Seeking the elusive genes associated with varicocele: a step forward

It is well established that varicocele can harm testicular function and fertility, mainly through thermal damage and oxidative stress (1). However, the reasons why the disease affects each individual's fertility in differing ways are not entirely understood. Moreover, the exact genetic background of familial varicocele and whether genetic factors increase the predisposition to varicocele occurrence are unknown. The multiplicity of phenotypes associated with varicocele suggests a complex multifactorial disorder in which genetic, epigenetic, and environmental factors seem to play a decisive role (1, 2).

Genetic expression alterations and epigenetic changes are associated with varicocele (1–4). For example, infertile men with varicocele differentially express proteins related to sperm mitochondrial structure and function (vs. fertile controls), suggesting that sperm mitochondrial dysfunction (e.g., excessive reactive oxygen species, reduced ATP synthesis) is involved in the varicocele pathophysiology (3). Furthermore, in a study including 26 infertile men with varicocele and 26 fertile men without varicocele, we found that the former exhibited global sperm hypomethylation compared with the controls (4). We identified 59 differentially methylated CpG sites and 1,695 differentially methylated DNA regions in sperm of men with varicocele. These regions relate to spermatogenesis, meiotic and meiosis cell cycle, and semen quality based on gene ontology analysis (4). Thus, we hypothesize that sperm methylation changes, particularly hypomethylation, may decrease semen quality of men with varicocele, ultimately leading to infertility. The above examples illustrate the clinical significance of transcriptome studies in elucidating possible new venues for varicocele diagnosis and treatment.

In a recent report, Yang et al. (5) used whole-transcriptome RNA sequencing (RNA-seq) and whole-exome sequencing (WES) to improve our understanding of varicocele occurrence and development. The authors first created a varicocele animal model to identify candidate genes with differential expression and sought potential deleterious variants in blood samples of 11 men with an early-onset varicocele and their fathers who also had a varicocele. The latter is critical to evaluate the role of genetic background on familial varicocele. Subsequently, they genotyped a large group of patients with clinical (palpable) varicoceles and controls without varicocele to assess the variants related to varicocele risk and disease development. Yang and colleagues identified three candidate genes associated with varicocele risk: *AAMP*, *SPINT*, and *MKI67*. These genes are involved in angiogenesis, tumorigenesis, and cell proliferation. Moreover, they described four biological pathways possibly involved in varicocele development, all related to angiogenesis. Their data support the notion that varicocele family history has a genetic background and that genetic factors are associated with varicocele occurrence and development.

Yang and coworkers' candidate genes were obtained from RNA-seq analysis of blood samples extracted from left spermatic veins of rats with induced varicocele, whereas the WES analysis was conducted with peripheral blood from men with varicocele. It is, therefore, unknown whether their results would still hold had a human model been used. Although establishing a human model might be challenging, we feel that future studies should analyze testicular blood samples of men with varicocele undergoing scrotal surgeries (e.g., vasectomy) to check if the candidate genes, as shown by these authors, remain valid.

Moreover, Yang and colleagues' study primarily involved WES and RNA-seq analyzes of blood samples. Therefore, it is impossible to establish whether the changes in gene expression found in blood samples also occur in sperm. The WES analysis of sperm (vs. blood) should be preferred as it might identify germline variants eventually missed in an analysis of somatic cells that would be transmitted to the offspring. Further studies are certainly warranted to fill these gaps in knowledge.

Unfortunately, Yang and coworkers' study was not designed to compare exome and transcriptome profiles between infertile versus fertile varicocele patients. Although the authors provided some gene expression data for these men, the results were not conclusive. The reasons may relate to the fact that only a small subset of the studied individuals was infertile, and the population of men with varicocele and presumed fertility was not adequately characterized. Because the authors did not formulate a hypothesis to explain how exome changes could impact gene expression, further studies are warranted to elucidate the relationship between these genes and infertility in men with varicocele.

Infertility remains the primary concern of reproductive urologists providing care to patients with varicocele. We need reliable markers to identify young adults (and possibly adolescents) in whom a varicocele will harm testicular function and fertility and for whom an early intervention may be beneficial.

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