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.

**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.
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

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