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Mutation-in-Brief

KRAS Analysis in 34 Noonan Syndrome Patients without *PTPN11* Mutation

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Introduction

Noonan syndrome (NS) is an autosomal dominant disorder characterized by short stature, cardiovascular lesions, and a constellation of minor anomalies including hypertelorism, webbed neck, and cubitus valgus (1). Mental retardation and hearing difficulty are also often observed in affected individuals, as are hypoplastic external genitalia and cryptorchidism in affected males. Furthermore, malignant disorders such as juvenile myelomonocytic leukemia and neuroblastoma have occasionally been reported in NS (2).

Recent molecular studies have successfully revealed genetic causes in NS. It is known that mutations of *PTPN11* (protein-tyrosine phosphatase, nonreceptor type 11) (3), *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) (4, 5), and *SOS1* (son of sevenless homolog 1) (6, 7) account for roughly 45%, <5% and 5–10% of NS patients, respectively, although the underlying genetic factors are still unknown in a substantial fraction of NS patients. Since such genes are involved in the mitogen-activated protein kinase signaling pathway, this explains the occasional occurrence of malignant disorders in NS (2). Here, we report mutation analysis of *KRAS* in *PTPN11* mutation negative NS patients.

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Patients and Methods

Patients

This study consisted of 34 NS patients (22 males and 12 females) aged 0.1–34.5 years who met the diagnostic criteria proposed by van der Burgt *et al.* (8). All patients were found to have no discernible mutations in the coding exons 1–15 of *PTPN11* by direct sequencing; the clinical and molecular data in *PTPN11* mutation positive patients have been reported previously (9). The karyotype was normal in all the patients.

Mutation analysis of *KRAS*

This study was approved by the IRB at the National Center for Child Health and Development. After obtaining informed consent, leukocyte genomic DNA of each patient was amplified by PCR for all the 5 exons (exons 1–4b) and their flanking splice sites of the *KRAS* gene (isoforms A and B). Subsequently, the PCR products were subjected to direct sequencing from both directions on a CEQ 8000

Table 1 Primer sequences, product sizes and annealing temperatures

	Forward primer Reverse primer	PS AT
Exon 1	GGTGGAGTATTTGATAGTGTATTAACC	246
	AATGGTCCTGCACCAGTAATATG	58
Exon 2	TCTTTGGAGCAGGAACAATG	402
	TGCATGGCATTAGCAAAGAC	58
Exon 3	TGACAAAAGTTGTGGACAGG	391
	AGAAGCAATGCCCTCTCAAG	58
Exon 4a	CAAACTTCTTGCACATGGCTTTCCC	298
	CACCTAAGTAGTTCTAAAGTGGTTGCC	58
Exon 4b	CTGTGCCATTGGTTATCCTTGTCTTTTG	488
	GCTAACAGTCTGCATGGAGCAG	58

PS: product size (bp); AT: annealing temperature (C).

autosequencer (Beckman Coulter, http://www.beckman.com/). The primer sequences and PCR conditions are shown in Table 1.

Results

No mutations were identified in any of the patients, while the previously known silent SNP on exon 4b (519T>C, Asp173Asp, *rs17473423*) was found in 12 patients.

Discussion

No mutations were found in the *KRAS* gene in the 34 NS patients who had no *PTPN11* mutations. This would be consistent with the previous finding that *KRAS* mutations are rare in NS patients (<5%) (2, 4, 5). However, since *KRAS* mutations are frequently associated with malignant lesions (2, 4, 5), *KRAS* appears to be worth analyzing in NS patients.

At present, the underlying genetic cause(s) remains to be clarified in the NS patients examined in this study. Although some of them may have mutations in *SOS1* or in unexamined promoter regions or intronic sequences of *PTPN11* or *KRAS*, most, if not all, of them would be classified as a group of NS patients in whom a causative gene(s) remains to be determined. Thus, when a novel candidate or demonstrated gene for NS has been identified, these patients should be examined for mutations of the gene.

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References

1. Mendez HM, Opitz JM. Noonan syndrome: a review. Am J Med Genet 1985;21:493–506.
2. Tartaglia M, Gelb BD. Noonan syndrome and related disorders: genetics and pathogenesis. Annu Rev Genomics Hum Genet 2005;6:45–68.
3. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, *et al.* Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001;29:465–8.
4. Schubbert S, Zenker M, Rowe SL, Boll S, Klein

C, Bollag G, et al. Germline KRAS mutations cause Noonan syndrome. Nat Genet 2006;38:331–6.

5. Carta C, Pantaleoni F, Bocchinfuso G, Stella L, Vasta I, Sarkozy A, *et al.* Germline missense mutations affecting KRAS isoform B are associated with a severe Noonan syndrome phenotype. Am J Hum Genet 2006;79:129–35.
6. Roberts AE, Araki T, Swanson KD, Montgomery KT, Schiripo TA, Joshi VA, *et al.* Germline gain-of-function mutations in SOS1 cause Noonan syndrome. Nat Genet 2007;39:70–4.
7. Tartaglia M, Pennacchio LA, Zhao C, Yadav KK, Fodale V, Sarkozy A, *et al.* Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. Nat Genet 2007;39:75–9.
8. van der Burgt I, Berends E, Lommen E, van Beersum S, Hamel B, Mariman E. Clinical and molecular studies in a large Dutch family with Noonan syndrome. Am J Med Genet 1994;53:187–91.
9. Yoshida R, Hasegawa T, Hasegawa Y, Nagai T, Kinoshita E, Tanaka Y, *et al.* Protein-tyrosine phosphatase, nonreceptor type 11 mutation analysis and clinical assessment in 45 patients with Noonan syndrome. J Clin Endocrinol Metab 2004;89:3359–64.