



Whole Exome Sequencing Identifies a Rare Nonsense Mutation in FAM47C as a Possible Cause of Severe Oligospermia in Brothers With Varicocele

Willy Chertman¹, Himanshu Arora¹, Anthony J. Griswold, Diana M. Lopategui, and Ranjith Ramasamy

Varicocele is a common cause of impaired semen parameters in men with infertility. Here, we investigated genetic variants as possible causes of varicocele with impaired semen parameters using whole exome sequencing in a family with 2 brothers with severe oligospermia, 1 unaffected brother, father, and the mother. Results showed a premature stop codon alteration on Chromosome X (37028866 CT) in the gene FAM47C. The affected brothers were found to be hemizygous for the variant, while the mother was a heterozygous carrier. In conclusion, identifying men with varicocele that would have impaired spermatogenesis, using approaches like whole-exome sequencing, can be paradigm shifting. *UROLOGY* 129: 71–73, 2019. © 2019 Elsevier Inc.

Varicocele is the pathologic enlargement of veins of the pampiniform plexus within the spermatic cord and is a common cause of impaired semen parameters. Varicocele occurs far more commonly in the left spermatic vein, which drains at a 90° fashion into the renal vein and is longer than the right spermatic vein, causing it to experience higher venous pressures.¹

There are several hypotheses of how varicocele may impact semen parameters, higher levels of Reactive Oxygen Species, local ischemia, increased scrotal temperature, and Leydig Cell dysfunction causing subsequent hypogonadism.¹ The prevalence of varicocele in the general population is 15%. However, only 1% of men with varicocele have fertility problems. Nevertheless, up to 35% of men with primary infertility have varicocele, 85% of men with secondary infertility have varicocele, and 25% of men with abnormal sperm have varicocele.² Because of the high prevalence of varicocele in the general population and because only a small proportion of men with varicocele have impaired spermatogenesis, it is imperative to determine which of the men with varicocele will have impaired semen parameters.

Varicocele is a multifactorial disorder³ with a genetic component. A variety of genetic factors have been

proposed, among them genes that are susceptible to oxidative stress, and genes affecting calcium homeostasis within the testes.⁴ First-degree relatives (fathers and brothers) of patients with varicocele are 4 to 8 times more likely than controls to have varicocele.^{5,6} There are several genetic variants associated with varicocele, ranging from Y-chromosome microdeletions⁷ to mtDNA deletions⁸ to a supernumerary minute chromosome 14.⁹

Whole exome sequencing (WES) has been used to identify a potentially causal mutation in a consanguineous Turkish family with multiple cases of nonobstructive azoospermia.^{10,11} No study has evaluated WES in a family with varicocele and impaired semen parameters. In this study we utilized WES in 2 brothers with severe oligospermia and varicocele and compared the results to an unaffected brother with similar physical findings. We hypothesized that WES may have value in evaluation of men with varicocele and severe oligospermia given the genetic heterogeneity of varicocele.

MATERIALS AND METHODS

Patient Ascertainment

Two brothers were referred to our clinic for varicocele repair of left grade II varicocele. After severe oligospermia (<1 million/cc) was observed in 2 separate semen analyses in both brothers, we brought their other brother, mother, and father in for consultation and obtained blood for DNA extraction. All 3 brothers had left grade II varicocele. Approval for this study was obtained through our Institutional Review Boards (IRB# 20150740). All participants provided written informed consent, and the study

¹ Contributed equally.

Disclosure: The authors report no conflicts of interest in this work.

From the Department of Urology, University of Miami Miller School of Medicine, Miami, FL; and the John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL

Address correspondence to: Ranjith Ramasamy, M.D., Department of Urology, University of Miami, 1120 NW 14th floor #1560, Miami, FL 33136. E-mail: ramasamy@miami.edu

Submitted: December 26, 2018, accepted (with revisions): March 19, 2019

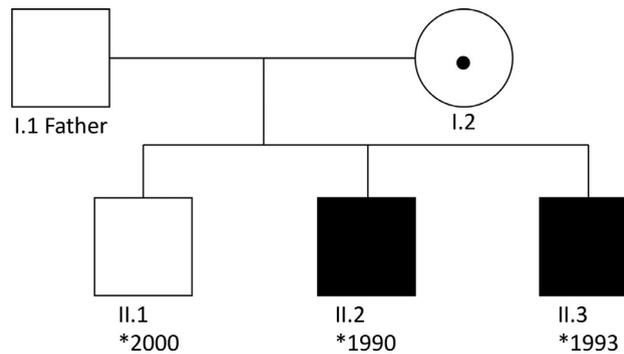


Figure 1. Pedigree chart of family. They had 3 male offspring of which 2 were oligospermic and 1 (youngest) was normozoospermic.

***WES data available.**

was performed in accordance to the World Medical Association's Declaration of Helsinki.

Whole Exome Sequencing

WES, DNA reads, and variant interpretation performed according to previously published protocols¹² at a cost of \$670.00 per sample for a total of \$2,010.00.

Data Analysis

Assuming a genetic defect with Mendelian heritability, we sought variants that were shared by severely oligospermic brothers, not present in brother with normozoospermia, and as described in a previously published protocol, fall within protein-coding regions as well as fall below a prevalence threshold.¹² Validation of an identified mutation was performed by targeted Sanger sequencing of the surrounding 265 base pairs in the variant of interest (Genewiz, South Plainfield, NJ). Primers were designed using the online application Primer3web (<http://bioinfo.ut.ee/primer3/>). Sequencing was performed with the primers 3' TTTCCAGTCTCCGCCAG and TGTGCTTGATGTACCTTCTG, elaborated by SIGMA-Aldrich (Darmstadt, Germany).

RESULTS

Clinical Characteristics

Pedigree chart of the study family is provided in [Figure 1](#). Clinical examination was available for all 3 brothers in this study and clinical information is provided in [Table 1](#).

Whole Exome Sequencing Results

WES found 44,789 single-nucleotide variants and small insertion-deletions with variation in at least one of the 3 brothers sequenced. After filtering for shared positions in the 2 affected brothers, 22,854 variants were left. Filtering for theoretically functional coding variants left 20,572 variants, of which 9809 were synonymous variants, 9283 were nonsynonymous

nonframeshift variants, and 307 were either frameshift deletion, frameshift insertion, stop-gain, or stop-loss variants. We further filtered for variants rarer than 1% in ExAC and 1000 Genomes, which left 59 variants. Since only 2 brothers were severely oligospermic, we expected that the normozoospermic brother would not share the variant, so we filtered for variants shared by the 2 oligospermic brothers but not by the normozoospermic brother, which left 17 variants.

The best candidate for the severe oligospermia phenotype is the C>A variant at chrX:37028866 (rs140378751) which caused a stopgain mutation in the FAM47C gene, probably causing a premature stop codon and inducing production of a shortened protein. The variant has been reported in ExAC at an overall frequency of 0.000313.

Mutation Confirmation and Segregation in the Family

Sanger sequencing was performed to screen the 5 members of the family (2 affected brothers, 1 unaffected brother, mother, and father) for the selected variant in FAM47C. The presence of this variant was confirmed in the 2 affected brothers (hemizygous) and in mother (heterozygous). No variant was found in unaffected brother and father ([Fig. 2](#)).

Comment

There are several known genetic causes of severe oligospermia, though only a few studies have used WES to explore this phenotype. There are theoretical reasons to suspect that the X chromosome is enriched for spermatogenesis genes, and a recent study found substantial X chromosome gene expression in mouse testis tissue.¹³ Another study examined Copy Number Variants (CNVs) in infertile men¹⁴ and found an increased number of CNVs in infertile men compared to controls in parts of the X chromosome. In that study, the FAM47C gene was part of the DUP26 CNV region that was enriched in an infertile patient, but that did not reach statistical significance across all cases. The FAM47C gene is highly expressed in the testis but its function is unknown.

Table 1. Clinical characteristics of 3 brothers with varicocele

Age	FSH (miU/mL)	LH (miU/mL)	Total testosterone (ng/dL)	Testis volume (bilaterally)	Sperm Count (Million/cc)	Ejaculate volume (cc)	Motile Sperm Count fraction	Motile Sperm Count
28	18.2	3.9	405	10 cc	0.12	1.8	5%	10,800
25	23	6.4	446	6 cc	0.75	3.2	24%	576,000
18	5	5.9	530	14 cc	21	1.7	58%	20,706,000

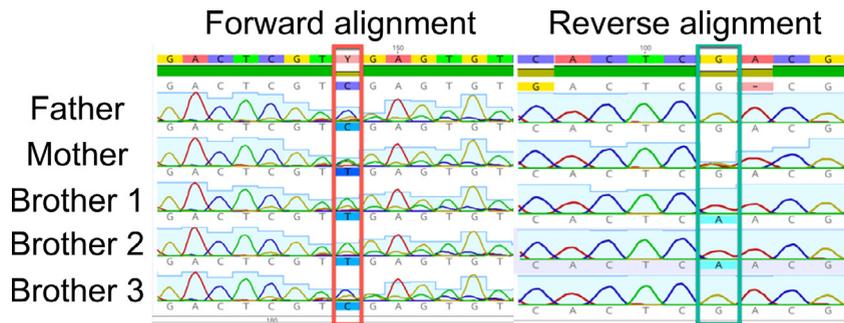


Figure 2. Sanger Sequencing of FAM47C variant. The mother was heterozygous for the variant while the father was normal. Two sons were hemizygous for the variant while 1 son and father were normal. (Color version available online.)

The brothers with severe oligospermia and the variant FAM47C gene underwent varicocele repair with no improvement in sperm count or changes in gonadotropin levels as measured at 12-month follow-up, as shown in Table 1. This variation could be accounted for by differences in gonadotropin levels but all 3 brothers have similar testosterone levels. Since sperm counts tend to increase in 50% of men with severe oligospermia after varicocele repair,¹⁵ this lack of response suggests that the underlying genetic variant likely causes severe oligospermia. WES could be used to predict response to varicocele repair in the future.

CONCLUSION

Using WES, we identified a nonsense mutation in FAM47C that segregates with the varicocele—severe oligospermia phenotype. The mutation was present in the severely oligospermic brothers and not in the normozoospermic brother despite all 3 brothers having a similar varicocele clinical grade. Because varicocele is prevalent (15%) in the population, identifying men that have impaired spermatogenesis using approaches like WES can be paradigm shifting.

References

1. Clavijo RI, Carrasquillo R, Ramasamy R. Varicoceles: prevalence and pathogenesis in adult men. *Fertil Steril.* 2017;108:364–369.
2. The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organization. *Fertil Steril.* 1992;57:1289–1293.
3. Santana VP, Miranda-Furtado CL, de Oliveira-Gennaro FG, Dos Reis RM. Genetics and epigenetics of varicocele pathophysiology: an overview. *J Assist Reprod Genet.* 2017;34:839–847.
4. Sheehan MM, Ramasamy R, Lamb DJ. Molecular mechanisms involved in varicocele-associated infertility. *J Assist Reprod Genet.* 2014;31:521–526.
5. Mohammadali Beigi F, Mehrabi S, Javaherforooshzadeh A. Varicocele in brothers of patients with varicocele. *Urol J.* 2007;4:33–35.
6. Raman JD, Walmsley K, Goldstein M. Inheritance of varicoceles. *Urology.* 2005;65:1186–1189.
7. Song NH, Wu HF, Zhang W, et al. Screening for Y chromosome microdeletions in idiopathic and nonidiopathic infertile men with varicocele and cryptorchidism. *Chin Med J.* 2005;118:1462–1467.
8. Gashti NG, Salehi Z, Madani AH, Dalivandan ST. 4977-bp mitochondrial DNA deletion in infertile patients with varicocele. *Andrologia.* 2014;46:258–262.
9. Stahl BC, Patil SR, Syrop CH, Sparks AE, Wald M. Supernumerary minute ring chromosome 14 in a man with primary infertility and left varicocele. *Fertil Steril.* 2007;87:1213.e1211–1213.
10. Ramasamy R, Bakircioglu ME, Cengiz C, et al. Whole-exome sequencing identifies novel homozygous mutation in NPAS2 in family with nonobstructive azoospermia. *Fertil Steril.* 2015;104:286–291.
11. Clavijo RI, Arora H, Gibbs E, et al. Whole Exome Sequencing of a consanguineous Turkish family identifies a mutation in GTF2H3 in brothers with spermatogenic failure. *Urology.* 2018;120:86–89.
12. Lopategui DM, Griswold AJ, Arora H, Clavijo RI, Tekin M, Ramasamy R. A rare ANOS1 variant in siblings with Kallmann syndrome identified by whole exome sequencing. *Andrology.* 2018;6:53–57.
13. Mueller JL, Mahadevaiah SK, Park PJ, Warburton PE, Page DC, Turner JM. The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. *Nat Genet.* 2008;40:794–799.
14. Chianese C, Gunning AC, Giachini C, et al. X chromosome-linked CNVs in male infertility: discovery of overall duplication load and recurrent, patient-specific gains with potential clinical relevance. *PLoS One.* 2014;9:e97746–e97746.
15. Masterson TA, Greer AB, Ramasamy R. Time to improvement in semen parameters after microsurgical varicocelectomy in men with severe oligospermia. *Can Urol Assoc J.* 2018;13(3):E66–E68.