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A novel mutation in the *PHF8* gene is associated with X-linked mental retardation with cleft lip/cleft palate

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Abstract

Recently, two truncating mutations in the *PHF8* (plant homeodomain finger protein 8) gene have been found to cause X-linked mental retardation associated with cleft lip/cleft palate (CL/P). One of the truncating mutations was found in the original family with Siderius–Hamel CL/P syndrome where only two of the three affected individuals had mental retardation (MR) with CL/P and one individual had mild MR. The second mutation was present in a family with four affected men, three of whom had MR and CL/P, while the fourth individual had mild MR without clefting. Here, we report a novel nonsense mutation (p.K177X) in a male patient who has MR associated with CL/P. The mutation results in a truncated PHF8 protein lacking the Jumonji-like C terminus domain and five nuclear localization signals. Our finding further supports the hypothesis that the PHF8 protein may play an important role in cognitive function and midline formation.

Keywords

cleft lip; cleft palate; PHF8; X-linked mental retardation; XLMR

X-linked mental retardation (XLMR) is a heterogeneous condition affecting approximately 1.6/1000 men, which can be divided into syndromic (MRXS) and non-syndromic (MRX) forms (1,2). To date, 50 genes responsible for MRXS and 25 genes responsible for MRX have been identified (2,3). In several cases, mutations in the same gene are responsible for the MRXS and the MRX forms of XLMR (4–11). Recently, two truncating mutations in the *PHF8* gene have been found to cause XLMR associated with cleft lip/cleft palate (CL/P) (12).

Mutation screening of the *PHF8* gene was performed in a panel of 27 unrelated patients (26 men and 1 woman) with mental retardation (MR), 26 of whom had cleft lip, cleft palate or related phenotypes and the 27th was a member of the MRX81 family, which shows linkage to Xp11.2-Xq12 (13).

A novel nucleotide change (c.529A>T), resulting in a nonsense mutation (p.K177X), was found in a patient with cleft lip. This mutation is in exon 6 of the *PHF8* gene and results in the premature truncation of the protein and the removal of the Jumonji-like C terminus (JmjC) domain and five nuclear localization signals (NLS).

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Materials and methods

Patients and control individuals

The patient panel consisted of 26 unrelated individuals (25 men and 1 woman) with MR associated with cleft lip (2), cleft palate (6), CL/P (9), submucous cleft palate (7) and high palate (2), plus one patient from MRX81 that is linked to Xp11.2-Xq12 (13). The proband from the MRX81 family had MR, but no CL/P. Of the 26 probands without linkage information, 2 had a family history consistent with XLMR, 4 had a family history of MR that was consistent with either XLMR or autosomal MR and 20 had no family history of MR.

The control panel consisted of 261 men [Caucasian (219), African American (39) and Hispanic (3)] and 278 women [Caucasian (198), African American (68), Hispanic (5), Asian (1) and unknown (6)] with normal intelligence and no cleft lip or cleft palate. Of the 261 men, 173 were college students and 88 had a known mutation in a non-X-linked gene. Of the 278 women, 187 were college students and 91 were normal mothers from a non-X-linked study.

Mutation screening

Mutation analysis of the *PHF8* gene was performed by incorporation PCR single strand conformation polymorphism (IPS) and by direct sequencing (14). Amplicons for exons 1, 2, 13, 18, and 21 were greater than 350 bp and were analyzed by direct sequencing. The remaining 17 exons were screened by IPS. The sequence of the primer pairs used for screening were the same as that described by Laumonnier et al. (12), except the primers used for sequencing were synthesized with M13-tails (M13F: 5′-GTAAAACGACGGCCAG-3′ and M13R: 5′-GTAAAACGACGGCCAG-3′).

IPS and sequence analysis

One hundred nanograms of genomic DNA was amplified in a total volume of 10 μl containing 1× GoTaq™ buffer (Promega, Madison, WI), 1 μM of each primer, 50 μM of deoxynucleoside triphosphates and 0.05 μCi of α^{32}P dCTP (3000 Ci/mmole; PerkinElmer™ Life Sciences, Waltham, MA) (14). One unit of GoTaq™ DNA polymerase (Promega) and 0.2 μg of TaqStart antibody (Clontech, Mountain View, CA) were incubated at 22°C for 5 min before addition to the polymerase chain reaction (PCR) mixture. Amplification was performed in a PTC-200 thermocycler (Bio-Rad, Hercules, CA) in the following conditions: 95°C for 5 min; 30 cycles of 95°C for 30 s, 65°C for 30 s and 72°C for 30 s; and 7 min extension at 72°C. Following PCR, 8 μl of the IPS loading dye (95% formamide, 10 mM NaOH, 0.25% bromophenol blue, and 0.025% xylene cyanol) was added. The samples were denatured at 96°C for 5 min and resolved on a 0.5× MDE gel (FMC, Philadelphia, PA) prepared in 0.6× TBE (53.4 mM Tris, 53.4 mM borate and 1.2 mM EDTA). The gel was run at 8 W for 16 h at room temperature. After the run, the gel was dried and the radioactive signal was visualized using BIO-MAX™ MS films (Eastman Kodak, New Haven, CT). DNA fragments exhibiting an abnormal pattern on the IPS gel were sequenced in both directions.

For sequencing, 100 ng of genomic DNA was amplified in a total volume of 50 μl using the M13-tailed primers. PCR products were purified using the GFX™ PCR purification kit (Amersham Biosciences, Pittsburgh, PA). The sequencing reaction was performed using M13-primers in both directions on the MegaBACE™ (Amersham Biosciences) using the DYEnamic™ dye terminator kit according to the manufacturer's protocol. The alignment and analysis of the sequence were performed by using the DNASTAR program (Lasergene, Madison, WI).

Allele-specific PCR and polymorphism study

The c.529A>T alteration did not create or destroy any restriction enzymatic site. Therefore, an allele-specific forward primer was designed to test the carrier status of the mother and to screen the normal control samples. The forward primer was designed as follows: KIEx6 MSPF primer: 5′-CAAGATGAAGCTTGGTGATTTTGAGT-3′. The last base of the primer was T instead of A to make it specific for the c.529A>T alteration. Furthermore, by changing the third base from the 3′ end from T to an A, the primer was made more specific. The normal sequence in this region reads as follows: 5′-CAAGATGAAGCTTGGTGATTTTGTGA-3′. The PRIMER3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used to select a reverse primer, KIEx6 MSPR (5′-TCAGGCACTTTGTCAGGTTTT-3′), about 137 base pairs away. The 'T_m' of the reverse primer was 4°C lower than that of the forward primer. The above primer pairs were used in a temperature gradient (55–65°C) PCR in a thermocycler (Bio-Rad) using the DNA from the patient carrying the c.529A>T change and a control individual as template DNA. The annealing temperature of 60°C was chosen because a 137 bp amplification product was seen only in the patient and not in the control individual. To ensure that all the DNAs from the normal individuals were amplifying, the above primers were multiplexed with primer set from a control gene (MID1IP1 AF-M13F/R), which generates a PCR product of 403 bp.

A polymorphism study for the c.2720G>A alteration was performed by allele-specific PCR (AS-PCR) using the following primers – KIAA1111-Ex21MSF: 5′ AAGGAGGTAGAACAGCCGCA 3′ and KIAA1111-Ex21MSR: 5′ AGCGGTGTACTCATGGTCAG 3′.

Results

We have screened 26 unrelated individuals with MR associated with cleft lip, cleft palate or related clinical phenotypes and one patient from a family with linkage to Xp11.2-q12 for mutations in the *PHF8* gene. The entire coding region and adjacent splice sites were screened either by IPS or by direct sequencing. An abnormal migration pattern was observed on the IPS gel in proband CMS12076 for exon 6 (Fig. 1). Sequence analysis showed an A to T substitution at position 529 (c.529A>T). This substitution resulted in a nonsense mutation (p.K177X). This mutation causes a premature truncation of the PHF8 protein, resulting in a protein that lacks the JmjC domain and five NLS. Sequencing of exon 6 in the proband's mother showed that she is not a carrier of the mutation and additional testing confirmed her biological relationship to the child (data not shown). Therefore, this mutation is a *de novo* event. Seven hundred and twenty-seven normal X chromosomes (243 men and 242 women) were analyzed for the c. 529A>T alteration by AS-PCR, and none of them were found to carry this alteration indicating that it is not a rare polymorphism.

Sequencing of exon 21 in another proband (CMS6748) led to the identification of a change from G to A at position 2720 (c.2720G>A). This alteration resulted in the substitution of an arginine with a histidine residue at position 907 (p.R907H). As this alteration was not reported in the single nucleotide polymorphism database, a polymorphism study was performed by AS-PCR to investigate if the alteration could be pathogenic. A total of 817 normal X chromosomes (261 men and 278 women) were analyzed and two of them (1 man and 1 woman) carried the alteration. Therefore, the c.2720G>A alteration is a polymorphism found in 0.2% (2/817) of the normal X chromosomes.

The panel of patients screened for mutations in the *PHF8* gene also included a proband from the MRX81 family, which shows linkage to this region. No mutation in the coding sequence of the *PHF8* gene was found in this individual.

Discussion

The *PHF8* gene, located in Xp11.21, has recently been found to cause XLMR associated with CL/P (12). The gene is composed of 22 exons with an open-reading frame of 3075 bp. It encodes a protein of 1024 amino acids and contains a plant homeodomain (PHD) zinc finger domain (amino acid positions 7–53) and a JmjC domain (amino acid positions 195–294). It also has six NLS. The *PHF8* transcript is ubiquitously expressed with a relatively high expression in the embryonic and early post-natal stages of brain development (12). The PHF8 protein is a new member of the PHD finger protein family. These proteins function as transcriptional regulators affecting eukaryotic gene expression by chromatin remodeling. The two truncating mutations previously reported in this gene are present either in or after the JmjC domain (12).

In this study, we identified a novel *de novo* nonsense mutation (p.K177X) that results in the premature truncation of the PHF8 protein and the loss of the JmjC domain and five NLS. Unfortunately, because no cell line was available from the patient, it was not possible to determine if the truncating mutation resulted in nonsense-mediated decay of the altered transcript. The clinical phenotype of the proband (CMS12076) consisted of cleft lip, cleft palate, MR and microcephaly.

The observation of this mutation further confirms that *PHF8* gene is associated with XLMR with CL/P. It also supports the hypothesis that the PHF8 protein may be involved in the development of cognitive function and midline formation.

The two families with *PHF8* mutations reported by Laumonnier et al. (12) had at least one affected individual, in whom MR without clefting was the clinical finding. One possible explanation for this could be reduced penetrance in these families. Based on this possibility, we decided to screen family MRX81 although the screening of other linked families by Laumonnier et al. (12) had been negative. Our screening detected no alteration in the *PHF8* gene. However, this gene cannot be excluded as causative for this family because only the coding sequence of the gene was screened.

In the present study, 1 of 25 of the male individuals with MR and oral cleft-related phenotypes showed a mutation in the *PHF8* gene. Although the yield of mutations identified was relatively low, finding a mutation could be of considerable significance to the families concerned in terms of genetic counseling and the identification of other family members potentially at risk, such as carrier women. Thus, we consider that, based on our findings, men with MR and cleft lip or cleft palate and patients with a family history of XLMR with cleft lip or cleft palate should be considered for screening for mutations in the *PHF8* gene.

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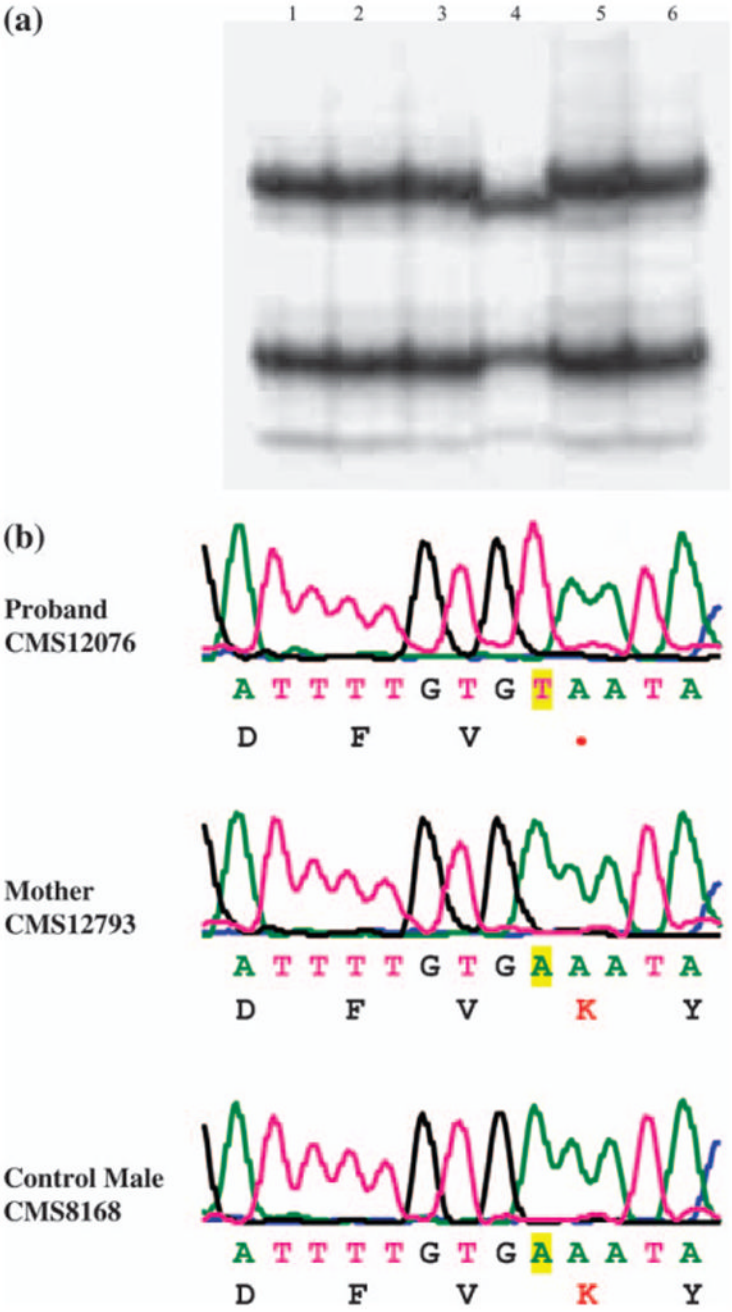

Fig. 1.

Mutation analysis of the *PHF8* gene. (**a**) An incorporation PCR single strand conformation polymorphism gel of exon 6 of the *PHF8* gene showing the abnormal migration pattern in the proband CMS12076. Lanes 1, 2, 3, 5, and 6: Probands with cleft lip with or without cleft palate. Lane 4: Proband CMS12076. (**b**) Sequence analysis of exon 6 of the *PHF8* gene from CMS12096, his mother, CMS12793, and a control male, CMS8168. The A>T substitution (c. 529A>T) is highlighted in yellow and the amino acid change (p.K177X) is shown in red.