

76 Molecular analysis of the *PTPN11* gene in Japanese patients with Noonan syndrome

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Noonan syndrome (NS) is an autosomal dominant disorder characterized by dysmorphic facial features, short stature, cardiac defect and skeletal malformations. The missense mutations in *PTPN11* (protein-tyrosine phosphatase, nonreceptor-type 11), the gene encoding the nonreceptor-type protein tyrosine phosphatase SHP-2 (Src homology region 2-domain phosphatase-2) is identified in only 45-50% of cases, suggesting genetically heterogeneous disorder. We analyzed the *PTPN11* gene in 20 Japanese patients by direct sequencing. The mutations were identified in 10 out of 20 patients, indicating the prevalence to 50%. All identified mutations were heterozygous missense mutations (Asp61Gly, Ala72Ser, Ala72Val, Leu74Phe, Ala75Ser, Glu76Asp, Gln79Arg in exon 3, Asn308Asp, Asn308Ser in exon 8, and Gly464Ala, Met504Val in exon 13). The novel clustered mutations of Ala72Val, Leu74Phe and Ala75Ser were identified in one patient.

Genotype-phenotype analysis revealed that mental retardation was less prevalent in the *PTPN11* mutation positive group than in the negative group (1/10 vs. 6/10; $P < 0.05$). Webbed neck was less prevalent (4/10 vs. 9/10; $P = 0.07$), and atrial septal defect was more prevalent in those with *PTPN11* mutations (3/10 vs. 0/10; $P = 0.11$). But these were not significant. The prevalence of other cardiac defect, short stature did not differ between the two groups.

78 The Missense Mutation at the Cleavage Site of Insulin-like Growth Factor-1 Receptor (arg709gln) in a Family with Short Stature Born Intrauterine Growth Retardation.

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We hypothesized that mutations of IGF-1R gene might predispose short stature born with intrauterine growth retardation (IUGR). Therefore, we determined the nucleotide sequence of the IGF-1R gene in 24 proband of short stature with IUGR.

1) A heterozygous mutation (R709Q) changing the cleavage site from Arg-Lys-Arg-Arg to Arg-Lys-Gln-Arg was identified in a 6-year-old Japanese girl and her mother who were short stature born with IUGR. 2) IGF-1 binding to fibroblasts from mother was reduced than normal control. 3) Growth of fibroblasts was significantly decreased compared to that of control. 4) These fibroblasts produced more IGF-1R proreceptors than that of normal control, and less β subunits than that of normal.

These findings strongly suggest that this mutation induce the failure of processing proreceptor in the same manner as a reported mutation at cleavage site of insulin receptor, and cause IGF-1 resistance and short stature born with IUGR. This study provides new and important information on the mechanism of short stature born with IUGR.

77 PTPN11 GENE AND NOONAN/LEOPARD SYNDROME, MUTATIONS ANALYSIS AND COMPARISONS OF THE CLINICAL FEATURES OF 45 JAPANESE PATIENTS.Sasaki R¹, Hasegawa T¹, Matsuo N¹, Sato S², Takiyama N³, Hasegawa Y⁴, Nakagome Y⁵, Ooyama K⁵, Kinoshita E⁶, Yoshimoto M⁶, Oonishi T⁷, Nagai T⁸, Tanaka Y⁹, Horikawa R¹⁰, Tanaka T¹⁰, Ogata T¹⁰

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[Aim] The *PTPN11* gene has been reported to cause Noonan syndrome and its related disorder, LEOPARD syndrome. We analyzed the *PTPN11* gene in 45 Japanese patients, and compared clinical features between mutation positive and negative patients.

[Methods] Mutation analysis was performed by direct sequencing of the 15 coding exons of the *PTPN11* gene in 41 patients with Noonan syndrome and 4 patients with LEOPARD syndrome.

[Results] Ten different missense mutations were identified in 17 patients, and a 3 bp deletion (G60del) was found in one patient. There was no statistically significant difference in the prepubertal height-SDS between mutation positive and negative patients ($P = 0.93$). The frequency of cardiac anomalies was higher in mutation positive patients ($P = 0.01$).

[Discussion] The results indicate that *PTPN11* mutations accounts for roughly 40% of patients with Noonan/LEOPARD syndrome, and first show the presence of a deletion mutation of the *PTPN11* gene in a Noonan syndrome patient. Comparison of clinical features imply that the degree of prepubertal short stature is similar but the prevalence of cardiac anomalies is significantly different between mutation positive and negative patients.

79 STUDIES ON INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR IN FETAL RATS WITH INTRAUTERINE GROWTH RETARDATION

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This study was conducted to detect the localization of the insulin-like growth factor 1 receptor (IGF-1R) in the cells and tissues in the rats with intrauterine growth retardation (IUGR) and normal growth rats. 21 pregnant rats were randomly divided into the experimental group (IUGR) ($n = 12$) and the control group ($n = 9$). Animal models for IUGR were established by clamping the uterine vasculature of pregnant rats for 20 minutes on day 14 of gestation. The control group rats were subjected to Sham-operation. On day 22 of gestation, fetal rats were delivered by cesarean section. Immunohistochemical assay was performed to detect the IGF-1R expression on liver and lung using specific polyclonal antibodies to rat IGF-1R. The results showed that in the experimental group, the IUGR rats' body weight, height, and the weight of their liver, lung and the placenta were significantly lower than those in the control group. In the IUGR rats' liver, the area ratio of IGF-1R was increased and the average density was increased, compared with those in the control group. In the IUGR rats' lung, the area ratio of IGF-1R was increased, compared with that in the control group, but no difference of average density was observed between the two groups. These suggest that the increased expression of IGF-1R in rats with IUGR, may be due to the decrease of IGF-1 and the compensative mechanism of the body.