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Research Article

SCREENING FOR THE HOMOZYGOUS C.144DELC MUTATION IN *AURKC* GENE IN ALGERIAN INFERTILE MEN

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ABSTRACT

Objective: Male infertility is a major health problem worldwide. Despite recent advances, the etiopathogenesis of spermatogenic failure remains largely uncertain. Aurora kinases, a family of serine/threonine kinases, consisting of Aurora A (AURKA), Aurora B (AURKB), and Aurora C (AURKC), are essential kinases for cell division through regulating mitosis and meiosis. The aim of this study was to investigate the frequency of c.144delC mutation in *AURKC* gene in infertile Algerian patients with abnormal sperm parameters.

Methods: In this study, 40 infertile men with impaired spermatogenesis (Azoospermia "AZOs,"" oligoasthenoteratospermia "OATs,"" Asthenospermia "ASTs") were recruited from Ibn Rochd clinic and Ibn Sina Laboratory, between 2008 and 2014. All men were of Algerian origin. DNA was extracted from peripheral blood. The third exon of the *AURKC* gene was amplified using polymerase chain reaction (PCR). Then, PCR products were sequenced using the big dye V1.1 terminator cycle sequencing in forward and reverse directions, and the results of sequenced segments were analyzed.

Results: Sequencing of the third exon of *AURKC* gene revealed the absence of c144delC mutation in all of the 40 patients screened.

Conclusion: Our data indicate that the AURKC c.144delC mutation must be investigated in infertile men with Macrozoospermia.

Keywords: AURKC, c144delC mutation, Male infertility, Sequencing, Algerian patients.

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INTRODUCTION

In recent decades, infertility has been considered to be a major public health problem worldwide. At present, male factors account for approximately 50% of cases which may exist alone or in conjunction with female factors [1,2]. Male infertility could be due to genetic and/or environmental factors. It is a complex problem where not only the genes but also the epigenetic factors play a key role. The alterations of epigenome have the same consequences as DNA mutations [3,4].

About 40% cases of male infertility are classified as idiopathic. These cases may be linked to genetic and genomic abnormalities. Chromosomal abnormalities and polymorphisms, microdeletions of the Azoospermia factor regions of the Y chromosome and cystic fibrosis transmembrane conductance regulator mutations have been demonstrated to be recurrent genetic causes of male infertility [2,5-8]. During the last years, more genes are implicated in male infertility leading to decline in the prevalence of idiopathic etiology [8,9].

The genetic association between the c144delC mutation of the *AURKC* gene and male infertility with macrozoospermia is well established especially in North African patients [10,11].

Aurora kinase family is comprised regulators controlling chromosome segregation and condensation, centrosome duplication, G2/M transition, kinetochore attachment, and cytokinesis [12]. Three types of Aurora kinases are present in mammalian cells, AURKA, AURKB, and AURKC which have specific subcellular localizations and functions during the cell cycle. Aurora kinase C (AURKC) is a serine/threonine protein kinase and is expressed in both somatic neoplastic and germ cells [13-15].

Expression of Aurora C was first described in the testis [16] and is involved in chromatin condensation and proper attachment of homologous chromosomes during the first meiotic division [17-19]. In humans, the absence of a functional protein of AURKC is associated with male infertility [10,11]. Macrozoospermia in men with AURKC mutations is due to a failure to complete meiosis I (MI) [20].

On the basis of these considerations, we constructed this study to investigate the prevalence of c.144delC frameshift mutation in *AURKC* gene in a cohort of Algerian patients with impaired spermatogenesis and to clarify that this mutation is correlated with macrozoospermic phenotype.

METHODS

This study included a total of 40 subjects of Algerian origin referred to our laboratory and clinic for routine semen analysis. Peripheral blood samples were collected after explaining the study objectives. All patients gave their written informed consent. The study was approved by the local Ethics Committee.

DNA extraction and DNA sequencing

Leukocyte genomic DNA was extracted from blood samples using the standard method of salting out. DNA was quantified using a Nanodrop Spectrophotometer.

Polymerase chain reaction (PCR) was performed in a mixture of the following composition: 20–50 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5.0U/µL of Taq DNA polymerase (Bioline), and 100 ng/µL of each primer. The reaction was processed in a thermocycler as follows: Denaturation for 5 min, at 95°C, followed by 40 cycles of 30 s, at 95°C (denaturation), 30 s at 66°C, and 30 s at 72°C (extension).

Oligonucleotide primers used for PCR and minisequencing of *AURKC* exon 3: Forward, 5'–GACTTTCCCTCCGCCTACCCTAC 3' and reverse 5'GCTGGGCTCAGACGTCAAAGA 3'.

Table 1: Sperm parameters and karyotype of analyzed patients (*n*=40)

Spermatic anomalies	Number of patients	Sperm parameters (average)			Karyotype
		Sperm count (million/mL)	Motility %	Abnormal morphology %	
Azoospermia	26	0	-	-	46, XY
Oligoasthenoteratozoospermia	12	3.1±1.7	4±0.06	96±3.38	46, XY
Asthenozoospermia	2	36.11±0.015	08.25±0.25	47.5±0.5	46, XY

The PCR products were submitted to agarose gel electrophoresis 2%, and afterward, the PCR products were purified using exonuclease I and phosphatase alkaline. These products were then sequenced (10–15 ng DNA template reaction) on automated DNA sequencher using the big dye V1.1 terminator cycle sequencing. To reveal the electrophoresis data, the peak signal was analyzed with sequencher 4.7.

RESULTS

Of the 40 cases, there were 26 cases with Azoospermia, 12 cases with Oligoasthenoteratozoospermia, and 2 cases with Asthenozoospermia (Table 1).

Direct sequencing of AURKC exon 3 revealed no mutation or c.144delC recurrent deletion in all patients screened (Fig. 1).

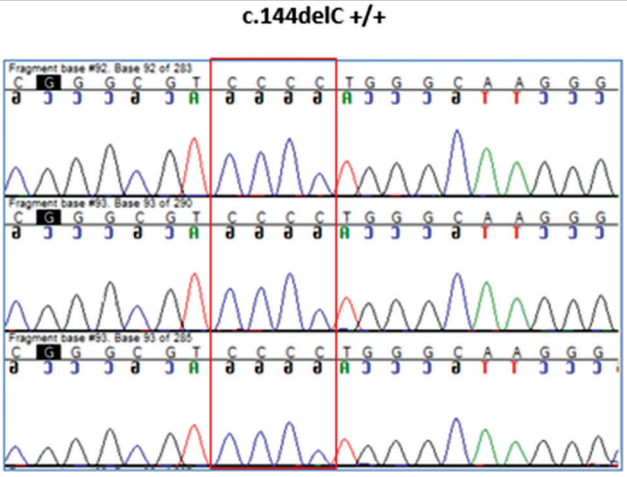

Fig. 1: Electrophoregram showing part of AURKC exons 3 from 3 subjects

DISCUSSION

To knowledge, the present study is the first to investigate the frequency of C144del in a cohort of patients (40) with abnormal sperm parameters.

The c.144delC frameshift mutation is expected to have a severe impact on the protein function. Translation of the mutated gene produces a short 71–amino acid peptide that lacks nine-tenths of its kinase catalytic domain which makes it inactive leading to a defective meiosis with a blockage of spermatogenesis before the first meiotic divisions and the production of tetraploid large-headed multiflagellate spermatozoa [10].

Few studies examined the association of c.144del C of *AURKC* gene with male infertility. All of them concerned North African populations [10,11,21-25]. Therefore, further studies in different populations are essential.

Our study revealed no mutation in the *AURKC* exon 3 gene in all of the patients who were analyzed.

Previously, Dieterich *et al*., [10,11] through two studies examined infertile men presenting typical spermatozoa mainly characterized by large heads, a variable number of tails and an increased chromosomal content and identified a common region of homozygosity harboring the aurora kinase C gene with a single nucleotide deletion in the AURKC coding sequence (c.144Cdel). All AURKC mutated patients either lived or originated from North Africa (Morocco, Tunisia, and Algeria). In agreement with our results, the same researchers [10,11] investigated the frequency of this genetic defect by analyzing 30 patients (among these patients: 19 were Iranian patients, and nine were principally of French origin) who did not fit the criteria defined for typical large-headed patients. No mutations were identified in any of these 30 patients after double strand sequencing of the entire coding sequence and intron boundaries of the *AURKC* gene.

Moreover, Molinari *et al*. [26] reported a case of recurrent miscarriage in a patient affected by a variant phenotype of sperm macrocephaly syndrome. However, no mutations detectable by *AURKC* sequencing have been observed.

Two studies have been conducted in Morocco and confirmed the high prevalence of the homozygous mutation c.144delC in the teratozoospermia with large-headed spermatozoa [22,24]. Eloualid *et al.* screened 326 idiopathic infertile patients for the presence of the AURKC c.144delC mutation and found a frequency of 2.14% in patients at homozygous and heterozygous states. Homozygous patients were characterized by macrocephalic and multiflagellate spermatozoa.

In 2011, Ben Khelifa *et al.* [21] studied two brothers from Tunisian descent who presented typical macrozoospermia with a few non-megaloheaded spermatozoa. Molecular analysis revealed the presence of a new heterozygous variant, c.436-2A>G, identified in both patients. This mutation is located in the acceptor consensus splice site of exon 5. These results indicate that AURKC molecular analysis of patients with large-headed spermatozoa should not be stopped in the absence of a homozygous recurrent mutation on exon 3 but complete sequence analysis should be performed. The same authors in 2012 have identified a novel nonsense mutation in the AURKC exon 6 gene, p.Y248*, in 10 unrelated individuals of European (*n*=4) and North African origin (*n*=6) [23].

In Tunisia, Ghédir *et al.* [25] evaluated the frequency of c.144delC mutation among infertile and control populations and showed that this mutation is relatively less frequent in the Tunisian population (0.4%).

Ounis *et al.* [27] have also carried out molecular analysis for the *AURKC* gene in patients with typical macrozoospermia and found 11 patients with this frameshift mutation among the 14 Algerian patients studied.

Furthermore, Chianese *et al.* [28] screened 3 patients with macrozoospermia for c144del C mutation and found that the two macrocephalic patients with the North African origin (Moroccan) carried the c.144delC mutation. The other patient (Spanish origin), as well as his brother, were homozygous for the p.Tyr248* (c.744C>G) mutation in exon 6.

Recently, Carmignac *et al.* [29] found by Sanger sequencing c.144delC homozygous mutations in the *AURKC* gene in two monozygotic twins presenting macrozoospermia and have recommended that the *AURKC* gene should be sequenced when the sperm contains 30% or more of enlarged head spermatozoa, and when a mutation is found, artificial reproductive technology should not be performed.

CONCLUSION

No mutations have been found in Algerian patients with abnormal spermogramms. However, we were limited by the small sample of patients, and the criteria of selection of patients who must have

macrozoospermia with a majority of large-headed spermatozoa with up to four flagella, which might decrease the power of our study, and thus, we recommend further larger studies to evaluate the frequency of this mutation in patients and control groups.

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AUTHORS CONTRIBUTION

Rezgoune Mohamed Larbi: PCR, Sequencing and writing manuscript. Chellat Djalila: recruitment of patients, DNA extraction and critical revision of the manuscript. Abadi Noureddine: Data analysis, Director of the biology and molecular genetics laboratory. Satta Dalila: Director of the cellular and molecular biology laboratory and final approval of the version to be published.

CONFLICT OF INTEREST

No conflict of interest exists.

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