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GENETICS

Deleterious mutation in *SYCE1* is associated with non-obstructive azoospermia

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Abstract
Purpose To determine the molecular basis of familial, autosomal-recessive, non-obstructive azoospermia in a consanguineous Iranian Jewish family.
Methods We investigated the genetic cause of non-obstructive azoospermia in two affected siblings from a consanguineous family. Homozygosity mapping in the DNA samples of the patients and their normospermic brother was followed by exome analysis of one of the patients. Other family members were genotyped for the mutation by Sanger sequencing. The mutation effect was demonstrated by immunostaining of the patients' testicular tissue.
Results The two patients were homozygous for a splice site mutation in *SYCE1* which resulted in retention of intron three in the cDNA and premature stop codon. *SYCE1* encodes a Synaptonemal Complex protein which plays an essential role during meiosis. Immunostaining of patient's testicular tissue with anti-Syce1 antibody revealed an undetectable level of Syce1. Histological examination of the patients' tissue disclosed immature-stages spermatocytes without mature forms, indicating maturation arrest.
Conclusion The significance of most synaptonemal complex proteins was previously demonstrated in a mutant mouse model. The present report underscores the importance of synaptonemal complex proteins in spermatogenenesis in humans. Our new approach, combining homozygosity mapping and exome sequencing, resulted in one of the first reports of an autosomal-recessive form of NOA.

Keywords Meiosis · Azoospermia · Maturation arrest · Exome sequencing

Capsule Splice site mutation in the SynaptonemaL Complex central Element 1 resulted in non obstructive azoopsermia in two siblings. The results confirmed by immunohystochemistry on testicular samples.

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Introduction

Male infertility accounts for 30–55 % of infertile couples. This is manifested by the high rate of men of reproductive age, 1 in 13, who require medical assistance in order to have a child [1]. Azoospermia, which is the complete absence of sperm in the ejaculate, accounts for 10–15 % of male infertility cases and generally affects 1 % of the male population [2]. Azoospermia is classified as obstructive or non obstructive (NOA). Obstructive azoospermia can result from blockage anywhere in the ductal system as a consequence of severe infection, iatrogenic injury or congenital anomalies.

Non obstructive azoospremia accounts for 60 % of cases of azoospermia and is caused by intrinsic testicular disease (primary testicular failure) or secondary testicular failure (endocrinopathy or other condition suppressing sperm production).

The evaluation for azoospermia includes endocrine tests and urologic and genetic evaluation. Currently the three most relevant genetic causes of azoospermia are: mutation in the CFTR gene (associated with congenital bilateral absence of the vas deferens and obstructive azoospermia), chromosomal abnormalities (such as Klinefelter syndrome) and microdeletions on chromosome Y, causing NOA [3]. Even after complete evaluation most cases of azoospermia are defined as idiopathic. Unfortunately, no medical treatment has proven reliably effective for improving semen parameters or fertility in men with idiopathic subfertility.

It has been previously proposed that azoospermia may result from single-gene disorders including haploinsufficiency of the Synaptonemal Complex protein SYCP3 [4]. Ayhan et al. recently published their findings regarding two mutations causing azoospermia in two consanguineous families [5]. It is reasonable to assume that additional genetic abnormalities might also explain male infertility.

Synaptonemal Complex Central Element 1 (Syce1) is a major component of the synaptonemal complex (SC), which is formed between homologous chromosomes during meiotic prophase and exists only during the first meiotic division [6]. Male *Syce1* null mice are infertile, have abundant primary spermatocytes, and lack postmeiotic cells [7]. In women, haploinsufficiency of *SYCE1* has been implicated in cessation of menstrual cycles in premature ovarian failure [8] while nonsense homozygous mutation in *SYCE1* was shown to cause premature ovarian failure in two daughters of a consanguineous family [9].

We report the results of molecular investigation of two azoospermic males originating from a consanguineous family linking human mutation in *SYCE1* with NOA. The results are followed by immunostaining of testicular tissue to support our findings.

Materials and methods

Patients

The subjects of the study, individuals II-2 and II-3, originated from a consanguineous (first cousins) Iranian-Jewish couple (Fig. 1). Both were referred for evaluation because of failure to reproduce. Their physical examination was unremarkable: height, weight and hair distribution were normal; testes were of normal size, volume and texture and no varicocelle was noted. Hormone profile including FSH, LH, and testosterone was also within normal limits. Sperm testing disclosed complete azoospermia. Further evaluation showed normal karyotype and analysis for deletions in the Y chromosome was negative. In both patients, testicular fine needle aspiration (TEFNA), repeated 20 times for each testis, revealed only primary spermatocytes without mature forms, indicating

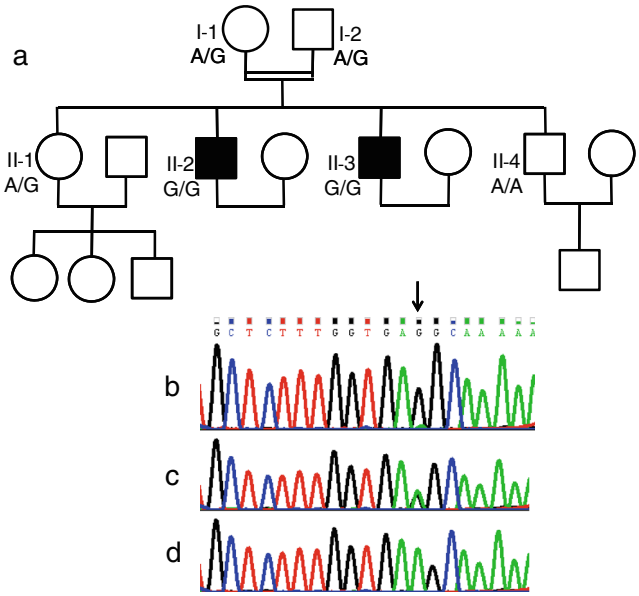

Fig. 1 Family pedigree and the effect of the *SYCE1* mutation. The patients are represented by filled symbols and the genotype at the mutation site is shown. The DNA sequence flanking the c.197-2 A>G mutation (*arrow*) in the *SYCE1* gene is shown in the DNA samples of a patient (**b**), a carrier (**c**), and a healthy control (**d**)

azoospermia due to maturation arrest (Fig. 2). In view of the complete absence of viable mature sperm both couples required sperm donation.

The family includes another healthy brother (II-4) and sister (II-1). The pedigree is shown in Fig. 1. Interestingly, the mother and sister in the family gave birth to their first offspring at the ages of 19 and 22, respectively, and the mother entered menopause at the age of 53.

All family members provided informed consent to participate in the study. The study protocol was approved by the Hadassah Medical Center and Ministry of Health Ethical Committees.

Homozygosity mapping

A search for common homozygous regions in the DNA of the affected siblings and their unaffected brother (II-4) was performed using the Affymetrix GeneChip Human Mapping 250 K SNP Array, as previously described [10]. In essence, this chip allows genotyping of SNPs with an average distance of ~ 50 kb between the markers. Homozygous regions >5.0 cM were manually detected. We therefore searched for common homozygous regions in the patients' DNA that were not shared by their fertile brother.

Whole exome sequencing

Whole exome sequencing, a technique for sequencing all the protein-coding genes in a genome (known as the exome), was performed on DNA from patient II-2 using the SureSelect

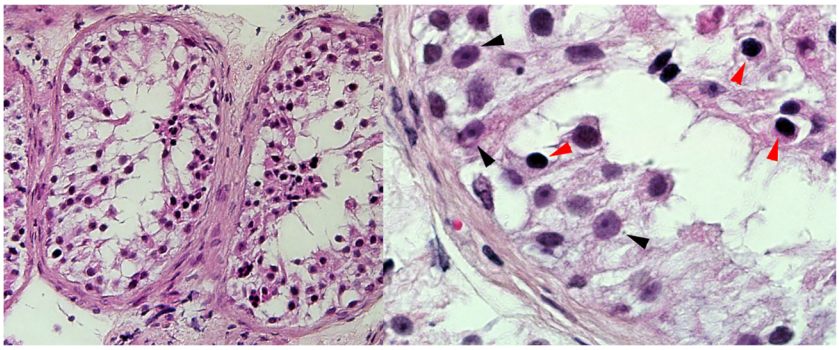

Fig. 2 H&E of patient II-3. H&E staining on paraffin sections of TEFNA taken from patient II-3 showing complete meiotic maturation arrest. On the right, high magnification showing Sertoli cells (*black arrowheads*) and arrested spermatogonia (*red arrowheads*). As a result of the TEFNA procedure, the specimens were overall poor of cells; therefore the sections were assessed by two different pathologists to conclude the diagnosis

Human All Exon V.2 Kit (Agilent Technologies, Santa Clara, California, USA) on HiSeq2000 (Illumina, San Diego, California, USA) as 100 bp paired-end runs. Reads alignment and variant calling were performed with DNAnexus software (Palo Alto, CA) using the default parameters with the human genome assembly hg19 (GRCh37) as a reference.

Immunohistochemistry

TEFNA of three controls and patients II-2 and II-3 were embedded in paraffin and 2 μm sections were immunostained using the BenchMark XT automated staining instrument (Ventana). The antibody used was anti SYCE1 (NBP1-88971, Novus) diluted 1:200.

Results

The two infertile brothers, II-2 and II-3, were affected with NOA; histopathological examination disclosed maturation arrest (Fig. 2). In light of parental consanguinity, we assumed a founder mutation transmitted in an autosomal-recessive manner. We therefore searched for common homozygous regions in the patients' DNA, which were not shared by their unaffected brother, II-4. This analysis, performed with DNA SNP array, resulted in the identification of four regions larger than 2 Mb, chr3:133172767-147368416, chr7:7344050-9178462, chr10:127938705-135422505 and chrX:2690310-33162851 (numbering according to HG19). The regions, spanning a total of 53.9 Mb, encompassed hundreds of protein-coding genes. We therefore opted for whole exome sequencing in the DNA sample of patient II-2. Average exome coverage was x86.2 and 92.5 % of the exons were covered to>x7. The resultant variants were filtered and heterozygous, synonymous, low-covered (<x7), off target, and those present in dbSNP132 or the in-house dbSNP were removed. Thirty-seven variants survived this process, however only three of them resided within the shared homozygous regions and only one, chr10:135372457, c.197-2 A>G in the *SYCE1* gene, was predicted pathogenic by Mutation Taster software [11]. The mutation was examined by Sanger sequencing in all family members and was found to segregate with the disease (Fig. 1). The mutation was absent from 55 Iranian Jewish fertile males and was not present in the 6503 exome analyses of healthy individuals available through the Exome Variant Server [NHLBI Exome Sequencing Project, Seattle, Washington, USA (http://evs.gs.washington.edu/EVS-v.0.0.21) (Aug 30, 2013)].

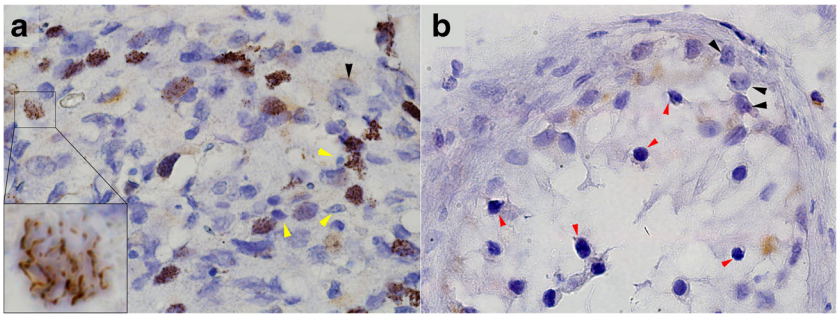

Fig. 3 Immunohistochemistry staining with anti-Syce1 antibody showing cross paraffin sections through the testicular seminiferous tubules of control (**a**) and patient II-2 (**b**). While in control sample Syce1 is localized to the chromatin at various spermatocytes maturation stages (inset in A highlighting a nucleus during pachytene stage), in the patient sample it is completely absent (arrested spermatogonia are marked by red arrowhead in B). *Black arrowheads* indicate Sertoli cells both in control and patient samples. *Yellow arrowheads* indicate late and mature spermatids, which do not express Syce1

The *SYCE1* gene consists of 13 exons encoding 351 amino acids protein (GI:118573896). The c.197-2 A>G mutation disrupts the acceptor site of intron 3. Our findings could be confirmed either by generating cDNA from the patients' testes, or employing immunohistochemistry at the protein level. Since the biopsy samples were embedded in paraffin in 2006, during clinical the evaluation of the patients, we were unable to generate cDNA from the testes. cDNA was generated from peripheral leukocytes (data not show) but was inconclusive. Immunostaining of testicular tissue from our patients using anti-Syce1 antibody disclosed undetectable levels of the Syce1 protein (Fig. 3). For validation purposes Western blot analysis on testis total protein extract yielded a single band at the predicted size of SYCE1 - ~50 kDa (Fig. 4).

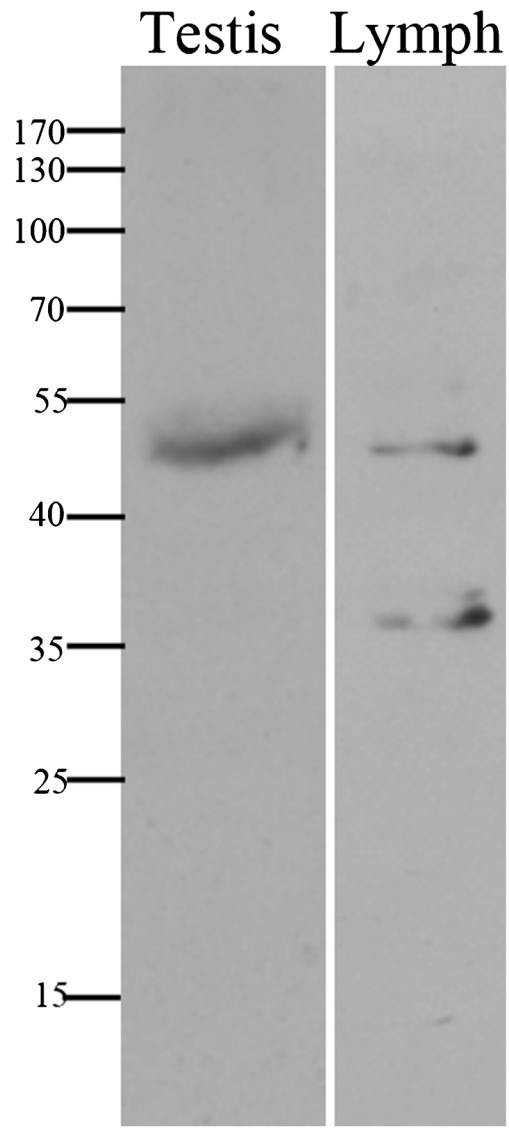

Fig. 4 Western blot. Normal testis total protein was extracted from TEFNA biopsy using radioimmunoprecipitation assay (RIPA)buffer. 40ug of protein was separated and analyzed by Western blot on 10 % SDS-PAGE and probed with anti-Syce1 Ab (1:1000; NBP1-88971, Novus). A single band at the predicted size of Syce1, ~ 50 kDa, is evident, underscoring the specificity of this antibody to Syce1 on testicular tissue

Discussion

Here we show for the first time that the human SYCE1 gene plays an essential role during meiosis. Syce1 is a major component of the synaptonemal complex (SC), which is formed between homologous chromosomes during meiotic prophase and exists only during the first meiotic division [6].

The significance of most SC proteins was previously demonstrated in mutant mouse models. These mice were invariably infertile, with the exception of female mutants in *Sycp2* and *Sycp3*, which had litters of reduced size and increased aneuploidy [12]. The importance of *SYCE1* for the more advanced stages of meiosis was underscored by the presence of early forms of spermatocytes with absence of mature forms in the testicular tissue of our patients (Fig. 3), consistent with maturation arrest. The male *Syce1* null mice had abundant primary spermatocytes and lacked postmeiotic cells; arrested cells were likely eliminated by accelerated apoptosis [7]. However, in mice, and likely in humans, SYCE1 is essential for stable homologue interactions mediated by the SC and crossover formation.

Notably, apart from NOA, our patients, just like the *Syce1* null mutant mice, were healthy and had no systemic symptoms, indicating that SYCE1 is required for meiosis only.

Haploinsufficiency of *SYCE1* was implicated in very early cessation of menstrual cycles in one case of premature ovarian failure [8]. While nonsense homozygous mutation in *SYCE1* was shown by Varies et al. to cause premature ovarian failure in two daughters of a consanguineous family [9], the normal menstrual cycles observed in the mother and sister of our patients, both heterozygous for the *SYCE1* null allele, do not support haploinsufficiency of *SYCE1* as a cause of premature ovarian failure.

The present report underscores the importance of *SYCE1* for SC formation, completion of meiosis and fertility in humans. In order to estimate the contribution of *SYCE1* mutations to human NOA, we determined the DNA sequence of the *SYCE1* coding region in ten unrelated NOA patients of various ethnic origins. This analysis did not detect additional mutations in the gene, suggesting that *SYCE1* mutations are not a common cause of NOA.

The main limitation of our study is our inability to support our finding by cDNA from the testes.

Our study is one of the first to present genetic causes linked to the mechanism of NOA in humans. Moreover it highlights the power of exome sequencing as a primary diagnostic tool that could be used to elucidate the genetic etiology of NOA in general.

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Conflict of interest The authors have no conflict of interest to declare.

Contributor statement YC, BY, AS, HG, SZ, CR, OE and AF conceived and designed the experiments; EMS, YC, BY, AS, HG, and SZ performed the experiments; all the coauthors analyzed the data and participated in the writing of the paper; EMS undertook patient management, collection of samples, and delineation of the phenotype. The research was supported in part by a grant from the Joint Research Fund of the Hebrew University and Hadassah.

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