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Review

Autosomal mutations and human spermatogenic failure ☆

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ABSTRACT

Infertility, defined as the inability to conceive after 1 year of unprotected intercourse, is a healthcare problem that has a worldwide impact. Male factors are involved in at least half of these cases of infertility. Despite 33 years of assisted reproductive activities, a considerable number of cases (25–30%) remain idiopathic. This situation can be explained by a poor understanding of the basic mechanisms driving male and female gametogenesis. Compared to multi-organ pathologies, only a few non-syndromic genetic causes of human infertility have been described so far, despite the fact that it is estimated that some infertility cases could be explained by genetic causes and that over 200 infertile or subfertile genetic mouse models have been described. So far, very little has been discovered in the field of human male reproductive genetics. Consequently, genetic tests proposed to infertile couples are limited, although worldwide efforts devoted to the field of human genetics of infertility are expected to provide new genetic tests in the near future. We present the requirements for performing informative genetics studies in the field of infertility, the techniques used and the results obtained so far. This article is part of a Special Issue entitled: Molecular Genetics of Human Reproductive Failure.

1. Introduction

Infertility, defined as the inability to conceive after 1 year of unprotected intercourse [1], is a healthcare problem that has a worldwide impact. Based on a recent report, the prevalence of infertility was estimated to be 9%. This represents more than 70 million infertile women, of whom more than 40 million are seeking infertility treatment [2]. Male factors are involved in at least half of these cases of infertility [3]. Infertility in men can be due to many causes. Among these cases, some of them are clearly part of genetic syndromes. Indeed, infertility is associated with other complex phenotypes such as sex reversal syndrome (involving genes such as *SOX9*, *SRY*, and *NROB1*), hypogonadotropic–hypogonadism defects (involving genes such as *GNRH*, *KAL*, *PC1*, *GNRHR*, *LEP*, and *PCSK1*), myotonic dystrophy (gene *DMPK*), a mild form of cystic fibrosis and many others [4]. For example, mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene are detected in infertile men exhibiting azoospermia with congenital bilateral absence of vas deferens (CBAVD) [5]. Men with CBAVD undergo normal or slightly reduced spermatogenesis and present clinical cystic fibrosis symptoms, suggesting that CBAVD is a mild form of this syndrome [5]. Compared to these multi-organ pathologies, only few non-syndromic genetic causes of human infertility have been described so far.

Despite 33 years of assisted reproductive activities, a considerable number of cases (25–30%) remain idiopathic [1,6]. This situation can be explained by a poor understanding of the basic mechanisms driving male and female gametogenesis, despite the fact that some infertility cases could be explained by genetic causes [1,7] and that over 200 infertile or subfertile genetic mouse models have been described [4]. This number gives an idea of the number of genes that could be involved in male and female gametogenesis and, consequently, the complexity of the process. So far, very little has been discovered in the field of human male reproductive genetics. This slow progress has two main causes. (i) Clinical evaluations are preliminary and often limited to the presence or absence of spermatozoa in the ejaculate. This has been reinforced by the advent of the intracytoplasmic sperm injection (ICSI) technique, which requires a very limited number of spermatozoa, even immotile ones, to fertilize the cohort of oocytes. (ii) Spermatogenesis is a highly sophisticated process involving complex molecular pathways requiring hundreds of genes. Consequently, genetic tests proposed to infertile couples are limited to

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karyotype analysis, CFTR mutation screening and Y-chromosome deletion screening [1]. The worldwide efforts devoted to the field of human genetics of infertility are expected to provide new genetic tests in the near future. At the present time the field is mainly, but not exclusively, concentrating its effort on male genetics of infertility. Indeed, because of the easy access to male gametes, diagnoses are of better quality, allowing a better categorization of the different phenotypes, which is fundamental for carrying out genetic studies.

In this review, we will focus on non-syndromic genetic defects affecting human male gametogenesis, presenting a monogenic autosomal recessive mode of transmission. We present the requirements for performing informative genetics studies in the field of infertility, the techniques used and the results obtained so far.

2. Requirements for the identification of mutated genes in human infertility

The first major requirement for identifying mutated genes in infertile patients is the quality of the clinical diagnosis. Indeed, any improper diagnosis will bias the selection of patients to be included in the study and introduce confusion in the analysis, rendering the genetic analysis difficult or even impossible.

Different causes have been described for male infertility. Defective sperm can be due to the male partner not producing any spermatozoa at all (azoospermia) or in insufficient numbers (oligozoospermia), without adequate motility (asthenozoospermia), without normal morphology (teratozoospermia) or a combination of these defects. Thus, the clinical features of male infertility vary from azoospermia to oligoasthenoteratozoospermia.

In order to eliminate frequent causes of oligozoospermia or azoospermia, a pre-screen of the recruited patients has to be performed. Indeed, chromosomal abnormalities including both numerical (aneuploidy) and structural aberrations (translocations/inversions) account for 5% of oligozoospermia and 15% of non-obstructive azoospermia (NOA) [8]. For instance, Klinfelter syndrome (XXY), the most common abnormality found in NOA [9], causes a maturation arrest of spermatogenesis at the primary spermatocyte stage. Furthermore, microdeletions of the Azoopermia factor region (AZF) of the Y chromosome are frequently observed in oligozoospermic to azoospermic men [10]. It should be noted that external factors, such as environmental pollutants on human gametogenesis, may also have potential deleterious effects.

The second major requirement is the ability to study informative patients. Indeed, nonetheless geneticists need well diagnosed patients, but they need whenever possible large families or cohorts of patients.

Two approaches are commonly used to identify genetic causes of pathologies, and these can also be applied to the genetics of infertility: reverse and forward genetics [7]. The reverse approach, also known as the candidate gene approach, is initiated by selecting genes from infertile animals, mainly mouse models, assuming that the gene function is conserved through evolution. Such an approach needs many well characterized patients and a large amount of time. So far, the discoveries resulting from this approach have been limited [11–17]. This is probably due to the great genetic heterogeneity and the limited number of patients tested.

Forward genetics, also known as positional cloning or descent studies, benefit from the recent and ongoing evolutions in molecular biology. Indeed, new powerful tools are continuously being developed and have reached the scale of whole genome analysis, facilitating the identification of recessive autosomal mutations. The main approach, homozygosity mapping, is based on genotyping studies and aims to investigate, for a single patient, thousands of single nucleotide polymorphisms (SNPs) simultaneously on a microarray. The success of homozygosity mapping studies strictly depends on the availability of patients and their rigorous selection (Fig. 1).

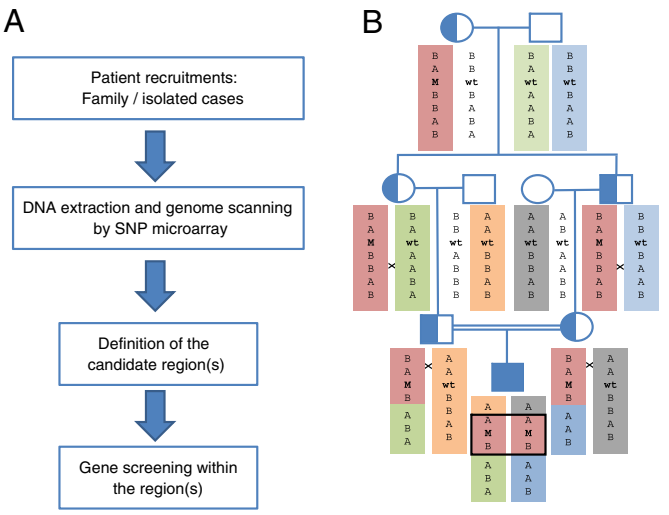

Fig. 1. Linkage analysis. (A) Positional cloning procedure. The positional cloning procedure used to identify candidate genes. A first critical step is the recruitment of the patients. Two groups of patients are of interest: (1) patients presenting a familial infertility, where the families contain at least two infertile members, and (2) patients from a small isolated geographic area or tribes, where the abnormality has a relatively high occurrence. In both cases, patients should present a non-syndromic infertility. A whole genome scan by SNP microarray is performed in order to select a locus shared only by the patients and absent in non-affected ones. Once the region is defined, a bioinformatics screen is performed to select candidate genes within the region. (B) Principle of homozygosity mapping. A patient with a rare genetic disorder resulting from consanguineous parents inherited a mutation (M) on both chromosomes, which was present at the heterozygous state in a common ancestor (great-grandmother in this diagram). The polymorphic markers located in the chromosomal region around the gene are represented by different colors and letters depending on the alleles. Because of various events of meiotic recombination (represented by crosses), the alleles of markers far from the gene change and are therefore heterozygous in the patient, while markers close to the mutated gene are transmitted in a block (called haplotype) from the great-grandmother and are therefore in the homozygous state in the patient. They define a region of homozygosity by descent (framed region).

3. Technologies

As mentioned above, the reverse or candidate genes approach is initiated by selecting candidates genes based, mainly, on mouse models. As soon as a convergence of phenotypes is observed between a specific mutated mouse gene and a human phenotype, mutations in the human ortholog gene can be screened for [7].

Forward genetics or positioning cloning is based on the whole genome scan by SNP microarray technology, which allows the identification of homozygous regions shared by affected siblings of the same family and absent in non-affected siblings [18,19]. The candidate regions, selected on the basis of consecutive homozygous SNPs, are then screened using a genome browser in order to list the candidate genes (Fig. 1). The genes are ranked according to their expression profile, which in this case must be predominant in the testes, and their potential function. Once genes are chosen, PCR and sequencing of all exons and exon/intron junctions are performed in order to highlight the mutation involved in the patient infertility.

SNP microarrays have tremendous advantages over previous genotyping methods as they are very dense and cover the whole genome [18,19]. It is a fast technique that requires only a small amount of genomic DNA (250 ng). In contrast, genomic regions generated by homozygosity mapping are generally multiple megabases in size and can contain multiple genes. Therefore, the identification of the mutated gene from a large number of candidates is expensive and time consuming. This is where the number of patients or the size of the family is of great importance. Indeed, the higher the number of siblings in the family or the larger the patient cohort, the smaller the homozygous region should be, limiting the number of candidate genes to be screened.