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Mutation N308T of protein tyrosine phosphatase SHP-2 in two Senegalese patients with Noonan syndrome

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Noonan syndrome is a genetic autosomal dominant disorder characterized by facial dysmorphy, short stature, delayed puberty and congenital heart defects. The first gene implicated in this syndrome is PTPN11, encoding protein tyrosine phosphatase SHP-2. Several studies worldwide have identified missense mutations in this gene in patients with Noonan syndrome. Our objective focused on mutations screening of PTPN11 on a Senegalese population with Noonan syndrome. Six patients clinically diagnosed with Noonan syndrome were included in this study. DNA was extracted from whole blood by phenol chloroform. Mutation screening was performed by bidirectional sequencing of amplified polymerase chain reaction (PCR) products of PTPN11 exons frequently mutated in Noonan syndrome. This study identified in two patients, a c.923A>C mutation in exon 8, predicting Asn308Thr (N308T) on SHP-2 protein. This is the first time that this mutation is described in Noonan syndrome in Africa, while codon 308 was reported as a hot spot mutation site in other populations. Frequently reported amino acid substitutions were Asn308Asp and Asn308Ser. All these mutations affected the protein tyrosine phosphatase domain (PTP) of SHP-2 protein exerting a gain of function which would likely explain observed phenotypes in patients.

Key words: Mutation, N308T, protein tyrosine phosphatise (PTP), SHP-2 protein, Noonan syndrome, Senegal.

INTRODUCTION

Noonan syndrome (NS, MIM 163950) is an autosomal dominant dismorphic syndrome described first by Noonan (1968). The prevalence of NS is estimated to be 1 in 1000 to 2500 births. The disease is characterized by proportionate short stature, delayed puberty, congenital heart defects and multiple minor anomalies such as hypertelorism, malrotated ears, webbed neck, bleeding diathesis, cryptorchidism in males, mental retardation, and hearing difficulties (Marino et al., 1999; Roberts et al., 2013; van der Burgt et al., 2007). The most common congenital heart defect is pulmonary valve stenosis with displastic leaflets followed by hypertrophic obstructive cardiomyopathy (HC), atrial septal defects (Marino et al., 1999; Musante et al., 2003; Tartaglia et al., 2006).

Jamieson et al. (1994) mapped a gene for NS to the long arm of chromosome 12 (12q24). Tartaglia et al. (2001) reported that NS is caused by heterozygous missense mutations of the gene protein-tyrosinephosph-

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atase nonreceptor type 11 (PTPN11) located on 12q24. PTPN11 mutations were detected in 45% of unrelated individuals with sporadic or familial NS (Tartaglia et al., 2002).PTPN11 gene consists of 15 exons and the expressed protein encodes cytoplasmic tyrosine phosphatase with two tandemly arranged Src homology 2 (SH2) domains (N-SH2 and C-SH2) at the N terminal region and a C terminal protein-tyrosine phosphatase domain (PTP) (Chan and Feng, 2007). The PTPN11 gene is widely expressed in various human tissues, especially in the heart, brain, and skeletal muscle (Ahmad et al., 1993). The protein plays a critical role in regulating the response of eukaryotic cells to extracellular signals through the RAS/MAPK pathway (Roberts et al., 2013).

All PTPN11 missense mutations associated with NS were clustered in the interacting portions of the N-SH2 domain and the PTP domains are involved in switching the protein from its inactive to the active conformation. Functional studies by energetic-based structural analysis of two N-SH2 mutants revealed that those mutations favoured the active conformation of PTPN11 protein, resulting in a gain-of-function effect (Tartaglia et al., 2001; Uhlen et al., 2006). Also, other studies have reported enhanced phosphatase activity of NS mutants located in the SH2 and PTP domains (Niihori et al., 2005; Tartaglia et al., 2003).

Understanding of the molecular genetic causes of NS, enable the study of the pathophysiological mechanisms underlying the varied medical and developmental features of NS. PTPN11 belongs to the RAS-MAPK pathway which is an important signal transduction pathway. Mutations that cause NS deregulated this pathway leading to the clinical features observed. Furthermore, all the other genes implicated in NS including SOS1, RAF1, and KRAS encode proteins integral to this pathway (Roberts et al., 2013).

Several studies have reported mutation analysis of PTPN11 gene and genotype-phenotype correlation in NS in different geographical regions (Bertola et al., 2004; Pierpont et al., 2009; Sznajer et al., 2007; Tartaglia et al., 2006; Yoshida et al., 2004). Mutation screening in NS patients from United States showed that all mutations are exonic changes with the majority clustering in exon 3 and 8. The most common mutation was a c.922A>G in exon 8, leading to the Asn308Asp substitution within the PTP domain (Tartaglia et al., 2002). The occurrence of an adjacent c.923A>G mutation predicting an Asn308Ser change and c.923A>C (Asn308Thr) indicated that codon 308 is a hot spot site for NS with a frequency of 36% in PTPN11 mutated patients (Tartaglia et al., 2002; Tartaglia et al., 2006; Tartaglia et al., 2003). Other studies have reported the occurrence of different mutations types of PTPN11 in European and Asian populations. In Germany, the most common mutation is c.188A>G (Tyr63Cys) followed by c.922A>G (Musante et al., 2003), while in Japan the most common mutation is c.236A>G (Gln79Arg) (Yoshida et al., 2004). In Africa, few studies have focused on mutation screening in NS. Only one report from Morroco identified the c.922A>G mutation in two affected siblings with normal parents (Elalaoui et al., 2010) and c.182A>G (Ratbi et al., 2008). In sub-Saharan Africa, no report from PTPN11 mutation is available to date. The objective in this study was to screen for PTPN11 gene mutations in 6 Senegalese patients with NS and summarized observed clinical features.

POPULATION AND METHODS

Patients and clinical assessment

After informed consent, 6 patients clinically diagnosed with NS were included in this study. Patients were examined by clinicians from the Cardiology Unit of Fann Hospital in Dakar (Senegal), who have experience with NS. Electrocardiograms, echocardiograms and clinical photographs were obtained from each patient. NS was diagnosed on the basis of the presence of the following major characteristics: typical facial dysmorphy, pulmonary valve stenosis or hypertrophic cardiomyopathy, chest deformity, developmental delay and cryptorchidism in male patients. To be diagnosed for NS, patient with facial dysmorphy had to have at least two of the major characteristics (van der Burgt et al., 1994).

Mutation screening of PTPN11 gene

Genomic DNA was isolated from peripheral blood lymphocytes by classic phenol/chloroform method. The most frequently mutated exons reported in NS (3, 4, 7 and 8) and flanking introns of PTPN11 gene were amplified by PCR with sets of primers as described previously (Tartaglia et al., 2002). All PCR products were purified with QIAquick PCR purification kit from Qiagen™ as described by the manufacturer. Mutations were screened by direct bidirectional sequencing of the purified PCR products with Big Dye Terminator chemistry (Perkin Elmer Biosystem™) on an ABI 3100 auto sequencer (ABI™, Foster City, CA). Obtained sequences were analysed by BioEdit software (Hall, 1997).

RESULTS AND DISCUSSION

Clinical features of studied NS patients are summarized in Table 1. In this study, 4 females and 2 males were recruited with ages ranging from 1 to 31 years. None of the patients had known family history of NS. Growth was delayed in all cases. Dysmorphic features were present with variable severity in all cases. Figure 1 illustrates some of these features (hypertelorism, low-set ears, webbed neck, chest deformity, and ptosis) in a male and a female patient. The most frequent cardiac abnormalities were hypertrophic cardiomyopathy (HCM) and pulmonary valve stenosis (PS). One of the male cases had cryptorchidism.

The phenotypes observed in NS are heterogeneous and vary in different ages. The diagnosis of Noonan syndrome is primarily clinical and is guided by the most common dysmorphic signs such as hypertelorism, low-

Table 1. Clinical features in 6 Senegalese patients with NS.

Parameter	NS1	NS2	NS3	NS4	NS5	NS6
Age (years)	17	31	4	15	1	4
Sex	F	F	M	M	F	F
Growth	delayed	delayed	delayed	delayed	delayed	delayed
Craniofacial dysmorphy	Hypertelorism webbed neck	Hypertelorism	Hypertelorism webbed neck ptosis	Hypertelorism	Hypertelorism	Hypertelorism webbed neck low set ears
Chest	Deformity	-	Deformity	Deformity	-	Deformity
Cardiovascular defects	HC	-	HC	PS	HC	PS
Genital defects	-	-	Cryptorchidism	-	-	-

NS: Noonan syndrome; HC: hypertrophic cardiomyopathy; PS: pulmonary valve stenosis.

Table 2. PTPN11 codon 308 mutations in Senegalese patients with NS compared to other populations around the world.

Exon 8	rs number	AA change	Senegal	USA	USA	Brazil	Japan
			Ndiaye et al	Tartaglia et al. (2006)	Pierpont et al. (2009)	Bertola et al. (2006)	Yoshida et al. (2004)
c. 922A>G	rs28933386	N308D	-	40/204	4/33	-	2/18
c. 923A>G	rs121918455	N308S	-	13/204	2/33	1/21	-
c. 923A>C	ND	N308T	2/6	2/204	1/33	-	-

ND: Not determined.

set ears, chest deformities and short stature, associated cardiac abnormalities (Tartaglia et al., 2002; van der Burgt, 2007; van der Burgt et al., 1994). These morphological abnormalities were observed in most of our patients and were the key elements of diagnosis.

In addition to these morphological abnormalities, alterations in several genes have been implicated in NS. This is the case of the PTPN11 gene which is mutated in 40 to 50% of patients with NS (Tartaglia et al., 2001). Mutation screening of PTPN11 gene in studied exons have identified a heterozygote substitution in exon 8, c.923A>C, in two unrelated patients NS5 and NS6 (Figure 2). This missense mutation led to N308T substitution on the PTP domain of SHP-2 protein. This mutation has not been detected in 15 healthy Senegalese controls.

The mutation rate found in our study (2 of the 6 studied patients) is lower than reported in other populations (Hung et al., 2007; Tartaglia et al., 2002). This difference could be explained, first by the small number of patients enrolled, due to the absence of a clinical consultation in dysmorphology at health facilities in Senegal. The six patients recruited are indeed followed up in the Cardiology Unit of Aristide Le Dantec Hospital for their associated heart abnormalities. Secondly, this study only have sequenced the exons 3, 4, 7 and 8 which are most frequently mutated in NS but mutations may be observed in other exons not studied (Musante et al., 2003; Yoshida et al., 2004). Thirdly, PTPN11 is not the only gene associated with NS. Mutations have also been reported in genes such as KRAS, RAF, SOS1 all involved in the same signaling RAS/MAP pathway (Roberts et al., 2013).

The c.923A>C mutation observed in our study is heterozygous and confirms the autosomal dominant transmission of NS. This mutation leads to the substitution of N308T in SHP-2 protein. It is the first time that this mutation is reported in African patients with NS. Two previous studies have reported this mutation in USA in 3 individuals without any details about their Caucasian or African-American ethnic origin (Pierpont et al., 2009; Tartaglia et al., 2006) (Table 2). It was hypothesized that N308T as de novo mutation may

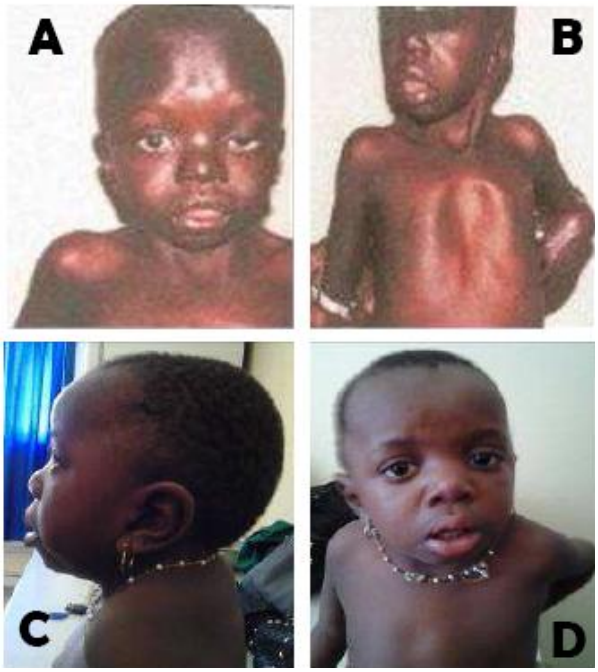

Figure 1. Two NS patients: (A and B) a 4 year old boy (NS3) without mutations in studied exons of PTPN11 gene, typical signs of dysmorphy are ptosis, hypertelorism, webbed neck, chest deformity; (C and D) a 4 year old girl (NS6) with mutation c.923A>C in exon 8 of PTPN11 gene, typical signs of dysmorphy are low set ears, hypertelorism, webbed neck.

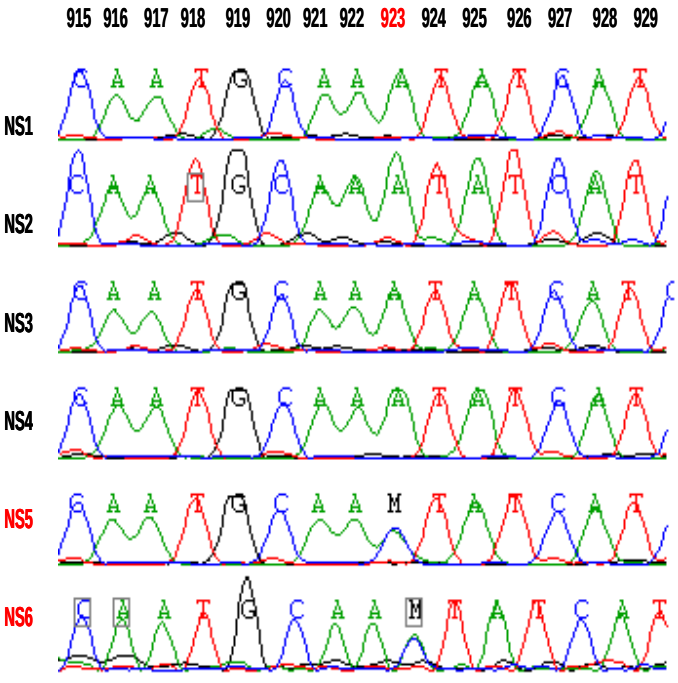

Figure 2. Sequence plots alignment around position 923 of exon 8 of PTPN11 gene in studied patients. NS1 to NS4 are homozygous for the wild type allele at position 923 (genotype A/A); NS5 and NS6 have a heterozygous mutation c.923A>C (genotype A/C).

arise first in Africa. It may be confirmed by investigating the ethnic origin of US patients bearing this mutation.

Although, position 308 of SHP-2 protein have been reported to be the most frequently mutated site in NS, amino-acid substitution reported were mostly N308D and N308S (Keren, 2006; Musante et al., 2003; Tartaglia et al., 2001). These mutations are located in the PTP domain of SHP-2. Crystallographic data have shown that the PTP domain as the SH2 domain play an important role in the stability and function of the SHP-2 protein (Musante et al., 2003; Tartaglia et al., 2001). Amino-acid changes in interaction sites between PTP and N-SH2 domains or near these sites may lead to the switch of SHP-2 protein from its inactive form to an active form, which result in a dominant positive effect on the activation of the RAS/MAPK signaling pathway effect. This activation is responsible for the morphological and cardiac anomalies in Noonan syndrome as reported in mouse model (Chen et al., 2010; Lapinski et al., 2013). Although inactivation of this pathway can rescue congenital heart defects and craniofacial malformations in Noonan mouse model (Nakamura et al., 2007, 2009).

It is also established that N308D mutation lead to a milder hyperactivation of the RAS/MAPK pathway compared to other described mutations in the PTPN11 gene (Oishi et al., 2006). Similarly, patients with Noonan syndrome and carrying this mutation have normal psychomotor development (Sznajer, 2009). This is not the case with the mutated patients in our study (NS5, NS6), which showed growth delay. This could be explained by the difference of involved amino acids. Indeed, it would be appropriate to consider functional studies on mutation N308T in order to evaluate its effect on the SHP-2 protein activity.

Conclusions

This study focused on finding the PTPN11 gene mutations involved in Senegalese patients with Noonan syndrome. Two patients in six had a heterozygous mutation, c.923A>C in exon 8 of PTPN11 gene, resulting in amino acid change Asn308Thr in SHP-2 protein. This is the first time that this mutation is described in Noonan syndrome in Africa although position 308 is considered as a "hot spot" site. The results presented are preliminary results of a pilot study of the PTPN11 gene in Senegalese patients with Noonan syndrome and currently followed in the health services in Dakar.

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ABBREVIATIONS

NS, Noonan syndrome; **PTPN11,** protein tyrosine phosphatase non receptor 11; **PTP,** protein tyrosine phosphatase domain; **HC,** hypertrophic cardiomyopathy; **PS,** pulmonary valve stenosis.

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