

## SHORT COMMUNICATION

# Exome sequencing identifies a *de novo* mutation in HDAC8 associated with Cornelia de Lange syndrome

Lei Feng<sup>1,5</sup>, Daizhan Zhou<sup>2,5</sup>, Zhou Zhang<sup>2</sup>, Yun Liu<sup>3,4</sup> and Yabo Yang<sup>1</sup>

Cornelia de Lange syndrome (CdLS) is a clinically and genetically heterogeneous developmental disorder. The clinical features of CdLS include growth retardation, intellectual disability, limb defects, typical facial dysmorphism and other systemic involvement. Here, we present the clinical and genetic characterization of a sporadic CdLS trio. The proband is a 7-year-old girl with typical CdLS, and both parents are apparently healthy. Whole-exome sequencing of the patient and of both her unaffected parents revealed a previously unobserved *de novo* mutation in exon 6 of the *HDAC8* gene (chrX: 71684483, c.586 A>T; p.M196K). Thus, we have further founded that the p.M196K mutation in *HDAC8* is a relevant causal mutation for CdLS.

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## INTRODUCTION

Cornelia de Lange syndrome (CdLS, MIM #610759, 300882, 300590, 614701 and 122470) is a congenital multisystemic genetic disorder with variable clinical expression.<sup>1</sup> It is characterized by typical facial dysmorphism, growth retardation, intellectual disability, limb defects, ocular disorders and other systemic involvement.<sup>1,2</sup> The worldwide prevalence of CdLS is estimated to be between 1:10 000 and 1:30 000 live births.<sup>3</sup>

Most CdLS cases are due to spontaneous genetic mutations. To date, mutations in three core cohesin subunits (SMC1A, SMC3 and RAD21) and in two cohesin-regulatory proteins (NIPBL and HDAC8) have been identified in CdLS probands.<sup>3</sup> *NIPBL*, which is located on chromosome 5p13 and encodes a cohesin regulatory protein, is the most common genetic cause of CdLS. Heterozygous mutations in *NIPBL* are found in at least 60% of CdLS probands.<sup>4,5</sup> Mutations in the core cohesion complex proteins, encoded by the *SMC1A*, *SMC3* and *RAD21* genes, together account for ~5% of subjects, often with atypical CdLS features.<sup>6–8</sup> Recently, mutations in *HDAC8* have been found to be associated with CdLS. The *HDAC8* gene encodes the lysine deacetylase for the cohesin subunit SMC3 in humans. Mutations in *HDAC8* are found in ~5% of individuals with CdLS.<sup>9,10</sup>

Here, we report a patient with typical CdLS possessing a previously unreported *de novo* *HDAC8* mutation that was identified via trio exome sequencing.

## MATERIALS AND METHODS

### Case report

A 7-year-old girl was brought to our eye clinic with a confirmed diagnosis of CdLS for ocular anomalies. She was the firstborn child of healthy nonconsanguineous parents, and her family history was negative regarding genetic disease. She was delivered at 41 weeks of gestational age by cesarean section because of maternal problems. Her birth weight was 3.5 kg, birth length 52 cm and head circumference 35 cm at 33 days after birth. She had a documented history of developmental delay and exhibited characteristic facial features: synophrys, long curly eyelashes, a depressed nasal bridge, anteverted nares (Figure 1a) and widely spaced teeth. Her hands and feet were small, and she exhibited brachydactyly in her forefinger, midfinger and pinkie finger (Figure 1b). Upon ophthalmic examination, cycloplegic refraction demonstrated severe myopia (–10.25D) in the right eye but no refractive error in the left eye. She was also diagnosed with myopia, anisometropia and amblyopia.

### Whole-exome sequencing

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), captured using the Truseq Human All Exome 62 Mb kit (Illumina, San Diego, CA, USA) and sequenced on an Illumina HiSeq2000 (Illumina), generating 2 × 100 bp paired-end reads. Exome data processing, variant calling and variant annotation were performed as previously described.<sup>11</sup> From these variants, we identified putative *de novo* mutations as sites where both parents were homozygous for the reference sequence, while the offspring was

<sup>1</sup>Eye Center, The Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, PR China; <sup>2</sup>Bio-X Center, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai, PR China; <sup>3</sup>Institutes of Biomedical Sciences, Fudan University, Shanghai, PR China and <sup>4</sup>Key Laboratory of Molecular Medicine, The Ministry of Education, Department of Biochemistry and Molecular Biology, Fudan University Shanghai Medical College, Shanghai, PR China

<sup>5</sup>These authors contributed equally as first authors.

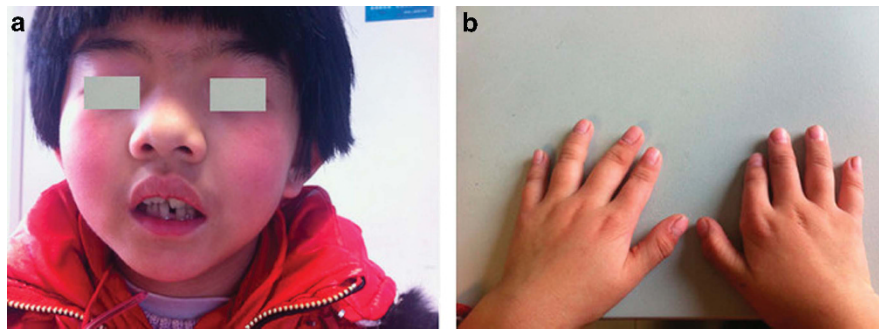
Correspondence: Dr Y Liu, Key Laboratory of Molecular Medicine, Ministry of Education, Department of Biochemistry and Molecular Biology, Fudan University Shanghai Medical College, 303 Mingdao Building, 138 Yixueyuan Road, Shanghai 200032, PR China.

E-mail: superliuyun@gmail.com.

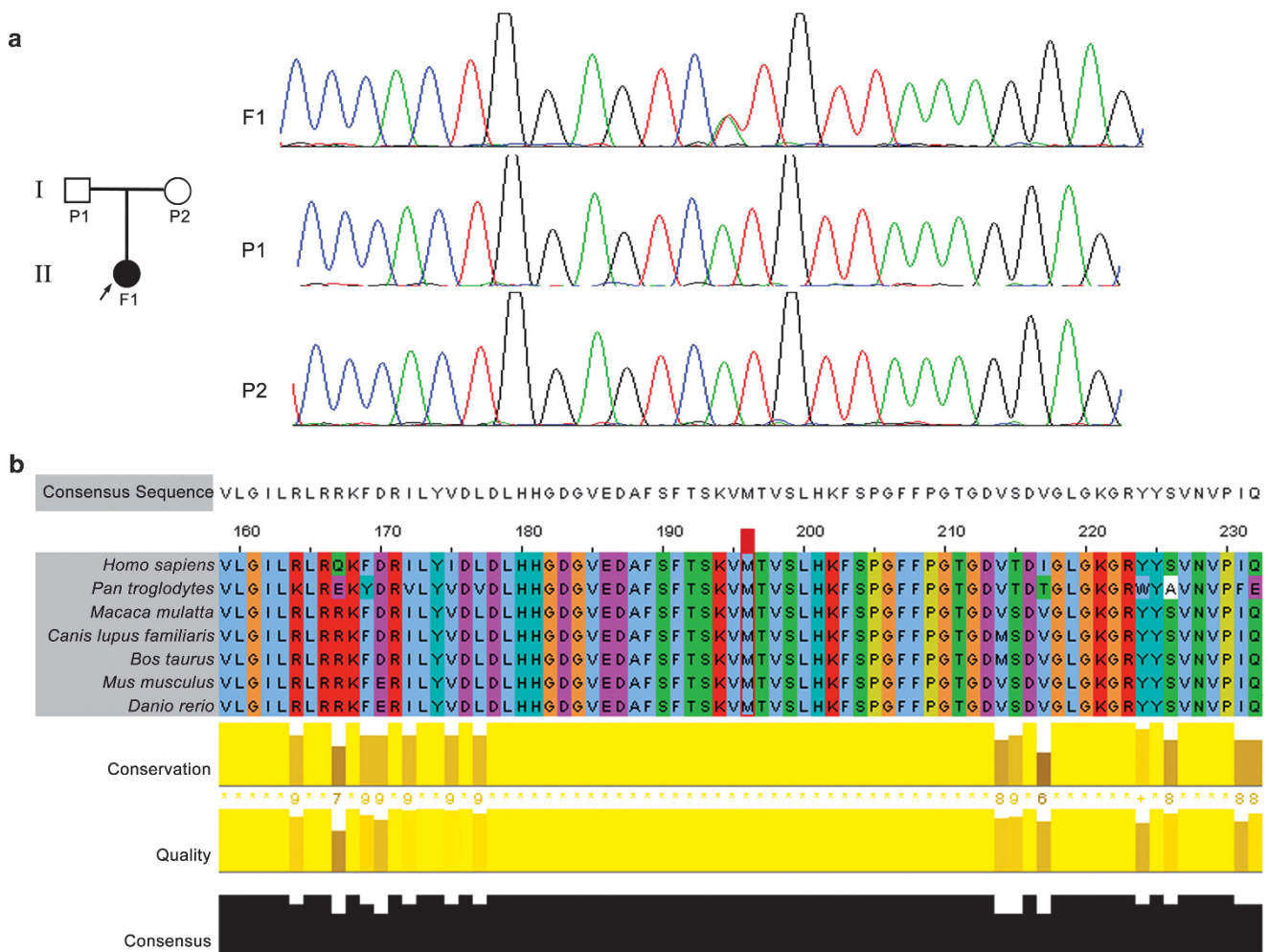
or Professor Y Yang, Eye Center, The Second Affiliated Hospital, College of Medicine, Zhejiang University, No. 88 Jiefang Road, Hangzhou 310009, PR China.

E-mail: yabyang@hotmail.com

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**Figure 1** Features of the proband. (a) Characteristic facial features of the proband, including synophrys, long curly eyelashes, a depressed nasal bridge, anteverted nares and widely spaced teeth. (b) Upper-limb anomalies observed in the patient, including brachydactyly in her forefinger, midfinger and pinkie finger.



**Figure 2** Mutation in HDAC8. (a) Sequence of the *de novo* HDAC8 gene mutation (c.586 A>T, p.M196K) in all subjects in the sporadic CdLS trio. (b) Evolutionary conservation analysis of the *de novo* HDAC8 gene mutation (c.586 A>T, p.M196K).

heterozygous, without considering the genotype depth for each candidate variant.

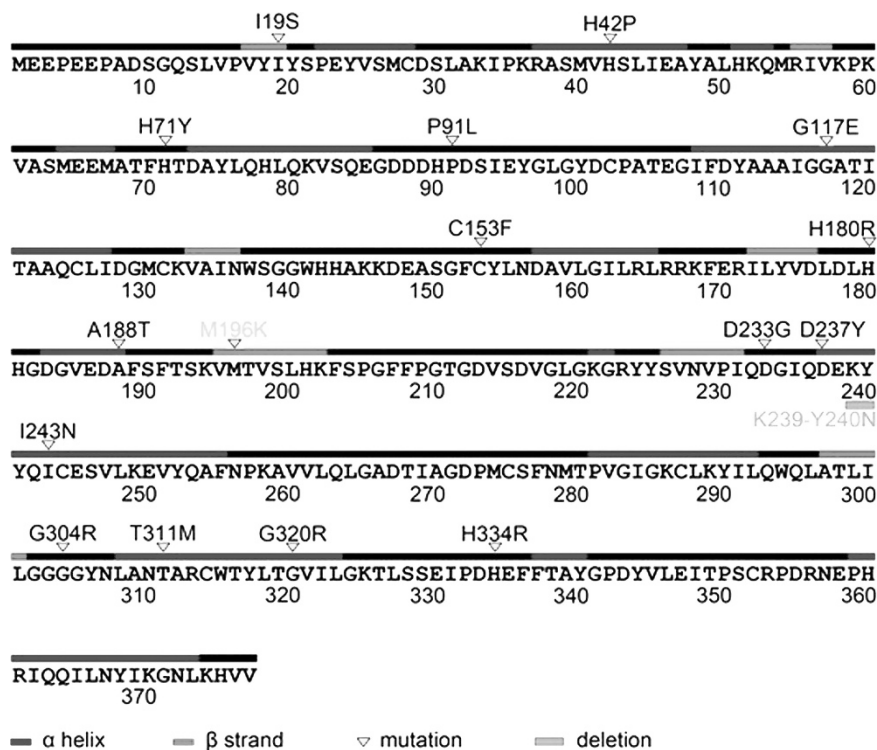
### Sanger sequencing

The putative *de novo* mutations were validated via Sanger sequencing of the carrier and both parents. The PCR primers for the 16 putative *de novo* mutations are summarized in Supplementary Table 1. Sequencing was performed using BigDye Terminator v3.1 cycle sequencing, and the obtained

sequences were analyzed on an ABI 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA).

### Prediction of functional impacts

The pathogenicity of the identified missense mutations was tested using PROVEAN<sup>12</sup> (<http://provean.jcvi.org/index.php>), SIFT<sup>13</sup> (<http://sift.jcvi.org/>) and Mutation Taster<sup>14</sup> (<http://www.mutationtaster.org/>) online software.



**Figure 3** Schematics of protein alterations in HDAC8 detected by the previous and present study. A helix in HDAC8 is colored blue, the  $\beta$  strand in HDAC8 is colored red. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

#### Analysis of evolutionary conservation

Data on HDAC8 orthologous genes were downloaded from the homogene database (<http://www.ncbi.nlm.nih.gov/homogene>). Multiple-sequence alignment was carried out using ClustalW (<http://www.ebi.ac.uk/clustalw>).<sup>15</sup>

#### RESULTS

For each sample, >8.5 Gbp of raw data were obtained through exome sequencing, showing a mean coverage of >50-fold (see Supplementary Table 2), and a total of 143 177 SNVs were detected. The exome sequencing data covered all exons of known CdLS-related genes (SMC1A, SMC3, RAD21, NIPBL and HDAC8), and no known functional mutations were identified in these genes (see Supplementary Table 3). For *de novo* mutation filtering, 16 putative *de novo* mutations were identified according to the standard that both parents were homozygous for the reference sequence and the offspring was heterozygous (see Supplementary Table 4). Only two sites were confirmed to be totally consistent with the exome sequencing data of each family member (*KPRP*, rs17612167; *HDAC8*, c.586 A>T, p.M196K) (Figure 2a and Supplementary Figure 1). All the other 14 variants were unconfirmed because all these variants showed homozygosity in the exome sequencing data of one of the parents and heterozygosity in Sanger sequencing. The single-nucleotide polymorphism (SNP) rs17612167 (A/T) was further excluded because it is a common SNP in the Chinese population (minor allele frequency (MAF) = 0.189). Moreover, by using Sanger Sequencing, the p.M196K mutation in HDAC8 was detected to be absent in 250 among the unrelated Chinese control population. Therefore, we further confirmed that the *de novo* mutation (c.586 A>T, p.M196K) in HDAC8 was the causal mutation in the proband of this CdLS trio.

In addition, the p.M196K mutation was predicted to be pathogenic by the algorithms of PROVEAN, SIFT and Mutation Taster (categorized as 'deleterious', 'damaging' and 'disease causing', respectively).

The identified mutation (p.M196K) is highly conserved throughout evolution across species (Figure 2b).

#### DISCUSSION

In this study, we identified two *de novo* mutations (*KPRP*, rs17612167; *HDAC8*, c.586 A>T, p.M196K) in a CdLS trio by both exome sequencing and Sanger sequencing. There are two reasons that rs17612167 was excluded from the candidate causal mutations of CdLS: first, rs17612167 is a common SNP with a MAF of 0.189 in the Chinese population; second, although the A>T base change of rs17612167 variant can result in a p. Q14H mutation in *KPRP* gene, the effect of this mutation was predicted to be moderate on the gene function. Hence, according to the results in the present study, the *de novo* p.M196K mutation in HDAC8 was the most recognized candidate variant associated with CdLS.

Recently, Deardorff *et al.*<sup>10</sup> identified four HDAC8 mutations in six individuals with typical CdLS or an overlapping phenotype. Subsequently, Kaiser *et al.*<sup>9</sup> screened an internationally assembled cohort of 586 patients with typical CdLS and overlapping clinical presentations and found 35 affected individuals whom they identified as having mutations in *HDAC8*. In both studies, the authors found six different chromosomal microdeletions or microduplications, three nonsense mutations, one splice site and 16 different missense mutations (p.I19S, p.H42P, p.H71Y, p.P91L, p.G117E, p.C153F, p.H180R, p.A188T, p.D233G, p.D237Y, p.K239-Y240N, p.I243N, p.G304R, p.T311M, p.G320R and p.H334R) (Figure 3). Thus, this is the first study to identify the *de novo* p.M196K mutation in HDAC8 in a sporadic CdLS patient (Figure 3). HDAC8, which is located on chromosome Xq13.1, encodes a histone deacetylase that deacetylates SMC3, which has been removed from chromatin to establish the cohesiveness of chromatin-loaded cohesin during both prophase and anaphase. Both Deardorff *et al.* and Kaiser *et al.* found that HDAC8

mutations in CdLS patients could cause reduced HDAC8 enzymatic activity. According to the structure of the HDAC protein (Figure 3), most of the CdLS-related mutations detected in the previous studies are located at the  $\alpha$  helix and  $\beta$  strand's second structure region; the p.M196K mutation is just located at a  $\beta$ -strand region of HDAC8. M196 is in a hydrophobic environment, and a mutation resulting in a larger, partially hydrophilic residue might subtly affect the packing interactions. Thus, the identified mutation (p.M196K) may also reduce HDAC8 deacetylase activity, which should be further functionally studied.

In summary, we applied whole-exome sequencing and functional prediction methods in this study and identified a *de novo* mutation (c.586 A>T, p.M196K) in HDAC8 in our sporadic CdLS trio that has not been detected in previous studies. Thus, we have further confirmed that HDAC8 is a relevant gene for CdLS.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)