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Advances in understanding the genetics underlying male infertility and evolving diagnostic and treatment options

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The 5th Florence-Utah International Symposium on Genetics of Male Infertility was held in Florence, Italy, in September 2013. This meeting has offered a unique opportunity for large, in-depth discussions on the genetic and epigenetic aspects of male reproduction. Thirty oral presentations and 56 poster presentations provided a global view on recent advances in the field. The identification and characterization of genetic and epigenetic factors of male infertility is not only important for diagnostic purposes but also for their potential implications to future offspring. Despite efforts, genetic testing in male infertility is still restricted to karyotype analysis, Y chromosome microdeletion screening and monogenic mutation screening in case of specific phenotypes such as hypogonadotrophic hypogonadism and congenital absence of the vas deferens. While new epigenetic tests of sperm are on the horizon, genetic testing of sperm is currently limited to sperm DNA fragmentation testing, a topic that was discussed in a special affiliated session of the meeting that included the formulation of plans for standardization and inter-laboratory comparisons of testing protocols (see Robaire *et al.*, this issue of *Andrology*).

Concerning the routine genetic testing of the male, a special session was dedicated to AZF deletions of the Yq, with the aim of discussing the novel European Academy of Andrology (EAA)/European Molecular Genetics Quality Network (EMQN) Guidelines. It was reported that the original protocol based on two multiplex PCR reactions remains fully valid and appropriate for accurate diagnosis of complete AZF deletions and requests only a minor modification in populations with specific Y chromosome background. It was emphasized that novel methods and kits with excessively high numbers of markers do not only not improve the sensitivity of the test, but may even lead to misinterpretation of the results. The important definition of the extension of the AZFa and AZFb deletions has been largely debated with the panel of experts and precise indications for novel 'extension' markers are now given in the new guidelines (Krausz *et al.*, 2014). The long-lasting discussion about the clinical significance of gr/gr deletions was also addressed with special emphasis on the meta-analysis data, reporting it as a significant genetic risk factor for impaired sperm production, and on the importance of Y chromosome background in the phenotypic expression of this genetic anomaly. The 12 year experience of the EMQN/EAA external quality control scheme showed a steep decline in diagnostic genotyping error rate and a simultaneous improvement on reporting practice.

Spermatogenesis requires the concerted action of >2000 genes and mutation of any of these genes may be responsible for infertility. Similar to other polygenic complex diseases, the promising approach using genome-wide association studies (GWAS) to search for disease-causing variants has not led to a substantial advancement in identifying novel infertility-associated variants. GWAS studies have been performed in the United States (Kosova *et al.*, 2012; Zhao *et al.*, 2012) and in China (Aston *et al.*, 2012; Hu *et al.*, 2012; Xu *et al.*, 2013) and despite the large study population size of the Chinese studies, no overlapping genetic risk factors were identified. The reason for the lack of validated disease-associated variants is probably related to two major factors. First, due to the individual low effect size of distinct single nucleotide polymorphisms (SNPs), only an extremely large study population (>10 000 subjects) would be able to detect their association with the disease. Second, the phenotypic heterogeneity of the study group is an important limitation, as differences in severity of spermatogenic impairment (azoospermia vs. severe and moderate oligozoospermia) and even 'azoospermia' with different testis histology (Sertoli cell-only syndrome, spermatogenic arrest and hypospermatogenesis) represent distinct 'pathologies' and therefore should be studied as separate disease groups.

The initial enthusiasm for SNPs arrays originates from the 'common SNPs – common disease' hypothesis, which would fit also with the relatively high frequency of male infertility. However, if common SNPs play any role in spermatogenic impairment, it is more likely that their combined effect rather than the effect of single SNPs has a pathogenic relevance. Again, the testing of this hypothesis requires very large studies based on homogeneous infertile phenotype groups.

The role of rare variants in male infertility has been proposed already in the first GWAS study, in which a higher load of rare

SNP variants in azoospermic men in respect of oligozoospermic or normozoospermic men was observed (Zhao *et al.*, 2012). This observation has been further confirmed by studies focusing on structural variations, such as copy number variations (CNVs). All three studies dealing with CNVs at the whole genome and at the X-chromosome level reached the same conclusion, that is a significantly higher deletion load in patients in respect of controls was observed (Tuttelmann *et al.*, 2011; Krausz *et al.*, 2012; Lopes *et al.*, 2013). This observation was particularly evident on the X chromosome, where most observed CNVs were rare, private variants. The follow-up study of X-chromosome-linked deletions identified the first recurrent, patient-specific deletion (Lo Giacco *et al.*, 2014) with potential diagnostic value. X chromosomes have acquired a significant number of genes involved in male reproduction (Mueller *et al.*, 2013) and it is expected that mutations in X-linked genes would contribute to a proportion of impaired sperm production. Clearly, the X chromosome is among the most promising targets for future mutation analyses by next generation sequencing.

In addition to these basic questions, the conference also addressed many other timely topics. Those topics included various aspects of the growing field of sperm epigenetics, including novel data regarding the establishment of the sperm epigenome throughout the stages of spermatogenesis, the role of the sperm epigenome in embryogenesis and the role of environmental factors on the sperm epigenome. It is increasingly clear that environmental influences on fertility may largely be mediated through epigenetic modifications. Such changes may be temporary, but some evidence points to a more permanent establishment of epigenome modifications that may influence offspring and future generations. It is likely that this area of study will be productive and may lead to profound insights into male infertility. Other key areas of discussion and presentation included the risks of environmental factors on gamete quality, including the effects of paternal aging, as well as the potential for personalized follicle stimulating hormone therapy through pharmacogenetic studies.

The Florence-Utah Symposium on the Genetics of Male Infertility provides a highly interactive forum to address the advances and translational impact of our increasing understanding of genetic and epigenetic mechanisms and factors that affect male infertility. We are pleased to include in this issue of *Andrology* a focus on some of the relevant topics in this important field.

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