

GENES PREDISPOSING TO ERECTILE DYSFUNCTION AND MANAGEMENT: A REVIEW

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ABBREVIATIONS

cGMP - cyclic guanosine monophosphate.
ED - erectile dysfunction.
GWAS - genome wide association studies.
SD - sexual dysfunction.
LH - luteinizing hormone.
PDE5I - phosphodiesterase 5 inhibitors.
PGE1 - prostaglandin E1.
SNP - single nucleotide polymorphism.
VED - vasculogenic erectile dysfunction.
VNTR - variable number of tandem repeats.

ABSTRACT

Background: The burden of erectile dysfunction (ED) is rising worldwide due to unresponsiveness of some affected individuals to existing drugs and treatment strategies. Fortunately, improvement in biological techniques has led to the understanding that some cases of the disorder may have a genetic etiology, which, when fully understood, may lead to improved treatment. **Objective:** This review articulated established ED candidate genes and pathophysiology to assist researchers and medical practitioners to formulate effective drugs and treatment procedures. **Methods:** The Google search engine was used to retrieve relevant information on the topic from reputable academic databases, including PubMed, Medline, Google Scholar, Scopus, and SpringerLink. **Results:** The search discovered 10 ED candidate genes, which are *SIM1*, *SLC6A4*, *5-HTTLPR*, *TGFBI*, *DAT1*, *MC4R*, *NOS3*, *GNB3*, *AR*, and *MTHFR*. Polymorphisms or mutations in these genes may disrupt erectile activities of the hypothalamus, neurotransmitters such as dopamine, serotonin, and nitric oxide as well as relaxation of penile tissues. Clinical presentations of ED include loss of erection, weak vaginal penetration, premature ejaculation, and anejaculation. Each gene has a distinct mechanism, which, if targeted in the affected may reverse the disorder or reduce the effects. **Conclusion:** Some cases of ED are genetic, which, when fully understood, may give an insight into new treatment procedures or improve on the current ones. Medical practitioners are advised to formulate treatment procedures that target the affected gene (s) in individuals.

Keywords: Erectile dysfunction, Dopamine, Hypothalamus, Neurotransmitter, Premature ejaculation



BACKGROUND

Sexual dysfunction (SD), the commonest form of which is erectile dysfunction (ED), occurs in about 52% of men aged 40 to 70 years [1, 2]. Erectile dysfunction weakens penile erection and prevents normal vaginal penetration [3]. Normal penile erection involves neurological and vascular activities, resulting in increased blood flow to the penis – the greater the blood flow, the more the penis's rigidity [4]. Penile erection is regulated by a balance between elevated arterial blood infusion and decreased venous outflow, leading to swelling of the cavernous sinus of the penis [1]. Disruption of these biological activities may affect blood availability to the corpus cavernosum, resulting in ED [5].

Erectile dysfunction is a major public health problem, affecting mature men across all age groups with a global incidence rate between 3 to 76.5% [6]. Worldwide, about 100 million men suffer from ED [7], and is expected to increase to 322 million men by the year 2025 [3]. Erectile dysfunction affects the psychological well-being, as well as the quality of life and marital relationship of the affected [8]. The global expenditure on ED treatments is also rising. For instance, the United States spent \$330 million on ED in 2000, compared with \$185 million spent in 1994 [6]. To stem the rising burden of ED, an in-depth understanding of the etiology of the disorder becomes imperative. Studies showed that ED may have organic and psychogenic origins [9]. The organic causes may be vascular, traumatic, neurological, endocrine-induced, and drug-induced, while the psychogenic causes include depression, performance anxiety, and relationship problems [9]. Studies have also associated ED with common chronic disorders such as diabetes mellitus, lower urinary tract infections, and obesity as well as cardiovascular and neurological diseases [10, 11]. Though the findings mentioned above had helped formulate some therapies that target the neurophysiology and the penile tissues, a large proportion of the affected did not respond to such therapies. This showed that there are some etiological aspects of the disorder yet unknown. To this end, advanced biological techniques have led to the discovery that some cases of ED may be genetic and, when fully understood, may give an insight into the development of new treatment strategies and drugs, or improvement on the existing drugs. The genetic etiology may also serve as a biomarker of ED. Therefore, this study was initiated to articulate established ED predisposing genes and pathophysiology for further actions by all stakeholders.

METHODS

Databases Searched

Academic databases searched for relevant information on the topic include Scopus, Medline, PubMed, SpringerLink, and Google Scholar. The articles retrieved from each database were pooled together and double citations were removed using EndNote software.

Search Terms

The search terms used to retrieve articles are 'sexual dysfunction', 'erectile dysfunction', 'gene predisposing to sexual dysfunction', and 'erectile dysfunction candidate genes'. Other search terms used include 'risk factors of sexual dysfunction', 'burden and prevalence of sexual dysfunction', 'premature ejaculation', and 'penile erection'.

Article Inclusion/Exclusion Criteria

Inclusion Criteria

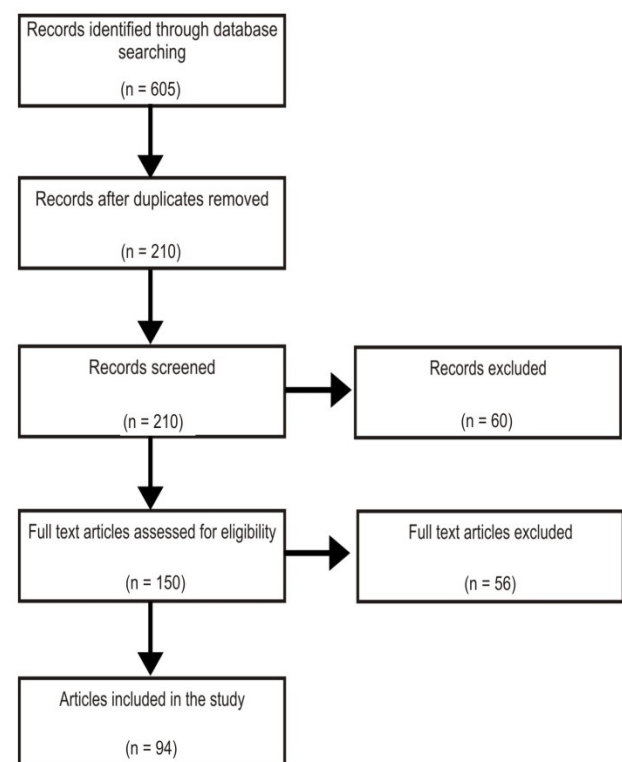
- Research published in the English language.
- Research that focused on SD.
- Studies that focused on the genetic basis of ED.
- Articles that centered on the burden and prevalence of SD.

Exclusion Criteria

- Studies that are not available in the English language.
- Studies with only abstract available.
- Research that described SD, but with no clear genetic mechanisms.

In all, 605 articles were retrieved from the databases searched (Figure 1). However, 210 articles were retained after removing duplicates. The 210 articles were tested for eligibility of which 150 articles passed the test. Ninety-four (94) of the eligible articles fit the study aim and thus made the final selection.

Figure 1. PRISMA flow diagram of article selection





RESULTS

Genetic Etiology of ED

At least 10 genes are being suspected in the pathogenesis of ED. Polymorphisms or mutations in these genes may affect sexual functions of the hypothalamus, neurotransmitters, leptin signalling pathways and penile tissues (figure 2). The descriptions of these genes and their cytogenetic locations are presented in Table 1.

Figure 2. Erectile dysfunction candidate genes and pathophysiology

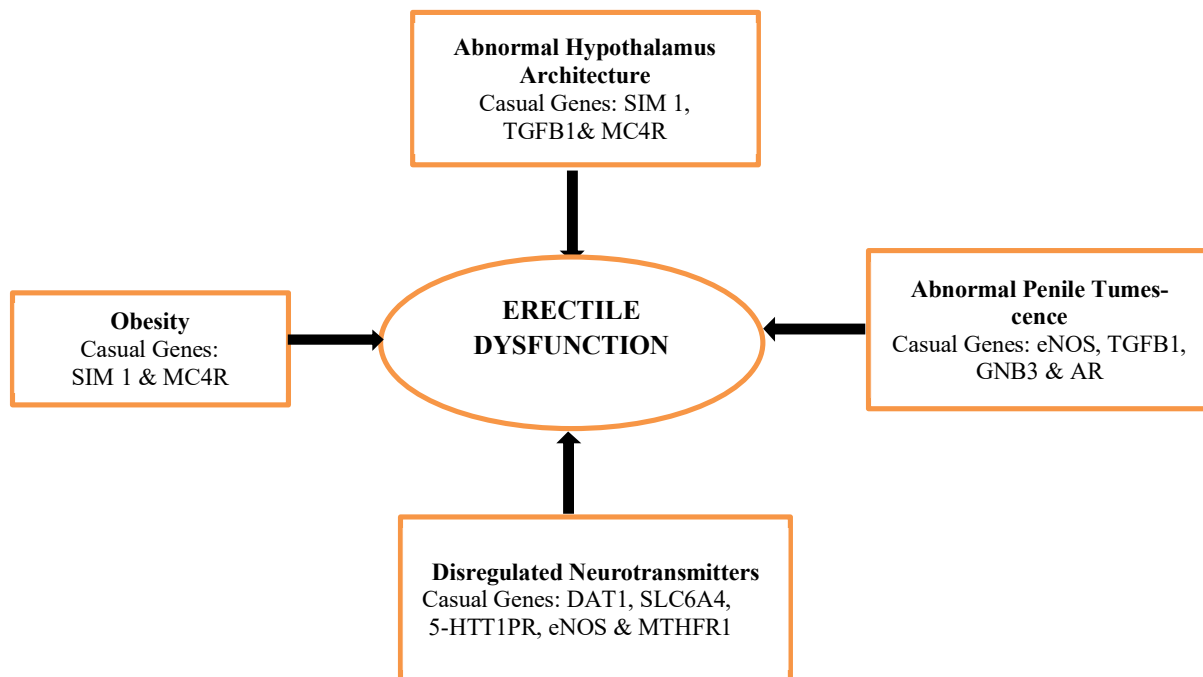


Table 1. Suspected erectile dysfunction genes and descriptions

Gene	Locus	Full Name	Function	Variants	ED Mechanism	Ref
<i>SIM1</i>	16q16.3	Single-minded 1	involves in leptin-melanocortin signaling and maintains normal hypothalamus architectures	rs17185536-T, rs57989773	obesity and abnormal hypothalamus	[12, 13]
<i>MC4R</i>	18q21.32	melanocortin-4 receptor	regulates blood leptin levels and takes part in nerve cell signaling	deletion (–/– and +/–)	disruption of leptin-mediated energy balance, resulting in hyperphagia and obesity	[14, 15]
<i>DAT1/SLC6A3</i>	5p15.33	dopamine transporter gene/solute carrier family 6 member 3	transmits and regulates dopamine levels in the neurons	VNTR (3-12 repeats)	alters <i>DAT1</i> expression, resulting in abnormal levels of dopamine	[16, 17, 18]



Gene	Locus	Full Name	Function	Variants	ED Mechanism	Ref
<i>SLC6A4</i>	17q11.12	Solute carrier family 6 member 4	regulates serotonin levels in the neurons	VNTR (9, 10, 12 repeats)	alters <i>SLC6A4</i> expression, leading to abnormal serotonin levels	[19, 20]
<i>5-HTTLPR</i>		serotonin transporter-linked promoter region	regulates serotonin levels in the neurons	VNTR (14 and 16 repeats)	alters <i>5-HTTLPR</i> expression, leading to abnormal serotonin levels	[21, 22]
<i>TGFB1</i>	19q13.2	transforming growth factor beta 1	cell signaling and regulation of male and female reproductive processes	deletion (–/– and +/–)	compromises the penile skin architecture and tissue compliance	[23, 24, 25]
<i>eNOS</i>	7q35-36	endothelial nitric oxide synthase	produces nitric oxide, a neurotransmitter that regulates relaxation and contraction of penile tissues	G894T, VNTRs in intron 4, and T-786C	reduces nitric oxide production, which interferes with the smooth muscle-regulated relaxation and contraction of the penis	[26, 27, 28]
<i>GNB3/GB3</i>	12p13	guanine nucleotide-binding protein/G protein subunit beta 3	relays signals from several receptors to diverse intracellular signaling pathways, including erectile function	C825T	induces obesity and down-regulates penile vasomotor tone	[29, 30, 31]
<i>AR</i>	Xq12	androgen receptor	switches androgen-responsive genes on or off to direct male sexual development and regulate sex drive	CAG and GCC repeats	causes androgen insensitivity syndrome, which affects fetal and adolescent sexual development	[32, 33]
<i>MTHFR</i>	1p36.3	methylenetetrahydrofolatereductase	involves in the metabolism of folate and homocysteine	C677T and A1298C	increases serum levels of homocysteine, which impairs endothelial function and neurotransmitters production	[34, 35, 36]

Detailed explanation of the listed genes and the role of their variants or mutations in the onset of ED are outlined below. Figures 3 and 4 also described biological activities leading to penile erection and disruption, respectively.



Figure 3. Initiation and maintenance of penile erection

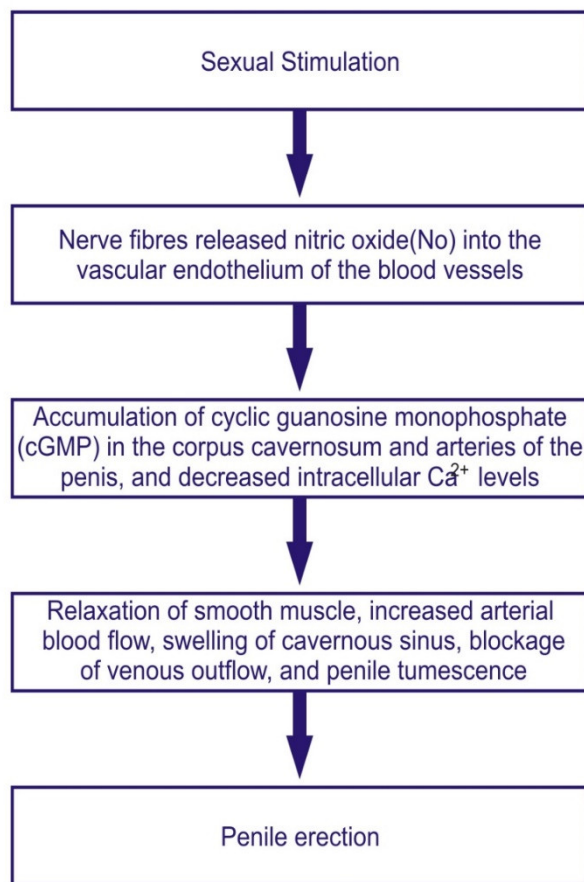
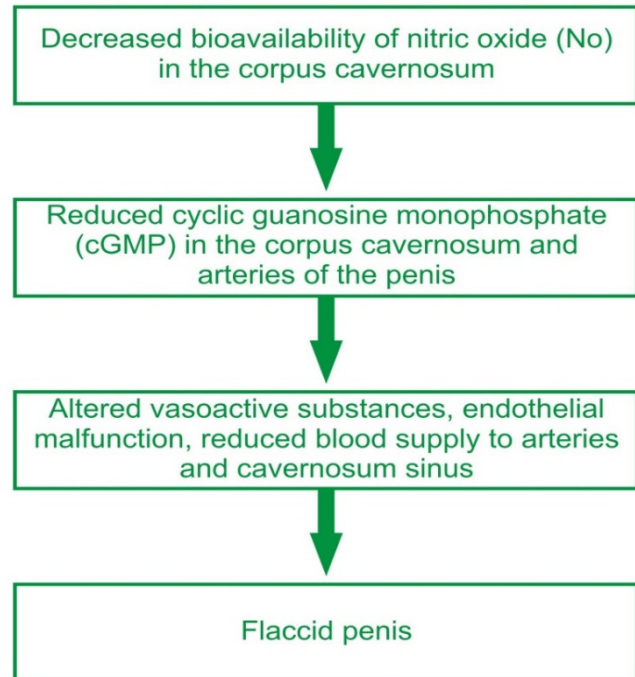


Figure 4. Disruption of penile erection (ED)



SIM1

The single-minded 1 gene (SIM1) encodes a protein, which mediates the development and function of the paraventricular nucleus of the hypothalamus, a region that helps maintain energy balance [8]. Studies suggest that SIM1 gene mediates energy homeostasis by participating in the central leptin-melanocortin signalling [13, 37]. In a mice experiment, homologous deletion ($-/-$) of the SIM1 gene caused abnormal hypothalamus architecture and death during the perinatal period [38]. In the same experiment, mice with the heterozygous deletion (SIM1 $+/-$) survived, but were obese related to an imbalance between calorie intake and energy expenditure [38]. In humans, point mutations in the SIM1 gene predispose to SIM1 haplo-insufficiency, resulting in obesity [39]. Recently, SIM1 gene was reported to be involved in sexual function.

A region near the SIM1 gene regulates sexual function by interacting with the promoter of SIM1 gene [40]. However, certain variants of this region alter the function of the promoter, affecting the expression of SIM1 gene and predisposing humans to ED [40]. SIM1 gene was first implicated in ED by a Genome-wide association study (GWAS) of 36,649 Northern California men and replication analyses in 222,358 men from the UK Biobank. In the study, a single nucleotide

polymorphism (SNP), rs17185536-T, in the implicated region near the SIM1 gene was related to ED [37]. The risk caused a differential enhancement of the promoter of SIM1 gene, causing ED independent of other risk factors such as weight and obesity [37]. In another GWAS study of 6,175 European men with ED, a SNP rs57989773 between *MCHR2* and SIM1 gene was observed to be predominant [12]. An examination of the affected men indicated that the SNP disrupted the expression of the SIM1 gene through its promoter, resulting in abnormal hypothalamus and ED [12]. This shows that brain cells deficient of SIM1 gene may disrupt sexual function by affecting the nervous system, especially the hypothalamus that mediates involuntary actions.

MC4R

The melanocortin-4 receptor gene (MC4R) encodes melanocortins, a membrane-bound receptor that regulates food intake and energy expenditure [15]. Melanocortins is released by pro-opiomelanocortin nerve cells in the arcuate nucleus of the hypothalamus in the brain [41]. Rats lacking functional MC4R expressed high body weight, adiposity, obesity, high blood leptin and insulin, and preference for energy-dense diets [42]. Humans with homozygote and heterozygote MC4R deficiency showed obesity with homozygote more severe [43]. Additionally, MC4R deficient humans gain



weight from childhood and showed increased fat and lean mass as well as increased linear growth, making them to appear big-boned [44]. Increased appetite (hyperphagia) was also reported in humans and rats expressing MC4R deficiency [44]. The severity of the hyperphagia was associated with the zygosity of the MC4R deficiency [44]. These observations underscored the role of the MC4R gene in regulating energy homeostasis, which determines the functions of many biological processes.

There are reports that melanocortins also control sexual function. The MC4R mRNA is expressed in the nerve fibers, mechanoreceptors in the penis, spinal cord, as well as pelvic ganglion, which is the main autonomic relay center to the penis [45]. Melanocortins agonists increase penile erection induced by electrical stimulation of the cavernous nerve in normal, but not MC4R deficient mice [45]. Copulatory behaviour was also enhanced by MC4R agonists in normal, but not in MC4R mutant mice [45]. In a study, male mice with MC4R deletion developed ED which was corrected with exercise, indicating a possible involvement of obesity [46, 47]. Melanocortins controls energy homeostasis through leptin action, which also controls reproductive activity, thus, its deficiency may affect leptin-mediated reproductive activity [48]. It has been shown that MC4R gene influences the expression and sexual activities of the SIM1 gene in the neurons. In a study that compared the sexual performance of mice expressing MC4R alone on SIM1 neurons with MC4R mutant mice, the mutant mice showed mounting and intromission problem as well as anejaculation [49]. These sexual disorders were reversed when MC4R alone was expressed in the SIM1 neurons of the mutant mice [49]. These observations highlighted the role of melanocortin signalling in SIM1 neurons in the control of male sexual function [49]. An examination of the hormone profiles of female MC4R mutant mice showed irregular estrous cycles, fewer corpora lutea, and abnormal luteinizing hormone (LH) levels compared with the control [50]. These indicate that MC4R plays an important role in regulating LH release, ovulation, and reproductive ability in females [50].

DAT1

The dopamine transporter gene (DAT1), also known as solute carrier family 6 member 3 gene (SLC6A3), codes for a protein called the dopamine transporter [51]. This protein is embedded in the membrane of certain nerve cells in the brain, where it transports a molecule called dopamine (DA, short for 3, 4-dihydroxyphenethylamine) into the cell after a synaptic transmission [52]. Dopamine transporters pump DA into the cytosol, where other transporters sequester it into vesicles for storage and reuse [17]. Dopamine re-uptake through DAT1 is the main mechanism through which the body clears DA from the synaptic cleft [17]. Dopamine is a chemical messenger (neurotransmitter) that transmits signals from one neuron to another [52]. To transmit a signal, the body releases DA into the space between neurons (the synaptic cleft), and binds to receptors on the surface of neighbouring neurons [52]. The DAT1 then returns DA from the

synaptic cleft into the neurons for reuse, thus, it helps regulate the levels and duration of DA in the synaptic cleft [52]. Dopamine is vital in signalling pathways involving sleeping, learning, thought, mood, memory, and movement, among others [17, 52]. However, variable number of tandem repeats (VNTRs) exist in DAT1, which causes differential levels of DA that are linked with some disorders such as bipolar disorder, depression, alcoholism, and substance abuse, among others [53]. Findings also suggest that DAT1-VNTRs, which result in high DA levels, are associated with PE [53].

A VNTR (usually 3 to 12 repeats) resides on the 3' untranslated region (3' UTR) of the DAT1 with the most common being the nine-repeat (9R) and the ten-repeat (10R) [18]. The 10R allele is associated with an increased expression of the DAT1 [18], while the 9R allele decreases the expression of DAT1 [54]. In a study that examined the relationships between DAT1 gene and sexual function among an Iranian population, polymorphisms in the gene were linked with PE. The study reported a link between the 9R and 7R alleles of the gene and PE [55]. The study also found a significant association between the DAT1 10R allele and PE when the 7R allele was absent [55]. Dopamine agonists fasten ejaculation, which again shows that high levels of DA promote PE [55]. These studies showed that decreased expression of DAT1 results in poor DA clearing, culminating in high bioavailability of the molecule and PE. Therefore, men with high concentrations of DA might be genetically predisposed to PE [55].

SLC6A4 and 5-HTTLPR

Solute carrier family 6 member 4 (SLC6A4) encodes serotonin transporter (5-HTT), a neurotransmitter that transports serotonin (5HT, short for 5-hydroxy-tryptamine) from synaptic spaces into presynaptic neurons in the brain [19, 56]. Serotonin transporter terminates the action of serotonin and recycles it in a sodium-dependent manner to ensure the availability of 5HT to other receptors of serotonergic systems [57]. The 5HT recycling or reuptake rate of 5-HTT is regulated by its promoter named serotonin transporter-linked promoter region (5-HTTLPR) and the transcriptional activity of its gene (SLC6A4) [20]. However, both the 5-HTTLPR and SLC6A4 genes have some polymorphisms, which may cause differential expressions [20]. For instance, a deletion in the 5-HTTLPR results in a short (S) allele containing 14-repeat elements, while an insertion creates a long (L) allele containing a 16-repeat element [58, 59]. The S allele represses 5HTT, reducing its 5HT reuptake activity compared with L allele [60, 61]. The L allele is subdivided into L_A and L_G , with L_A showing a high expression of 5HTT mRNA [62]. The SLC6A4A gene contains a polymorphism known as STin2, which is a VNTR in the second intron of the gene [60, 62]. The three most common alleles of this polymorphism are named STin2.9, STin2.10, and STin2.12 having 9, 10 and 12 repeats, respectively [61, 62]. The transcriptional regulatory activity of this polymorphism depends on the number of the repeats, with the STin2.12 allele having a higher expression than other alleles [59, 63].



Studies have shown that polymorphisms in 5-HTTLPR and levels of 5-HT are associated with ejaculation latency time. In a study of ejaculation time in men with lifelong premature ejaculation (PE), polymorphism in 5-HTTLPR were significantly related to latency to ejaculate [22]. Men with the LL genotype ejaculated 100% faster than men with the SS genotype [22]. In three Chinese men with anejaculation, the 5-HTTLPR and SLC6A4A polymorphisms were again implicated. The three men had low expressing alleles (S and STin2.10 alleles), which could have reduced the function of 5-HTT, resulting in high 5-HT bioavailability [64]. The serotonergic system takes part in the neurobiogenesis of ejaculation and PE is mediated mainly through disturbances in serotonergic neurotransmission and serotonin receptors in the central nervous system [55]. It was suggested that the ejaculatory threshold for men with low 5HT levels may be genetically programmed at a very low point, resulting in rapid ejaculation [65]. Men with a very high programmed point may experience delayed or absence of ejaculation despite prolonged sexual stimulation and achieving a full erection [65].

TGFB1

The transforming growth factor beta1 gene (TGFB1) codes for a protein called transforming growth factor beta-1 [25]. The protein triggers chemical signals that regulate cellular activities, such as cell growth and proliferation, cell movement, cell maturation and differentiation, and apoptosis [25]. The TGFβ1 protein is present throughout the body, but is more abundant in the skeletal tissues, where it regulates the formation and growth of bone and cartilage [25]. The TGFβ1 is also involved in the formation of blood vessels, development of muscle tissue and body fat, wound healing, immune system maintenance, and prevention of tumour growth [25]. Studies also associate TGFB1 with the development and function of some male and female reproductive processes, and its polymorphisms or deletion may cause sexual SD [24, 66].

In a scanning electron microscopy of some mice expressing SD with TGFB1 deletion, abnormal superficial coating of the penis' surface in keratinized epithelial cells was observed [23]. Some mice in the study showed a reduced protrusion of penile spines through the superficial tissue, while some other mice had spines almost completely embedded [23]. Histologic analysis of the mice revealed a reduced penis's skin thickness and excess collagen deposits [23]. Further observations showed that the mutant mice expressed sexual interests in the opposite sex, including anogenital investigation, mounting attempts, and a brief intromission, but failed to exhibit sustained intromission and ejaculation [23]. Electrical stimulation of penile function in the affected mice produced nonphysiologic erectile activity and ejaculation of motile sperm [23]. According to the researchers, TGFB1 homozygous deletion may disrupt copulatory behaviour by compromising the penile skin architecture and tissue compliance, culminating in ED [23]. These defects might disrupt male-female anchoring or cause reduced sensory stimulation

or pain, preventing the progression from mounting to intromission and ejaculation [23]. The excess collagen deposition could impair tissue compliance, resulting in reduced blood flow, while the reduced physiologic stimulation may cause an insufficient penile erection for intromission and ejaculation [23]. These observations showed that TGFB1 is necessary for the establishment of normal hypothalamic-pituitary-gonadal axis signalling [23]. Loss of TGFB1 function may impair LH secretion, ultimately affecting oestrous cycles, ovulation, and progesterone production in females as well as testosterone secretion in males [23]. In a study that investigated the involvement of TGFB1 and its type II receptor in patients with ED, expression of TGFB1 was significantly greater in vasculogenic ED (VED) [67]. TGFB1 type II receptor expression was also significantly increased in VED [67]. There was an abundance of collagen deposits in the ED patients, which correlated with the expression of both TGFB1 and its type II receptor [67].

NOS3

The nitric oxide synthase 3 gene (NOS3), otherwise known as endothelial nitric oxide synthase (eNOS) and constitutive nitric oxide synthase (cNOS), produces nitric oxide (NO) in the vascular endothelium, a one-layered flat cells lining the inner surface of blood vessels [26]. Nitric oxide regulates vascular tone, cell multiplication, leukocyte adhesion, platelet aggregation, and many other biological processes [26]. Nitric oxide is also a neurotransmitter that maintains sexual function by regulating relaxation and contraction of smooth muscle of the penis (penile tumescence) [27]. Upon sexual stimulation, NO is synthesized, which results in the accumulation of cyclic guanosine monophosphate (cGMP) and erection [68]. Termination of erection comes with the production of phosphodiesterase type 5 (PDE5), which degrades the cGMP and promotes smooth muscle tone [68]. Insufficient NO after sexual stimulation, resulting from down-regulation of eNOS may disrupt sexual activity [27]. Specifically, three SNPs in the eNOS gene, namely G894T, VNTRs in intron 4, and T-786C, are being suspected in the disruption of sexual function [27].

In a study that examined the associations between polymorphisms in G894T, intron 4 VNTRs, and T-786C with ED, G894T and T-786C were associated with the disorder [26]. Repressed eNOS mRNA and low serum NO were observed in carriers of the CC allele of T-786C [69]. The G894T polymorphism caused an amino acid substitution in eNOS, producing a protein prone to proteolytic cleavage, ultimately resulting in reduced NO production [70]. The eNOS enzyme activity in T allele of the G894T polymorphism was significantly lower compared with the G allele homozygotes [71]. The G894T polymorphism interferes with the smooth muscle-regulated relaxation and contraction of the penis, predisposing to ED [28]. In a study of eNOS intron 4 VNTR susceptibility to ED among Turkish men, a/a allele repeat was associated with severe ED, while a/b was associated with mild effects [72]. In the study, the frequency of intron 4 VNTR gene polymorphism was significantly higher than a



matched control [72]. Generally, a SNP in the eNOS gene affects either its transcription rate or activity of its enzyme, resulting in low levels of NO [73]. Low NO results in reduced cGMP levels, culminating in altered vasoactive substances, endothelial function, and insufficient blood pressure to sustain a penile erection [26].

GNB3/GB3

GNB3, known as guanine nucleotide-binding protein or G protein subunit beta 3 belongs to the class of heterotrimeric guanine nucleotide-binding proteins [29, 30]. The protein transmits signals from several receptors to diverse intracellular signalling pathways, including erectile function [29]. A SNP in this gene, named C825T is associated with high blood pressure, obesity and ED [75, 76]. The C825T polymorphism shortens the gene, creating an alternative splice form referred to as Gβ3-s [74]. The GB3-s increases intracellular calcium concentration by activating phospholipase C, resulting in an increased inositol trisphosphate level and down-regulation of penile vasomotor tone [74]. As explained earlier, erection begins after NO and acetylcholine are released from certain nerve fibres which raises cGMP levels, decreases intracellular Ca^{2+} levels and relaxes smooth muscle cells [75]. As the smooth muscle relaxes, blood fills the lacunar spaces in the corpora cavernosa, compressing the subtunical venules, and blocking the venous outflow [75]. The erection produced is terminated when PDE5 hydrolyzes the cGMP [75]. The GB3-s variant can disrupt the activities leading to penile erection by initiating mitogen-activated protein kinases [76, 77]. The protein kinases are vasoconstrictors that are capable of impairing smooth muscle cell relaxation, preventing penile erection [74, 77]. The GB3-s can also inhibit the adenylyl cyclase, resulting in a reduced cGMP concentration in penile cavernosal cells, culminating in weak penile erection [74, 77]. The GNB3 may interact with eNOS to maintain erectile function, suggesting that polymorphism in the gene may repress eNOS, causing insufficient NO to maintain penile erection [78].

AR

The androgen receptor gene (AR) provides instructions for making a protein called androgen receptor [32]. Androgens are a group of hormones such as testosterone that are important for normal male sexual development during intrauterine life and at adolescence [32]. Androgen receptors allow the body to respond appropriately to these hormones [32]. The receptors are present in many tissues, where they bind to androgens to form an androgen-receptor complex, which binds to DNA and regulates the activity of androgen-responsive genes [32]. The AR switches these genes on or off to direct male sexual development as well as regulate hair growth and sex drive [32]. However, polymorphisms in the AR gene are associated with androgen insensitivity syndrome, which affects fetal and adolescent sexual development [32]. Some polymorphisms shortened the AR, while others deformed it, preventing it from binding androgens or DNA [32]. Consequently, the androgen sensitive cells

become less responsive or unable to use these hormones [32]. Affected individuals are genetically male (XY), but because of their non-responsiveness to male sex hormones, they may express mainly female external sex characteristics or a mixture of both sexes [79].

The most frequent polymorphisms on AR gene are the CAG and GCC repeats, which ranges from less than 10 to 36 repeats [32]. The number of CAG and GCC repeats on AR gene influences sexual function in men and women [80]. In men, CAG and GCC affect the transcriptional activity of AR in which fewer repeats increase AR sensitivity and more repeats decrease its sensitivity, resulting in SD [81]. Longer polymorphic repeats of AR are associated with high testosterone and low oxytocin levels in diabetic patients with ED [81]. In females, long CAG repeats enhance sexual performance, while low CAG repeats result in the inability to reach orgasm [80]. Fewer repeats, resulting in low levels of serum testosterone in human, cause hypogonadism, which can be congenital or may develop at adulthood [82]. Congenital male hypogonadism may delay puberty and disrupt normal development, while adulthood hypogonadism may alter masculine physical characteristics, decrease muscle mass, and cause ED [82].

MTHFR

The methylenetetrahydrofolate reductase gene (MTHFR) encodes methylenetetrahydrofolate reductase, which is involved in the metabolism of folate and homocysteine (Hcy) [34, 36]. Homocysteine is a sulfur-containing amino acid produced from the de-methylation of methionine, which, during methionine deficiency, can be re-methylated to form methionine [83]. Thus, Hcy is an important component of the methylation cycle [84]. However, mutations in MTHFR gene are associated with high serum Hcy levels, which impair endothelial function and cause occlusive vascular disease [34]. High Hcy levels reduce the body's ability to detoxify the system and boost immunity as well as produce enzymes and neurotransmitters, with homozygote more severe [35]. Mutations in the MTHFR gene are also linked with ED and two variants, the C677T allele and the A1298C allele, are frequently reported [36].

The C677T allele is characterized by a point mutation at position 677 of the MTHFR gene that converts a cytosine (C) into a thymine (T), which results in an amino acid substitution (alanine to valine) in the enzyme [36]. In the A1298C allele, a point mutation in exon 7 results in an amino acid substitution (glutamate for alanine) in the enzyme [36]. In a study that examined the involvement of C677T, A1298C, and G1793A polymorphisms of MTHFR in the pathogenesis of early onset VED, individuals with 677TT and A1298C were at an increased risk [34]. Carriers of both 677TT and A1298C alleles were particularly at higher risk [34]. In a study that determined the effects of plasma concentrations of Hcys on ED, elevated concentrations of Hcys beyond the normal levels correlated with mild and severe ED, depending on the



levels [85]. Thus, Hey levels can be used to predict ED development [85].

APPLICATION OF THE IDENTIFIED LOCI IN THE CURRENT TREATMENT OF ED

The identified loci are used as targets to formulate new drugs or improve on the existing ones. Available ED drugs include selective phosphodiesterase 5 inhibitors (PDE5I) such as sildenafil and tadalafil, which bind to PDE5 site, preventing enzymatic degradation of cGMP to GMP [86]. This results in an intracellular build-up of cGMP in the smooth muscle cells in the corpus cavernosum and in the walls of the supplying arteries, ultimately resulting in the relaxation of the smooth muscle, increased arterial blood flow, and penile tumescence [86]. Worldwide, PDE5I is the most used ED drugs, partly because of its efficacy and safety compared to other ED drugs. However, the efficacy of the drugs depends on the availability of cGMP, which is mediated by NO pathway, thus the drugs may not have much impact in ED patients in whom this pathway is disturbed or defective [2]. Some disease conditions that deplete NO are erectile tissue degeneration after prostatectomy, diabetes neuropathy, endothelial dysfunction, and repression of *eNOS* due to atherosclerosis, ageing and hypogonadism [87].

Apomorphine is another ED drug, which is a non-selective DA agonist that binds to dopaminergic receptors of hypothalamic neurons [2]. However, it is not as effective as PDE5I [2]. Intracavernous and intra-urethral injection of vasoactive substances is another ED treatment procedure, of which the most often used vasoactive substance is prostaglandin E1 (PGE1) [2]. PGE1 binds to specific G-protein-coupled receptors (GPCR) in the cell membrane of smooth muscle and activates adenylyl cyclase (AC), resulting in the cleavage of ATP to cAMP [2]. Melanocortin receptor agonists and serotonin antagonists are also being used for treating ED [2].

Generally, the proportion of ED patients that respond negatively to these drugs is high. The poor response to the drugs may be due to the diverse etiology of ED, thus a single drug may not reverse all the diverse mechanisms leading to ED. Hence, there is a need to formulate new drugs and improve on the existing ones to cater for the specific need of each patient, bearing in mind the causal genes and mechanisms. The identified loci in this review may be used as a guide to formulate new drugs and treatment procedures for each pathophysiology leading to ED. For instance, a drug may be formulated to enhance the promoter region of the *SIM1* gene in men with ED expressing *SIM1* repression. For individuals with recalcitrant ED, the synergistic effects of these drugs may be tested.

APPLICATION OF THE IDENTIFIED LOCI FOR FUTURE TREATMENT OF ED

Gene and stem cell therapy are gaining momentum in medicine, and the identified loci in this review may facilitate

their effective application in reproductive health. Genes that produce the same proteins or products as the identified genes may be delivered to their respective locus to treat ED in both men and women. Gene therapy strategies that enhance NO production or NO-mediated signalling pathways, growth factor-mediated nerve regeneration or K⁺ channel activity in the smooth muscle are under investigation [88]. There are NO-releasing polymers capable of delivering NO in a pharmacologically useful way. Such compounds include compounds that release NO upon being metabolized and compounds that release NO spontaneously in aqueous solution. Some animal studies suggest that cavernosal injections of NO polymers can significantly improve erectile function [89]. Adenoviral-mediated CGRP gene transfer into corpora cavernosa have also been shown to enhance erectile responses in aged rats with ED. In a study, AdRSVprepro-CGRP (an adenoviral vector containing prepro-CGRP gene) significantly increased cAMP level in corpora cavernosa and maintained cGMP level, resulting in increased erectile responses (Deng et al 2005). Similarly, intracavernosal extracellular superoxide (SOD) gene transfer was demonstrated to reduce superoxide formation, increase cAMP level, and restore age-associated erectile function (Bivalacqua et al 2003).

The identified loci in this study may enable the stem cells expressing the loci to be regenerated in sexually dysfunction humans. Currently, there are several studies evaluating the efficacy of stem cell in the treatment of ED and a few had reached clinical trials [90]. The first clinical trial of such studies was published in 2010 in which umbilical cord stem cells were injected in seven diabetic men expressing ED. In the mentioned study, most of the participants regained morning erections within one month and maintained this for more than six months (Bahk et al 2010). A review study has also demonstrated that, platelet-rich plasma (PRP) therapy has the potential for treating male sexual dysfunction (Epifanova et al 2020).

CONCLUSION

From the articles reviewed, at least 10 genes are suspected in the pathogenesis of ED. These genes are *SIM1*, *SLC6A4*, *5-HTTLPR*, *TGFB1*, *DAT1*, *MC4R*, *NOS3*, *GNB3*, *AR*, and *MTHFR*. Variants of these genes can disrupt erectile activities in the brain, neurons, and penile tissues, leading to loss of erection, weak vaginal penetration, premature ejaculation, and anejaculation. Each gene has a distinct mechanism and pathophysiology, which, if targeted in the carriers may reverse the disorder or reduce the effects. Researchers and healthcare providers are advised to develop treatment procedures or drugs that target these genes and their pathophysiology.

AUTHORSHIP STATEMENT

TY conceptualized, did the literature search, drafted the manuscript, and did correspondence. **EO** designed the article and did the literature search. **MS** did the article selection and proofreading.



CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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