Further contributions towards the molecular analysis of *NIPBL* and *SMC1A* genes in a cohort of patients with Cornelia de Lange Syndrome

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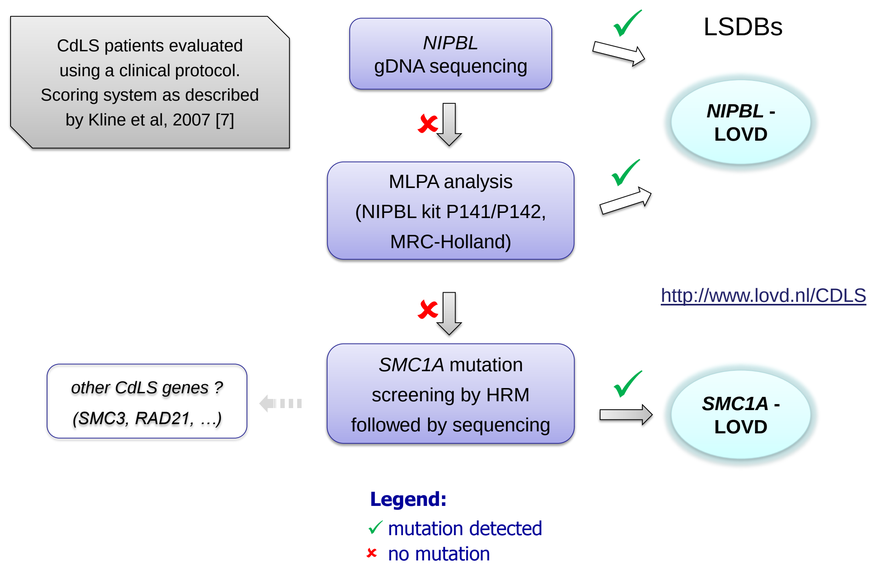
Background

Cornelia de Lange Syndrome [CdLS (MIM#122470)] is a rare multisystemic developmental disorder with an estimated incidence of 1/10,000 - 30,000 births. It is characterized by a typical phenotype that includes distinctive facial dysmorphism, hirsutism, growth and psychomotor developmental delay, limb abnormalities, and relatively frequent gastrointestinal and congenital heart defects [1,2].

Mutations in the *NIPBL* gene [5p13.1 (MIM*608667)] have been identified in ~50% of patients, while mutations in *SMC1A* gene [Xp11.2 (MIM*300040)] account for ~5% of individuals with CdLS. Mutations have also been described in *SMC3* [10q25 (MIM*606062)] [3,4]. More recently, individuals with alterations in the *RAD21 gene* [8q24.11 (MIM*606462)] were also found to develop a cohesinopathy, with a milder cognitive impairment. These genes encode proteins linked to the cohesin complex that mediates sister-chromatid cohesion. This complex is involved in global transcriptional regulation and repair of DNA double strand breaks [5].

The molecular and clinical characterization of Portuguese CdLS patients has been previously described, as well as the development of a Locus Specific Database for *NIPBL* [6]. This presentation is an update on the molecular findings of our patient cohort; in particular, new *NIPBL* mutations which include large deletions, and screening for mutations in *SMC1A* by high resolution melting curve analysis (HRM).

Patients and Methods



Results

Table 1: Mutations found in a subset of our patient cohort

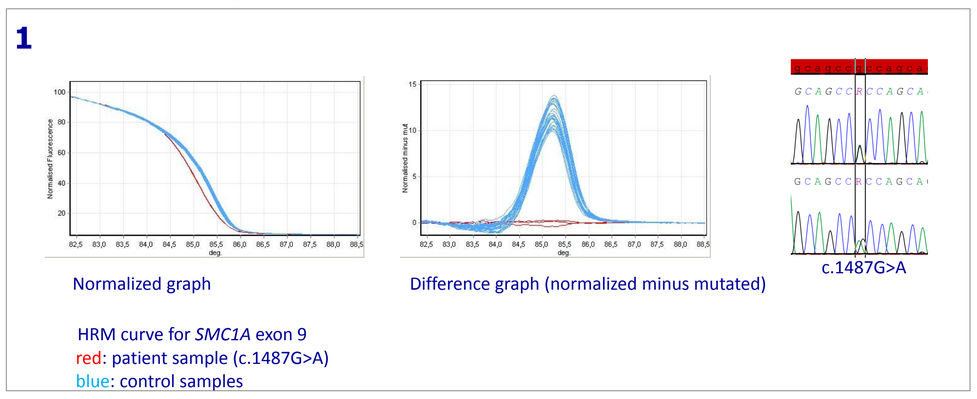
Patient	Mutations	Location	Predicted polypeptide change	Variant/Origin	Detection	Type	Clinical Phenotype [7]	References
			SMC1A gene [a]					
1	c.1487G>A	Exon 9	p.(Arg496His)	de novo	HRM/SEQ	missense	n.a.	Deardorff et al 2007
			NIPBL gene [b]					
2	c.64+1G>A	Exon 2	p.(?)	*de novo*	SEQ.	splicing	mild	Borck et al 2004 [8]
3	**c.86del**	**Exon 3**	**p.Pro29Hisfs*18**	**unknown**	**SEQ.**	**frameshift**	**severe**	**Novel**
4	c.1885C>T	Exon 10	p.(Arg629*)	unknown	SEQ.	nonsense mosaicism?	moderate	Myake et al 2005 [9]
5	c.3316C>T	Exon 12	p.(Arg1106*)	*de novo*	SEQ.	nonsense	mild	Miyake et al 2005
6	c.4422G>T	Exon 21	p.(Arg1474Ser)	*de novo*	SEQ.	missense	mild	Oliveira et al 2010
7	c.5471C>T	Exon 29	p.(Ser1824Leu)	unknown	SEQ.	missense	mild	Oliveira et al 2010
8	c.6653_6655delATA	Exon 39	p.(Asn2218del)	*de novo*	SEQ.	deletion	moderate	Borck et al 2004
9	c.6763+5G>T	Intron 39	p.(?)	unknown	SEQ.	splicing	mild	Krantz et al 2004
10	**c.6983C>G**	**Exon 41**	**p.(Thr2328Arg)**	**unknown**	**SEQ.**	**missense**	**n.a.**	**Novel**
11	c.7168G>A	Exon 42	p.(Ala2390Thr)	*de novo*	SEQ.	missense	moderate	Gillis 2004 [10]
12	**c.7307C>T**	**Exon 43**	**p.(Ala2436Val)**	***de novo***	**SEQ.**	**missense**	**n.a.**	**Novel**
13	**c.(?_-481)_(*927_?)del**	**Exon 1 to 47**	**p.(?)**	***de novo***	**MLPA**	**large deletion**	**severe**	**Novel**
14	**c.(5710-?)_(*927+?)del**	**Exon 31 to 47**	**p.(?)**	**unknown**	**MLPA**	**large deletion**	**severe**	**Novel**

[a] - *SMC1A* Accession number of cDNA reference sequence: NM_006306

[b] - *NIPBL* Accession number of cDNA reference sequence: NM_133433.3

Seq. – Sequencing; HRM – High Resolution Melting;

SMC1A mutation



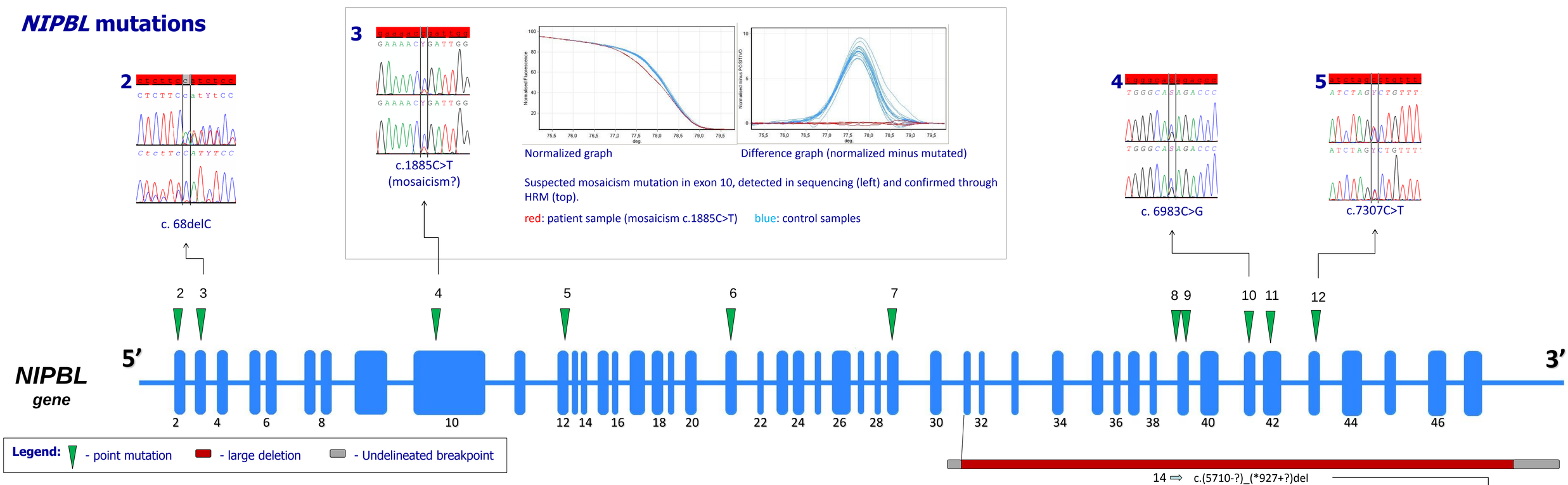
***SMC1A* mutation**

We identified a previously described mutation in *SMC1A* c.1487G>A (p.Arg496His) in patient 1 (Fig. 1). The HRM technique was used for mutation scanning, 3 replicates of each sample were used to prevent false positives/negative results. Samples with consistent abnormal melting curves were sequenced.

***NIPBL* mutations**

11 point mutations were identified upon sequencing including 3 novel mutations: c.68delC (causing a frame-shift), missense mutations c.6983C>G (p.Thr2328Arg) and c.7307C>T (p.Ala2436Val) (Fig. 2, 4 and 5). We suspect the existence of somatic mosaicism in case 4, since the c.1885C>T mutation is underrepresented as compared with the WT allele (Fig. 3). Two novel gross rearrangements were found by MLPA (Fig. 7 and 8): one deletion covering the complete *NIPBL* gene in patient 13; and the other deletion spanning exons 31 to 47 of in case 14.

NIPBL mutations



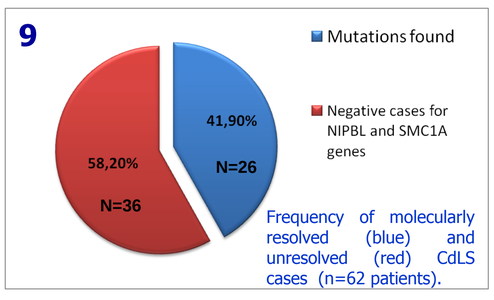
Concluding remarks

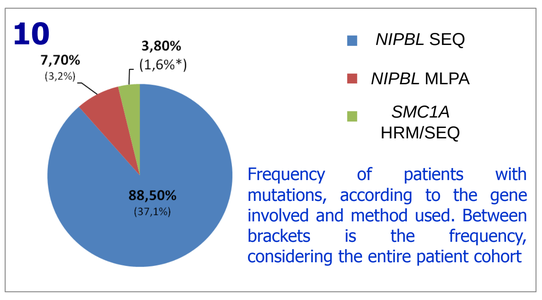
The mutation detection rates in our cohort correspond to those cited in the literature (Fig. 9 and 10). Our results also corroborate the importance of using different techniques (especially MLPA for *NIPBL*) to attain higher mutation detection rates.

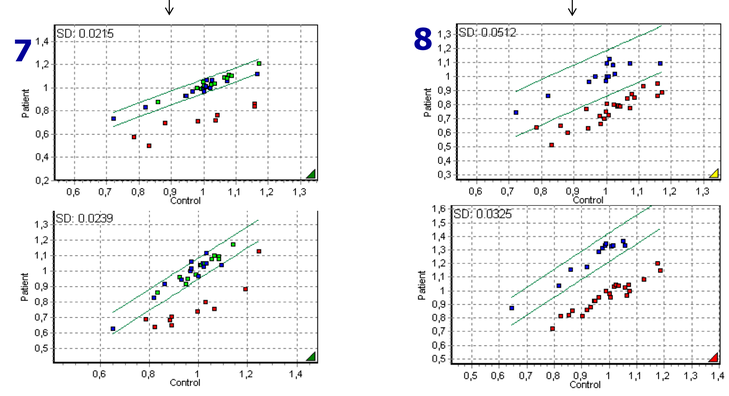
The relatively small size of *SMC1A* exons and the limited number of SNPs allowed the use of HRM as a preliminary mutation screening method. This approach is more cost effective and time saving as compared to sequencing, especially considering the reduced number of cases with *SMC1A* mutations reported.

Somatic mosaicism is probably underestimated in the literature and might explain some degree of phenotypic variability in some patients. This holds true for the c.1885C>T mutation that was originally reported in a Japanese CdLS patient with a severe phenotype - our case (with suspected somatic mosaicism) was classified as moderate.

Routine Sanger sequencing may not be sufficiently sensitive to detect low levels (<20%) of mutated alleles, leading to false negative results. We have shown that the use of HRM might be helpful to detect mutations in cases with somatic mosaicism.







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