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Short communication

Germline *PTPN11* missense mutation in a case of Noonan syndrome associated with mediastinal and retroperitoneal neuroblastic tumors

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Abstract Noonan syndrome (NS) is an autosomal dominant disorder characterized by short stature, typical craniofacial dysmorphism, skeletal anomalies, congenital heart defects, and predisposition to malignant tumors. In approximately 50% of cases, the disease is caused by missense mutations in the *PTPN11* gene. To date, solid tumors, and particularly brain tumors and rhabdomyosarcomas, have been documented in patients with NS; however, few cases of neuroblastoma associated with NS have been reported. Here we report an unusual case of neuroblastoma with mediastinal, retroperitoneal, and medullar locations associated in a NS patient carrying a *PTPN11* germline missense mutation (p.G60A). This missense mutation occurs within the N-SH2 domain of the *PTPN11* gene and has been reported to be associated with acute leukemia in NS patients. The association of this p.G60A *PTPN11* mutation with neuroblastoma provides new evidence that gain of function *PTPN11* mutations may play an important role in the pathogenesis of solid tumors associated with Noonan syndrome.

1. Introduction

Noonan syndrome (NS; MIM #163950) is an autosomal dominant disorder characterized mainly by short stature, hypertelorism, downward eye slant, low-set posteriorly rotated ears, epicanthic folds, wide-spaced nipples, and short neck with webbing or redundancy of skin. Other typical features include a characteristic chest deformity with a pectus carinatum and pectus excavatum, mild mental retardation, predisposition to malignant tumors, and congenital cardiac anomalies [1,2].

In ~50% of cases, NS is caused by missense mutations in the *PTPN11* gene (protein-tyrosine phosphatase, nonreceptor-type 11), coding for SHP-2. The SHP-2 gene product is a nonreceptor protein tyrosine phosphatase containing two SH2 domains (N-SH2, C-SH2), a PTP domain, and a C-tail with tyrosine phosphorylation sites and a proline-rich motif [3]. SHP-2 participates in signal transduction downstream of growth factor receptors to regulate multiple cellular responses, including proliferation, differentiation, and migration [4].

SHP-2 is the first known tyrosine phosphatase that functions as an oncogene in human cancer [5]. Indeed, germline and somatic mutations are reported to enhance the function of SHP-2. For instance, N-SH2 mutations disrupt inactivating interactions with the PTP domain and upregulate phosphatase activity, causing an inappropriate activation of the RAS/MAPK cascade (RAS-mitogen activated protein kinase). Commonly, germline mutations in SH2/PTP are observed in NS patients, whereas somatic mutations are often identified in hematological myeloid malignancies, including juvenile myelomonocytic leukemia, myelodysplastic syndromes, acute lymphoblastic leukemia, and acute myeloid leukemia [6]. Both myeloid leukemias and a variety of solid tumors including astrocytoma, glioblastoma, glioma, medulloblastoma, rhabdomyosarcoma, melanoma, lung adenocarcinoma, breast and colon cancers have been described in patients with NS [7–9]. To date, few cases of neuroblastic tumors have been reported in NS patients [10–12].

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Here we report a *PTPN11* germline missense mutation in a patient with a NS phenotype, a patient in whom multiple neuroblastic tumors were detected.

2. Materials and methods

2.1. Case report

The propositus was the second child of unrelated white parents. She was born at 39 weeks gestation with a birth weight of 2,725 g (10th to 25th percentile), length of 48 cm (25th percentile), and occipitofrontal circumference of 34 cm (50th to 90th percentile). The results of antenatal fetal ultrasound were not significant, apart from neck translucency (crown–rump length of 95 mm) and agenesis of the venosus ductus. At birth, she presented a severe respiratory distress due to inhalation of meconium, and had decreased tone and feeding difficulties. She remained 2 days in the neonatal intensive care unit.

During this time, a heart murmur was noted and X-radiography of the chest revealed a cardiomegaly (cardiothoracic index of 0.67). Echocardiography revealed a ventricular septal defect, a secundum atrial septal defect with left-to-right shunting, and moderate pulmonary stenosis without evidence of dysplastic pulmonary valve. Abdominal echography revealed a hepatic steatosis. Clinically, she presented an excessive nuchal skin (cystic hygroma), hypertelorism, microcephaly, small nasal bridge, long smooth philtrum, webbing of the neck, wide-spaced nipples, and the persistence of a large anterior fontanelle. A systolic murmur was best heard in the upper left sternal border. NS diagnosis was suspected and mutational analysis of *PTPN11* gene was carried out.

At 5 months of age, a computed tomography scan and radiographic examinations of the chest and abdomen revealed several posterior mediastinal and retroperitoneal masses with calcifications. The diagnosis of neuroblastoma was confirmed by scintigraphy with meta-iodobenzylguanidine, dosage of tumor markers, and histological analysis. Further tests revealed bone marrow infiltration and cranial metastases. The *MYCN* gene was not amplified. Urinary catecholamine metabolites analysis showed increased vanillylmandelic acid and homovanillic acid values (273.8 and 285.08 μg/mg creatinine, respectively). The patient received chemotherapy treatment including four cycles of carboplatin and VP16 followed by three cycles of hydroxyurea, cyclophosphamide and doxorubicin (SIOP Europe Neuroblastoma 99.03 protocol). The patient achieved complete remission.

2.2. Mutation analysis of the PTPN11 gene

Patient genomic DNA sample was extracted from peripheral lymphocytes using a Qiagen kit (Qiagen, Valencia, CA; Courtaboeuf, France). The *PTPN11* mutational analysis on the genomic DNA sample was performed on exons 2, 3, 4, 7, 8, 12, and 13 and their flanking intronic boundaries, because these coding regions encompass the mutational hot spot sites [13].

The PCR reactions were performed as previously described [14]. PCR products were analyzed by denaturing high-performance liquid chromatography (DHPLC), using a WAVE HPLC and DNASep column (Transgenomic, Elancourt, France) as previously described [15]. The abnormal DHPLC profiles were directly sequenced bidirectionally using an ABI 3100 capillary array sequencer (Applied Biosystems, Foster City, CA).

3. Results and discussion

Most commonly, NS is associated with a variety of pediatric hematologic malignancies, including juvenile myelomonocytic, lymphoblastic, and acute myelogenous leukemias [16,17]. Moreover, solid tumors (including brain and thyroid tumors, rhabdomyosarcoma, cutaneous melanoma, and colon cancer) are frequent in NS patients.

There have been few reported cases of neuroblastoma in association with NS, and all of these neuroblastic tumors were mediastinal masses [10–12]. In the present case, however, the neuroblastic tumors were located in both mediastinal and retroperitoneal sites and also showed medullary and cranial dissemination. Neuroblastoma is a malignant childhood tumor of neuroectodermal cells derived from neural crest and migrating to the adrenal medulla and the sympathetic nervous system [18].

About half of the NS patients have germline missense mutations in the *PTPN11* gene [19]. The *PTPN11* gene, located in 12q24.1 (MIM 163950; http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim), encodes the SHP-2 protein. This protein is a member of a subfamily of cytoplasmic Src homology-2 (SHP-2) domain-containing protein tyrosine phosphatases (PTPs), which play an important role in several intracellular signal transduction pathways and control a number of developmental processes, including cardiac semilunar valvulogenesis [3]. Mutations in tyrosine kinase or phosphatase can result in malignant transformation by intracellular accumulation of tyrosine-phosphorylated proteins [20]. However, gain-of-function mutations in PTP including SHP-2 have recently been associated with tumorigenesis.

In the present case, the *PTPN11* mutational analysis performed on genomic DNA of the proband, identified a heterozygous transition G→C at position 179 within exon 3, predicting the substitution of glycine by an alanine residue (p.Gly60Ala) within the N-SH2 domain of the *PTPN11* gene. Most of the *PTPN11* mutations are recurrent and cluster in the N-SH2 (exon 3) and PTP-domains (exons 7, 8, and 13) [17,21]. The *PTPN11* mutations identified in patients with NS destabilize the catalytically inactive conformation and activate the SHP-2 protein. In addition, almost all of the mutations occurring within exon 3 of *PTPN11*

activate phosphatase activity by altering N-SH2 amino acids that interact with the PTPase domain [19].

The missense mutation p.G60A identified in the N-SH2 domain of the *PTPN11* gene has been reported in 4% of cases to be associated with acute myeloid leukemia and acute lymphoblastic leukemia in NS patients [6,22]. Previous data suggest that low levels of SHP2 activation result in NS, whereas higher levels of activity may be required for leukemogenesis [5]. Indeed, SHP2 mutants promote myeloid cell survival and proliferation, and preferentially enhance monocytoid differentiation [23].

Recently, Bentires-Alj et al. [7] reported a series of solid tumors associated with somatic *PTPN11* mutations. Among them, the vast majority were located within the N-SH2 at the N-SH2/PTP interface, and all of these mutations contribute to oncogenesis by increased basal PTP activity. In 89 patients with neuroblastomic tumors, the authors identified only three *PTPN11* mutations: the missense p.Y62C (exon 3), p.E69K (exon 3), and p.T507K (exon 13) mutations. Two of these were somatic mutations, but one (p.Y62C) was germline, suggesting an undiagnosed, and thus probably mild, NS in that patient [7].

The present findings suggest that the *PTPN11* mutations, thought to be implicated in leukemogenesis by gain of function in SHP-2 and in inappropriate activation of SHP-2, may play an important role in the pathogenesis of solid tumors associated with NS. To our knowledge, the present patient is the only one in whom a germline *PTPN11* c.G179>C (p.G60A) missense mutation affecting exon 3 has been reported to be associated with neuroblastoma.

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