Am. J. Hum. Genet. 75:492–497, 2004

Report

Paternal Germline Origin and Sex-Ratio Distortion in Transmission of *PTPN11* Mutations in Noonan Syndrome

Marco Tartaglia,[1,2] Viviana Cordeddu,[1] Hong Chang,[4] Adam Shaw,[5] Kamini Kalidas,[5] Andrew Crosby,[5] Michael A. Patton,[5] Mariella Sorcini,[1] Ineke van der Burgt,[6] Steve Jeffery,[5] and Bruce D. Gelb[2,3]

[1]Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, Rome; Departments of [2]Pediatrics and [3]Human Genetics, Mount Sinai School of Medicine, New York; [4]Health Institute, Division of Clinical Care Research, Tufts–New England Medical Center, Boston; [5]Division of Medical Genetics, St. George's Hospital Medical School, London; and [6]Department of Medical Genetics, University Hospital Maastricht, Maastricht, The Netherlands

Germline mutations in *PTPN11*—the gene encoding the nonreceptor protein tyrosine phosphatase SHP-2—represent a major cause of Noonan syndrome (NS), a developmental disorder characterized by short stature and facial dysmorphism, as well as skeletal, hematologic, and congenital heart defects. Like many autosomal dominant disorders, a significant percentage of NS cases appear to arise from de novo mutations. Here, we investigated the parental origin of de novo *PTPN11* lesions and explored the effect of paternal age in NS. By analyzing intronic portions that flank the exonic *PTPN11* lesions in 49 sporadic NS cases, we traced the parental origin of mutations in 14 families. Our results showed that all mutations were inherited from the father, despite the fact that no substitution affected a CpG dinucleotide. We also report that advanced paternal age was observed among cohorts of sporadic NS cases with and without *PTPN11* mutations and that a significant sex-ratio bias favoring transmission to males was present in subjects with sporadic NS caused by *PTPN11* mutations, as well as in families inheriting the disorder.

Noonan syndrome (NS [MIM 163950]) is a developmental disorder with clinical features that include facial dysmorphism, proportionate short stature and growth retardation, as well as skeletal, hematologic, and heart defects (Noonan 1968; Allanson 1987). The prevalence of NS is estimated to be 1 in 1,000–2,500 births (Nora et al. 1974), which makes it the most common nonchromosomal syndrome with cardiac involvement. NS is genetically heterogeneous (Jamieson et al. 1994), and ~50% of affected individuals are heterozygous for missense mutations in the *PTPN11* gene (Tartaglia et al. 2001, 2002; Kosaki et al. 2002; Maheshwari et al. 2002; Musante et al. 2003; Zenker et al. 2004). *PTPN11* encodes SHP-2, a cytoplasmic SH2-domain–containing protein tyrosine phosphatase with relevant roles in signal transduction and development. Molecular modeling, energetics-based structural analyses, and biochemical characterization studies of SHP-2 mutants have documented that these mutations destabilize the catalytically inactive conformation of the protein, resulting in a gain of function (Tartaglia et al. 2001, 2003; Fragale et al. 2004). Germline *PTPN11* lesions have been identified as the underlying cause of other developmental disorders closely related to NS: Noonan-like/multiple giant cell lesion syndrome (MIM 163955) (Tartaglia et al. 2002) and LEOPARD syndrome (MIM 151100) (Digilio et al. 2002; Legius et al. 2002). A distinct class of somatic mutations, appearing to have even higher gain-of-function levels, contributes to leukemogenesis (Tartaglia et al. 2003, 2004; Loh et al. 2004). The identification of these mutations explains the higher prevalence of myeloproliferative disorders and acute leukemia among children with NS.

PTPN11 mutations appear to be more prevalent among families segregating NS than among sporadic cases (Tartaglia et al. 2002; Zenker et al. 2004). Like

Received April 29, 2004; accepted for publication June 10, 2004; electronically published July 9, 2004.

Address for correspondence and reprints: Dr. Marco Tartaglia, Istituto Superiore di Sanità, Dipartimento di Biologia Cellulare e Neuroscienze, Viale Regina Elena, 299, 00161 Rome, Italy. E-mail: mtartaglia@iss.it

0002-9297/2004/7503-0014$15.00

many autosomal dominant disorders, however, a significant percentage of NS cases arise from de novo mutations. The genetic mechanisms underlying de novo gene mutations have been contemplated for nearly a century (Weinberg 1912). Epidemiological and statistical genetic approaches established a correlation between advanced parental age and several autosomal dominant disorders. This led to the prevailing view, first proposed by Penrose, that attributes this phenomenon to the increased opportunity for mitotic errors in spermatogonia, which cycle continuously throughout the reproductive life of a male, compared to that in oogonia, which do not (Penrose 1955). More recently, the availability of molecular approaches has permitted investigators to determine the parental origin for de novo mutations. Studies of disorders that arise from lesions affecting the fibroblast growth-factor receptor (FGFR) gene family have demonstrated the paternal germline origin of point mutations (Moloney et al. 1996; Wilkin et al. 1998; Glaser et al. 2000). Complete or high prevalence of paternally derived gene mutations have also been documented for Rett syndrome (Trappe et al. 2001), type I neurofibromatosis (Jadayel et al. 1990), multiple endocrine neoplasia 2A and 2B (Carlson et al. 1994; Schuffenecker et al. 1997), and retinoblastoma (Zhu et al. 1989; Dryja et al. 1997). On the other hand, de novo mutations of both paternal and maternal origin have been demonstrated in other autosomal dominant disorders: von Hippel-Lindau syndrome, hemophilia B, type II neurofibromatosis, and Treacher Collins syndrome (Richards et al. 1995; Ketterling et al. 1999; Kluwe et al. 2000; Splendore et al. 2003). These apparently conflicting findings suggest that the ratio of paternal to maternal origin of mutation is variable and depends on the gene and the type of mutational event. Heterogeneity has also been observed in the degree of the paternal-age effect associated with the paternal origin of mutations (Jadayel et al. 1990; Moloney et al. 1996; Dryja et al. 1997; Ketterling et al. 1999; Glaser et al. 2000; Splendore et al. 2003).

To explore this genetic phenomenon further, we investigated the parental origin of de novo *PTPN11* mutations in NS cases—using rare flanking polymorphisms with which alleles were phased—and explored the effect of parental age. A total of 49 families were included in the study. Each family consisted of an affected individual heterozygous for a *PTPN11* mutation in exon 3 or 8 and both unaffected parents. All families were of European origin and were part of two large NS cohorts recently screened for *PTPN11* mutations (Tartaglia et al. 2002; M.T. and B.D.G., unpublished data). Consent for genetic analyses was obtained from all families. For the majority of families, the de novo occurrence of mutation had been verified by analyzing genomic DNA of both parents. In seven families, DNA was available from only one parent, but phenotypic information on the untested parent indicated that he or she was unambiguously clinically unaffected. Since nonpenetrance of *PTPN11* mutations has been documented to be extremely rare, and since parental mosaicism—gonadal or somatic—has not been reported for NS to date, we assumed the de novo occurrence of the mutation in these families.

To trace the parental origin of mutations, portions of the genomic region flanking the disease-causative lesions (~6,000 and ~4,000 bases flanking exons 3 and 8, respectively) were analyzed for the presence of polymorphic sites in affected individuals. Several short overlapping stretches, located upstream and downstream of those exons, were PCR amplified (primer pairs, annealing temperatures [T_{ann}], and sizes of PCR products are listed in table A1 [online only]), and unpurified PCR products were denatured, slowly reannealed, and analyzed by denaturing high-performance liquid chromatography, with the use of the Wave Nucleic Acid Fragment Analysis System (Transgenomic) at column temperatures recommended by WaveMaker v. 4.1.31 (Transgenomic). Amplimers with abnormal denaturing profiles were purified (with Microcon PCR [Millipore]) and were sequenced bidirectionally with the use of the ABI BigDye Terminator Cycle Sequencing Kit v. 3.1(Applied Biosystems) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems). We identified a heterozygous condition for intronic polymorphisms in 15 individuals, and genotyping of their parents (by restriction analysis or by direct sequencing) indicated that 14 of the 15 families were informative. The amplification and cloning (by the TA Cloning Kit [Invitrogen]) of genomic fragments encompassing the exonic mutation and the intronic polymorphic site allowed the determination of haplotypes in affected individuals. As shown in table 1, segregation analysis demonstrated the paternal germline origin of the mutation in all cases (81%–100% CI). Compared with the expected 1:1 ratio, this distribution was significantly different (Fisher exact test, $P < .025$). The identification of only two haplotypes among five or more sequenced clones for each PCR product argued against the presence of somatic mosaicism in affected individuals. Parental sex assignment was confirmed in all informative families by *amelogenin* gene (*AMELX* [UniGene accession number Y040206] and *AMELY* [UniGene accession number X14439]) amplification, as described by Nakahori et al. (1991). The genotyping of markers D2S406, D4S1625, D7S1817, D11S1392, and D19S253 (Research Genetics) proved paternity in all families.

To determine whether advanced paternal age was a factor in the mutagenesis of NS, the ages of the British fathers and their marital statuses were compared with the population data (from the Office of Population Cen-

Table 1

Parental Origin of De Novo *PTPN11* Mutations

Case	Mutation[a]	SNP/STR	Intron	SNP/STR Genotype[b]			Haplotype	Origin
				Father	Mother	Case		
NS59	188A→G	−790 A/C	2	A/A	A/C	A/C	188G, −790A	Paternal
NS44	215C→G	+105 $(ATTT)_{7/8}$	4	$(ATTT)_{7/7}$	$(ATTT)_{7/8}$	$(ATTT)_{7/8}$	215G, +105$(ATTT)_7$	Paternal
NS4	218C→T	+376 C/A	3	C/C	A/C	A/C	218T, +376C	Paternal
NS3	922A→G	−223 C/T	7	C/C	C/T	C/T	922G, −223C	Paternal
NS55	922A→G	−132 T/C	7	NA	T/T	C/T	922G, −132C	Paternal
NS5	922A→G	−21 C/T	7	C/C	C/T	C/T	922G, −21C	Paternal
NS1	922A→G	−21 C/T	7	C/T	C/C	C/T	922G, −21T	Paternal
NS6	922A→G	−21 C/T	7	C/C	C/T	C/T	922G, −21C	Paternal
NS2	922A→G	−21 C/T	7	C/C	C/T	C/T	922G, −21C	Paternal
NS17	922A→G	−21 C/T	7	NA	C/C	C/T	922G, −21T	Paternal
NS28	922A→G	+966 T/G	9	T/T	NA	T/G	922G, +966T	Paternal
NS38	922A→G	+1330 A/G	9	A/A	A/G	A/G	922G, +1330A	Paternal
NS64	922A→G	+1330 A/G	9	A/A	G/G	A/G	922G, +1330A	Paternal
NS34	923A→G	−21 C/T	7	C/C	C/T	C/T	923G, −21C	Paternal

[a] No mutation occurred in the context of a CpG dinucleotide.
[b] NA = genomic DNA not available for molecular analyses.

suses and Surveys). Since the mean paternal age for the British population varied over the period of the study and consistently showed a skew toward younger ages (i.e., failed a test of normality), statistical comparison of the raw data would not have been valid. Instead, a log transformation was performed with the population data for each year, which produced a normal distribution. The ages of the fathers were then expressed as Z scores. One-tailed T tests were performed, with the significance threshold of $P < .05$, to determine if the cohorts of fathers whose offspring had NS with and without *PTPN11* mutations were older than the underlying population. For those British men whose children had a *PTPN11* mutation ($n = 15$), the mean Z score was 0.93 ($P < .001$). For the fathers of children with NS but without a *PTPN11* mutation ($n = 43$), the mean Z score was 0.62 ($P < .001$). For comparison with other studies of advanced paternal age, we noted that the average paternal age of the *PTPN11*-related cohort was 35.6 years, which was 6.1 years older than the population average for the children's average year of birth (1980). For the *PTPN11*-negative cohort, the average paternal age was 33.4 years, which was 4.0 years older than the population average for the children's average year of birth (1981).

Finally, we examined the transmission of sporadic *PTPN11* mutations to look for a sex-ratio bias. Our analysis was performed with the use of available published (Tartaglia et al. 2001, 2002, 2003; Kosaki et al. 2002; Maheshwari et al. 2002; Kondoh et al. 2003; Musante et al. 2003) and unpublished (M.T. and B.D.G., data available upon request) records and indicated a statistically significant sex bias in favor of males (66 vs. 31; $\chi^2 = 12.63$, $P < .001$). To consider whether that bias was also present among families inheriting NS with *PTPN11* mutations, we reviewed all published (Tartaglia et al. 2001, 2002; Kosaki et al. 2002; Maheshwari et al. 2002; Schollen et al. 2003) and unpublished (M.T. and B.D.G., data available upon request) records. With the exclusion of founders, NS occurred in 37 males and 21 females ($\chi^2 = 4.41$, $P < .05$). Examination of maternal and paternal transmission did not suggest that the distortion arose in a strongly preferential way from one or the other (the affected mother transmitted to 27 affected boys and 14 affected girls; the affected father transmitted to 10 affected boys and 7 affected girls). Among these families, there were 19 unaffected males and 21 unaffected females born to affected parents. There were also more transmitting mothers ($n = 31$) than fathers ($n = 10$), a significant difference ($\chi^2 = 10.76$, $P < .01$) that can be ascribed to the reduced fertility of male individuals with NS (Elsawi et al. 1994).

The data presented here provide the first evidence for a paternal origin of de novo *PTPN11* mutations in NS and for their association with advanced paternal age. This finding confirms previous studies supporting a predominance of paternal origin of point mutations in the majority of autosomal dominant diseases. It is clear that this predominance does not reflect some genetic quirk isolated to the *FGFR* genes, nor does it necessitate a restricted molecular diversity of mutations, as observed in some disorders (e.g., achondroplasia). The higher level of DNA methylation in spermatagonia—compared with that in oogonia—which would predict increased substitutions at CpG dinucleotides, has been suggested as an important contributing factor. This was bolstered by studies of Apert syndrome, for which the *FGFR2* mutation that affects a CpG dinucleotide is twice as prevalent as the one that does not (Moloney et al. 1996). On the basis of the 100–1,000-fold differences between

FGFR3 mutation rates at CpG dinucleotides causing achondroplasia and hypochondroplasia, it was understood that the context of the CpG dinucleotide must interact with other paternal factors (Bellus et al. 1995; Wilkin et al. 1998). Here, we have documented that exclusive paternal origin can occur without any substitutions at CpG dinucleotides. Our finding is consistent with data reported elsewhere by Glaser et al. (2000) concerning Crouzon and Pfeiffer syndromes. Since NS is believed to be relatively prevalent (Nora et al. 1974), methylation status appears not to be central to the phenomenon of paternal origin of sporadic mutations in autosomal dominant disorders.

The phenomenon of paternal origin of sporadic mutations remains perplexing. Penrose's cycling theory, remarkably insightful for its time, does not readily explain why sporadic point mutations in the *VHL* gene that cause von Hippel-Lindau syndrome arise with about equal frequency in gametogenesis for either parent (Richards et al. 1995), nor does it explain the dramatic variability of paternal bias associated with transitions, transversions, and small deletions and insertions (Ketterling et al. 1999). Similarly, it is unclear why *NF1* point mutations causing type I neurofibromatosis are almost entirely paternal in origin but are not associated with advanced paternal age (Jadayel et al. 1990). We considered the possibility that genes for which homologous sequences exist in the human genome might be prone to mutagenesis through gene conversion, a process that might not depend on paternal inheritance or age. BLAT searches using sequences for all of the autosomal dominant disease genes for which parental origins have been studied, failed to identify any genes with such highly homologous sequences (see UCSC Genome Browser Web site).

There are other problems with Penrose's cycling theory. First, Risch et al. statistically examined the patterns of advanced paternal age (Risch et al. 1987). If mitotic cycle number were the principal driving factor underlying the predominance of paternal origin, then prevalence of sporadic mutations ought to be linear with paternal age. They observed that this was not the case, the available data being more consistent with an exponential process. Second, recent work from D. Page's group indicates that the ratio of mutation rates for homologous sequences on the X and Y chromosomes (that were physically distant from any gene and were not under selection pressure) over the past 3–4 million years of hominid evolution is only 1.7 (Bohossian et al. 2000). These data support the notion that the global rate of nucleotide substitutions in the human genome is higher in males and may be attributable, at least in part, to the cycling disparity noted by Penrose. That mechanism, however, cannot explain the exponential process driving the appearance of new mutations for relatively prevalent autosomal dominant disorders like NS. Consistent with this, recent studies demonstrated that the increased prevalence of *FGFR* mutations in sperm from cohorts of men of various ages is not sufficient to sustain the exponential rise of sporadic achondroplasia and Apert syndrome births associated with advanced paternal age (Tiemann-Boege et al. 2002; Glaser et al. 2003). This suggests that additional contributing mechanisms, such as selective advantage of mutant spermatogonial cells, cell-specific DNA-repair efficiency, and/or decreased apoptotic control with age, might also play a role (Glaser et al. 2003; Goriely et al. 2003; Singh et al. 2003). Glaser and Jabs raised the possibility that gain-of-function mutations in *FGFR* genes might result in a selective advantage in sperm motility and capacitation (Glaser and Jabs 2004). It is interesting, therefore, to note the possibility that the *PTPN11* mutations in NS could have similar effects, since SHP-2 is a positive regulator of signal transduction downstream from the FGFRs.

We observed a distorted ratio in the sex of subjects with sporadic NS and *PTPN11* mutations. Sex-ratio distortion has also been observed in retinoblastoma and multiple endocrine neoplasia type 2B, occuring more often in males and females, respectively (Carlson et al. 1994; Naumova and Sapienza 1994). Possible explanations for our findings would include chance, patient-selection bias, adverse effects on embryonic development in a sex-specific manner (poorer survival for female embryos, in this case), preferential fertilization by sperm with a *PTPN11* mutation and a Y chromosome, and nonrandom segregation of chromosomes 12 and Y when the former bears a *PTPN11* mutation. Our data on sporadic NS were based on two independent cohorts, and the sex ratio was distorted in both, providing some confirmation. As Sapienza noted in an editorial (Sapienza 1994), adverse effects on embryos should operate in the transmission of mutant alleles in familial cases as well as sporadic ones. Our observation that the sex-ratio distortion was also present in familial cases suggests that chance and patient-selection bias are unlikely to be operative. Since the distortion was present when the affected allele was transmitted by the mother and was not present among the unaffected siblings, preferential fertilization and nonrandom chromosome assortment also seem unlikely. Thus, we would favor sex-specific developmental effects as the explanation for the sex-ratio distortion in *PTPN11*-associated NS, because fetal lethality has been documented in this disorder.

Acknowledgments

We are indebted to the families who participated in the study and to the physicians who referred the subjects. The study was supported in part by grants from Telethon (GGP04172 to M.T.), Ricerca Finalizzata 1% FSN2002 "Valutazione molec-

olare e funzionale delle malformazioni e disfunzioni cardiache su base genetica" (M.T.), the National Institutes of Health (HL71207 and HD01294 to B.D.G.), the March of Dimes (FY03-52 to B.D.G.), the Birth Defects Foundation-UK (S.J.), and the British Heart Foundation (S.J.).

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

UCSC Genome Browser, http://www.genome.ucsc.edu/cgi-bin/hgBlat/ (for April 2003 freeze used for BLAT searches)

Online Mendelian Inheritance of Man (OMIM), http://www.ncbi.nlm.gov/Omim/ (for Noonan syndrome, Noonan-like/multiple giant cell lesion syndrome, and LEOPARD syndrome)

UniGene, http://www.ncbi.nlm.nih.gov/entrez/ (for *PTPN11* genomic sequence [NT_009775], *PTPN11* cDNA sequence [NM_002834], AMELX genomic sequence [Y040206], and AMELY genomic sequence [X14439])

References

Allanson JE (1987) Noonan syndrome. J Med Genet 24:9–13

Bellus GA, McIntosh I, Smith EA, Aylsworth AS, Kaitila I, Horton WA, Greenhaw GA, Hecht JT, Francomano CA (1995) A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. Nat Genet 10:357–359

Bohossian HB, Skaletsky H, Page DC (2000) Unexpectedly similar rates of nucleotide substitution found in male and female hominids. Nature 406:622–625

Carlson KM, Bracamontes J, Jackson CE, Clark R, Wells SA Jr, Goodfellow PJ (1994) Parent-of-origin effects in multiple endocrine neoplasia type 2B. Am J Hum Genet 55:1076–1082

Digilio MC, Conti E, Sarkozy A, Mingarelli R, Dottorini T, Marino B, Pizzuti A, Dallapiccola B (2002) Grouping of multiple-lentigines/LEOPARD and Noonan syndromes on the *PTPN11* gene. Am J Hum Genet 71:389–394

Dryja TP, Morrow JF, Rapaport JM (1997) Quantification of the paternal allele bias for new germline mutations in the retinoblastoma gene. Hum Genet 100:446–449

Elsawi MM, Pryor JP, Klufio G, Barnes C, Patton MA (1994) Genital tract function in men with Noonan syndrome. J Med Genet 31:468–470

Fragale A, Tartaglia M, Wu J, Gelb BD (2004) Noonan syndrome–associated SHP2/PTPN11 mutants cause EGF-dependent prolonged GAB1 binding and sustained ERK2/MAPK1 activation. Hum Mutat 23:267–277

Glaser RL, Broman KW, Schulman RL, Eskenazi B, Wyrobek AJ, Jabs EW (2003) The paternal-age effect in Apert syndrome is due, in part, to the increased frequency of mutations in sperm. Am J Hum Genet 73:939–947

Glaser RL, Jabs EW (2004). Dear old dad: paternal age and the origin of spontaneous mutations in humans [review]. Sci Aging Knowledge Environ 3:1–11

Glaser RL, Jiang W, Boyadjiev SA, Tran AK, Zachary AA, Van Maldergem L, Johnson D, Walsh S, Oldridge M, Wall SA, Wilkie AO, Jabs EW (2000) Paternal origin of FGFR2 mutations in sporadic cases of Crouzon syndrome and Pfeiffer syndrome. Am J Hum Genet 66:768–777

Goriely A, McVean GA, Rojmyr M, Ingemarsson B, Wilkie AO (2003) Evidence for selective advantage of pathogenic FGFR2 mutations in the male germ line. Science 301:643–646

Jadayel D, Fain P, Upadhyaya M, Ponder MA, Huson SM, Carey J, Fryer A, Mathew CG, Barker DF, Ponder BA (1990) Paternal origin of new mutations in von Recklinghausen neurofibromatosis. Nature 343:558–559

Jamieson CR, van der Burgt I, Brady AF, van Reen M, Elsawi MM, Hol F, Jeffery S, Patton MA, Mariman E (1994) Mapping a gene for Noonan syndrome to the long arm of chromosome 12. Nat Genet 8:357–360

Ketterling RP, Vielhaber E, Li X, Drost J, Schaid DJ, Kasper CK, Phillips JA III, Koerper MA, Kim H, Sexauer C, Gruppo R, Ambriz R, Paredes R, Sommer SS (1999) Germline origins in the human F9 gene: frequent G:C→A:T mosaicism and increased mutations with advanced maternal age. Hum Genet 105:629–640

Kluwe L, Mautner V, Parry DM, Jacoby LB, Baser M, Davis K, Stavrou D, MacCollin M (2000) The parental origin of new mutations in neurofibromatosis 2. Neurogenetics 3:17–24

Kondoh T, Ishii E, Aoki Y, Shimizu T, Zaitsu M, Matsubara Y, Moriuchi H (2003) Noonan syndrome with leukaemoid reaction and overproduction of catecholamines: a case report. Eur J Pediatr 162:548–549

Kosaki K, Suzuki T, Muroya K, Hasegawa T, Sato S, Matsuo N, Kosaki R, Nagai T, Hasegawa Y, Ogata T (2002) PTPN11 (protein-tyrosine phosphatase, nonreceptor-type 11) mutations in seven Japanese patients with Noonan syndrome. J Clin Endocrinol Metab 87:3529–3533

Legius E, Schrander-Stumpel C, Schollen E, Pulles-Heintzberger C, Gewillig M, Fryns JP (2002) *PTPN11* mutations in LEOPARD syndrome. J Med Genet 39:571–574

Loh M, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH, Cheng JW, Lee CM, Stokoe D, Bonifas JM, Curtiss NP, Gotlib J, Meshinchi S, Le Beau MM, Emanuel PD, Shannon KM (2004) Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 103:2325–2331

Maheshwari M, Belmont J, Fernbach S, Ho T, Molinari L, Yakub I, Yu F, Combes A, Towbin J, Craigen WJ, Gibbs R (2002) PTPN11 mutations in Noonan syndrome type I: detection of recurrent mutations in exons 3 and 13. Hum Mutat 20:298–304

Moloney DM, Slaney SF, Oldridge M, Wall SA, Sahlin P, Stenman G, Wilkie AO (1996) Exclusive paternal origin of new mutations in Apert syndrome. Nat Genet 13:48–53

Musante L, Kehl HG, Majewski F, Meinecke P, Schweiger S, Wieczorek D, Hinkel GK, Tinschert S, Hoeltzenbein M, Ropers HH, Kalscheuer VM (2003) Spectrum of mutations in PTPN11 and genotype-phenotype correlation in 96 patients with Noonan syndrome and five patients with cardiofacio-cutaneous syndrome. Eur J Hum Genet 11:201–206

Nakahori Y, Hamano K, Iwaya M, Nakagome Y (1991) Sex identification by polymerase chain reaction using X-Y homologous primer. Am J Med Genet 39:472–473

Naumova A, Sapienza C (1994) The genetics of retinoblastoma, revisited. Am J Hum Genet 54:264–273

Noonan JA (1968) Hypertelorism with Turner phenotype: a new syndrome with associated congenital heart disease. Am J Dis Child 116:373–380

Nora JJ, Nora AH, Sinha AK, Spangler RD, Lubs HA (1974) The Ullrich-Noonan syndrome (Turner phenotype). Am J Dis Child 127:48–55

Penrose L (1955) Parental age and mutation. Lancet 269:312–313

Richards FM, Payne SJ, Zbar B, Affara NA, Ferguson-Smith MA, Maher ER (1995) Molecular analysis of de novo germline mutations in the von Hippel-Lindau disease gene. Hum Mol Genet 4:2139–2143

Risch N, Reich EW, Wishnick MM, McCarthy JG (1987) Spontaneous mutation and parental age in humans. Am J Hum Genet 41:218–248

Sapienza C (1994) Parental origin effects, genome imprinting, and sex-ratio distortion: double or nothing? Am J Hum Genet 55:1073–1075

Schollen E, Matthijs G, Gewillig M, Fryns JP, Legius E (2003) *PTPN11* mutation in a large family with Noonan syndrome and dizygous twinning. Eur J Hum Genet 11:85–88

Schuffenecker I, Ginet N, Goldgar D, Eng C, Chambe B, Boneu A, Houdent C, Pallo D, Schlumberger M, Thivolet C, Lenoir GM, Le Groupe d'Etude des Tumeurs a Calcitonine (1997) Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. Am J Hum Genet 60:233–237

Singh NP, Muller CH, Berger RE (2003) Effects of age on DNA double-strand breaks and apoptosis in human sperm. Fertil Steril 80:1420–1430

Splendore A, Jabs EW, Félix TM, Passos-Bueno MR (2003) Parental origin of mutations in sporadic cases of Treacher Collins syndrome. Eur J Hum Genet 11:718–722

Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, van der Burgt I, Brunner HG, Bertola DR, Crosby A, Ion A, Kucherlapati RS, Jeffery S, Patton MA, Gelb BD (2002) *PTPN11* mutations in Noonan syndrome: molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. Am J Hum Genet 70:1555–1563

Tartaglia M, Martinelli S, Cazzaniga G, Cordeddu V, Iavarone I, Spinelli M, Palmi C, Carta C, Pession A, Arico M, Masera G, Basso G, Sorcini M, Gelb BD, Biondi A (2004) Genetic evidence for lineage- and differentiation stage-related contribution of somatic *PTPN11* mutations to leukemogenesis in childhood acute leukemia. Blood 104:307–313

Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, van der Burgt I, Crosby AH, Ion A, Jeffery S, Kalidas K, Patton MA, Kucherlapati RS, Gelb BD (2001) Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 29: 465–468

Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, Hahlen K, Hasle H, Licht JD, Gelb BD (2003) Somatic mutations in *PTPN11* in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 34:148–150

Tiemann-Boege I, Navidi W, Grewal R, Cohn D, Eskenazi B, Wyrobek AJ, Arnheim N (2002) The observed human sperm mutation frequency cannot explain the achondroplasia paternal age effect. Proc Natl Acad Sci USA 99:14952–14957

Trappe R, Laccone F, Cobilanschi J, Meins M, Huppke P, Hanefeld F, Engel W (2001) *MECP2* mutations in sporadic cases of Rett syndrome are almost exclusively of paternal origin. Am J Hum Genet 68:1093–1101

Weinberg W (1912) Zur Vererbung des Zwergwuches. Arch Rassen Gesel Biol 9:710–718

Wilkin DJ, Szabo JK, Cameron R, Henderson S, Bellus GA, Mack ML, Kaitila I, Loughlin J, Munnich A, Sykes B, Bonaventure J, Francomano CA (1998) Mutations in fibroblast growth-factor receptor 3 in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome. Am J Hum Genet 63:711–716

Zenker M, Buheitel G, Rauch R, Koenig R, Bosse K, Kress W, Tietze HU, Doerr HG, Hofbeck M, Singer H, Reis A, Rauch A (2004) Genotype-phenotype correlations in Noonan syndrome. J Pediatr 144:368–374

Zhu XP, Dunn JM, Phillips RA, Goddard AD, Paton KE, Becker A, Gallie BL (1989) Preferential germline mutation of the paternal allele in retinoblastoma. Nature 340:312–313