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A Novel Nonsense CDK5RAP2 Mutation in a Somali Child With Primary Microcephaly and Sensorineural Hearing Loss

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Primary microcephaly is a genetically heterogeneous condition characterized by reduced head circumference (−3 SDS or more) and mild-to-moderate learning disability. Here, we describe clinical and molecular investigations of a microcephalic child with sensorineural hearing loss. Although consanguinity was unreported initially, detection of 13.7 Mb of copy neutral loss of heterozygosity (cnLOH) on chromosome 9 implicated the CDK5RAP2 gene. Targeted sequencing identified a homozygous E234X mutation, only the third mutation to be described in CDK5RAP2, the first in an individual of non-Pakistani descent. Sensorineural hearing loss is not generally considered to be consistent with autosomal recessive microcephaly and therefore it seems likely that the deafness in this individual is caused by the co-occurrence of a further gene mutation, independent of CDK5RAP2. Nevertheless, further detailed clinical descriptions of rare CDK5RAP2 patients, including hearing assessments will be needed to resolve fully the phenotypic range associated with mutations in this gene. This study also highlights the utility of SNP-array testing to guide disease gene identification where an autosomal recessive condition is plausible.

Key words: CDK5RAP2; MCPH3; microcephaly; deafness; SNP array

INTRODUCTION

Mutations in ASPM and WDR62 account for the majority of patients with primary microcephaly [Bond et al., 2002; Nicholas et al., 2010]. Five other causative genes are known but have only been reported in a few individuals (for recent review see [Mahmood et al., 2011]). A recent study suggests CEP135 may be responsible for an eighth microcephaly locus on chromosome 4q12 [Hussain et al., 2012]. The fact that the majority of these microcephaly genes were mapped using consanguineous kindreds from Pakistan highlights the importance of the autozygosity mapping strategy in the

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understanding of this rare condition. However, as a consequence, it is unclear what clinical relevance these genes have in other populations.

CDK5RAP2 is responsible for one of the rarest forms of primary microcephaly (MCPH3), with only two different mutations published to date, in three independent families originating from northern Pakistan [Bond et al., 2005; Hassan et al., 2007] (Table I). A recent study of primary microcephaly patients from 112 consanguineous Iranian pedigrees did not show any linkage to this locus, indicating that CDK5RAP2 mutations may be rare even in consanguineous families [Darvish et al., 2010]. In common with other microcephaly genes, CDK5RAP2 appears to be involved with centrosomal function: an inversion mutation in mice leads to abnormal spindle poles, spontaneous aneuploidy and neurogenic defects, resulting in microcephaly in some strains [Lizarraga et al., 2010]. There is also evidence that CDK5RAP2 has undergone positive selection and may have been a genetic factor leading to the evolutionary increase in human brain size [Montgomery et al., 2011].

Here, we describe an individual of Somali descent with primary microcephaly and sensorineural hearing loss. Although the family was initially reported to be non-consanguineous, detection of two regions of copy neutral loss of heterozygosity (cnLOH) led to further molecular investigation of the CDK5RAP2 gene.

**CLINICAL REPORT**

The patient was born to Somali parents at 40 weeks gestation, weighing 2.37 kg (−2.39 SDS) with an occipito-frontal head circumference (OFC) of 30 cm (−3.68 SDS) and length of 43 cm (−3.68 SDS). Auditory brainstem response testing at 3 days of life was normal. Although there were no neonatal problems, she experienced significant gastro-oesophageal reflux during the first 4 months and was fed via a gastrostomy (aged 9 months). Early developmental milestones were delayed. A brain MRI performed at 15 months showed microcephaly but no structural abnormalities (Fig. 1A). Development was assessed aged 3 using the Vineland Adaptive Behaviour scale: function was between the 1st and 6th percentiles for various domains of development with social and daily living skills on the 6th and 5th centiles, respectively. Moderate-to-severe bilateral sensorineural hearing loss was diagnosed at 3 years, 10 months. Molecular testing of GJB2 and GJB6 was normal. Although language skills progressed following the introduction of hearing aids, at 6 years she communicates largely by gestures. She is described as a happy, sociable child with no reported behavioral problems. At 6 years, growth parameters were: weight 17.6 kg (−1.12 SDS), height 107.5 cm (−1.61 SDS), and OFC 41.5 cm (−8.91 SDS), with a sloping forehead (Fig. 1B). There was mild joint laxity, hypotonia, and decreased muscle bulk. No maternal or environmental causes were identified that may have contributed to the microcephaly or deafness. Given the absence of associated malformations or neurological deficits, a clinical diagnosis of primary microcephaly was made. ASPM was sequenced but no mutations were identified.

**MATERIALS AND METHODS**

### Genotyping

With appropriate ethical approval and consent, DNA from the patient and mother were genotyped using the CytoSNP-12 v2.1 array (Illumina Inc., San Diego, CA). DNA from the patient’s father could not be obtained. Data analysis was performed with Nexus v5.1 Discovery Edition (BioDiscovery, Hawthorne, CA) and GenomeStudio V2009.2 (Illumina).

### Sequencing

All 38 exons and intron–exon boundaries of the CDK5RAP2 gene were amplified using the FastStart Taq DNA polymerase (Roche, Burgess Hill, UK) and primers from a previous study [Hassan et al., 2007]. PCR products were purified using exonuclease I (NEB, Ipswich, MA) and shrimp alkaline phosphatase (USB, Cleveland, OH). Bidirectional Sanger sequencing was then performed using BigDye chemistry (Applied Biosystems, Foster City, CA) and run on a 3730xl DNA Analyzer (Applied Biosystems).

**RESULTS**

The genome-wide SNP analysis did not reveal any copy number variants (CNVs) in the patient, other than those noted already in the Database of Genomic Variants. However, two large tracts of cnLOH involving chr7p15.2–p21.3 and chr9q33.1–q34.12 (Fig. 1C) were noted of greater than 5 Mb. These were not observed in the mother. Co-occurrence of two independent cnLOH regions led us to consider an unreported consanguineous parental relationship. The coefficient of inbreeding (fraction of the genome showing cnLOH) was estimated to be ~1/95, consistent with parents who are second-cousins or second-cousins-once-removed. Subsequent re-evaluation of family-history established that parents were indeed second-cousins (Fig. 1D).

Of the eight known microcephaly genes, one (CDK5RAP2) is situated within the candidate region on chr9q33.1–q34.12. Targeted sequencing of this gene revealed three homozygous coding changes. Two of these were common missense polymorphisms (rs4837768 and rs4836822), whereas the third was a novel nucleotide transversion in exon 8 (c.700G>T, NM_018249.4) that predicts a premature stop at codon 234 (Fig. 1E). The variant was heterozygous in the mother but was not seen in >5,000 Caucasian and African-American samples in the Exome Variant Server (http://evs.gs.washington.edu/EVS/; v.0.0.10).

We next considered the possibility that one of the cnLOH loci might harbor a second rare mutation, in a deafness gene. In addition to the large regions of cnLOH on chr7p and chr9q, five other cnLOH loci of intermediate size (2–5 Mb) were identified. These were located at 3p24.3, 4q26, 5q21.1, 16p11.2 (pericentromeric region), and Xq22.3. We searched these seven loci for genes noted in OMIM as being linked with deafness and although we identified four genes (DFNA5, HOXA2, COL4A5, and PRPS1), none of these appeared to be a likely candidate based on their inheritance pattern or because the phenotype of our patient did not match.
<table>
<thead>
<tr>
<th>Refs.</th>
<th>Mutation based on NM_018249.4 (predicted effect on protein)</th>
<th>Ethnicity/level of parental consanguinity</th>
<th>Patient ID/gender</th>
<th>Degree of microcephaly</th>
<th>Learning disability</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond et al. [2005]</td>
<td>Pedigree 1 has c.246T&gt;A (p.Y82X)</td>
<td>Northern Pakistan/first cousin^a</td>
<td>VI-2/male</td>
<td>Microcephaly present at birth; 6–8 SDS below age- and sex-related means</td>
<td>Mild</td>
<td>Moderate Profound congenital sensorineural deafness and infrequent tonic/clonic fits</td>
</tr>
<tr>
<td></td>
<td>Pedigree 1 given by Moynihan et al. [2000])</td>
<td></td>
<td>VI-3/female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pedigree 2 has c.4005-15A&gt;G (novel splice acceptor—addition of four new amino acids and then a termination codon)</td>
<td>Two female cousins (no IDs given)</td>
<td>Both had congenital microcephaly with late closing fontanelles. One patient was −7 SDS below age- and sex-related means at 11y. The other was −5 SDS below age- and sex-related means at 4y^c</td>
<td>Moderate^c</td>
<td>Low birth weight: 1.9 kg at term. Subsequent growth normal. No deafness, fits or spasticity in either individual^c</td>
<td></td>
</tr>
<tr>
<td>Hassan et al. [2007]</td>
<td>c.246T&gt;A (p.Y82X)</td>
<td>Northern Pakistan/Kashmir/first cousins^a</td>
<td>V-2/female</td>
<td>Microcephaly present at birth; 4–7 SDS below age- and sex-related means</td>
<td>Mild to moderate, with</td>
<td></td>
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<td></td>
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<td></td>
<td>V-3/female</td>
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<td></td>
<td>V-4/male</td>
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<td></td>
<td></td>
<td>V-5/male</td>
<td></td>
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</tr>
</tbody>
</table>

*WISC-R, Weschsler Intelligence Scale for Children-Revised; SDS, standard deviations; OFC, occipito-frontal head circumference. For the patient described here, it appears that the microcephaly shows some degree of progression; the other published patients do not present OFC measurements at multiple time points and so it is not possible to determine whether this is a common feature of CDK5RAP2 patients.

^aAdditional consanguineous loops present in the pedigree.

^bIQs reported by Heney et al. [1992].

^cPersonal communication, Professor C.G. Woods.
DISCUSSION

To date, only two disease-causing mutations in CDK5RAP2 have been described, both in consanguineous families; a Y82X mutation [Bond et al., 2005; Hassan et al., 2007] and a IVS26-15A>G splicing mutation [Bond et al., 2005] (Table I). All three published families are from Northern Pakistan. Therefore, our report of a Somali child with primary microcephaly and a novel E234X mutation confirms CDK5RAP2 as a disease gene with clinical relevance outside the Pakistani population.

Common features of patients with CDK5RAP2 mutations include microcephaly from birth (−4 to −8 SDS), with mild-to-moderate learning disability (Table I). All have a short sloping forehead but otherwise there do not appear to be any other dysmorphic features or associated malformations in common. Notably, our patient has significant bilateral sensorineural hearing loss. This manifestation is not generally considered to be consistent with autosomal recessive microcephaly and therefore it is highly possible that the deafness may be caused by a further gene mutation, independent of CDK5RAP2. We speculated that such a mutation, in a consanguineous family like this, might be recessive, caused by an additional homozygous mutation. However, when we scanned the seven cnLOH regions for phenotypes entered in OMIM, we identified only one autosomal recessive hearing loss phenotype (OMIM #612290: microtia, hearing impairment, and cleft palate caused by HOXA2 mutations), which did not match the phenotype of our patient. There were two deafness genes on Xq22.3; COL4A5 which is implicated in Alport syndrome with variable sensorineural hearing loss [Barker et al., 1990] and PRPS1 which is mutated in non-syndromic X-linked deafness-1 [Liu et al., 2010]. However, since all five siblings of the proband (two males and three females) and her parents are clinically normal, we believe that both genes are
unlikely candidates for the progressive sensorineural hearing loss noted in the proband. We also identified an autosomal dominant non-syndromic sensorineural deafness phenotype (OMIM #600994 caused by DFNA5 mutations) and we have not ruled out the possibility of a de novo mutation in this or other dominant deafness genes in our patient. Other explanations for the deafness include the existence of a novel recessive deafness gene located in one of the cnLOH regions. Alternatively, there is a possibility of compound heterozygous mutations elsewhere in the genome which would not have been detected by our autozygosity mapping approach. Interestingly, a recent study unexpectedly detected multiple disease alleles at the DFNB3 locus within a single consanguineous pedigree [Lezirovitz et al., 2008]. The increasing use of exome sequence data now allows filtering for genetic variants that are compatible for multiple disease mechanisms and a recent study used this method to resolve a complex case of Miller syndrome to be a combination of two different genetic disorders [Ng et al., 2010].

The hearing loss present in individual VI:3 published previously [Moynihan et al., 2000] prompted us to also consider the possibility that CDK5RAP2 mutations might have variable expressivity and that this might extend to include sensorineural hearing loss. However, in the case described previously, the deafness was congenital, whereas our patient passed a hearing test at birth, suggesting different etiologies. Nevertheless, we cannot rule out a link based on these cases alone and therefore further detailed clinical descriptions of rare CDK5RAP2 patients, including hearing assessments, will be needed to help resolve the full phenotypic range associated with mutations in this gene.

In a recent array-CGH study, 22% of subjects with brain malformations carried rare CNVs, many of which are likely to have etiological relevance [Kariminejad et al., 2011]. In our study, insufficient DNA was available for standard array-CGH. The decision to test the patient using a SNP-array (requiring significantly less DNA) instead proved fortuitous and highlights the advantage of SNP-arrays over array-CGH platforms in helping to guide targeted sequencing efforts, especially in cases where a condition is suspected to be recessive and parental consanguinity is a possibility. Whilst both platforms can detect pathogenic CNVs, only the SNP platform can detect all forms of cnLOH [Bruno et al., 2011], inform subsequent targeted sequencing strategies and if needed, confirm family relationships through SNP genotypes. In this way, SNP-array testing can also act as an invaluable prescreening and supportive tool when embarking on expensive whole genome and exome sequencing studies. However, it should be cautioned that using genomic data to infer family relationships can be a sensitive issue [Schaaf et al., 2011] and appropriate genetic counseling should be provided prior to testing.

In summary, we identified a novel CDK5RAP2 mutation, the first in a patient of non-Pakistani descent. Our study confirms CDK5RAP2 is a rare primary microcephaly disease gene and emphasizes that when no consanguinity is reported but is suspected, SNP-array testing can reveal cnLOH that may infer distant relationships between parents and guide disease gene identification.

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