 Int J Hum Genet, 16(1,2): 61-69 (2016)

Heterozygous Complete *NIPBL* Gene Deletion in Cornelia de Lange Syndrome: First Case Report from India

Shailesh Bajaj[1], Sheela Nampoothiri[2], Dhanya Yesodharan[2], Prakash Gambhir[3] and Suvidya Ranade[1*]

[1]Department of Chemistry, Biochemistry Division, Savitribai Phule Pune University, Pune 411 007, Maharashtra, India
[2]Amrita Institute of Medical Sciences & Research Center, Cochin, Kerala, India
[3]Birthright Genetic Clinic, Erandwane, Pune, Maharashtra, India

KEYWORDS Cornelia de Lange Syndrome. MLPA. *NIPBL*. RT PCR

ABSTRACT Cornelia de Lange Syndrome-1 (CdLS; OMIM # 122470) is a multisystem, congenital, developmental disorder caused by heterozygous mutation in *NIPBL* gene on chromosome 5p13. CdLS is characterized by growth and developmental delay, facial dysmorphism, limb abnormality and other organ defects. The condition is mainly caused due to mutation in one of the cohesin ring forming genes. Among *NIPBL*, *SMC1A* and *SMC3*; *NIPBL* is mainly responsible for causing CdLS. To date molecular data for Indian CdLS patients is not available. Entire *NIPBL* gene has been screened in 12 children showing CdLS using MLPA in this study. The study reports entire gene deletion in one proband and partial gene deletion in the second proband. The observed deletion was in heterozygous condition in both the cases. The finding was validated by real time PCR.

INTRODUCTION

Cornelia de Lange Syndrome (CdLS) has typical gestalt which include bushy arched eyebrows with synophrys, long eye lashes, depressed nasal bridge with anteverted nose, thin upper lip with downturned angles of mouth, hypertrichosis, microcephaly, partial fusion of 2nd and 3rd toes and global developmental delay (Boyle et al. 2015). The overall prevalence of CdLS is estimated to be 1/10, 000 (Krantz et al. 2004). It has been observed that most cases appear to be sporadic. 50 percent of CdLS cases are associated with mutations in *NIPBL* (Nipped B like) gene affecting the cohesin complex (Rohatgi et al. 2010). Growth retardation is almost universal in CdLS patients. Although clinical features in CdLS can be easily recognized a wide range of phenotypic variation is observed. The milder phenotypes may remain undiagnosed many times (Gillis et al. 2004). The prevalence of autism spectrum disorder (ASD) is comparatively high in CdLS (Parisi et al. 2015). Earlier studies on *NIPBL* gene have reported several mutations in exons using traditional approach like PCR and sequencing (Gillis et al. 2004). Screening of *NIPBL* gene in CdLS probands by using MLPA (Multiplex Ligation dependant Probe Amplification) method has been carried out by researchers in different countries. The MLPA is the robust and highly sensitive method in modern research. The MLPA method has detected large deletions, insertions and rearrangements in *NIPBL* gene in the past (Bhuiyan et al. 2007; Ratajska et al. 2010; Russo et al. 2012; Gervasini et al. 2013; Cheng et al. 2014). Recently, Teresa et al. 2016 reported two intronic *NIPBL* mutations causing loss of exon 37 resulting in premature translation and synthesis of a shorter polypeptide (Teresa et al. 2016). The findings obtained on screening of *NIPBL* gene by other researchers have been summarized in Table 1. In the present study, the gene *NIPBL* was completely screened for 12 Indian CdLS patients for the first time by using MLPA method.

Objectives of the Study

The main objective of the present study was to screen entire *NIPBL* gene in CdLS affected probands and to find any rearrangement in the *NIPBL* if it exists.

[*]Address for correspondence:
Dr. Suvidya H. Ranade,
Department of Chemistry,
Biochemistry Division,
Savitribai Phule Pune University,
Pune 411 007, Maharashtra, India.
Phone: 020-25691728
E-mail: suvidya@chem.unipune.ac.in

Table 1: Short summary for the large rearrangement or deletion found earlier by MLPA

Author/ Year	*Reference*	*Findings*	*Method adopted for screening NIPBL*	*Method adopted to reconfirm the finding*
Bhuiyan et al. 2007	Large genomic rearrangements in *NIPBL* are infrequent in Cornelia de Lange syndrome.	5.2 kb deletion encompassing exons 41–42 of *NIPBL*	MLPA	Normal PCR
Ratajska et al. 2010	Cornelia de Lange syndrome case due to genomic rearrangements including *NIPBL*	A large deletion encompassing exons 35 to 47 of the *NIPBL* gene	MLPA	aCGH
Russo et al. 2012	Intragenic and large *NIPBL* rearrangements revealed by MLPA in Cornelia de Lange patients single-exon deletions and one single exonduplication	Two large gene deletions extending beyond the gene, four intragenic multi- or	MLPA	RT PCR, Sequencing
Gervasini et al. 2013	Molecular characterization of a mosaic *NIPBL* deletion in a Cornelia de Lange patient with severe phenotype	The partial *NIPBL* deletions occur in a mosaic condition (exon 2 to 32)	MLPA	FISH, aCGH
Cheng et al. 2014	Copy number analysis of *NIPBL* in a cohort of 510 patients reveals rare copy number variants and a mosaic deletion	One intragenicduplication and a deletion in mosaic state.	MLPA	Whole Genome Microarray

MATERIAL AND METHODS

In the present study, peripheral blood samples of 12 CdLS patients were collected after taking consent from family and following information was noted: Name, Age, Address, Community, Pedigree, and family history of CdLS, consanguinity, and clinical phenotype. The research was prospectively reviewed and approved by a duly constituted ethics committee of Savitribai Phule Pune University. The CdLS cases were recruited from Birthright Genetic clinic based at Pune and Amrita Institute of Medical Sciences & Research Center, Cochin, Kerala. DNA was isolated using Stratagene kit (200600) from 5 ml of peripheral blood leukocytes. The quality and quantity of DNA was checked and MLPA (Multiplex Ligation dependant Probe Amplification) protocol was standardized using P141 and P142 probe sets. The MLPA protocol included DNA denaturation at 98^0C for 5 minutes, the hybridization at 60^0C for 16 to 20 hours followed by ligation of probes at 54^0C for 15 minutes and the multiplex PCR was the final step. The obtained fragments of different sizes were separated by capillary electrophoresis on ABI 3130XL Genetic Analyzer. MLPA data were normalized by the Coffalyser software v. 9.4 (MRC-Holland, the Netherlands). According to the diagnostic threshold, a peak was considered abnormal when the probe ratio was <0.7 (deletion) or >1.3 (duplication) compared to the reference peak.

To confirm the observations obtained by MLPA; RT PCR assay with SYBR-Green I detection was performed. Primers were designed for following exons; exon 11, 16, 22, 28 and 34. For confirmation of complete gene deletion by RT PCR, primers were also designed for 5' and 3' UTR regions of *NIPBL*. The primer details are given in supplement 1.

RESULTS

In the present study, screening of *NIPBL* gene (size 189655bp) in 12 CdLS probands from India by MLPA method has been carried out for the first time. A heterozygous deletion of *NIPBL* gene was observed in two cases. In one proband, entire *NIPBL* gene (187690bp) deletion including UTRs in heterozygous condition was seen whereas in the other case, deletion of exon 11 to exon 34 (43020bp) in heterozygous condition was observed. The MLPA Dosage Quotient value in the range 0.40 to 0.65 indicated heterozygous gene deletion (Fig. 1 A-D).

To confirm the results, RT PCR was carried out using specific primers for the test and control. The decrease in threshold cycle (Ct) value

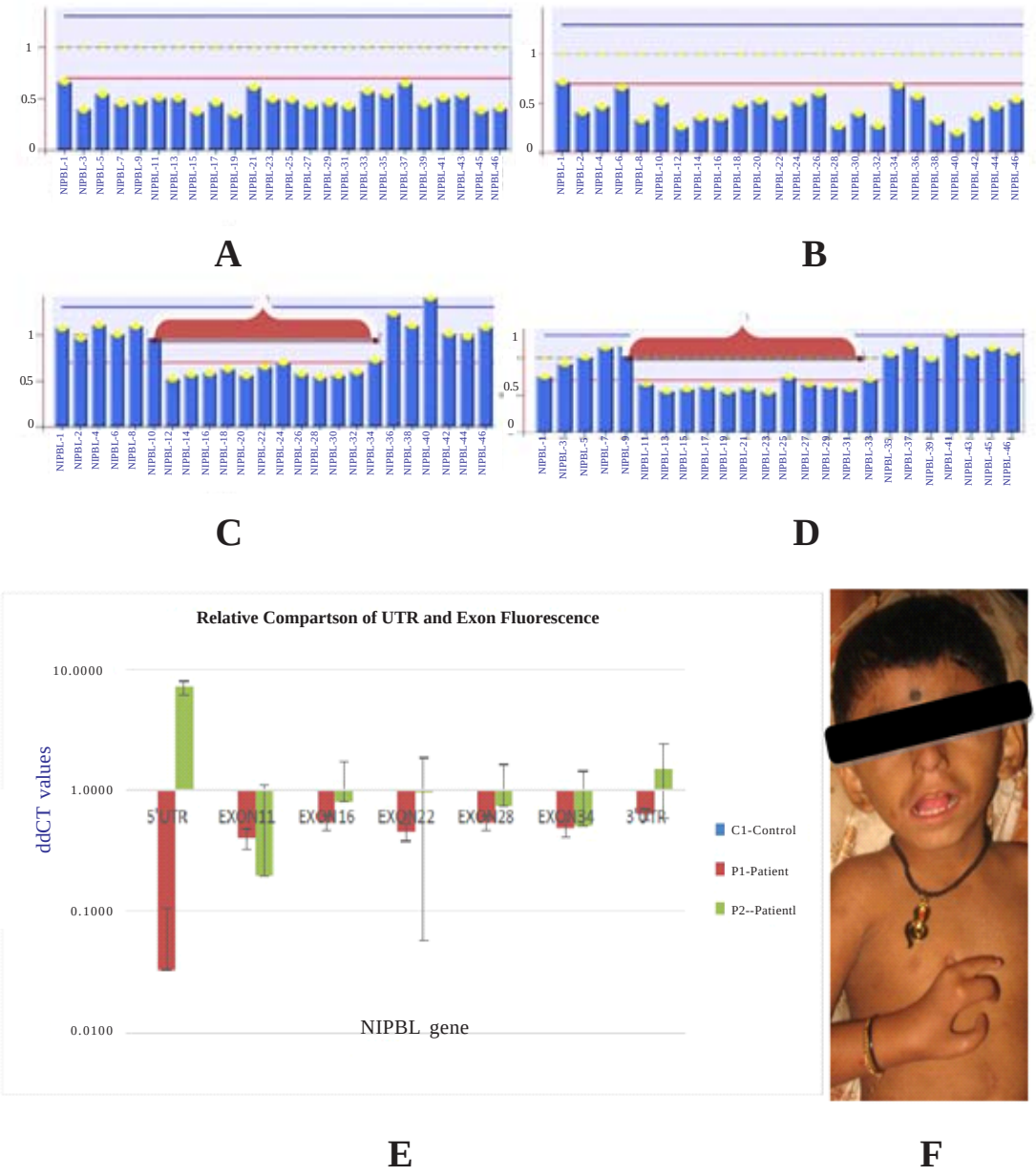

Fig. 1. MLPA Results (A to D) exon number on X axis and final probe ratio on Y axis: A and B shows complete *NIPBL* gene deleation in heterozygous manner in patient 1. C and D indicate heterozygous deletion of *NIPBL* gene middle region (Exon 11 to 34) in patient 2. R T PC Results: (E) The ddCT values on logarithmic plot in comparison with C1-control sample. ddCT values P1; 3' and 5' UTR along with studied exons <1, ddCT values P2 for studies exons<1(F) Photograph of proband 1.
Source: Author

in test (N) as compared to control (2N) was observed; whereas, the threshold value (Ct) of control for specific target was equal to housekeeping gene threshold value. The results of MLPA and RT PCR where ddCT values for P1 and P2 for studied region were <1 are given in Figure 1 E. Detailed table for calculated RQ is given in supplement 2.

DISCUSSION

Proband 1 (Fig. 1F) was a 4 year old boy with classical CdLS phenotype with synophrys, bushy arched eyebrows, thin upperlip, long philtrum, bilateral ptosis, hypertrichosis, prefixed thumb and micropenis. He also had lobster claw hand anomaly on right hand with four fingers. Surgical correction of ptosis was performed bilaterally and he also underwent surgery for malrotation at 2 years of age. Proband 2 was a one year old girl. She also had the classical phenotype of CdLS. In addition she had camptodactyly of 2,3,4,5 fingers bilaterally and cleft of the soft palate. She was on feeding tube as she had severe failure to thrive. Both patients were born to nonconsanguineous couple and had normal

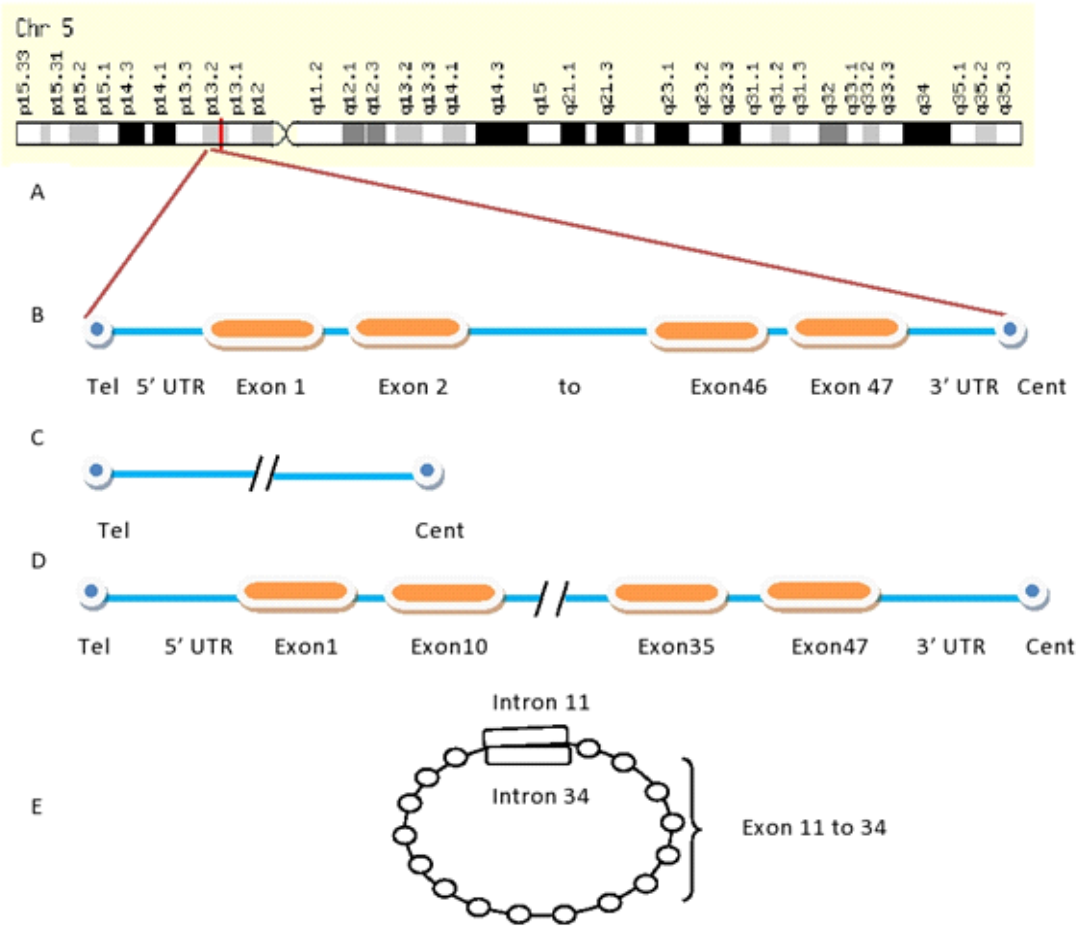

Fig. 2. Schematic representation of proposed mechanism of deletions found in probands. A: *NIPBL* location on chromosome 5 at 5p13.2. B: Exon 1 to 47 with 5' and 3' UTR, Telomere and Centromere in control, C: Heterozygona deletion of all 47 exons with 5' and 3' UTR in patient I, D: Eleterozygous deletion of exon 11 to 34 in patient 2, E: A region looped out by recombination event occurred between Intron 11 and Intron 34 as highly similar nucleotides (85%) presents in this region identity was found between these introns..
Source: Author

karyotype. They were born to phenotypically normal parents. Table 2 shows clinical characteristics of other studied CdLS probands. Parental genotype was checked for *NIPBL* gene by MLPA and was found to be normal. Thus the disease cause or change in both the cases was found 'de novo'. To find out the pathogenesis of partial deletion of a region in second proband; the intronic sequences (Intron 11 and 34) were collected from NCBI. The obtained sequences were aligned together by Align Sequences Nucleotide BLAST tool at NCBI. As shown in sup-

Table 2: Details of CdLS patients recruited: Genotype, phenotype and research findings

Patient number	*Sex*	*Age*	*Karyo type*	*Pheno-type*	*Consanguinity*	*Research Findings*
1	F	4 year	46,XX	Arched Eyebrows, long eyelashes, Hypoplstic uvula, Thin lowerlip, Long Philtrum, Microcephaly, Small hands and feet	Non-consanguinous	No change in *NIPBL*
2	M	4 year	46,XY	Microcephaly, Hoarse Voice, small sloping forehead, synophrys, sharp nose, arched eyebrows, long philtrum, thin upperlip, hypertrichosis, hand anomaly	Non-consanguinous	Entire *NIPBL* deletion in heterozygous condition
3	F	1 Year	46,XX	Synophrys, hypertension, Narrow palpebral fissure, small hands and feet	Non-consanguinous	No change in *NIPBL*
4	M	2 Year	46,XY	Microcephaly, Low set ears, long eyelashes, long philtrum, mesognalthic, thin upper lip, small hand and feet	Non-consanguinous	No change in *NIPBL*
5	F	1 Year	Awaited	Microcephaly, hypertrichosis, synophrys, hairy forhead, thin upperlip, long philtrum, microstomia, micrognathic	Non-consanguinous	Exon 11 to 34 deletion in heterozygous condition
6	F	4 year	46,XX	Microcephaly, Arched eyebrows, long eyelashes, long philtrum, thin lips	Non-consanguinous	No change in *NIPBL*
7	M	8 Year	Awaited	Lower hairline, synophrys, arched eyebrows, hypertrichosis, Brachydactyly	Non-consanguinous	No change in *NIPBL*
8	F	5 Year	46,XX	Microcephaly, Synophrys, hypertrichosis, thin upper lip	Non-consanguinous	No change in *NIPBL*
9	M	1 Year	Awaited	Arched eyebrows, hypertrichosis, synophrys, long philtrum, thin lips	Non-consanguinous	No change in *NIPBL*
10	F	1 Year	Awaited	Hairy forehead, synophrys, arched eyebrows, Brachydactyly	Non-consanguinous	No change in *NIPBL*
11	M	1 Year	Awaited	Microcornea, Ectrodactyly, thin upper lip, hypoplastic thumb	Non-consanguinous	No change in *NIPBL*
12	F	5 Year	46, XX	Microcephaly, Arched eyebrows, long eyelashes, thin lips, long philtrum.	Non - consanguinous	No Change in *NIPBL*

plement 3, 85% identity was found after the BLAST for 329 nucleotide between these introns. Thus the researchers hypothesize that the recombination event may have occurred in between intons 11 and 34 where sequence identity exists. This region between introns 11 and 34 may loop out and thus lead to partial gene deletion in proband 2 (Fig. 2). Deletion of one or few exons usually shows mild phenotype except for deletion of exon 32. The deletion of exon 32 results in a more severe phenotype presumably because the loss of this exon, encoding a portion of the H3 repeat of HEAT domain, the ability of affects *NIPBL* to interact with other proteins (Linda et al. 2013). In the present study deletion of exon 32 was observed in both probands. Most deletions have been predicted to result in haploinsufficiency due to heterozygous loss-of-function mutations, which may result in a more severe CdLS phenotype (Pehlivan et al. 2012). As reported in OMIM (122470); CdLS-1 is associated with heterozygous *NIPBL* gene (OMIM#608667) deletion. Further the genotype and phenotype correlation of the two probands where large deletions were found showed the similar phenotypic features (as mentioned earlier) like the other CdLS probands. These observations suggested that the other probands may contain some point mutation in *NIPBL* which are not covered in MLPA probeset or there may be a mutation in some other CdLS causing gene. To date there appear to be no reports on the complete *NIPBL* gene deletion. This study reports the complete and partial heterozygous *NIPBL* gene deletion for the first time in Indian CdLS.

CONCLUSION

In the present study, the researchers have found a partial and complete heterozygous *NIPBL* deletion in two CdLS probands. These mutations are 'de novo' as the parents did not show any NIPBL rearrangement. The researchers are reporting here, the first case of complete *NIPBL* gene deletion in heterozygous manner from India.

RECOMMENDATIONS

The *NIPBL* deleted in heterozygous manner causes CdLS. MLPA can find large reaarangements in *NIPBL* which is more commonly seen in CdLS. Thus, the prenatal screening of foetus for *NIPBL* with MLPA method is highly adviced in CdLS positive families.

ACKNOWLEDGEMENTS

Grant Sponsored by University Grants Commission (UGC), New Delhi, India. (File no. 41-833/2012 (SR). The researcher are very thankful to University Grants Commission (UGC) F. No. 41-833/2012 (SR) for funding, to the parents and patients for giving blood samples and clinical information generously and to Department of Chemistry, Savitribai Phule Pune University for providing infrastructure.

REFERENCES

Bhuiyan ZA, Stewart H, Redeker EJ, Mannens MM, Hennekam RC 2007. Large genomic rearrangements in *NIPBL* are infrequent in Cornelia de Lange syndrome. *Eur J Hum Genet,* 15: 505-508.

Boyle MI, Jespersgaard C, Brøndum-Nielsen, Bisgaard AM, Tümer Z 2015. Cornelia de Lange Syndrome. *Clin Genet,* 88: 1-12.

Cheng YW, Tan CA, Minor A, Arndt K, Wysinger L et al. 2014. Copy number analysis of *NIPBL* in a cohort of 510 patients reveals rare copy number variants and a mosaic deletion. *Mol Genet Genomic Med,* 2: 115-123.

Gervasini C, Parenti I, Picinelli C, Azzollini J, Masciadri M et al. 2013. Molecular characterization of a mosaic *NIPBL* deletion in a Cornelia de Lange patient with severe phenotype. *Eur J Med Genet,* 56: 138-143.

Gillis L A, McCallum J, Kaur M, DeScipio C, Yaeger D et al. 2004. *NIPBL* mutational analysis in 120 individuals with Cornelia de Lange syndrome and evaluation of genotype-phenotype correlations. *Am J Hum Genet,* 75: 610-623.

Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA et al. 2004. Cornelia de Lange syndrome is caused by mutations in *NIPBL*, the human homolog of Drosophila melanogaster Nipped-B. *Nat Genet,* 36: 631-635.

Linda M, Francesco C, Valentina Q, Krantz ID, Antonio M 2013. Mutation spectrum and genotype-phenotype correlation in Cornelia de Lange Syndrome. *Hum Mutat,* 34: 10.

Parisi L, Di Flippo T, Roccella M 2015. Behavioral phenotype and autism spectrum disorder in Cornelia de Lange Syndrome. *Ment Illn,* 7: 5988.

Pehlivan D, Hullings M, Carvalho CM, Gonzaga-Jauregui CG, Loy E et al. 2012. *NIPBL* rearrangements in Cornelia de Lange syndrome: Evidence for replicative mechanism and genotype-phenotype correlation. *Genet Med,* 14: 313-322.

Ratajska M, Wierzba J, Pehlivan D, Xia Z, Brundage EK et al. 2010. Cornelia de Lange syndrome case due to genomic rearrangements including *NIPBL*. *Eur J Med Genet,* 53: 378-382.

Rohatgi S, Clark D, Kline AD, Jackson LG, Pie J 2010. Facial diagnosis of mild and variant CdLS: Insights from a dysmorphologist survey. *Am J Med Genet A,* 152A: 1641-1653.

Russo S, Masciadri M, Gervasini C, Azzollini J, Cereda A et al. 2012. Intragenic and large *NIPBL* rearrangements revealed by MLPA in Cornelia de Lange patients. *Eur J Hum Genet*, 20: 734-741.

Teresa-Rodrigo ME, Eckhold J, Puisac B, Pozojevic J, Parenti I et al. 2016. Identification and functional characterization of two intronic *NIPBL* mutations in two patients with Cornelia de Lange Syndrome. *Biomed Res Int*, 8742939.

Paper received for publication on August 2015
Paper accepted for publication on May 2016

Supplement 1: RT PCT Primer details

Locus NIPBL	*Primer Name*	*5'-3'SEQ*	*Size of Amplicon (bp)*
5'UTR	5UF	CCAAAGGTTGGGTTCTTATTCG	130
	5UR	GCGATGATGCCCGTTTTAAATG	
EXON 11	11EF	CCTGAACTCCTGGCAGAAAT	83
	11ER	TTAACTGTGCTACGCTTGCG	
EXON 16	16EF	CTGTGAAAGTCTTAAATATCTTGGAGA	60
	16ER	AAGTGGAAAGCTTTGACCCATC	
EXON 22	22EF	TGTCATTACTAACTCTTATGAAACAGC	63
	22ER	GAAGATGGAGAGGAAGTTTTGGG	
EXON 28	28EF	TCGAACAAAAGCCATGAAGTGT	61
	28ER	CCTTGCTAGAATACTGGGGTCT	
EXON 34	34EF	TGGTAGCTTGCATAACCACTT	83
	34ER	TGGTTGCATAGTCATTGCATGT	
3'UTR	3UF	TGTTGTCCAGCAGAAACAGA	180
	3UR	CCGCTGCTAGGTTAAACTCC	

Source: Author

Supplement 2: ΔΔCt Results

Sample	5'UTR	EXON11	EXON16	EXON22	EXON28	EXON34	3'UTR
C1- Control	25.7090	22.3110	23.3390	22.9990	22.8390	21.5790	21.8620
GAPDH	20.1020	20.1020	20.1020	20.4090	20.1020	20.1020	20.1020
dCt	5.6070	2.2090	3.2370	2.5900	2.7370	1.4770	1.7600
ddCt	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RQ	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
P1 – Patient	30.6400	23.6340	24.2380	23.8430	23.7290	22.6400	22.5360
GAPDH	20.1120	20.1120	20.1120	20.1120	20.1120	20.1120	20.1120
dCt	10.5280	3.5220	4.1260	3.7310	3.6170	2.5280	2.4240
ddCt	4.9210	1.3130	0.8890	1.1410	0.8800	1.0510	0.6640
RQ	0.0330	0.4025	0.5400	0.4534	0.5434	0.4826	0.6311
P2- Patient	23.4200	25.1650	24.1960	23.2720	23.7960	23.0740	21.8140
GAPDH	20.6240	20.6240	20.6240	20.6240	20.6240	20.6240	20.6240
dCt	2.7960	4.5410	3.5720	2.6480	3.1720	2.4500	1.1900
ddCt	-2.8110	2.3320	0.3350	0.0580	0.4350	0.9730	-0.5700
RQ	7.0177	0.1986	0.7928	0.9606	0.7397	0.5094	1.4845

Source: Author

Supplement 3: Result for Nucleotide Sequence Alignment by BLAST tool.

Score	Expect	Identities	Gaps	Strand
326 bits(176)	3e-91	279/329(85%)	6/329(1%)	Plus/Minus

```
Query  6434  atttatttatt-tatttatttatttattGAGATGGAGTCTCGCTGTGTTGCCCAGGCTGG  6492
             ||||  ||  |||  |  |||  |||  |||  ||||||   ||||||  |||  ||||||||||||||||
Sbjct  4816  ATTTTTTAATTGTCTTT-TTTTTTTTTTGAGACAGAGTCTTGCTCTGTTGCCCAGGCTGG  4758

Query  6493  AGTGCAGTGGCGTGATCTTGGCTCACTGCAGACTCCGCCTTCCGGGCTCATGCCATTCTC  6552
             ||||||||||| ||||||||||||||||||   |||  ||||  |||||  |||  ||  |  | |
Sbjct  4757  AGTGCAGTGGCATGATCTTGGCTCACTGCAACCTCTGCCTCCCGGGTTCAAGC-AACCCC  4699

Query  6553  CTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCACCCGCCACCATGCCTGGCTAATTT  6612
             |||  |||||||  ||||||||||||||||  |||||||  |||  ||||||||||||||||||||
Sbjct  4698  CTGACTCAGCCACCCGAGTAGCTGGGATTACAGGCGCCCACCACCATGCCTGGCTAATTT  4639

Query  6613  TTGTATTTTTAGTAGAGACGGGGTTTCACTGTGTTAGCCAGGATGGTCTCGATCTCCTGA  6672
             ||||||||||||||||||||||||||||||  |  ||  ||||||  ||||||  |  |||  ||
Sbjct  4638  TTGTATTTTTAGTAGAGACGGGGTTTCACCATCTTGGCCAGGCTGGTCTAGGACTCACGA  4579

Query  6673  CCTCGTGATCCACCCACCTCGGCCTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACTAC  6732
             ||||||||||||||  ||||||||||||||||||||||||||||||||||||||||||  |||  |
Sbjct  4578  CCTCGTGATCCACCTGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGTGTGAGAAACTGC  4519

Query  6733  GCCTGGCCCCTTTATTTCTTATCTTTGTA  6761
             |  |  ||||   ||  ||||  |  ||  ||||||
Sbjct  4518  GTCCGGCC--TTAATTT-TCATTTTTGTA  4493
```

Source: Created by Author by using NCBI BLAST tool