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

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Conglict of interest statement

The authors declare that they have no competing interests.

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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

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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 agenesia, 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

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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

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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 [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 [14–17]. Almost 50% of patients negative for *NIPBL* mutation in leucocytes are predicted to have a somatic mosaicism [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 [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.

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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 sugery 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.

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Table 1. Molecular and clinical features of 38 novel patients presenting with Cornelia de Lange syndrome.

Patient	Gender	Nucleotide mutation	Protein mutation	Exon/ intron	Parents	Sample	Age	Intrauterine growth failure	Feeding difficulties	Gastro-oesophageal reflux	Limb abnormalities	Malformations	Short stature	Microcephaly	Intellectual disability	Behaviour trouble	Deafness	Facial dysmorphism
1	F	c.5_12delins11	p.Asn2Lysfs*18	exon 2	NA	leukocytes	23 years	NA	NA	yes	pes cavus	aortic stenosis	-5 SD	NA	moderate	severe	NA	classical
2	F	c.19del	p.His7Metfs*19	exon 2	NA	buccal cells only	6 years	<-3 DS	moderate	NA	brachymetacarpy I	NA	-2 SD	-2 SD	moderate	moderate	yes	NA
3	F	c.143_147del	p.Glu48Glyfs*8	exon 3	*de novo*	leukocytes	2 months	NA	NA	NA	distal phalanges hypoplasia	diaphragmatic hernia	NA	<-3 SD	mild	absent	NA	NA
4	F	c.275del	p.Ile92Asnfs*30	exon 4	*de novo*	leukocytes	6 years	< -3 DS	enteral nutrition	severe	brachymetacarpy I	atrial septal defect, aortic coarctation, bifid uterus, Pierre Robin sequence	<-3 SD	-4 SD	moderate	absent	NA	classical
5	M	c.403del	p.Ser135Valfs*81	exon 5	NA	tissue	TOP at 20 GW	<-2 DS	-	-	limb defects	tetralogy of Falot, Pierre Robin sequence	-	absent	-	-	-	classical
6	F	c.1342insC	p.Pro448Profs*19	exon 9	*de novo*	tissue	TOP at 32 GW	<-3 SD	-	-	limb defects	diaphragmatic hernia	-	absent	-	-	-	classical
7	M	c.1821_1824del	p.Glu607Aspfs*6	exon 10	*de novo*	lung tissue	TOP at 24 GW	<-3 SD	-	-	brachymetacarpy I	diaphragmatic hernia, hypospadias	-	absent	-	-	-	classical
8	M	c.1984A>T	p.Lys662*	exon 10	*de novo*	leukocytes	3 years	-3 SD	enteral nutrition	yes	limb defects	Pierre Robin sequence, cryptorchidy	-2 SD	-6 SD	severe	absent	yes	classical
9	M	c.2178del	p.Lys726Asnfs*68	exon 10	*de novo*	tissue	TOP at 31 GW	<-2 DS	-	-	brachymetacarpy I, phalanges hypoplasia	ventricular septal defect	-	-2 SD	-	-	-	classical
10	M	c.2259del	p.Arg754Aspfs*40	exon 10	*de novo*	tissue	TOP at 25 GW	<-2 DS	-	-	brachymetacarpy I, ectrodactyly	absent	-	-2 SD	-	-	-	classical
11	M	c.2412_2413delinsGA	p.Pro805Thrfs*10	exon 10	*de novo*	leukocytes	6 months	<-3 SD	enteral nutrition	yes	ectrodactyly	hypospadias	-4 SD	-4 SD	moderate	absent	yes	moderate
12	M	c.2773_2774del	p.Lys925Glufs*2	exon 10	NA	leukocytes	13 years	<-3 SD	moderate	NA	limb defects	hypospadias, cryptorchidy	-8 SD	-6 SD	severe	absent	NA	classical
13	F	c.3172_3184del	p.Glu1058Profs*8	exon 11	Father NA	leukocytes	9 months	<-2 SD	?	NA	NA	NA	<-2 SD	<-2 SD	NA	NA	NA	classical
14	M	c.3316C>T	p.Arg1106*	exon 12	*de novo*	leukocytes	2,5 years	-2 SD	moderate	yes	brachymetacarpy I	NA	absent	absent	mild	absent	NA	NA
15	NI	c.3439C>T	p.Arg1147*	exon 12	*de novo*	tissue	TOP at 14 GW	absent	-	-	limb defects	diaphragmatic hernia, ventricular septal defect, cleft palate	-	-	-	-	-	NA
16	M	c.3445C>T	p.Arg1149*	exon 12	*de novo*	leukocytes	13 years	<-3 SD	enteral nutrition	NA	limb defects	cardiac defect, hypospadias	-4 SD	<-3 SD	moderate	severe	yes	classical
17	M	c.4020_4024delinsAGTGTA	p.Lys1341Valfs*13	exon 17	NA	buccal cells only	18 months	absent	absent	absent	brachydactyly	micropenis, cryptorchidy, pyelo ureteral duplication	NA	-2 SD	mild	mild	yes	classical
18	F	c.6516_6517insA	p.Val2173Serfs*3	exon 38	*de novo*	leukocytes	8 years	-3 SD	moderate	yes	Clinodactyly V	absent	-2 SD	-4 SD	moderate	mild	absent	classical
19	M	c.6694C>T	p.Gln2232*	exon 39	*de novo*	leukocytes	2 years	<-3 SD	enteral nutrition	yes	limb defects	hypospadia, cryptorchidy, ventricular septal defect	<-8 SD	<-8 SD	severe	absent	absent	moderate
20	F	c.6987_6994delinsA	p.Asp2329Glufs*15	exon 41	NA	leukocytes	1 year	<-2 SD	enteral nutrition	yes	moderate	NA	<-2 SD	NA	severe	severe	NA	classical
21	F	c.7969delinsTC	p.Gly2657Serfs*4	exon 46	*de novo*	leukocytes and buccal cells	18 months	-3 SD	moderate	yes	brachymetacarpy I	NA	-2 SD	-2.5 SD	moderate	absent	yes	classical
22	M	c.8275_8276dup	p.Leu2759Leufs*6	exon 47	*de novo*	leukocytes	6 years	absent	enteral nutrition	severe	absent	absent	-2 SD	-2 SD	moderate	mild	absent	moderate
23	F	c.8361insA	p.Val2788Metfs*32	exon 47	NA	leukocytes	7 years	NA	moderate	NA	NA	NA	NA	<-2 SD	mild	absent	NA	NA
24	F	c.8377C>T	p.Arg2793*	exon 47	NA	leukocytes	4 years	<-3 SD	absent	absent	distal phalanges hypoplasia	absent	-3 SD	-4 SD	mild	absent	NA	moderate
25	F	c.64+2T>A	skipping ATG initiation codon	intron 2	Father NA	leukocytes	3 years	-2 SD	moderate	yes	syndactyly	absent	-3.5 SD	-4 DS	mild	absent	yes	moderate
26	M	c.358+1G>A	unknown	intron 4	NA	buccal cells only	14 years	<-2 SD	moderate	yes	brachymetacarpy I	cryptorchidy	NA	<-2 SD	moderate	absent	yes	NA
27	M	c.772-7_772-4delinsA	p.Asp258Tyrfs*13	intron 7	*de novo*	tissue	TOP at 23 GW	<-2 SD	-	-	brachydactyly	ventral septal defect, cystic kydneys	-	absent	-	-	-	classical
28	M	c.5225_5225+4del	unknown	intron 26	NA	leukocytes and buccal cells	3 months	<-2 SD	moderate	NA	brachydactyly, syndactyly	micropenis	<-2 SD	<-3 SD	moderate	NA	yes	classical
29	F	c.5329-15A>G	p.Ile1777_Arg1809del	intron 27	NA	leukocytes	10 years	NA	absent	absent	brachydactyly	NA	no	-3 SD	NA	NA	NA	moderate
30	M	c.5329-15A>G	p.Ile1777_Arg1809del	intron 27	*de novo*	leukocytes	7 years	<-3 SD	absent	NA	brachymetacarpy I	NA	-3 SD	-4 SD	mild	absent	NA	NA
31	F	c.6250-1G>A	unknown	intron 35	*de novo*	leukocytes	21 months	<-3 SD	enteral nutrition	NA	brachymetacarpy I, phalanges hypoplasia	Pierre Robin sequence	-4 SD	-6 SD	moderate	absent	yes	classical
32	M	c.80_89delins16	p.Pro27_Pro29delinsLeuGlnLeuProAsn	exon 3	*de novo*	leukocytes	18 months	<-3 SD	enteral nutrition	absent	Brachydactyly, clinodactyly V	pulmonar stenosis, cryptorchidy	-5 SD	-4 SD	moderate	absent	absent	moderate
33	M	c.6148A>T	p.Ile2050Phe	exon 35	Father NA	leukocytes	19 years	<-3 SD	moderate	severe	clinodactyly V, syndactyly	micropenis, cryptorchidy	NA	<-3 SD	severe	absent	NA	NA
34	F	c.6221T>A	p.Met2074Lys	exon 35	*de novo*	leukocytes	14 years	-2 SD	absent	absent	brachymetacarpy I	NA	absent	-2,5 SD	mild	absent	NA	moderate
35	M	c.6275T>C	p.Leu2092Pro	exon 36	*de novo*	tissue	TOP at 15 GW	absent	-	-	limb defects	absent	-	no	-	-	-	moderate
36	F	c.6430C>T	p.Leu2144Phe	exon 37	NA	leukocytes	1 month	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
37	M	c.6817G>A	p.Gly2273Arg	exon 40	*de novo*	leukocytes	20 years	<-3 SD	NA	NA	brachymetacarpy I	ventricular septal defect	-4 SD	-2 SD	mild	moderate	NA	NA
38	M	c.7012G>C	p.Ala2338Pro	exon 41	*de novo*	leukocytes	24 years	NA	NA	yes	camptodactyly	NA	absent	-2 SD	moderate	absent	NA	moderate