

MUTATION IN BRIEF

Detection of 53 Novel DNA Variations Within the Tyrosinase Gene and Accumulation of Mutations in 17 Patients with Albinism

Sven Opitz^{1†}, Barbara Käsmann-Kellner^{2†}, Markus Kaufmann^{1†}, Eberhard Schwinger¹, and Christine Zühlke^{1*}

¹Institut für Humangenetik der Universität Lübeck, 23538 Lübeck, Germany; ²Augenklinik der Universität des Saarlandes, Homburg (Saar), Germany

*Correspondence to: Ch. Zühlke, Institut für Humangenetik, Ratzeburger Allee 160, Universität Lübeck D23538 Lübeck, Germany; E-mail: zuehlke@uni-luebeck.de

†These authors contributed equally to this work

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Oculocutaneous albinism (OCA) in man may be caused by mutations within the tyrosinase gene (TYR) resulting in OCA1. Analysing patients with recessively inherited albinism we found DNA variations in 82 unrelated individuals. 53 out of 78 mutations and polymorphisms revealed by this study are not published previously. The changes include 68 nucleotide substitutions resulting in amino acid changes, stop mutations and polymorphisms as well as four nucleotide insertions and six deletions. Furthermore, we found an accumulation of three to five mutations in 17 patients with OCA1. © 2004 Wiley-Liss, Inc.

KEY WORDS: albinism; tyrosinase gene; TYR; OCA; OCA1

INTRODUCTION

Human oculocutaneous albinism (OCA) is a clinically and genetically heterogeneous disorder that affects approximately 1 in 20,000 individuals. Defects in the melanin biosynthesis or transport result in little or missing pigment. To date, four OCA genes have been identified including the tyrosinase gene (TYR, MIM# 606933; OCA1, MIM# 203100), the P gene (OCA2, MIM# 203200), the gene for the tyrosinase related protein-1 (TYRP1; MIM# 115501; OCA3, MIM# 203290), and the MATP gene (MIM# 606202; OCA4, MIM# 606574).

Oculocutaneous albinism type 1 (OCA1) is inherited in an autosomal recessive trait and characterized by absence of pigment in hair, skin, and eyes. Severe nystagmus, photophobia, and reduced visual acuity are common features. This phenotype has been detected in all human races and numerous animal species.

Human OCA1 can be divided into two general phenotypes based on tyrosinase activity. Individuals with absence or low activity of tyrosinase, a 529-amino acid copper-binding protein that catalyses the initial conversion

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of tyrosine to dopaquinone, are born without pigmentation of the skin, hair and eyes at birth. Tyrosinase-negative OCA (OCA1A) is associated with complete loss of tyrosinase activity, missing melanin synthesis, and white hair, skin and blue eyes throughout life. In contrast, hair of individuals with OCA1B turns to yellow or blond with age. Pigmentation in these patients can vary between slight and approximate normal levels in adults (King et al., 1995).

The gene for tyrosinase has been mapped to the long arm of chromosome 11 at 11q14-q21 (Barton et al., 1988). The five exons of the gene span 50 kb (Giebel et al., 1991). Electronic mutation databases (Albinism Database and Human Gene Mutation Database, www.hgmd.org/ and www.cbc.umn.edu/tad/) list over 100 mutations and polymorphisms in the tyrosinase gene. The majority of mutations have been detected by the groups of Oetting & King (1992, 1993; Oetting et al., 1998; King et al., 2003) and Spritz (Fukai et al., 1995; Spritz et al., 1997; Park et al., 1997).

However, less is known concerning the number of sequence variations within a single individual. First cases with three mutations have been reported in German patients (Passmore et al., 1999).

In the study presented here, we looked for DNA variations - mutations and polymorphisms - in about 150 patients with OCA. Here, we report the results for 82 patients with one to six nucleotide changes in the tyrosinase alleles.

MATERIALS AND METHODS

After having obtained written informed consent for genetic analyses, total genomic DNA was isolated from proteinase K/SDS digest of blood samples. For PCR amplification of exons intronic and overlapping primers according Passmore et al., 1999 were used.

PCR was performed in a volume of 25 µl containing 50 ng genomic DNA, 10 pmol of each primer, 5 pmol dNTP and 0.5 units Taq polymerase (Ampligene). Products were separated by agarose (1.5%) gel electrophoresis and visualized by ethidium bromid staining.

To search for mutations, SSCP analyses were performed using exon-specific primers. Products were mixed with 1 volume formamide and heated for 5 min to 95°C followed by cooling on ice. Samples were resolved on 6% polyacrylamide gels containing 10% urea at 30 W and room temperature for 2 to 4 hours. The SSCP banding pattern was detected by silver staining.

PCR products were sequenced using the dideoxy chain termination method on double stranded DNA templates in presence of IRD800 labeled universal M13 primers on a Licor 4200 automated sequencer and compared to sequence GenBank NM_000372.2.

RESULTS

Molecular investigations of 163 patients with oculocutaneous albinism revealed changes, deletions and insertions of single nucleotides within the tyrosinase gene in 82 non-related individuals. Using SSCP and sequencing strategies, we found 191 DNA sequence variations in these patients. The variations include mutations as well as polymorphisms that do not change the encoded amino acid.

Table 1: Number of DNA Variations and Amino Acid Changes within the Tyrosinase Gene in Unrelated Individuals

DNA variations	Individuals	Amino acid changes	Individuals
1	27	1	27
2	26	2	38
3	14	3	10
4	7	4	5
> 4	8	5	2

In 33% of cases (27 persons) just one nucleotide change was detected, but in 29 persons three and more changes at the DNA level were present (Table 1). We could detect 78 specific differences at the DNA level (Table 2). The changes include 68 nucleotide substitutions resulting in amino acid changes, stop mutations and polymorphisms. Furthermore, four nucleotide insertions and six deletions were identified. Of the 78 DNA variations listed in Table 2, only a small part (25) was described previously. Fifty-three mutations and polymorphisms revealed in this study are not published to date.

Forty-nine of 78 DNA changes are unique in single individuals and can be denoted as “private” mutations. Two mutations will affect the splicing of the gene, nine will result in a frame shift during translation. A total of 21 mutated alleles in this study is affected by splice or frame shift mutations.

In 29 patients with albinism three to six DNA variations causing three to five amino acid changes (Table 3) were found. In addition to point mutations, insertion and deletions, 15 of these patients are carriers of DNA polymorphisms at codon 471 (c.1413G>A) and 482 (c.1446G>C). At both positions the amino acid alanine is present and not altered by the DNA variation. In total, the polymorphisms c.1413G>A + c.1446G>C were detected in 20 persons. In none of our samples polymorphism c.1413G>A without polymorphism c.1446G>C was found and vice versa. This linked polymorphism has a frequency of 0.12 (20 out of 164 alleles) in our sample collection. Furthermore, this polymorphism was shown for all patients carrying the frame shift mutation c.1467insT.

Four additional DNA polymorphisms that do not change the amino acid are marked in Table 2. Furthermore, eleven alleles have been found with the variation p.Glu398Ala and p.Ala416Ser. These variations may present high frequent mutation alleles or polymorphisms in the German population (11 out of 164 alleles = 6.7%).

Neglecting DNA polymorphisms that do not change the corresponding amino acid, we found two patients with five mutations, five patients with four mutations and ten patients with three mutations (Table 3). The frequency of multiple mutations in 20% of the patients with sequence variations within the tyrosinase gene (17 out of 82) is unexpected high.

Comparing clinical findings of patients with two mutations (38) to patients with three and more mutations (17) within the tyrosinase gene revealed no significant differences between both groups even though phenotypic variability between single patients is present.

Table 2: Identified Mutations and Polymorphisms

Alteration in cDNA	Alteration in protein	Reference	Alleles
c.73G>T	p.Val24Phe	Z	1
c.74_75insT	p.Ser26fsX2	King 03	1
c.96A>C	p.Thr29Pro	Z	1
c.130A>C	p.Ser44Arg	Z	1
c.130A>G	p.Ser44Gly	Z	1
c.139G>T	p.Gly47Val	Z	1
c.140G>A	p.Gly47Asp	King 03	1
c.204A>T	p.Gln68His	Z	1
c.230G>A	p.Arg77Gln	King 03	5
c.236C>T	p.Ser79Leu	Z	3
c.242C>T	p.Pro81Leu	King 03	1
c.344_345delGA	p.Arg115fsX52	King 03	1
c.346C>T	p.Arg116X	King 03	1

Alteration in cDNA	Alteration in protein	Reference	Alleles
c.463A>T	p.Thr155Ser	Z	1
c.529G>T	p.Val177Phe	Z	3
c.535A>T	p.Met179Leu	Z	1
c.538C>A	p.His180Asn	Z	2
c.595G>A	p.Asp199Asn	Z	1
c.601G>T	p.Ala201Ser	Z	1
c.601delG	p.Ala201fsX24	Z	1
c.649_650CG>TC	p.Arg217Ser	Z	1
c.707G>T	p.Trp236Leu	Z	1
c.719A>T	p.Asp240Val	Z	1
c.728A>C	p.Lys243Thr	Z	1
c.766C>T	p.His256Tyr	Camand 01	1
c.813G>A	p.Asp271Asn	Z	1
c.820-2 delA		Z	2 ^{*1)}
c.842delA	p.Glu281fsX37	Z	1
c.865T>C	p.Cys289Arg	Oetting 98	1
c.953T>A	p.Val318Glu	Z	1
c.985T>C	p.Ser329Pro	Z	4
c.995T>C	p.Met332Thr	King 03	1
c.1034A>G	p.Glu345Gly	Z	1
c.1063G>C	p.Ala355Pro	Z	2
c.1075C>T	p.Gly359X	Spritz 97	1
c.1087C>T	p.His363Thr	Z	1
c.1118C>A	p.Thr373Lys	King 03	6
c.1132C>A	p.Gln378Lys	Z	2
c.1147G>A	p.Asp383Asn	King 03	1
c.1164delT	p.His389fsX95	Tripathi 92	2
c.1167_1168insT	p.His390fsX4	Z	1
c.1177G>T	p.Val393Phe	Oetting 94	2
c.1180delG	p.Asp394fsX90	Z	1
c.1183A>C	p.Ser395Arg	Z	2
c.1184 +3T>G		Z	1 ^{*2)}
c.1194A>T	p.Glu398Val	Z	2
c.1194A>C	p.Glu398Ala	Z	11 ^{*3)}
c.1204C>T	p.Arg402X	King 03	1
c.1205G>T	p.Arg402Leu	Z	1
c.1205G>A	p.Arg402Gln	Morell 97	3

Alteration in cDNA	Alteration in protein	Reference	Alleles
c.1209G>C	p.Arg403Ser	Tripathi 92	1
c.1209G>T	p.Arg403Ser	Tripathi 92	1
c.1210C>A	p.His404Asn	Z	2
c.1214G>T	p.Arg405Leu	Z	7
c.1217C>T	p.Pro406Leu	King 03	1
c.1224A>C	p.Gln408His	Z	2
c.1227A>C	p.Glu409Asp	Z	1
c.1237G>T	p.Glu413X	Z	1
c.1246G>T	p.Ala416Ser	Z	11 ^{*3)}
c.1250C>A	p.Pro417His	Z	1
c.1255G>A	p.Gly419Arg	King 03	1
c.1265G>A	p.Arg422Gln	Giebel 91b	2
c.1271C>T	p.Ser424Phe	Z	1
c.1265T>A	p.Met426Lys	Z	2
c.1280T>G	p.Val427Gly	Z	2
c.1302G>T	p.Arg434Ile	Z	1
c.1303A>G	p.Asn435Asp	Z	1
c.1331A>G	p.Asp444Gly	Z	6
c.1342G>A	p.Asp448Asn	King 03	1
c.1467_1468insT	p.Ala490fsX19	King 03	7
c.1501_1502insC	p.Arg501fsX8	Giebel 91	3

Polymorphisms: predicted protein change (codon affected)			
c.575C>A	p.Ser192Tyr	*4)	4
c.1137A>T	(p.Gly379)	Z	1
c.1206A>C	(p.Arg402)	Z	1
c.1338C>G	(p.Gly446)	Z	1
c.1368A>T	(p.Gly456)	Z	3
c.1413G>A	(p.Ala471)	Z	20
c.1446G>C	(p.Ala482)	Z	20

Nucleotide +1 is the A of the ATG translation initiation codon, GenBank NM_000372.2.

¹ Splice site mutation.

² Splice site mutated in 1 family with 2 affected children.

³ Frequency may direct to polymorphism.

⁴ Polymorphism according to Giebel & Spritz, 1990.

Z: not previously published.

References: First author et al. 19xx, 20xx

Table 3: Amino Acid Changes in Patients with Albinism and Three to Five DNA Variations within the Tyrosinase Gene

Sample	Amino acid changes				
397 (5M)	p.A201fsX24	p.A355P	p.P406L	p.S424F	p.N435D
504 (5M)	p.D394fsX90	p.V393F	p.E398A	p.R402X	p.R403S
405 (4M)	p.G47D	p.S192Y	p.H256Y	p.D271N	
484 (4M)	p.H180N	p.S192Y	p.D199N	p.E398V	
509 (4M)	p.A490fsX19	p.R501fsX8	p.T373K	p.Q378K	
528 (4M)	p.R77Q	p.P81L	p.E398A	p.D444G	
631 (4M)	p.A490fsX19	p.R501fsX8	p.E398A	p.R405L	
424 (3M)	p.W236L	p.C289R	p.D383N		
406 (3M)	p.H390fsX4	p.R501fsX8	p.R77Q		
448 (3M)	p.S44R	p.S395R	p.R405L		
551 (3M)	p.T29P	p.S44G	p.S79L		
412 (3M)	p.S79L	p.S395R	p.R405L		
417 (3M)	p.R116X	p.E398A	p.D444G		
497 (3M)	p.V24F	p.G47V	p.M332T		
534 (3M)	p.V177F	p.H180N	p.R217S		
535 (3M)	p.V177F	p.A201S	p.E281fsX37		
550 (3M)	p.G68K	p.R77Q	p.S79L		

(..M) describes the number of mutations

DISCUSSION

In this study, we performed a mutation analysis on patients with albinism. Here, we present a wide spectrum of mutations and DNA polymorphisms within the tyrosinase gene. We found 78 different sequence variations in 82 unrelated individuals representing app. 50% of the samples. For 55 patients (34%) two to five mutations were detected. The description of 53 unknown mutations and DNA polymorphisms expands the albinism database significantly and represents the broadest spectrum published so far.

One of the most important results of this study is the identification of patients with up to five mutations (Table 3). Regarding the missense mutations, it is not possible to differentiate disease-causing mutations from protein polymorphisms which - for example - are correlated with skin colors within the "normal" range. Extensive molecular DNA analyses with control populations would be necessary to answer this question. However, the slightly more severe phenotype in some individuals with multiple changes in the tyrosinase cDNA sequence in comparison to other patients with OCA1 points to a possible accumulation of mutation effects.

Furthermore, the linkage of specific mutations e.g. c.1467insT with questionable polymorphisms as c.1413G>A + c.1446G>C should be investigated. Unfortunately, no DNA of the parents is available to determine the segregation of tyrosinase DNA variations.

For 27 individuals with oculocutaneous albinism, alterations have been detected in only one allele of the tyrosinase gene. Mutations within the P gene associated with OCA2 as well as within the MATP gene associated with OCA4 have been excluded for 20 of these patients by molecular analyses. Two of these 20 individuals are heterozygous for the frequent amino acid variation at codon 398. Possibly, this change is a polymorphism that is not correlated with OCA1.

For the remaining 18 patients it seems likely that the second mutation was not detected by the used SSCP conditions or occurring in gene regions not presented in the PCR products. E.g. the promoter and extended intronic parts have not been investigated. For OCA4 it is known that mutations in the splice-acceptor sequences are associated with the disease phenotype (Newton et al., 2001). This points to disease-causing DNA changes localized in untranslated regions. In addition, deletions or duplications of the complete gene or single exons are not seen by the SSCP procedure but may be important in mutational analyses (Hedrich et al., 2001).

On the other hand, it is not excluded that these patients are affected due to mutations in further genes involved in pigmentation. In fact, one of the patients is homozygous for a mutation within the OCA4 gene (Rundshagen et al., 2004).

Interestingly, heterozygous mutations in the P gene have been found in six patients. Therefore, pathologic effects of heterozygous mutations in non-allelic genes should be discussed. Recently, in cases of ocular albinism and Waardenburg syndrome digenic mutation types have been described (Morell et al., 1997; Ming & Muenke, 2002). Thus it seems quite imaginable that heterozygous mutations in non-allelic genes encoding proteins or enzymes involved in pigment synthesis and transport might produce an additional negative effect resulting in OCA phenotypes.

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