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Waardenburg Syndrome (WS): The Analysis of a Single Family with a WS1 Mutation Showing Linkage to RFLP Markers on Human Chromosome 2q

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Summary

Waardenburg syndrome type I (WS1; MIM 19350) is caused by a pleiotropic, autosomal dominant mutation with variable penetrance and expressivity. Of individuals with this mutation, 20%–25% are hearing impaired. A multilocus linkage analysis of RFLP data from a single WS1 family with 11 affected individuals indicates that the WS1 mutation in this family is linked to the following four marker loci located on the long arm of chromosome 2: ALPP (alkaline phosphatase, placental), FN1 (fibronectin 1), D2S3 (a unique-copy DNA segment), and COL6A3 (collagen VI, α3). For the RFLP marker loci, a multilocus linkage analysis using MLINK produced a peak lod ($\hat{Z}$) of 3.23 for the following linkage relationships and recombination fractions ($\hat{\theta}_i$): (ALPP←.000→FN1)←.122→D2S3←.267→COL6A3. A similar analysis produced a $\hat{Z}$ of 6.67 for the following linkage relationships and $\hat{\theta}_i$ values among the markers and WS1: (FN1←.000→WS1←.000→ALPP)←.123→D2S3←.246→COL6A3. The data confirm the conclusion of Foy et al. that at least some WS1 mutations map to chromosome 2q.

Introduction

Waardenburg syndrome type I (WS1; MIM 19350) is an autosomal dominant mutation with variable penetrance and expressivity. Pleiotropic effects include deafness, hypopigmentation, optic abnormalities, and alterations of the skeletal system, particularly the structure of the face (Waardenburg 1951; Divekar 1957; DiGeorge et al. 1960; Stoller 1962; Calinikos 1963; Ahrendts 1965; Goldberg 1966; Arias 1971; Pantke and Cohen 1971; Delleman and Hageman 1978; Wang et al. 1981; Preus et al. 1983; Arias 1984; McKusick 1988). WS1 is responsible for hearing impairment in approximately 2% of congenitally deaf individuals (Waardenburg 1951; Partington 1964). Approximately 98% of WS1 subjects exhibit dystopia canthorum, while only 25% have hearing impairment (Waardenburg 1951; Hageman and Delleman 1977). The population frequency of WS1 is 1/42,000, with new mutations arising with a frequency of 1/270,000 births (Waardenburg 1951; Partington 1964).

From a comparison of the syntenic relationships of homologous genes found in the house mouse and in man, Asher and Friedman (1990) suggested that the WS1 phenotype could be caused by mutations at one or more of three different loci. The phenotypes of heterozygotes for the mouse mutations *Sp* (Splotch), Mi^{or} (Microphthalmia–Oak Ridge), and *Ph* (Patch) are similar to the phenotypes associated with WS1. Syntenic relationships involving the *Sp* gene predict a human homologue on the long arm of human chromosome 2 near the genes FN1 (fibronectin 1), COL6A3 (collagen VI, α3), ALPP (alkaline phosphatase, placental), and D2S3 (a single-copy DNA segment). Syntenic relationships involving the Mi^{or} gene predict a human homologue either near the gene RAF1 (proto-oncogene c-raf-1) on the short arm of human chromosome 3 or near the gene RHO (rhodopsin) on the long arm of chromosome 3. Syntenic relationships involving the *Ph* gene predict a human homologue on the long arm of human chromosome 4 near KIT (a proto-oncogene).

Three recently reported observations support the hy-

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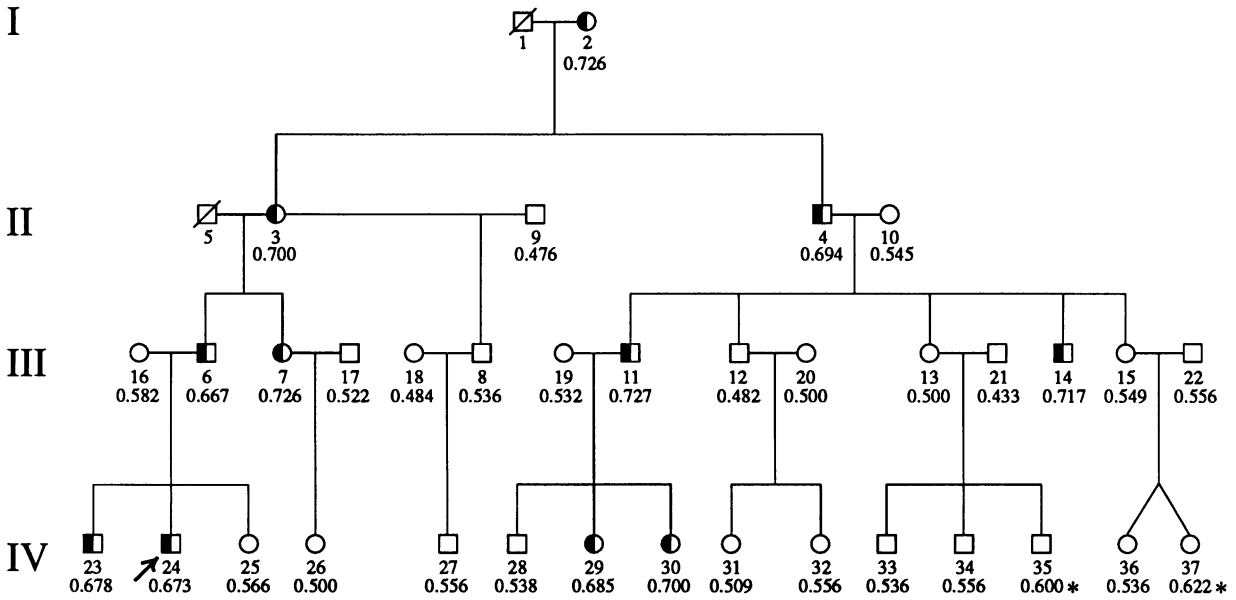

Figure 1 Single family segregating for a WS1 mutation. Half-filled symbols are affected individuals. The fraction presented below the identification number is the a/b ratio. Individuals 11, 14, and 24 are hearing impaired. Individual 14 shows the largest number of pleiotropic effects of WS1 expressed in this family. The arrow indicates the propositus. The asterisk (*) indicates that the a/b ratio is in the zone of overlap between the unaffected and affected populations.

pothesis that certain WS1 mutations are located on human chromosome 2. First, a sporadic inversion was observed in a child with classical WS1 features (Ishikiriyama et al. 1989). The breakpoints of this inversion are at 2q35 near FN1 and at 2q37.3 near COL6A3 and ALPP. Second, an interstitial deletion including 2q32.2-q33.1, near FN1, was found in a child who, in our opinion, expressed many of the symptoms found in Waardenburg-Klein syndrome (Glass et al. 1989). Last, Foy et al. (1990) reported a peak lod score ($\hat{Z}$) = 4.76 at a peak recombination fraction ($\hat{\theta}$) of .023, demonstrating close linkage between WS1 and ALPP in five families. Here we present a multilocus analysis of data from a single WS1 family that demonstrates linkage between the mutant WS1 gene and four RFLP markers located on chromosome 2q.

Material and Methods

Subjects

A WS1 family spanning four generations with 35 living members and 11 affected individuals (fig. 1) was analyzed for linked RFLP markers on human chromo-

Table 1

Probes Used to Identify RFLPs Exhibiting Linkage to Human Chromosome 2q and 3p

Chromosome Location	Gene Symbol	Gene Name	Probe	Source[a]	Reference
2q34-q36	FN1	Fibronectin 1	pFH154	ATCC	Colombi et al. 1987
2q35-q37	D2S3	Single-copy DNA	p5-1-25	ATCC	Litt 1986
2q37	ALPP	Alkaline phosphatase, placental	ALP-1	Trowsdale	Martin et al. 1987
2q37	COL6A3	Collagen VI alpha 3	p24	Chu	Weaver et al. 1988
3pter-p21	D3S17	Single-copy DNA	CRI-L892	CRI	Donis-Keller et al. 1987
3p25	RAF1	Proto-oncogene c-raf-1	p627	Rapp	Seizinger et al. 1988

[a] ATCC = American Type Culture Collection; Trowsdale = Dr. John Trowsdale, Imperial Cancer Research Fund Laboratories; Chu = Dr. Mon-Li Chu, Jefferson Medical College; CRI = Collaborative Research, Inc.; Rapp = Dr. Ulf Rapp, National Cancer Institute.

Table 2

Genes, Enzymes, Alleles, RFLP Sizes, and Allelic Frequencies for RFLPs Used in the Linkage Analysis

Gene	Enzyme	Alleles	Size of RFLP (kb)	Frequencies
FN1	*Hae*III	A1, A2	1.5, 2.0	.39, .61
D2S3	*Pst*I	J3, J4, J6, J7	2.6, 2.5, 2.3, 2.2	.13, .24, .31, .32
ALPP	*Rsa*I	B1, B2	1.6, 1.8	.38, .62
COL6A3	*Bgl*I	A1, A2	6.7, 6.0	.61, .39
D3S17	*Taq*I	A2, A3, A4, A5	8.6, 8.4, 8.0, 4.1	
RAF1	*Taq*I	A1, A2	6.8, 6.3	.74, .26

SOURCE.—Kidd et al. (1989).

somes 2 and 3 (table 1). RFLP alleles and their frequencies are presented in table 2. Figure 2 illustrates the facial features of some affected and nonaffected members of this family. Table 3 summarizes the phenotypes of affected individuals in the WS1 family. Individuals 11 and 24 exhibit profound bilateral congenital deafness (figs. 1 and 2). Individual 14 exhibits profound unilateral congenital deafness along with many other features usually associated with WS1, including dystopia canthorum, heterochromia iridis, white forelock, premature graying, fused eyebrows, broad nasal root, and prominent patches of hypopigmentation surrounding both wrists.

Given the variable expressivity and penetrance of WS1 exhibited within both the family illustrated in figure 1 and six other families examined by us (data not shown), the following criteria were established to assign an affection status. To be considered affected, an individual must have two of the following four characteristics: (1) hearing impairment; (2) dystopia canthorum; (3) pigmentation anomalies represented by premature graying of head hair, heterochromia iridis, hypopigmentation of the fundus, skin, or hairy structures; or (4) a first-degree relative exhibiting WS1.

The quantitative measure of dystopia canthorum used in this analysis was the ratio of the inter-inner canthal distance divided by the interpupillary distance, defined as the a/b ratio. Individuals having a/b $\geq$ 0.600 are 2 SDs above the mean for nonaffected individuals (Partington 1964). An a/b ratio of 0.600 is also approximately 2 SDs below the mean for affected individuals. Individuals 35 and 37 (fig. 1), who have unaffected parents, normal hearing, and a/b ratios of .600 and .622, respectively, fall within the zone of overlap between the distributions of the affected and nonaffected populations. The a/b ratios and affection status of each individual within the family are presented in figure 1 and table 4.

Preparation of Genomic DNA and Probes

DNA was obtained from 20 ml of heparinized blood samples. Blood cells were lysed by the addition of an equal volume of 0.1 M Tris-HCl pH 7.9, 1 mM EDTA, 20 mM NaCl, 4% SDS. After a 30-min incubation at room temperature, the lysate was extracted with an equal volume of salt-saturated-phenol (SS-phenol); (Davis et al. 1986) with gentle shaking for 10 min. Following a 20-min centrifugation at 1,800 *g*, the aqueous phase was removed and the DNA was precipitated with the addition of 1/10 vol of 3 M Na_4OAc and 2.5 vol of 100% ethanol. Following a brief centrifugation (2 min at 1,800 *g*), the DNA was dissolved in 1.6 ml of 50 mM Tris-HCl pH 7.9, 0.5 mM EDTA, 10 mM NaCl. The samples were digested with 9 units RNase A (R-5503; SIGMA) for 30 min (37°C), brought to 0.5% SDS, and digested with 17 units of pronase (P-6911; SIGMA) for 2 h (37°C). The samples were then reextracted with SS-phenol, ethanol precipitated, washed in 70% ethanol, and solubilized in 200–400 μl of 10 mM Tris-HCl pH 8.0, 1 mM EDTA, yielding 200–600 μg of high-molecular-weight DNA.

The probes used to identify RFLPs in this linkage analysis are listed in table 1. Inserts were removed from their vectors by digestion with the appropriate restriction enzymes, separated by agarose gel electrophoresis (Maniatis et al. 1982), purified with an Elutip (Schleicher and Schuell), and labeled with ^{32}P-dATP to a specific activity of 1×10^8–2×10^9 CPM/μg by using random primers (Feinberg and Vogelstein 1983, 1984).

Southern Blots

Human genomic DNA (3–5 μg) was digested using

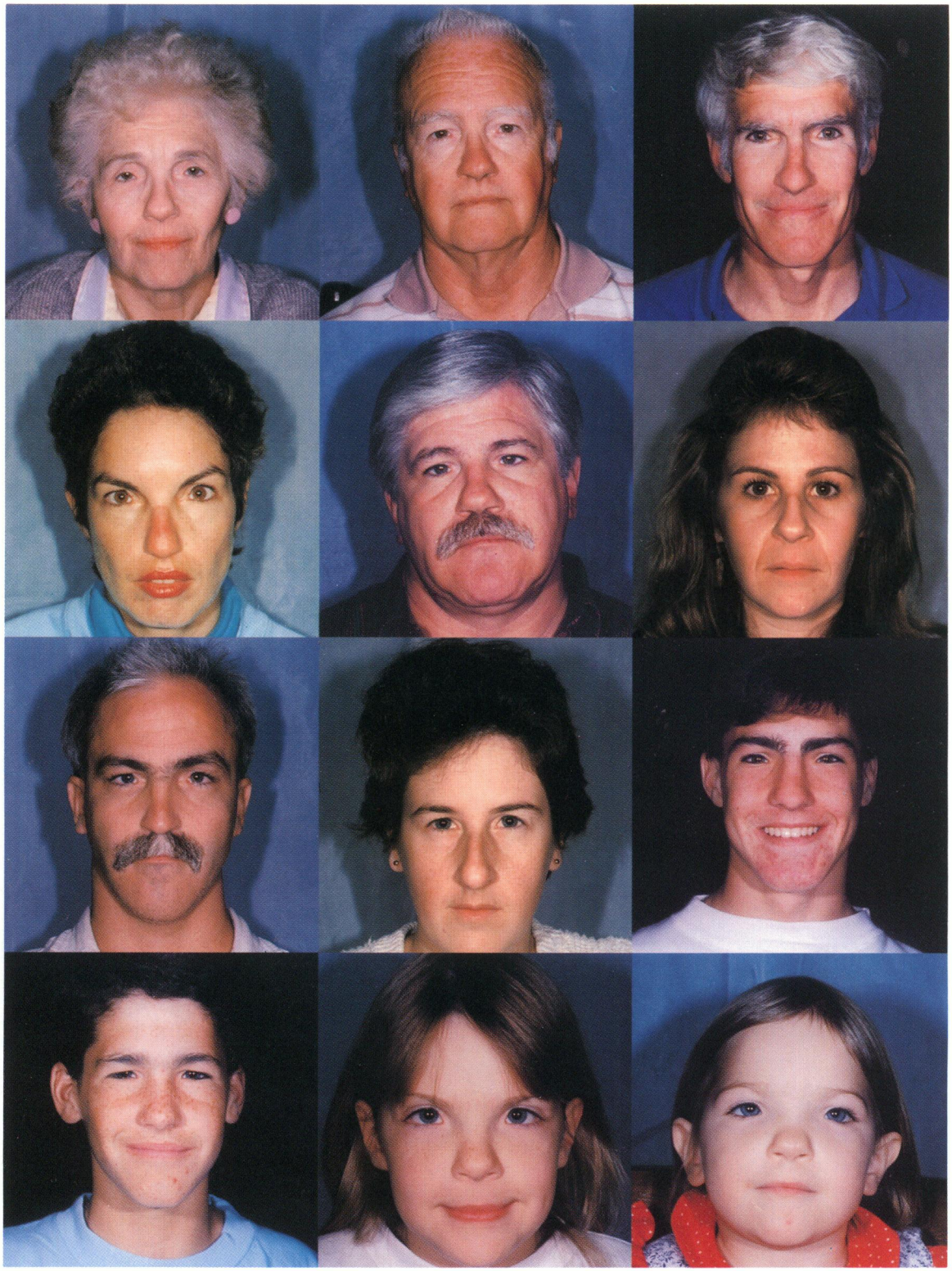

Figure 2 Some affected and unaffected individuals from family illustrated in fig. 1. The identification numbers of individual subjects, from left to right and top to bottom, are as follows: 3, 4, 6; 7, 11, 13; 14, 15, 23; and 24, 29, 30.

Table 3

Phenotypes of Affected Individuals in the Single WS1 Family Illustrated in Figures 1 and 2

Individual (Sex)	Phenotype[a]						
	DysC	SN	HetI	HypE	WF/EG	Skin	Eyb
2 (F)	+	−	−	−	+	−	−
3 (F)	+	−	−	−	+	−	−
4 (M)	+	−	−	−	+	−	−
6 (M)	+	−	−	−	+	−	−
7 (F)	+	−	−	−	+	−	+
11 (M)	+	+	−	+	+	−	−
14 (M)	+	+	+	−	+	+	+
23 (M)	+	−	−	−	−	−	+
24 (M)	+	+	−	−	−	−	−
29 (F)	+	−	−	+	−	−	−
30 (F)	+	−	−	+	−	−	−
35 (M)[b]	+	−	−	−	−	−	−
37 (F)[b]	+	−	−	−	−	−	−

[a] According to Foy et al. (1990), with modification. DysC = dystopia canthorum with an a/b ratio ≥ 0.600 (Partington 1964); SN = sensorineural hearing loss; HetI = heterochromia iridis; HypoE = hypoplastic blue eyes; WF/EG = white forelock and/or early graying; Skin = hypo- or hyperpigmented skin patches; Eyb = bushy confluent eyebrows; a plus sign (+) denotes that the feature is present; a minus sign (−) = denotes that the feature is absent.

[b] Individual has an a/b ratio which is in the zone of overlap between the unaffected and affected populations.

the restriction endonucleases listed in table 2. The digested DNA was separated by electrophoresis using 0.8% or 2.0% agarose gels, depending on the size and number of restriction fragments to be resolved. After denaturation and neutralization, the gels were blotted onto Hybond-N nylon (Amersham) or Immobilon-N (Millipore) membranes by using 6 × SSC (90 mM citrate-HCl pH 7.0, 0.9 M NaCl). DNA was bound to the membranes by UV irradiation and/or baking at 80°C for 1 h. Membranes were prehybridized at 42°C overnight in 50% formamide, 50 μg sheared salmon sperm DNA/ml, 0.1 M PIPES pH 7.04, 0.1 M NaCl, 0.1% Sarkosyl, 0.1% Ficoll, 0.1% PVP-40 (molecular wt. 360,000), 0.1% BSA. Since D2S3 contains a sequence highly repeated in the human genome, sonicated human placental DNA (60 μg/ml [D-7011; SIGMA]) was added to the prehybridization solution. For genomic Southern blots, 1–5 × 10^7 CPM of ^{32}P-dATP-labeled DNA was used per hybridization. Hybridization was carried out at 42°C overnight in 40% formamide, 20 μg sheared salmon sperm DNA/ml, 0.1 M PIPES at pH 7.04, 0.1 M NaCl, 0.1% Sarkosyl, 0.1% Ficoll, 0.1% PVP-40 (molecular wt. 360,000), 0.1% BSA, and 10% dextran sulfate. The blots were then washed briefly three times in 2 × SSC, 0.05% N-laurylsarkosine, 0.02% NaPPi at 50°C; and were washed two times, 1 h each, with 0.1 × SSC, 0.05% N-laurylsarkosine, 0.02% NaPPi at 50°C; and were exposed to X-ray film for 3 h–5 d at −70°C with an intensifying screen.

Linkage Analysis

The WS1 mutation segregating in the family illustrated in figure 1 was evaluated for two possible chromosomal locations: linkage to RAF1 and D3S17 on chromosome 3p (table 1) or linkage to ALPP, FN1, COL6A3, and D2S3 on chromosome 2q. Single and multipoint linkage analyses (Lathrop et al. 1984, 1985) were performed using LIPED (Ott 1974) and LINKAGE v4.9 (Lathrop and Lalouel 1984). The a/b ratio for dystopia canthorum was used as a quantitative character in the multipoint linkage analysis. The means and variances for normal and WS1 subjects were taken from the literature and are (1) $\bar{X} = 0.532$, $S^2 = 0.0011556$ ($N = 516$) for the normal population and (2) $\bar{X} = 0.695$, $S^2 = 0.00252507$ ($N = 61$) for the WS1 population (Waardenburg 1951; DiGeorge et al. 1960; Partington 1964). In the actual analysis using MLINK, the means and individual values were multiplied by 10, whereas the variances were multiplied by 100.

Table 4

Pedigree and Phenotypic Data from the WS1 Family Illustrated in Figure 1

Identification Number			Affection					
Subject (sex)	Father	Mother	Status[a]	ALPP	D2S3	(a/b) × 10	FN1	COL6A3
1 (M)	0	0	U	0 0	0 0 0 0	.00	0 0	0 0
2 (F)	0	0	A	1 1	0 0 1 1	7.26	0 1	1 1
3 (F)	1	2	A	1 1	0 0 1 0	7.00	0 1	0 1
4 (M)	1	2	A	1 1	0 0 1 0	6.94	0 1	0 1
5 (M)	0	0	U	0 0	0 0 0 0	.00	0 0	0 0
6 (M)	5	3	A	1 0	1 0 1 0	6.67	0 1	1 1
7 (F)	5	3	A	1 0	1 0 1 0	7.26	0 1	1 1
8 (M)	9	3	U	0 1	1 0 1 0	5.36	1 1	1 1
9 (M)	0	0	U	0 1	1 1 0 0	4.76	1 1	1 1
10 (F)	0	0	U	1 1	1 0 1 0	5.45	1 0	1 0
11 (M)	4	10	A	1 1	1 0 1 0	7.27	1 1	1 1
12 (M)	4	10	U	0 1	1 0 1 0	4.82	1 1	1 1
13 (F)	4	10	U	1 1	0 0 1 0	5.00	1 1	1 1
14 (M)	4	10	A	1 1	1 0 1 0	7.17	1 1	1 1
15 (F)	4	10	U	0 1	1 0 1 0	5.49	1 1	1 1
16 (F)	0	0	U	1 1	1 0 1 0	5.82	1 1	0 1
17 (M)	0	0	U	0 1	0 0 1 0	5.22	1 0	1 0
18 (F)	0	0	U	0 1	0 0 0 0	4.84	0 0	0 0
19 (F)	0	0	U	1 1	0 1 0 1	5.32	1 0	1 1
20 (F)	0	0	U	0 1	0 0 1 1	5.00	1 0	1 1
21 (M)	0	0	U	1 1	0 1 0 1	4.33	1 1	1 0
22 (M)	0	0	U	1 1	0 1 1 0	5.56	1 0	1 1
23 (M)	6	16	A	1 1	0 0 1 0	6.78	1 1	0 1
24 (M)	6	16	A	1 0	1 0 1 0	6.73	0 1	0 1
25 (F)	6	16	U	1 0	0 0 1 0	5.66	0 1	0 1
26 (F)	17	7	U	1 1	1 0 1 0	5.00	1 1	1 0
27 (M)	8	18	U	0 1	0 0 0 0	5.56	0 0	0 0
28 (M)	11	19	U	0 1	0 0 0 0	5.38	0 0	0 0
29 (F)	11	19	A	1 0	0 1 1 0	6.85	1 1	1 0
30 (F)	11	19	A	1 1	0 0 1 1	7.00	1 1	1 1
31 (F)	12	20	U	0 1	1 0 0 1	5.09	1 1	1 0
32 (F)	12	20	U	0 1	0 0 1 0	5.56	1 1	1 1
33 (M)	21	13	U	0 1	0 0 1 1	5.36	0 1	1 1
34 (M)	21	13	U	0 1	0 0 1 1	5.56	0 1	1 0
35 (M)	21	13	U	0 0	0 0 0 0	6.00	0 0	0 0
36 (F)	22	15	U	0 0	0 1 1 0	5.36	1 1	0 0
37 (F)	22	15	U	1 1	1 0 1 0	6.22	1 0	1 1

Note.—Allelic designations (table 2) are according to the factor union system.

[a] U = Unaffected; A = affected.

Using pedigree and phenotypic data presented in table 4, linkage analyses were conducted in three different ways. The first analysis was conducted using the quantitative character for dystopia canthorum (the a/b ratio) as the marker for WS1. Possible misclassifications caused by problems of penetrance were then evaluated by MLINK as outlined by Ott (1974, 1977, 1985) and subsequently used by Lange et al. (1976) and Ott (1977). No additional assumptions were made regarding penetrance, nor were assignments made for affection status.

For the second analysis, dystopia canthorum was assumed to have a penetrance of 98% and the affection status of each individual was assigned assuming that individuals 35 and 37 were unaffected. For the third analysis, the affection status of each individual was assigned assuming that individuals with $a/b \geq 0.600$ are

Table 5

$\hat{Z}$ Values for Linkage between a WS1 mutation Segregating within a Single Family and RFLPs for the Genes ALPP (A), FN1 (F), D2S3 (D), and COL6A3 (C)

Comparison	$\hat{\theta}_1,\hat{\theta}_2,\ldots,\hat{\theta}_i$	$\hat{Z}$[a]
A–F	.000	1.249874
F–D	.196	.627575
D–C	.224	.430370
F–D–C	.206, .257	1.021752
A–F–D–C	.000, .112, .268	3.232445
WS1–A	.000	2.095071
WS1–F	.000	.591169
WS1–D	.112	.649086
WS1–C	.331	.085271
A–WS1–F	.000, .000	4.166710
A–WS1–F–D	.000, .000, .122	6.156597
A–WS1–F–D–C	.000, .000, .123, .246	6.667686

[a] The linkage analyses involving WS1 were carried out using the quantitative measure of dystopia canthorum (the a/b ratio). The mean and standard deviation of unaffected and affected individuals were multiplied by 10 and were 5.32 ± 0.341 (N = 516) and 6.95 ± 0.503 (N = 61), respectively. For these analyses, affection status of individuals was not chosen.

affected. In this case, parents 13 and 15 are phenotypically normal and nonpenetrant for WS1 while their offspring, individuals 35 and 37, are affected.

Results

We first tested the possible homology between the WS1 mutation segregating in the family illustrated in figure 1 and the mouse mutation *Mi*^{or}. The mutation *Mi*^{or} is located on mouse chromosome 6 approximately 1 cM from the proto-oncogene *Raf-1* and produces phenotypic effects similar to WS1. The linkage analysis rejected tight linkage between WS1 segregating in this family and the chromosome 3p RFLP markers for RAF1 (Z = −2.6 at θ = .15) and D3S17 (Z = −2.0 at θ = .10).

Having rejected linkage of WS1 to markers on chromosome 3p, we next began a test of the possible homology between the WS1 mutation segregating in this family and the mouse mutation *Sp*. The mutation *Sp* is located on mouse chromosome 1 approximately 3 cM from the fibronectin gene (*fn-1*). Linkage relationships among human chromosome 2q RFLP markers for ALPP, FN1, D2S3, and COL6A3 were examined. A $\hat{Z}$ of 3.23 was obtained, indicating significant linkage between these four loci, with gene order and $\hat{\theta}_i$ values as follows: (ALPP←.000→FN1)←.112→D2S3←.268→COL6A3 (table 5). Linkage relationships among WS1, as determined by the a/b ratio, and the above RFLP markers were then examined. A $\hat{Z}$ of 6.67 was obtained from a five-point linkage analysis (table 5). The linkage order and $\hat{\theta}_i$ values are (ALPP←.000→WS1←.000→FN1)←.123→D2S3←.246→COL6A3. The WS1 gene segregating in this family is thus closely linked to ALPP and FN1, exhibits moderate linkage to D2S3, and is loosely linked to COL6A3.

Linkage analyses were also performed using assigned affection status assuming that individuals 35 and 37 are unaffected in spite of their a/b ratios. These analyses produce $\hat{Z}$ values nearly identical to those obtained using the quantitative character (table 5) with a $\hat{Z}$ of 6.67. A third linkage analysis was performed assuming, on the basis of their borderline a/b ratios, that individuals 35 and 37 are affected. In this case, a positive though not significant $\hat{Z}$ of 1.03 was obtained for linkage between WS1 and ALPP, with $\hat{\theta}$ = .079. Comparisons using all other loci rejected their tight linkage with WS1. Ott (1977, 1985) and Lathrop and Lalouel (1984) point out the importance of using quantitative characters in avoiding this type of misclassification and increasing the strength of the linkage analysis.

Discussion

Asher and Friedman (1990), analyzing the syntenic relationships that exist between mouse and human homologous genes, predicted that mutations causing WS1 phenotypes might map to one of the following three different human chromosomal locations: on chromosome 2q near FN1, on chromosome 3p near RAF1, or on 3q near RHO, and on chromosome 4q near KIT. The human chromosome 2 probes for genes FN1, COL6A3, and D2S3 and the chromosome 3 probes for genes RAF1 and D3S17 used in our analyses were chosen initially on the basis of their homology with genes in close proximity to *Sp* on mouse chromosome 1 and *Mi*^{or} on mouse chromosome 6. Following discussions with Dr. Andrew Read (St. Mary's Hospital, Manchester, England) concerning his linkage analysis of WS1 and after receipt of the manuscript of Foy et al. (1990), we included ALPP in our chromosome 2 analysis.

Our linkage analyses indicate that the dominant WS1 mutation segregating in the family described here is not located on chromosome 3p near RAF1 (Z = −2.6 at θ = .15) but is located on chromosome 2q near ALPP,

FN1, D2S3, and COL6A3 ($\hat{Z}$ = 6.67; table 5). These data illustrate the use of a quantitative character in avoiding misclassification by the assignment of affection status. Misclassifications occur because of the variability associated with certain mutant phenotypes (Ott 1977). The calculation procedures outlined by Ott (1974, 1977, 1985) and used in MLINK employ quantitative characters and minimize this effect. Because of the large deviation between the means of normal subjects and those of subjects with the WS1 phenotype ($\Delta\overline{X}$ = 4.53 SD), the relative efficiency of our linkage analysis should be near 90% (Ott 1985), in spite of the variable penetrance of the WS1 mutation.

The use of the a/b ratio as a quantitative character thus provides an objective means of evaluating linkage between WS1 and marker loci when the assignment of affection status may be difficult because of phenotypic variability, as is the case with individuals 35 and 37. This procedure, however, cannot be used in performing linkage analyses of mutations causing Waardenburg syndrome type II (WS2), as these mutations do not affect the a/b ratio.

The results presented in table 5 confirm the linkage analysis of Foy et al. (1990), which indicates that some WS1 mutations map to 2q. Our data also support the hypothesis, based on syntenic relationships and phenotypic similarities, that the mutant gene segregating in this WS1 family is homologous to the mouse mutation *Sp*. The data described here, from a single WS1 family, were analyzed using a multilocus approach and keeping in mind our hypothesis that the WS1 phenotype might be caused by any one of at least three unlinked mutant genes (Asher and Friedman 1990).

For at least two other human abnormalities, similar phenotypes can be caused by unlinked, nonallelic dominant autosomal mutations. Wilms tumor can result from mutations at more than one locus (Francke 1990), while at least two unlinked mutations can cause Charcot-Marie-Tooth disease (Patel et al. 1990). We are now performing additional single-family multilocus linkage analyses to determine whether WS1 mutations map to a single locus on chromosome 2q or to one of two other loci predicted from a consideration of mouse mutants with similar phenotypes (Asher and Friedman 1990). Once these single-family analyses have been completed, data from families with identical linkage profiles can be pooled to establish an accurate linkage map of the markers surrounding WS1.

Once the map position of a WS1 locus has been identified, it is possible to begin a molecular analysis of the WS1 region. To understand how WS1 mutations produce their pleiotropic effects, the normal WS1 allele must be cloned and sequenced. Because of the syntenic relationships revealed by our linkage analyses, cloning and characterizing the *Sp* region in the mouse may be helpful in understanding the normal function of the Splotch locus as well as that of the WS1 gene. The deficiency and inversion breakpoints of patients described by Glass et al. (1989) and Ishikiriyama et al. (1989) also may be useful in cloning and determining the primary function of mutant WS1 genes.

WS1 mutations appear to affect the neural crest cells. Sensorineural deafness, pigmentation anomalies, malformation of the facial bones, and megacolon can all be attributed to defects in neural crest cell migration (Erickson and Weston 1983; Le Douarin and Smith 1988; Noden 1988). Fibronectins and collagens, constituents of the extracellular matrix (ECM), are known to influence neural crest cell migration (Erickson 1987; for review, see Perris and Bronner-Fraser 1989) and, if altered, might lead to the production of the WS1 phenotype. Barker et al. (1990) demonstrated that Alport syndrome, which causes deafness and nephritis, is associated with mutations in the collagen gene COL4A5 in three families. It is worth noting that genes coding for the following five ECM proteins have been mapped to the tip of chromosome 2q: collagen III α1, collagen V α2, collagen VI α3, fibronectin, and elastin (Weil et al. 1988). Because of the loose linkage existing between WS1 and COL6A3 ($\hat{\theta}$ = .331) in the family described here, the WS1 mutation segregating in this family is probably not a mutant COL6A3 gene; however, the remaining ECM genes, including FN1, on chromosome 2q are plausible candidates for genes which, when mutated, might cause the WS1 phenotype.

In summary, we have tested the linkage of a WS1 mutation segregating in a single family to two of the four chromosome locations predicted by Asher and Friedman (1990), on the basis of syntenic relationships of human and mouse genes. For the single family analyzed here, the WS1 mutation is absolutely linked to FN1 and ALPP, moderately linked to D2S3, loosely linked to COL6A3 ($\hat{Z}$ = 6.67; table 5), and is possibly homologous to the mouse mutation Splotch. It is now of interest to determine whether other WS1 mutations map to the same locus or to different loci as predicted by Asher and Friedman (in press) and whether other Waardenburg syndromes (WS2 [MIM 19351], WS3 [MIM 14992], and WS4 [MIM 27758]) are allelic or nonallelic to WS1.

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