REVIEW

Genetics and epigenetics of varicocele pathophysiology: an overview

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Abstract Varicocele is found in approximately 20% of adults and adolescents and in 19–41% of men seeking treatment for infertility. It is associated with a decrease in sperm count as well as sperm motility and morphology. The currently accepted description of the pathophysiology of varicocele does not explain all its clinical manifestations; therefore, other factors such as genetic and epigenetic changes, associated with the environment, might be involved in causing infertility and decrease in sperm quality. It has been reported that the varicocele-induced deterioration of testicular function is progressive and interferes with fertility; hence, early and efficient assessment of the genetic manifestations in patients would be important for developing future medical interventions. Chromosomal disorders, mutations, polymorphisms, changes in gene expression, and epigenetic changes have all been reported to be associated with varicocele. Several studies are underway to unravel the genetic basis of this disease, as it is important to understand the origin and the aggravating factors to ensure appropriate guidance and intervention. Here, we review the available literature regarding the genetic and epigenetic changes associated with varicocele, and how these alterations are related to the different clinical manifestations of the disease.

Keywords Varicocele · Male infertility · Genetics · Epigenetics · Genome

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Introduction

The term “varicocele” was introduced by Curling in 1843 [1] and refers to dilatation of testicular veins in the scrotal portion of the pampiniform plexus. Varicocele is found in approximately 20% of adults and adolescents and in 19–41% of men seeking treatment for infertility [2]. Clinical varicocele can be diagnosed through physical examination, while the subclinical form can only be observed via Doppler ultrasound [3].

Studies have shown that varicocele causes a progressive decline in fertility; one study reported that 69–81% of men with secondary infertility have the disease [4, 5]. In 1965, MacLeod [6] observed that most semen samples of patients with varicocele showed low sperm count, decreased motility, and morphological abnormalities. A significant decrease in sperm motility and abnormal sperm morphology were reported by Xue et al. [7] and Kadioglu et al. [8] as well. Surgical correction has been suggested as a possible treatment option to reduce damage to sperm and to improve the seminal parameters [9–11].

The testicular damage has been attributed to increased scrotal temperatures and venous pressure, accumulation of toxic substances, hypoxia, hormonal dysfunction, autoimmunity, oxidative stress, and apoptosis [12–14]. However, the currently accepted description of the pathophysiology of varicocele does not explain all its clinical manifestations [15]. Thus, other effects must interact with the disease to produce infertility.

One study suggested that infertility in patients with varicocele has a complex and multifactorial etiology, in which genetic alterations and environmental factors contributed to the disease progression, further reducing the spermatozoa quality and leading to infertility [16]. It has also been demonstrated that men with a familial history of varicoceles, especially in first-degree relatives, have an increased risk of disease occurrence [17, 18], indicating that the genetic factors may play an important role in the disease development.

Some patients with varicocele were reported to have normal a sperm count and were fertile, while others with the same severity of varicocele showed no improvement in fertility even after varicocelectomy [19]. This variability of clinical phenotypes related to varicocele also suggests the presence of unknown associated genetic factors. Chromosomal alterations and genetic and epigenetic modifications may be important in the etiology of the disease. Understanding the origin and the factors that cause complications in varicocele is important for developing a treatment and for appropriate guidance and intervention. This review focuses on the major genetic and epigenetic factors reported to be associated with varicocele (Table 1) in order to provide a better understanding of the mechanisms involved in the pathophysiology of this disease.

Sperm DNA fragmentation

Sperm DNA damage is the major cause of male infertility, miscarriages, abnormalities in the offspring, and failures in assisted reproduction cycles [44]. This damage has

multifactorial etiology and may occur spontaneously or by environmental causes, such as toxins and pollutants, drugs, chemo-radiation, cigarette smoking, febrile illness, and advanced age [45–47]. Besides that, failures in the spermatogenesis process may result in the generation of spermatozoa with high levels of DNA damage [44], especially DNA fragmentation [48]. DNA fragmentation also may be a result of elevated reactive oxygen species (ROS) production, together with decreased antioxidant capacity [49]. This imbalance in ROS levels causes lipid peroxidation of the sperm plasma membrane, deficiencies in the protamination process, and damage to nuclear and mitochondrial DNA [12, 50–52].

Oxidative stress is one of the major causes of infertility in men with varicocele [12, 53–57]. In the pathophysiology of the disease, the nitric oxide released by the endothelial cells of dilated veins and the peroxynitrite generated by the reaction with the superoxide radical are a major cause of oxidative damage in gametes [58]. Therefore, the spermatozoa of patients with varicocele exhibit high ROS production and deficiency in the pro-oxidant defense system [12, 59, 60], further increasing oxidative DNA damage, base modification, DNA strand breaks, and chromatin cross-linking [5].

Table 1 (Epi)Genetic alterations reported in varicocele

Article	Age	Grade	Diagnosis	Alteration
[20]	*	*	*	Microdeletions in Y
[21]	33	I, II, III	*	Chromosomal abnormalities, microdeletions in Y
[22]	*	*	*	Microdeletions in Y
[23]	32	Small	*	Chromosome 14 ring
[24]	33	Severe	Sonography	46,X,idi(Y)(q11.22)[58]/45,X[12]
[25]	*	*	*	Abnormal frequency of disomy and diploidy
[26]	29	I, II, III	Palpation and Doppler duplex ultrasound	Abnormal frequency of <i>GSTM1</i> and <i>GSTT1</i>
[27]	*	I, II, III	Palpation	Abnormal frequency of <i>GSTM1</i> and <i>GSTT1</i>
[28]	27	*	Doppler duplex ultrasound	Polymorphism in <i>ACPI</i> gene
[29]	27	I, II, III	Palpation	Polymorphic allele A1298C
[30]	28	I, II, III	Palpation	Abnormalities in <i>TNP1</i>
[31]	28	I, II, III	Palpation and Doppler duplex ultrasound	Abnormal frequency of G894 T
[32]	31	I, II, III	Palpation	Deletions in the mtDNA
[33]	32	I, II, III	Palpation	Polymorphism of <i>POLG</i>
[34]	17	II, III	Palpation	Abnormal <i>HSPA2</i> expression
[35]	—	—	Induced in rats	Abnormal <i>PK2</i> mRNA expression
[36]	31	I, II, III	Palpation and Doppler duplex ultrasound	Abnormal <i>BAX</i> and <i>BCL2</i> expressions
[37]	*	*	*	Abnormal <i>AR</i> expression
[38]	—	—	Induced in rats	Abnormal <i>AR</i> expression
[39]	24	III	Palpation	Abnormal <i>MT1M</i> , <i>PHLDA1</i> , <i>CCIN</i> , and <i>PRM2</i> expressions
[40]	32	I, II, III	Palpation and Doppler duplex ultrasound	Abnormal <i>Ropporin</i> expression
[41]	*	II, III	*	Abnormal methylated DNA
[42]	31	II, III	Palpation and Doppler duplex ultrasound	Abnormal methylated DNA
[43]	29	II, III	*	Abnormal miR-15a expression

*Information not reported by the authors; — studies in models or rats

It has been suggested that patients with infertility and varicocele were observed to have significantly increased DNA-damaged sperm [61–65]. Seminal samples from patients with varicocele contain a higher proportion of spermatozoa with abnormal and immature chromatin than that in samples from fertile and infertile men without varicocele [66]. An overall estimate by Wang et al. [67] indicated that patients with varicocele have significantly more sperm DNA damage than fertile controls, with a mean difference of 9.84%. Enciso et al. [68] reported that the frequency of spermatozoa with fragmented DNA was 32.4% in patients with varicocele, which is 2.6 times higher than that obtained in fertile individuals.

The DNA quality in men with varicocele seems to be positively influenced by the varicocelectomy since a decrease in DNA fragmentation has been observed after treatment of the disease [8, 67, 69–72]. This improvement in the DNA fragmentation percentage can be explained by the reduction of oxidative stress and the normalization of ROS production after surgical treatment, which has been observed even in patients who did not have other changes in seminal quality [73–75].

Somatic chromosomal alterations

Chromosomal alterations are the most common genetic abnormalities in infertile men; these are present in 2.1–15.5% of infertile men, a higher frequency than that found in the general population (0.65%) [20, 76]. Some studies in patients with varicoceles have reported an association between the disease and chromosomal abnormalities [20–24].

Rao et al. [21] reported that chromosomal abnormalities and microdeletions in the Y chromosome were associated with varicocele. Among the men analyzed, 19.3% showed changes in autosomal chromosomes, including inversions on chromosomes 9 and 2, translocations between chromosomes 4 and 15, a deletion in chromosome 4, and insertion on chromosome 9. Additional chromosomal material on chromosomes 21 and 22 was observed in 10.5% of the patients, while the karyotype 46,XY add(22)(p13) was reported in half of them. In addition, 5.3% of patients with varicocele had microdeletions in sY153, sY158, and sY254 regions of the Y chromosome. Although no significant differences were found in the frequencies of these microdeletions on the Y chromosome, this study was the first to demonstrate a direct association between genetic defects and varicoceles and subsequent infertility.

Another study that investigated the frequency of microdeletions on the Y chromosome in patients with varicocele reported microdeletions in regions of the azoospermia factors AZFa, AZFb, and AZFc on the long arm of the Y chromosome in 6.5% of individuals [22]; similar deletions were found in a study by Dada et al. [20]. Because Y-linked

genes are important in testicular determination and can affect sperm production [77], these microdeletions on the Y chromosome may be responsible for the severe bilateral testicular damage observed in patients with varicocele.

The presence of a chromosome 14 ring was reported in a patient with primary infertility and a left-sided varicocele [23]. The exact breakpoint could not be identified, but seemed to be in the paracentric region, between 14p11.1 and 14q11.2 [47,XY,r(14)(p11.2q11.2)]. Another study reported a patient with varicocele and an isodicentric Y chromosome and a karyotype of 46,X, idic(Y)(q11.22)[58]/45,X[12], a structural rearrangement commonly found in azoospermic men, resulting in the exclusion of a part of the long arm and the doubling of the short arm of chromosome Y [24]. However, these studies did not determine if the varicocele was a phenotypic expression of these chromosomal alterations or a genotypic variation that may or may not be associated with other diseases.

Sperm chromosomal alterations

The gametes of infertile men with altered sperm morphology and motility show high rates of chromosomal abnormalities [78], which originate mostly from meiotic errors [79]. However, even men with normal karyotypes have been reported to have altered spermatozoa [80].

Abnormal meiotic segregation in spermatozoa of men with varicoceles was observed by Baccetti et al. [25]. The authors observed that the mean frequency of disomy and diploidy of the sex chromosomes in sperm samples of 46,XY patients with varicocele was higher than that in samples from the control group. These data support the hypothesis that varicocele exert a negative effect on spermatogenesis, which may also affect the chromatin structure and, consequently, the chromosome segregation during meiosis.

Acar et al. [81] compared the frequency of chromosomal aneuploidy in the spermatozoa nuclei of patients with varicocele before and after varicocelectomy. Chromosomes 1, 16, 17, and 18 were evaluated, and a lower frequency of chromosomal aneuploidy was detected in spermatozoa after surgical treatment, although this difference was not statistically significant.

Mutations and gene polymorphisms

Given the high number of genes linked with human spermatogenesis, it is possible that idiopathic testicular insufficiency in infertile men has genetic origins. Mutations and gene polymorphisms are frequently observed in infertile men and may be associated with several diseases. Several studies have demonstrated an association between varicocele and

polymorphisms, including single-nucleotide polymorphisms (SNPs) [30, 82].

Glutathione *S*-transferases (GSTs) are enzymes that play an important role in the detoxification of products subjected to oxidative damage or exposure to carcinogens [83]. The genes that encode two GST isozymes, *GSTM1* and *GSTT1*, are known to be highly polymorphic in humans. In both cases, a deletion of the gene is responsible for the presence of a null allele, and homozygous individuals for this allele do not have functional enzymes [84]. Because men with varicocele have elevated levels of oxidative stress, it has been proposed that the higher susceptibility to ROS may be related to these polymorphisms [84].

Tang et al. [26] reported a frequency of 47.7% for null *GSTM1* and 44.6% for null *GSTT1* in patients with varicocele, while in control subjects, the frequencies were 43.3 and 50.0%, respectively. Similar results were reported by Acar et al. [27]; men with higher grades of varicoceles showed a higher frequency of these two polymorphisms than control individuals; however, the difference was not statistically significant. It has also been reported that these genetic variations could negatively affect the result of varicocelectomy in individuals with a mutated allele of the *GSTT1* gene [84].

Another polymorphism associated with varicocele is of the *ACP1* gene (acid phosphatase locus 1), which has three alleles *A, *B, and *C [28]. The *ACP1* protein causes a decrease in the activity of platelet-derived growth factors (PDGFs) through the dephosphorylation of PDGF receptors [85]. PDGFs are known to regulate the development of gonads during the embryonic and post-natal period [86]. Gentile et al. [28] reported that men with varicocele and the *B/*C genotype showed alterations in seminal parameters. This genotype is associated with a higher enzyme activity, and therefore reduced sperm count and abnormal sperm morphology, than individuals who do not have this genotype.

The *MTHFR* (methylenetetrahydrofolate reductase) gene encodes an enzyme involved in the conversion of homocysteine to methionine, releasing a methyl radical that is used by DNA-methyltransferase [87]. Thus, *MTHFR* plays a fundamental role in regulating the addition of methyl groups during DNA replication, an important mechanism of regulation during spermatogenesis [88]. The most well-known polymorphisms of the *MTHFR* gene are the SNPs C677T and A1298C, which reduce the activity of the enzyme [82]; these SNPs are also associated with low semen quality and infertility [89, 90]. According to a study by Ucar et al. [29], men with a homozygous genotype (1298AA) for the polymorphic allele A1298C have a 2.3-fold higher risk of having varicocele compared with men who do not have this genotype.

In another study, a base substitution was identified in the intronic region of *TNP1* (transition nuclear protein-1) in individuals with varicocele; although still controversial, the results suggested that this SNP might have been related to infertility in these patients [30]. *TNP1* encodes nuclear transition

proteins that function in chromatin remodeling [91], and these proteins maintain the integrity of the DNA in the sperm head, thus protecting it against attacks from exogenous and endogenous agents [92]. Mutations in *TNP* have also been reported to be associated with DNA damage in patients with azoospermia [93], probably by causing abnormal chromatin condensation and increased frequency of DNA breaks [94].

High concentrations of nitric oxide (NO) and high activity of nitric oxide synthase (NOS) have been observed in the seminal plasma of patients with varicocele [31]. The release of NO leads to a relaxation of smooth muscle cells and vascular dilation [95]; when released in excess, it causes an increase in ROS [96]. *NOS3* (nitric oxide synthase) gene has three main polymorphisms: T-786C, G894T, and 4b/a. The frequency of the genotype G894T in patients with varicocele was higher than that in the control group (varicocele, 47.1%; control, 33%), suggesting that this polymorphism may contribute to the pathophysiology of the disease [31].

Changes in sperm mitochondrial DNA

Deletions in the mitochondrial DNA (mtDNA) of spermatozoa have been reported in men with varicocele [97–99]. A study conducted by Gashti et al. [32] showed that 81.7% of patients with varicocele had a deletion of 4977 base pairs in the mtDNA of spermatozooids, while only 15.5% of the controls had the same deletion. In comparison with the nuclear DNA, mtDNA is highly sensitive to oxidative damage associated with the varicocele phenotype. Thus, high rates of ROS may induce deletions in mtDNA in spermatozoa. In addition, the deletions are frequently present in regions with multiple structural genes that would be transcribed into enzymes of the electron transport system, causing defects in ATP production, sperm immobilization, and infertility [32].

Polymorphisms in the polymerase gamma gene (*POLG*) have been associated with varicocele. *POLG* is a nuclear catalytic subunit located in the mitochondria and is involved in the replication and repair of mtDNA [100]. A CAG repeat polymorphism of *POLG* has been reported in cases of oligospermia in infertile men, in which the altered allele displays more than ten CAG repeats [101]. In patients with varicocele, although no significant differences were found in the groups studied, they proposed that *POLG* CAG repeat extensions is a contributing genetic risk factor that affects varicocele grade, and the possible mechanisms remain elusive and require further studies [33].

Gene expression

An important feature of the human semen is the high amount of messenger RNAs (mRNAs) stored during spermatogenesis.

mRNAs are important tools for identifying genes related to the production of spermatozooids and, consequently, genes related to infertility [102–104]. Using mRNAs, several studies have reported altered levels of expression of different genes and proteins in patients with varicoceles [105, 106].

Lima et al. [34] observed the decreased expression of *HSPA2* (heat shock protein A2) gene in adolescents with varicocele and oligozoospermia, compared with those with varicocele and normal sperm counts. HSPs have a protective function during cellular self-regulation in response to heat [107] as well as in homeostasis mechanisms. The activity of *HSPA2* has been reported in spermatocytes during meiosis and gamete maturation [108]. Such findings may be associated with the fact that if a defense mechanism against thermal stress fails to develop, compromised spermatozoa are produced, which possibly undergo apoptosis, causing low sperm counts in oligozoospermic men [34]. Consistent with these findings, Afyani et al. [109] noted that the production of *HSPA2* in rats with induced varicocele was higher than that in rats without the disease.

PK2 (prokineticin 2) is a multifunctional protein, which, along with vascular endothelial growth factors and other cytokines, promotes testicular growth and survival [110]. The expression of *PK2* mRNA is induced by hypoxic stress and occurs predominantly in primary spermatocytes [111]. Increased expression of *PK2* mRNA was observed in rats with induced varicocele, showing that changes in the expression of this gene may be linked to varicocele and, consequently, to male infertility [35].

Increased *BAX* (BCL2-associated protein X) and decreased *BCL2* (B-cell lymphoma protein 2) gene and protein means levels have also been observed in patients with varicocele [36]. These proteins are involved in the regulation of apoptosis in several cell types, either inhibiting it (*BCL2*) or promoting it (*BAX*) [112]. Apoptosis has an important role in spermatogenesis throughout the mitotic division of spermatogonia, meiotic division of spermatocytes, morphological differentiation, and maturation of spermatids for final sperm formation [113]. Thus, these results are also associated with decreased number and motility, as well as abnormal morphology of spermatozoa [36].

Varicocele may also impair Leydig cell function by increasing apoptosis and suppressing the expression of the StAR (steroidogenic acute regulatory) protein [114]. A decrease in the expression levels of *StAR* mRNA compromises the transport of cholesterol, a substrate for androgen biosynthesis, to the mitochondria, thus interfering with the synthesis of testosterone in the Leydig cells. Varicocele appears to be related to changes in *AR* (androgen receptor) gene expression [37, 38] as well. The expression levels of *AR* have been correlated with sperm count, motility, morphology, velocity, and acrosome reaction, and its decrease seemed to be responsible for the decrease in fertility of patients with varicoceles [37].

Other studies have demonstrated that varicocelectomy can alter the expression of genes important for semen quality. The expression of the gene Ropporin may be related to sperm motility, given the fact that the protein encoded by this gene is a component of the fibrous sheath in the flagella of the mammalian sperm [115]. In a study by Amer et al. [40], an abnormal expression of the Ropporin protein was reported in asthenozoospermic men with varicocele, and varicocelectomy seemed to improve both sperm quality and the expression level of this protein. Oliveira et al. [39] observed an increase in the expression of various genes after varicocelectomy, including *MTIM* (metallothionein-1M), associated with protection against oxidative stress; *PHLDA1* (pleckstrin homology like domain family A member 1), a mediator of apoptosis; *CCIN* (calicin), which preserves the integrity of the sperm nuclei; and *PRM2* (protamine-2), associated with DNA condensation.

Epigenetic disorders

Epigenetic modifications are tightly regulated mechanisms that promote stable regulation of gene expression necessary to ensure normal development [116]. Epigenetic changes involve changes in gene expression that are not related to direct changes in the DNA sequence. Epigenetic mechanisms such as DNA methylation, histone modifications, and the functions of non-coding RNAs are essential for a number of biological processes, including genomic imprinting and X chromosome inactivation [117–119].

In spermatozoa, epigenetic marks, which play a fundamental role in the viability of the gamete, are acquired during cell growth and differentiation [120]. Failure to establish these epigenetic marks are associated with various diseases in humans, including infertility. Changes in the methylation pattern on genes associated to male infertility in sperm DNA have been reported in several studies [41, 42, 121, 122].

Tavalaee et al. [42] reported that the percentage of methylated sperm DNA was lower in individuals with varicocele than that in fertile individuals. The authors did not observe any change in the DNA methylation patterns after varicocelectomy. In a study by Bahreinian et al. [41], similar results were obtained; a low percentage of methylated DNA was found in men with varicocele. They also demonstrated a negative correlation between methylation and DNA fragmentation. The authors observed that patients with varicocele showed increased susceptibility to DNA damage when the DNA was hypomethylated.

MicroRNAs (miRNAs) regulate a variety of physiological and developmental processes, including cell differentiation, proliferation, and apoptosis [123, 124]; they also play important roles in spermatogenesis [125]. miRNAs have also been reported to help in the restoration of homeostasis following

cellular changes such as hyperthermia or oxidative stress [126, 127].

Ji et al. [43] evaluated the expression of several miRNAs associated with oxidative stress in the spermatozoa of patients with varicocele and observed reduced expression of miR-15a in these individuals. miR-15a is also known to repress the expression of *HSPA1B*. The regulation of the *HSPA1B* by miR-15a may play an important protective role against cell stress throughout sperm maturation. Thus, these results help elucidate spermatogenic involvement in the pathology of varicocele [43].

Conclusion

Spermatogenesis is an essential process that determines male fertility and is tightly regulated by several genes. The reproductive potential of men with varicocele is highly variable. Different clinical phenotypes have been observed, which may or may not lead to infertility in these patients. This phenotypic variability suggests an unknown associated genetic factor that, regardless of the severity of the disease, compromises fertility. Given the progressive deterioration of testicular function caused by varicocele, early and efficient assessment of the causes of these changes is important for developing techniques for medical intervention and improving the treatment conditions [128].

Because of the multifactorial nature of varicocele, there are still no known biomarkers that could be identified in the early stages of the disease. The routine use of specific gene sequences, mutation analyses, and studying the sperm epigenome are limited by several factors, including cost, availability of equipment, and clinical relevance. The advent of high-throughput platforms in the “omics era”, such as next-generation sequencing, making possible the identification of whole genome, transcriptome, and methylome information, as well as proteomics studies, may add important insights about the genetic and epigenetic basis of the varicocele, especially in the prevention or treatment of infertility in these patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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