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Gene panel analysis of literature-based infertility genes in Sertoli cell-only syndrome patients

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Abstract

Sertoli cell-only syndrome (SCOS) is a condition of male infertility characterized by a total absence of spermatogenic cells in nearly all seminiferous tubules. Apart from well-established genetic changes such as Klinefelter syndrome, CFTR variants, and Y-chromosome microdeletions, several hundred candidate genes were reported as associated with male infertility. We selected 92 evidence-based genes associated with infertility and investigated data from whole-exome sequencing in 6 individuals with clinically diagnosed SCOS. Eight heterozygous variants passed our filtering criteria, including population frequency ≤ 0.1% and high functional impact indicated by Sift, Polyphen, and CADD scores. Out of them, we considered only variants with putative autosomal dominant effects on infertility that were subsequently validated by Sanger sequencing. This filtering pipeline has led to the final likely causative variants detected in CHD7 and SCYP3 genes that potentially explain SCOS in two of our patients. Our discoveries suggest that gene panel testing of patients with SCOS could improve the diagnostic outcome; however, assembling a gene panel consisting of only genuine causative genes is crucial.

Introduction

Male infertility affects approximately 7% of men worldwide (Lotti *et al.*, 2014; Kumar & Singh, 2015). Sertoli cell-only syndrome (SCOS) is a form of male infertility manifested by a complete lack of spermatogenic cells in almost all seminiferous tubules, with only Sertoli cells lining them (Levin, 1979; Paduch *et.*,2019). Y chromosome microdeletions, chromosome abnormalities, undescended testis, and exposure to environmental factors such as radiation are underlying causes of SCOS (Ghanami Gashti et al., 2021). However, many patients with SCOS diagnosis are left with unknown aetiology.

It is known that more than 2000 genes are involved in spermatogenesis (Krausz, Escamilla and Chianese, 2015), while 334 genes are expressed in Sertoli cells (Uhlén et al., 2015), and many of these genes have already been associated with infertility in different genetic studies (Schultz, Hamra and Garbers, 2003; Djureinovic *et al.*, 2014; Kasak *et al.*, 2018). In recent years, next-generation sequencing (NGS) has sped up the discovery phase, and many rare variants have been detected in family studies. However, it remains still elusive to what extent those candidate genes explain infertility on a population scale. The construction of an infertility panel that could increase the diagnostic yield in male infertility has been of much interest lately (Tüttelmann, Ruckert and Röpke, 2018; Cariati, D'Argenio and Tomaiuolo, 2019; Oud *et al.*, 2019). A recent systematic review by Oud et al. (2019) classified infertility-related genes according to currently available evidence as non-existing, limited, moderate, strong or definitive based on 1337 publications (Oud *et al.*, 2019). Thirty-eight genes were classified as definitive, 22 as strong, and 32 as moderate, permitting the construction of an evidence-based infertility panel that could potentially facilitate the genetic diagnosis in infertile men. In this study, we sought to investigate the implication of definitive, strong and moderate genes as classified by Oud et al. in our sample of 6 SCOS individuals that had undergone whole-exome sequencing.

Material and methods

Sample

Six SCOS patients assessed for infertility treatment at the Department of Urology at Clinical Hospital Center, Zagreb, were included in the study. All patients signed the informed consent, and the Ethics Committee of the University Hospital Zagreb, Croatia, approved the study with the reference number 02/21 JG. Testicular samples were taken for a histopathological checkup during an open surgical testicular biopsy for testicular sperm extraction (TESE). Blood samples were taken for subsequent genetic analyses (Y-chromosome AZF microdeletions, *CFTR* mutations, karyotype) at the Department of Urology at the Clinical Hospital Center. The criteria for enrollment in our study were the diagnosis of Sertoli cell-only syndrome, which manifested in both testes and the exclusion of all known genetic variants (Y-chromosome AZF microdeletions, *CFTR* mutations, Klinefelter syndrome). All participants gave written, informed consent according to the study protocol.

Analysis

Genomic DNA was extracted from participants' peripheral blood samples using the standard extraction procedure (Invitrogen™ iPrep™ PureLink™ gDNA Blood Kit). Whole exome sequencing was performed in Macrogene Inc. using the NovaSeq6000 platform and Agilent SureSelect XT_V5+UTR library preparation kit. Obtained sequences were aligned to the human reference genome version hg38 (Schneider *et al.*, 2017) using the BWA-MEM software (Li, 2013). Variants were called with the HaplotypeCaller algorithm from GATK (McKenna *et al.*, 2010) and annotated with Jannovar (Jäger *et al.*, 2014) using the transcript definition database from UCSC (Karolchik, Hinricks and Kent, 2012).

For the purpose of this study, variants in strong, definitive and moderate genes classified by Oud et al. were assessed (Oud *et al.*, 2019). Variants with population frequency higher than 1 % in the ExAC database 0.3 (Karczewski *et al.*, 2017) and GnomAD database release 2.1.1 (Karczewski *et al.*, 2020) were not considered. Variant effect predictions were assessed using SIFT (Sim *et al.*, 2012), PolyPhen (Adzhubei, Jordan and Sunyaev, 2013) and CADD (Mahmood *et al.*, 2017). Only variants predicted as deleterious by SIFT and possibly damaging or probably damaging by PolyPhen and with a CADD score greater than 20 were considered and classified as likely causative. These variants were verified by Sanger sequencing. Briefly, target regions were amplified by PCR using the following primers: CHD7F: CTGTTCCCAAACAACTAGACATTG; CHD7R: AAGGAAATGCATAACGCGCA, SYCP3F: TCCCAATTTACCATTTCCTGTCT, SYCP3R: GGTCACGAAACGGAAAACCA. After amplification, PCR products were sent to Macrogen Inc. for Sanger sequencing.

Results and Discussion

Six patients diagnosed with SCOS were analyzed for possible causative variants in 92 infertility genes reported by Oud et al. (Oud *et al.*, 2019). Patients' characteristics - age, reproductive hormone levels and testicular volumes (where available) - are presented in Table 1.

Overall, eight variants passed our specified criteria for functional impact: *PKD1* (c.3098C>A), *PKD1* (c.3061G>T), *CYP21A2* (c.844G>T), *CHD7* (c.7702C>T), *FANCM* (c.1874G>C), *WDR66* (c.2123T>C), *CEP290* (c.1079G>A) and *SYCP3* (c.521C>T). All listed variants were detected in heterozygous form in our patients. Thus, we further down considered only variants in genes consistent with an autosomal dominant effect on infertility - *CHD7*, *SYCP3* and *PKD1* (Oud *et al.*, 2019). Among these, we could not verify *PKD1* variants by Sanger sequencing. *PKD1* gene has six pseudogenes on chromosome 16 that share 97.7% sequence identity, which can cause false-positive calls of *PKD1* pathogenic variants (Tan *et al.*, 2014). Since pathogenic *PKD1* variants are primarily associated with polycystic kidney disease, and our patients did not have any recorded manifestation of the disease, we did not attempt to further verify *PKD1* variants in our patients, assuming that those were false-positive NGS results. The variant c.7702C>T in *CHD7*, detected in the patient SCOS_03, had the following characteristics: MAF 0.01, SIFT prediction deleterious (0.02), PolyPhen prediction possibly damaging (0.807), and CADD value 29.5. This patient was 39 years old at the time of diagnosis. Follicle-stimulating hormone (FSH) values were 30 U/l, testosterone values were 15,7 nmol/L, and testicular volumes were 7,3 cm3. The patient did not have any male gland abnormalities, varicocele or any other acquired cause of infertility documented in his medical records. The variant c.7702C>T in *CHD7* has been reported in ClinVar as a variant with uncertain significance.

CHD7 gene encodes for chromodomain helicase DNA binding protein 7 gene (MIM 608892). This protein is a member of the CHD protein family, which has the common function of hydrolyzing ATP and changing the structure of the nucleosome (Marfella & Imbalzano, 2007). Mutations in the *CHD7* gene primarily cause CHARGE syndrome, which is characterized by many different features such as coloboma, heart defects, choanal atresia, retardation of growth and development, genital hypoplasia, and ear anomalies (Vissers *et al.*, 2004; Aramaki *et al.*, 2006; Zhou *et al.*, 2018). Milder mutations in *CHD7* are responsible for hypogonadotropic hypogonadism (HH) and Kallmann syndrome (KS) (Li *et al.*, 2020). *CHD7* mutations have a dominant effect in all these conditions. In a published review by Oud et al. 2019; *CHD7* is classified in the category definitive because male infertility is a common feature of HH and KS. However, our patient had no symptoms of hypogonadism or

other characteristics that could point to the conditions mentioned. His elevated FSH levels probably reflect his decreased testicular function (Gudeloglu & Parekattil, 2013). However, in a recent study, *CHD7* variants were identified in three patients who suffered from isolated severe oligozoospermia. The authors of the study reported them as variants of uncertain significance and concluded that *CHD7* variants might be associated with male infertility (Li *et al.*, 2020). The variant detected in *CHD7* was successfully verified by Sanger sequencing (Figure 1).

Table 1. Clinical characteristics of the sample

Sample	Age (years)	Testis volume (cm^3/mL)	FSH (U/L)	LH (U/l)	Testosterone (nMol/L)
SCOS_01	32	No data	13.18	3.34	9.03
SCOS_03	39	L 7, D 7.3	30	5.76	15.7
SCOS_04	35	L 7.5, D 10	27.3	8	7.1
SCOS_05	47	L 9.8, D 10	24	11.8	11.8
SCOS_07	34	L 7.1, D 2.04	18.1	5.6	14.4
SCOS_08	35	No data	29.3	10.2	5.4

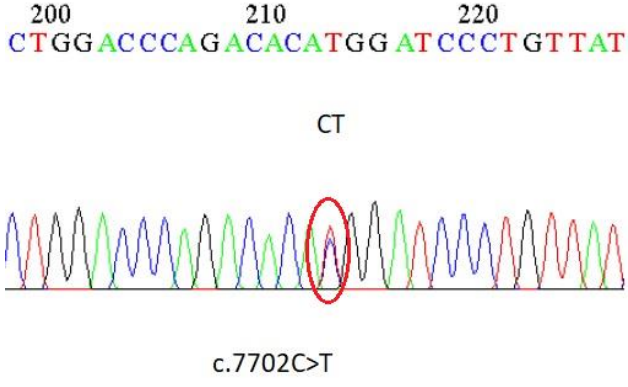

Figure 1. The *CHD7* variant detected in a male patient with SCOS is shown on the forward sequence electropherogram diagram after experimental validation using Sanger sequencing.

Furthermore, we detected variant c.521C>T in the *SYCP3* gene in the patient SCOS_05 with MAF value < 0.01, SIFT prediction deleterious (0), PolyPhen prediction probably damaging (0.999), and CADD value 29.5. This patient was a 47-year-old at the time of diagnosis. Follicle-stimulating hormone (FSH) values were 24 U/l, testosterone values were 11,8 nmol/L, and testicular volumes were 9,8 cm3 left and 10 cm3 right. This patient had cryptorchidism documented in his medical history.

The *SYCP3* gene encodes a DNA-binding protein, a component of the synaptonemal complex that regulates the synapsis between homologous chromosomes during the meiosis of germ cells (Yuan *et al.*, 2000).

SYCP3 involvement in male fertility has been previously established by research on knockout mice (Yuan *et al.*, 2000; Kolas *et al.*, 2004). Its participation in human infertility was first reported in 2003 by Miyamoto et al. (Miyamoto *et al.*, 2003), where the authors suggested that *SYCP3* has an essential meiotic function in human spermatogenesis that heterozygous mutations could compromise. Oud et al. classified this gene as moderately linked to isolated male infertility in the autosomal dominant way (Oud *et al.*, 2019).

Our findings could add to the evidence of *SYCP3* involvement in male infertility. The variant detected in *SYCP3* was successfully verified by Sanger sequencing (Figure 2).

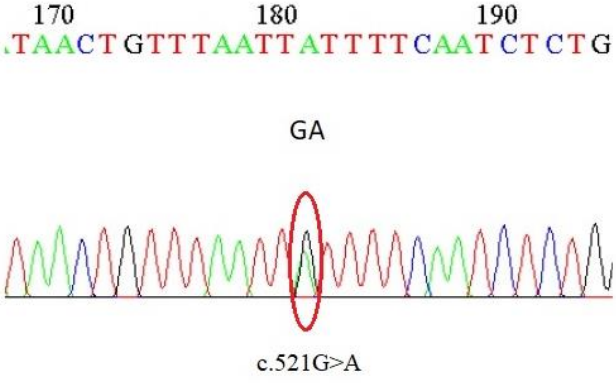

Figure 2. The *SYCP3* variant detected in a male patient with SCOS is shown on forward sequence electropherogram diagrams after experimental validation using Sanger sequencing.

Conclusion

In this study, we examined only genes with definitive, strong, and moderate effects on male infertility and detected likely causative variants in 2 out of 6 men with SCOS. There is emerging evidence that *CHD7* and *SYCP3* variants could have a causative role in male infertility. Our results support these genes' role in the isolated form of male infertility with an autosomal dominant effect. However, further studies will be needed to determine the real causative effects of these genes in SCOS.

Our results underscore the complexity of genetic diagnosis in infertile men. They suggest that careful selection of genes with an apparent causative effect on infertility is crucial, as well as a complete clinical picture of studied patients.

Hopefully, a growing number of studies will clarify the real causative impact of genes still in candidate status, ultimately leading to higher diagnostic yield in panel testing for male infertility.

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