CLINICAL GENETICS An International Journal of Genetics, Molecular and Personalized Medicine

Clin Genet 2013: 83: 181–186
Printed in Singapore. All rights reserved

Published by Blackwell Publishing Ltd
CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2012.01875.x

Short Report

Clinical and molecular analysis of RASopathies in a group of Turkish patients

Şimşek-Kiper PÖ, Alanay Y, Gülhan B, Lissewski C, Türkyılmaz D, Alehan D, Çetin M, Utine GE, Zenker M, Boduroğlu K. Clinical and molecular analysis of patients with RASopathies in Turkish patients. Clin Genet 2013: 83: 181–186. © John Wiley & Sons A/S. Published by Blackwell Publishing Ltd, 2012

The 'RASopathies' are a group of disorders sharing many clinical features and a common pathophysiology. In this study, we aimed to clinically evaluate a group of Turkish patients and elucidate the underlying genetic etiology. Thirty-one patients with a clinical diagnosis of one of the RASopathy syndromes were included in the study. Of these, 26 (83.8%) had a clinical diagnosis of Noonan syndrome, whereas 5 had a clinical diagnosis of either Costello, LEOPARD or cardio-facio-cutaneous syndromes. Twenty of 31 (64.5%) patients were found to be mutation positive. Mutations in *PTPN11*, *SOS1* and *SHOC2* genes were detected in patients with Noonan syndrome (57.6%). Mutations in *MEK1*, *PTPN11*, *BRAF* and *HRAS* genes were detected in the remaining. Pulmonary stenosis was the most common (61.5%) cardiac anomaly. Among Noonan syndrome patients with a confirmed mutation, mild intellectual disability tended to be more common in patients with *PTPN11* mutation than in those with *SOS1* mutation. Hematologic evaluation revealed coagulation defects in three Noonan syndrome patients with a mutation. This is currently the largest clinical and molecular study in Turkish RASopathy patients. Our findings indicate that molecular epidemiology and genotype–phenotype correlations in RASopathies are relatively independent from the ethnic population background.

Conflict of interest

The authors declare that they have no conflict of interest.

PÖ Şimşek-Kiper[a], Y Alanay[b], B Gülhan[a], C Lissewski[c], D Türkyılmaz[d], D Alehan[e], M Çetin[f], GE Utine[a], M Zenker[c] and K Boduroğlu[a]

[a]Pediatric Genetics Unit, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey, [b]Pediatric Genetics Unit, Department of Pediatrics, Faculty of Medicine, Acibadem University, Istanbul, Turkey, [c]Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany, [d]Audiology Unit, Department of Ear-Nose-Throat, Faculty of Medicine, Hacettepe University, Ankara, Turkey, [e]Pediatric Cardiology Unit, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey, and [f]Pediatric Hematology Unit, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Key words: *BRAF* – cardio-facio-cutaneous – Costello – *HRAS* – LEOPARD – Noonan – *PTPN11* – *SHOC2* – *SOS1*

Corresponding author: Pelin Özlem Şimşek-Kiper, MD, Pediatric Genetics Unit, Hacettepe University İhsan Doğramacı Children's Hospital, 06100 Sihhiye, Ankara, Turkey.
Tel.:: +90 312 311 55 22;
fax: +90 312 311 55 22;
e-mail: pelinozlem@hacettepe.edu.tr

Received 11 December 2011, revised and accepted for publication 12 March 2012

Recent genetic studies have showed that Noonan syndrome (NS) and related disorders (now collectively called 'RASopathies') are caused by germline mutations in a number of genes interacting in the same Ras/mitogen-activated protein kinase pathway (1–3). The overlapping clinical features, wide spectrum of phenotypic expression within each trait, and the absence of clinical features with pathognomonic value and consensus on diagnostic criteria can make diagnosis of NS and related disorders challenging (4). Therefore, molecular analysis has become an important key for the verification of clinical diagnosis and establishment of individual follow-up plans. The most consistent genotype–phenotype correlations that have

been confirmed in independent cohorts are: *PTPN11*, *KRAS*, *SOS*, *RAF1*, and *NRAS* gene mutations in NS (1); *SHOC2* gene mutations in NS-like disorder with loose anagen hair (1); *CBL* gene mutations in disorder with variable NS-like features and propensity to JMML (1); *HRAS* gene mutations in Costello syndrome (CS) (5); specific *PTPN11* gene mutations in LEOPARD syndromes (LS) (6, 7); *BRAF*, *KRAS*, *MEK1*, *MEK2* gene mutations in cardio-facio-cutaneous (CFC) syndrome (1, 8, 9). Studies on clinical features and molecular etiology of RASopathy syndromes in additional cohorts with various ethnic background will contribute not only to our understanding of clinical variability and molecular epidemiology but also to refinement of genotype–phenotype correlations in these disorders.

Materials and methods

Clinical evaluation

Clinical diagnosis of NS was made according to the NS scoring system (10), whereas for CFC Kavamura index was utilized (11). All patients were examined by experienced clinical dysmorphologists (Y. A. and K. B.). Ethical approval and informed consent were received from patients or their legal guardians. Anthropometric measurements, cardiovascular evaluation with echocardiography and cardiac catheterization when necessary, hematological evaluation including total blood count, peripheral blood smear and coagulation parameters (aPTT, INR, vWF, FII, FV, FVII-FXIII levels), hearing assessment and psychometric evaluation using Stanford–Binet test and Wechsler Intelligence Scale for Children-Revised (WISC-R) in 2–6 and 6–16 years of age of patients, respectively were performed.

Molecular screening

DNA from patients and their parents were extracted from peripheral blood lymphocytes by using standard extraction procedures. National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) was used to obtain gene information. All exons harboring known mutations in Noonan, CFC, Costello and LEOPARD syndromes were analyzed for each gene studied. Initially the genes *PTPN11*, *SOS1*, *KRAS*, *RAF1*, *SHOC2*, *NRAS* and *CBL* were analyzed for patients with NS and LS. For this purpose exons and flanking intronic regions of the genes including *PTPN11* Exons 2–4, 7, 8, 11–14 (NM_002834), *RAF1* Exons 7, 12, 14, 17 (NM_002880), *KRAS* isoform B Exon 2, 3, 5 (NM_004985), *SOS1* Exons 3–11, 13,14, 16 (NM_005633), *SHOC2* Exon 2 partial (NM_007373), *NRAS* Exons 2, 3 (NM_002524), *CBL* Exons 7–9 (NM_005188) were amplified by PCR and analyzed by high resolution melting on a Light Cycler (Roche, LC-480; Roche Diagnostics, Grenzach-Wyhlen, Germany). Oligonucleotide primer sequences and PCR protocols are available on request. Melting graphs were compared using the Light Cycler 480 Gene Scanning Software (Roche). The curves differing from the wild type, indicated samples with mutation. For variants from standard curves, the PCR products were then purified and sequenced using an automated capillary sequencer. Sequence analysis was performed using 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA). For the patient with a clinical diagnosis of CS only the *HRAS* (NM_005343) gene was analyzed using bidirectional sequencing. Patients with negative results upon high resolution melting (HRM) screening and targeted sequencing were subsequently sequenced for the genes *BRAF* (NM_004333), *MEK1* (NM_002755), *MEK2* (NM_030662) and *HRAS* as well as the remaining exons of *PTPN11*.

Statistical analysis

Statistical analysis of clinical findings of patients with mutation *vs* patients without mutation was performed at Hacettepe University Ihsan Dogramaci Children's Hospital using SPSS version 19.0, with a statistical significance set at $p < 0.05$.

Results

Clinical evaluation

Craniofacial features and a summary of clinical findings of mutation-positive and mutation-negative patients are shown in Fig. 1 and Table 1, respectively. In total, 31 patients with a suspected RASopathy were included in the study. Of these, 26 (83.8%) had a clinical diagnosis of NS. The remaining patients had two Costello, two LEOPARD and one CFC diagnoses. Overall, 27 patients were sporadic; however, four cases had a parent with suggestive features of NS. Audiological assessment was performed and found to be normal in 10 patients with NS. Hematologic evaluation revealed thrombocytopenia in a mutation-negative patient who is still under investigation and thrombocyte aggregation abnormality in three patients each with a mutation in one of the three genes (*SOS1*, *PTPN11* and *SHOC2*) and in two other patients with no mutation.

Mutation screening

All exons of *PTPN11*, *SOS1*, *SHOC2*, *RAF1*, *KRAS*, *BRAF*, *MEK1*, *MEK2*, *HRAS*, *NRAS* and *CBL* genes harboring known mutations in Noonan, CFC, Costello and LEOPARD syndromes were analyzed. We identified heterozygous point mutations in 20 of 31 patients with an overall detection rate of 64.5% (Table 2). Mutations were detected in 16 (80%) sporadic patients and in 4 (20%) patients with a family history of RASopathy. The remaining patients were negative regarding *PTPN11*, *SOS1*, *SHOC2*, *RAF1*, *KRAS*, *BRAF*, *MEK1*, *MEK2*, *HRAS*, *NRAS* and *CBL* mutations. All of the detected mutations in this study have been previously described as causative changes for NS and related disorders (5, 6, 8, 12–17).

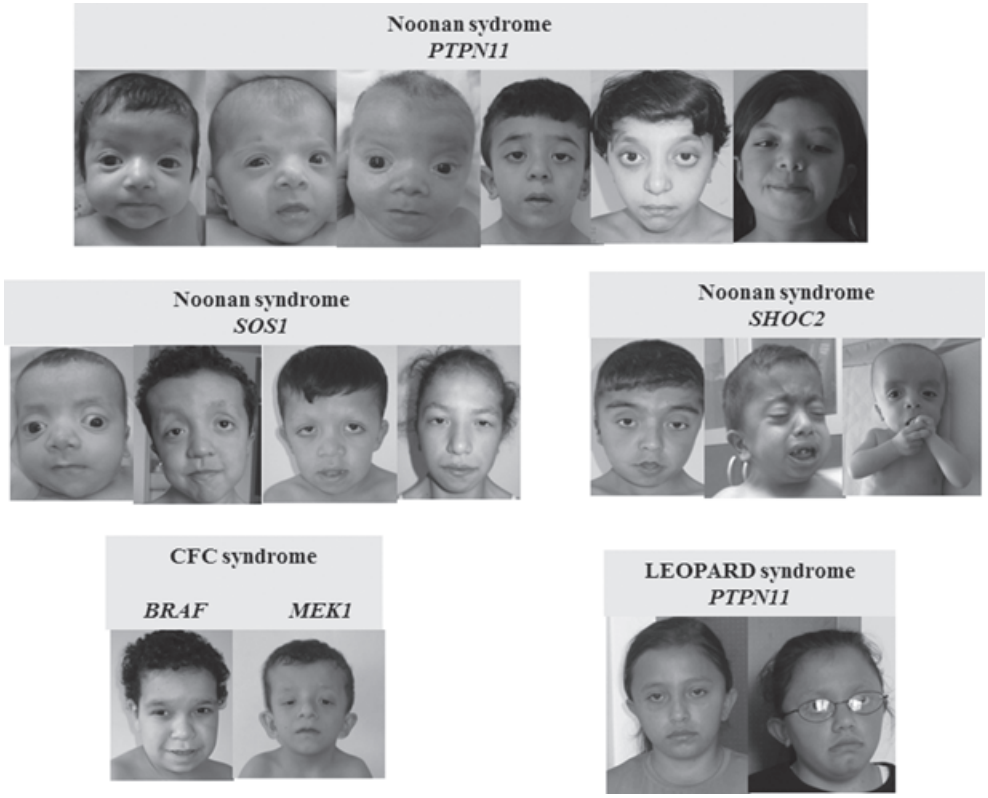

Fig. 1. Craniofacial features of patients with molecularly confirmed RASopathy.

Discussion

In this report, we describe our RASopathy patients both clinically and molecularly. Heterozygous point mutations were identified in 20 of 31 patients, most of which represented sporadic cases, probably due to *de novo* mutations. NS was confirmed in 58% of patients whereas LEOPARD and CFC syndromes were confirmed molecularly in all presumed patients. In two patients with CS, one was found to have a *BRAF* mutation (Exon 12: c.1495A>G), therefore, changing the diagnosis to CFC syndrome whereas the other one was found to have a *HRAS* (Exon 2: c.35G>C) mutation confirming the clinical diagnosis.

The majority of patients with NS had *PTPN11* mutations while *SOS1* and *SHOC2* mutations were also detected. The lack of *RAF1* mutations and the apparent over representation of *SHOC2* mutations is most likely a bias due to relatively small size of the cohort.

Mutations in *PTPN11*, the first described and predominant gene in NS (13), are reported in 33–50% of patients in literature. In this study, 27% of patients with NS had *PTPN11* mutations which were almost exclusively present in Exons 3 and 8. Interestingly, Exon 8 mutation (c.922A>G) previously described as the most common mutation in NS (12) was not detected in any of our patients.

SOS1 mutations are known to cause NS in about 20% of affected individuals without *PTPN11* mutations (13, 14). *SOS1* mutation detection rate in our cohort was 19% and all except one were sporadic. Phenotypically patients with *SOS1* mutation often present with ectodermal abnormalities with normal stature, growth and intellectual development (14, 15, 18). Such a trend could also be reproduced in our cohort which was, however, too small for statistical significance.

SHOC2 mutations have only recently been reported among genes responsible for a recognizable subtype of NS (16). A unique feature of the *SHOC2* mutation is its association with 'loose anagen hair' (1). In this study, the recurrent *SHOC2* mutation p.S2G was detected in three NS patients. All displayed loose anagen hair phenotype. One of them had systemic lupus erythematosis (SLE). However, the causal interrelationships remain speculative and more systematic studies and long-term follow-up data are needed to clarify the possible association.

CFC shows considerable phenotypic overlap with Noonan and Costello syndromes, especially in young children (19). Patients with CFC syndrome most frequently have *BRAF* or *MEK* mutations, whereas specific *HRAS* mutations are regarded as definitive for CS (20, 21). In our cohort, one of the two patients with a clinical diagnosis of CS was found to have a *BRAF* mutation (Exon 12: c.1495A>G) leading to a change in diagnosis toward CFC syndrome.

The most common congenital heart disease in NS is pulmonary valve stenosis with dysplastic leaflets (22, 23). Prevalence of pulmonary stenosis in NS patients with *PTPN11* mutations is reported to be as high as 74% (24). In this study, 71.4% of patients

Table 1. Clinical features of patients with RASopathy syndromes

Gene RASopathy syndrome	*PTPN11* NS	*SOS1* NS	*SHOC2* NS	*HRAS* CS	*BRAF* CFC	*MEK1* CFC	*PTPN11* LS	RASopathy patients with no molecularly confirmed mutation
Number of patients	7	5	3	1	1	1	2	11
Gender (f, m)	4f, 3m	2f, 3m	3m	f	F	m	2f	3f, 8m
Age (range in months)	3–204	24–192	7–204	6	126	42	138–162	42–348
Prenatal findings	4/7	5/5	1/3	+	+	+	1/2	3/11
Polyhydramnios	2/7	1/5	–	+	–	–	–	2/11
Preterm event	2/7	1/5	–	+	–	–	–	2/11
Neonatal lymphatic anomalies	1/7	2/5	1/3	+	+	–	–	2/11
Small gestational age	–	1/5	–	–	–	+	1/2	–
Large gestational age	2/7	2/5	–	–	–	–	–	–
Growth and development								
Short stature (<3‰)	5/7	3/5	2/3	+	+	+	2/2	4/11
Macrocephaly (>2SD)	–	2/5	2/3	–	+	+	–	4/11
Feeding difficulties	3/7	1/5	–	+	–	+	–	1/11
Growth retardation	4/7	4/5	1/3	+	–	+	–	8/11
Developmental delay	4/7	4/5	1/3	+	+	+	–	9/11
Muscular hypotonia	4/7	4/5	1/3	+	+	+	–	7/11
Speech delay	2/7	2/5	1/3	–	+	+	–	3/11
Intellectual disability	4/7	2/5	1/3	–	+	+	–	5/11
Epilepsy	–	–	1/3	–	–	–	–	1/11
Cerebral anomalies	–	–	–	–	+	+	–	3/11
Craniofacial findings								
Hypertelorism	6/7	5/5	3/3	+	+	+	–	8/11
Down-slanting palpebral fissures	7/7	3/5	3/3	+	+	+	–	6/11
Ptosis	7/7	5/5	3/3	+	–	+	2/2	10/11
Broad forehead	6/7	5/5	3/3	+	–	+	2/2	7/11
Short neck	6/7	5/5	3/3	+	+	+	2/2	7/11
Low set and/or posteriorly rotated ears	5/7	2/5	3/3	–	–	+	–	6/11
Webbed neck	4/7	3/5	3/3	–	–	+	–	6/11
Low posterior hair line	4/7	3/5	3/3	–	–	+	–	7/11
Ectodermal findings								
Curly hair	2/7	3/5	–	+	+	+	–	2/11
Sparse and/or slow growing hair	2/7	1/5	–	+	–	+	1/2	–
Sparse eye brows	4/7	4/5	–	+	+	+	2/2	4/11
Keratosis pilaris	1/7	–	–	–	–	–	–	1/11
Deep palmar/plantar crease	–	–	1/3	+	+	+	–	–
Nevus	–	–	–	–	–	+	–	1/11
Lentigines	–	–	–	–	+	+	2/2	–
Hemangioma	–	–	–	–	–	+	–	2/11
Cafe au-lait macules	–	–	–	–	+	–	2/2	1/11
Congenital heart defects	5/7	5/5	2/3	+	+	–	–	7/11
Pulmonary valve stenosis	5/7	5/5	1/3	–	–	–	–	5/11
Atrial septal defect	3/7	2/5	1/3	+	–	–	–	5/11
Ventricular septal defect	–	1/5	–	–	–	–	–	2/11
Hypertrophic cardiomyopathy	1/7	1/5	–	+	–	–	–	–
Mitral valve anomaly	1/7	–	–	–	+	–	–	2/11
Renal anomaly	–	1/5	–	–	–	+	–	1/11
Cryptorchidism (males)	2/7	1/5	2/3	–	–	+	–	4/8
Cubitus valgus	3/7	3/5	2/3	–	+	–	2/2	7/11
Pectus deformity	3/7	4/5	1/3	–	+	–	2/2	6/11
Wide chest	1/7	1/5	2/3	–	+	–	–	3/11
Scoliosis	–	2/5	–	–	–	–	–	1/11
Ocular anomalies	2/7	1/5	1/3	–	+	+	2/2	5/11
Strabismus	–	1/5	–	–	+	+	–	2/5
Refractory error	1/7	–	1/3	–	–	–	2/2	3/5
Glocoma	1/7	–	–	–	–	–	–	–
Coagulation defects	1/7	1/5	1/3	–	–	–	–	3/11
Deafness	–	–	–	–	–	–	–	1/11
Family history	1/7	1/5	–	–	–	–	2/2	–

CFC, cardio-facio-cutaneous syndrome; CS, Costello syndrome; LS, LEOPARD syndrome; NS, Noonan syndrome.

Table 2. Molecular characterization and clinical diagnosis of patients included in the study

Gene	Exon	Nucleotide substitution	Amino acid substitution	Disorder	Number of patients	Novel	Sporadic/familial
PTPN11	3	c.181G>A	p.D61N	NS	1	–	Sporadic
PTPN11	3	c.182A>G	p.D61G	NS	1	–	Sporadic
PTPN11	3	c.184T>G	p.Y62D	NS	1	–	Sporadic
PTPN11	3	c.188A>G	p.Y63C	NS	1	–	Sporadic
PTPN11	3	c.317A>C	p.D106A	NS	1	–	Familial
PTPN11	8	c.854T>C	p.F285S	NS	2	–	Sporadic
SOS1	6	c.797C>A	p.T266K	NS	1	–	Sporadic
SOS1	6	c.806T>G	p.M269R	NS	2	–	Sporadic
SOS1	10	c.1642A>C	p.S548R	NS	1	–	Familial
SOS1	10	c.1655G>A	p.R552K	NS	1	–	Sporadic
SHOC2	2	c4A>G	p.S2G	NS	3	–	Sporadic
HRAS	2	c.35G>C	p.G12A	CS	1	–	Sporadic
BRAF	12	c.1495A>G	p.K499E	CFC	1	–	Sporadic
MEK1	3	c.389A>G	p.Y130C	CFC	1	–	Sporadic
PTPN11	12	c.1403C>T	p.T468M	LS	2	–	Familial

CFC, cardio-facio-cutaneous syndrome; CS, Costello syndrome; LS, LEOPARD syndrome; NS, Noonan syndrome.

with NS had pulmonary valve stenosis. Hypertrophic cardiomyopathy was a rare finding among *PTPN11* and *SOS1* mutation positive NS patients in our cohort, which was a parallel finding with the lack of *RAF1* mutations.

In this study, mutation detection rate in patients with Noonan and CFC syndromes is not significantly different from previous reports (8, 12, 13). We were unable to identify the molecular cause in about 35% of our patients with NS but we believe that these patients still have this syndrome, yet through undiscovered genes and mutations. This study might have had minor limitations because of the fact that for some genes, only selected exons were screened for mutations. However, this selection included all exons in which germline mutations have been described, to date. As RASopathy-associated mutations have been shown to cause gain-of-function and cluster in specific domains of the gene products (5, 6, 8, 12–17), the chosen approach is very unlikely to miss a relevant number of mutations. The variations detected in our cohort were very heterogeneous. Therefore, a statistically significant comparison between patients with specific genotypes could not be made.

In conclusion, this is the largest Turkish cohort of patients with RASopathies. We found that mutation spectrum and phenotypic expressions are not significantly different from cohorts with other ethnic background. We found an interesting association of SLE with a *SHOC2* mutation. More subtle phenotype associations and differences in molecular epidemiology would require much larger cohorts.

Acknowledgements

We thank all family members who participated in this study. This work was supported by a grant from the European Research Area Network for research programs on rare diseases (E-Rare) 2009 to M. Z. (European Network on Noonan Syndrome and Related Disorders). This work was also supported by Hacettepe University Faculty of Medicine Scientific Research Unit (Project number: LUT 09/118 with LUT 09/118-176).

References

1. Tartaglia M, Zampino G, Gelb BD. Noonan syndrome: clinical aspects and molecular pathogenesis. Mol Syndromol 2010: 1: 2–26.
2. Tidyman WE, Rauen KA. The rasopaties: developmental syndromes of Ras*Mapk pathway dysregulation. Curr Opin Genet Dev 2009: 19: 230–236.
3. Zenker M. Genetic and pathogenetic aspects of NS and related disorders. Horm Res 2009: 72 (Suppl 2): 57–63.
4. Tartaglia M, Gelb BD, Zenker M. Noonan syndrome and clinically related disorders. Best Pract Res Clin Endocrinol Metab 2011: 25: 161–179.
5. Aoki Y, Niihori T, Kawame H et al. Germline mutations in HRAS proto-oncogene cause Costello syndrome. Nat Genet 2005: 37: 1038–1040.
6. Digilio MC, Conti E, Sarkozy A et al. Grouping of multiple lentigines/LEOPARD and Noonan syndromes on the PTPN11 gene. Am J Hum Genet 2002: 71: 389–394.
7. Legius E, Schrander-Stumpel C, Schollen E et al. PTPN11 mutations in LEOPARD syndrome. J Med Genet 2002: 39: 571–574.
8. Niihori T, Aoki Y, Narumi Y et al. Germline KRAS and BRAF mutations in cardiofacio-cutaneous syndrome. Nat Genet 2006: 38: 294–296.
9. Zenker M. Clinical manifestations of mutations in RAS and related intracellular signal transduction factors. Curr Opin Pediatr 2011: 23: 443–451.
10. Van der Burgt I, Berends E. Lommen E et al. Clinical and molecular studies in a large Dutch family with Noonan syndrome. Am J Med Genet 1994: 1: 187–191.
11. Kavamura MI, Peres CA, Alchorne MM, Brunoni D. CFC index for the diagnosis of cardiofaciocutaneous syndrome. Am J Med Genet 2002: 112: 12–16.
12. Tartaglia M, Kalidas K, Shaw A et al. PTPN11 mutations in Noonan syndrome: molecular spectrum, genotype–phenotype correlation, and phenotypic heterogeneity. Am J Hum Genet 2002: 70: 1555–1563.
13. Tartaglia M, Mehler EL, Goldberg R et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001: 29: 465–468.
14. Roberts AE, Araki T, Swanson KD et al. Germline gain-of-function mutations in SOS1 cause Noonan syndrome. Nat Genet 2007: 39: 70–74.
15. Tartaglia M, Pennacchio LA, Zhao C et al. Gain-of-function SOS1 mutations cause a distinctive form of Noonan syndrome. Nat Genet 2007: 39: 75–79.

16. Cordeddu V, Di Schiavi E, Pennacchio LA et al. Mutation of SHOC2 promotes aberrant protein N-myristoylation and causes Noonan-like syndrome with loose anagen hair. Nat Genet 2009: 41: 1022–1026.
17. Rodriguez-Viciana P, Tetsu O, Tidyman WE et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science 2006: 311: 1287–1290.
18. Zenker M, Horn D, Wieczorek D et al. SOS1 is the second most common Noonan gene but plays no major role in cardio-facio-cutaneous syndrome. J Med Genet 2007: 44: 651–656.
19. Tartaglia M, Gelb BD. Disorders of dysregulated signal traffic through the RAS- MAPK pathway: phenotypic spectrum and molecular mechanisms. Ann N Y Acad Sci 2010: 1214: 99–121.
20. Gripp KW, Stabley DL, Nicholson L et al. Somatic mosaicism for an HRAS mutation causes Costello syndrome. Am J Med Genet A 2006: 140: 2163–2169.
21. Gripp KW, Lin AE, Nicholson L et al. Further delineation of the phenotype resulting from BRAF or MEK1 germline mutations helps differentiate cardio-facio-cutaneous syndrome from Costello syndrome. Am J Med Genet A 2007: 143: 1472–1480.
22. Pernot C, Marçon F, Worms AM et al. Cardiovascular dysplasia in Noonan's syndrome. Apropos of 64 cases. Arch Mal Coeur Vaiss 1987: 80: 434–443.
23. Lin AE. Noonan syndrome. J Med Genet 1988: 25: 64–65.
24. Zenker M, Buheitel G, Rauch R et al. Genotype–phenotype correlations in Noonan syndrome. J Pediatr 2004: 144: 368–374.