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Waardenburg Syndrome: Clinical Differentiation Between Types I and II

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Here we present the results of a study performed on 59 patients affected by Waardenburg syndrome (WS), 30 with the I variant, 21 having the type II, and 8 of them being isolated cases without telecanthus. These patients belong to 37 families; the main contributions and conclusions are based on the detailed study of 25 of these families, examined using standard procedures. All patients were examined as to the presence of eight cardinal signs important for the diagnosis of the condition; from each patient, from many of his/her normal relatives, and from a control sample of 300 normal individuals stratified by age and sex, 23 different craniofacial measurements were obtained. We also estimated, using our own data as well those collected from the literature, the frequencies of the cardinal signs, based on a total sample of 461 affected individuals with WSI and 121 with WSII. In order to originate discriminant functions to separate individuals affected by one of the two variants, both metric (from craniofacial measurements) as well as categoric data (based on the frequencies of the cardinal signs or symptoms) were used. Discriminant analysis based on the frequency of the eight cardinal signs can improve the separation of WSI patients without telecanthus from those presenting the variant II. We present also a Table with the conditional probabilities favoring the diagnosis of WSI for suspect subjects without telecanthus and any combination of the other seven signs/symptoms. The discriminant function based on the four ocular measurements (inner and outer intercanthal, interpupillary, and inferior lacrymal distances), on the other side, perfectly classifies patients affected by one of the variants of WS, the same taking place when the average values of the W index of all affected individuals per family are used. The discriminant function based solely in the individual W index values of patients correctly classifies 93% of WSII subjects, but only 60% of the patients with the I variant of WS. © 2003 Wiley-Liss, Inc.

KEY WORDS: Waardenburg syndrome; genetic heterogeneity; discriminant analysis

INTRODUCTION

The Waardenburg syndrome (WS), first comprehensively described in 1951, is a genetically heterogeneous condition, each of its forms having a wide clinical spectrum with a very high degree of phenotypic expressivity. In the present paper, we will consider only the two most frequent variants (WSI and WSII) out of the four described so far. These two forms, together accounting for a prevalence of 2 to 3 affected individuals/100,000 in the general population, are determined by non-allelic autosomal dominant mutant genes with a high penetrance. WS is characterized clinically by the association of craniofacial dysmorphim, pigmentation defects, and severe sensorineural congenital hearing impairment. The craniofacial dysmorphisms most commonly seen in affected individuals include telecanthus (in WSI only), broad and high nasal root, hypoplasia of the alae nasi, lower lacrimal dystopia, and synophrys. Telecanthus (dystopia canthorum lateroversa) is classically described as an increase of inner ocular intercantal distance (IID)

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with preservation of both interpupillary (IPD) and outer intercantal (OID) distances. WS patients with this sign, however, commonly present larger values of the other two measurements, so that they exhibit a certain degree of hypertelorism. Patients frequently display conspicuous pigmentary defects of the irides (totally or partially heterochromic and bright hypochromic blue irides), hypopigmented skin spots, and partial hair albinism (white forelock or early graying).

The first variant (WSI), with telecanthus, is caused by mutations at the PAX3 gene located in 2q35, while the second (WSII) is determined by other non-allelic autosomal dominant mutations located in the region 3p12.3 → 3p14.1 of the MITF gene. Many of these are point mutations involving single-base substitutions and the number of different mutations described so far for both loci is so large that the molecular screening for them in WS can not be routinely performed in most laboratories. Because of all this, the differential diagnosis between variants I and II still relies largely on classic clinical methods.

Clinical signs and symptoms are similar in both conditions, but telecanthus is known to occur only in WSI; the other characteristics have contrasting frequencies in both forms, especially iris and hair pigmentary disturbances and deafness. The penetrance of the last trait is higher in the second variant of WS, which has therefore a poorer clinical prognosis.

Telecanthus (sometimes hypertelorism) is the most important sign for the differentiation between both forms, because it is present in the vast majority (95–99%) of WSI patients and virtually absent in those with the WSII variant. The presence of conspicuous craniofacial dysmorphisms in WS explains why the condition has been widely studied anthropometrically. The first of these studies was performed on Waardenburg's original data by Cotterman [1951], who derived, by means of linear discriminant analysis, an efficient index for the diagnosis of WS using the three measurements just mentioned. Cotterman's index is given by the formula $L = IID\text{-}0.2497 \times IPD\text{-}0.2119 \times OID\text{-}3.909$. After Arias [1971] recognized the existence of two different forms of WS (with and without telecanthus), this index was used for differentiating between them and, in this context, other indexes have also been proposed, such as the one introduced by Partington [1964], expressed by the formula IID/IPD. In 1978, finally, Arias and Mota introduced the W index, given by the formula $W = (2 \times IID\text{-}0.2119 \times OID\text{-}3.909)/OID + (2 \times IID\text{-}0.2479 \times IPD\text{-}3.909)/IPD + IID/IPD$, an expression thus combining the information contained in the discriminant functions derived by Cotterman and Partington. The average values of W in sets of individuals affected by WSI are generally larger than 2.07, while typically corresponding values are smaller than 1.95 for sets of non-affected controls and for sets of WSII patients from families with no cases of telecanthus. Only in a few instances does W present average values within those two limits in which case the diagnosis of the variant is doubtful [Farrer and Arnos, 1994]. In families with typical cases of WSI there exist, however, some affected individuals without telecanthus, who, as expected, present W indexes smaller than 2.07 [Arias and Mota, 1978]; the incomplete penetrance of the trait has been recently confirmed by molecular studies, which demonstrated that some WS patients without telecanthus and with a W index less than 2.07 carried mutations of the PAX3 gene, a typical finding in WSI [Farrer and Grundfast, 1992, Farrer and Arnos, 1994].

Since the penetrance of the telecanthus trait and consequently the efficiency of the W index—although generally high—are both incomplete [Arias and Mota, 1978], the clinical diagnosis of the variant in an isolated affected individual is troublesome; on a routine basis, such cases are generally labelled as WSII. On the other hand, if the affected individual without telecanthus belongs to a family with typical cases of WSI (with telecanthus), the conflicting situation is solved without any problems. In isolated cases without telecanthus, other methods of differential diagnosis should be used, and these include multivariate methods based on the frequencies of major signs occurring in the two variants, or discriminant functions that take into account a larger number of metric variables, or a combination of both, such as the ones derived by Silva et al. [1993] and [Silva and Batista, 1994], which anthropometric cephalic data were obtained from the study of a Brazilian kindred with a large series of type I WS patients.

In this paper, we describe 59 individuals affected by the Waardenburg syndromes WSI and WSII, belonging to 25 Brazilian families. A detailed craniofacial phenotypic description of all affected individuals is presented, as well as the values of several measurements taken in these patients. The relative frequencies of cardinal signs and the values of craniofacial measurements are used to compare, through discriminant analysis, WSI and WSII affected individuals.

MATERIALS AND METHODS

Out of the 25 families studied personally, 18 were ascertained in the Laboratory of Human Genetics (LGH, Departamento de Biologia, IB USP, São Paulo) and seven were examined at the Hospital de Reabilitação de Anomalias Cranio-Faciais (HRAC, Faculdade de Odontologia, USP, Bauru). For this, we used a standardized routine for physical examination that included the investigation, in all affected individuals (with the exception of a few instances in which one measurement could not be recorded and the corresponding feature could not be evaluated objectively), of the following eight cardinal signs and symptoms of WS: telecanthus, synophrys, iris pigmentation disturbances, localized albinism on hair (white forelock and early graying), hearing impairment, nasal root hyperplasia, hypopigmented skin spots, and lower lacrimal dystopia.

We selected also, through review of the international literature, 44 different papers published from 1951 to 1995 with complete clinical presentation of cases of WS [Waardenburg, 1951; DiGeorge et al., 1960; Partington, 1964; McDonald and Harrison, 1965; Goldberg, 1966; Cant and Martin, 1967; Feingold et al., 1967; Jensen, 1967; Reed et al., 1967; Fanaroff and Levin, 1968; Oliveira and Garcia, 1968; Univelli and Silenzi, 1969;

Amini-Elihou, 1970; Bwibo and Mkono, 1970; Rappoport, 1970; Roux et al., 1970; Arias, 1971; Char, 1971; Conde and Quesada, 1971; David, 1971; Mallardi and Calzaretti, 1971; Murdoch and Mengel, 1971; Pantke and Cohen, 1971; Penchaszadeh and Char, 1971; Pryor, 1971; Viswanathan, 1973; Hageman and Delleman, 1977; Arias and Mota, 1978; Bard, 1978; Parry and Safyer, 1978; Dodinval and Lhussier-Grodos, 1981; De Saxe et al., 1984; Goodman et al., 1988; Narod et al., 1988; Ishikiriyama et al., 1989; Asher and Friedman, 1990; Foy et al., 1990; Kapur and Karam, 1991; Silva, 1991; Winship and Beighton, 1992; Chatkupt and Johnson, 1993; Hol et al., 1995; Lalwani et al., 1995; Liu et al., 1995]. Since the presence of telecanthus establishes the diagnosis of WSI with certainty, in many papers presenting cases of WSI, the authors do not mention some of the above-mentioned eight cardinal charateristics; in relation to WS patients without telecanthus, on the other hand, since the diagnosis will rely on the association of the other seven characteristics, with very few exceptions were all cardinal signs thoroughly researched. Sometimes, a cardinal sign or symptom is not mentioned in the description of a given affected individual simply because it is not present; in other cases, however, they were not mentioned because they were not investigated. There exist some heuristic criteria to distinguish between the options just mentioned, but in any case, if X, Y, and Z (with $X + Y + Z = N$) are the observed numbers of a given sign or symptom described respectively as present or absent, or non-mentioned, in a series of N cases collected from the literature, under the hypothesis (a) that the

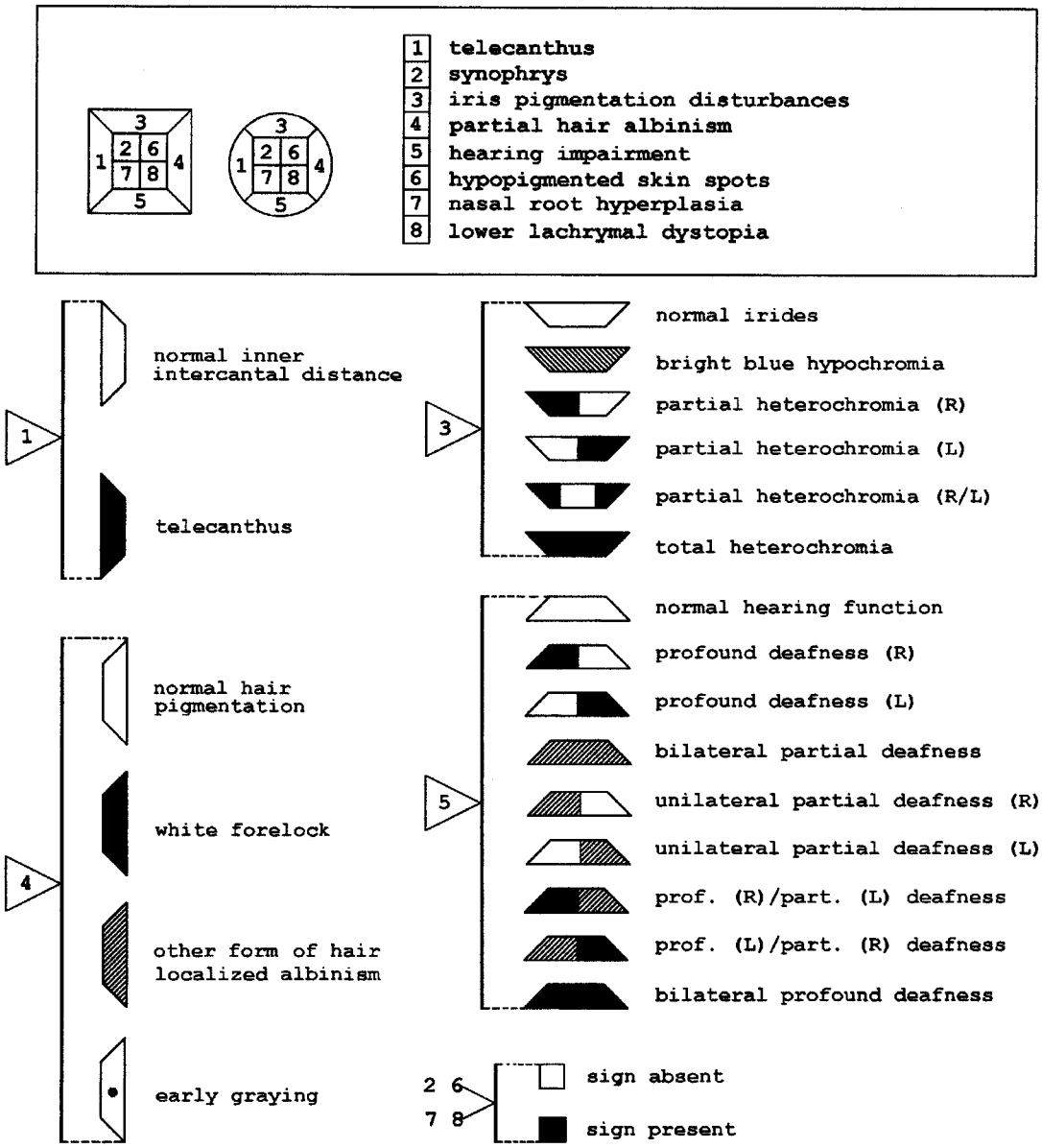

Fig. 1. Symbols used for indicating the presence of cardinal signs/symptoms.

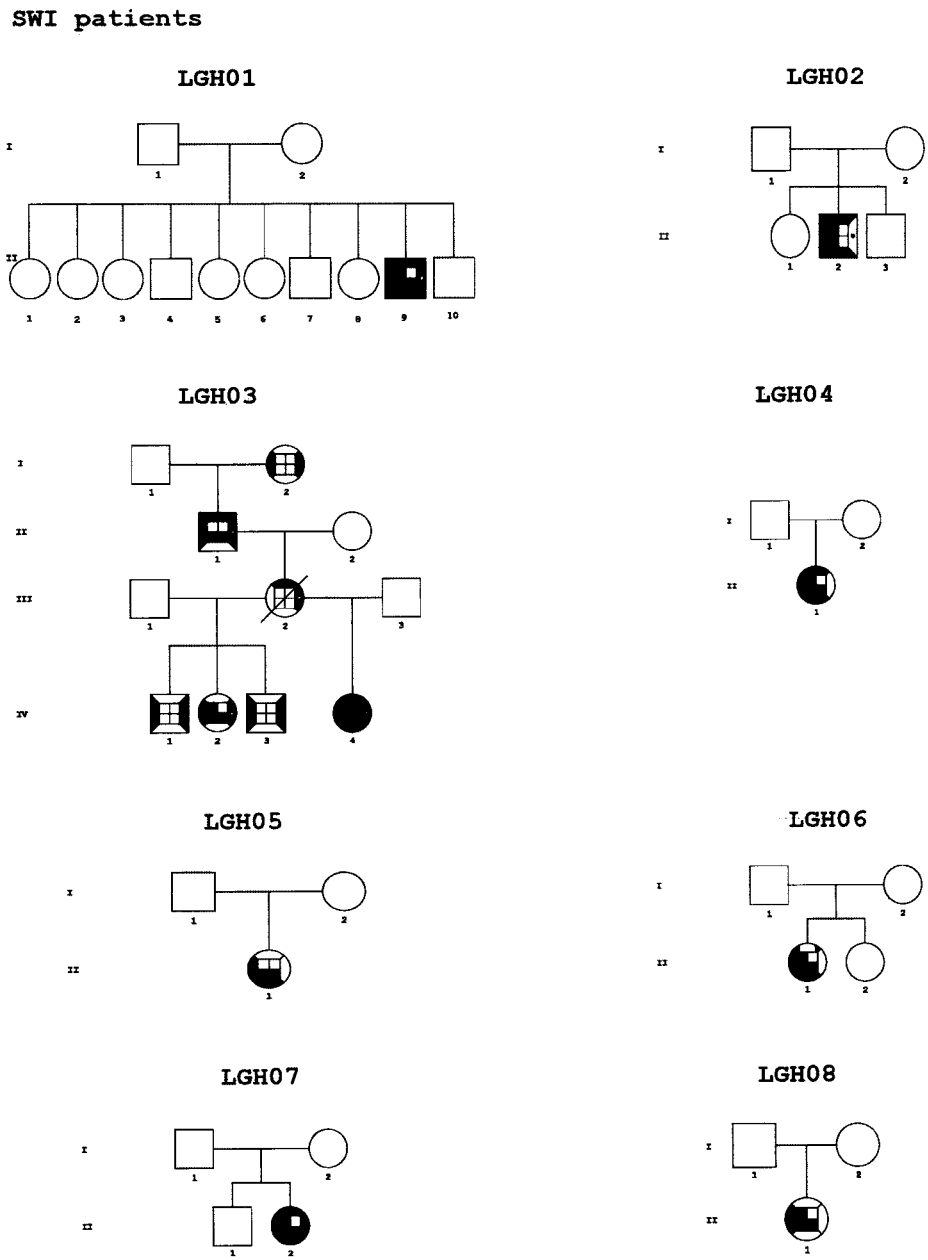

Fig. 2. Genealogies of affected individuals from our sample. The question mark in some symbols representing affected relatives of the index case indicates that the characteristic could not be investigated properly because they were referred to by relatives or retrieved from hospital records. **a**: SWI patients. **b**: SWII patients. **c**: SWII? patients.

non-mentioned characteristic was absent, the estimate for its frequency is given by $x' = X/(X + Y + Z) = X/N$, with expected binomial variance $\mathrm{var}(x') = x'(1 - x')/N$; under the hypothesis (b) that the non-mentioned sign/symptom was not investigated, its frequency estimate is given by $x'' = X/(X + Y) = X/(N - Z)$, with expected binomial variance $\mathrm{var}(x'') = x''(1 - x'')/(N - Z)$. Obviously, the true estimate of the frequency is given by an unknown quantity within an interval with lower und upper limits given by x' and x''. If there is no additional information enabling us to choose one out of the two hypotheses above, an estimate of the true frequency x can be obtained by weighing the estimates x' and x'' by the reciprocal of their expected binomial variances. This estimate will be used throughout this work to contrast the frequencies of the cardinal signs in a sample of WSI and WSII patients combining our data with those from the literature.

We also determined—in random samples of Caucasian individuals stratified by sex and age (total of 300 individuals) and in affected individuals and in their relatives belonging to ten of our 25 families—23 different craniofacial measurements of interest in the diagnosis of WS. Some of these measurements were used for comparing controls and patients as well as types I and II of WS.

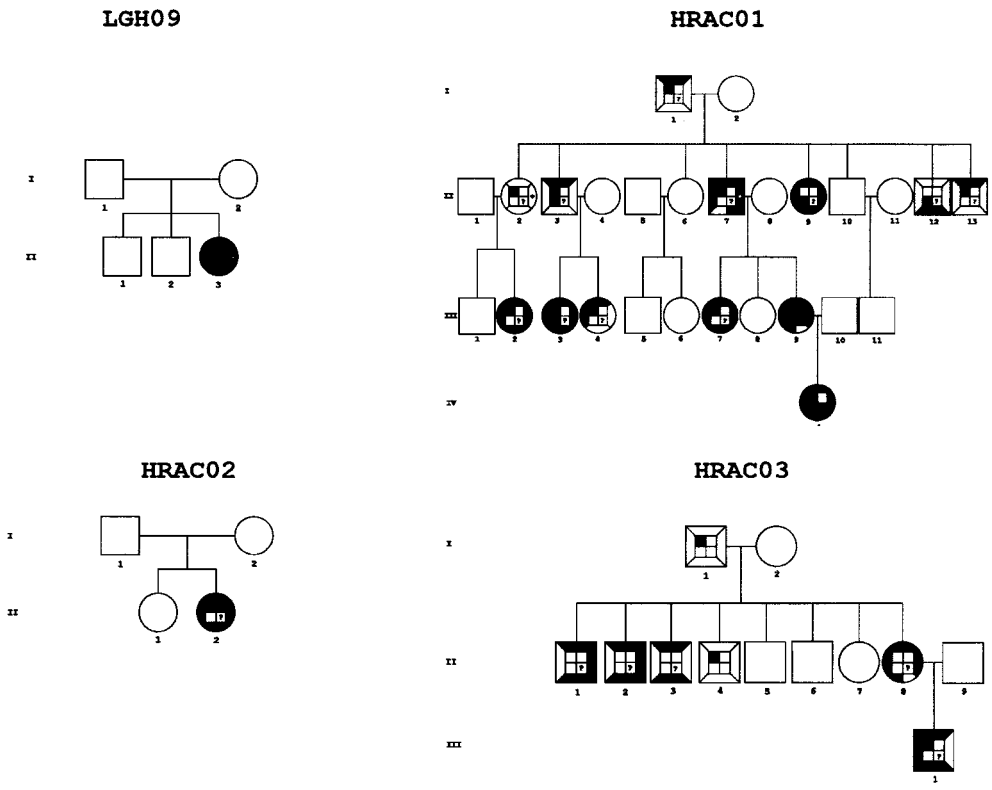

Fig. 2. (*Continued*)

We have classified as WSI all the patients, familial or isolated, that presented conspicuous telecanthus. In order to classify as WSI a case of WS without telecanthus, this affected individual should always belong to a family with at least one typical case of WSI (with telecanthus). Therefore, all cases of WSI without telecanthus presented here are familial, whereas all isolated cases of WS classified as WSI present with the sign. Inversely, all cases of WS classified as WSII are necessarily familial, that is, they belong strictly to families with at least one more affected individual, none of them presenting telecanthus. In the cases selected from literature, we applied the same classification criteria, systematically disregarding the classification of isolated cases of WS without telecanthus as being WSII. In the presentation of our cases in the Results and Discussion section, all isolated WS patients without telecanthus were grouped in a group labelled as WSII?, but their data were not used in the statistical analyses described below.

For the study of cardinal characteristics, the application of the above-mentioned stringent criteria to the cases from literature enabled us to consider a total of 461 WSI patients (29 of them not presenting telecanthus) and 121 carriers of the WSII variant. With the addition of our own data to those from the literature, the discriminant analysis performed with categorical data was based, therefore, on totals of 491 WSI and 142 WSII patients, respectively.

The techniques of statistical analysis used throughout this paper are detailed in standard textbooks (e.g., Zar [1999]). Those on linear and non-linear discriminant analysis in particular are detailed in Smith [1947, 1969], Penrose [1947], and Karn and Penrose [1951].

RESULTS AND DISCUSSION

Description of Cases

Using a modification of the genealogy symbols proposed by Cotterman [1951] and adapted by Arias [1971], shown in Figure 1, we present in Figure 2 and Table I the summary of cardinal characteristics presented by all affected individuals studied here. In families with isolated cases, the corresponding genealogy is ommited and only the first degree relatives are shown besides the index case. Figure 3 summarizes the findings of the orbitary region in 12 patients.

Discriminant Analysis Using the Frequencies of Cardinal Signs and Symptoms

The estimated frequencies of the eight cardinal characteristics of WS were calculated from reliable case descriptions in the literature and are shown in Table II combined with estimates from our own data. The rightmost column shows the weighed average of estimates a (obtained under the hypothesis that the non-referred sign was investigated and was not present) and b (obtained under the alternative hypothesis that the non-referred sign was not investigated).

(b) SWII patients

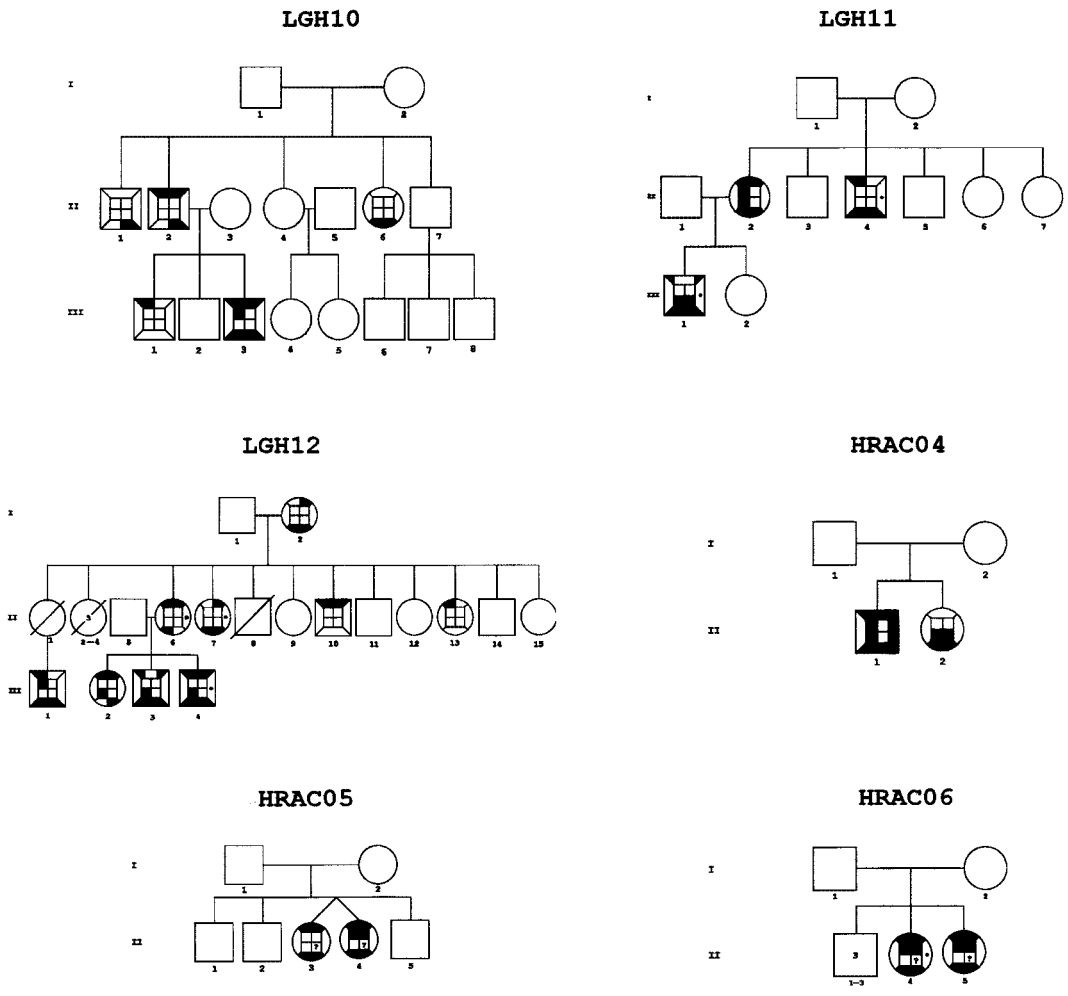

Fig. 2. (*Continued*)

Comparing the observed frequencies of each sign in the groups of WSI and WSII patients through chi-squared tests in 2×2 contingency tables, we obtained in all cases test figures that were significant at least at the 1% level.

The elements necessary for performing a simplified categoric discriminant analysis, together with an application example, are summarized in Table AI (Appendix).

Since there are only seven other possible signs besides telecanthus, all the possible combinations of these seven signs/symptoms (presence or absence) reduce to $2^7 = 128$. 38 out of these 128 combinations generate probability figures larger than 95% or less than 5% favoring the diagnosis of WSI and are shown in Table III. These sign arrays are, therefore, useful for the clinical differentiation between forms I and II; besides this, its inspection enables us to draw, without any difficulties, an interesting conclusion: the most important characteristics for the diagnosis of the WS variants in patients without telecanthus are hearing impairment, the presence of which favors the diagnosis of WSII, and defects of fusion of the medial region of the face (synophrys, nasal root hyperplasia, and lower lacrimal dystopia), which favors the diagnosis of WSI.

We could obtain, combining our data with those from the literature, complete individual phenotypic descriptions of 111 patients affected by WSII out of the 142 used for deriving the probabilities shown in Table AI. The total number of WSI patients without telecanthus whose individual data we could collect was 29, all of these having been included in the group of WSI patients used for generating our probability figures. For each of these, we obtained a final probability figure favoring the diagnosis of WSI. The analysis of the distribution of these values showed that $6/29 = 0.207 \pm 0.075$ of WSI and $110/111 = 0.991 \pm 0.009$ WSII patients were classified correctly. The total misclassification rate revealed by this simplified categoric discriminant analysis was thus $(23+1)/(29+111) = 24/140 = 0.171 \pm 0.032$. In spite

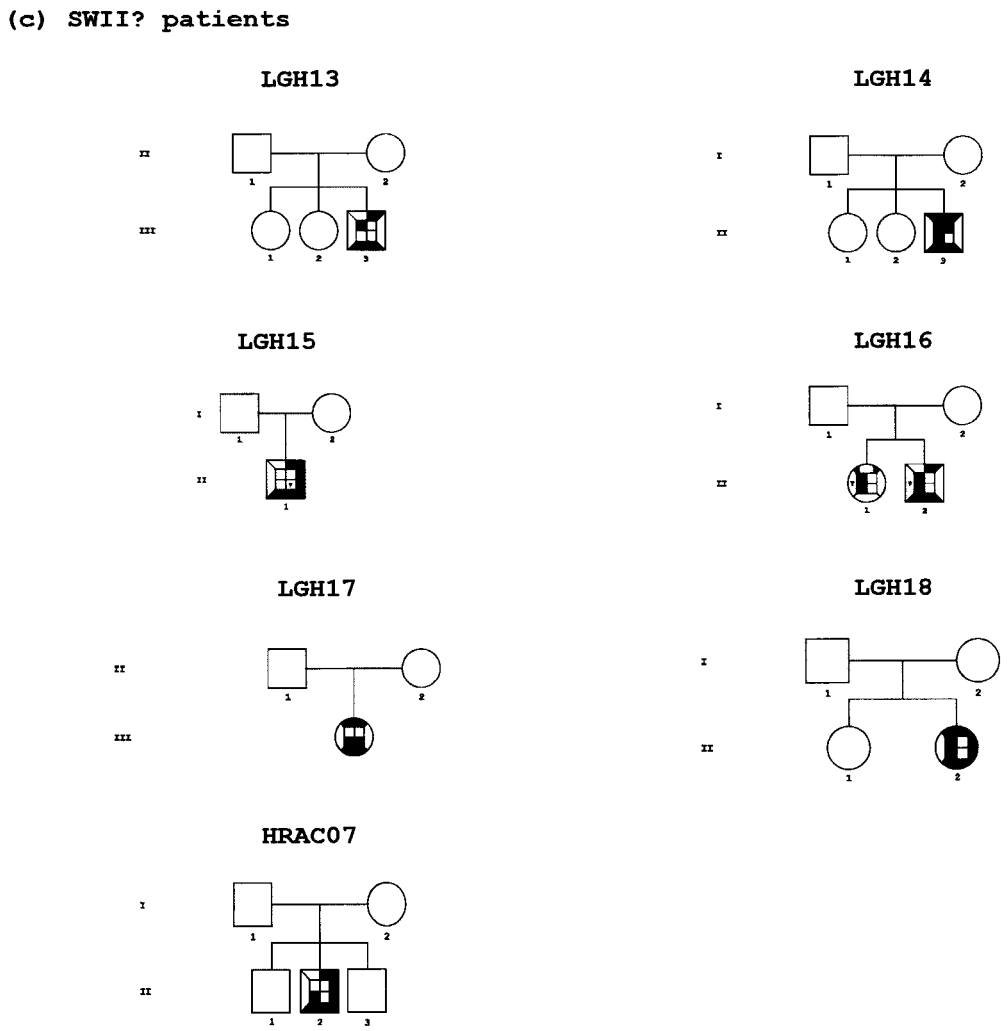

Fig. 2. (*Continued*)

of correctly classifying virtually all WSII patients, categoric methods fail to correctly classify WSI patients without telecanthus. Only two out of 29 WSI patients generated conditional probability figures larger than 90% favoring the correct diagnosis, whereas the same was true for 101 of 111 WSII patients.

Discriminant Analysis Based on Craniofacial Measurements

First we compared the craniofacial measurements between WSI and WSII patients, and between WS patients and controls through t tests with allowance for variance heterogeneity. Using as selection criterion all variables that were statistically different between any of the two comparison groups at least at the 0.001 significance level, we chose the following variables to be used on discriminant analysis: inner intercanthal distance (IID); outer intercanthal distance (OID); interpupillary distance (IPD); lower interlacrimal distance (LID); nose interalar distance (IAD); mean length of ear (EML), obtained by averaging the longitudinal length of both auricles; and the W index (WI), a composite measure used in the literature for separating WSI and WSII patients and described in the introduction section. We decided to also include the variables facial length or morphological face height (MFH) and the mean width of ear (EMW), a measurement obtained by averaging the transversal length of both auricles. These two measurements, in spite of not showing statistical significance at the 0.001 level, exhibited differences at a critical level much less than 0.01. The statistical parameters of these nine measurements, estimated in the groups of WSI and WSII patients and controls, are shown in Table IV, grouped over three different age ranges. We then applied to the set of variables standard methods of stepwise quadratic discriminant analysis, using commercial software (Minitab, Inc.) or programs

TABLE I. Cardinal Signs/Symptoms Presented by the Waardenburg Syndrome (WS) Patients From Our Sample

Identification	1	2	3	4	5	6	7	8
				(a) WSI patients				
LGH01 II-9	+	+	+	+	+	−	+	+
LGH02 II-2	+	+	+	+	+	−	+	−
LGH03 II-1	+	−	+	+	−	−	+	+
LGH03 IV-2	+	+	−	+	−	−	+	+
LGH03 IV-4	+	+	+	+	+	+	+	+
LGH04 II-1	+	+	+	−	+	−	+	+
LGH05 II-1	+	−	−	−	+	−	+	+
LGH06 II-1	+	+	+	−	+	−	+	+
LGH07 II-2	+	+	+	+	+	−	+	+
LGH08 II-1	+	+	−	−	−	−	+	+
LGH09 II-3	+	+	+	+	+	+	+	+
HRAC01 I-1	−	+	+	−	−	−	−	?
HRAC01 II-2	−	+	−	+	−	−	−	?
HRAC01 II-3	−	+	+	−	−	−	+	?
HRAC01 II-7	+	+	+	+	+	−	−	?
HRAC01 II-9	+	−	+	+	+	−	+	?
HRAC01 II-12	−	−	−	−	+	−	+	?
HRAC01 II-13	−	+	+	−	−	−	−	?
HRAC01 III-2	+	+	+	+	+	−	−	?
HRAC01 III-3	+	+	+	+	+	−	+	?
HRAC01 III-4	+	+	+	−	−	−	−	?
HRAC01 III-7	+	+	+	+	+	−	−	?
HRAC01 III-9	+	+	+	+	+	+	+	+
HRAC01 IV-1	+	+	+	+	+	−	+	+
HRAC02 II-2	+	+	+	+	+	+	−	?
HRAC03 II-1	−	−	+	+	+	−	−	?
HRAC03 II-2	−	−	+	+	+	−	−	?
HRAC03 II-3	−	−	+	−	+	−	−	?
HRAC03 II-8	+	−	+	+	+	−	−	?
HRAC03 III-1	+	+	+	−	+	−	−	?
Frequency	22/30	22/30	25/30	19/30	22/30	4/30	17/30	12/13
	0.733	0.733	0.833	0.633	0.733	0.133	0.567	0.923
				(b) WSII patients				
LGH10 II-1	−	−	−	−	+	−	−	−
LGH10 II-2	−	−	+	−	+	−	−	−
LGH10 II-6	−	−	−	−	+	−	−	−
LGH10 III-3	−	+	+	−	+	−	−	−
LGH11 II-2	−	+	+	−	+	−	+	−
LGH11 III-1	−	−	+	+	+	−	+	+
LGH12 I-2	−	−	+	−	+	−	−	−
LGH12 II-6	−	−	+	+	+	−	−	−
LGH12 II-7	−	−	+	+	+	−	−	−
LGH12 II-10	−	−	+	−	−	−	−	−
LGH12 II-13	−	−	+	−	−	−	−	−
LGH12 III-1	−	+	+	−	+	−	−	−
LGH12 III-2	−	−	+	−	+	−	+	−
LGH12 III-3	−	−	+	−	+	−	+	−
LGH12 III-4	−	−	+	+	+	−	+	−
HRAC04 II-1	−	+	+	+	+	−	+	−
HRAC04 II-2	−	−	−	−	+	−	+	+
HRAC05 II-3	−	−	+	−	+	−	−	?
HRAC05 II-4	−	+	+	−	+	+	−	?
HRAC06 II-4	−	+	+	+	+	+	−	?
HRAC06 II-5	−	+	+	−	+	+	−	?
Frequency	0/21	7/21	18/21	6/21	19/21	3/21	7/21	2/17
	0.000	0.333	0.857	0.286	0.905	0.143	0.333	0.118
				(c) WSII? patients				
LGH13 III-3	−	+	+	−	+	−	−	−
LGH14 II-3	−	+	+	−	+	+	+	−
LGH15 II-1	−	−	+	+	+	−	−	?
LGH16 II-1	?	+	+	−	−	−	+	−

TABLE I. (*Continued*)

Identification	1	2	3	4	5	6	7	8
LGH16 II-2	?	+	+	−	+	−	+	−
LGH17 III-1	−	−	+	−	+	−	+	+
LGH18 II-2	−	+	+	+	+	−	+	−
HRAC07 II-2	−	−	+	+	+	−	+	−
Frequency	0/6	5/8	8/8	3/8	7/8	1/8	6/8	1/7
	0.000	0.625	1.000	0.375	0.875	0.125	0.750	0.143

In this and in the tables that follow, the characteristics are identified by the numbers 1–8, as in the genealogy symbols [1, telecanthus; 2, synophrys; 3, iris pigmentation disturbances; 4, localized albinism (hair); 5, deafness/hearing impairment; 6, hypopigmention skin spots; 7, nasal root hyperplasia; 8, inferior lacrimal dystopia].

we developed in Basic. The results obtained made it clear that the craniofacial measurements, especially those from the orbitary region, are important for separating patients from controls and Waardenburg variants I and II. In fact, by combining values of IID, OID, IPD, and LID, a virtually complete discrimination between variants I and II is obtained, without any case of misclassification. As to the W index, a composite measurement that aggregates the values of IID, OID, and IPD, when applied individually, we verified that it correctly classifies 60% WSI and 93% WSII patients. On the other hand, the simultaneous use of the eight selected craniofacial measurements enabled the correct classification of 89% of WS patients and 97% of controls.

Since we had taken the measurements in all available non-affected first-degree relatives of affected familial cases, we applied the discriminant analysis to both groups (patients and relatives) and verified that, although the method was able to correctly classify 83% of the patients, the rate of correct classifications among the relatives was poor, of the order of only about 67%. Since the survey of normal control samples was performed concomitantly to the examination of our families, we can conclude that the results obtained probably reflect the obvious observation bias in a research like the present, given that the investigated material was not unidentified. A heuristically correct procedure would include the collection and analysis of data from affected and control individuals in a masked fashion. Its application involves, however, virtually unsurmountable difficulties. Taking into account all these observations, and adding to them the differences that are usually detected when the measurements are taken by different observers, we come to the conclusion that generally the separation between groups based on metrical characteristics should be considered with reservations. At any rate, the metrical analysis performed here makes it clear that the measurements of the orbitary region are important for distinguishing between variants I and II of WS, as already pointed out by the very first studies performed on the subject. However, when one associates the LID measurement to the other three that compose the W index, the discrimination between the two variants seems to improve.

TABLE II. Frequencies (±1 Standard Binomial Error of Estimates) of the Eight Cardinal Signs/Symptoms Among Patients Affected by Variants I and II of WS

Sign/symptom	Estimate a	Estimate b	Weighed estimation
	(a) WSI		
1	$462/491 = 0.941 \pm 0.011$	$462/491 = 0.941 \pm 0.011$	0.941
2	$338/491 = 0.688 \pm 0.021$	$338/437 = 0.773 \pm 0.020$	0.733
3	$226/491 = 0.460 \pm 0.022$	$226/482 = 0.469 \pm 0.023$	0.465
4	$256/491 = 0.521 \pm 0.023$	$256/469 = 0.546 \pm 0.023$	0.533
5	$227/491 = 0.462 \pm 0.023$	$227/472 = 0.481 \pm 0.023$	0.471
6	$91/491 = 0.185 \pm 0.018$	$91/320 = 0.284 \pm 0.025$	0.218
7	$348/491 = 0.709 \pm 0.021$	$348/437 = 0.796 \pm 0.019$	0.755
8	$47/491 = 0.096 \pm 0.013$	$47/58 = 0.810 \pm 0.051$	0.140
	(b) WSII		
1	$0/142 = 0.000 \pm 0.000$	$0/142 = 0.000 \pm 0.000$	0.000
2	$18/142 = 0.127 \pm 0.028$	$18/142 = 0.127 \pm 0.028$	0.127
3	$85/142 = 0.599 \pm 0.041$	$85/142 = 0.599 \pm 0.041$	0.599
4	$47/142 = 0.331 \pm 0.039$	$47/141 = 0.333 \pm 0.040$	0.332
5	$111/142 = 0.782 \pm 0.035$	$111/135 = 0.822 \pm 0.033$	0.803
6	$14/142 = 0.099 \pm 0.025$	$14/139 = 0.101 \pm 0.026$	0.100
7	$13/142 = 0.092 \pm 0.024$	$13/139 = 0.094 \pm 0.025$	0.093
8	$2/142 = 0.014 \pm 0.010$	$2/17 = 0.118 \pm 0.078$	0.016

The column headings are explained in the text above and the characteristics are identified by the same numbers as in Table I.

TABLE III. Conditional Probabilities Favoring the Diagnosis of WSI in Patients With WS Without Telecanthus (First Element of Signs/Symptoms in the Lists AAAAAAAA to APPPPPPP, Where the Letter A Indicates That the Characteristic is Absent and P that it is Present) With any out of the 128 Possible Combinations of the Other Cardinal Signs/Symptoms (2–8)

Signs	P(WSI)	Signs	P(WSI)	Signs	P(WSI)
AAPAPAAA	0.00118	AAAAPAAP	0.02030	APAPPAPP	0.96452
AAAAPAAA	0.00202	AAAPAAAA	0.02081	APAAPPPP	0.96748
AAPPPAAA	0.00270	APPAPAAA	0.02175	APAPAPPA	0.96827
AAPAPPAA	0.00295	AAAAAPAA	0.02273	APPAAAPP	0.96919
AAAPPAAA	0.00463	AAPPPAAP	0.02695	APPPPPPP	0.97547
AAAAPPAA	0.00506	AAPAPPAP	0.02941	APAAAAPP	0.98184
AAPAAAAA	0.00535	AAPPAPAA	0.03015	APAPPPPP	0.98558
AAPPPPAA	0.00676	AAPAPAPA	0.03438	APPPAAPP	0.98635
AAAAAAAA	0.00917	APAAPAAA	0.03681	APPAAPPP	0.98751
AAAPPPAA	0.01156	AAAPPAAP	0.04544	APAPAAPP	0.99202
AAPAPAAP	0.01191	APPPPAAA	0.04860	APAAAPPP	0.99270
AAPPAAAA	0.01221	AAAAPPAP	0.04952	APPPAPPP	0.99453
AAPAAPAA	0.01335			APAPAPPP	0.99681

The order of the characteristics in the lists is the same used in the preceding tables and text, so that the second element indicates absence (A) or presence (P) of synophrys, the third refers to iris pigmentation disturbances, and so on.

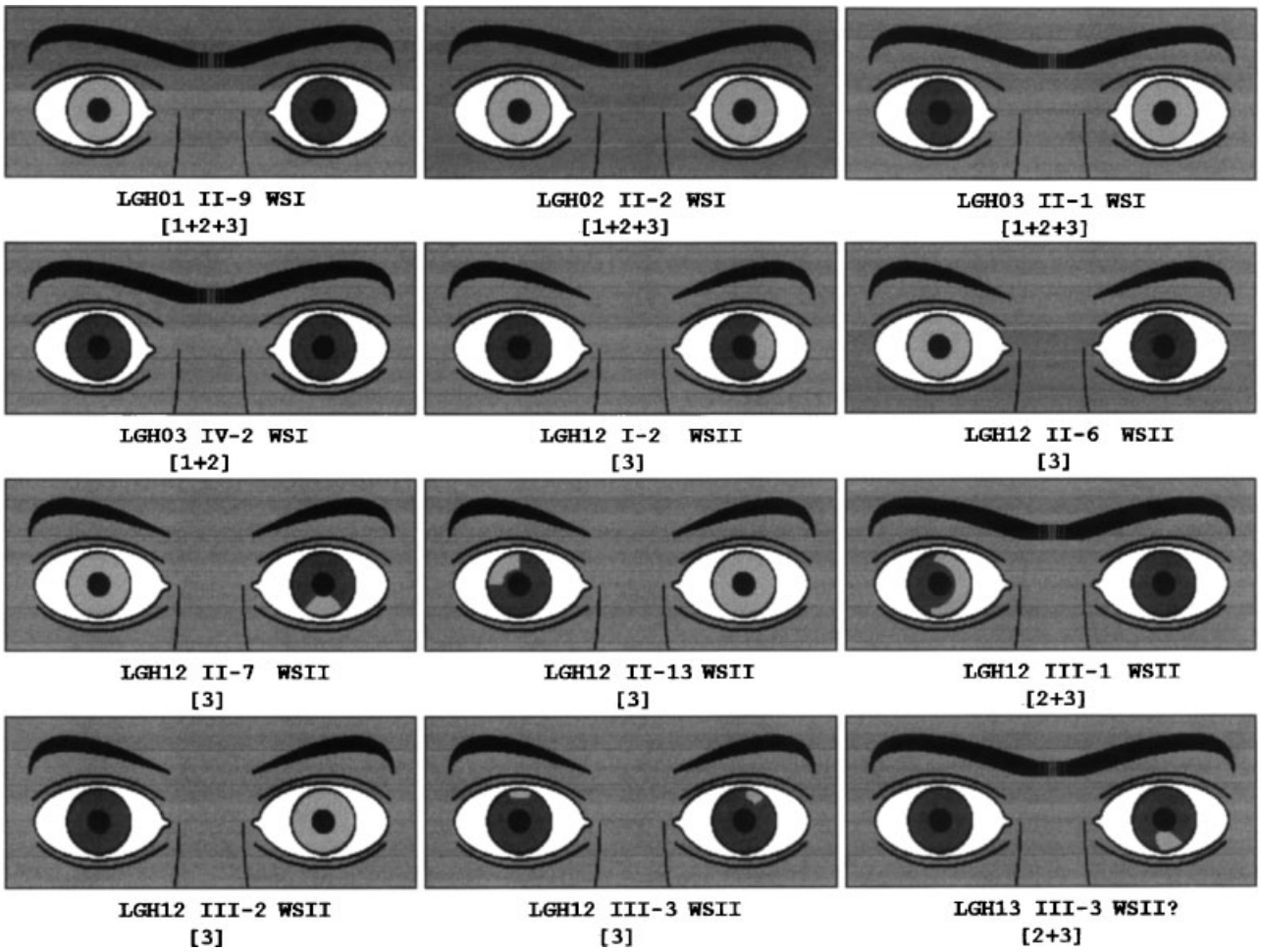

Fig. 3. Schematic representation of the orbitary region from 12 differently affected individuals of our sample, exhibiting at least one out of the following conspicuous characteristics: telecanthus [1], synophrys [2], and iris pigmentation defects [3], which includes heterochromia (total or partial) or hypopigmented bright blue irides.

TABLE IV. Summaries of Descriptive Statistics of Eight Craniofacial Measurements and the W Index (WI) in Patients Affected by the Waardenburg Syndrome Types I and II, and in Normal Controls

	<10a			10–20a			>20a		
Measurements	n	Mean	SD	n	Mean	SD	n	Mean	SD
				WSI patients					
MFH	2	101.500	3.540	2	113.350	2.330	2	108.000	0.000
EML	2	57.500	2.120	2	67.550	1.344	2	64.750	2.470
LID	5	46.180	2.960	4	49.300	4.660	4	53.625	1.887
IPD	6	53.000	3.740	5	62.720	3.740	5	62.600	2.880
IAD	5	28.400	6.110	4	32.870	4.130	4	30.700	1.913
OID	6	87.830	6.960	5	96.620	8.530	5	105.000	6.320
IID	6	38.000	4.050	5	39.800	5.810	5	44.300	4.090
EMH	2	35.750	3.180	2	34.225	1.096	2	31.870	1.590
WI	6	2.437	0.210	5	2.176	0.420	5	2.410	0.252
				WSII patients					
MFH	2	96.250	13.790	5	107.740	2.120	8	112.500	7.310
EML	2	58.300	4.530	5	61.630	6.360	8	64.912	2.488
LID	3	40.500	3.280	6	42.750	2.361	8	40.170	3.730
IPD	4	53.925	1.135	7	58.570	4.030	8	60.640	6.310
IAD	3	32.367	1.185	6	35.500	2.074	8	36.125	1.481
OID	4	83.820	5.140	7	90.260	5.630	8	86.040	6.400
IID	4	30.500	3.870	7	34.214	2.233	8	32.060	3.980
EMH	2	35.375	0.530	5	33.520	5.610	8	31.080	4.970
WI	4	1.905	0.371	7	1.986	0.137	8	1.788	0.162
				Controls					
MFH	120	88.287	7.408	120	107.210	7.070	60	120.220	7.280
EML	120	50.462	3.558	120	55.718	4.551	60	64.414	4.272
LID	120	33.444	3.241	119	37.803	3.347	60	40.645	2.999
IPD	120	46.297	3.615	120	53.762	3.469	59	57.697	3.391
IAD	120	26.714	2.709	120	31.418	2.912	60	37.048	3.411
OID	119	72.313	4.297	118	81.921	4.511	60	87.205	4.144
IID	120	25.913	2.453	117	29.029	2.266	59	30.663	2.791
EMH	120	26.290	2.454	120	27.899	2.317	60	30.579	3.079
WI	119	1.800	0.196	116	1.756	0.161	58	1.728	0.174

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APPENDIX

We summarize in Table AI below the elements necessary to perform a simplified categoric discriminant analysis between types I and II of Waardenburg syndrome (WS). In this Table, the i column indicates the cardinal signs/symptoms, identified, as before, by the numbers 1–8; fr_{11} and fr_{21} are the frequencies of WSI carriers, respectively with and without the particular characteristic i; fr_{12} and fr_{22} are the corresponding quantities for WSII patients; fr_{11} and fr_{12} were taken directly from Table II, L_1w and L_2w are the odds or likelihood ratios WSI/WSII in a given carrier of WS, respectively, with and without the sign/symptom identified in i, obtained directly from $L_1w = fr_{11}/fr_{12}$ and $L_2w = fr_{21}/fr_{22}$. $\log_1w$ and $\log_2w$ are the natural logarithms of L_1w and L_2w. The conditional probabilities P_1(WSI) and P_2(WSI) favoring the diagnosis of the variant WSI in a suspected patient with and without the

TABLE AI. Elements Used for Calculating the Conditional Probabilities Favoring the Hypothesis of WSI as Function of the Signs/Symptoms Presented by any Suspected Individual

i	fr_{11}	fr_{21}	fr_{12}	fr_{22}	L_1w	$\log_1w$	L_2w	$\log_2w$	P_1(WSI)	P_2(WSI)
1	0.941	0.059	0.000	1.000	—	—	0.059	−2.829	1.000	0.056
2	0.733	0.267	0.127	0.873	5.778	1.754	0.306	−1.184	0.852	0.234
3	0.465	0.536	0.599	0.401	0.776	−0.254	1.334	0.288	0.437	0.572
4	0.533	0.467	0.332	0.668	1.606	0.474	0.699	−0.359	0.616	0.411
5	0.471	0.529	0.803	0.197	0.587	−0.533	2.683	0.987	0.370	0.729
6	0.218	0.782	0.100	0.900	2.185	0.781	0.869	−0.140	0.686	0.465
7	0.755	0.245	0.093	0.908	8.165	2.100	0.270	−1.311	0.891	0.212
8	0.140	0.860	0.016	0.984	8.936	2.190	0.873	−0.135	0.899	0.466

characteristic, respectively are shown in the last two columns. The quantities $P_1(WSI) = P(WSI \mid \text{sign present})$ and $P_2(WSI) = P(WSI \mid \text{sign absent})$ are obtained by normalizing L_1w and L_2w: $P_i(WSI) = L_iw/(1 + L_iw) = e^{\log_i w}/(1 + e^{\log_i w})$.

The values shown in Table AI enable us to determine the conditional probability favoring the diagnosis of WSI in a suspected individual presenting any constellation of signs/symptoms. Taking as example an individual suspected of WS without telecanthus but presenting all other cardinal characteristics and adding all the logarithms shown in the column $\log_1 w$ and the first value of column $\log_2 w$, we obtain 4.205; by applying this value in $e^{4.205}/(1 + e^{4.205})$ we obtain the figure of $67.02/68.02 = 0.9855$ (or about 99%) favoring the diagnosis of WSI. If we had taken into consideration only the absence of telecanthus, without investigating the other signs, the conditional probability of this individual being affected by WSI would be evaluated as 0.056/1.056 or approximately 5.3%.

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