LEOPARD syndrome (LS) is a rare autosomal dominant disorder characterised by lentigines and café au lait spots, EKG anomalies, ocular hypertelorism, pulmonary stenosis (PS), abnormal genitalia, retardation of growth, and deafness, and has been successively considered as a distinct syndrome and later as a clinical variant of Noonan syndrome (NS). Some months after the discovery of heterozygous mutations in the PTPN11 gene in roughly 40% of clinically typical NS patients, Digilio et al reported the presence of PTPN11 mutations in nine out of 10 unrelated patients with LS or NS with multiple lentigines or café au lait spots, confirming that both disorders are allelic variants. PTPN11 encodes SHP-2, a ubiquitously expressed non-receptor-type tyrosine phosphatase involved in a variety of cytokine and growth factor initiated signal transduction processes. SHP-2 contains two tandem SH2 domains encoded by exons 1 to 4 at the N terminus, and a phosphatase domain (PTP) encoded by exons 7 to 13 at the C terminus. Three different mutations have been described so far in LS, all located in the PTP domain. These mutations are believed to disrupt the interaction between the N-SH2 and PTP domains, leading to increased phosphatase activity as similarly observed in NS. However, mutations described in LS seems to be highly specific for this syndrome.

In an attempt to better define the pattern of PTPN11 mutations responsible for LS and their correlation with clinical presentation, we here report results obtained in 14 families.

METHODS

DNA samples obtained from peripheral leucocytes of 14 unrelated propositi with a clinical diagnosis of LS were referred to our laboratory by confirmed clinician geneticists for PTPN11 mutation screening. For eight of them, parental DNA was also collected. Bi-directional direct sequencing of PTPN11 exons 2, 3, 4, 7, 8, 12, and 13, and their flanking intron-exon boundaries was performed for each patient using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). Reaction products were run on an ABI 3100 Genetic Analyzer (Applied Biosystems). After analysis using ABI PRISM DNA Analysis software (Applied Biosystems), sequences were aligned using Seqscape analysis software (Applied Biosystems) and compared with the reference sequences for genomic DNA (GenBank accession number NT_009775.14) and mRNA (GenBank accession number NM_002834.3).

Clinical data were gathered with a standard datasheet used for all NS/LS patients referred to our centre. Frequencies of clinical features for the two major mutations were compared using Fisher's exact test.

RESULTS

Molecular data

A PTPN11 heterozygous sequence variation of the coding sequence was detected in 13 of the 14 unrelated index cases tested, and five relatives. Four different nucleotide changes were found. A missense variation c.836A→G/p.Y279C in exon 7 was identified in seven subjects (six unrelated probands and one mother). A c.836A→C/p.Y279S was found in one sporadic proband. A missense variation c.1403C→T/p.T468M was identified in two unrelated patients. Two new mutations were identified. A substitution T/p.T468M was found in two unrelated patients. Among eight parents whose parental DNA was available, three mothers and one father were carriers and clinically affected. No mutations were found in one atypical, familial case further exhibiting marfanoid habitus. Comparison of T468M and Y279C phenotypes showed a significant difference for EKG anomalies, and possible (but not statistically significant) trends for heart defect, growth retardation, and deafness. AML, probably a not so rare complication of Noonan syndrome, is reported here for the first time with LS.

Key points

- LEOPARD syndrome (LS) is an autosomal dominant disorder characterised by lentigines and café au lait spots, EKG anomalies, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and deafness. So far, three distinct mutations of the PTPN11 gene have been reported in 15 patients with LS.
- Fourteen unrelated propositi with a clinical diagnosis of LS from the French Multicentric Noonan Study were screened for PTPN11 mutations.
- The two previously described recurrent mutations, Y279C in exon 7 and T468M in exon 12, were identified, respectively, in six and four unrelated patients.
- Two new mutations were identified. A substitution Y279S was found in one child who developed acute myeloid leukaemia (AML). Missense variation Q510P was found in two unrelated patients.
- Among eight parents whose parental DNA was available, three mothers and one father were carriers and clinically affected.
- No mutations were found in one atypical, familial case further exhibiting marfanoid habitus.
- Comparison of T468M and Y279C phenotypes showed a significant difference for EKG anomalies, and possible (but not statistically significant) trends for heart defect, growth retardation, and deafness.
- AML, probably a not so rare complication of Noonan syndrome, is reported here for the first time with LS.

Abbreviations: AML, acute myeloid leukaemia; CMP, cardiomyopathy; LS, LEOPARD syndrome; MVP, mitral valve prolapse; NS, Noonan syndrome; PS, pulmonary stenosis

Clinical data
Pool ed clinical data, sorted by mutation, are presented in
Table 1 for all probands and four out of five related carriers
with classical mutations and sufficient clinical data. Many
patients showed heart defects. Among the Y279C patients,
one had PS and cardiomyopathy (CMP), another had mitral
valve prolapse (MVP), and two had, respectively, septal and
generalised myocardial hypertrophy without structural
anomalies. In the T468M group, two patients had PS, MVP,
and septal hypertrophy, one had aortic stenosis, and one pure
MVP. The three patients with the Q510P mutation had
classical, mild LS phenotypes, with normal growth and
intelligence (patients 1 to 3, Table 1). One of those children
had mild hearing impairment, whereas another was severely
affected. Patient 3 (Figs 1 and 2) had MVP. Her lentigines
were profuse and associated with café au lait spots. Patient 4
developed acute myeloid leukaemia (AML) of the FAB-M2
subtype with normal cytogenetics. Two affected patients (one
proband and his father), for whom no mutations were
detected, had lentigines and multiple naevi associated with a
marfanoid habitus (patients 5 and 6, Table 1). Their facial
appearance was thought to compatible with LS or NS.
Interestingly, the father had aortic root dilatation but no eye
anomalies, whereas the boy, despite pectus deformity and
scoliosis, was 186 cm tall.

We compared the phenotypes for Y279C and T468M for
patients of our series grouped with those previously reported
in the literature by non-parametric test (Table 1). No
statistically significant differences were observed for most
parameters except for the frequency of abnormal EKG. Other
apparent differences between the two mutations (a trend for
growth retardation and deafness in Y279C patients, and an
excess of heart defects other than PS in T468M patients) did
not reach significance. Further study will be necessary to
clarify if these trends are valid or just stochastic sampling
effects (Table 1).

DISCUSSION
NS causative defects are not randomly distributed in SHP-2.
Most (75%) affect residues located in or close to the N-SH2
and protein tyrosine phosphatase interacting surfaces. To
date, all molecular lesions have been heterozygous missense
changes suspected of inducing a gain of function effect. In
contrast to NS, LS is highly specific for PTPN11 and
mutations have been identified in 15 out of the 17 patients
reported in the literature.3–5 Defects reported in LS are
restricted in diversity since only two recurrent mutations,
one in exon 7 (c.836A→G/p.Y279C) and one in exon 12 (c.1403C→T/p.T468M), have been reported (a clinical sum-
mary of those patients with sufficient clinical data are
presented in Table 1). A third mutation, c.1517A→G/p.Q506P,
has been reported in a single patient.8 Only two typical LS
patients reported so far have been negative for PTPN11
mutation screening.3 With only one of 14 LS cases not having
a detectable mutation, our data confirm the high frequency of
the PTPN11 mutation in LS.

If we combine our patients with those reported in the
literature,2–5 taking into account the fact that patients briefly
reported by Digilio2 were later described in more detail by
Sarkozy4 (E Conti, personal communication), we reach a total
of 28/31 (90%) LS patients with PTPN11 mutations. Exon 7,
12, and 13 mutations are, respectively, found in 53, 36, and
10% of the 28 cases with mutations. Although mutations
responsible for NS affect either the N-SH2 or PTP domain,
those responsible for LS seem to be restricted to the PTP
domain. LS mutations are distinct from NS mutations. The
specific association of LS mutations with skin pigmentation
abnormalities is the most obvious difference, and is not
limited to lentigines. Café au lait spots may herald lentigines,
and are much more common in LS than in NS (15/24 subjects
with LS in our review). As lentigines are often undetectable
in infancy, distinction between NS and LS may be difficult in
young children, and may explain why both Y279C and
T468M substitutions were reported in patients without
lentigines. The age of the patient with NS and Y279C1 was
not given. The two children with the T468M mutation, who
were free of lentigines at the ages of 5 and 8.5, respectively,
both showed café au lait spots, and the carrier mother
displayed multiple lentigines. Zemker et al excluded other
genotype/phenotype associations for the T468M mutation:
less adverse effect on body growth, and lower prevalence of
PS but more CMP. A low prevalence of mental handicap
seems another characteristic. Our data combined with the
literature confirm these trends, and indicate that the Y279C
and T468M mutations may have slightly different expres-
sion. Comparison of T468M and Y279C phenotypes showed a
significant excess of EKG anomalies in Y279C, and trends
toward more frequent deafness and growth retardation, and
less frequent heart defects. A trend for café au lait spots
cannot be distinguished, as patients harbouring each of these
mutations are relatively different in age. The Y279C mutation
appears to lead to a more characteristic LS phenotype,
whereas T468M seems to result in a mixed NS-LS phenotype.
However, these data need to be confirmed by further studies.

Two previously undescribed PTPN11 mutations were found
in our series of patients with LS. These mutations are both
located within the cluster of activating mutations in exons 7
and 13. The first affects Y279 and changes it into a serine
instead of a cysteine. This mutation has been observed in a
boy with typical LS syndrome who also had AML. NS is
known to be associated with a higher risk of developing

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caused by PTEN hamartoma syndrome-1 mutations. Nat Genet
3. Sarkozy A, E Conti E. Mutation screening in Noonan syndrome
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3053–61.
juvenile myelomonocytic leukaemia (JMML) and possibly AML. Moreover, somatic PTPN11 mutations have been reported in sporadic JMML, AML, and acute lymphoblastic leukaemia, strengthening the link between SHP-2 and leukaemogenesis. AML with somatic mutations in LS and the specificity of the mutations, which preferentially exhibits a monocytic subtype (FAB-M5), which is not the case in our patient. Whether or not LS or mutations represent atypical LS or a distinct entity remains open to correlation is described. Whether the non-mutated family members’ siblings or parents are affected is at present unclear.

**Table 1** Poooled clinical data sorted by mutation for 13 literature cases and 19 LS from this study

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Y279C</th>
<th>T468M</th>
<th>Fisher exact test (two-tailed)</th>
<th>Q510P</th>
<th>Y279S</th>
<th>No mutation</th>
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<tbody>
<tr>
<td>Mean age at report</td>
<td>12 years</td>
<td>13 years</td>
<td>11 years</td>
<td>13 years</td>
<td>11 years</td>
<td>25 years</td>
</tr>
<tr>
<td>Mean BW (g)</td>
<td>850</td>
<td>850</td>
<td>850</td>
<td>850</td>
<td>850</td>
<td>850</td>
</tr>
<tr>
<td>Mean BLM (cm)</td>
<td>50.6</td>
<td>50.6</td>
<td>50.6</td>
<td>50.6</td>
<td>50.6</td>
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<tr>
<td>Mean birth OCP (cm)</td>
<td>35.1</td>
<td>35.1</td>
<td>35.1</td>
<td>35.1</td>
<td>35.1</td>
<td>35.1</td>
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<td>Lenticanes</td>
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<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Café au lait spots</td>
<td>2/4</td>
<td>3/6</td>
<td>5/10</td>
<td>7/7</td>
<td>12/14</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary stenosis</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Heart defect (not PS)</td>
<td>1/5</td>
<td>2/6</td>
<td>3/11</td>
<td>4/7</td>
<td>3/14</td>
<td>1</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>3/6</td>
<td>3/6</td>
<td>6/12</td>
<td>2/7</td>
<td>6/14</td>
<td>1</td>
</tr>
<tr>
<td>EKG conduction anomalies</td>
<td>2/2</td>
<td>3/5</td>
<td>6/7</td>
<td>1/7</td>
<td>6/14</td>
<td>0.02*</td>
</tr>
<tr>
<td>Retardation of growth</td>
<td>2/6</td>
<td>2/6</td>
<td>4/11</td>
<td>1/7</td>
<td>6/7</td>
<td>0.13</td>
</tr>
<tr>
<td>Deafness</td>
<td>0/3</td>
<td>0/3</td>
<td>2/9</td>
<td>0/7</td>
<td>2/4</td>
<td>0.14</td>
</tr>
<tr>
<td>Abnormal genitalia</td>
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<td>1/1</td>
<td>1/7</td>
<td>1/7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cryptorchidism</td>
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<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
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<tr>
<td>Mental retardation</td>
<td>1/6</td>
<td>2/6</td>
<td>3/12</td>
<td>2/7</td>
<td>3/14</td>
<td>1</td>
</tr>
<tr>
<td>OFC-p75</td>
<td>3/6</td>
<td>0/3</td>
<td>3/9</td>
<td>4/7</td>
<td>1/5</td>
<td>5/12</td>
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<tr>
<td>Triangular face</td>
<td>1/2</td>
<td>1/2</td>
<td>4/6</td>
<td>–</td>
<td>4/7</td>
<td>1</td>
</tr>
<tr>
<td>Ocular hypertelorism</td>
<td>5/6</td>
<td>3/6</td>
<td>8/12</td>
<td>7/7</td>
<td>3/10</td>
<td>0.66</td>
</tr>
<tr>
<td>Ptosis</td>
<td>6/6</td>
<td>3/6</td>
<td>9/12</td>
<td>5/7</td>
<td>3/10</td>
<td>0.43</td>
</tr>
<tr>
<td>Prognathism</td>
<td>2/6</td>
<td>2/6</td>
<td>2/6</td>
<td>2/6</td>
<td>2/7</td>
<td>1</td>
</tr>
<tr>
<td>Pterygium coli</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0.57</td>
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<tr>
<td>Pectus excavatum/ carinatum</td>
<td>2/5</td>
<td>6/7</td>
<td>8/12</td>
<td>5/7</td>
<td>5/7</td>
<td>10/14</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>1/2</td>
<td>2/2</td>
<td>3/9</td>
<td>–</td>
<td>1/7</td>
<td>0.58</td>
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<tr>
<td>Congenital abnormalities</td>
<td>1/1</td>
<td>1/4</td>
<td>2/5</td>
<td>–</td>
<td>2/3</td>
<td>2/3</td>
</tr>
</tbody>
</table>

*Comparison of Y279C with T468M, *t* original LEOPARD acronymic keywords.

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PTPN11 mutations in patients with LEOPARD syndrome: a French multicentric experience

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