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CASE REPORT

A HOMOZYGOUS DELETION OF THE *DPY19L2* GENE IS A CAUSE OF GLOBOZOOSPERMIA IN MEN FROM THE REPUBLIC OF MACEDONIA

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ABSTRACT

Globozoospermia is a rare but severe teratozoospermia, characterized by ejaculates consisting completely of round-headed spermatozoa that lack an acrosome or, in partial globozoospermia, containing a variable proportion (20.0-90.0%) of acrosomeless spermatozoa. Men that are affected with total globozoospermia are infertile, and even the application of intracytoplasmic sperm injection (ICSI) has met with disappointingly low success rates.

In humans, several case reports of globozoospermia have demonstrated that two or more siblings were affected in each family, which suggested a genetic component to this disease. Currently, three genes are known to be associated with total globozoospermia in humans, *SPATA16*, *PICK1* and *DPY19L2* genes. Mutations in *SPATA16* and *PICK1* are rare causes of globozoospermia, found in only one patient each. Several studies have suggested that DPY19L2 mutations are the major cause of globozoospermia in patients from different ethnic origins and different geographic regions. The most common *DPY19L2* mutation is the 200 kb deletion arising from a nonallelic homologous recombination (NAHR) between the flanking low copy repeats (LCRs). Here we describe the presence of a homozygous deletion of the *DPY19L2* gene in two infertile Macedonian patients with 100.0% round headed spermatozoa, thus suggesting that this deletion represents a major cause of globozoospermia among Macedonian men.

Keywords: Globozoospermia; *DPY19L2* gene; Intra cytoplasmic sperm injection (ICSI); Nonallelic homologous recombination (NAHR); Low copy repeats (LCRs); Deletion(s); Male infertility

INTRODUCTION

Globozoospermia is a rare but severe form of teratozoospermia, accounting for less than 0.1% of male infertility [1]. It is characterized by ejaculates consisting completely of round-headed spermatozoa that lack an acrosome (total globozoospermia) or, in partial globozoospermia, containing a variable proportion (20.0-90.0%) of acrosomeless spermatozoa.

The genetic component to globozoospermia was suggested many years ago by several case reports which demonstrated that two or more siblings were affected in each family [2-5]. Furthermore, genetic studies in mice have provided direct evidence that disruption of several genes, including *GOPC* (Golgi-associated PDZ and coiled-coil motif containing protein), *HRB* (HIV-1 Rev binding protein) and *CSNK2A2* (casein kinase 2, α prime polypeptide) results in a globozoospermia phenotype with decreased fertility. However, there has not been a clear link between homozygous mutations in these genes and globozoospermia in humans [6].

Currently, three genes are known to be associated with total globozoospermia in humans. In 2007, a

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genome-wide study of six brothers from a consanguineous Ashkenazi Jewish family suggested that a homozygous mutation (c.848G>A or R283Q) in *SPATA16* (spermatogenesis-associated 16) was associated with male infertility in human globozoospermia [7].

Later, using the candidate gene screening strategy, a homozygous missense mutation (G198A) in exon 13 of the *PICK1* (protein interacting with C kinase 1) gene was identified in a Chinese family. The family member affected by this homozygous missense mutation showed a complete lack of acrosome [8]. However, no mutations in these genes were detected in other men with globozoospermia, thus suggesting that *SPATA16* and *PICK1* genes are not the main loci associated with this condition [9].

In 2011, a large homozygous deletion, encompassing ~200 kb, including the entire *DPY19L2* (protein dpy-19 homolog 2) gene was identified in four (out of 21 screened) unrelated patients [10]. At the same time, a study of 20 patients with globozoospermia originating mainly from Tunisia identified the *DPY19L2* deletion, at a much higher rate (75.0%), thus suggesting that deletions involving this gene might be a major cause of globozoospermia [11]. This was confirmed when a larger cohort including 64 globozoospermic patients was screened. This study showed that the *DPY19L2* gene was mutated in 66.7% (36 out of 64) globozoospermic patients [12]. In addition to the deletion, several point mutations were also identified. Out of 36 patients with the mutated gene, 69.4% were homozygotes, 19.4% were compound heterozygotes for both this deletion and a point mutation and 11.1% showed a homozygous point mutation. Molecular analysis of the *DPY19L2* gene among Chinese globozoospermic patients revealed that a genetic defect was present in nine (60.0%) of the 15 unrelated patients [13]. Four patients were homozygous for the deletion and five were homozygous for a point mutation. This study confirmed that DPY19L2 mutations are the major cause of globozoospermia in patients from different ethnic origins and different geographic regions.

MATERIALS AND METHODS

Among more than 200 infertile men, two patients with 100.0% of globozoocephalic spermatozoa with no acrosome in their semen were recruited for genetic study at the Macedonian Academy of Sciences and Arts, Research Center for Genetic Engineering and Biotechnology (RCGEB) "Georgi D. Efremov", Skopje, Republic of Macedonia laboratories. Patient 1 was a 30-year-old man with two unsuccessful intra cytoplasmic sperm injection (ICSI) attempts, while patient 2 was a 35-year-old man who has previously experienced three unsuccessful ICSI attempts. Both patients were of Macedonian ethnic origin, living in Tetovo, a town in the western part of the Republic of Macedonia. Although they originated from the same town, they were not related and no consanguinity was reported in their families.

The search for mutations in the *SPATA16* and *PICK1* genes was performed on genomic DNA by sequencing of all exons and exon/intron boundaries using BigDye™ Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM™ 3100 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA, USA). The oligonucleotide primers used for polymerase chain reaction (PCR) and sequencing were designed by Primer 3 software [14] and synthesized by Integrated DNA Technologies, Coralville, IA, USA.

The search for copy number variants (CNVs) was performed by oligonucleotide array comparative genomic hybridization (aCGH) using the 180K Agilent Human Genome CGH Microarrays and Genomic Workbench software (Agilent Technologies, Santa Clara, CA, USA). The deletion at 12q14.2, including the entire *DPY19L2* gene, was confirmed with classical PCR using seven loci, of which three were intragenic, located on exons 1, 11, and 22 of the *DPY19L2* gene, while the other four were located in the low copy repeat (LCR) regions. The primers in the centromeric LCR1 region, named LCRa and LCRb, were localized approximately 25 and 9 kb 3' of DPY19L2, respectively, and the primers in the telomeric LCR2 region, named LCRc and LCRd, were localized approximately 62 and 77 kb 5' of DPY19L2, respectively [11]. For further confirmation of the deletion we performed a gap-PCR, using primers flanking the DPY19L2 deletion [10]. This PCR generates a fragment of 1700 bp only in the patients carrying the *DPY19L2* deletion.

RESULTS

We have sequenced the *SPATA16* and *PICK1* genes in the first globozoospermic patient (patient 1). However, we found no mutations that might be responsible for the infertility in this patient. Only two polymorphisms in the *SPATA16* gene were identified:

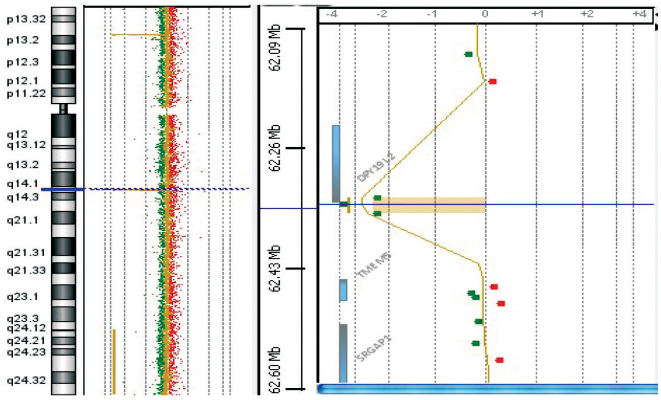

Figure 1. Oligonucleotide array CGH of patient 1 showing the homozygous deletion of three probes in the telomeric region of DPLY19L2 gene.

rs 115897458 or codon 18 (C*A*T>C*G*T) (His→Arg) and rs508508 or codon 225 (A*C*T>A*G*T) (Thr→Ser).

Then, aCGH analysis was carried out in patient 1 and showed the presence of a homozygous deletion including the *DPY19L2* gene (Figure 1). Because of the low number of probes in that region, classical PCR was performed for narrowing down the breakpoints and confirming the deletion. Patient 2 was studied by PCR analysis only. Both patients showed the same pattern of deletion breakpoints with PCR amplification only of outer loci of LCR region and no amplification of the inner loci LCR regions and the three exons in the *DPLY19L2* gene (exons 1, 11 and 22), (Figure 2), with the maximum size of the deleted region being approximately 210 kb. The gap-PCR analysis generated a PCR fragment of approximately 1700 bp in both patients with globozoospermia, thus confirming the presence of a *DPLY19L2* gene deletion.

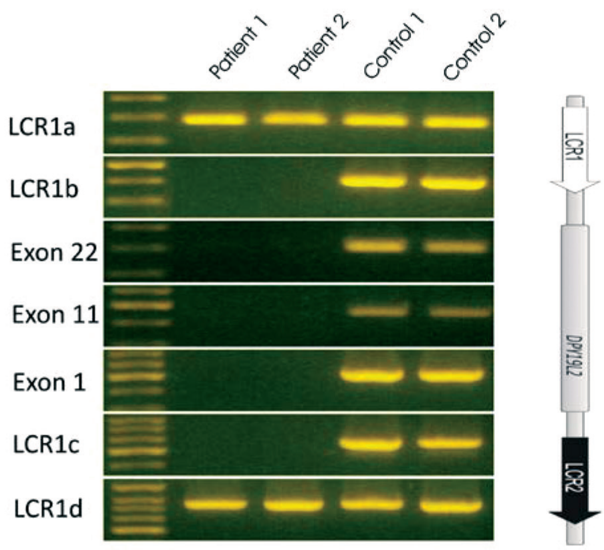

Figure 1. Polymerase chain reaction analysis of the DPY19L2 gene in two patients and controls showing that the deletion breakpoints are localized extragenically within the LCR regions.

DISCUSSION

Here we present the genetic analysis of two patients with 100.0% round-headed spermatozoa. A homozygous deletion of the *DPY19L2* gene was detected in both patients, thus suggesting that this deletion represents a major cause of globozoospermia among Macedonian men.

The mechanism underlying the recurrent *DPY19L2* gene deletion is a nonallelic homologous recombination (NAHR) between the two highly homologous 28 kb LCRs present on each side of *DPY19L2* [10]. Thus far, nine breakpoint zones have been identified in patients from different regions [12,13]. The fact that the same breakpoints are shared by patients from different regions and ethnic origin, and that patients from the same country have different breakpoints, excludes the founder effect and strongly suggests that the deletion results from recurrent events linked to the specific architectural feature of this region.

Intracytoplasmic sperm injection is the only treatment for patients with globozoospermia. However, fertilization rates after ICSI in these patients are severely reduced [7,15]. Fertilization failures in the patients with globozoospermia have been attributed to a deficiency in oocyte activation capacity [16]. A recent study of a large cohort of globozoospermic patients has shown that the fertilization rates after ICSI with assisted oocyte activation (AOA) are restored to normal when compared with conventional ICSI in globozoospermic patients with and without a mutations in the *DPY19L2* gene [17]. Thus, it was proposed that the first-line therapeutic approach for complete globozoospermia should include ICSI with AOA regardless of the *DPY19L2* status. Although at present the molecular diagnosis does not influence the choice of treatment in patients with globozoospermia, it is very important for adequate genetic counseling of couples with this rare form of male infertility.

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