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Letter to the Editor

Somatic mosaicism in a Cornelia de Lange syndrome patient with *NIPBL* mutation identified by different next generation sequencing approaches

To the Editor:

Cornelia de Lange Syndrome (CdLS) is an autosomal dominant (*NIPBL*, *SMC3* and *RAD21*) or X-linked (*SMC1A* and *HDAC8*) congenital disorder, characterized by distinctive craniofacial appearance, growth retardation, intellectual disability and limb malformations (1). Currently, mutations in about 70% of the patients studied have been identified (1). However, recent studies have found limitations in mutation detection efficiency by classical sequencing approaches using DNA derived from peripheral blood samples (2). Thus, Huisman et al. identified *NIPBL* mutations in buccal epithelial cells of patients with classic CdLS phenotype who were previously negative by classical sequencing approaches using DNA of blood leukocytes (3). This finding supported the hypothesis that somatic mosaicism might contribute to the wide clinical expressivity and to explain the non-detectability of mutations in CdLS patients by classical molecular testing (4, 5).

Here we report on a patient with classic CdLS (Fig. 1a i–iii) in whom previous molecular analyses of all known CdLS genes by Sanger sequencing did not identify any mutation. Interestingly, subsequent exome and panel sequencing approaches were able to disclose a somatic mosaicism for a mutation in *NIPBL*. In addition, pyrosequencing assays were performed to precisely quantify the percentage of the mutated allele in DNA samples from multiple tissues.

Sequencing analyses of the five genes associated with CdLS were performed on DNA from blood leukocytes, but Sanger sequencing failed to identify pathological variants in these genes. Thereafter exome sequencing was performed in the affected individual. This technique found a *de novo* heterozygous missense mutation in exon 39 of the *NIPBL* gene [c.6647A > C; p.(Tyr2216Ser)]. The mutated allele was present in 5 of 21 (24%) reads in exome sequencing and a weak signal was visible in the chromatogram of the second test of direct sequencing (Fig. 1b). Together, these data prompted us to suspect mosaicism for this *NIPBL* mutation.

Additionally genomic DNA was sequenced using an Ion Ampliseq™ (Life Technologies, Grand Island, NY) custom designed gene panel to amplify the coding exons of 16 genes including the known CdLS genes. After data analysis and database filtering using the Torrent Variant Caller and SEQNEXT software in parallel, only the c.6647A > C mutation in exon 39 of the *NIPBL* gene was identified (Fig. 1c).

Subsequently, pyrosequencing was used for detailed quantification comparing the wild-type (wt) and mutant allele in genomic DNA samples from different tissues. The results revealed the tissue-specific levels of heterozygosity (Fig. 1b).

It has been suggested that a significant number of CdLS individuals with negative standard molecular analyses may have somatic mosaicism (3). Among the viable explanations are, the limited sensitivity of Sanger sequencing for detection of somatic mosaicism (lowest level of identifiable mosaicism is between 10% and 20%) and more recently Huisman et al. has proposed the loss of mutations in leukocytes due to reversion and leukocyte specific selection against mutant cells (2, 3). Interestingly, somatic mosaicism in other CdLS causative genes has not been reported so far. Although no elucidation can be stated here, this finding is probably based on the limited diversity of tissues investigated but possibly could also be explained by a *NIPBL* specific molecular mechanism that is currently unknown.

Currently the exome sequencing as well as gene panel enriched sequencing, has been described as very sensitive tools for mosaic mutation detection. Whole exome sequencing has emerged as a novel technique for the identification of mutations even for diagnostic purposes. Although the last years show a dramatic decrease in cost of the exome sequencing, subsequent bioinformatics analysis are limiting for most of the diagnostic laboratories (2). Molecular analysis by gene panel specific sequencing represents a less cost intensive alternative for gene specific sequencing with a very high coverage, and will allow the identification of somatic mosaicism as shown here.

On the basis of the data available and the findings presented here, we agree with the recommendation made by Huisman et al., in any patient with clinical

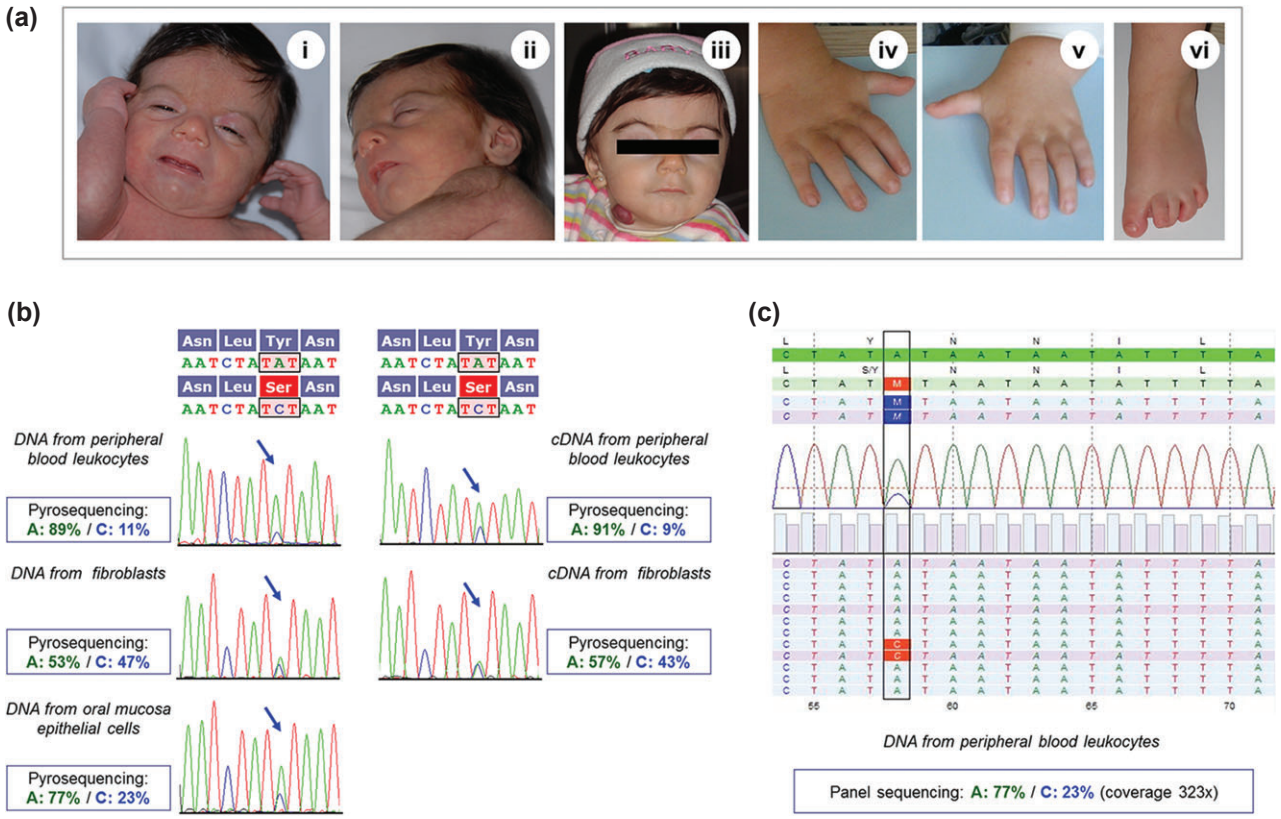

Fig. 1. (a) Phenotype of the patient. (b) Partial electropherograms of exon 39 of the *NIPBL* gene and pyrosequencing results. (c) High coverage gene panel sequencing on genomic DNA from patient's peripheral blood leukocytes, that could identify the missense mutation.

diagnosis of CdLS, the molecular study should begin with the analysis of *NIPBL* in DNA derived from buccal epithelial cells (3). Nevertheless a gene panel enriched sequencing analysis, when available, should be considered for diagnostic purposes.

Supporting Information

The following Supporting information is available for this article:

Appendix S1. Material, methods and results details.
Fig. S1. 3-D modeling of Tyr2216Ser substitution in the NIPBL HEAT structure domain.

Additional Supporting information may be found in the online version of this article.

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