Review

Hearing loss in Waardenburg syndrome: a systematic review

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Waardenburg syndrome (WS) is a rare genetic disorder characterized by hearing loss (HL) and pigment disturbances of hair, skin and iris. Classifications exist based on phenotype and genotype. The auditory phenotype is inconsistently reported among the different Waardenburg types and causal genes, urging the need for an up-to-date literature overview on this particular topic. We performed a systematic review in search for articles describing auditory features in WS patients along with the associated genotype. Prevalences of HL were calculated and correlated with the different types and genes of WS. Seventy-three articles were included, describing 417 individual patients. HL was found in 71.0% and was predominantly bilateral and sensorineural. Prevalence of HL among the different clinical types significantly differed (WS1: 52.3%, WS2: 91.6%, WS3: 57.1%, WS4: 83.5%). Mutations in *SOX10* (96.5%), *MITF* (89.6%) and *SNAI2* (100%) are more frequently associated with hearing impairment than other mutations. Of interest, the distinct disease-causing genes are able to better predict the auditory phenotype compared with different clinical types of WS. Consequently, it is important to confirm the clinical diagnosis of WS with molecular analysis in order to optimally inform patients about the risk of HL.

Conflict of interest

The authors declare that they have no conflict of interests.

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Waardenburg syndrome (WS) is the most common type of autosomal dominant syndromic hearing loss (HL) (1), mainly characterized by the association of HL and pigmentation abnormalities, including depigmented patches of the skin and hair, brilliant blue eyes or heterochromia irides. WS incidence is 1/212,000 (2), but due to clinical variability of the syndrome (3) it is speculated that the actual population incidence is 1/42,000. Estimates suggest that the syndrome is found in approximately 2–5% of all congenitally deaf persons, and in 0.9–2.8% of the deaf population (4–8).

WS has been classified into four main phenotypes based on physical characteristics. WS type 1 (WS1, OMIM #193500) and type 2 (WS2, OMIM #193510) have very similar features but are distinguished by dystopia canthorum, which is present only in WS1. WS type 3 (WS3, OMIM #148820) has dystopia canthorum and upper limb abnormalities. WS type 4 (WS4, OMIM #277580), also known as Waardenburg-Shah syndrome, has the additional feature of Hirschsprung disease (4, 9). A clinical subset of WS4 is PCWH (peripheral demyelinating neuropathy, central dysmyelination, WS and Hirschsprung disease), in which patients may suffer from peripheral neuropathy, mental retardation, cerebellar ataxia and spasticity.

The syndrome is genetically heterogeneous: the *PAX3* gene (OMIM #606597) is associated to WS1 and WS3 (10–12), *MITF* (OMIM #156845) (13) and *SNAI2* (OMIM #602150) to WS2 (14), and genes involved in the endothelin pathway, *EDNRB* (OMIM #131244) and *EDN3* (OMIM #131242), were found to be involved in the WS4 (15, 16). Mutations in *SOX10* (OMIM #602229) are responsible for WS2 and WS4 (17–19) (Table 1). For each of these genes, mutations are largely private, and a

Table 1. Genes related to Waardenburg syndrome

Type of WS	Gene involved	Chromosome located
Waardenburg type I	*PAX3*	2q35–2q37 (20)
Waardenburg type II	*MITF*	3q12.3–p14.1 (13, 21)
	SNAI2	8q11 (14)
	SOX10	22q13.1 (19)
	unknown	–
Waardenburg type III	*PAX3*	2q35–2q37 (19)
Waardenburg type IV	*EDNRB*	13q22 (22)
	EDN3	20q13.2–13.3 (23, 24)
	SOX10	22q13.1 (17)

high intra- and interfamilial variability is reported (25). However, still a number of cases remain unexplained at the molecular level.

A Dutch ophthalmologist, Jan van der Hoeve, described two deaf twin girls with a special type of blepharophimosis in 1916. In 1951, the syndrome was thoroughly delineated by Dr Waardenburg (2) and bears his name. Waardenburg described it as a disorder combining anomalies of the eyelids, eyebrows, and nasal root with congenital deafness. HL, mostly sensorineural, is one of the most prevalent features in WS. A review on WS reported that 69% of patients with WS1 and 87% with WS2 had a sensorineural HL (25). Its severity is largely variable within and between families, ranging from profound deafness to a progressive post-lingual HL (25, 26). Bilateral HL appears more frequent than unilateral, and asymmetric HL is also described (25). In WS2, Hildesheimer et al. reported of a high rate of progression of HL (27). This notion has not been confirmed in other articles. There appears to be no typical audiogram shape. The reported prevalence of temporal bone abnormalities varies from 0% to 50% (28, 29). The most frequent inner ear malformations are enlarged vestibular aqueduct and semicircular canal malformations (dilatation, absence) (28, 30). However, the prevalence of vestibular dysfunctions is not well established (31, 32). Vertigo is rare, although vestibular function abnormalities at caloric or rotation tests were described (27, 31, 32).

Our objective was to provide an up-to-date overview of the literature on HL in WS, mentioning prevalences and other auditory features for the different subtypes and the different genes.

Materials and methods

Search strategy

We intended to find all articles describing the phenotype with regard to hearing in WS patients, along with their genotype. The Pubmed database was searched for relevant articles published from inception to 11 August 2014. In order to select relevant articles, the following search string was used: 'Waardenburg syndrome [Title/Abstract]'. EndNote X4 (Thomson Reuters, New York, NY) was used to create a bibliography with the citations retrieved from the above-mentioned search.

Non-relevant papers, which were defined as not focusing on the topic based on the abstract, were excluded as well as non-English articles.

Eligibility of the articles was based on two main inclusion criteria: the description of HL in WS and WS-like patients, and the specification of the causative mutation. To avoid reporting bias, we also included papers in which hearing features were not extensively described or studied. Patients in whom only linkage to a gene was showed were excluded. Discrepancies in the identification and interpretation of data were discussed and resolved by mutual consensus of all authors.

Statistical analysis

The following data were extracted from relevant articles which met the inclusion criteria: study characteristics (authors, year of publication, type of the article, study design and methods, original data or described elsewhere), patient attributes (age and gender, ethnicity and type of the syndrome), auditory features (hearing impairment, type and severity of HL, additional auditory data and inner ear malformation) and mutation (gene involved, mutation type and location and mutation effect). When a patient or family was described in different papers, the most informative paper was used for data collection.

Calculations were performed using SPSS version 22 (SPSS Inc., Chicago, IL). Where appropriate statistical tests were used to assess statistical significance. Bonferroni correction was applied in case of pairwise comparisons out of larger groups.

The study was conducted taking into account the instructions of the PRISMA statement for reporting systematic reviews (33) and of the meta-analysis of observational studies in epidemiology (MOOSE) group for reporting a meta-analysis of observational studies (34).

Results

Search results

In Fig. 1, a flow diagram of the search process is depicted. Out of the 246 potentially eligible articles based on title and abstract, 73 articles met the inclusion criteria and were included in the analysis (10, 12–19, 21, 35–98). One article fulfilled the inclusion criteria, but was not included in the analysis because the patient suffered from a second genetic disorder that could as well result in HL (99). One individual patient was also excluded from the analysis because he exhibited two heterozygous disease-causing Waardenburg mutations in different genes (91). Quality of the included papers was assessed, but none of them were rejected based on quality properties alone, in order to obtain a large population in which it is possible to draw firm conclusions. In the 73 included articles, a total of 417 individual patients were found to meet the inclusion criteria.
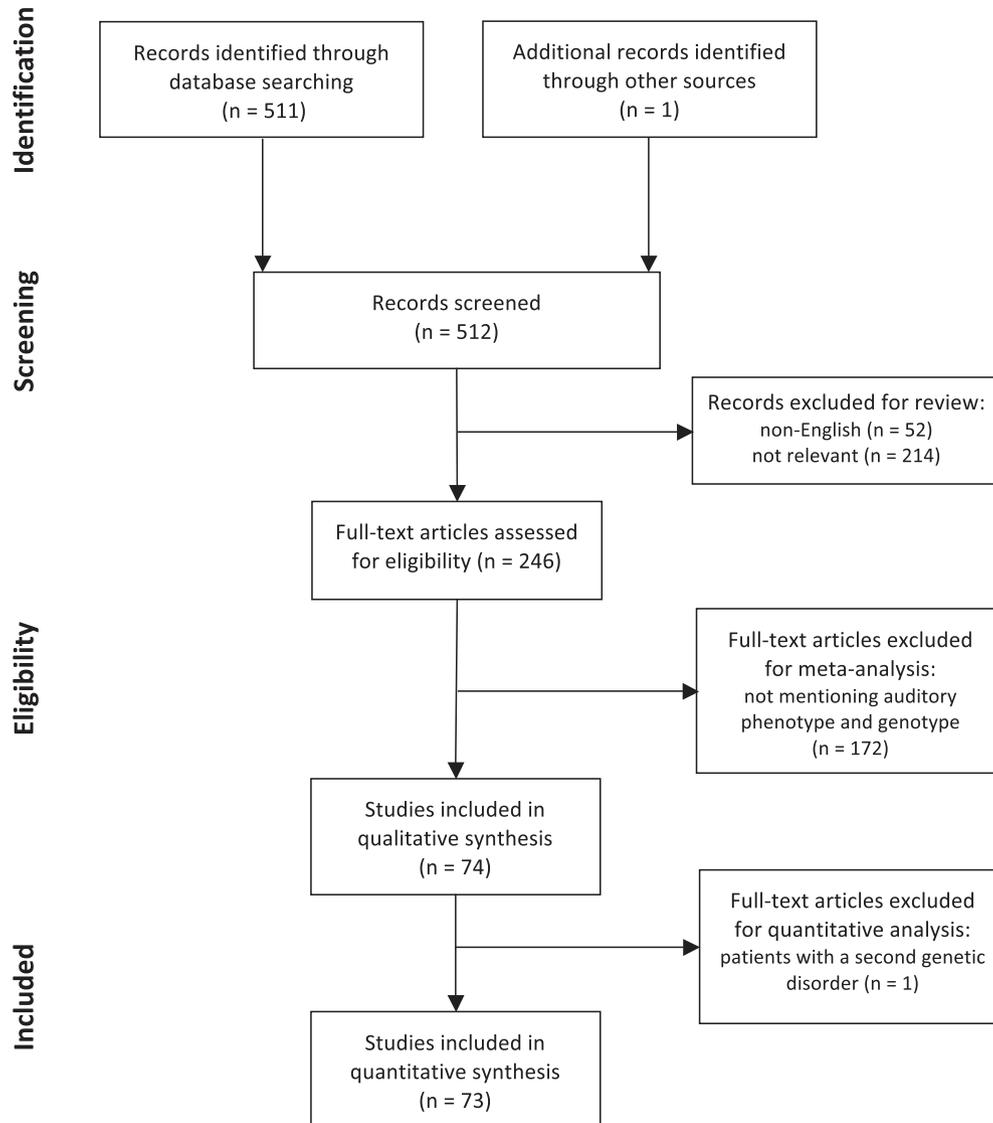
Fig. 1. The PRISMA flow diagram, showing the overview of the search process.

Auditory phenotype in WS

Gender distribution was 46.6% female *vs* 53.4% male persons (gender was provided in 352 patients) and mean age of the included patients was 11.7 years (standard deviation 13.6, exact age was provided in 136 patients). More frequently, patients were subdivided in categories (young children (≤6 years) or older children/adults (>6 years)): 29.5% were young children whereas 70.5% were older than 6 years (information provided in 281 patients).

HL was reported in 296 out of the 417 included patients (71.0%). Of interest, when only selecting patients who objectively underwent audiometric testing as described in the methods of the original articles, we obtained a HL prevalence of 90.2% (110/122). The type of HL was exclusively sensorineural (data available in 174 patients), except for one child with mixed HL (75). No explanation for the conductive component was provided. HL was bilateral in 93 patients *vs* unilateral in 11 persons

(89.4% *vs* 10.6%, data available in 104 persons), and stable in 57 patients *vs* progressive in 5 individuals (91.9% *vs* 8.1%, data available in 62 patients), although few studies followed patients in a longitudinal way. Three of the five patients with progressive HL were diagnosed with bilateral moderate to severe sensorineural hearing loss (SNHL) at initial diagnosis (below 1 year of age), and progressed into profound HL (final diagnosis at age 8 and 13 years, respectively, unclear in one patient). Another patient was tested with ABR (auditory brainstem response) at the age of 5 years, showing moderate SNHL in the right ear and severe SNHL in the left ear. Subsequent ABR at the age of 6 years revealed progression to profound SNHL in both ears. The fifth patient had bilateral mild SNHL at 4 years, with progression to moderate SNHL at 16 years.

Analysis of the age of onset revealed HL being mostly pre-lingual (≤4 years, 65 out of 72 patients, 90.3%). In the remaining seven patients, HL was first
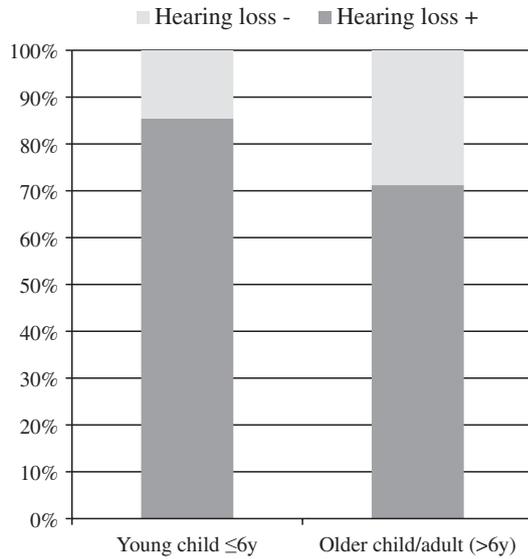
Fig. 2. Prevalence of hearing loss in age group ≤6 years and age group >6 years.

detected between the age of 5 and 11 years (five bilateral profound, one unilateral profound, one unilateral moderate), but could be present at younger age. No significant relationship between the presence or absence of HL and gender could be detected (p = 0.18). However, the comparison of HL and young children reached statistical significance (p = 0.011), with HL being more prevalent in the group ≤6 years (Fig. 2).

We also looked at the different Waardenburg phenotypes. Of the included patients, 195 had type I (46.8%), 136 exhibited type II (32.6%), 7 showed a type III phenotype (1.7%) and 79 had type IV WS (18.9%). Fifteen of the 79 patients with type IV WS (19.0%) were diagnosed with PCWH based on several neurological symptoms. Most patients had a positive family history (75.1%). HL significantly differed among the different types of WS [p < 0.001 for group comparison, significant pairwise comparisons: p(I vs II) < 0.001 and p(I vs IV) < 0.001]. Severity and laterality were also significantly different among the types (p = 0.027 for severity and p = 0.040 for laterality).

Auditory phenotype in different Waardenburg genotypes

Mutations in six different genes have been described in WS. Mutations in *PAX3* (202 patients, 48.4%), *MITF* (115 patients, 27.6%) and *SOX10* (75 patients, 18.0%) account for the majority, whereas mutations in *EDNRB* (15 patients, 3.6%), *EDN3* (8 patients, 1.9%) and *SNAI2* (2 patients, 0.5%) have been found infrequently. The relationship between the different Waardenburg types and involved genes can be found in Table 2. Type I and type III WS are exclusively caused by *PAX3* mutations. Type II WS is associated with *MITF* mutations (84.6%), *SOX10* mutations (14.0%) and *SNAI2* mutations (1.5%). Type IV WS is caused by *SOX10* mutations (70.9%), *EDNRB* mutations (19.0%) and

EDN3 mutations (10.1%). When comparing the prevalence of HL among the different genes, we obtained a significant difference [p < 0.001 for group comparison, significant pairwise comparisons: p(PAX3 vs SOX10) < 0.001, p(PAX3 vs MITF) < 0.001, p(SOX10 vs EDNRB) < 0.001 and p(MITF vs EDNRB) < 0.001]. Severity and laterality were again significantly different among the types (p < 0.001 for severity and p = 0.009 for laterality).

In order to evaluate the importance of Waardenburg type *vs* gene concerning HL, we made a combination of these factors and compared them (Fig. 3). No difference in prevalence of HL between *PAX3* mutations resulting in WS1 *vs PAX3* mutations resulting in WS3 could be found (p = 0.80), nor between *SOX10* mutations resulting in WS2 *vs SOX10* mutations resulting in WS4 (p = 0.54). However, hearing significantly differed among the genes causing WS4 [p = 0.001 for group comparison, significant pairwise comparison: p(SOX10/type IV vs EDNRB/type IV) < 0.001]. The results of the significant comparison between type/gene and severity can be found in Fig. 4, and that of type/gene and laterality in Table 3.

The disease-causing variants included nonsense mutations (35.9%), missense mutations (27.1%), deletions (18.3%), frameshifts (13.2%), insertions (2.4%), splice site mutations (2.2%) and duplications (0.7%). Only a minority of the included individuals had a *de novo* mutation (23.1%).

Imaging in WS

Temporal bone imaging was performed in 24 patients with a *PAX3* or *SOX10* mutation [3 Magnetic resonance imaging (MRI), 11 Computed tomography (CT), 10 both] all presenting with profound HL (19, 59, 64, 68, 85, 88, 92, 93, 97). Five of them showed normal inner ear morphology. In 19 patients, temporal bone abnormalities were reported, mostly bilateral. All of these patients exhibited a *SOX10* mutation (4 WS2 and 15 WS4). The most frequently reported aberrations were agenesis or hypoplasia of the semicircular canals (19/19), an enlarged or malformed vestibule (18/19), and a cochlea with a reduced size and occasionally abnormally shaped (12/19). In three patients, cochlear nerve aplasia could be found, which was bilateral in two of them. Agenesis of the olfactory bulbs, hypoplastic parotid and lacrimal glands and white matter signal abnormalities are among the most frequent imaging abnormalities besides the inner ear.

Specific vestibular symptoms or examinations were unfortunately not mentioned in the included articles.

Discussion

Sensorineural HL is a common clinical feature in WS, with a prevalence of 71%. Bilateral hearing impairment is more prevalent than unilateral, and progression of HL is rare although few patients are followed-up in a longitudinal way. In addition, the higher prevalence

Table 2. Distribution of the Waardenburg syndrome patients included in this study over the genes and the specific WS phenotype

	Type I	Type II	Type III	Type IV	Total
PAX3	195[a]	–	7[a,b]	–	202 (48.4%)
MITF	–	115[a]	–	–	115 (27.6%)
SOX10	–	19[a]	–	56[a]	75 (18.0%)
EDNRB	–	–	–	15[a,b]	15 (3.6%)
EDN3	–	–	–	8[a,b]	8 (1.9%)
SNAI2	–	2[b]	–	–	2 (0.5%)
Total	195 (46.8%)	136 (32.6%)	7 (1.7%)	79 (18.9%)	417

[a]Dominant inheritance.
[b]Recessive inheritance.
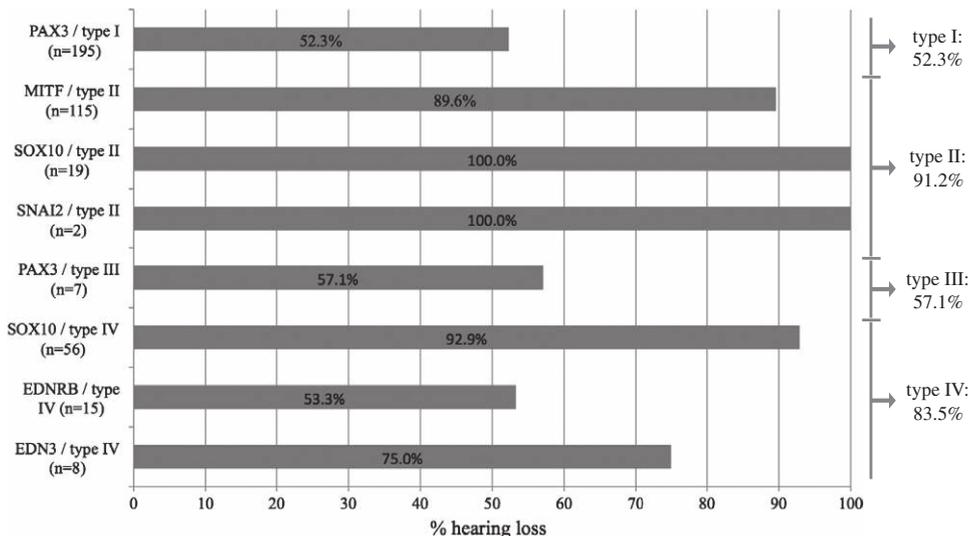

Fig. 3. The prevalence of hearing loss regarding the different type/gene of Waardenburg.

of HL in young children (≤6 years) does not support a high occurrence of progressive HL. All degrees of severity could be observed, but profound HL (>90 dB) appears to be most common. There is no difference in HL prevalence between women and men, whereas age appears to have some influence. However, a publication bias might contribute to this difference. Moreover, quality analysis of the included articles revealed that HL appears to be more frequently found in studies with auditory testing compared with history alone. Up to 90% of the patients, who underwent audiometric testing, were found with HL. This may be explained by a selection bias, because hearing-impaired patients are more probably to undergo hearing tests. Consequently, regular hearing tests in Waardenburg patients are recommended, regardless of symptom reporting. Our findings are in compliance with other authors studying the auditory phenotype in Waardenburg patients (9, 25, 100, 101).

Differentiation into the different clinical types as well as into the different genes is possible. WS1 is caused by heterozygous PAX3 mutations in the majority of cases, if not all (4, 102). In WS3, a phenotype with upper limb abnormalities, PAX3 mutations could also be detected, but this is a rather rare phenotype. The WS2 phenotype is genetically heterogeneous: about 15% is caused by MITF mutations, another 15% can be explained by SOX10 mutations and a minority of WS2 patients exhibits SNAI2 mutations (4, 19, 102). Consequently, in more than half of WS2 patients no disease-causing mutation could be detected yet. The WS4 phenotype is caused by SOX10 mutations (about 50%) or by EDN3/EDNRB mutations (20–30%) (102). Thus, a minority of WS4 patients still remains molecularly unraveled (19). As shown in our results, the different disease-causing genes can better predict the auditory phenotype than the different clinical types of WS. Consequently, the hearing results are discussed for the distinct genes below.

PAX3

A PAX3 mutation had been found in almost half of the patients included in this study, most of them exhibiting WS1, but also some with a WS3 phenotype. Sensorineural HL is found in 52.5% of the PAX3 patients (52.3% in WS1, 57.1% in WS3). The degree of HL differs from mild to profound, even intrafamilially. Of interest, all PAX3 mutations leading to WS3 resulted in severe to profound HL. Unilateral HL is present in about a quarter of the patients. HL due to PAX3 mutations is also described in cranio-facial-deafness-hand syndrome (OMIM #122880).
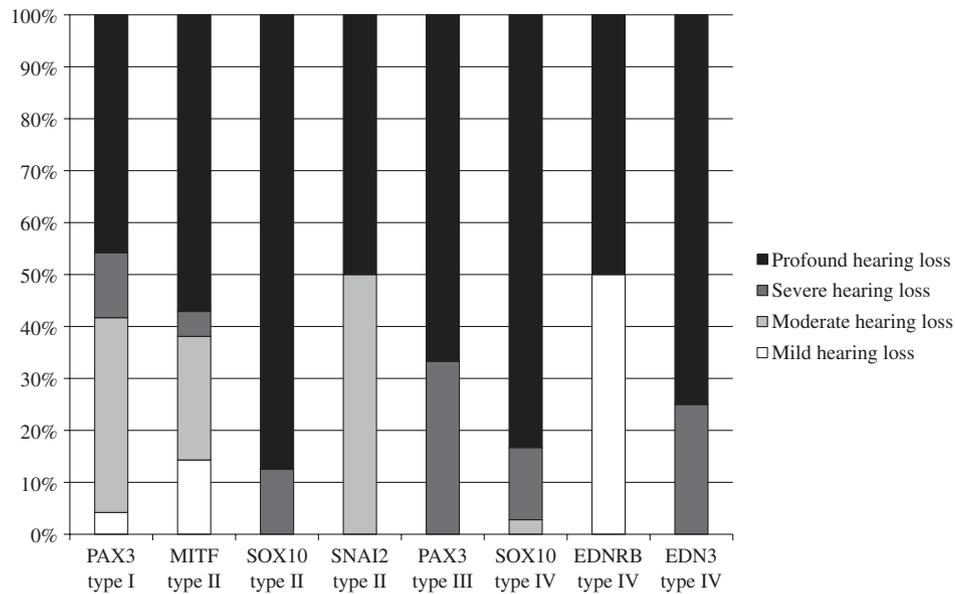
Fig. 4. Severity of hearing loss in the hearing-impaired patients regarding the different type/gene (mild = 26–40 dB, moderate = 41–70 dB, severe = 71–90 dB, profound >90 dB).

Table 3. Laterality of hearing loss in the hearing-impaired patients regarding the different type/gene

	Bilateral hearing loss (%)	Unilateral hearing loss (%)
PAX3/type I	72.2	27.8
MITF/type II	89.5	10.5
SOX10/type II	100.0	0.0
SNAI2/type II	100.0	0.0
PAX3/type III	75.0	25.0
SOX10/type IV	100.0	0.0
EDNRB/type IV	71.4	28.6
EDN3/type IV	80.0	20.0

Pax3 mutations result in inner ear dysfunction as shown in Splotch mutant mice. The gene is thought to be mainly involved in the development of neural crest (NC) cells in the inner ear. However, recent studies have shown that Pax3-expressing cells contribute extensively to multiple inner ear structures, some of which are considered to be derived from the otic epithelium. Lately, Pax3 function is shown to be specifically required for inner ear components with melanogenic fates (103). Therefore, due to these various contributions to cochlear structures, *PAX3* mutations might be directly causal to HL.

MITF

In our series, *MITF* is the second most common gene causing WS (28%) and mutations consistently result in WS2. Sensorineural HL is found in about 90% of these patients, of which severe to profound HL in more than 60%. HL in *MITF* appears to be more prevalent and more severe than in *PAX3*. Only a minority has

unilateral HL. The allelic Tietz syndrome (albinism-deafness syndrome, OMIM #103500) also results in bilateral profound congenital sensorineural HL in most patients.

MITF participates and regulates the growth process of NC source cells, especially survival, proliferation and differentiation of the melanocyte (104). In the stria vascularis of the adult rat, the persisting expression of Mitf can be detected in the melanocytes. Also, the phenotype of deafness can be observed in mice containing particular homozygous mutations in the *Mitf* gene (105). Few functional tests have been performed on human *MITF* mutations. So far, the phenotype of WS2 is most probably caused by haploinsufficiency due to loss-of-function mutations (20, 45, 104).

SOX10

In the *SOX10* mutation-positive group of the included patients, HL was found in 96.4%, of which the vast majority has profound HL. Progression of hearing impairment was described in four patients with a *SOX10* mutation, and generally evolved to profound HL. As for *PAX3*, mutations in *SOX10* leading to a different type of WS exhibited about the same hearing prevalence (92.9% in WS4 *vs* 100% in WS2). Neurological symptoms might be present in a subset of type IV WS, namely PCWH. In addition, heterozygous mutations in *SOX10* can also cause other diseases such as susceptibility to Hirschsprung disease (OMIM #142623) and yemenite deaf-blind hypopigmentation syndrome (YDBS, OMIM #601706), the latter resulting in HL as well.

SOX10 is a key transcription factor of NC development, playing a crucial role in the development of the melanocyte and intestinal ganglia. It could promote the development of the embryonic neural cells and the

peripheral nervous system (106). Besides, *SOX10* is extensively expressed in the early development of the inner ear (107). These results are consistent with animal studies in which heterozygous mutant *Sox10$^{+/mut}$* mice show a HL phenotype (106, 108) due to inner and outer hair cells and supporting cells (Deiters' cells) being absent in the cochlea (109). These results are consistent with the observation of abnormal morphology of the human inner ear according to the findings of MRI/CT scans in patients with *SOX10* mutations (59, 68, 73, 110).

EDNRB/EDN3

Dominant or recessive mutations in *EDNRB* and *EDN3* resulting in a WS4 phenotype have been described in 23 patients. A large variation of hearing phenotype among these patients exists: some had no HL and others had mild to profound hearing impairment. Both unilateral and bilateral HLs have been described, but progression has never been observed. A homozygous *EDNRB* mutation has also been observed in the autosomal recessive ABCD (albinism, black lock, cell migration disorder of the neurons of the gut and deafness) syndrome. It can be hypothesized that the ABCD syndrome is not a separate entity, but an expression of Shah-WS (111).

EDN3 belongs to the group of endothelins, mainly mediating its effect as a physiological ligand for the G protein-coupled heptahelical receptor named Ednrb, which is supported by the observation of similar phenotypes in *EDN3* and *EDNRB* human and mouse mutants (112). A large number of studies have established that the signaling mediated by endothelins plays an essential role in the development of NC-derived cell lineages. Cochlear disorders have been found in the homozygous WS4 mice model (113).

SNAI2

We could include only two unrelated WS2 patients from one article caused by *SNAI2* mutations in our study (14). Both of them showed HL, from moderate to profound.

SNAI2 is expressed in migratory NC cells and is necessary for melanoblast migration and/or survival. The Snai2-null mice are viable but small, with minor craniofacial defects, pigmentary abnormalities, macrocytic anemia and infertility (114, 115). Hyperactivity and circling can also be observed in some animals, and this behavior is suggestive for hearing impairment (14). However, to date, it is not clear how mutations in this gene can cause HL. As only one research group reported *SNAI2* involvement in WS, we can conclude that this gene only has a minor link with WS.

Inner ear malformation in WS

In WS patients, inner ear malformations have been a matter of interest for many years. Until now, there is a debate whether WS is related to any anatomical inner ear anomalies that could be seen on imaging. Previous studies show very heterogeneous results with an incidence of temporal bone anomalies varying from 0%

to 100% among patients with WS (28, 29, 116, 117). Recently, a retrospective case review showed that inner ear malformations were not found in any of the 20 studied patients (40 ears) with WS, suggesting that inner ear malformations are not characteristic for any type of WS (118). However, these patients were not molecularly confirmed. Imaging was performed in 24 molecularly confirmed WS patients and showed that *SOX10* mutations are strongly associated with inner ear malformations including hypoplasia or agenesis of the semicircular canals, enlarged vestibules and cochlear deformities (85). Moreover, the neurological symptoms of PCWH, a subset of type IV WS due to *SOX10* mutations, frequently have imaging correlates (85). Consequently, imaging of temporal bone and brain is of particular interest in patients with *SOX10* mutations. We would also like to stress the lack of results of vestibular examination in WS patients based on this literature overview.

Conclusion

Sensorineural HL is a very common finding in WS affecting 71% of the patients. The different disease-causing genes are associated with different prevalences of hearing impairment and are better in predicting the auditory phenotype compared with the different clinical Waardenburg types. HL in *PAX3* mutations is present in nearly half of the patients, regardless the clinical type. HL in *SOX10* mutations is more common and more severe. Consequently, it is important to confirm the clinical diagnosis of WS with molecular analysis and to perform thorough genotype–phenotype comparisons in order to optimally inform patients about the risk of HL.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Acknowledgements

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