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Polymorphism of Xenobiotic Detoxification Genes and Male Infertility

Nguyen Thi Trang and Vu Thi Huyen

Abstract

Infertility is a multifactorial disease caused by both genetic and environmental factors. It is observed in 10–15% of couples, among which male infertility contributes for half the cases. Thus, identifying underlying causes of male infertility and for proper methods for treating and/or preventing sperm damage is of paramount importance. It is found that one of the factors that has been recently implicated in male infertility is oxidative stress, mediated by reactive oxygen species (ROS) that are produced during the metabolic process, as well as during the exposure to environmental chemical agents and their interaction with tissue-specific enzymes. Several studies have identified genetic variations at different loci, connected with male infertility, that may shed light on some idiopathic cases of seminal fluid abnormalities. In this chapter, we make an effort to decipher the contribution of polymorphisms in xenobiotic detoxification genes in the male infertility development.

Keywords: male infertility, xenobiotics, arylamine N-acetyltransferase 2, GSTM1, GSTT1, GSTP1, cytochrome P4501A1, genetic polymorphism, oxidative stress, DNA fragmentation, detoxification

1. Introduction

Deterioration of male reproductive health has become a serious problem in most countries [1]. Contributing factors for male infertility comprise genital infections, ejaculatory duct obstruction (EDO), hypogonadism, varicoceles, or exposure to environmental factors (e.g., xenobiotics, ionizing radiation), lifestyle factors (e.g., alcohol, smoking and obesity), genetic causes, systemic diseases, and abnormal ejaculation [2]. However, in approximately 30–45% of male infertility cases, the etiology remains undetermined and is called the idiopathic infertility [3]. Genetic factors make a significant contribution to the development of idiopathic male infertility. For example, oligozoospermia and azoospermia have been recently determined to be tied with such genetic deviations as translocations, microdeletions and mutations [4] in genes that play a role in testicular development, gametogenesis and metabolism of xenobiotics associated with reproductive system disorders. Xenobiotic metabolism causes the main damage to the organism by creating covalent bounds with cellular macromolecules (DNA or protein). This indicates that the expression regulation and activity of xenobiotic-metabolizing enzymes may play the crucial role in determination of male reproductive system

susceptibility to chemically induced damages. Thus, xenobiotic-mediated adverse effects of male reproductive system are associated with the polymorphisms in xenobiotic detoxification genes. Different variants of one gene are categorized as polymorphism if their frequency in the population exceeds 1% [5]. In contrast to mutations, polymorphisms have indirect connections with particular seminal fluid abnormalities but may be indispensable during the investigation of multifactorial diseases. Polymorphic variants may change the expression or function of encoded protein, leading to its conformational changes that would result in different pharmacokinetics, chemical reaction capacities and efficiency of the xenobiotic-detoxifying enzymes. In this chapter, we discuss polymorphisms in the main enzymes capable of maintaining the oxidants/antioxidants balance in reproductive tissues, focusing mainly on phase I cytochrome P4501A1 (CYP1A1) and phase II (GSTM1, GSTT1, GSTP1 and arylamine N-acetyltransferase 2 (NAT2)) detoxifying enzymes.

2. Xenobiotic metabolism

The foreign environmental chemicals are known as xenobiotics (Gk. *xenos*—“stranger”) and include drugs, drug metabolites and environmental pollutants (such as synthetic pesticides, herbicides and industrial pollutants). Xenobiotics may cause damages in the innate state (alkyl iodides, acyl halides and nitrogen mustards) or after activation in the metabolizing process.

Xenobiotic metabolism is performed in three stages (**Figure 1**):

1. Phase I enzymes initiate the detoxification process, during which the lipophilic xenobiotics become more polar and acquire sites for subsequent conjugation reactions. Phase I enzymes comprise mainly the cytochrome P450 (CYP) superfamily of microsomal enzymes, which include the 36 gene families. It is considered that CYP1, CYP2, CYP3, CYP4 and CYP7 families play the key roles in hepatic and extra-hepatic metabolism and in the elimination of xenobiotics and drugs in human [6].
2. Phase II enzymes catalyze the conjugation process. These enzymes can interact with xenobiotics either directly or, more generally, interact with the metabolites produced by phase I enzymes. Phase II enzymes include a lot of superfamilies, namely, the sulfotransferases (SULT), UDP-glucuronosyltransferases (UGT), DT-diaphorase or NAD(P)H quinone oxidoreductase (NQO) or NAD(P)H menadione reductase (NMO), glutathione S-transferases (GST) and N-acetyltransferases (NAT). Each superfamily consists of families and subfamilies of genes encoding the various isoforms with different substrate and tissue specificities [7].
3. Phase III elimination via transporters or passive transport. Phase III transporters involve P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), which belongs to the subfamily of the ATP binding cassette (ABC) transporters and organic anion transporting polypeptide 2 (OATP2).

3. Potential mechanisms for idiopathic male infertility

Male infertility is a complex, multifactorial disorder and often its etiology remains poorly understood. Increasing volume of data suggests that oxidative

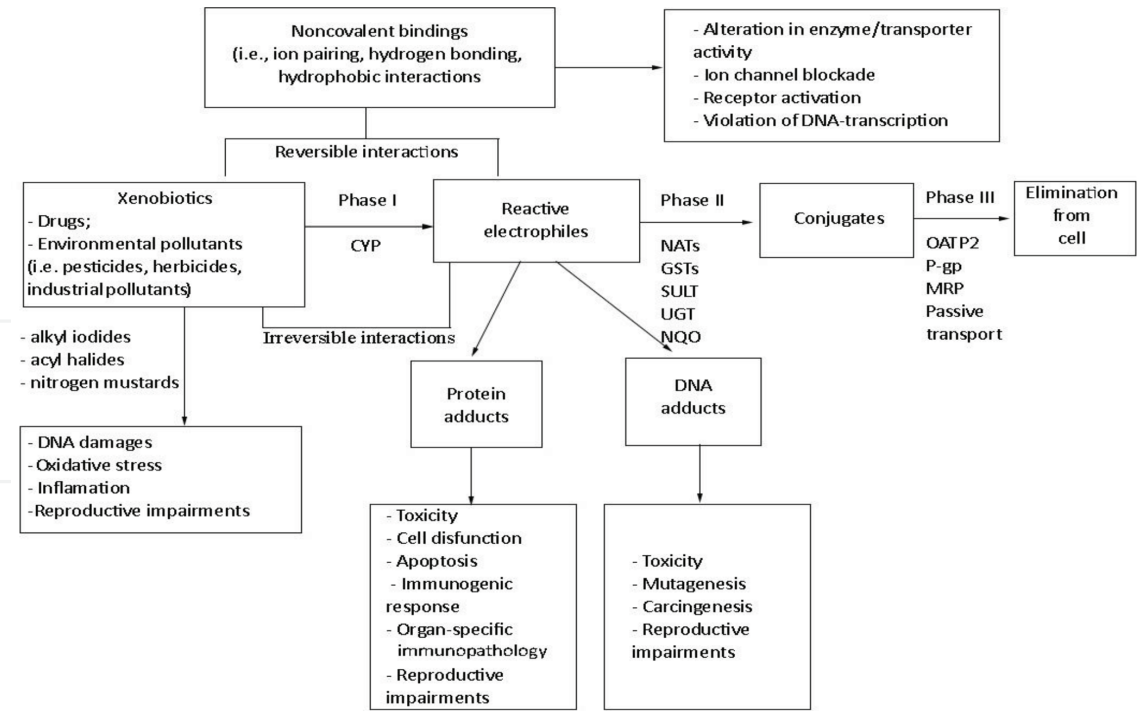


Figure 1.
The three-step detoxification of xenobiotics. Lipophilic substances subjected to metabolic activation by phase I enzyme cytochrome P450 (CYP) acquire reactive center for phase II conjugation reactions that lead to formation of hydrophilic compounds, following elimination by phase III transporters or passive transport. Most of the noxious effects are caused by reactive electrophiles after phase I activation, but some xenobiotics pose a threat before metabolic activation. CYP—cytochrome P450; NATs—N-acetyltransferases; GSTs—glutathione S-transferases; SULT—sulfotransferase; UGT—UDP-glucuronosyltransferase; NQO—NAD(P)H quinone oxidoreductase; OATP2—organic anion transporting polypeptide 2; P-gp—P-glycoprotein; MRP—multidrug resistance-associated protein.

stress is the most probable cause of idiopathic male infertility. Oxidative stress is mediated by reactive oxygen species (ROS), if their level exceeds antioxidant capacity of the organism. An increased ROS level is observed in 40–80% of infertile men and in 11–78.5% of infertile men with normal sperm count [8]. Elevated ROS levels can be explained by several reasons such as increased leukocytes' activity due to inflammation in the genital tract, varicocele or presence of immature spermatozoa as well as external causes like exposure to noxious chemical compounds, radiation and lifestyle factors [9]. A small amount of ROS is necessary in some physiological processes such as capacitation [10], while excessive ROS may damage sperms. Spermatozoa have restricted volume of cytoplasm, therefore the quantity of ROS-metabolizing enzymes is also limited and make them more vulnerable to ROS compared to other cells [9]. ROS cause sperm damages in several ways: first, ROS are capable of interacting with the sperm plasma membrane, which is rich in polyunsaturated fatty acids, promoting the decrease of its flexibility and, hence, tail motility [11]. Second, ROS may lead to the sperm's acrosome membrane peroxidation and decline in acrosin activity, thus making fertilization less probable [12]. Lastly, an increased ROS level is associated with the increase in sperm DNA fragmentation (that is used as an assessment tool for unexplained male infertility) and diminished sperm motility [13]. Moreover, considering that ROS are intensively produced in the mitochondria of stressed spermatozooids, they may cause mutations of mitochondrial DNA of different cells which participate in spermatogenesis. As a result, sperm maturation and functioning could be violated. Thus, the development of effective antioxidant treatment and antistress strategies has become one of the paramount tasks for the scientific society [10].

Mutations (that include both chromosomal and single-gene alterations) in several hundred genes are the other important and significant factor that potentially may lead to male infertility. For instance, Y-chromosome microdeletions (YCM), which influence genes responsible for spermatogenesis, are one of the best-studied types of mutations that may cause male infertility. Thus, recent meta-analysis has shown that oligospermic men with sperm concentration > 1 million/mL had significantly higher rates of YCM compared to normospermic men [14]. Among the other examples of mutations, there are the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations, which lead to the absence of vas deferens; deletions of the autosomal Doublesex and Mab-3 Related Transcription factor 1 (DMRT1) gene in the azoospermic men (which is critical for germ cells development), ranging in size from 54 kb to over 2 Mb; and alterations in autosomal PLEC1 (plectin) and microRNA 661 (MIR661) genes in an azoospermic and oligozoospermic men and various single-nucleotide polymorphisms (SNPs) [15]. The latter is one of the most promising candidates for the elucidation of “hidden” genetic factors responsible for idiopathic male infertility. For example, rs1801133 (677C > T) variant in the methylenetetrahydrofolate reductase (MTHFR) (NAD (P) H) gene found in males with impaired spermatogenesis [16], rs4986938 (1406 + 1872G > A) polymorphism of estrogen receptor 2 (ESR2) gene, rs2070744 (786C > T in the promoter region) and rs61722009 (27 bp Variable Number Tandem Repeat (VNTR) polymorphisms in the intron 4, also known as 4a4b polymorphisms) variants of NOS3 (nitric oxide synthase 3) are the potential predisposal factors of male infertility [15].

Still, male infertility depends not only on mutations alone but also on the complex system of gene–environmental interactions and epigenetic factors. A bright example of environmental factors, which may cause seminal fluid abnormalities in the corresponding genetic background, is xenobiotics, which is discussed in the following sections.

4. Association of xenobiotics with male infertility

Xenobiotics may interact with macromolecules in reversible (forming non-covalent bindings, for example, ion pairing, hydrogen bonding, hydrophobic interactions, etc.) or irreversible ways through covalent bond formation between electrophilic xenobiotics or their active metabolites and endogenous molecules. The first variant may lead to the alteration in enzyme and transporter activity, ion channels blockade, activation of specific receptors and violation in DNA transcription, if the specific structure of the xenobiotic fits to macromolecule-binding sites. Irreversible covalent interaction does not demand such fitness of structures and leads to the formation of endogenous adducts of nucleophiles (such as nucleophilic amino acid or nucleic acids). As a result, such reactions may cause genetic mutations, carcinogenesis (if the violated gene participates in regulation of cell reproduction and differentiation) and protein malfunctions, which consequently may promote cell death and tissue toxicity.

It is worth mentioning that xenobiotics injure the male reproductive system not only by themselves and their metabolites but also via defensive reactions of the organism, such as innate and specific acquired immune responses. Thus, locally available reactive metabolites may cause disruption of testis immunoprivilege through the immunocompetent cells activation. This would lead to organ-specific immunopathology. Xenobiotics may have a toxic effect due to the malfunction of both the first and the second phase enzymes because of their hyperactive or inhibited functioning. In the case of increased phase I enzyme activity, or decreased

phase II enzyme activity, electrophilic intermediates of the xenobiotic metabolism are accumulated in the cells and mediate the abovementioned damages. At the same time, the reduced activity of the first phase enzymes leads to metabolism retardation and accumulation of noxious chemical compounds in different tissues of the organism, including in those of the reproductive system. For example, decreased activity of GST (phase II enzyme that transfers glutathione (GSH) to activated environmental chemicals) will probably lead to the formation of reactive intermediates that will mediate cell damage mechanisms. On the other hand, phase I enzyme CYP1A1 is able to metabolize the polycyclic aromatic hydrocarbons (PAHs) to intermediate substances, which prompt genotoxic and mutagenic effects before they are further detoxified by phase II enzymes. This indicates that the increased activity of CYP1A1 may contribute to the accumulation of these compounds in the organism and to the elevation of the PAH-DNA adducts level. Although the liver performs the main functions of detoxification, reactive metabolites can also be generated in a particular organ, mediating organ specific toxicity. For example, experiments on laboratory animals such as rats and mice showed the presence of both phase I and phase II enzymes in their testicles. [17, 18]. The possibility of PAHs (benzo(a)pyrene) detoxification was also shown on the rats' Leydig cells [19]. The xenobiotic-detoxification enzymes are much needed in the reproductive system, since some endocrine-deteriorating agents (phthalates, dioxins, polychlorinated biphenyls (PCBs) and pesticides) have been shown to exert a negative influence on the reproductive tissues [20]. Thus, stable and lipophilic chlorinated hydrocarbons are capable of penetrating into the male reproductive tract, promoting idiopathic sterility and other reproductive impairments. One of the well-studied examples of male testicular toxins is occupational xenobiotic nematocide 1,2-dibromo 3-chloropropane (DBCP) that causes a partially reversible damage to the seminiferous epithelium, leading to diminished sperm counts and sterility [21]. Another occupational and environmental toxin dichlorodipenyldichloroethylene also reduces sperm count and mediates male infertility [22].

5. Polymorphism of genes that affect spermatogenesis

Except xenobiotic detoxification gene polymorphisms, another target group of genes that are most probably involved in development of male infertility is genes that take part in spermatogenesis. This group includes genes with different functions such as endocrine regulation of spermatogenesis (i.e., androgen receptor (AR), follicle-stimulating hormone receptor (FSHR) and Estrogen receptor α and β), specific spermatogenic functions (i.e., deleted azoospermia-like (DAZL), Ubiquitin carboxyl-terminal hydrolase 26 (USP26), protamine-1 (PRM1) and gonadotrophin-regulated testicular helicase (GRTH)), regulation of cell functions such as metabolism, cell cycle and mutation repair (i.e., mtDNA polymerase γ (POLG) and Methylene tetrahydrofolate reductase (MTHFR)) and Y-chromosome haplogroups. Y-chromosome abnormalities are the best studied, while data about association of male infertility with the other spermatogenesis regulation genes remain contradictory. For example, the spermatogenesis locus azoospermia factor c (AZFc) region partial deletion (gr/gr deletions, which include DAZ (Deleted in azoospermia) genes) is regarded as classical Y-chromosome mutation. Its role in male infertility was proven by several studies, although it is present in normospermic men as well [16, 23]. Here are some examples of the other probable risk factors obtained by meta-analysis [23]. One of gene candidates, which is regarded as the most probable risk factor of male infertility, is MTHFR, which encodes methylenetetrahydrofolate reductase—one of the key enzymes in folate metabolism. The C \rightarrow T substitution in

the position 667 changes an alanine to a valine (Ala222Val) and decreases activity of enzyme, thus leading to the decrement of spermatogenesis. FSHR SNP showed a significant association with seminal fluid abnormalities only in the case when both the exon 10 SNPs and SNP of promoter at position 29 are present. G197 T SNP in PRM1 gene, which encodes DNA-binding protein, responsible for packing of DNA into the sperm head, may be a novel risk factor for patients with a normal sperm count but elevated levels of sperm DNA fragmentation and/or teratozoospermia. Studying of the genes affecting spermatogenesis also demonstrated significant influence of ethnicity on the association of polymorphisms and the risk of male infertility. Thus, AR repeat length polymorphism with repeat number more than 23 is a risk factor for Asiatic but not the European population. T54A mutation in exon 3 of DAZL gene was significantly associated with oligospermia or azoospermia in the Chinese population, while in Japanese or Caucasian populations, such an association was not established. The POLG gene, which encodes mtDNA polymerase γ and thus important for mitochondria functioning, has common 10-fold repeated CAG motif in the first exon. It is known that mitochondria are one of the main determinants of sperm motility, and their altered regulation may be connected with asthenozoospermia (which is characterized by reduced sperm motility). Nevertheless, only one study [24] among four considered [24–27] established that the absence of this allele was significantly associated with male infertility. These results may not be relevant enough, as far as the sample size was not sufficiently big, (verum group included 99 infertile men, while control, 98 fertile men). In spite of the increased interest in the genetic causes of male infertility, there are no clear predictive and diagnostic criteria, except few polymorphisms such as AZF gr/gr deletions (OR 1.81, 1.46–2.24 CI, $P < 0.00001$) and MTHFR 677C \rightarrow T (OR 1.39, 1.15–2.69 95% CI, $P = 0.0006$) [16]. The only way to build an adequate picture of reasons standing behind idiopathic male infertility is application of methods based on whole genome analysis, taking into account environmental factors.

6. Polymorphism of xenobiotic detoxification genes

The reasons behind the idiopathic cases of male infertility can probably be explained by the existence of unidentified genetic abnormalities involving several hundred genes, environmental interaction and epigenetics. Each of these genes is accountable for only a small part of the cases. In the late 1990s, the polymorphisms-based approach to searching for genetic factors in patients with idiopathic infertility was reinforced because of the findings of other investigations related to multifactorial impairments. Potential polymorphic genes that participate in male infertility development were determined from the increasing data on model organisms, expression analyses (transcriptome and proteome analyses) associated with poor germ cells quality and from data available from the Genome-Wide Association Studies (GWAS). In total, 2000 genes (housekeeping and germ cell specific genes) are engaged in spermatogenesis [28], but 314 single-nucleotide polymorphisms (SNPs) have been reported only in 123 genes for the year 2015 [15]. Nearly 70% of these SNPs are responsible for general cell functions (regulation of apoptosis, DNA repair, xenobiotic metabolism and detoxification of reactive oxygen species), while the rest are involved in specific processes associated with spermatogenesis, meiosis and endocrine regulation of the reproductive system. However, available data are often contradictory and only a small amount of genetic polymorphisms are well studied. Meta-analyses were conducted for such genes as AR, CYP1A1, GSTP1, GSTM1, GSTT1, DAZL, ESR1, ESR2, MTHFR, NOS3, POLG, TP53 and USP26, among which CYP1A1, GSTP1, GSTM1 and GSTT1 detoxify the environmental

chemicals and are of great interest. In addition to the abovementioned genes, NAT2 will also be regarded as an important candidate carrying SNPs that alter xenobiotic detoxification phenotypes. Readers considering the represented data should always bear in mind that determination of connections between xenobiotic detoxification genes polymorphisms and male infertility is significantly complicated by the existence of intricate interactions among phase I and phase II metabolizing enzymes, as well as among the phase III transporters and their nuclear receptors (aryl hydrocarbon receptor (AhR), orphan nuclear receptors, nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), constitutive androstane receptor (CAR) and pregnane X receptor (PXR)). These receptors can act as transcriptional factors for some xenobiotic metabolizing enzymes. For instance, CYP1 genes can be induced by AhR in response to PAHs, while CYP2B and CYP3A genes are activated by CAR and PXR in response to phenobarbital-like compounds and dexamethasone and rifampin-type of agents, respectively. It was also assumed that Nrf2 is the most likely transcriptional factor for phase II detoxification enzymes (such as GST) [29]. Thus, mutations or polymorphisms of these genes may contribute to alteration of xenobiotic metabolism indirectly that can also lead to a damage of the male reproductive system.

6.1 Cytochrome P4501A1 polymorphism

Cytochrome P4501A1 (CYP1A1) is a heme-thiolate containing monooxygenase that plays a central role in phase I extra-hepatic metabolism of lipophilic xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs) that are produced during the combustion of fossil fuels, protein pyrolysis in well-fried meat, as well as from aromatic amines, which are present in cigarette smoke, pesticides, dyes, drugs and industrial products. CYP1A1 is expressed in male reproductive organs. Thus, its polymorphisms may define susceptibility to male infertility [30]. In addition to PAHs metabolism, CYP1A1 participates in the synthesis of cholesterol, steroids and other lipids, as well as in steroid hormone metabolism. Thus, its polymorphic variants can intervene in the endocrine and paracrine regulation of testicular function [31]. Cytochrome P4501A1 gene is located on 15q22-q24 chromosome. It is 5987-bp long and encodes a 512 amino acid protein. Benzo(a)pyrene, 3-methylcholanthrene, PCBs or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) activate its expression. Although the CYP1A1 is a polymorphic gene, only two common SNPs have been found to be functionally significant, namely, CYP1A1*2A (3801 T > C in the 3'-flanking region, rs4646903) and CYP1A1*2C (Ile462Val, 2455A > G (exon 7), rs1048943) [32]. The T > C substitution in CYP1A1*2A promotes the creation of a new MspI restriction site in the noncoding 3'-flanking region that causes elevation of transcript half-life and consequently enzyme activity, leading to increased levels of reactive metabolites [33]. The A > G substitution prompts an isoleucine-valine exchange in the heme-binding region of the enzyme, and according to some studies, it changes the enzyme's kinetics during the generation of diol metabolites of benzo[a]pyrene or causes a -threefold increase in transcripts quantity and microsomal enzyme activity in Asians [34]. Such data can be explained by the fact that the CYP1A1*2A and CYP1A1*2C allelic variants appear much more frequently in Asians compared with other ethnic groups, namely, Caucasians and African Americans [35]. However, these data are contradictory. A recent meta-analysis of 1060 cases and 1225 controls from 7 published case-control studies revealed that the homozygous CYP1A1*2A genotype was significantly associated with the susceptibility to idiopathic male infertility in Asians (CC vs. TT: OR = 2.88, 95% CI: 1.20–6.89, TC vs. TT: OR = 1.42, 95% CI: 1.03–1.98, recessive model: OR = 1.63, 95% CI: 1.08–2.45.) but not in Caucasians, while no association with male infertility

risk was found in the case of CYP1A1*2C genotype (GG vs. AA: OR = 1.03, 95% CI: 0.53–1.99, GA vs. AA: OR = 1.51, 95% CI: 0.51–4.43, dominant model: OR = 0.81, 95% CI: 0.42–1.54, recessive model: OR = 1.57, 95% CI: 0.55–4.47) [36]. Still, these results seem to be preliminary, since such individual characteristics as age, body mass index, smoking status and environmental factors, as well as the sperm concentration, motility and other semen parameters were not taken into account during the data analysis. Another meta-analysis of 6 studies including 1060 cases and 1225 controls also confirmed a valid association between CYP1A1*2A genotype and the male infertility risk with the highest figures for the homozygous variant (OR = 2.18, 95% CI: 1.15–4.12) [37]. Subgroup analysis based on ethnicity, sample size and quality assessment score failed to expose any significant level probably due to limited studies and population numbers discussed in the meta-analysis. Moreover, these conclusions may be irrelevant and need to be confirmed, as only two of six considered studies showed significant correlation and the abovementioned meta-analysis did not account the differences in exposure to environmental factors. The latter factor is of prime importance in the case of xenobiotic detoxification genes. Thus, Yarosh et al. [38] revealed a significant association between CYP1A1*2C and idiopathic male infertility risk only among smokers but not among nonsmokers. Finally, CYP1A1 may participate in male infertility through induction of the oxidative imbalance in cells as far as together with ligand-dependent transcription factor—Aryl hydrocarbon receptor (AhR)—it is capable of generating local reactive oxygen species (ROS) [39]. Considering that morphologically abnormal semen has reduced antioxidant capacity and increased ROS production [40], CYP1A1 may play a crucial role in male infertility development.

6.2 GSTM1, GSTT1 and GSTP1 polymorphisms

Human semen is rich in Glutathione S-transferases (GSTs) [41], which belongs to phase II superfamily of antioxidant enzymes involved in the cellular detoxification of various physiological substances (e.g., excessive ROS) or exogenous electrophiles. Detoxification process depends on the gene family class. At least seven gene classes of GSTs can be allocated: alpha (α), mu (μ), pi (π), sigma (ς), theta (θ), kappa (κ) and zeta (ζ). They are coding GSTA, GSTM, GSTP, GSTS, GSTT, GSTK and GSTZ enzymes, respectively. Polymorphisms are encountered most frequently in GSTM1, GSTT1 and GSTP1 subfamilies. According to the available data, GSTM1 and GSTT1 null polymorphic variants are the most studied GST SNPs and are associated with the increased susceptibility to several diseases and hypersensitivity to toxic xenobiotics [42]. For instance, GSTM1 was shown to have homozygous deletion in nearly half of the people from various ethnicities. Such deletion results in decreased enzyme function [43]. In general, inhibited GST activity leads to diminished semen motility via membrane damage. Considering that unmetabolized toxic substances, which accumulate in the cellular matrix of the testis, cause spermatogenesis deterioration, another probable reason for sperm impairment in subjects with GSTM1 or GSTT1 null genotypes may be considered to be the insufficient functioning of seminiferous tubules and fibrosis developed in the testicular tissue [44].

GSTM1 is located on 1p13.3 chromosome; three alleles have been identified at its locus: GSTM1*A, GSTM1*B (causing the lysine 172 replacement by aspartic acid (534C > G) and not exhibiting alterations in enzyme activity) and GSTM1 null genotype (gene deletion) [45].

GSTT1 is located on 22q11 chromosome and has one polymorphism (GSTT1 null genotype) that leads to the inhibition of enzyme production in homozygote [46].

Several studies have suggested that GSTM1, as well as GSTT1, might be crucial isozymes in the metabolism of ROS [47, 48]; meanwhile, diminished antioxidant capacity of seminal plasma turned out to promote subfertility.

Numerous studies reported the connection between the polymorphisms of GST genes and the DNA fragmentation increased due to the impaired defense against oxidative stress [49], which may result in some kinds of cancers. Considering that surplus of reactive oxygen species is a damaging factor for spermatozoa, GST SNPs most likely contribute to idiopathic male infertility. A possible explanation for the spermatogenesis impairment in GSTT1 and GSTM1 null allele carriers was proposed by Wu et al. [50]. Considering that null genotypes eliminate the binding site for some transcription factors, such as nuclear factor 1 (NF-1) (may act as transcriptional repressor or activator, determined by target gene expression), specificity protein 1 (SP1) (involved in cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage and chromatin remodeling) and serum response factor (SRF) (participates in transforming extracellular signals into specific nuclear responses), GSTT1 and GSTM1 null variants may cause changes in gene expression through the removal of transcription factors. Such alterations in gene expression may entail deterioration of sperm maturation and impaired fertility.

Another polymorphic gene (GSTP1) is approximately 4 kb in length and is located on 11q13.2 chromosome. It includes 7 exons and encodes a 210 amino acid protein. Two polymorphisms of GSTP1 rs1138272 (341C > T (exon 6) leading to the alanine 114 replacement by valine) and rs1695 (313A > G (exon 5) encoding the amino acid exchange (replacement of isoleucine in the position 105 by valine) were associated with a decreased heat stability and the detoxification ability of this enzyme [51].

Most of the current data on the polymorphisms of xenobiotic detoxification gene are devoted to GST SNPs. A pooled analysis of 11 studies (7 involve Asians and 4 involve Caucasians), which included 1323 cases and 1054 controls, revealed that GSTM1 (OR = 2.75, 95%; CI: 1.72–3.84, P = 0.003) and GSTT1 null (OR = 1.54, 95%; CI: 1.43–3.47, P = 0.02) genotypes showed significant association with strong/moderate risk of impaired male fertility, respectively. At the same time, GSTP1 Ile/Val genotype was proved to mediate the protective effect on male reproductive system (OR = 0.48, 95%; CI: 0.27–0.77) [52]. These results are reliable enough, as far as researchers took into account the age, ethnicity and the smoking status. Interestingly, that in contrast to CYP1A1 polymorphism, there was no difference between smokers and nonsmokers (P = 0.26). At the same time, GSTM1 null genotype, considered in the research on Indian subjects, showed 8.6 times increment of infertility risk if the subject was a smoker. Going back to the pooled analysis, synergistic effects of GSTM1 null allele, GSTT1 null allele and GSTP1 Ile/Ile polymorphism on male infertility was recognized as a valuable feature of this research [53]. However, further analysis showed that GSTM1 null genotype was associated with male infertility only at a borderline level of significance [53]. Such inconsistency may be explained by more rigid selection criteria in the latter meta-analysis. Since 161 of 168 studies were excluded, only 6 case-control studies concerning the GSTM1 genotype and 5 studies concerning the GSTT1 were eligible. Moreover, most studies referred to Caucasian subjects (except one, concerning the GSTM1 polymorphism, and two, concerning the GSTT1 polymorphism). On this basis, no ethnic variations were analyzed. Another doubtful issue touches upon the possibility of association between GSTP1 Ile/Ile polymorphism and the susceptibility to idiopathic male infertility. The fact is that in human population, Ile allele is encountered more frequently than the Val allele is. Thus, its positioning as a risk factor for male infertility contradicts the principles of evolutionary genetics, as Ile allele should have disappeared from the population. Furthermore, recent meta-analysis,

devoted to the association between GSTP1 polymorphism and cancer susceptibility, revealed that Val allele was a risk factor for carcinogenesis, and no accumulation effect of Ile/Ile genotype was revealed [54]. All this indicates the necessity of additional researches that would comprise GSTP1 Ile/Ile polymorphism.

Another GSTP1 polymorphism—Ala/Val (rs1138272) substitution—was studied even less extensively than the Ile/Val polymorphism. Recently, this SNP was shown to be associated with the increased risks of infertility in Vietnam male subjects (OR = 7.42, 95%; CI: 3.86–14.30) [55], while previously, another work, devoted to the oxidative damage in infertile men with varicoceles, revealed no significant differences between the indices recorded in the patient and control groups [56]. Further meta-analysis that focused on the relationship between the prostate cancer and the GSTP1 Ala/Val polymorphism revealed no significant associations [57]. Although Aydemir et al. did not report such a correlation, they revealed a connection between the GSTM1 null polymorphic variant with markers of oxidative stress in patients and the idiopathic male infertility [58]. Thus, most investigations, including five meta-analyses, confirmed the GSTM1 association with the risk of male infertility [52, 59–61].

A meta-analysis of 15 researches (8 involve Asian people, 6—European people, and 1 comprises a mixed population) that included 1897 cases and 1785 controls showed that GSTM1 null genotype was significantly associated with susceptibility to idiopathic male infertility, but not with sperm concentration. In the case of GSTT1 null genotype, no association with oligoasthenoteratozoospermia and sperm concentration was revealed. Subgroup analysis on ethnicity did not show any reliable association between the idiopathic male infertility and the GSTM1 null or GSTT1 null genotype [59].

Contradicting data were obtained via the case-control study of Han people from East China, which included 1476 infertile men with normozoospermia, oligozoospermia and nonobstructive azoospermia and 895 healthy controls, matched by age, drinking and smoking status, body mass index and semen volume [50]. The research revealed that GSTT1 null genotype is a predisposing factor for idiopathic male infertility (OR = 1.26; 95%; CI: 1.07–1.50; $P = 0.007$), while GSTM1 null genotype showed no significant association with the idiopathic male infertility risk (OR = 1.15; 95%; CI: 0.97–1.36; $P = 0.116$). At the same time, GSTM1 null variant was prevalent in oligozoospermic patients (OR = 1.55; 95%; CI: 1.15–2.08; $P = 0.004$), while the GSTT1 null polymorphism was associated with normozoospermia (OR = 1.23; 95%; CI: 1.03–1.48; $P = 0.025$) and azoospermia (OR = 1.58; 95%; CI: 1.18–2.11; $P = 0.002$). Interestingly, no differences were found in GSTM1 expression between the present and deleted genotypes, but such were found in the GSTT1 null polymorphism, which expression was significantly decreased in comparison with the present variant.

The authors also conducted a meta-analysis of 19 case-control studies in 2002–2013. The meta-analysis included 3981 cases and 2953 controls involving the Asian and Caucasian ethnic groups [50]. As a result, GSTM1 null allele carriers were subjected to the risk of male infertility (OR = 1.39; 95%; CI: 1.14–1.70; $P = 0.001$) and to oligoasthenoteratozoospermia (OR = 1.53; 95%; CI: 1.25–1.89; $P < 0.001$). This association persisted in the case of subgroup analyses that involved Asians (OR = 1.51; 95%; CI: 1.13–2.10; $P = 0.005$) and Caucasians (OR = 1.24; 95%; CI: 1.00–1.52; $P = 0.046$). The GSTT1 null variant, however, was significantly associated with male infertility only among Asian people (OR = 1.44; 95%; CI: 1.10–1.90; $P = 0.009$). Such results may be explained by the fact that the GSTT1 null genotype is most frequently encountered in individuals of Asian origin than in other populations [62]. On the whole, differences from the previous meta-analysis [59] may be explained by the fact that they have added some recent studies.

Data of numerous studies revealing the GST polymorphism effect on the male infertility development remain contradictory. The list of the most probable

underlying causes includes the relatively small number of participants, ignoring some gene-environment and gene-gene interactions and the possible small influence of the GST SNPs on the risk of idiopathic male infertility.

6.3 Arylamine N-acetyltransferase 2 polymorphism

Arylamine N-acetyltransferase 2 (NAT2) is a phase II xenobiotic detoxification enzyme metabolizing such chemicals as arylamines, aromatic and heterocyclic amines and hydrazines via N-acetylation and O-acetylation. In other words, it transfers the acetyl group from acetyl-coenzyme A to the nitrogen or oxygen of the substrate. It takes part in the metabolism of such drugs as sulfadimidine, sulfamethazine, isoniazid, nitrazepam, dapsone or caffeine [63]. Therefore, it determines human susceptibility to cancer and the side effects of drugs [64].

Biological significance of NAT2 could be demonstrated through the association between its polymorphisms with susceptibility and different types of cancer—lung, colon or bladder cancer [65, 66].

NAT2 is expressed in the male reproductive organs (genital ducts, testicular tissues, exocrine and prostate glands), promoting protective effect against the environmental chemicals that may lead to male urogenital diseases [67].

NAT2 is a 290 amino acid protein, encoded by intronless 9.9 kb gene, located on the 8p22 chromosome and consists of 3 exons. Scientists identified the acetylation polymorphism more than 60 years ago in tuberculosis patients, who reacted to isoniazid toxicity in different ways [68]. NAT2 is characterized by a high number of polymorphic genes, comprising more than 66 alleles [69]. Most of these polymorphisms are synonymous and do not always cause variations in enzyme activity [70]. The most common and important variants of SNPs are rs1799929 (481C > T that does not cause leucine alteration at amino acid 161, L161 L) marked as NAT2*11A and rs1799930 (590G > A that leads to the charged arginine replacement by polar glutamine at the codon 197 (R197Q)) marked as NAT2*6 [71]. NAT2*6 corresponds to a slow acetylator phenotype, while the NAT2*11A to a rapid one that may differ from the NAT2*6 by a threefold incensement in the metabolic rate [72]. These two extreme acetylator phenotypes are considered as risk factors for disease development after the subjection to arylamines and other NAT2 targets. For instance, NAT1 and NAT2 slow acetylator phenotypes were shown to be predisposing factors for prostate cancer [73], while the rapid NAT2 phenotypes had higher frequencies in contact-allergic patients [74]. It is considered that the existence of canonical isoniazid slow acetylator phenotype is caused by the reduction in NAT2 protein [75]. Among the other reasons, there are low levels of expression, instability or reduced catalytic activities [76].

There are substantial interethnic variations of the slow acetylator phenotype. Thus, it can be found in 40–70% of Caucasian and African people. At the same time, its frequency ranges from 10 to 30% in Japanese, Chinese, Korean and Thai people [77].

There is a lack of evidence to confirm the role of NAT2 polymorphism in the development of male infertility. However, recently it was proved that gene-environment interactions play a very important role in the case of NAT2 polymorphism and determine whether it will predispose male infertility. Thus, several data have showed that rs1799929 and rs1799929 SNPs themselves were not associated with the increased risks of idiopathic male infertility [78]. However, if the subject was exposed to cigarette smoking (OR = 1.71, 95% CI: 1.02–2.87, P = 0.042), alcohol abuse (OR = 2.14, 95% CI: 1.08–4.27, P = 0.029) and low fruit/vegetable intake (OR = 1.68, 95% CI: 1.01–2.79, P = 0.04), the risk of male infertility significantly increased in the case of slow acetylator phenotype rs1799930.

Contrariwise, rapid acetylator phenotype was found to cause higher DNA-fragmentation levels after 2 days of meat diet [79]. Considering that the level of

DNA fragmentation is significantly higher in infertile men [80], this study also proves that NAT2 polymorphism is involved in the process of impaired reproductive development in men.

The latest study, conducted on Vietnam males, revealed that idiopathic male infertility is associated with both the rs1799930 (OR = 3.10, 95%; CI: 1.92–5.01) and the rs1799929 (OR = 3.74, 95%; CI: 2.26–6.18) alleles. The current research also shows that GSTP1 and NAT2 have a synergetic effect—they cause the biggest risk of infertility only when both polymorphisms are present. Namely, the 481C > T rs1799929 (NAT2) and the 341C > T or 341 T > T rs1138272 (GSTP1) cause the 17-fold increase in the risk of idiopathic male infertility (OR = 17.24, 95%; CI: 7.30–40.74, $P = 0.0001$) [55].

Thereby, the NAT2 involvement in the process of male infertility development is highly probable, but more data are needed to confirm its role in mediating the impaired male reproduction.

7. Conclusions

Male infertility is a worldwide health problem with multifactorial etiology, showing an upward trend during the last decade. One of the most significant reasons behind this trend is the exposure to environmental factors—xenobiotics, hypo/hyperthermia, stress or harmful radiations (such as X-rays). All this phenomena may lead to oxidative stress, treated as one of the most common trigger of male infertility and found in nearly half of all the infertile men [81]. It causes impairment of sperm maturation, testis injury, sperm motility reduction and DNA damages. Research on the polymorphisms of xenobiotic detoxification genes may be helpful for determining the interethnic and interindividual peculiarities of noxious chemicals metabolism (including possible risks of male infertility development) based on gene-environment interactions. Overall, knowledge about the SNPs of xenobiotic metabolizing genes associated with male infertility is rather inconsistent or even contradictory. Thus, more comprehensive analysis is required that would be stratified according to the age, body mass index, ethnic background, diet, smoking and drinking status, environmental exposures and other lifestyle factors. Moreover, many studies were carried out on small samples. This factor increases the probability of overestimating the association. Numerous studies indicated that such polymorphisms as CYP1A1, GSTM1, GSTT1, GSTP1 and NAT2 are most likely to be involved in male infertility development. Their polymorphic transcripts were shown to change the xenobiotic metabolism. In some cases, they failed to provide sufficient antioxidant defense. Knowledge of the role that the polymorphisms of xenobiotic detoxification genes have in male infertility development could be useful for providing sufficient diagnostic methods, as well as for providing reliable recommendations for infertile men on disease prevention and treatment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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