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Original Article

Whole genome genotyping mapped regions on chromosome 2 and 18 in a family segregating Waardenburg syndrome type II

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Abstract

Objectives: Waardenburg syndrome is a rare genetic disorder. It is characterized by sensorineural hearing impairment and pigment defects of the skin, hair and iris. In some cases abnormalities in the tissues derived from neural crest have also been reported. Mutations in several genes have been reported as an underlying cause of Waardenburg syndrome. Objective of this study is to identify the chromosomal region(s) associated with Waardenburg syndrome in an extended Saudi family.
Methods: Genomic DNA was extracted from fifteen individuals of a Saudi family segregating Waardenburg syndrome. Whole genome SNP genotyping was performed to identify common identity by descent chromosomal region(s) shared by affected individuals.
Results: Pedigree analysis confirm autosomal dominant inheritance of Waardenburg syndrome type II in a family. Whole genome SNP genotypes were analyzed using AutoSNPa and DominantMapper tools. Shared identity by descent chromosomal regions were identified on chromosome 2 and chromosome 18. Regions were checked for known Waardenburg syndrome genes. No known gene is present in both regions.
Conclusions: In summary, we identified novel chromosomal regions associated with Waardenburg syndrome type II in a Saudi family. Deep sequencing of a complete candidate regions are required to identify the gene underlying Waardenburg syndrome in this family.

Keywords: Waardenburg syndrome, SNP genotyping, Gene, Chromosomal regions

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Introduction

Waardenburg syndrome (WS) is a rare autosomal dominant disorder. It was first described by Waardenburg in 1951.[22] WS is characterized by sensorineural deafness, pigmentation defects of the skin, hair and iris along with several other abnormalities in the tissues derived from neural crest.[22,3] WS is clinically and genetically heterogeneous disorder with an incidence rate ranging from 1/20000 to 1/42000.[11] Clinical manifestations usually observed in WS patients are due to loss of melanocytes in hair, skin, eye and stria vascularis of the cochlea.

According to the Waardenburg consortium, WS diagnosis is established if an individual is presented with a 2 major features or one major and two minor features.[7] Major clinical features observed in WS are (a) congenital, non-progressive sensorineural deafness, (b) pigmentary abnormality of the iris, (c) hypopigmentation of hair such as white forelock or

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white hairs at other body site, (d) dystopia canthorum and (e) a first degree relative with WS. The minor diagnostic features for WS are (i) congenital leukoderma, (ii) synophrys-connected eye brows (iii) broad high nasal root (iv) hypoplasia of the nostrils and (v) premature gray hair before the age of 30 years.[16,23,21,1,18] Based on presence of combination of clinical features, WS is divided into 4 major types including WSI, WSII, WSIII and WSIV.[17,14]

Mutations in several genes have been reported as an underlying cause of WS and a genotype-phenotype correlation exists between a causative gene mutation and various clinical features in WS. Mutations in *EDN3, EDNRB, MITF, PAX3, SNAI2* and *SOX10* are the six currently reported genes implicated in the pathogenesis of WS.[20,12,15] Mutations in *PAX3* gene is mainly responsible for the clinical features of WS type I and III, whereas mutations in genes *MITF, SOX10* and *SNAI2* are identified in WS type II. Genetic mutations in *EDN3* and *EDNRB* have been implicated in WS type IV.[8,6,10,5,9,19]

Presence or absence of certain clinical features in an individual are required to establish clinical diagnosis of WS. All types of WS need 2 major, or 1 major and 2 minor features to confirm diagnosis except in type II which needs 2 major clinical characteristics out of 5 in the absence of dystopia canthorum which can be identified by calculation of the W index.[13]

Here we report a Saudi family with eleven members diagnosed with Waardenburg syndrome type II based on the presence of two or three major diagnostic criteria. Whole genome SNP genotyping data analysis identified two shared regions on chromosome 2 and 18.

Materials and methods

Recruitment of family members

Affected members of the family visited Magrabi Eye and Ear Hospital Almadinah Almunawwarah, Saudi Arabia. A consultant ophthalmologist examined all individuals. Family members were interviewed and pedigree was drawn based on the information taken from elders of the family (Fig. 1). EDTA containing vacutainers were used to collect 3 ml blood from each participant.

Genomic DNA extraction and genotyping

Nucleic acid was extracted from the blood samples of all available family members using QIAamp DNA mini kit (Qiagen, Venlo, Netherlands). DNA was quantified using spectrophotometer (MaestroGen, Hsinchu City 30091, Taiwan).

Whole genome SNP genotyping was performed using DNA from 13 individuals using Affymetrix GeneChip Human Mapping 250 K Nsp array containing probes for genotyping 262,000 SNPs. 250 ng genomic DNA was digested with Nsp I restriction enzyme. The digested samples were ligated using Nsp adaptor and T4 DNA ligase followed by dilution of samples with AccuGENE water. Ligated samples were PCR amplified using PCR master mix and thermal cycler. Amplified products were resolved on agarose gel followed by purification and elution. The purified products were quantified and normalized in RB buffer. The purified and normalized PCR products were fragmented using Fragmentation reagent, DNase I. The fragmented samples were labelled with fluorescent dyes using GeneChip DNA labelling reagent followed by loading of sample onto GeneChip Human Mapping 250 K Nsp array. The arrays were placed into a hybridization oven for 16–18 hours at 49℃. Fluidics Satation 450 was used for washing and staining GeneChip Mapping 250 K array. The GeneChip® Scanner 3000 7G was used for scanning arrays. The scanned probe array image (.dat file) was obtained and analyzed. Affymetrix genotyping console software (Affymetrix, Santa Clara, California, United States) was used to call genotypes. DominantMapper[4] was used to identify shared chromosomal regions in affected individuals.

Results

Clinical details of patients

All of the eleven affected family members universally had the major criteria of sensorineural hearing loss with variable degrees of intensities ranging from profound to mild hearing impairment (HI). Hearing loss was affecting one (unilateral HI)

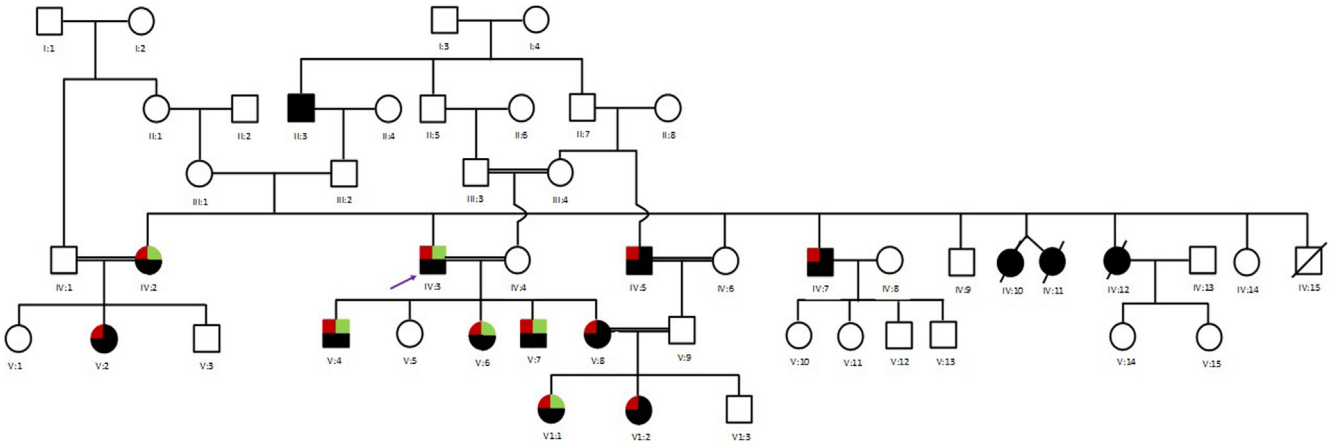

Fig. 1. An extended six generation pedigree chart of a Saudi family segregating Waardenburg syndrome in an autosomal dominant pattern. Clear symbols represent unaffected individuals, whereas filled symbols represent affected individuals. Double lines are indicative of consanguineous unions. The index patient (proband) IV-3 is indicated by an arrow. Red and green colors shows deafness and iris atrophy/ heterochromia, respectively.

or both ears (bilateral HI). Second major criteria of positive family history of hearing loss and variable presence of the third major criteria of ocular hypopigmentation were instrumental in establishing diagnosis. Ocular hypopigmentation ranged from severe hypopigmentation in six members (IV:2, IV:3, V:4, V:6, V:7 and VI:1) (55%), sectoral hypopigmentation in either OS or OD in two members (V:4 and V:6) (20%). Five affected individuals (IV:5, IV:7, V:2, V:8, VI:2) (45%) had no ocular hypopigmentary changes. Dystopia canthorum was not observed in any affected member of the family as their individual calculated W index was less than 1.95 mm for each member thereby excluding WS type I.

All members had no integumentary hypopigmentation in the form of white forelock or leukoderma. Other minor criteria were absent such as synophrys, broad nasal root, hypoplasia of alae nasi or premature greying of the hair. Detailed clinical examination of all family members excluded constipation or skeletal abnormality in the hand and arm. Absence of constipation or skeletal abnormality rule out WS type III and IV. Therefore, this family segregates WS type II in an autosomal dominant manner.

Clinical details of proband and his affected progeny

A 58 years old male individual (IV:3) was presented with gradual diminution of vision of both eyes and congenital deafness. A family history revealed congenital deafness with variable ocular discoloration in ten other members. Unaided visual acuity (UAVA) was 0.1 OD and 0.2 OS. Ocular examination revealed a leukoma adherent scar at the peripheral cornea of the left eye with bilateral iris hypopigmentation and sphincter pupillae and dilator muscle iris atrophy. He had posterior subcapsular cataract OU, OD more than OS. Fundus view was hazy with a depigmented reddish reflex. Audiological test assessment revealed profound bilateral sensorineural hearing loss (Fig. 2E). His final best corrected visual acuity (BCVA) post phacoemulsification with posterior chamber intraocular lens implantation improved to 0.9 OD and 0.7 OS because of presumed bilateral mild amblyopia.

A 16 years old male son (V:4) of proband had severe iris hypopigmentation OS together with a generalized hypopigmented fundus OS (Fig. 2A, B). Ocular examination OD showed no abnormalities. His BCVA was 0.6 and 0.4 for OD and OS respectively due to amblyopia. Audiological testing assessment revealed profound bilateral sensorineural hearing loss. A 28 years old female daughter (V:6) of proband had sectoral iris atrophy and hypopigmentation OD (from one to 7 o'clock) together with a normally pigmented fundus OD. Her left eye showed severe iris hypopigmentation and atrophy associated with a severe generalized hypopigmented fungus (Fig. 2C, D, E). Her BCVA was 0.8 OD and 0.7 OS due to amblyopia. Audiological testing assessment revealed profound bilateral sensorineural hearing loss. A 22 years old male son (V:7) of proband had sectoral patchy iris atrophy and depigmentation OU at 11 o'clock OD and one o'clock OS. Fundus examination was normal in both eyes. His BCVA was 0.1 OU and his audiological testing revealed profound sensorineural hearing loss in the right ear and moderate in the left ear. A 26 years old female daughter (V:8) of proband did not show any ophthalmological abnormalities. Ocular and fundus examination was normal OU but her audiological test assessment revealed mild sensorineural hearing loss in right ear and moderate in the left ear.

Clinical features of granddaughters of proband

A 5 years old girl (VI:1), a granddaughter of a proband, had bilateral severe iris hypopigmentation and defects at the far periphery evident on retro illumination. She did fix and follow and with no refractive error. She has severe bilateral sensorineural hearing loss managed by hearing aid. Patient 6 and patient 7 are sisters. Patient 7 is a 3 years old girl (V1:2). She had normal ocular and fundus examinations

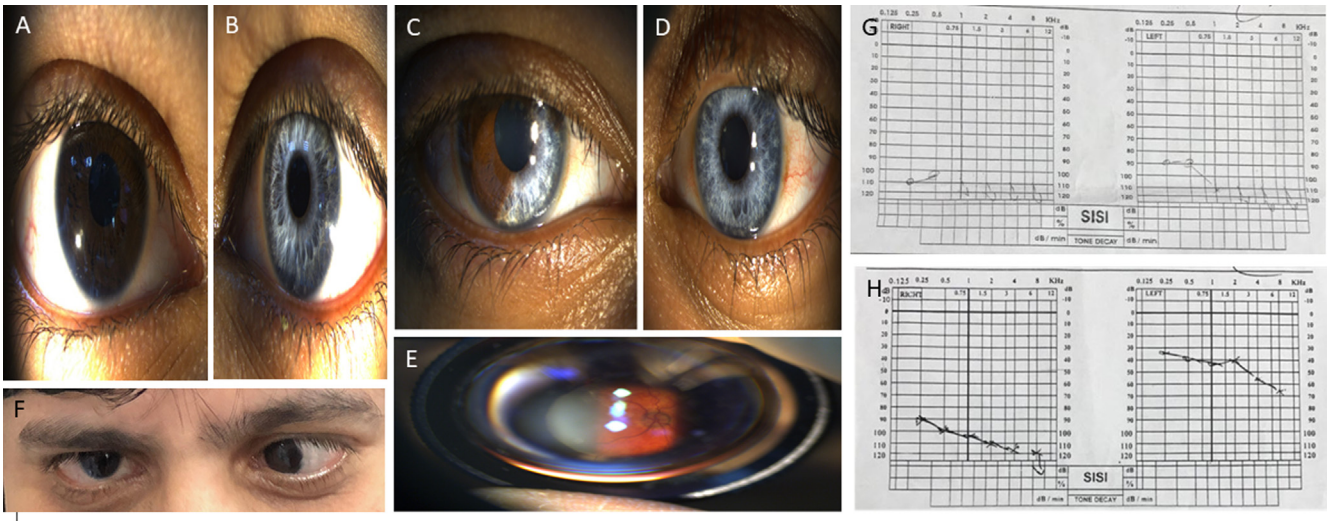

Fig. 2. Clinical features of affected individuals with Waardenburg syndrome type II. (A, B) A 16 years old son (V:4) of a proband with severe iris hypopigmentation OS together with a generalized hypopigmented fundus OS. (C, D, E) A 28 years old female daughter (V:6) of proband had sectoral iris atrophy and hypopigmentation OD together with a normally pigmented fundus OD. Her left eye showed severe iris hypopigmentation and atrophy associated with a severe generalized hypopigmented fundus. (F) A 25 years old niece (IV:5) showed normal ocular and fundal examinations, however, a large angle esotropia OS secondary to dense amblyopia was observed. (G, H) Audiological test assessment of a proband (IV:3) and his son (V:7), respectively, revealed profound bilateral sensorineural hearing loss.

Fig. 3. Graphical representation of DominantMapper output displaying regions linked with the disease phenotype on chromosome 2 and chromosome 18. This result window is composed of two regions which display the analysis results. The upper region (orange bar to the right of figure) shows the results of the rule-based analysis for each SNP, while the lower region (black bar to the right of figure) shows a graph of an empirically derived score, plotted against chromosome position. The chromosomal physical map position is shown between the two regions. The discontinuous thick blue line below the scale represents the positions of the SNPs, with gaps identifying regions with no SNP coverage. Green are SNPs that do not exclude linkage, Orange represent SNPs that are excluded by affected relatives, Yellow show SNPs that are excluded by unaffected sibs, Red are SNPs that are excluded by affected sibs.

OU but has severe bilateral sensorineural hearing loss managed by hearing aid as her sister.

Clinical features of a niece of proband

A 25 years old female (IV:5), a niece of proband, was assessed clinically. She was found to be mentally retarded and resides in rehabilitation center. She suffers from bilateral absolute deafness since birth and her ocular and fundal examinations were within normal limits except for a large angle esotropia OS secondary to dense amblyopia (Fig. 2F).

Clinical presentation of brother and sister of proband

A 49 years old brother (IV:7) of proband had no iris atrophy or hypopigmentation in both eyes. His fundus examination was unremarkable in both eyes. His BCVA was 1.0 OU. He suffers from bilateral deafness since birth and audiological testing assessment revealed profound bilateral sensorineural hearing loss. A 60 years old sister (IV:2) of proband had iris atrophy in both eyes. Her fundus examination was unremarkable in both eyes. Her BCVA was 1.0 OU. She suffers from bilateral deafness since birth and audiological testing assessment revealed profound bilateral sensorineural hearing loss.

Genotyping data analysis identified shared regions

Analysis of SNP genotypes using DominantMapper identified two regions on chromosome 2p16.3-p15 (50 Mb−63 Mb) and chromosome 18q21.33-q22.1 (61 Mb–65 Mb). Region on chromosome 2 is 13 Mb in size and contain 24 genes. Region on chromosome 18 is 4b in size and harbor 14 genes (Fig. 3). Further analysis of these regions revealed that genotypes are identical in all affected individuals. Functional and expression data failed to implicate any gene in both regions as a potential WS candidate gene (Fig. 4).

Discussion

WS is a rare inherited disorder. The phenotype segregates in an autosomal recessive as well as dominant form. The penetrance is incomplete and variable phenotype expressivity is found within members of a same family as well as between families. WS is classified into 4 types (WSI, WSII, WSIII and WSIV) based on the presence of a combination of certain clinical features and their underlying genetic cause.[17,14] ***WS type I*** is characterized by dystopia canthorum, congenital sensorineural hearing loss, pigmentation abnormalities of the eyes, hair and/or skin. ***WS type II,*** however, is characterized by varying degrees of deafness and pigmentation abnormalities of the eyes, hair and/or skin colour. Types I and II can be clinically distinguished by dystopia canthorum (W index > 1.95) which is restricted to type I.[2,24] ***WS type III*** is clinically similar to type I and the difference between two types is the presence of musculoskeletal abnormalities of upper limbs in type III. WS type III is also called Klein-Waardenburg syndrome. ***WS type IV*** is known as

Fig. 4. Coding genes in the chromosome 2 (A) and chromosome 18 regions in the UCSC genome browser.

Shah-Waardenburg syndrome or Waardenburg-Hirschsprung disease as it is associated with intestinal obstruction and megacolon.[21] Mutations in 6 genes have been reported as an underlying cause of WS. This includes *PAX3, SOX10, SNAI2, EDN3, EDNRB,* and *MITF*. *PAX3* mutations have been identified in both WSI and WSIII. Similarly, *SOX10* mutations have been reported in both WS type II and WS type IV. *SANI2* mutations are reported only in WS type II.[20]

In this study, we report an extended Saudi family with 11 individuals presented with clinical picture of WS type II. The clinical diagnosis is based on the fact that all affected individual exhibit variable expressivity of hearing impairment along with ocular hypopigmentation. We performed whole genome SNP genotyping to localize the disease linked region (s). Analysis of SNP genotypes revealed regions on chromosome 2 and 18 (Fig. 3). Both regions have similar genotypes in all affected individuals. We searched for known WS associated genes in both regions but failed to identify any known gene. All other genes present in the linked regions were searched in literature and in UCSC genome browser for their function and expression. No relevant gene was identified (Fig. 4). Therefore we recommend to carry out whole exome sequencing to identify the disease causing mutation(s) in this family.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

References

1. Akal A, Göncü T, Boyaci N, Yılmaz ÖF. Anisometropic amblyopia in a case of type 2 Waardenburg syndrome BMJ. *Case Rep* 2013;**18**, 2013. pii: bcr2013201140.
2. Arias S, Mota M. Apparent non-penetrance for dystopia in Waardenburg syndrome type I, with some hints on the diagnosis of dystopia canthorum. *J Genet Hum* 1978;**26**:103–31.
3. Bondurand N, Pingault V. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. *Hum Mol Genet* 2006;**9**:1907–17.
4. Carr IM, Johnson CA, Markham AF, Tomes C, Bonthron DT, Sheridan EG. DominantMapper: rule-based analysis of SNP data for rapid mapping of dominant diseases in related nuclear families. *Hum Mutat* 2011;**32**:1359–66.
5. Choi EY, Choi W, Lee CS. A novel PAX3 mutation in a Korean patient with Waardenburg syndrome type 1 and unilateral branch retinal vein and artery occlusion: a case report. *BMC Ophthalmol* 2018;**18**:266.
6. Doubaj Y, Pingault V, Elalaoui SC, et al. A novel mutation in the endothelin B receptor gene in a moroccan family with shah-waardenburg syndrome. *Mol Syndromol* 2015;**6**:44–9.
7. Farrer A, Kenneth M, Amos GJ. Waardenburg syndrome (WS) Type I is caused by defects at multiple loci, one of which is near ALPP on chromosome 2: first report of the WS consortium Lindsay. *Am J Hum Genet* 1992;**50**:902–13.
8. Hazan F, Ozturk AT, Adibelli H, Unal N, Tukun A. A novel missense mutation of the paired box 3 gene in a Turkish family with Waardenburg syndrome type 1. *Mol Vis* 2013;**19**:196–202.
9. Jalilian N, Tabatabaiefar MA, Yazdanpanah M, et al. A Comprehensive genetic and clinical evaluation of waardenburg syndrome Type II in a set of Iranian patients. *Int J Mol Cell Med* 2018;**7**:17–23.
10. Jalilian N, Tabatabaiefar MA, Bahrami T, et al. A novel pathogenic variant in the MITF gene segregating with a unique spectrum of ocular findings in an extended Iranian Waardenburg syndrome kindred. *Mol Syndromol* 2017;**8**:195–200.
11. Mahmoudi A, Rami M, Khattala K, Elmadi A, Afifi MA, Youssef B. Shah-Waardenburg syndrome. *Pan Afr Med J* 2013;**14**:60.
12. Mohan SC. Case of Waardenburg Shah syndrome in a family with review of literature. *J Otol* 2018;**13**:105–10.
13. Nasser LS, Paranaíba LM, Frota AC, Gomes A, Versiani G, Martelli Júnior H. Waardenburg syndrome–ophthalmic findings and criteria for diagnosis: case reports. *Arq Bras Oftalmol* 2012;**75**:352–5.
14. Ortonne JP, Mosher DB, Fitzpatrick TB. Waardenburg syndrome. In: Lotti T, Hercogova J, editors. *Vitiligo and other hypomelanoses of hair and skin*. New York: Plenum Medical Book; 1983. p. 337–68.
15. Pang X, Zheng X, Kong X, et al. A homozygous MITF mutation leads to familial Waardenburg syndrome type 4. *Am J Med Genet A* 2018. https://doi.org/10.1002/ajmg.a.60693.
16. Read AP, Newton VE. Waardenburg syndrome. *J Med Genet* 1997;**34**:656–65.
17. Reed WB, Stone VM, Boder E, Ziprkowski L. Pigmentary disorders in association with congenital deafness. *Arch Dermatol* 1967;**95**:176–86

18. Sharma K, Arora A1. Waardenburg syndrome: a case study of two patients Indian. *J Otolaryngol Head Neck Surg* 2015;**67**:324–8.
19. Shi Y, Li X, Ju D, Li Y, Zhang X. Zhang Y. A novel mutation of the MITF gene in a family with Waardenburg syndrome type 2: A case report. Exp Ther. *Med.* 2016 Apr;**11**(4):1516–8.
20. Song J, Feng Y, Acke FR, Coucke P, Vleminckx K, Dhooge IJ. Hearing loss in Waardenburg syndrome: a systematic review. *Clin Genet* 2016;**89**:416–25.
21. Şuhani RD, Şuhani MF, Muntean A, Mesaroş MF, Badea ME. Waardenburg syndrome type 2: an orthodontic perspective. *Rom J Morphol Embryol* 2015;**56**(2 Suppl):879–83.
22. Waardenburg PJ. A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. *Am J Hum Genet* 1951;**3**:195–253.
23. Wang Li, Qin Li, Li T, et al. Prenatal diagnosis and genetic counseling for Waardenburg syndrome type I and II in Chinese families. *Mol Med Rep* 2018;**17**:172–8.
24. Yang S, Dai P, Liu X, et al. Genetic and phenotypic heterogeneity in Chinese patients with Waardenburg syndrome type II. *PLoS One* 2013;**8**:e77149.