Exclusion of putative CATSPER2 and STRC gene deletion and FOXII gene mutations in a unique cohort with sensorineural deafness and male infertility from south India

Justin Margret Jeffrey1, Jayasankaran Chandru1, Amritkumar Pavithra1,2, Murugesan Kalaimathi1, Nagarathinam Indhumathi3, Pangadan Ashraf1 and C. R. Srikumari Srisailapathy1,4

1Department of Genetics, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, India
2Department of Medical Genetics, Apollo Hospitals, Chennai 600 006, India
3Present address: Post Graduate and Research Department of Biotechnology, Women’s Christian College, College Road, Chennai 600 006, India

Prelingual genetic deafness and male infertility can appear as isolated findings or as part of a syndrome. Deafness-Infertility Syndrome (DIS) was previously reported to be caused due to a rare contiguous gene deletion of CATSPER2 and STRC genes on chromosome 15q15.3. We tested this contiguous gene deletion in a unique cohort of 15 probands with deafness and male infertility, who were partners in assortative mating from south India. Screening for this alleged contiguous gene deletion did not test positive. Given high parental consanguinity, it is possible that infertility and deafness may not be part of a contiguous gene deletion, but two independent events. As a next option we screened another candidate gene FOXII (5q35.1), known to independently influence sperm maturation and also encode transcriptional factor of a deafness gene SLC26A4, to implicate for this DIS phenotype. However none of the probands had any pathogenic mutations in FOXII gene. Having excluded (i) DIS contiguous gene deletion and (ii) FOXII gene mutations’ role in this phenotype, we conclude that this unique cohort’s genetic etiology can be resolved using high-throughput NGS and CNV assessment. This approach may also identify potential linkage to any novel genes.

Keywords: Assortative mating, contiguous gene deletion, CATSPER2, STRC, p.1355.

Deafness may occur by itself as an isolated phenotype or as a part of a syndrome in which the hearing loss is associated with other medical conditions. One such autosomal recessive syndrome is sensorineural deafness with male infertility (DIS) which is due to a contiguous gene deletion of the CATSPER2 and STRC genes on chromosome 15q15.3 (ref. 1). Cation Channel Sperm Associated 2 (CATSPER2) encodes calcium channels required for hyperactive motility of the sperm tail to push through the egg cell1,2. Adjoining CATSPER2 is the stereocilin (STRC) gene, expressed in the stereocilia of the outer hair cells of the inner ear, involved in mecanoreception of sound waves3. Hence when the deletion is present both deafness and anomaly in morphology and motility of the sperm exist in males; whereas females with this deletion have only hearing loss but are fertile3. Similarly, FOXII (Forkhead box 11) has a functional role in hearing, fertility and male acidoisis. It is a potential transcriptional activator of an auditory gene SLC26A4 (ref. 6) which also has a role in epididymal expression that is required for male fertility. Mutations in FOXII gene (5q35.1) cause sensorineural deafness syndrome with distal renal tubular acidoisis and male infertility4.

So we tested for this contiguous gene deletion in a unique cohort of assortatively mating families, where the male partners have both sensorineural deafness and infertility as a phenotype. Furthermore we improvised the approach by adding another candidate gene FOXII which has not been concurrently screened in DIS probands. So far this gene has only been screened in prelingual deaf without infertility, although FOXII has a role in sperm maturation. So this adds to the novelty of the study design.
Fifteen probands and their families were included for this study after informed consent and Institutional Human Ethical Committee approval. A total of 25 healthy fertile males with normal hearing were included as control. Genomic DNA was extracted by standard phenol chloroform isoamylalcohol method\(^8\). Initially cytogenetic analysis was performed on the probands using GTG banding\(^9\). A minimum of 20 metaphase spreads of 450–500 bands per haploid set were analysed. Karyotypes were described according to the International System for Human Cytogenomic Nomenclature\(^10\). Normal karyotypes were observed in all the probands (Supplementary Figure 1). All the probands were initially screened for the common genetic etiology due to deafness causing \(GJB2\) gene mutation and male infertility related \(Y\) chromosome microdeletion; both were majorly ruled out. Although one proband tested positive for \(GJB2\) pathogenic mutation (p.I35S, homo mutant), he was still taken forward to resolve the genetic basis for his infertility. Following this, all the probands were screened for \(DIS\) contiguous gene deletion by multiplex PCR using STS markers D15S784 (ref. 11), REN37386 and beta actin control primers (Supplementary Table 1 and Supplementary Figure 2). On exclusion for this deletion, \(FOXI1\) gene (exon 1 and 2) mutational analysis was performed for all the probands and controls (Supplementary Table 2). Using bidirectional sequencing the chromatograms obtained were compared with appropriate gene sequences in the National Center for Biotechnology Information (NCBI: http://www.ncbi.nlm.nih.gov/) and UCSC genome browser (https://genome.ucsc.edu/) to identify the nucleotide base-pair changes.

It is important to mention how this large cohort of 15 probands, a rare (1 in 40,000) syndrome like \(DIS\) was ascertained. Originally 115 assortatively mating (AM) prelingual deaf families comprising 66 Deaf on Deaf (DXD) and 49 Deaf on Normal hearing (DXN) combinations were screened for a spectrum of deafness mutations during the period 2010–2016. In these mating types, 103 spouses were males with prelingual deafness; among them 14 turned out to be infertile as well. An additional unmarried deaf proband was included in this study as well, who was a referred case with proven infertility. So this study focuses on these 15 probands and their families originating from Kerala (8), Tamil Nadu (3), Karnataka (2) and Andhra Pradesh (2).

Pedigree analysis showed nine simplex cases (60%) with deafness and infertility phenotype. Only one proband had recurrence of this phenotype in his brother. In
The remaining 5 probands’ families, deafness and infertility seem to be segregating independently. Sixty per cent of the probands (9/15) had parental consanguinity. However all the probands’ marriages were non-consanguineous; 11 (79%) probands showed positive AM (DXD) and 3 (21%) showed negative AM (DXN) (Supplementary Figure 3).

Audiometry revealed severe to profound hearing loss; semen analysis disclosed reduced volume, deformity in sperm motility and morphology establishing infertility. Despite the co-segregation of deafness and infertility, due to the absence of any putative DIS deletion and pathogenic FOXI1 gene mutations, this molecular genetic etiology cannot be implicated for the observed phenotype. The two synonymous changes, p.R93R and p.Y348Y residing in FOXI1 gene (Figure 1; Table 1) observed in this study transmitted together in the patients and control group. The allele frequency among patients and controls of p.R93R (23.33% and 22%) and p.Y348Y (90% and 98%) respectively did not show any statistically significant difference between the patients and controls. A similar frequency was reported in the ExAC database for the south Asian population (Table 2). However considerable evidence has accumulated over the past decades to show that synonymous mutations can result in aberrant mRNA splicing which can lead to human disease.12,13

This is the first study to screen for DIS deletion and FOXI1 gene mutations in an exclusive cohort with both deafness and infertility; whereas previous studies targeted either completely hearing impaired or male infertile population. The novelty of this work lies in assembling a unique and large cohort of a rare syndrome that has been reported to be 1 in 40,000 (ref. 1). So far there are 10 studies available, worldwide described as case reports (Table 3).

Contiguous gene syndromes (CGS) are primarily seen in males with X chromosome deletions. CGS are much less common on autosomes because of the usual presence of the normal homologue.14 The autosomal CATSPER2 and STRC gene deletions did not account for the deafness infertility phenotype observed in our study. We conducted a meta-analysis of 10 studies reporting DIS deletion, identified 32 individuals from 22 families worldwide (Table 2) along with carrier frequency in controls varying from 1.09% (ref. 15) to 1.6% (ref. 1). Avidan et al.16 first reported on 3 siblings born to...
Table 3. Compilation of so far globally reported DIS contiguous gene deletion cases

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Size</th>
<th>Gender</th>
<th>Age</th>
<th>Phenotype</th>
<th>Size of the deletion</th>
<th>Technique used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>1 Family</td>
<td>Male-3</td>
<td>58, 56 and 54 years</td>
<td>Moderate SNHL, Asthenoteratozoospermia, CDAI</td>
<td>~70 kb</td>
<td>Genotype analysis</td>
<td>16</td>
</tr>
<tr>
<td>Iran</td>
<td>3 Families</td>
<td>Male-4</td>
<td>23 and 35 years; age not provided for rest</td>
<td>All have SNHL and no syndromic features; one male – asthenoteratozoospermia</td>
<td>~100 kb</td>
<td>Linkage analysis</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female-2</td>
<td>Females 35 and 20 years; for male age not provided</td>
<td>All have SNHL and no syndromic features; sperm motility assessment not performed</td>
<td>~100 kb</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male-1</td>
<td>Males: 26 and 21 years; female 17 years</td>
<td>All have SNHL and no syndromic features; both males have asthenoteratozoospermia</td>
<td>90 kb</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1 Family</td>
<td>Male-1</td>
<td>10 years</td>
<td>Moderate Bilateral SNHL, MR, short stature, dysmorphic features, normal HC, sperm motility assessment not performed</td>
<td>90 kb</td>
<td>Array CGH &amp; MLPA</td>
<td>15</td>
</tr>
<tr>
<td>Caucasian, Asian</td>
<td>659 HI children</td>
<td>n = 4</td>
<td>Not provided</td>
<td>Hearing Loss</td>
<td>~77 kbp</td>
<td>SNP Genotype array</td>
<td>4</td>
</tr>
<tr>
<td>USA</td>
<td>1</td>
<td>Female</td>
<td>13 months</td>
<td>Mild/moderate bilateral SNHL, macrocephaly</td>
<td>62 kbp</td>
<td>Array CGH</td>
<td>1</td>
</tr>
<tr>
<td>USA</td>
<td>686 HL patients</td>
<td>Female-4</td>
<td>0–10 years range</td>
<td>Hearing Loss</td>
<td>–</td>
<td>TGE and MPS (OtoSCOPE panel)</td>
<td>19</td>
</tr>
<tr>
<td>North India</td>
<td>2 Infertile patients</td>
<td>Male</td>
<td>35 years</td>
<td>Severe oligospermia Oligospermia</td>
<td>193 kbp</td>
<td>Microarray analysis</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>(1 family)</td>
<td></td>
<td>31 years</td>
<td></td>
<td>174 kbp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>94 NSHL + 1 Syndromic SNHL</td>
<td>Male-2, female-1</td>
<td>Pre-puberty age</td>
<td>One male and female had only SNHL whereas another male had SNHL; facial anomalies, atrial septal defect, hydroxylysinuria/lysemia, recurrent infections</td>
<td>30.1 kbp and 45.1 kbp</td>
<td>CNV analysis</td>
<td>18</td>
</tr>
<tr>
<td>China</td>
<td>63 Sporadic NSHL</td>
<td>Male-1</td>
<td>3 years</td>
<td>Moderate HL at high frequency</td>
<td>–</td>
<td>TGE &amp; MPS</td>
<td>20</td>
</tr>
<tr>
<td>Japan</td>
<td>194 NSHL probands</td>
<td>Female-1, Male-2</td>
<td>26 years, 12 years</td>
<td>Mild HL and tinnitus Mild HL</td>
<td>–</td>
<td>TGE &amp; MPS (OtoSCOPE panel), array CGH</td>
<td>21</td>
</tr>
</tbody>
</table>

SNHL, Sensorineural hearing loss; CDAI, Congenital dyserythropoietic anemia type I; MR, Mental retardation; HL, Hearing impaired; NSHL, Non-syndromic hearing loss; HI, Hearing impaired; TGE, Targeted genome enrichment; MPS, Massively parallel sequencing.
non-consanguineous parents with type I congenital anemia, athenoteratozoospermia and nonsyndromic deafness, with a homozygous ~70 kb deletion removing the entire functional STRC and the last 2 exons (225 bp) of CATSPER2 gene, explaining the observed deafness and male infertility phenotypes. Following this, Zhang et al. investigated 3 consanguineous families and reported ~100-kb deleted region involving KIAA0377, CKMT1B, STRC and CATSPER2 genes. They further identified a marker D15S784 between the STRC and CATSPER2 genes and used this for rapid screening of 300 deaf probands. Various deletion sizes (90 kb and 62 kb) with additional co-morbidities like mental retardation, short stature; dysmorphic features and macrocephaly were also reported earlier. A first Indian report identified this deletion (193 kb and 174 kb) in two infertile brothers but without deafness phenotype. Using NGS on diverse hearing impaired population, additional DIS deletion cases were reported. In all the previous studies, individuals who tested positive for DIS deletion had variable severity in hearing loss. Hence infertile males with normal hearing should be screened for this deletion to avoid false negatives. However we did not find any DIS deletion in 50 normal hearing infertile males that was recently reported.

If the rearrangements due to the deletion are quite complex, there are chances of having missed some positive cases. Given high parental consanguinity (60%), it is possible that infertility and hearing loss are not part of a contiguous gene deletion, but two independent events.

Having excluded (i) DIS contiguous gene deletion and (ii) FOX11 gene mutations’ role in this phenotype, we conclude that this unique cohort’s genetic etiology can be resolved using high-throughput NGS and CNV assessment. This approach may also identify potential linkage to any novel genes.


ACKNOWLEDGEMENTS. We thank all the families for their participation in our study. This study was supported by UGC-UPE Phase II Biomedical Research Project (No. 2013/PFEP/C3/199); Ad-hoc research project (5/8-10-17/Oto/CPF/2011-NCD-I) from Indian Council of Medical Research, Government of India, UGC-SAP, DST-FIST II and UICIC Program of University of Madras sanctioned to CRS. J.M.J. was a project fellow in UGC-UPE II Biomedical Research Project and currently CSIR SRF; J.C. was supported by UGC-BSR; A.P. and M.K. were supported by ICMR. There is no conflict of interest among the authors.

Received 21 July 2017; revised accepted 27 February 2018

doi: 10.18520/cvs/v14/i12/2538-2542

CURRENT SCIENCE, VOL. 114, NO. 12, 25 JUNE 2018