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

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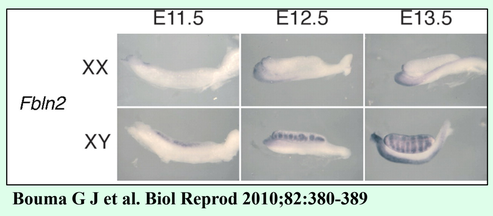
INTRODUCTION



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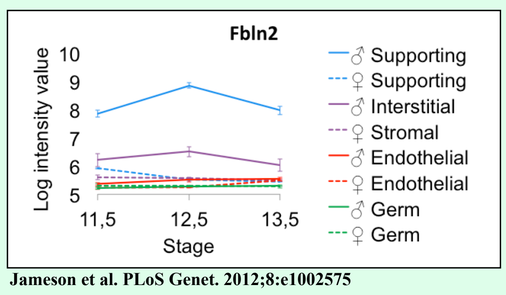

Jameson et al. PLoS Genet. 2012;8:e1002575

Fig 1. Expression of murine *Fbln2* in the XX and XY gonads at sex-determination. *Fbln2* expression is strongly upregulated in the male gonad.

FIBULIN2 is a 180 kD 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 fibulin2 are detected at sites of epithelial-mesenchymal transformation (1). Fbln2 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-cellules-souches) 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/~bioiuser/gpp/tools.php) as well as a manual interogation 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. 3 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).

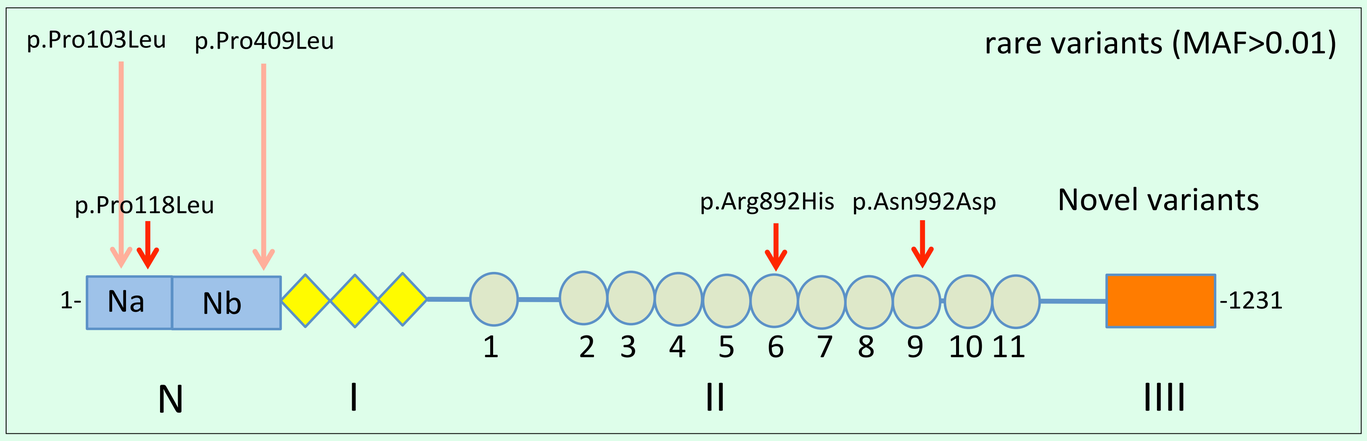

Fig 2. Distribution of *FBLN2* mutations in relation to the functional domains of the protein. The domains I, II and III are extracellular modules. Blue rectangle - N domain divided into cysteine-rich (Na) and cysteine-free (Nb) regions; yellow diamonds – anaphylatoxin-like modules; green circles – EGF-like modules; red rectangle – fibulin-type module.

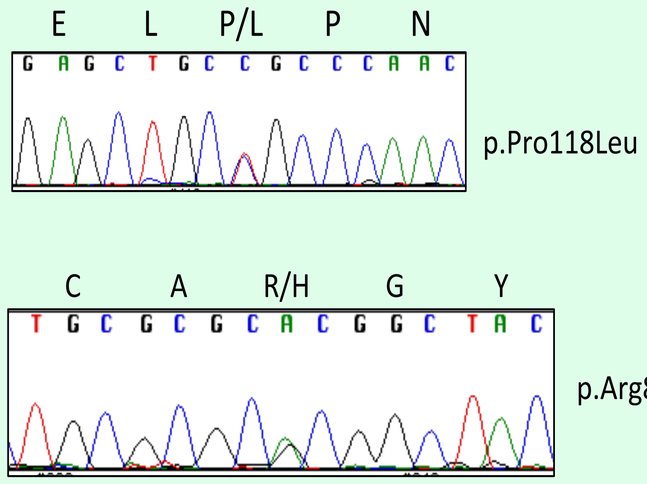

Fig 3. Representative chromatograms of the mutations identified by exome sequencing

Patient	Age at presentation	External genitalia	Gonads	Mullerian structures	FSH	LH	T	*FBLN2* mutation	Comments
1	16.8 yr	Female, hypertropic clitoris. Tanner stage 5. No breast development.	L: absent R: Some testicular tissue present	Present	elevated	elevated	undetectable	Novel, heterozygous c.353C>T, p.Pro118Leu	46,XY. Height 182 cm. Mutation inherited from normal mother.
2	15.2 yr (sister of 1)	Female, hypertropic clitoris. Tanner stage 2. No breast development	Dysgenetic testis with rare Leydig cells. Sertoli & germ cells absent	Present, small uterus, duplicated vagina	elevated	elevated	undetectable	Novel, heterozygous c.353C>T, p.Pro118Leu	46,XY. Mutation inherited from normal mother.
3	16 yr	Female, Tanner stage 2.	Streak gonads. Absence of Sertoli and Leydig cells.	Rudimentary fallopian tubes. Uterus and vagina absent.	elevated	elevated	low	Novel, heterozygous, c.2675G>A, p.Arg892His	46,XY. Height 165 cm. Cardiac murmur at birth.
4	18 yr	Female	Bilateral streak gonads	Present	elevated	elevated	undetectable	Novel, heterozygous c.2975A>G p.Asn992Asn	46,XY
5	17 yr	Female	Bilateral streak gonads	Present	elevated	elevated	undetectable	rs113265853 c.1226C>T, p.Pro409Leu (rare variant)	46,XY
6	18 m	Ambiguous	Not papable	Absent	NA	NA	low	rs113057429 c.308C>T, p.Pro103Leu (rare variant)	46,XY. AMH levels undetectable

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

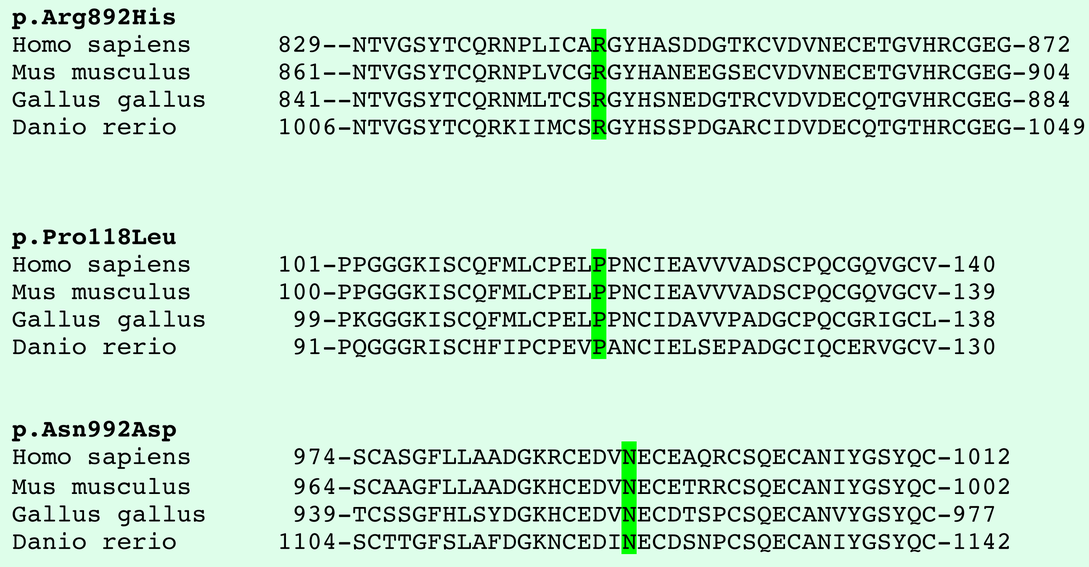

Fig 4. Evolutionary conservation of amino acids that are mutated in *FBLN2* in association with 46,XY gonadal dysgenesis. These mutations have not been reported in any public database and are absent from appropriate ancestry-matched control samples.

CONCLUSIONS

For the first time, we provide evidence to indicate that mutations involving the *FBLN2* gene contribute to 46,XY DSD. *Fbln2* was proposed as a testis-determining gene since its expression is highly up-regulated both in the somatic cells of the mouse 11.5 d.p.c. XY gonad and in *Foxl2*$^{-/-}$ XX and *Wnt4*$^{-/-}$ XX ovaries suggesting that *FBLN2* is repressed by, or competitive to FOXL2/WNT4 pathways (4, 5). Our data are consistent with these findings and indicate that *FBLN2* mutations are a new cause of the severe forms of DSD, 46,XY partial and complete gonadal dysgenesis.

References:
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