Mutations involving FIBULIN2 are a novel cause of 46,XY DSD

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INTRODUCTION

FIBULIN2 is a 180 kDa extra cellular matrix protein (ECM) protein that can interact with a wide range of ECM proteins and be incorporated into various extracellular structures, including the elastin/fibrillin fiber system, fibronectin microfibrils, basement membranes, and proteoglycan aggregates. During embryonic development, high levels of fibulina2 are detected at sites of epithelial-mesenchymal transformation (1). Fbn2 is transiently expressed in the basal membrane (BM) of newborn rat testis (2) and its expression is highly upregulated in the XY gonad at the moment of testis-determination leading to the suggestion that it may be involved in sex-determination (3, 4; Fig 1). Here, as part of the DSD-NGS project at the Institut Pasteur (http://www.pasteur.fr/fr/recherche/biologie-du-developpement-cellsouches) we identified a series of mutations in FBLN2 associated with otherwise unexplained 46,XY complete or partial gonadal dysgenesis.

METHODOLOGY

Exon enrichment was performed using Agilent SureSelect Human All Exon V4. Paired-end sequencing was performed on the Illumina HiSeq2000 platform. Sequencing was performed at x50 coverage. Read files were generated from the sequencing platform via the manufacturer’s proprietary software. Reads were mapped using the Burrows–Wheeler Aligner and local realignment of the mapped reads around potential insertion/deletion (indel) sites was carried out with the GATK version 1.6. SNP and indel variants were called using the GATK Unified Genotyper. SNP novelty was determined against dbSNP138. Datasets were filtered for novel or rare (MAF<0.01) variants. Pathogenic mutations were confirmed by Sanger sequencing.

RESULTS

Analyses of the exome sequencing datasets revealed heterozygous missense mutations in the FBLN2 gene in a series of patients with 46,XY gonadal dysgenesis (Table 1). Analyses of the variant datasets from these patients using a panel of gene prioritization software (http://homes.esat.kuleuven.be/~bioiusseri/gp/tools.php) as well as a manual interrogation of each variant did not reveal any other candidate genes involved in gonadal development. Of the six individuals carrying mutations, two are sisters who inherited the mutation from their healthy mother. Three mutations are novel and 2 mutations are known rare variants. The rare variants may not be pathogenic but may contribute to the phenotype. The novel mutations were not observed in ancestry-matched healthy control samples (>400/mutation).

CONCLUSIONS

For the first time, we provide evidence to indicate that mutations involving the FBLN2 gene contribute to 46,XY DSD. Fbn2 was proposed as a testis-determining gene since its expression is highly up-regulated both in the somatic cells of the mouse testis at 11.5 d.p.c., XY gonad and in Foxd3−/− XX ovaries suggesting that FBLN2 is repressed by, or competitive to FOXL2/WTN4 pathways (4, 5). Our data are consistent with these findings and indicate that FBLN2 mutations are a new cause of the severe forms of 46,XY partial and complete gonadal dysgenesis.

References


Fig 1. Expression of murine Fbn2 in the XX and XY gonads at sex-determination. Fbn2 expression is strongly upregulated in the male gonad.

Fig 2. Distribution of FBN2 mutations in relation to the functional domains of the proteins. The domains I, II and III are extracellular modules. Blue rectangle - N domain divided into cysteine-rich (Na) and cysteine-free (Nb) region; yellow diamonds - anaplastilithy-like modules; green circles - EGF-like module; red rectangle - fibulin-type module.

Fig 3. Representative chromatograms of the mutations identified by exome sequencing.

Fig 4. Evolutionary conservation of amino acids that are mutated in FBLN2 in association with 46,XY gonadal dysgenesis. These mutations have not been reported in any public database and are absent from appropriate ancestry-matched control samples.

Table 2. Clinical description of 46,XY DSD patients carrying heterozygous mutations in FBLN2.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Hypothalamic</th>
<th>Gonadal</th>
<th>Clinical description</th>
<th>FBLN2</th>
<th>Genotype</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>F</td>
<td>Normal</td>
<td>M</td>
<td>height 150 cm, gonadal dysgenesis</td>
<td>c.1011A&gt;G, p.Pro338Arg</td>
<td>heterozygous, 46,XY</td>
<td>Height 150 cm, hypogonadism, normal karyotype.</td>
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<td>2</td>
<td>6</td>
<td>M</td>
<td>Hypothalamic</td>
<td>M</td>
<td>height 160 cm, height 180 cm</td>
<td>c.1011A&gt;G, p.Pro338Arg</td>
<td>heterozygous, 46,XY</td>
<td>Height 160 cm, height 180 cm, hypogonadism, normal karyotype.</td>
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<tr>
<td>3</td>
<td>8</td>
<td>F</td>
<td>Normal</td>
<td>M</td>
<td>height 150 cm, gonadal dysgenesis</td>
<td>c.1011A&gt;G, p.Pro338Arg</td>
<td>heterozygous, 46,XY</td>
<td>Height 150 cm, hypogonadism, normal karyotype.</td>
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<tr>
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<td>M</td>
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<td>Height 150 cm, height 180 cm, hypogonadism, normal karyotype.</td>
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<tr>
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<td>Normal</td>
<td>M</td>
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<td>M</td>
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