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Mutation analysis in a family with oculocutaneous albinism manifesting in the same generation of three branches

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Purpose: To elucidate the molecular basis of oculocutaneous albinism with variable expressivity in a family from The Netherlands in which no consanguinity was reported.
Methods: Three affected family members were screened for mutations in tyrosinase (*TYR*) and the pink-eye-dilution gene (*P*) by using SSCP. The melanocortin receptor gene (*MC1R*) and amplimers of *P* showing an aberrant banding pattern in SSCP were analyzed by direct sequencing. All participants underwent ophthalmologic examination including funduscopy, and visually evoked potentials were recorded in two cases.
Results: The pedigree had three branches A, B, and C. We identified three mutations in *P* (V443I, N476S, C793F) that cause a compound heterozygous situation in cases from branch A (N476S/C793F) and B (V443I/C793F), who showed oculocutaneous albinism. Hair and skin color followed the light Nordic complexion that was also present in other affected and unaffected members of this family. Descendants of branches A and B showed light complexion with iris translucency and peripheral fundus hypopigmentation independent from the genotype identified. A single descendant had red hair, carrying a well known compound *MC1R* mutation combination for red hair color and a single heterozygous *P* mutation.
Conclusions: *P* mutations underlie oculocutaneous albinism in this family. Two known mutations in *MC1R* caused red hair color in one family member. No modifier effect of *MC1R* on *P* mutations could be deduced from the results of this study.

Pigmentation of skin, hair, iris, and ocular fundus is a concerted activity of pigment production and distribution. Pigment is produced in specialized organelles-melanosomes-recruited from the lysosomal cycle [1]. Pigment occurs in two forms: black-brown eumelanin and yellow-red pheomelanin [2]. Both forms depend on the activity of tyrosinase (*TYR*) which controls the major steps in pigment production. TYR is guided through the cell from the endoplasmic reticulum to the late endosomal compartments, where it is essential for the production of melanin in the developing early-stage melanosomes.

The second important protein involved is encoded by the pink-eye dilution gene (*P*) and is thought to control the melanosomal pH value thus regulating *TYR* activity and melanosomal distribution [3,4].

The melanocortin-1 receptor (MC1R) determines the relative production of eumelanin and pheomelanin in the melanosome. Reduced activity of MC1R prevents the formation of eumelanin, thus causing red hair coloring and limiting the skin's ability to tan [5,6]. *MC1R* mutations have been characterized as strong (*D84E, R142H, R151C, R160W, D294H*) and weak alleles (*V60L, V92M*) in causing red hair as well as involvement in blond and light hair phenotypes [7-9].

Mutations acting in an autosomal recessive mode in any of these genes have been associated with phenotypes of reduced pigmentation. Oculocutaneous albinism (OCA) is a complete lack of pigmentation of any kind. This disorder presents with hypopigmented skin, white hair, and various ocular anomalies such as nystagmus, reduced visual acuity, macular hypoplasia, fundus hypopigmentation, and misrouting of the optic nerve fibers. This phenotype is usually classified as OCA1A1 [10]. A milder phenotype is present in patients carrying mutations in *P*. Patients may have red hair during the first weeks of life then develop a light blond hair color later on. This phenotype is usually classified as OCA2 [11]. Depending on the mutation type, the phenotype varies for both genes from OCA to autosomal recessive ocular albinism (AROA; OA3) characterized by macular hypoplasia and fundus hypopigmentation only. Recently, persistent red colored hair was reported in patients carrying mutations in *P* causing OCA modified by mutations in *MC1R* to cause red hair color [12]. *MC1R* is a highly polymorphous gene with variable influence of its various mutants on skin, hair, and even iris color [13].

In this study we report on the identification of *P* and *MC1R* mutations and exclusion of *TYR* mutations in two branches of

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a family with OCA, spanning three generations, in which no contribution of *MC1R* to the albinism phenotype was found.

METHODS

Patients and controls: We investigated a Dutch family consisting of 21 descendants from a non-consangiuneous couple. The couple had seven children (three male, two female). The male children established three branches with three affected children (two female, one male) and an unaffected female in the first branch, an affected female and an unaffected male in the second branch, and an affected male and an unaffected female in the third branch. The affected male from the first branch had a pair of children and his affected sister two male children. The affected female in the third branch also had a pair of children. (Figure 1) Two of the five affected family members had already died (one female in branch A and a male in branch C) and the members of branch C did not cooperate. We investigated the living three affected family members from the third generation and further 11 unaffected members of the family as indicated in Figure 1. All participants in this study were informed about its objectives, and each gave informed consent according to the tenets of the declaration of Helsinki. The study was approved by the Ethics Committee of the University of Regensburg. DNA samples of 50 unaffected German individuals were included as controls for mutation analysis.

Clinical examination: Ophthalmic examination was performed on three OCA cases who presented with nystagmus, macular hypoplasia, hypopigmented fundus, reduced visual acuity, and iris translucency. We also conducted exams on seven unaffected members: six descendants from affected members from branches A and B and the father of case III:8 (Figure 1 indicated by arrows). Examinations included indirect ophthalmoscopy of the peripheral fundus, best-corrected visual acuity, fundus biomicroscopy with a 90D lens, and slit-lamp examination for checking abnormal iris translucency. In this latter exam, we looked for a generalized translucency with at least part of the lens equator visible. In two cases where we suspected OCA, we recorded visually evoked potentials according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standard [14] to check if misrouting of the optic nerve fibers, pathognomonic in all forms of albinism, was present [15,16]. Medical records were available for case III:10, who was deceased.

Molecular genetic analysis: Peripheral blood was taken (Figure 1 indicated by DNA-IDs) from the three clinically affected participants as well as from 11 relatives who a regular macular reflex, normal visual acuity, and macular pigmentation. DNA was extracted by standard procedures using osmotic disruption of erythrocytes, protease digest of nucleated cells and erythrocyte membrane proteins followed by salting out of protein remnants and finally ethanol precipitation of DNA [17]. *TYR, P, and MC1R* were amplified in 34 amplimers using primers we designed (see Table 1) and those created by Lee et al. [18]. Amplimers were screened for mutations by single strand conformation polymorphism analysis in *TYR* and *P*. Amplimers of *MC1R* as well as amplimers of *P* that showed aberrant banding patterns were directly sequenced using the fluorescent dye termination method. Sequencing results were compared to standardized sequence listing of TYR, P, and *MC1R* (GenBank AY225228). *V443I* and *C793F* mutations within P^{V443I}, P^{C793F} were confirmed by *PsyI*, and *BsgI* restriction endonuclease assay, respectively.

RESULTS

A summary of the available clinical data is given in Table 2. Case III:10 (Figure 1) was said to have had albinism. He had markedly reduced vision, but his hair and skin were less affected than those of his cousins. No further information was available.

Mutation analysis was first performed in III:3 and III:8, who were from the first and second branch of the family and later confirmed in other family members (Figure 1). Mutations in *TYR* were excluded. Case III:3 instead carried two novel compound heterozygous missense mutations in *P* (N476S, C793F) which was also found in his affected sister (III:6).

P^{C793F} could be followed by *BsgI* restriction endonuclease assay to segregate through the family to an affected paternal cousin III:8 (Figure 1). In addition III:8 carried another well known mutation in *P* (V443I) that she inherited from her mother II:7. All three mutations did not appear in 100 alleles from control samples. In the fourth generation, mutations of *P* could be shown in the single heterozygous state only.

Direct sequencing of *MC1R* in this family revealed four missense mutations (V60L, G104S, R151C, R160W). The distribution of the *MC1R* alleles is given in Table 2 and Figure 1. The mutations present in the family were investigated in the most recent generation. As would be assumed from his red hair color and his skin's limited ability to tan we were able to identify compound heterozygous MC1R mutations in case IV:5 (R151C, R160W).

DISCUSSION

In this study we identified *P* mutations in a family with OCA and variable expressivity in five cases. The family had three branches with cases occurring in the same generation in all branches (Figure 1). Usually, such a situation can be observed in consanguineous families. Since the family was unaware of any consanguinity, monogenic inheritance seemed questionable; all spouses would have to be heterozygous carriers for a disease causing mutation, which is a rare condition. Family members from the fourth generation of the first branch (IV.1-4) consistently showed translucency of the irides, a history of easy burning when exposed to the sun, but no macular pathology.

These phenotypes made us consider the usual OCA genes, *TYR*, and *P*, as well as *MC1R* since King et al. reported on red hair, light tanning, and ocular signs of OCA due to mutations in *P* on various *MC1R* background in all eight cases investigated in his study [12]. No information was provided on the genotype of the carrier relatives in that publication.

We could assess the first and second branch of the family, and we conclusively showed *P* mutations (N476S/C793F/

V443I) to cause OCA in these branches (see Figure 1). N476S and C793F mutations are reported here for the first time. The corresponding amino acid positions in P-protein are conserved throughout phylogeny from pig by mouse, rat, and rabbit to fish (*Astyanax mexicanus*). Cytosine loss is usually deleterious to tertiary and quartenary structures. The conversion of asparagine to serine removes an ion-binding site and introduces an aminoacid of lower hydrophilicity, which should affect the tertiary structure of the protein.

The mutations identified in *MC1R* were conclusive for the red hair phenotype in case IV:5 and have been reported previously by King et al. (*MC1R*^{R151C}, *MC1R*^{R160W}) [12]. Like his sister (IV:6) he did not show any signs of albinism. IV.6 and other unaffected relatives (II:7 and III:9) carried different other mutations in *MC1R* (V60L and G104S) in a compound heterozygous state but did not have red hair. The *MC1R*^{G104S} mutation is unlikely to contribute to the red hair phenotype

Table 1. *MC1R* primers designed for this study

Amplimer	Forward	Reverse
5' part	AGGCCTCCAACGACTCCTTCCT	AGAAGACCACGAGGCACAGCAGGAC
3' part	GGTGCTGCAGCAGCTGGACAAT	ACTTAAAGCCGCGTGCACCG

This table contains the primers used to amplify and sequence the *MCR1R* gene. The gene was amplified in two amplimers (5' part and 3' part). The amplimers overlapped covering codons 111 to 196.

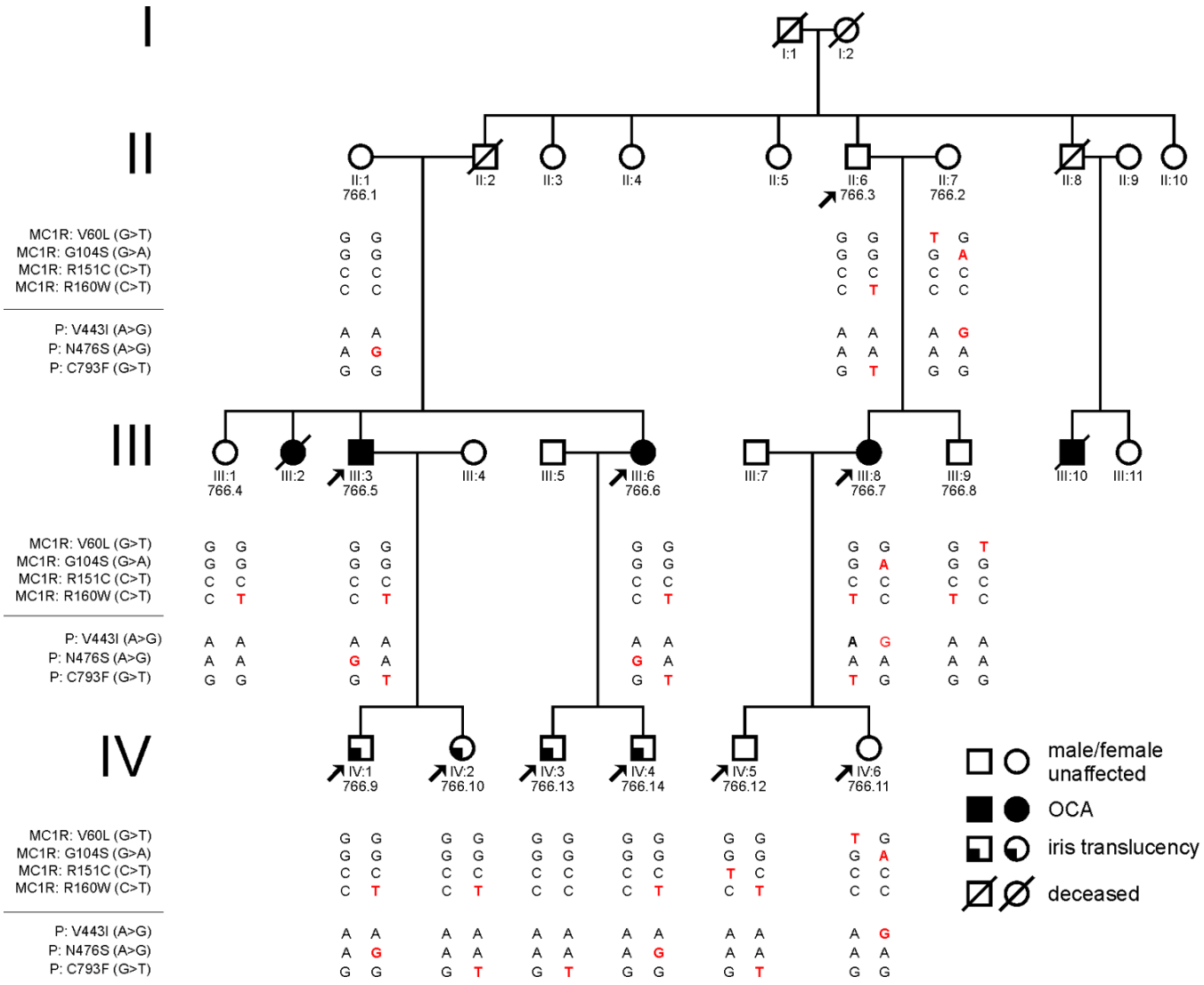

Figure 1. Pedigree of family 766 showing the distribution of mutations in *P* and *MC1R* segregating through the family. DNA-IDs of screened samples are given below pedigree IDs. Mutations were identified in the human pink eyed dilution gene (*P*) and the melanocortin receptor gene (*MC1R*) in the screened samples. Nucleotides (A: Adenine, C: Cytosine, G: Guanine, T: Thymine) of alleles are given below individuals. Corresponding amino acid changes are listed at left side (C: Cystein, F: Phenylalanine, G: Glycine, I: Isoleucine, L: Lysine: N: Asparagine, R: Arginine, S: Serine, V: Valine, W: Tryptophane) Nucleotide changes are given in brackets. Red font marks mutant alleles. OCA: oculocutaneous albinism.

since none of the persons carrying this mutation, together with a second *MC1R* mutation, could be shown to express the red hair phenotype. *MC1R*^{G104S} was reported in the population from The Netherlands at a frequency <0.5% without further associations with functional deficits [19]. All affected parents (III:3, III:6, and III:8) in this study showed *P* gene mutations that were sufficient to explain the phenotype of OCA. An influence of *MC1R* on the expression of OCA could not be deduced from the clinical data although these cases were at least heterozygous for *MC1R*^{R160W}. Our consideration of combinations of *MC1R* mutations (G104S/R160W) as a modifier of *P* mutations (*P*^{C793F}/*P*^{V443I}) in III:8 was inconclusive. In addition III:6, who did not have the MC1R^{G104S} allele, was similarly affected as III:8. Therefore, an influence of *MC1R*^{G104S} on the albino phenotype cannot be deduced.

Finally, a variation in *MC1R* (*V60L*) was present in the compound heterozygous state with other *MC1R* mutations in three unaffected individuals (II:7, III:9, IV:6; Table 2). *MC1R*^{V60L} has been reported to be a minor allele in red hair color [9]. Its influence cannot be assessed sufficiently in this family.

MC1R is a highly polymorphous gene with variable influence of its various mutations on skin, hair, and even iris color [13]. This may explain the iris translucency in the descendants of cases in branch 1 (IV:1, IV:2, and IV:4) who inherited a single heterozygous *MC1R*^{R160W} mutation from their parents III:3 and III:6 against a single heterozygous *P* background. However, since IV:3 did not carry any *MC1R* mutation, this reasoning seems invalid; the iris translucency has to be attributed to either mutations in other genes or the general

Table 2. Available phenotypic and genotypic data of family members

Pedigree ID	Age at Diagnosis	Age at Examination	Mutations in P	Mutations in MC1R	Nystagmus	VA	Fundus	VEP	Iris color	Iris translucency	Remarks
II.1	na		N476S/+	+/+							
II.6	na	74 y.	C793F/+	R160W/+	x	OD: 0.8 OS: 0.8	Normal		light blue	x	
II.7	na			V60L/ G104S							
III.1	na		+/+	R160W/+							
III.2	at birth	died at 10 d due to infection			o						
III.3	1 y,	54 y.	N476S/ C793F	R160W/+	o	OD: 0.1 OS: 0.2				o	- very light hair at birth
III.6	at birth	47 y.	N476S/ C793F	R160W/+	o	OD: 0.16 OS: 0.2	hypopigmented no macular reflex	badly differentiated but in accordance with misrouting	pinkish	o	- very light hair and skin at birth - light skin easily burning - blond at examination
III.8	at birth	45 y.	C793F/ V443I	G104S/ R160W	o	OD: 0.3 OS: 0.25	hypopigmented no macular reflex	moderately differentiated but in accordance with misrouting	light brown/green	o	- very light hair and skin at birth - light skin easily burning - blond at examination
III.9	na	40 y.	+/+	V60L/ R160W							
III.10	at birth	died at 34 y. due to cardiac heart attack				markedly reduced vision					less light skin than other affected of the family
IV:1	na	30 y.	N476S/+	R160W/+	x	OD: 1.6 OS: 1.6	- lightly hypopigmented - normal macular reflex - OS: choroidal nevus		blue-green	o	- blond hair - easily burning
IV:2	na	28 y.	C793F/+	R160W/+	x	OD: 1.6 OS: 1.6	- hypopigmented periphery - normal macular reflex		light blue	o	- very blond hair - light skin
IV:3	na	27 y.	C793F/+	+/+	x	OD: 0.8 OS: 0.8	- hypopigmented periphery - normal macular reflex		- light blue	o	- dark blond hair - easily burning
IV:4	na	15 y.	N476S/+	R160W/+	x	OD: 1.0 OS: 1.0	- hypopigmented periphery - normal macular reflex		- light blue	o	- dark blond hair - light skin - easily burning - congenital hypothyreoidism
IV:5	na	25 y.	C793F/+	R160W/ R151C	x	OD: 1.25 OS: 1.25	- normal pigment - normal macular reflex		- blue-brown	x	- red-blond hair - light skin - tans easily
IV:6	na	21 y.	V443I/+	V60L/ G104S	x	OD: 1.0 OS: 1.0	- normal pigment - normal macular reflex		- green-brown	x	- Dark blond

This table summarizes the data on the individuals of pedigree 766 screened for mutations in *P* and *MCR1R*. The data were obtained by ophthalmological examinations and by interview of relatives. Abbreviations: OD represents right eye; OS represents left eye; na represents not affected. o present, x absent, ID represents Identification code; VA represents visual acuity, VEP represents visually evoked potentials.

Nordic phenotype of blond hair and sun sensitivity in these individuals, in combination with a mutation in the *P* gene.

In conclusion we examined an exceptional family with OCA in three branches. Although monogenic inheritance seemed unlikely in this family, we showed autosomal recessive inheritance in two branches by finding compound heterozygosity for mutations in the *P* gene that were sufficient to cause the affectation. We also identified several *MC1R* variations in the compound and single heterozygous state and on a compound and single heterozygous *P* background. We did not find a modifying effect on the OCA phenotype, but we independently identified some *MC1R* mutations as the cause of the red hair phenotype in one person.

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