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A series of 38 novel germline and somatic mutations of NIPBL in Cornelia de Lange syndrome

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Conflict of interest statement
The authors declare that they have no competing interests.
ABSTRACT

Cornelia de Lange syndrome is a multisystemic developmental disorder mainly related to de novo heterozygous NIPBL mutation. Recently, NIPBL somatic mosaicism has been highlighted through buccal cell DNA study in some patients with a negative molecular analysis on leukocyte DNA.

Here, we present a series of 38 patients with a Cornelia de Lange syndrome related to an heterozygous NIPBL mutation identified by Sanger sequencing. The diagnosis was based on the following criteria: 1) intrauterine growth retardation and postnatal short stature, 2) feeding difficulties and/or gastro-oesophageal reflux, 3) microcephaly, 4) intellectual disability and 5) characteristic facial features.

We identified 37 novel NIPBL mutations including 34 in leukocytes and three in buccal cells only. All mutations shown to have arisen de novo when parent blood samples were available. The present series confirms the difficulty in predicting the phenotype according to the NIPBL mutation. Until now, somatic mosaicism has been observed for twenty cases which does not seem to be consistently associated with a milder phenotype. Besides, several reports support a postzygotic event for those cases.

Considering these elements, we recommend a first-line buccal cell DNA analysis in order to improve gene testing sensitivity in Cornelia de Lange syndrome and genetic counseling.

Key words: Cornelia de Lange syndrome, NIPBL, somatic mosaicism, buccal cells
INTRODUCTION

Cornelia de Lange syndrome [MIM #122470, 300590, 610759, 300882, 614701] is a rare and severe multisystemic developmental disorder. Main clinical features include intrauterine growth retardation and postnatal short stature, microcephaly, limb defects ranging from brachydactyly to agenesis, characteristic facial features – arched eyebrows, synophrys, long philtrum and thin upper lip –, hirsutism, feeding difficulties, heart or urogenital malformation, hearing loss and variable degree of intellectual disability 1.

Five genes have been identified so far in this syndrome. A heterozygous NIPBL [MIM #608667] mutation is observed in about 60% of the patients 2–4. The four other genes, SMC1A [MIM #300040], SMC3 [MIM #606062], HDAC8 [MIM #300269] and RAD21 [MIM #606462], represent about 10% of identified mutations 5–7. While a small fraction of cases are inherited, majority of them results from de novo events. All these genes encode proteins involved in the cohesin complex, which is decisive for cell division and gene expression regulation. Recently, NIPBL somatic mosaicism has been highlighted through buccal cell DNA study in some patients with a negative molecular analysis on leukocyte DNA 8.

MATERIALS AND METHODS

Here, we present a series of thirty-eight patients with a Cornelia de Lange syndrome caused by an heterozygous NIPBL mutation. Patients were recruited through national collaboration. The diagnosis of Cornelia de Lange syndrome was approved before molecular investigations based on the following criteria: 1) intrauterine growth retardation and postnatal short stature 2) feeding difficulties and/or gastro-oesophageal reflux 3) microcephaly, 4) intellectual disability and 5) characteristic facial features (see table 1). The study was approved by our hospital ethics board. Written informed patients and parent consents were obtained prior to genetic investigation.

NIPBL was analysed in first-line in all patients referred to our molecular genetics laboratory. DNA was isolated from leukocytes using the Puregene Blood Core Kit B (Qiagen), the Autopure automate (Qiagen) or the extraction blood kit Nucleon Bacc3 (GE Healthcare). DNA was additionally extracted from buccal cells in five patients with the Oragene saliva DNA collection Kit and according to the PrepIT protocol (DNA Genotek). cDNA was isolated from PAXgene blood RNA tubes (Qiagen) by a standard procedure for three patients with an intronic mutation. NIPBL coding region (GeneBank NG_006987.1) was sequenced with intronic primers designed by Primer3 Input (http://bioinfo.ut.ee/primer3-0.4.0/) and using the Big Dye Terminator cycle sequencing kit v3 (Applied Biosystems). Sequences were analysed on a 3130 or 3500 Genetic Analyser
sequencing machine (Applied Biosystems).

RESULTS

Among these thirty-eight patients, we identified thirty-four novel NIPBL mutations in leukocytes and three novel NIPBL mutations in buccal cells only. To note that nine saliva samples have been tested among NIPBL negative patients on leukocytes. Mutations included six nonsense mutations, eighteen frame shifts, six intronic mutations and seven missense mutations (see table 1). The c.5329-15A>G intronic mutation was identified in two unrelated probands. Patients 2, 17 and 26 had a distinct novel mutation only present in buccal cells and undetectable in leukocytes by Sanger sequencing. All mutations were shown to have arisen de novo when parent blood samples were available for DNA study. The missense mutations were predicted as damaging using Polyphen and Alamut software. The intronic mutations were predicted to alter a donor or acceptor splicing site according to the Human Splicing Finder predicting splicing software. To analyse the effect of intronic mutations, namely the c.64+2T>A, c.772-7_772-4delinsA and c.5329-15A>G mutations, we performed NIPBL cDNA analysis on patients 25, 27 and 29 RNA extracted from leukocytes. These mutations were found to respectively cause skipping of exon 2 containing the ATG translation initiation codon, partial retention of intron 7 leading to a frame shift with premature stop codon, and in frame deletion with exon 28 skipping responsible for the exclusion of thirty-three amino acids in the conserved HEAT domain H1 of the protein (data not shown). Finally, DNA extracted from buccal cells were tested in three patients with a known NIPBL mutation on leukocytes. The mutation was confirmed in the three buccal cell samples (data not shown).

DISCUSSION

Until now, over 300 distinct mutations have been reported in NIPBL (literature and our present series) including a proven somatic mosaicism for seventeen ones. Among them, 60% (196/325) are nonsense mutations, frame shifts or rearrangements, 16% (52/325) are splicing mutations and 24% (77/325) are missense mutations. All type of mutations have also been observed in patients with a somatic mosaicism. Mutations are spread all over the gene except for exons 13 and 16 as previously discussed, and most of them seem to be unique 4.

To date, various attempts have failed to bring out a reliable phenotype-genotype correlation in patients with an NIPBL mutation 9,10. Indeed, whereas a NIPBL mutation usually leads to a more severe phenotype than a mutation in one of the other genes involved in Cornelia de Lange syndrome, unrelated probands with a similar NIPBL mutation can have a severe, a classic or a mild phenotype 11,12. The present series confirms the difficulty
in predicting the phenotype according to the NIPBL mutation. To the same extent, a somatic mosaicism does not seem to be consistently associated with a milder phenotype. In this report, the three patients with somatic NIPBL mutation presented with the usual features namely short stature, microcephaly, feeding difficulty, brachymetacarpy and mild to moderate intellectual disability. Facial dysmorphism was also suggestive of the disease. However, additional reports of somatic NIPBL mosaicism is required to exclude a possible genotype-phenotype correlation. Other events are probably involved in the pathophysiology of the disease supporting the wide range of severity.

Recently, buccal cell analysis has shown its relevance for the molecular study of patients presenting with a Cornelia de Lange syndrome. Thereby, mutation in buccal cells has been identified in probands negative for NIPBL mutation in leukocytes.\textsuperscript{8,13} Other reports support a postzygotic event. Indeed, somatic mosaicism has already been identified on leukocytes for four different NIPBL mutations, namely a large intragenic deletion, a frame shift, a nonsense and a missense mutation, by array-comparative genomic hybridisation, pyrosequencing and exome sequencing, respectively.\textsuperscript{14–17} Almost 50% of patients negative for NIPBL mutation in leukocytes are predicted to have a somatic mosaicism.\textsuperscript{8} Embryological data are in agreement with these results. Indeed, oral epithelium derives from embryonic ectoderm, which is also the precursor for the neural tube leading to brain development. Besides, buccal cells can be performed less invasively than fibroblasts. Considering these elements, we recommend a first-line buccal cell DNA analysis in order to easily improve gene testing sensitivity in Cornelia de Lange syndrome. The exome sequencing extent will probably lead to a better detection of low levels of mosaicism in different cell types, setting out more arguments to refine genetic counseling, cutting down the germline mosaicism likelihood.\textsuperscript{18}

To conclude, NIPBL remains the major gene responsible for Cornelia de Lange syndrome either by germline or somatic mutation. The high frequency of somatic mosaicism supports the need for buccal cell DNA analysis. Further investigations should help understanding whether this is a disease or gene specific mechanism, or a more generalised one.
REFERENCES


TABLE LEGEND

Table 1. Molecular and clinical features of thirty-eight patients presenting with Cornelia de Lange syndrome. (F: female, M: male, NA: not available, SD: standard deviation, TOP: termination of pregnancy). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. Concerning clinical features, stature is represented as standard deviation compared to standard healthy population. Feeding difficulties are noticed as moderate when there is an impact on growth but without medical assistance; otherwise enteral nutrition device is indicated. Gastro-enteral reflux disease is considered as severe when a surgery was performed. Intellectual disability is represented according to OMS classification namely mild for IQ 55 to 70, moderate for IQ 40 to 55 and severe for IQ below 40. Facial dysmorphism is considered as classical when the diagnosis is obvious including fine arched eyebrows, synophris, long eyelashes, thin upper lip and anteverted nares.
Table 1. Molecular and clinical features of 38 novel patients presenting with Cornelia de Lange syndrome.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Nucleotide mutation</th>
<th>Protein mutation</th>
<th>Sample</th>
<th>Age</th>
<th>Malformations</th>
<th>Microcephaly</th>
<th>Intellectual disability</th>
<th>Behavioural trouble</th>
<th>Facial dysmorphism</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>c.2358delins11</td>
<td>p.Ala786Leu*18</td>
<td>ex2</td>
<td>NA</td>
<td>leukocytes 23 years</td>
<td>NA</td>
<td>NA</td>
<td>severe</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>c.614del</td>
<td>p.Thr205Met*19</td>
<td>ex2</td>
<td>NA</td>
<td>leukocytes only 9 years</td>
<td>- 2 SD</td>
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<td>yes</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>c.1451_1471del</td>
<td>p.Ile484Ile*8</td>
<td>ex5</td>
<td>de novo</td>
<td>leukocytes 2 months</td>
<td>NA</td>
<td>NA</td>
<td>severe</td>
<td>NA</td>
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<tr>
<td>4</td>
<td>F</td>
<td>c.275del</td>
<td>p.Ile92Met*30</td>
<td>ex3</td>
<td>de novo</td>
<td>leukocytes 6 years</td>
<td>- 3 SD</td>
<td>enteral nutrition</td>
<td>severe</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>c.405del</td>
<td>p.Ser135Pro*41</td>
<td>ex5</td>
<td>NA</td>
<td>tissue</td>
<td>TOP at 20 GW</td>
<td>- 2 SD</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>c.1345del</td>
<td>p.Pro448Pro*10</td>
<td>ex6</td>
<td>de novo</td>
<td>tissue</td>
<td>TOP at 12 GW</td>
<td>- 3 SD</td>
<td>-</td>
<td>-</td>
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<td>7</td>
<td>M</td>
<td>c.1421_1424del</td>
<td>p.Leu477Leu*7</td>
<td>ex7</td>
<td>de novo</td>
<td>leukocytes 5 years</td>
<td>- 3 SD</td>
<td>enteral nutrition</td>
<td>yes</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>c.1994del</td>
<td>p.Lys662*</td>
<td>ex7</td>
<td>de novo</td>
<td>leukocytes 6 years</td>
<td>&lt; -3 SD</td>
<td>moderate</td>
<td>yes</td>
<td>NA</td>
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<td>9</td>
<td>M</td>
<td>c.2174del</td>
<td>p.Lys726Asn*68</td>
<td>ex10</td>
<td>de novo</td>
<td>leukocytes</td>
<td>TOP at 51 GW</td>
<td>- 2 SD</td>
<td>-</td>
<td>-</td>
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<td>10</td>
<td>M</td>
<td>c.2252del</td>
<td>p.Arg754Asp*40</td>
<td>ex10</td>
<td>de novo</td>
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<td>moderate</td>
<td>yes</td>
<td>NA</td>
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<tr>
<td>11</td>
<td>M</td>
<td>c.2412_2413del</td>
<td>p.Pro805Pro*10</td>
<td>ex10</td>
<td>de novo</td>
<td>leukocytes 6 months</td>
<td>- 3 SD</td>
<td>severe</td>
<td>yes</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>c.2771_2774del</td>
<td>p.Lys925Glu*2</td>
<td>ex10</td>
<td>de novo</td>
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<td>13</td>
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<td>c.3172_3184del</td>
<td>p.Glu553Asp*8</td>
<td>ex11</td>
<td>de novo</td>
<td>leukocytes 9 months</td>
<td>- 3 SD</td>
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<td>NA</td>
<td>-</td>
</tr>
<tr>
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<td>M</td>
<td>c.3151del</td>
<td>p.Arg1066*</td>
<td>ex11</td>
<td>de novo</td>
<td>leukocytes 2.7 years</td>
<td>- 2 SD</td>
<td>severe</td>
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<td>NA</td>
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<tr>
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<td>M</td>
<td>c.3435del</td>
<td>p.Arg149*</td>
<td>ex12</td>
<td>de novo</td>
<td>leukocytes 14 years</td>
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<td>NA</td>
<td>NA</td>
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<td>M</td>
<td>c.3445del</td>
<td>p.Arg149*</td>
<td>ex12</td>
<td>de novo</td>
<td>leukocytes 15 years</td>
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<td>moderate</td>
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<td>NA</td>
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<td>c.5042del</td>
<td>p.Lys141del*15</td>
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<td>NA</td>
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<td>severe</td>
<td>yes</td>
<td>NA</td>
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<tr>
<td>18</td>
<td>M</td>
<td>c.6516_6517del</td>
<td>p.Met2178Met*5</td>
<td>ex14</td>
<td>de novo</td>
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<td>- 3 SD</td>
<td>severe</td>
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<td>-</td>
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<td>19</td>
<td>M</td>
<td>c.6694del</td>
<td>p.Gln2232*</td>
<td>ex15</td>
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<td>moderate</td>
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<tr>
<td>20</td>
<td>M</td>
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<td>p.Glu2492Glu*15</td>
<td>ex16</td>
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<td>leukocytes and buccal cells</td>
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<td>moderate</td>
<td>NA</td>
<td>-</td>
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<td>ex17</td>
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<td>- 2 SD</td>
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<td>leukocytes 7 years</td>
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<td>p.2896del</td>
<td>ex19</td>
<td>de novo</td>
<td>leukocytes 7 years</td>
<td>- 3 SD</td>
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<td>NA</td>
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<tr>
<td>24</td>
<td>F</td>
<td>c.5313del</td>
<td>p.Glu1713*</td>
<td>ex19</td>
<td>de novo</td>
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<td>- 3 SD</td>
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<td>NA</td>
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<td>25</td>
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<td>c.6143del</td>
<td>p.Pro27_Pro29delinsLeuGlnLeuProAsn</td>
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<td>de novo</td>
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<td>M</td>
<td>c.6430del</td>
<td>p.Val2144*2</td>
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<td>de novo</td>
<td>leukocytes 1 month</td>
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<td>NA</td>
<td>severe</td>
<td>NA</td>
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<td>c.6817G&gt;A</td>
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<td>ex22</td>
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<td>NA</td>
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<td>NA</td>
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<tr>
<td>28</td>
<td>M</td>
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<td>p.Ala2338Pro*12</td>
<td>ex23</td>
<td>de novo</td>
<td>leukocytes 24 years</td>
<td>- 2 SD</td>
<td>moderate</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: Microcephaly: -2 SD to -4 SD; Severe: -6 SD.