identification of one novel PTPN11 substitution, c.328G>A (p.E110K), which has not been previously described. The main weakness of

Table 1 Clinical characteristics of NS patients with PTPN11 mutations.

Patient (NS)	Age (gender)	Score	Birth length (SDS)	Current height (SDS)	Pectus	Cardiopathy	Face	Other	Mutation
12	20 a (F)	3>	−0.80	−2.5	+	−	+[1]	+[a]	c.1510 A>G (p.M504V)
14	9 a (F)	1> 2<	−0.18	−0.87	−	+[b]	+[1]	−	c.181 G>A (p.D61N)
15	13 a (F)	3> 1<	−0.29	−4.01	+/−	+	+[1]	+[c]	c.328 G>A (p.E110K)
21	11 a 2 m (F)	2> 1<	0.23	−2.08	+/−	−	+[2]	−	c.1510 A>G (p.M504V)

Score: > major criteria, < minor criteria (15). Pectus (*excavatum*):+, present; +/−, moderate; −, absent. Face 1 typical; 2 suggestive.
[a]Coagulation anomalies. [b]Pulmonary stenosis. [c]Psychomotor development delay.

Table 2 PTPN11 SNVs detected in NS patients without PTPN11 mutations.

Intron	SNVs[a]	Reference
2	c.137 +49 A/T	NR
3	c.332 +17 G/T	rs115658366
5	c.525 +12 G/C	rs41304351
7	c.854 -21 C/T	rs41279090
	c.854 -30 C/T	rs144391508
	c.854 -32 A/C	rs187389813

NR, no reported. [a]Position according to the NM_002834.3 sequence.

this study is the relatively small number of patients investigated. However, this molecular-based screening strategy allowed the laboratory confirmation of the clinical diagnosis of NS in four patients, which was very helpful for the genetic counseling and clinical management of these patients.

Acknowledgments: We are grateful to the patients and their families for helping us carry out this study. We thank the clinicians (Drs. Vivian Gallardo, Carolina Sepulveda, Ximena Gaete, Roberto García, Ana Rocha and Paulina Merino) who referred patients for this study, and the technical assistants (Patricia López, Alejandra Ávila, Verónica Maturana and Clara Aguilera) who collaborated with this study. We also thank Dr. Sara Benito-Sanz and Dr. Angel Campos Barros for the help they extended while we were completing this study.

Received May 8, 2013; accepted August 29, 2013; previously published online October 23, 2013

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