Mutation in Intron 6 of the Hamster *Mitf* Gene Leads to Skipping of the Subsequent Exon and Creates a Novel Animal Model for the Human Waardenburg Syndrome Type II

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ABSTRACT

In the course of analysis of ENU-induced mutations in Syrian hamsters, a novel dominant anophthalmic white mutant (Wh^{V203}) with hearing loss was recovered. Because of this phenotype and a close linkage to the *Tpi* gene, the *Mitf* gene was considered as a candidate gene. In the *Mitf* cDNA, a deletion of 76 bp covering the entire exon 7 was detected. Further molecular analysis revealed a T → A exchange 16 bp upstream of the end of intron 6, leading to skipping of exon 7. These 16 bp at the end of intron 6 are identical in hamster, rat, mouse, and humans, indicating high conservation during evolution and a functional importance in splicing. Since the loss of exon 7 changes the open reading frame of the *MITF* transcript, translation will be stopped after 10 new amino acids. The truncated protein is predicted to contain only a part of the basic region and will miss the two helical domains and the leucine zipper. The Wh^{V203} mutation in the Syrian hamster affects the same functional domains of the Mitf transcription factor as the human R124X mutation, causing human Waardenburg syndrome type II. Therefore, the Wh^{V203} hamster mutant provides a novel model for this particular syndrome.

SINCE the discovery of the mouse *microphthalmia* (*Mi*) mutation more than 50 years ago (Hertwig 1942), numerous mutant alleles have been identified and genetically characterized. The mutations affect particular cell types, which are derived from neural-crest melanocytes. The size of the mutant eyes is reduced because of the affected retinal pigmented epithelium. The mutants frequently develop deafness owing to the lack of inner ear melanocytes. The mutations detected in the mouse are mainly recessive, but semidominant or dominant phenotypes also have been reported. The wild-type allele encodes a basic-helix-loop-helix leucine zipper (bHLHzip) transcription factor and has been referred to as microphthalmia-associated transcription factor (mitf; Steingrímsson *et al.* 1994; Yajima *et al.* 1999; Hallsson *et al.* 2000; Thaung *et al.* 2002).

In the rat, only one mutation in the *Mitf* gene has been identified (*mib/mib*); it leads to depigmentation, microphthalmia, osteopetrosis, and neurological disorders. The mutation is recessive and was characterized as a deletion covering several kilobases of genomic DNA at the *Mitf* locus. Since no *Mitf* cDNA was detected, the mutation most likely represents a *Mitf*-null allele (Opdecamp *et al.* 1998).

In the zebrafish, a recessive mutation (*nacre; nac^{w2}*) also was described recently. The homozygous mutants lack melanophores throughout development, but the retinal pigment epithelium is normal. The mutation was characterized as a C → T exchange leading to a premature stop codon. The truncated protein lacks the basic DNA-binding domain and the helix-loop-helix/leucine zipper. It is suggested that the *nac^{w2}* mutation is a loss-of-function mutation in the *Mitfa* gene. Since the zebrafish genome possesses a second *Mitf* gene (*Mitfb*), the loss of *Mitfa* function can be compensated for at least in some tissues (*e.g.*, the retinal pigmented epithelium; Lister *et al.* 1999, 2001).

Mutations within the human *MITF* were estimated in ~20% of patients suffering from Waardenburg syndrome type II (Yajima *et al.* 1999). The mutations lead to dominant phenotypes and affect mostly the basic helix-loop-helix motif and the leucine zipper region (Tassabehji *et al.* 1994, 1995; Morell *et al.* 1997; Smith *et al.* 1997).

In the Syrian hamster, one dominant mutation in *Mitf* (*W241X*) has been reported and designated as *anophthalmic white* (*Wh*). It is predicted that this premature stop codon leads to a truncation of the protein in the loop between helix 1 and helix 2 of the bHLHzip region. It prevents the protein from dimerizing or from binding to its DNA target sites (Hodgkinson *et al.* 1998).

In this article, we describe a novel dominant allele (Wh^{V203}) in the Syrian hamster. The phenotype cosegregates with a point mutation in a highly conserved region

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. AJ458438 and AJ458439.

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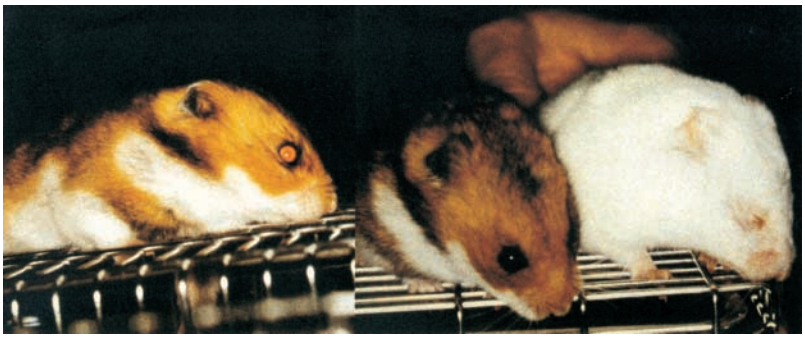

FIGURE 1.—Hamster mutant Mh^{V203}: microphthalmic eyes with an albino coat color. The wild-type hamster in the middle is flanked by the homozygous white and microphthalmic Wh^{V203}/Wh^{V203} mutant on the right and the heterozygous Wh^{V203} mutant with red eyes and a white belly on the left.

of intron 6. It leads to skipping of exon 7 of the *Mitf* gene during the maturation of the transcript.

MATERIALS AND METHODS

Animals: Three-month-old Syrian hamsters (*Mesocricetus auratus*) were treated with ENU (ethylnitrosourea; 160 mg/kg body weight). Immediately after treatment, the animals were mated with an untreated partner. The eyes of the hamsters were examined with a slit lamp after one drop of 1% atropine without anesthesia (KRATOCHVILOVA 1981). Homozygous mutants were obtained by intercrosses of the heterozygotes. In the homozygous mutants, the appearance of the microphthalmic eyes was obvious macroscopically.

General pathology: A standard pathological procedure was used to determine any morphological abnormalities in the homozygous mutants.

Histology: Four-day-old animals were killed and the dissected eyes were placed into Carnoy's solution. After 3 hr, the tissues were embedded into JB4 plastic medium (Polysciences, Eppelheim, Germany) according to the manufacturer's suggestion. Serial transversal sections (2–4 μm) were cut with a dry glass knife at an ultramicrotom (OMU4; Reichert, Walldorf, Germany), collected in water drops on glass slides, and stained with methylene blue and basic Fuchsin.

Hearing loss: Hamsters were exposed to the sound of a "click box" (1000 kHz, 102 dB; MRC Institute of Hearing Research, Nottingham, UK). Usually, the hamsters react immediately to this sound. If no reaction was observed, the hamsters were classified as deaf.

Linkage analysis: Microphthalmic hamster *V203* was mated to the mutant *Gapdh/Tpi* 4300 (PRETSCH *et al.* 2000). F_1 offspring were backcrossed to wild-type hamsters. F_2 animals were screened for the presence of microphthalmia and the *Tpi* mutation.

Molecular characterization: Eyes of wild-type hamster or remnants of the eyes from homozygous V203 hamster mutants were isolated from 1- or 2-day-old hamsters. RNA was isolated according to standard procedures and cDNA was prepared using the Ready-to-Go kit (Amersham-Pharmacia, Freiburg, Germany). The *Mitf* coding region was amplified using the meso-wh primers L1 and R1 (Table 1) spanning most of the hamster *Mitf* gene (according to EMBL accession no. AF020-900). The PCR amplification product was cloned into pCR-TOPO vector (Fermentas, St. Leon-Rot, Germany) and sequenced commercially (SequiServe, Vaterstetten, Germany). To confirm the 76-bp deletion at the cDNA level, cDNA of five mutants was prepared and sequenced.

Genomic DNA was prepared from spleen of wild-type and homozygous mutant hamster. Based on sequence homologies between the mouse and human *Mitf* sequences, the primer

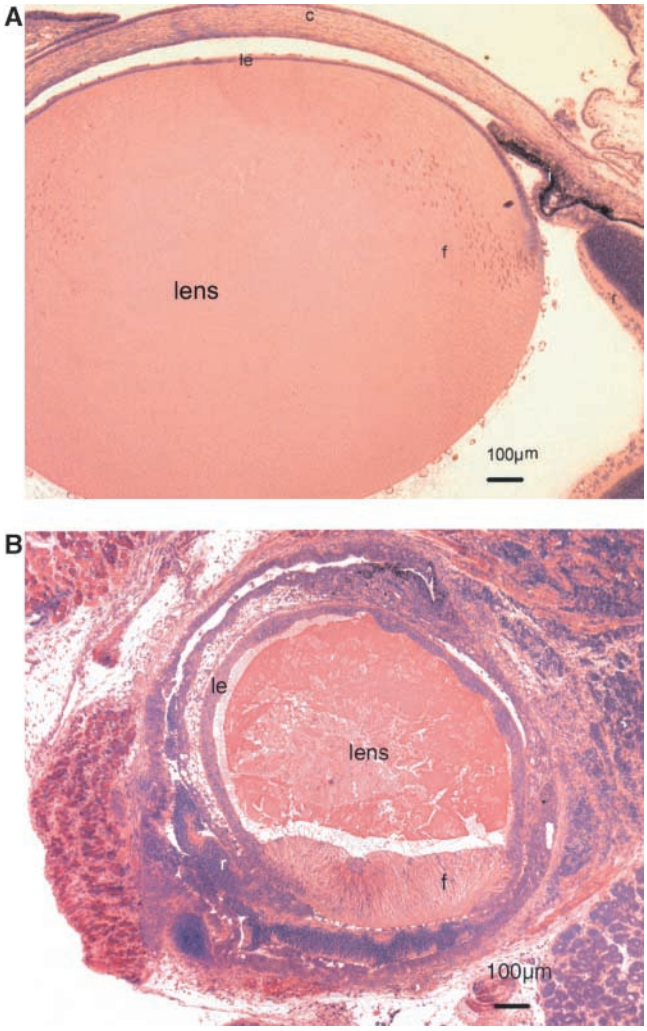

FIGURE 2.—Histology of hamster eyes at postnatal day 4. (A) A section of a wild-type hamster eye. The cornea (c) and the lens are clearly separated; the lens epithelium (le) consists of one cell layer; the lens fibers (f), the iris (i), and the retina (r) are normally developed. (B) The eye globe of a homozygous Wh^{V203} mutant at postnatal day 4 is smaller than that of the wild type. The lens epithelium (le) is multilayered; the lens contains mainly liquefacted mass, and in the posterior part shortened lens fibers are present. The development of the retinal layers (r) is disturbed; they exhibit excessive growth and folding.

TABLE 1

Primers used for PCR and sequencing

Designation	Lab no.	Accession no.	Sequence (5′ → 3′)	T_m
meso-wh-L1	19016	AF020900	ACATGCCAGCCAAGTCCTGAGC	60°
meso-wh-R1	19017	AF020900	GTAAATTATGAAGTCTACTGAAGAAGAGAGGAAGC	60°
meso-wh-LM	24069	AF020900	AACTCTTGTCCAGCCAACCTTCCC	54–61°
meso-wh-RM	24389	AF020900	CTTTCGGATGTAGTCCACAGAGGCC	54–61°
Ham-*mitf*-L3	24729	Z23066	CCTTGTTTATGGTGCCTTCTTTATGCC	58°
Ham-*Mitf*-R3	24730	AF020900	CACAGTTGGAGTTAAGAGTGAGCATAGCC	58°
Mitf-Intron6-L2	30917	Unpublished	CATCCCTTCTTAAAAGTATTCCCCTCTAGTATC	51–58°
Mitf-Exon7-R1	24769	AF020900	TGCGGTCATTTATGTTAAATCTTCTTCTCC	51–58°

Ham-Mitf-L3 was designed on the basis of the mouse sequence Z23066 and combined with a hamster-specific primer (Ham-Mitf-R3). To amplify the 3′ end of the hamster *Mitf* intron 6, together with its flanking part of exon 7, a primer based on the (unpublished) intron sequence of the mouse (kindly provided by E. Steingrimsson) was combined with the primer specific for the hamster exon 7 (Table 1).

The computer-aided analysis of deduced amino acid (aa) sequences used the proteomics tools from Expasy (http://www.expasy.ch).

RESULTS

General characterization: A novel hamster mutant characterized by red eyes and white belly was recovered in the first generation after paternal treatment with ENU and recorded under the laboratory number *V203*. Homozygous mutants resulting from intercross matings of heterozygotes are white and exhibit microphthalmia (Figure 1). Since this phenotype is similar to another hamster mutant, *Wh* (Hodgkinson *et al.* 1998), our new mutation has been referred to as microphthalmic white (*Wh*^{V203}).

Because of the phenotypic similarity to several *Mitf* mutants in the mouse and the known linkage between the *Tpi* and *Mitf* genes (http://www.informatics.jax.org), *Wh*^{V203} was tested for linkage with a recently detected *Tpi* mutation in hamster (Pretsch *et al.* 2000). We observed only three recombinants in 48 F_2 hamsters tested. This is statistically significantly different ($P \leq 0.001$, χ^2 test) from a random distribution of 1:1, which would be expected if two loci were at different chromosomes. The genetic distance calculated between *Tpi* and *Wh*^{V203} is 6.3 ± 3.6 cM.

Microphthalmia: In the wild-type eye of a 4-day-old hamster (Figure 2A), the cornea, iris, lens, and retina are well developed and regularly arranged. In contrast, severe defects in the eye of homozygous *Wh*^{V203} mutants (Figure 2B) are recovered. The eye globe is distorted and the cornea is malformed; the iris cannot be recognized. The residual lens shows degenerated fiber cells, which become liquefied in the part closed to the cornea. Except in agglomerated pigmented cells, no differentation of the retinal cell layers was observed.

Hearing loss: Hearing loss was tested using a click box. Wild-type animals demonstrated in all cases an immediate adverse reflex ($n = 15$); among the six homozygous mutants tested, none was able to hear the ultrasound. The response from the heterozygotes was intermediate; 12 out of 14 were positive, whereas 2 showed only a very weak reaction.

Viability and fertility: In the intercrosses of heterozygotes, normal numbers of offspring with the expected 1:2:1 ratio of homozygous and heterozygous mutants and wild types were found. There was a slight reduction in the number of homozygous females ($\chi^2 = 5.4$). The outcross of the homozygous mutants revealed a low fertility of the males. However, the analysis of their sperm cells revealed a normal number of spermatozoa in the epididymis and normal population of developing germ cells in the testes. Homozygous female mutants become pregnant very rarely and never bred any offspring. The pathological examination did not reveal any abnormalities except in the eye. In particular, no indications for osteopetrosis were found by X-ray examination (A. Luz, personal communication).

Molecular analysis: For a molecular characterization of the mutation, cDNA was prepared from the eye or its remnants within the first two days after birth. Previous *Mitf* sequence information in hamsters (Hodgkinson *et al.* 1998) covered only a part of the *Mitf* gene; this fragment was amplified using the primer pair L1/R1 (Table 1). On the basis of the sequence homology between mouse and human (accession nos. Z23066 and Z29678; primer pair L3/R3), we could amplify a fragment of an additional 400 bp containing exon 1m (exon nomenclature according to Hallsson *et al.* 2000), which overlaps with the main downstream fragment. Both fragments resulted in a complete cDNA sequence of the hamster *Mitf* gene starting at position 91; the regular stop codon is at position 1347 (Figure 3a). The newly identified 5′ end of the hamster *Mitf* mRNA corresponds to the mouse *Mitfa* mRNA, which is enriched in the retinal pigmented epithelium of the mouse embryo (Amae *et al.* 1998).

In the PCR products, we confirmed the alternative

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WT     GCTCTTGGAA TCGGACTTAC AGAAAGTAGA GGGAGGAAGA ATAAGTAGTC TGCCCTGTGT   60
V203   GCTCTTGGAA TCGGACTTAC AGAAAGTAGA GGGAGGAAGA ATAAGTAGTC TGCCCTGTGT

                                        M  L  E  M  L  E  Y   S  H  Y   10
WT     CCTTGGCTTG GGGCCGCCTG AGACGTTGCT ATGCTGGAAA TGCTAGAGTA CAGTCACTAC  120
V203   CCTTGGCTTG GGGCCGCCTG AGACGTTGCT ATGCTGGAAA TGCTAGAGTA CAGTCACTAC

        Q  V  Q  T  H  L  E   N  P  T   K  Y  H   I  Q  Q  A   Q  R  H   30
WT     CAGGTGCAGA CCCACCTGGA AAACCCCACC AAGTACCACA TACAGCAAGC CCAGAGGCAC  180
V203   CAGGTGCAGA CCCACCTGGA AAACCCCACC AAGTACCACA TACAGCAAGC CCAGAGGCAC

        Q  V  K  Q  Y  L  S   T  T  L   A  N  K   H  A  S  Q   V  L  S   50
WT     CAGGTAAAGC AGTACCTTTC TACCACTTTA GCAAATAAAC ATGCCAGCCA AGTCCTGAGC  240
V203   CAGGTAAAGC AGTACCTTTC TACCACCTTA GCAAATAAAC ATGCCAGCCA AGTCCTGAGC

        S  P  C  P  N  Q  P   G  D  H   A  M  P   P  V  P  G   S  S  A   70
WT     TCGCCATGTC CAAACCAGCC TGGCGACCAT GCCATGCCAC CAGTGCCGGG GAGCAGCGCA  300
V203   TCGCCATGTC CAAACCAGCC TGGCGACCAT GCCATGCCAC CAGTGCCGGG GAGCAGCGCA

        P  N  S  P  M  A  M   L  T  L   N  S  N   C  E  K  E   A  F  Y   90
WT     CCCAACAGCC CCATGGCTAT GCTCACTCTT AACTCCAACT GTGAAAAAGA GGCGTTCTAT  360
V203   CCCAACAGCC CCATGGCTAT GCTCACTCTT AACTCCAACT GTGAAAAAGA GGCGTTCTAT

        K  F  E  E  Q  S  R   A  E  S   E  C  P   G  M  N  T   H  S  R  110
WT     AAGTTTGAAG AGCAGAGCAG GGCAGAGAGT GAGTGCCCAG GTATGAACAC GCACTCTCGA  420
V203   AAGTTTGAAG AGCAGAGCAG GGCAGAGAGT GAGTGCCCAG GTATGAACAC GCACTCTCGA

        A  S  C  M  Q  M  D   D  V  I   D  D  I   I  S  L  E   S  S  Y  130
WT     GCGTCGTGCA TGCAGATGGA TGATGTAATT GATGACATCA TCAGCCTGGA GTCAAGTTAT  480
V203   GCGTCGTGCA TGCAGATGGA TGATGTAATT GATGACATCA TCAGCCTGGA GTCAAGTTAT

        N  E  E  I  L  G  L   M  D  P   A  L  Q   M  A  N  T   L  P  V  150
WT     AATGAAGAAA TCTTGGGCTT GATGGACCCT GCCTTGCAAA TGGCAAACAC GTTACCTGTC  540
V203   AATGAAGAAA TCTTGGGCTT GATGGACCCT GCCTTGCAAA TGGCAAACAC GTTACCTGTC

        S  G  N  L  I  D  L   Y  S  N   Q  G  L   P  P  P  G   L  T  I  170
WT     TCTGGAAACT TGATCGACTT ATACAGCAAC CAGGGCCTGC CACCCCCGGG CCTCACCATC  600
V203   TCTGGAAACT TGATCGACTT ATACAGCAAC CAGGGCCTGC CACCCCCGGG CCTCACCATC

        S  N  S  C  P  A  N   L  P  N   I  K  R   E  L  T  E   S  E  A  190
WT     AGCAACTCTT GTCCAGCCAA CCTTCCCAAC ATAAAAAGGG AGCTCACAGA GTCTGAAGCA  660
V203   AGCAACTCTT GTCCAGCCAA CCTTCCCAAC ATAAAAAGGG AGCTCACAGA GTCTGAAGCA

        R  A  L  A  K  E  R   Q  K  K   D  N  H   N  L  I  E   R  R  R  210
WT     AGAGCATTGG CTAAAGAGAG GCAAAAAAAG GACAATCACA ACTTGATTGA ACGGAGAAGA  720
V203   AGAGCATTGG CTAAAGAGAG GCAAAAAAAG GACAATCACA ACTTGA---- ----------

        R  F  N  I  N  D  R   I  K  E   L  G  T   L  I  P  K   S  N  D  230
WT     AGATTTAACA TAAATGACCG CATTAAAGAA CTAGGTACTT TGATTCCCAA GTCAAATGAT  780
V203   ---------- ---------- ---------- ---------- ---------- ----------

        P  D  M  R  W  N  K   G  T  I   L  K  A   S  V  D  Y   I  R  K  250
WT     CCGGACATGC GGTGGAACAA AGGAACCATT CTAAAGGCCT CTGTGGACTA CATCCGAAAG  840
V203   --GGACATGC GGTGGAACAA AGGAACCATT CTAAAGGCCT CTGTGGACTA CATCCGAAAG
         R  T  C  G  G  T  K   E  P  F   .

        L  Q  R  E  Q  Q  R   A  K  D   L  E  N   R  Q  K  K   L  E  H  270
WT     TTGCAACGAG AACAGCAGCG TGCAAAGGAC CTTGAAAACC GACAGAAGAA GCTGGAACAT  900
V203   TTGCAACGAG AACAGCAGCG TGCAAAGGAT CTTGAAAACC GACAGAAGAA GCTGGAACAT

        A  N  R  H  L  L  L   R  V  Q   E  L  E   M  Q  A  R   A  H  G  290
WT     GCTAACCGGC ATCTGTTGCT CAGAGTACAG GAGCTTGAGA TGCAGGCGAG AGCGCATGGA  960
V203   GCTAACCGGC ATCTGTTGCT CAGAGTACAG GAGCTTGAGA TGCAGGCGAG AGCGCATGGA
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FIGURE 3.—Comparison of wild-type and *V203* cDNA sequences. (a) The entire Mitf cDNA sequence of the hamster is given. Start codon ATG in exon 1m (according to Z23066; position 91–93) is underlined and in boldface type. Moreover, differences from the already published sequence (AF020900) at the 3′ end are also underlined and in boldface type. The deduced amino acid composition is given above the cDNA sequence. The skipped exon 7 in the Wh^{V203} mutant is indicated by a dashed line between positions 707 and 782; the changed open reading frame leads to 10 new amino acids, which are underlined and in boldface type. (b) Amplification of partial *Mitf* cDNA from wild-type and homozygous Wh^{V203} mutants. The PCR-amplified cDNA fragment from the wild type is obviously larger than the corresponding fragment from the homozygous V203 hamster. The size difference is due to the skipping of exon 7.

splicing at the beginning of exon 6 as observed in humans (HODGKINSON *et al.* 1993) or in mouse (STEINGRÍMSSON *et al.* 1994). According to the peak areas in the sequencing profiles, it is estimated that the additional 18-bp fragment in exon 6 is present in about half of the cDNA.

In the 3′ part of the *Mitf* gene we observed four polymorphic sites in our hamsters as compared to the database (AF020900). Two polymorphisms are silent (position 870 GAC → GAT encoding Asp; position 1179 AAA → AAG encoding Lys). In contrast, the change from GAC → GGC at position 1106 will lead to an exchange from Asp to Gly, and the AGC → GGC exchange at position 1165 is considered to switch Ser to Gly.

In general, the Mitf amino acid sequence is highly conserved between mouse and hamster. The first 302 amino acids are even identical, and among the next 67 amino acids only four substitutions were observed. All these alterations are downstream of the helix-loop-helix motif (aa 236–251) or the leucine zipper (aa 261–282); a PROSITE scan suggests that the putative phosphorylation sites are not affected.

The obvious difference between the wild-type hamster and the Wh^{V203} mutant is the reduced size of the L1/R1

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         L  S  L   I  P  S  S   G  L  C   S  P  D   L  V  N  R   I  I  K    310
WT     CTTTCCCTTA TCCCATCCTC TGGCCTCTGC TCGCCTGATC TGGTGAACCG GATCATCAAG 1020
V203   CTTTCCCTTA TCCCATCCTC TGGCCTCTGC TCGCCTGATC TGGTGAACCG GATCATCAAG

         Q  E  P   V  L  E  N   C  S  Q   E  L  V   Q  H  Q  T   D  L  T   330
WT     CAAGAACCAG TTCTTGAGAA CTGCAGCCAG GAACTTGTGC AGCACCAGAC AGACCTGACA 1080
V203   CAAGAACCAG TTCTTGAGAA CTGCAGCCAG GAACTTGTGC AGCACCAGAC AGACCTGACA

         C  T  T   T  L  D  L   T  D  G   T  I  T   F  T  N  N   L  G  T   350
WT     TGTACAACGA CCCTGGATCT CACGGACGGT ACCATCACCT TCACAAACAA CCTCGGCACC 1140
V203   TGTACAACGA CCCTGGATCT CACGGGCGGT ACCATCACCT TCACAAACAA CCTCGGCACC

         M  P  E   S  N  P  A   Y  S  I   P  R  K   M  G  S  N   L  E  D   370
WT     ATGCCAGAGA GCAACCCCGC CTATGGCATC CCCAGGAAGA TGGGCTCCAA CTTGGAAGAC 1200
V203   ATGCCAGAGA GCAACCCCGC CTATAGCATC CCCAGGAAGA TGGGCTCCAA CTTGGAAGAC

         I  L  M   D  D  A  L   S  P  V   G  V  T   D  P  L  L   S  S  V   390
WT     ATCTTGATGG ACGATGCCCT CTCGCCTGTT GGCGTCACTG ATCCACTGCT GTCATCAGTG 1260
V203   ATCTTGATGG ACGATGCCCT CTCGCCTGTT GGCGTCACTG ATCCACTGCT GTCATCAGTG

         S  P  G   A  S  K  T   S  S  R   R  S  S   M  S  A  E   E  T  E   410
WT     TCCCCAGGAG CTTCTAAGAC AAGCAGCCGG AGGAGCAGTA TGAGCGCAGA AGAAACGGAG 1320
V203   TCCCCAGGAG CTTCTAAGAC AAGCAGCCGG AGGAGCAGTA TGAGCGCAGA AGAAACGGAG

         H  A  C  .                                                        418
WT     CATGCATGTT AGCGAGCCCA CCTTGCTCTG CCTCTGCACA AACTGCTTCC TCTCTTCTTC 1380
V203   CATGCATGTT AGCGAGCCCA CCTTGCTCTG CCTCTGCACA AACTGCTTCC TCTCTTCTTC

WT     AGTAGACTTC ATAATTTAC                                              1399
V203   AGTAGACTTC ATAATTTAC
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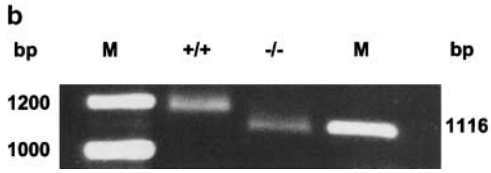

FIGURE 3.—*Continued.*

PCR product in the mutant (Figure 3b). Sequence analysis revealed a deletion of 76 bp between positions 725 and 800 (Figure 3a). On the basis of the human exon boundaries (TASSABEHJI *et al.* 1994), it is concluded that the missing 76 bp reflects the loss of exon 7 in the Wh^{V203} mutants.

The cause for skipping exon 7 was found in intron 6; from genomic DNA, we amplified a region covering the 3′ end of intron 6 and the 5′ region of exon 7 (primer pair intron6-L2/exon7-R1; Table 1). An exchange of T → A was observed in intron 6, 16 bp upstream of its boundary to exon 7 (Figure 4). The substitution was confirmed in several independent sequences from wild-type and homozygous mutant mice. Moreover, sequence comparison of this particular region showed that it is highly conserved among human, mouse, rat, and hamster; in particular, the last 16 bp are identical in these species, indicating a functional importance of this element in splicing. Therefore, this mutation is strongly suggested to be causative for the skipping of exon 7 and for the resulting phenotype.

DISCUSSION

The *Mitf* gene belongs to a group of genes, which are expressed during development of neural-crest-derived melanocytes. *Mitf* is activated by Pax3 (WATANABE *et al.* 1998; VACHTENHEIM and NOVOTNA 1999), Sox10 (BONDURAND *et al.* 2000), Wnt3a (DORSKY *et al.* 2000), and onecut-2 (JACQUEMIN *et al.* 2001). As a basic helix-loop-helix/leucine-zipper transcription factor, Mitf protein itself regulates other genes like *MyoD*, *Myf5*, *c-Met*, *c-Kit*, *tyrosinase*, *Trp-1*, *Qnr-71*, *Ednrb*, and *Edn3* (TACHIBANA 1999). Therefore, mutations in these genes may cause similar syndromes. To understand the molecular mechanisms underlying these syndromes, a detailed analysis of a variety of homologous mutations in different species is important. Moreover, the comparison of conserved regions *vs.* polymorphic sites will allow also more detailed knowledge of which part of a gene might be important for its functions.

In this article, we describe the entire *Mitf* gene in the Syrian hamster. The *Mitf* gene, in both mouse and humans, has a very complex structure in its 5′ region. The first four possible exons (1a, 1h, 1b, and 1m) in front of exon 2 lead to tissue-specific alternatively spliced transcripts (AMAE *et al.* 1998; HALLSSON *et al.* 2000). On the basis of the corresponding sequence information in the mouse, we amplified the 5′ part of the *Mitf* cDNA from the hamster retinal pigmented epithelium. It corresponds to mouse *Mitf-1m* and indicates a strong conservation of the tissue-specific alternative splice products at least for rodents. Additionally, a few polymorphic sites in the 3′ part of the coding region were found.

During this study, we characterized a novel dominant mutation in the hamster *Mitf* gene. An exchange of T → A 16 bp upstream of the splice donor site of exon 7 leads to a loss of this exon during splicing. Since exon

FIGURE 4.—Point mutation in a conserved region at the end of intron 6. A fragment covering the boundary between intron 6 and exon 7 was amplified from genomic DNA. A base-pair exchange from A → T was observed (in boldface type and underlined) 16 bp upstream of the 3′ end of intron 6; the beginning of exon 7 is in green. The comparison between the genomic sequence of hamster, mouse, rat, and humans demonstrates the identity of the last 16 bp in intron 6; the mutation in the hamster V203 affects the first of them, indicating a functional importance of this stretch during splicing. (*) Identical bases in all species.

7 consists of 76 bp, its loss changes the open reading frame and after 10 novel amino acids (after position 211) a stop codon occurs, truncating the protein just in front of the helix-loop-helix and leucine zipper motif.

The skipping of exon 7 as a consequence of the T → A substitution 16 bp upstream of the intron 6/exon 7 border demonstrates the importance of this particular base for the splicing mechanism even if the sequence at this position does not fit the conserved sequence in mammalian nuclear pre-mRNA introns, which is recognized by the U2 snRNA (STALEY and GUTHRIE 1998). However, the most downstream 16 bp of intron 6 are identical in human, mouse, rat, and hamster, indicating their functional importance. Farther upstream in the intron, the degree of homology is lower.

Heterozygous *Mitf*^{V203} mutants are normal agouti, but with a white belly and red eyes, whereas the homozygous mutants are white with closed eyes and suffer also from sterility and hearing loss. Two dominant mutations in humans [one of Indian origin (LALWANI *et al.* 1998) and the other from northern Europe (NOBUKUNI *et al.* 1996)] leading to a similar phenotype have been characterized recently. They affect the same functional region of the Mitf transcription factor by a stop codon at amino acid position 214 (R214X), which is also in front of the helix-loop-helix and leucine zipper motif. In both families, hearing loss was the most common finding, followed by ocular pigmentary disturbances. However, *heterochromia iridis* occurs more frequently in the Indian family than in the affected members of the European family, indicating additional effects of different genetic backgrounds. Since the new hamster mutation *Mitf*^{V203} affects the same region, it might be considered as an excellent model for this mutation in humans.

The other already described dominant hamster mutation, *Wh*, has been found downstream and leads to a stop codon between helix 1 and helix 2 (W241X). This nonsense mutation destabilizes the *Mh-Mitf* mRNA and prevents the encoded basic helix-loop-helix leucine zipper protein from dimerizing or binding to its DNA target sites (HODGKINSON *et al.* 1998). The mutation leads to a highly pleiotropic phenotype exhibiting dominant spotting coat colors and causing homozygotes to be deaf, blind, white, and sterile (ASHER and JAMES 1982).

In addition to the mutations mentioned above, several other mutations are described in mice and humans that affect the basic region and the first helix of the helix-loop-helix motif of the Mitf protein. In the mouse, the alleles *Mi*^{wh} (I212N), *mi* (ΔR215), *Mi*^{or} (R216L), and *mi*^{vt} (D222N) are reported to touch this particular region also (for a detailed overview see STEINGRÍMSSON *et al.* 1994; MOORE 1995). Recently, *Mitf*$^{Mi\text{-}H}$ and *Mitf*^{Rorp} were detected; in the *Mitf*$^{Mi\text{-}H}$ mutants a splicing defect also was shown to be causative for the phenotype (THAUNG *et al.* 2002).

The increasing list of mutations in the *Mitf* gene is a hint of its functional importance. However, more detailed comparative studies are necessary to deduce genotype-phenotype correlations, considering the mutations available even in different species.

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