

Mouse and hamster mutants as models for Waardenburg syndromes in humans

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Abstract

Four different Waardenburg syndromes have been defined based upon observed phenotypes. These syndromes are responsible for approximately 2% of subjects with profound congenital hearing loss. At present, Waardenburg syndromes have not been mapped to particular human chromosomes. One or more of the mouse mutant alleles, *Ph* (patch), *s* (piebald), *Sp* (splotch), and *Mr^{or}* (microphthalmia-Oak Ridge) and the hamster mutation *Wh* (anophthalmic white) may be homologous to mutations causing Waardenburg syndromes. In heterozygotes, phenotypic effects of these four mouse mutations and the hamster mutation are similar to the phenotypes produced by different Waardenburg syndrome mutations. The chromosomal locations and syntenic relationships associated with three of the four mouse mutant genes have been used to predict human chromosomal locations for Waardenburg syndromes: (1) on chromosome 2q near FN1 (fibronectin 1), (2) on chromosome 3p near the proto-oncogene *RAF1* or 3q near *RHO* (rhodopsin), and (3) on chromosome 4p near the proto-oncogene *KIT*. Waardenburg syndromes show extensive intrafamilial phenotypic variability. Results of our studies with the hamster mutation *Wh* suggest that this variability may be explained in part by modifier genes segregating within families.

Classical Waardenburg syndrome in humans (WS1, MIM 19350) is caused by an autosomal dominant mutation with extensive phenotypic variation observed both within and between families.¹⁻⁵ There have been no reported cases of persons homozygous for WS1. In heterozygotes, the WS1 mutation is highly pleiotropic, affecting at least 18 different

characteristics.² Six of these characteristics form the key to diagnosis: (1) dystopia canthorum, (2) a broad nasal root, (3) hypertrichosis of the medial ends of the eyebrows, (4) hypopigmentation of the skin and head hair, (5) total or partial heterochromia iridis, and (6) congenital unilateral or bilateral deafness.⁶ Some of the phenotypic effects caused by WS1 are illustrated by a 23 year old female who has significant hearing loss and her 5 year old daughter who is profoundly deaf (fig 1a, b).

Premature graying of head hair and beard occurs in approximately 33% of subjects with WS1,⁷ while optic abnormalities including iridial hypoplasia, hypopigmentation of the fundus, coloboma, and microphthalmia occur in approximately 51% of the cases reported.^{1 7 8-12} WS1 subjects may also exhibit cleft lip, cleft palate, or cleft face with a frequency of up to 10%,^{7 13 14} while cardiac abnormalities including septal defects are occasionally observed.^{2 13 15-18} In families where radiographical studies have been performed, high frequencies of minor skeletal abnormalities are observed.^{1 19-21}

In certain extended families, dystopia canthorum shows a penetrance of 98% and serves as a reliable indicator of Waardenburg syndrome.¹ In these families, penetrance of bilateral profound deafness in heterozygotes was initially reported as 17%,¹ but with a larger data set this frequency is closer to 25%.²² The frequency of Waardenburg syndrome among students from schools for the deaf in the Netherlands and Canada is 1.43% and 2.7%, respectively, with an estimate of affected persons in the general population of 1/42 000 and a mutation rate of 1/270 000.^{1 23} The WS1 mutation is a significant cause of congenital deafness and hearing impairment in humans.

Observing the range of phenotypes initially reported by Waardenburg,¹ investigators have defined four different subdivisions of Waardenburg syndromes. Arias,^{6 24} and later Hageman and Delleman,²² suggested that patients with Waardenburg syndrome but lacking dystopia canthorum should be classified as Waardenburg syndrome type II (WS2, MIM 19351). This syndrome is caused by an autosomal dominant mutation. The frequency of profound bilateral deafness among heterozygotes for WS2 is approximately 50%,²² twice the frequency observed among WS1 heterozygotes. This subdivision of

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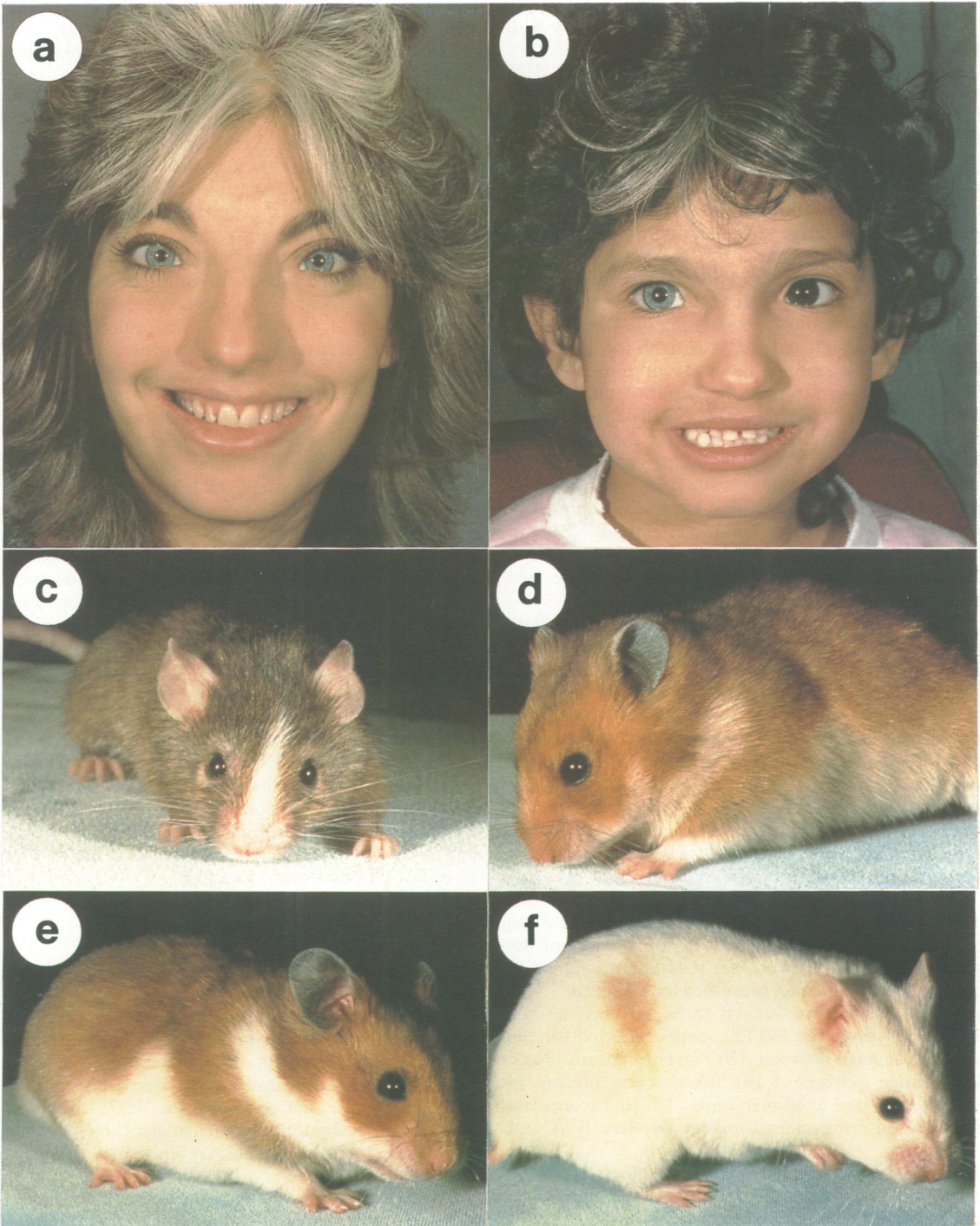


Figure 1 Two human subjects with Waardenburg syndrome (WS1) and mouse and hamster mutants showing similar phenotypes. (a) A 23 year old woman with dystopia canthorum, broad nasal root, white forelock, isochromia iridis, and premature graying. She has a flat auditory brainstem evoked response (ABR) at 100 dB normal hearing level (nHL) in the right ear and has an ABR threshold of 45 dB nHL in the left ear while normal thresholds are 20 to 25 dB nHL. When not shaved, her eyebrows are fused. (b) The 5 year old daughter of the subject in (a). The daughter has dystopia canthorum, broad nasal root, fused eyebrows, white forelock, total heterochromia iridis, and profound deafness. She has a 6 month old half sister who gives flat ABR responses in both ears at 130 dB nHL. (c) A C3Hf/R1 $Mi^{or}/+$ mouse with a white head blaze, light ears, light eyes and a white belly patch. Homozygotes (Mi^{or}/Mi^{or}) have a completely white coat and are profoundly deaf and eyeless. (d) A normal agouti Syrian hamster of the genotype $wh/wh;E/e$.

Waardenburg syndrome also contains patients with ocular albinism.²⁵

Waardenburg syndrome type III (WS3, MIM 14882, Klein-Waardenburg syndrome or Waardenburg syndrome with upper limb abnormalities) is a third subdivision of patients initially considered by Waardenburg¹ as belonging to WS1.²⁶ This mutation also behaves as an autosomal dominant. Heterozygotes with this disorder have the classical WS1 phenotype in addition to upper limb abnormalities, which include hypoplasia of the musculoskeletal system and syndactyly. Some patients also exhibit microcephaly and severe mental retardation.^{26 27}

Persons with a fourth subdivision of Waardenburg syndromes, designated as Waardenburg syndrome variant, Waardenburg-Shah syndrome, Shah-Waardenburg syndrome, or Hirschsprung disease with pigmentary anomaly (MIM 27758), exhibit many of the classical WS1 characteristics in addition to having megacolon.²⁸⁻³² This apparent variant of Waardenburg syndrome is caused by an autosomal recessive mutation. Megacolon has also been found in association with the phenotypic effects of WS1 and WS2, but Waardenburg-Shah syndrome is considered distinct from the syndromes caused by these two mutations.⁵

The complex phenotypic variation observed both within and between families with Waardenburg syndromes may be explained by one of at least three different genetic models: (1) a single locus with several mutant alleles, (2) a single locus with several mutant alleles each interacting with modifying genes, and (3), as suggested by Waardenburg,¹ more than one locus each with multiple mutant alleles which interact with modifying genes. Differences in the allelic state of modifying genes are invoked to explain the variation observed both among and within families with Waardenburg syndromes.

At present, there are no confirmed chromosomal assignments for Waardenburg syndromes and no biochemical or molecular data to support any of the above three models. WS1 was tentatively assigned to chromosome 9 based on presumed loose linkage^{15 33 34} with the ABO locus known to be on chromosome 9.^{34 35} However, the lod (Z) scores reported for the assumed linkage between ABO on chromosome 9 and WS1 range between 1.101 for absolute linkage to 0.201 for linkage at 40 map units.³⁶⁻³⁸ Lod scores less than 3.0 do not establish linkage between WS1 and ABO.^{39 40}

Two recent papers may provide clues as to the possible chromosomal location of the mutations causing Waardenburg syndromes. Ishikiriyama *et al.*⁴¹ reported a case of sporadic WS1 associated with a paracentric inversion on human chromosome 2 involving breakpoints in bands 2q35 and 2q37.3. The child showed typical features of WS1 without the abnormalities usually associated with duplications or

deletions involving 2q.⁴²⁻⁴⁶ So far, a causal connection has not been established between this paracentric inversion and the WS1 phenotype.

In a case involving a deletion of a nearby area, Glass *et al.*⁴⁷ described a 16 year old proband with craniofacial dysmorphology, extensive scalloped hypopigmentation of the skin, microcephaly, mental retardation, no comprehensible speech, short stature, beaked nose, bilateral corneal ectasia, divergent strabismus, bilateral ptosis, and cleft palate. The hearing capabilities of this child were not reported. The boy was heterozygous for an interstitial deletion involving 2q32.2-q33.1. Though not present in this subject, skeletal abnormalities and coloboma were noted in subjects with deletions involving the same genetic region.⁴⁸⁻⁵⁴ Hypopigmentation was not observed in these latter deletions. We note that the subject reported by Glass *et al.*⁴⁷ shares many of the features of Waardenburg syndrome type III. These reports suggest the possibility that Waardenburg syndrome mutations might map to human chromosome 2.

A genetic and developmental analysis of Waardenburg syndromes will determine: (1) the number of genetic loci responsible for Waardenburg syndromes, (2) the number of mutant alleles at a given locus with different phenotypic effects, (3) the primary functions of the loci involved, and (4) the mechanisms by which alterations of these primary functions produce abnormalities of the eye, inner ear, pigmentation, and skeleton. An obvious first step to obtain this information is to assign WS1 and WS2 to particular human chromosomes using RFLP markers with known map positions. To narrow the initial choice of autosomal RFLP markers to be used in such a linkage analysis, mutations in other mammals which may be homologous to WS1 and WS2 can be used to predict possible chromosomal locations of Waardenburg syndromes.

Mouse and hamster mutations

MOUSE MODELS

The phenotypic effects produced by WS1 are not unique to man as these same abnormalities are also caused by mutations in dogs, cats, mink, horses, cattle, house mice, deer mice, and Syrian hamsters.⁵⁵⁻⁶⁷ A house mouse heterozygous for *Mi*^{or} (microphthalmia-Oak Ridge) and Syrian hamsters heterozygous for *Wh* (anophthalmic white) are illustrated in fig 1c, d, e, f.

The use of chromosomal segments with conserved homologous linkage groups found in several different vertebrate species provides one method of predicting the location of genes in humans causing disease states. Comparing the genetic maps of man and house mouse, there are 241 known homologous autosomal genes found on 68 homologous chromosomal segments

encompassing all of the autosomes of man and mouse. The average length of a conserved segment in the mouse, homologous to a human chromosomal segment, is 10.1 ± 2.2 cM.⁶⁸ Of the 2138 mutant alleles of the house mouse listed by Peters,⁶⁹ 178 mutant alleles of 79 loci affect pigmentation. Twenty-one of these loci have alleles which produce pleiotropic effects including abnormalities of the skeleton, eye, inner ear, neural or neuromuscular system, internal viscera, reproductive system, haematopoietic system, and endocrine system. Nine of these 79 loci have mutant alleles which affect the development of the inner ear: *dr* (dreher), *mi* (microphthalmia), *mu* (muted), *pa* (pallid), *Ph* (patch), *s* (piebald), *Sp* (splotch), *Va* (varitint-waddler), and *W* (dominant spotting).

Of these nine loci, *pa*, *W* (*Kit*), *dr*, *mu*, and *Va* were eliminated as possible models for Waardenburg syndromes. The primary effect of *pa* appears to be upon the transport of Mn^{++} which alters the development of melanoblasts and otoliths.^{70 71} *W* (*Kit*) appears to alter the mobility and proliferation of neural crest cells, haematopoietic precursors, and primordial germ cells.⁷²⁻⁷⁴ The *W* locus has recently been shown to be equivalent to *Kit*, a proto-oncogene encoding a cell surface protein kinase receptor.⁷⁵ The earliest observed defect caused by *dr* appears to involve hindbrain development leading to deafness and abnormal behaviour including circling and head tossing,⁷⁶ while *mu* alters melanoblast development and causes a balance defect similar to the *pa* mutation.⁷⁷ *Va* causes severe abnormalities of vestibular development in addition to its effects on the

development of the cochlea and pigmentation which leads to circling behaviour, head tossing, and deafness.⁷⁸ Thus, *pa*, *W* (*Kit*), *dr*, *mu*, and *Va* are probably not good models for Waardenburg syndromes.

We suggest that *mi*, *Ph*, *s*, and *Sp* are possible models for Waardenburg syndromes because of the similarities among their pleiotropic phenotypes and Waardenburg syndromes. If one or more of the mouse genes *Ph*, *s*, *Sp*, and *Mi^{or}* are homologous to genes causing Waardenburg syndromes, the syntenic relationships between genes in man and mouse can be used to predict the chromosome locations of Waardenburg syndromes.^{68 79-81} Of these four loci, alleles of the *mi* locus (table) exhibit the widest range of phenotypic effects paralleling the variability observed both between and among families with Waardenburg syndromes. Before presenting a discussion of the *mi* locus, three loci which show some of the phenotypic variation of Waardenburg syndromes are described.

The Ph mouse model

The *Ph* locus of the house mouse is located on chromosome 5, approximately 37 map units from the centromere.^{80 81} Mapping at the same genetic position are *W* (dominant spotting) and *Rw* (rump-white).^{82 83} *Ph*, *W* (*Kit*), and *Rw* each affect pigmentation phenotypes and map 1 cM from the hyper-variable region *Ms15-6* (minisatellite 15-6).⁸⁴ The two alleles at the *Ph* locus cause patches of hypopigmentation and inner ear defects. *Ph/+* mice have skulls which are wider and shorter than normal and

Mutant alleles at the mi locus in the house mouse.

Allele	Name	Phenotypic effects		References
		Heterozygotes	Homozygotes	
<i>mi</i>	Microphthalmia	Pale ear pigmentation, white belly and head patches, premature gray, light eye pigmentation	White coat, absence of eye pigmentation, very small or no eyes, skeletal defects, abnormal cochlea, dies near weaning	91
<i>mi^{bw}</i>	Black eyed white	Normal genotype	White coat, black eyes, skin without melanocytes	96
<i>mi^{di}</i>	Microphthalmia-defective iris	Bright red eye reflex	White coat, skeletal abnormalities, abnormal retinal lamination, small eyes, abnormal iridial shape and pigmentation	97
<i>mi^{ew}</i>	Eyeless white	?	White coat, very small or no eyes	69
<i>mi^{rw}</i>	Red eyed white	Normal phenotype	White coat with some pigmentation around neck, small red eyes	98
<i>mi^{sp}</i>	Mi spotted	Normal visible phenotype, lowered tyrosinase activity, interacts with other <i>mi</i> alleles	Normal visible phenotype, lowered tyrosinase activity	99
<i>mi^{ws}</i>	White spot	White belly spot	White coat, some with small eyes	100
<i>Mi^b</i>	Microphthalmia-brownish	Dilute fur pigmentation, light ears and tail	White coat, reduced eye pigmentation	101
<i>Mi^{or}</i>	Microphthalmia-Oak Ridge	Dilute fur pigmentation, patches of white on head, belly, and tail, premature gray, reduced eye and ear pigmentation	White coat, very small or no eyes, incisors may fail to erupt, skeletal defects, deaf	102 103
<i>Mi^{wh}</i>	Microphthalmia-white	Dilute fur pigmentation, white belly patches, light ears, deaf, abnormal cochlea and vestibule	White coat, small eyes with slight pigmentation, cochlear and vestibular abnormalities with deafness	104

have enlarged interfrontal bones⁸² leading to a facial phenotype similar to that observed among Waardenburg syndromes. Homozygotes for *Ph* and *Ph^e* usually die in utero; however, survivors have cleft faces.⁸⁵ Comparisons of the known syntenic relationships between mouse and man identify extensive regions of homology between mouse chromosome 5 and human chromosome 4p where KIT is found.⁸⁶ Because of the likely homologies between the mouse mutant *Ph* and human WS1, a possible chromosomal position for the location of the gene(s) for Waardenburg syndromes is on human chromosome 4p closely linked to the proto-oncogene KIT.

The *s* mouse model

The *s* locus of the house mouse is located on chromosome 14, approximately 43 map units from the centromere.⁸⁰⁻⁸¹ Mutant alleles at the *s* locus of the mouse cause the production of patches of hypopigmentation and inner ear defects. Homozygotes may be completely white, suffer from megacolon,⁸⁷ and have structural defects of the iris.⁸⁸ In some respects, mice homozygous for mutants of the *s* locus are phenotypically similar to humans with dominant piebald trait (MIM 17280); however, they may represent a possible model for recessive Waardenburg-Shah syndrome which produces megacolon.³⁰⁻³¹ There are no reported homologous syntenic relationships between the genetic region containing *s* in the mouse and a defined chromosomal region in man.⁸¹ With the identification of additional mutations closely linked to *s* on mouse chromosome 14, these newly identified mutations could be used to locate a second possible chromosomal position for the location of Waardenburg syndromes.

The *Sp* mouse model

Sp is a semidominant mutation which exhibits profound effects upon the development of the neural crest.⁸⁹ Heterozygotes (*Sp*/+) usually exhibit small, irregular, ventral, white belly patches. Homozygotes usually die in utero, are frequently missing all neural crest derivatives, and exhibit cranioschisis, rachischisis, and occasionally microcephaly.⁸⁹ The *Sp* locus of the house mouse is located on chromosome 1, 36 map units from the centromere and 3 map units on the telomere side of *fn-1* (fibronectin 1).⁸⁰⁻⁸¹ Extensive homologies exist between this chromosomal region of the house mouse and human chromosome 2q. Eleven homologous syntenic genetic markers have been identified with fibronectin 1 (*fn-1*) located 33 map units from the centromere in mouse and FN1 (the human homologue) located in bands 2q34-q36.⁸¹⁻⁹⁰ Because of the similarities between effects caused by *Sp* and the deletion reported by Glass *et al*⁴⁷ at

2q32.2-q33.1, which causes severe developmental defects remarkably like the defects caused by WS3, these two genes may be homologous. The sporadic WS1 mutation reported by Ishikiriya *et al*⁴¹ associated with an inversion of genetic material from 2q35-q37.3 indicates that genetic alterations of this general chromosomal region may be capable of producing different Waardenburg syndromes. Thus, a third possible location of Waardenburg syndromes is on chromosome 2q near FN1.

The *Mi^{or}* mouse model

Mi^{or}, an allele of the *mi* locus of the house mouse, has been mapped to chromosome 6, 46 map units from the centromere (fig 2).⁸⁰⁻⁸¹ The first mutant allele of this locus, *mi* (*mi*=microphthalmia), was induced by a 1500 rad dose of x ray and is classified as a recessive lethal.⁹¹⁻⁹² Heterozygotes, however, have reduced ocular pigmentation, lightly pigmented ears, and frequent white head and belly patches as adults. Thus, +/+ and +/*mi* mice are easily distinguished. Homozygotes (*mi*/*mi*) usually die at the time of weaning and suffer from microphthalmia and coloboma,⁹³⁻⁹⁴ abnormal cochlear development producing deafness,⁹⁵ absence of pigment in all tissues,⁹³ and numerous skeletal abnormalities including the failure of incisors to erupt.⁹² Nine additional independently arising mutant alleles at the *mi* locus have been identified by complementation and linkage tests (table). Since some degree of complementation exists between certain of these nine alleles, Hollander¹⁰⁵ and West *et al*⁹⁷ proposed that the *mi* locus may be a large complex locus composed of several closely linked genes which are functionally related.

The range of phenotypes of the 10 mutant alleles of *mi* in heterozygotes (table) parallels much of the variation in phenotypes observed among heterozygotes for Waardenburg syndromes. The phenotypes of *mi*/+ and *Mi^{or}*/+ mice (fig 1c, table) resemble the phenotypes of persons with classical Waardenburg syndrome (WS1) because of white spotting, premature graying, reduction of eye pigmentation, skeletal defects, and less severe hearing abnormalities. The phenotype of *Mi^{wh}*/+ mice (table) resembles the phenotype of persons heterozygous for Waardenburg syndrome type II (WS2) because both lack skeletal effects but have increased penetrance for hearing defects.¹⁰⁴

Phenotypic similarities between mutations at the *mi* locus and mutations causing Waardenburg syndromes suggest a fourth possible chromosomal position for the location of Waardenburg syndromes. The *mi* locus is located on mouse chromosome 6 to which 47 other loci have been mapped.^{69,80} The syntenic relationships among homologous genes found on mouse chromosomes 6 and 9 and three human chromosomes, 3, 7, and 12, are illustrated in fig 2A and B. The

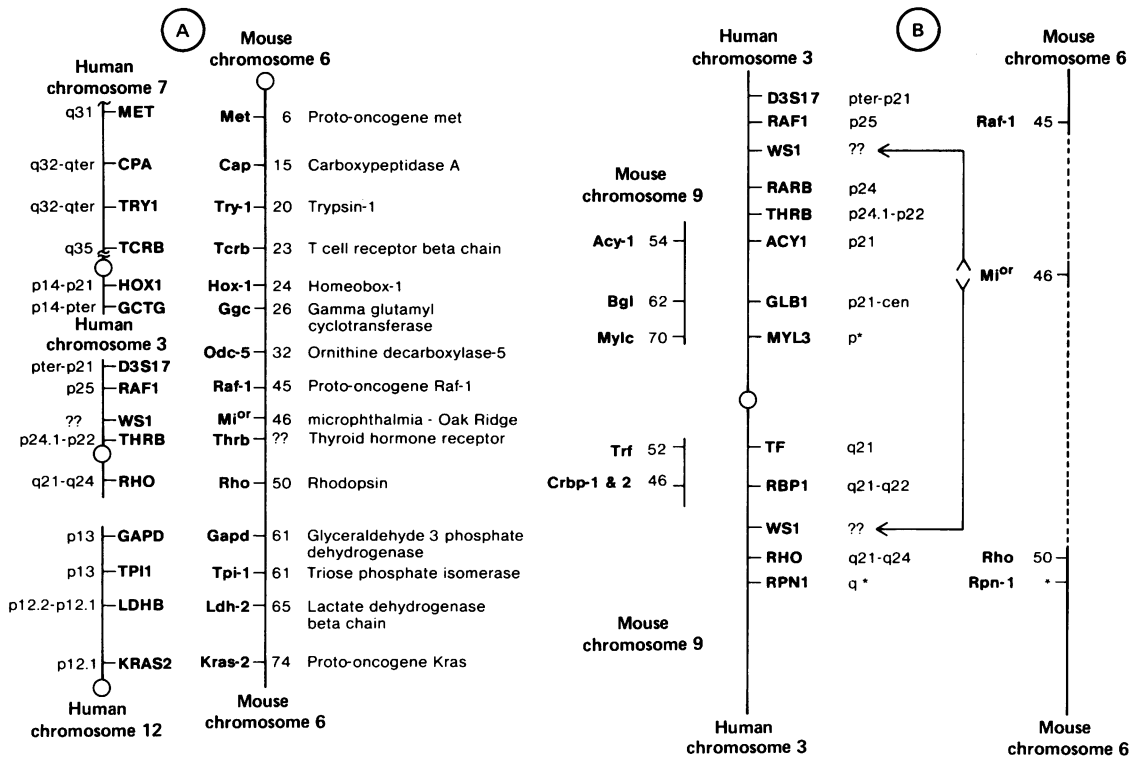


Figure 2 Syntenic relationships between mouse chromosomes 6 and 9 and human chromosomes 3, 7, and 12. (A) Major syntenic relationships between genes on mouse chromosome 6 and human chromosomes 3, 7, and 12.^{80 81 90} Note the tight linkage between *Mi^{or}* and *Raf-1*. The question marks (??) indicate predicted linkage relationships for *WS1* on human chromosome 3 and an *Erba-2* related gene on mouse chromosome 6. (B) Some syntenic relationships between genes on human chromosome 3 and mouse chromosomes 6 and 9. In mouse, the distance between *Raf-1* and *Rho* is 5 map units,¹⁰⁶ while these two markers are on opposite arms of human chromosome 3. For this reason, there are actually two predicted locations for *WS1* (??): near *RAF1* on 3q or between *RBP1* and *RHO* on 3q. An asterisk (*) next to a chromosome position indicates that the marker is on the chromosome but the exact position has yet to be determined.

closest marker to *mi* is *Raf-1* a proto-oncogene, reported to be 1 map unit from *mi* (fig 2A).¹⁰⁶ The human homologue *RAF1* has been cloned and mapped to human chromosome 3.¹⁰⁷ The observations supporting the possibility that *WS1* may be on the short arm of human chromosome 3 are: (1) the possible homology between *Mi^{or}* and *WS1*, (2) the close linkage between *mi* and *Raf-1*, (3) the average length of conserved chromosome segments of 10.1 ± 2.2 cM of mouse genes whose homologues are found in humans, and (4) the fact that there are at least three established syntenic relationships between mouse chromosome 6 and human chromosome 3 involving *RAF1*,^{107 108} *RHO* (rhodopsin),^{106 109} and *RPN1* (ribophorin 1).¹¹⁰ Given the syntenic relationships between mouse chromosomes 6 and 9 and human chromosome 3 (fig 2A and B), another possible location of *WS1* is on the long arm of chromosome 3 between the gene for the cellular retinol binding protein (*RBP1*) found at 3q21–q22 and the gene for rhodopsin (*RHO*) found at 3q21–q24.

Because of the complex nature of neural crest cell development, there may be several human and mouse loci each with multiple mutant alleles which are capable of producing phenotypic effects similar to Waardenburg syndromes. A study of mutant alleles in mouse and the syntenic relationships between genes in man and mouse suggests that there may be at least four different genetic loci affecting neural crest morphogenesis which could give rise to mutations with pleiotropic effects similar to Waardenburg syndromes. These proposed human genes homologous to specific mouse genes and their proposed locations are: a gene homologous to *Sp* on human chromosome 2q, a gene homologous to *Mi^{or}* on human chromosome 3p or 3q, a gene homologous to *Ph* on human chromosome 4p, and a gene homologous to *s* located on mouse chromosome 14 with an unknown syntenic relationship to a human chromosome.

Since the phenotypes of persons with Waardenburg syndromes vary between unrelated kindreds and vary within the same family, it is also possible that there

are genes at other loci which modify the expression of these major mutations. Epistatic interactions involving modifier genes of pigmentation phenotypes have been identified for some alleles of the four mouse loci discussed above.^{92 111 112} In the case of mice, epistatic interactions have not been described which involve the modification of hearing phenotypes. Such modifier genes for hearing deficit phenotypes have been identified in one of our laboratories (JA) for the hamster mutation *Wh*¹¹³ which appears to be homologous to *Mi*^{or}.

HAMSTER MODEL

The gene *Wh* (anophthalmic white) of the Syrian hamster, discovered independently by one of the authors (JA) in 1962 and by other laboratories,¹¹⁴⁻¹¹⁶ is a highly pleiotropic mutation causing numerous morphological, physiological, and behavioural abnormalities.^{67 113 117-123} The obvious morphological effects of the *Wh* mutation are to cause homozygotes to be deaf, blind, and white. In addition, *Wh* and *e* (cream, an unlinked autosomal mutation at the extension locus, *E*) show a strong epistatic interaction such that *Wh/wh;E/-* hamsters are white bellied agouti (Imperial hamster, fig 1d and e) while *Wh/wh;e/e* hamsters are black eyed whites (fig 1f). The skin and fur colour of the black eyed whites is thought to be one large white patch, a phenotype identical to *mi*^{bw} homozygotes in mice. Two other unlinked loci, *s* (piebald) and *Ba* (banded), interact with *Wh* to produce various sized dorsal white patches.¹²⁴ This variation in the pigmentation phenotype is similar to the range of hypopigmentation anomalies observed among Waardenburg syndromes patients.

While analysing hearing capabilities of hamsters using auditory brain stem evoked response (ABR) analyses, Amedofu¹¹³ showed that *wh/wh;e/e* (cream) hamsters have significantly shortened response latencies ($p < 0.05$) when compared to *wh/wh;E/-* (agouti) hamsters. Thus, *e* appears to improve hearing sensitivities. Both *Wh/wh;E/e* (white bellied agouti) and *Wh/wh;e/e* (black eyed white) hamsters have moderate to severe hearing impairment based on both latencies and response thresholds. However, *Wh/wh;e/e* hamsters have lower hearing thresholds than do *Wh/wh;E/e* hamsters. The interaction between the *e* locus and the *Wh* locus leads to improved hearing capabilities. Like the *e* gene in hamsters, there may be a gene in humans which segregates independently of but interacts with Waardenburg syndrome mutations. Thus, one way of explaining the intrafamilial variation observed among subjects with Waardenburg syndromes is the existence of modifying genes segregating within these families.

Discussion

Based upon the analysis of mouse and hamster

mutations, it is possible that as many as four loci may be involved in causing human Waardenburg syndromes. Since the phenotypes associated with the non-allelic mouse (*Ph*, *s*, *Sp*, and *Mi*^{or}) and hamster (*Wh*) mutations overlap extensively, as do the phenotypes of Waardenburg syndromes, linkage analyses performed to locate the chromosome position of Waardenburg syndrome mutations must use very large families defined by a single mutational event. If a given Waardenburg syndrome can be caused by mutations at different loci, analyses of pooled data from different families could result in the failure to localise the mutation to any human chromosome. Based on the mouse mutant phenotypes which are similar to Waardenburg syndromes and the syntenic relationships between mouse and human chromosomes, we propose that a single Waardenburg syndrome mutation might map to one of four possible chromosomal locations: (1) on chromosome 2q near FN1 (fibronectin 1), (2) on chromosome 3p near the proto-oncogene RAF1, (3) on chromosome 3q near RHO (rhodopsin), and (4) on chromosome 4p near the proto-oncogene KIT.

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Addendum

Recently we received a preprint from Dr Andrew Read (Foy C, Newton V, Wellesley D, Harris R, Read A. Assignment of the locus for Waardenburg syndrome type 1 to human chromosome 2q37 and possible homology to the Splotch mouse. *Am J Hum Genet*, 1990, in press) showing close linkage between *WS1* and *ALPP* assigned to 2q37. Our data from a multilocus linkage analysis of a large *WS1* family confirm the observation of Foy *et al* cited above (Asher *et al*, submitted). Because of the close linkage between *ALPP* and *FN1* in humans and the corresponding linkage of these genes in mouse, it appears, as we propose herein, that *Sp* (splotch) in mouse is a good model for Waardenburg syndrome type I.

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