EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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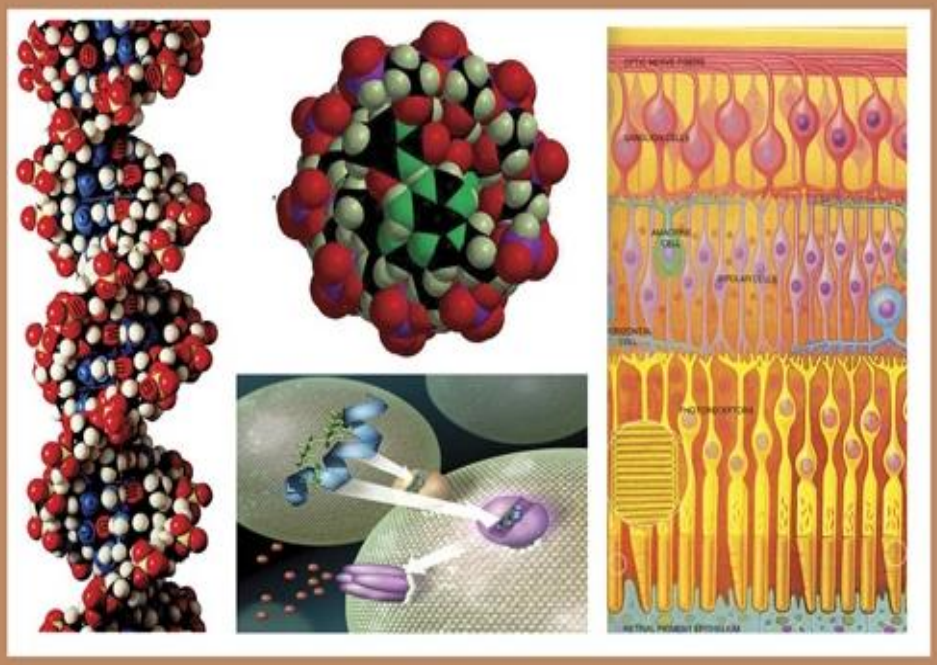

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

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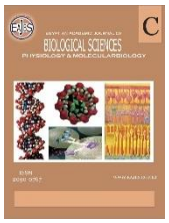
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(1) pp277-292 (2022)*
DOI: 10.21608/EAJBSC.2022.229309

Egypt. Acad. J. Biolog. Sci., 14(1):277-292 (2022)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Clinical and Molecular Genetic Characterization of Waardenburg Syndrome

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REVIEW INFO
Review History
Received:22/3/2022
Accepted:2/4/2022
Available:6/4/2022

Keywords:
Waardenburg syndrome, hearing loss, pigmentation, neural crest cells, transcriptional factor.

ABSTRACT

Waardenburg syndrome (WS) is a clinically and genetically heterogeneous rare genetic disorder encompassing a wide spectrum of anomalies. WS is divided into four primary categories based on clinical and genetic characteristics. WS exists in an autosomal dominant as well as autosomal recessive form. It is characterized by a range of clinical symptoms including pigmentation anomalies of hair, skin, and iris. In the majority of cases, congenital hearing loss is also present. Dystopia canthorum, limb deformities, and neurological impairment have also been associated with some forms of WS and these clinical impairments are used to classify WS. Up until now, mutations in *PAX3, MITF, EDN3, EDNRB, SOX10*, and *SNAI2* have been reported as the main cause of the disease. In this review, I will provide a brief knowledge about WS and its clinical features, prevalence, and types. In addition, I will summarize up-to-date information about WS-associated genes and their involvement in the disease complexity.

INTRODUCTION

Waardenburg syndrome (WS) is a rare heterogeneous condition. It is clinically diverse with genetic variations in multiple genes. WS shows autosomal dominant as well as, in some cases, autosomal recessive inheritance patterns (Pingault *et al*., 2010; Shelby, 2017). The syndrome was named and fully described for the first time by the Dutch ophthalmologist Petrus Johannes Waardenburg in 1951 (Waardenburg, 1951). The main clinical features recorded by Waardenburg in his first description were sensorineural deafness, associated with combining developmental anomalies in eyebrows and eyelids, and pigmentation defects of the hair, skin, iris, and nose root (Farrer *et al.*, 1992; Schultz, 2006). Since then, the syndrome has been reported in several ethnic groups, including Arabs, Asians, Blacks, and Caucasians (Sellars and Beighton, 1983; De Saxe *et al.*, 1984; Nayak and Isaacson, 2003; Wildhardt *et al*., 2013; Kassem *et al*., 2018).

During embryogenesis, the programmed migration of neural crest stem cells (NCC) at the border of the neural tube generates various cell types based on the expression of a subset of genes (Theveneau and Mayor, 2014). Among them are pigment-producing cells (melanocytes) of the glia, inner ear, skin, nervous system, and skeletal tissues (Pingault *et al.*, 2010). Mutation in genes involved in the development of NCC can cause abnormal differentiation, migration, survival, or proliferation of NCC-derived melanocytes (Koffler *et al.*, 2015; Pingault *et al.*, 2010). Mutations in these genes have been reported to associate with various clinical features in WS patients (Song *et al*., 2016; Huang *et al*., 2021).

1-Classification and Clinical Manifestations of WS:

WS is a neurocristopathy with clinical manifestations of various phenotypic features. WS was categorized into 4 distinct subtypes depending on distinguishing clinical characteristics (Doubaj *et al.*, 2015; Zaman *et al.*, 2015). WS-I (WS1, MIM193500) and WS-II (WS2, MIM193510) are the most common types of WS, whereas WS-III or Klein-Waardenburg syndrome (WS3, MIM148820), and WS-IV or Shah-Waardenburg syndrome (WS4, MIM277580), are relatively rare. Interfamilial and intrafamilial clinical variability have also been reported in the presence of the same mutation (Liu *et al.*, 1995; Zaman *et al.*, 2015).

Farrer *et al.* reported clinical diagnostic criteria based on the Waardenburg consortium, which is helpful to differentiate between type I and II WS. Based on these criteria, a patient is diagnosed as having WS if presented with two major symptoms or at least one major and two minor symptoms (Farrer *et al.*, 1992). The major clinical features required to establish WS diagnosis are: (a) abnormality of the pigmentation of the iris, (b) loss of pigmentation of hair-like white forelock or white hairs at any other body site, (c) congenital, non-progressive sensorineural deafness, (d) increased distance between the inner corners of the eyelids (dystopia canthorum), and (e) a familial incidence, such as having a first degree relative with WS. The minor features required for the diagnosis of WS are: (a) synophrys (connected eyebrows), (b) congenital leukoderma, (c) hypoplasia of the nostrils, (d) abnormally wide, high nasal bridge and narrow nostrils, and (e) premature whitening of hair (Liu *et al*., 1995; Zardadi *et al*., 2021). The main difference in clinical features between WS-I and WS-II is the presence of dystopia canthorum in nearly 97% of WS-I patients, but which is entirely lacking in type-II patients (Morell *et al.*, 1997). Sensorineural deafness is also frequent in WS-II patients, with an approximate incidence rate of 90%, compared to 60% in WS-I patients (Koffler *et al.*, 2015). WS-II is a more complex heterogeneous disease, and some patients show neurological impairment. Depending on the underlying genetics, WS-II is subdivided into five categories: 2A, 2B, 2C, 2D, and 2E (Selicorni *et al*., 2002; Liu *et al*., 2020).

WS-III shares primary clinical features with WS-I, but more prominent musculoskeletal abnormalities are observed in WS-III (Klein and Opitz, 1983). Some patients also display microcephaly and mental disability in addition to primary clinical features (Huang *et al*., 2021). WS-IV shares a similar phenotype with WS-II. However, WS-IV is a very rare condition and is normally associated with Hirschsprung disease and frequently results in congenital megacolon and gastrointestinal atresia (Shah *et al*., 1981; Huang *et al*., 2021). Based on the underlying mutations, WS-IV has been classified into three subtypes:4A, 4B, and 4C (Mohan, 2018). Furthermore, neurological features are also described in a group of WS-IV patients. These features include neuropathy of the peripheral nervous system, intellectual disability, ataxia of the cerebellum, and muscle stiffness (Table 1) (Pingault *et al.*, 2010).

Table 1. Clinical Manifestations used to diagnose and classify WS types

	Types	WS-I	WS-II	WS-III	WS-IV
Major clinical features	Hearing loss	+	+	+	+
	Pigmentary abnormality	+	+	+	+
	Dystopia canthorum (W> 1.95)	+	-	+	-
	Musculoskeletal abnormalities	-	-	+	-
	Aganglionic megacolon	-	-	-	+
Minor clinical features	Broad nasal root	+	-	+	-
	Synophrys (unibrow)	+	-	+	-
	Heterochromia	+	+	+	+
	Severe constipation and neurological impairment	-	-	-	+
	Premature gray hair (age <30 years)	+	+	+	+

2-Prevalence and Incidence of WS:

The distribution of WS in both genders is nearly equal but the prevalence of the disorder varies in different geographic regions (Pingault *et al.*, 2010). The most common types of the disease are WS-I and WS-II. The incidence rate of WS range from 1:20000 to 1:42000 (0.05-0.023 per 1000) among the general population (Zaman *et al.*, 2015). The highest incidence of WS has been reported among Kenyans, with nearly 1 in 20000 people affected by it (Nayak and Isaacson, 2003). WS-IV is the rarest type, with a prevalence of <1/1000000 (Mohan, 2018). In 2020, only about 80 cases worldwide were reported (Khan *et al.*, 2020). As far as hearing impairment is concerned, WS is responsible for approximately 2–5% of overall congenital hearing deafness (Read and Newton, 1997; Nayak and Isaacson, 2003; Newton and Read, 2003).

3-Genetic Variations Associated with WS Phenotype:

The WS phenotype appears as a result of mutations in at least six different genes. Typically, WS follows an autosomal dominant and, in some cases, autosomal recessive form of inheritance (Fig. 1). Reports of an incomplete dominant or incomplete recessive inheritance also exist. I will discuss the role of six genes (*PAX3*, *MITF*, *SOX10*, *EDN3*, *EDNRB*, and *SNAI2*) associated with WS and their mutation spectrums (Table 2).

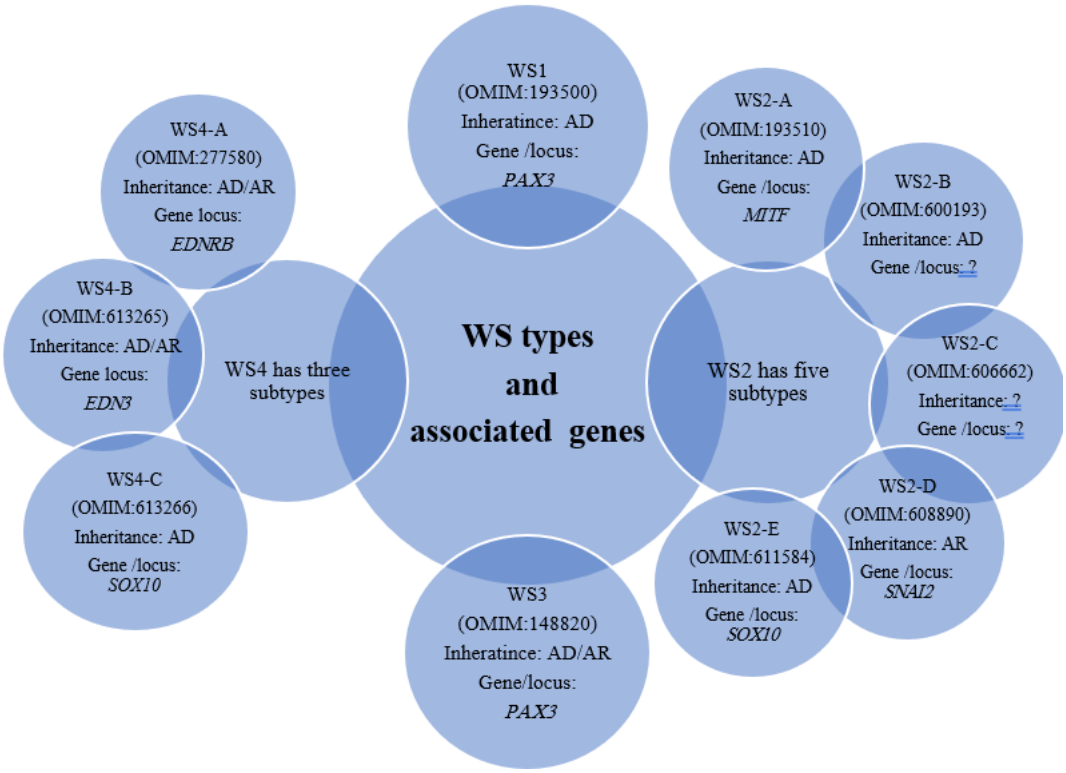

Fig. 1. Summary of WS types, modes of inheritance, and associated genes based on OMIM site.

Table 2. Summary of genes associated with WS and the clinical outcomes resulting from mutations

Gene Symbol	Gene Name	Encoded Protein Function	Chromosome Band	Total Variants	Mode of Inheritance in WS	WS Type	Other Associated Disease
PAX3	Paired box 3	Transcription factor	2q36.1	244	AD AD/AR	WS1 WS3	Craniofacial deafness hand syndrome (CDHS) Rhabdomyosarcoma, type 2 (RMS2)
MITF	Microphthalmia-associated transcription factor	Transcription factor	3p13	156	AD	WS2A	COMMAD syndrome Tietz albinism-deafness syndrome (TADS) Melanoma, cutaneous, malignant, susceptibility to, type 8 (CMM8)
EDN3	Endothelin 3	Secreted growth factor	20q13.32	38	AD/AR	WS4B	Hirschsprung disease, type 4 (HSCR-4)
EDNRB	Endothelin receptor type B	Transmembrane receptor: ligand is EDN3	13q22.3	112	AD/AR	WS4A	ABCD syndrome (ABCDS) Hirschsprung disease, susceptibility to, type 2 (HSCR-2)
SOX10	SRY (sex determining region Y)-box 10	Transcription factor	22q13.1	184	AD	WS2E, WS4C	Intellectual disability (ID) PCWH syndrome
SNAI2	Snail family transcriptional repressor 2	Transcription factor	8q11.21	5	AR	WS2D	Piebaldism

3.1-*PAX3* Gene:

PAX3 (the paired box 3 transcription factor) locus is mapped in chromosome 2q36.1 and consists of 10 exons with an approximal size of 10 kb that encodes 505 amino acids. This transcriptional factor was reported to be involved in several biological functions inside the cell, including the development of neural crest cells, muscle cells, and neural tubes (Boudjadi *et al.*, 2018). Heterozygous mutations in *PAX3* have been described as the common cause of both WS-I and WS-III. PAX3 mutations were first identified in WS-1 families (Tassabehji *et al.*, 1992). Nearly 80% of WS-I patients carry heterozygous point mutations in the *PAX3* gene. Partial or total deletions in *PAX3* are frequently seen in severe cases of WS-III. Also, homozygous or compound heterozygous mutations were reported in some WS-III patients (Boudjadi *et al.*, 2018).

More than a hundred sequence alterations in the *PAX3* gene have been linked to either WS-I or WS-III. The most common changes in the *PAX3* gene in WS cases are missense mutations, which make up 38% of total detected mutations, followed by small deletions, which account for about 20%. Nonsense mutations have also been found in 15% of total changes, including gross deletion (11%), small insertions (8%), and splicing mutations (8%) (Jalilian *et al*., 2015; Boudjadi *et al*., 2018).

The majority of *PAX3* mutations are present in exons 2 to 6, with exon 2 being the mutation hotspot (Pingault *et al*., 2010). Only a few mutations have been identified in exons 9 and 10. The commonly mutated regions alter the structure of the paired domain or homeodomain and thus affect the DNA binding function (Baldwin *et al*., 1995; Carey *et al*., 1998; Jalilian *et al*., 2015). There is no correlation between the mutation location, type, and the phenotype severity symptoms (Baldwin *et al*., 1995). In a subset of WS1 cases, such as those recently reported in Chinese and Korean populations, the proband's PAX3 mutation was not detected in either parent, suggesting the existence of a de novo mutational occurrence (Wang *et al*., 2010; Jang *et al*., 2015). However, the report of two WS1-affected siblings in which the shared PAX3 mutation was not present in either parent also points to the possibility of germinal mosaicism in rare cases (Chen *et al*., 2018).

3.2-*MITF* Gene:

MITF (Microphthalmia-associated transcription factor) belongs to the MYC supergene family. It encodes a transcriptional factor protein that contains a serine-rich transcriptional activation domain and a helix-loop-helix leucine zipper (bHLH-ZIP) domain (Steingrímsson *et al.*, 2004). Human MITF protein in association with closely related proteins TFEB, TFEC, and TFE3 regulates the expression of the target gene by binding to the E-box motif (CANNTG) within the promoter of the target gene, as a homodimer or heterodimer (Moore, 1995; Steingrímsson *et al.*, 2003)
MITF is located on 3p14p13 and spans 229 kbp with a 419 amino acid residue. It has nine distinct isoforms (-A, -B, -C, -D, -E , -J, -H, -M and -MC), each with a 5′ exon (Sun *et al*., 2017; Oliveira *et al*., 2021). MITF protein plays an important role in the differentiation, survival, and development of melanocytes via the regulation of downstream target genes tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and tyrosinase-related protein-2 (TYRP2/Dct) expression (Bertolotto *et al*., 1998; Wang *et al*., 2012).

In 1994, mutations in *MITF* were shown for the first time in two WS-II families (Tassabehji *et al.*, 1994). Consequently, several studies have shown mutations in *MITF* as an important cause of WS type 2A with an autosomal dominant inheritance. Nearly 15–20% of WS-II patients have heterozygous or de novo mutations in *MITF*. Heterozygous mutations in *MITF* also can cause Tietz albinism-deafness disorder (OMIM 103500), which has highly overlapping features with WS type 2A (Huang *et al.*, 2021).

There are more than 77 *MITF* mutations that cause WS2A or Tietz syndrome, with nearly half of them being missense variations. Point mutations are more common in exons 8 and 9 of the MITF gene, whereas, splice-site and truncating mutations of *MITF* are found throughout the gene (Thongpradit *et al.*, 2020). A frameshift variant in the *MITF* gene has also been reported co-segregating with the *C2orf74* gene in a large Saudi family with WS2. It has been hypothesized that the resultant phenotype in the Saudi family might be because of the interaction of *C2orf74* with the product of *MITF* (Albarry *et al.*, 2021). Functional analysis by Zhang et al. demonstrated that a missense variant (p.Arg217Ile) of MITF caused WS2A via a dominant-negative effect, but a frameshift mutation c.575delC (p.Thr192LysfsTer20) caused haploinsufficiency (Zhang *et al.*, 2012). Remarkably, it has been shown that a homozygous intronic mutation of the 5′ splice site sequence of *MITF* led to a severe WS2A phenotype in an Argentinean family (Rauschendorf *et al.*, 2019). Recently, a novel de novo frameshift mutation in *MITF* was identified in twins with WS type 1 (Li *et al.*, 2020).

Interestingly, a study of a Chinese Han family revealed a possible association between homozygous mutations in *MITF* and the development of WS-IV (Pang *et al.*, 2019). Furthermore, the homozygous mutations were also reported to cause autosomal recessive non-syndromic hearing impairment (ARNSHI). Heterozygous individuals of this family, however, were free from any clinical symptoms (Thongpradit *et al.*, 2020).

3.3-*SOX10* Gene:

The *SOX10* (SRY box 10) transcription factor belongs to the large SOX (SRY-related HMG-box) family, which consists of nearly 20 genes. The protein products of these genes are involved in a variety of developmental processes, including skeletogenesis, male differentiation, neurogenesis, and neural crest (NC) formation. Moreover, they also regulate neural stemness, differentiation, and cell fate (Pingault *et al.*, 2022). The human *SOX10* is located on chromosome 22q13.1 and it has five coding exons. It encodes an open reading frame of nearly 466 amino acids. The SOX10 protein has three main functional domains: a highly conserved HMG domain, a SOX Group E domain, and carboxy-terminal transactivation (TA) domain (Pingault *et al.*, 2010; Wang *et al.*, 2017). SOX10 is associated with the maintenance of NC stem cell multipotency and is essential in the formation and differentiation of melanocytes and the enteric nervous system (Pingault *et al.*, 2010; Bondurand and Sham, 2013). *SOX10*, together with *PAX3*, regulates the expression of the MITF gene in the melanocyte lineage. Moreover, SOX10 directly upregulates the expression of TYR, TYR1, and TYR2/Dct, which encode the enzymes necessary for melanin synthesis (Bondurand *et al.*, 2000; Wang *et al.*, 2017).

The first report of the involvement of *SOX10* in WS-VI came from mutated *SOX10* in Dominant megacolon (Dom) mice (Herbarth *et al.*, 1998). These mutated strains presented themselves through dominant megacolon, intestinal aganglionosis, and white spotting (Southard-Smith *et al.*, 1998). This finding prompted researchers to immediately investigate the association of *SOX10* with Waardenburg-Hirschsprung disorder. In 1998, there were the first reports of heterozygous mutations in *SOX10* in four families with WS-IV (Pingault *et al.*, 1998). Later, in 2007, Bondurand *et al.* detected a mutation of *SOX10* in patients with WS-II, verifying that *SOX10* is another important gene involved in WS-II (Bondurand *et al.*, 2007).

SOX10 mutations are responsible for roughly 45%–55% of WS-IV cases and ~ 15% of WS-II cases (Bondurand *et al.*, 2007; Wang *et al.*, 2017). Truncating mutations, which most often remove the main functional domains of a protein, are more frequent in *SOX10* (Chaoui *et al.*, 2011). These truncated mutations can cause a variety of severe neurological symptoms in

the NC (PCWH), such as outer peripheral demyelinating neuropathy, central myelination dysfunction, WS, and Hirschsprung's disease (Pingault *et al.*, 2010). A recent study by Pingault et al. showed that truncating mutations (frameshifts or stops) in *SOX10* genes represented 68% of WS-IV and 54% of WS-II cases. Whereas, non-truncation mutations such as missense, deletion, or small inframe insertions were found in 32% of WS-II and 19% of WS-IV cases (Pingault *et al.*, 2022).

Defects in the *SOX10* gene have also been associated with other human diseases such as Kallmann syndrome (KS) and deafness (Vaaralahti *et al.*, 2014; Izumi *et al.*, 2015). The main phenotypic features that appear in KS patients are anosmia and hypogonadotropic hypogonadism. However, Pingault et al. reported that loss of *SOX10* function in KS patients can cause further symptoms, including deafness, which displays in nearly one-third of cases (Pingault *et al.*, 2013). It has been reported that loss of *SOX10* function leads to agenesis of the olfactory bulb, not only in KS patients but also in some WS cases (Elmaleh-Bergès *et al.*, 2013). The association between WS, deafness, and KS have been reported in different studies. For instance, Suzuki et al. showed a de novo mutation in *SOX10* in a Japanese male patient with KS, sensory deafness, anosmia, and iris hypopigmentation (Suzuki *et al.*, 2015). This identified mutation was seen previously in a patient with WS and Hirschsprung disease (Chaoui *et al.*, 2011). Another study reported the first case of a female with both WS-II type C and KS (Hamada *et al.*, 2020). A recent report also found in a Chinese family a heterozygous mutation in *SOX10* leading to KS coexisting WS-II (Chen *et al.*, 2021).

3.4-*EDN3* and *EDNRB* Genes:

In 1988, Yanagisawa et al. isolated endothelin (EDN; known as ET) from porcine aortic endothelial cells. Endothelin was reported as one of the most potent vasoconstrictors of coronary artery strips (Yanagisawa *et al.*, 1988). Three distinct human endothelin-related genes, known as endothelin-1 (EDN1; ET-1), endothelin-2 (EDN2; ET-2), and endothelin-3 (EDN3; ET-3) were later identified (Inoue *et al.*, 1989). The endothelins (ETs) are mediated by two G protein-coupled receptors (GPCRs): EDNRA and EDNRB. EDNRB binds to all ET-1, ET-2, and ET-3, with comparable affinities, whereas EDNRA binds to ET-1 and ET-2 with a higher affinity than with ET-3 (Li *et al.*, 2020).

EDN3 is located on 20q13.2-13.3 and encompasses five exons that translate to several isoforms. *EDN3* initially translates as pre-pro-endothelin 3, cleaved by an endothelin-converting enzyme into a 21-residue peptide (Kurihara *et al.*, 1999). Whereas the endothelin receptor type B (EDNRB) gene is located on 13q22 and consists of seven exons (Pingault *et al.*, 2010). The interaction of *EDN3* with EDNRB is essential for vasoconstriction, proliferative activities, and the development of NC-derived cell lineages, such as melanocytes and enteric neurons (Bondurand *et al.*, 2018). In a cultured human melanocytes cell line, EDNRB signaling was shown to influence the expression and posttranslational modifications of the *MITF* gene (Sato-Jin *et al.*, 2008). Mice lacking the EDN3/EDNRB receptor-mediated signaling showed defects in enteric neurons and melanocytes derived from a trunk/vagal NC, resulting in megacolons and coat color spotting (Baynash *et al.*, 1994).

Homozygous and heterozygous mutations in *EDN3* and *EDNRB* have been shown to be associated with WS-IV (Puffenberger *et al.*, 1994). Homozygous mutations in these two genes are accountable for 20–30% of WS-IV cases (Attié *et al.*, 1995; Edery *et al.*, 1996). It is reported that *EDN3* and *EDNRB* mutations have a complicated transmission pattern and usually the phenotypic severity depends on the residual activity of the protein. For instance, severe phenotypes tend to appear in patients with a homozygous mutation of *EDN3* and *EDNRB*, whereas patients with heterozygotes mutations display one or more clinical manifestations of the disorder with

low or incomplete penetrance (Huang *et al.*, 2021).

Interestingly, mutations in *EDNRB* have been found in some sporadic WS-II cases. In 2017, Issa et al. screened a cohort of WS-II patients and identified six heterozygous *EDNRB* variations associated with the disease and estimated the mutation. It has been estimated that heterozygous mutations in *EDNRB* are responsible for 5%–6% of WS-II (Issa *et al.*, 2017).

Furthermore, defects in *EDNRB* have been reported in ABCD syndrome, an autosomal recessive disorder that shows clinical overlap with WS-IV (Verheij *et al.*, 2002).

3.5-*SNAI2* Gene:

SNAI2 (Snail family transcriptional repressor 2), formerly called SLUG, is a member of the superfamily Snail zinc finger protein, which encompasses the closely related Snail and Scratch families (Nieto, 2002). The Snail factors are well known for triggering the epithelial-mesenchymal transition (EMT) in mammals, which is caused in part by the direct repressor of E-*cadherin* expression throughout embryogenesis as well as tumorigenesis (Pingault *et al.*, 2010; Zhou *et al.*, 2019). The family of Snail genes encompasses *SNAI1*, *SNAI2*, and *SNAI3*, which are highly conserved among vertebrate species (Barrallo-Gimeno and Nieto, 2005).

The human *SNAI2* consists of three exons and is located on 8q11. The SNAI2 protein consists of consecutive C2H2 type zinc fingers at its C-terminus and a highly conserved SNAG (Snail/Gfi) domain at its N-terminus (Zhou *et al.*, 2019). SNAI2 binds to the E-box-containing promoter of its downstream target genes via its five C-terminus zinc finger domain, and functions as a transcriptional repressor relying on the N-terminus SNAG domain that interacts with a co-repressor (Nieto, 2002; Peinado *et al.*, 2004). SNAI2 is involved in the formation of the primitive streak, mediates EMT, left-right asymmetry and the morphogenesis of several tissues. Its expressed in migratory NCC, which plays an important role in melanoblast migration and survival but not in the formation of NC (Cobaleda *et al.*, 2007; Zhou *et al.*, 2019).

Sanchez-Martin et al. first described the association of *SNAI2* mutations with human disease in 2002. They reported homozygous deletions in *SNAI2* in two unrelated WS-II type D patients (Sánchez-Martín *et al.*, 2002). In 2003, the same group discovered that a deletion in the *SNAI2* gene leads to another melanocyte development disorder known as piebaldism (Sánchez-Martín *et al.*, 2003). WS and piebaldism are both hereditary neurocristopathies disorders and share pigmentation abnormalities, such as congenital patchy leukoderma and poliosis (Mirhadi *et al.*, 2020). However, no other published work discusses the involvement of *SNAI2* in WS development. More recently, all *SNAI2* related WS and albinism cases were re-analyzed for possible analysis errors (Huang *et al.*, 2021). It was concluded that the *SNAI2* mutation might cause WS-II with a minor involvement and further large-scale studies are required to determine the function of SNAI2 mutations in WS (Mirhadi *et al.*, 2020).

3.6-Other Variants Associated with WS:

In 2015, Zazo Seco et al. reported a heterozygous missense mutation in the tyrosine kinase receptor ligand (KITLG) that segregated in a patient with WS-II (Seco *et al.*, 2015). In addition, a homozygous mutation in *KITLG* was reported in a patient with WS-II (Ogawa *et al.*, 2017). Interestingly, both patients were suffering from WS-II which was accompanied by large, pigmented macules. It is known that mutation in *KITLG* can cause a very rare pigmentation disorder, called familial progressive hyper- and hypo-pigmentation (FPHH) (Ogawa *et al.*, 2017).

Recently, a study of a large Saudi family, segregating WS-II, showed a rare heterozygous mutation in *C2orf74* in association with a single nucleotide deletion in the MITF gene. However, the *C2orf74* variant was incompletely penetrant (Albarry *et al.*, 2021).

Conclusions:

WS has always been challenging in genetic counseling because of its clinical and genetic complexity. Most WS patients suffer from hereditary hearing loss, which strongly influences their social communication, cognitive development, speech, and lifestyle (Alzhrani *et al*., 2018; Huang *et al*., 2021). Genetic counseling is very important because the syndrome can pass to the next generation in autosomal dominant or autosomal recessive inheritance mode and de novo cases have been reported which makes it more complicated (Nusrat *et al*., 2018).

The genotype–phenotype correlation of WS remains elusive and further studies are required to fully understand the disease's pathogenesis. First, target gene sequencing for related WS genes should be used to determine the pathogen variants. Whole-exome and whole-genome sequencing approaches should then be used to facilitate the identification of novel variants in undiagnosed cases. WS-II is the most complex type of the disease because of the involvement of several genes in the development of the disease and large-scale studies are required to understand its genetic complexity.

Conflict of interest: The author declares that he has no competing financial interests.

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