INTRODUCTION

The World Health Organization estimates that one in every four couples in developing countries is confronted to infertility. Among couples with a desire for a child, male factor contributes approximately half of the cases. Despite long years of assisted reproductive activities, a significant number of cases remain idiopathic. It is likely that most ‘idiopathic’ forms may have a genetic origin. At present, only a dozen of genes have been identified as responsible of an infertility phenotype in men when mutated.

Using exome sequencing, we identified two new genes involved in non-obstructive azoospermia and oligozoospermia phenotype in consanguineous Turkish families.

RESULTS

Two Turkish consanguineous families having three well documented male infertility cases as well as at least two unaffected brothers were included in the study (figure 1). All affected males had a normal karyotype and no Y-chromosome microdeletions were found.

A whole exome sequencing of two affected males per family was performed by IGBMC microarray and sequencing platform.

Figure 1: Family pedigrees. Samples for which exome sequencing was performed indicated with blue arrow. Blue asterisk showed the mutated male with an unknown fertility status.

Given the known consanguinity in the family, we hypothesized that the disease should follow an autosomal recessive inheritance mode and we thus tracked homozygous variants shared by both samples.

Indeed, for family A, we found a stop-gained mutation in an autosomal gene, leading in early translocator arrest in the first 1/3 of the protein. Interestingly, the human phenotype is highly similar to the one observed in the mouse KO for the orthologous gene. Indeed, male KO mice are infertile with small testis and a meiotic arrest at the first wave of spermatogenesis is observed.

It should be noted that patient IV:5 in family A was diagnosed as severe oligozoospermia in 2010, however in 2014, a new semen analysis resulted in an azoospermia; no spermatid was detected even after concentration, suggesting a decrease of sperm count with time.

For Family B, no autosomal mutation was found. A deeper scanning of the genome reveals a no-stop mutation in an X-linked gene which causes the production of an extended protein by turning the stop codon into amino acid coding codon, leading to a 23 amino-acid extension of the protein.

Both identified genes are presenting a testis specific expression. Expression pattern was confirmed by RT-PCR on different human tissues and on testicular tissues with different histology (figure 2)

Analyzing the other family members, we could show that both mutations are co-segregating with the infertility phenotype. In addition, we could show the absence of two mutations in any of databases that collect human sequences and repertory polymorphisms among the human population and of both mutation in 107 healthy matched fertile Turkish controls were assessed. Altogether, this strongly suggest that the two identify mutations are responsible of the spermatogenic defect observed in the two families.

We also analyzed 92 unrelated Turkish individual infertile cases with non-obstructive azoospermia or severe oligozoospermia all exon and exon/intron boundaries of the two identified genes, no mutation was found.

Given the potential functional and biological consequences of both mutations, we hypothesized that both mutations could be present in the sample of a Turkish couple newly diagnosed with infertility.

CONCLUSION

We hypothesize that identified mutation in family A may correlate with a decrease on sperm count in time, while mutation in family B shall indicate a presence of focal spermatogenesis. A diagnostic test identifying the mutation in man could prompt patients to undertake measures of precaution such as sperm cryopreservation at an early age.

Our understanding of genetic basis of male infertility has large implications not only for understanding the cause of infertility but also in determining the prognosis and management of such couples, and to provide maximum adapted therapeutics and counseling to the couple.