A tyrosinase gene missense mutation in temperature-sensitive type I oculocutaneous albinism. A human homologue to the Siamese cat and the Himalayan mouse.

L B Giebel, … , R A King, R A Spritz

J Clin Invest. 1991;87(3):1119-1122. https://doi.org/10.1172/JCI115075.

Research Article

Type I oculocutaneous albinism (OCA) is an autosomal recessive disorder in which deficient synthesis of melanin pigment results from abnormal activity of melanocyte tyrosinase. A novel type I OCA phenotype in which hypopigmentation is related to local body temperature is associated with a missense substitution in tyrosinase, codon 422 CGG (Arg)----CAG (Gln). This substitution results in a tyrosinase polypeptide that is temperature-sensitive. This form of type I OCA thus is homologous to the temperature-related forms of albinism seen in the Siamese cat and the Himalayan mouse.

A Tyrosinase Gene Missense Mutation in Temperature-sensitive Type I Oculocutaneous Albinism

A Human Homologue to the Siamese Cat and the Himalayan Mouse

Lutz B. Giebel,* Ram K. Tripathi,* Richard A. King,‡ and Richard A. Spritz*§

*Departments of *Medical Genetics and §Pediatrics, Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706; and ‡Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota 55455*

Abstract

Type I oculocutaneous albinism (OCA) is an autosomal recessive disorder in which deficient synthesis of melanin pigment results from abnormal activity of melanocyte tyrosinase. A novel type I OCA phenotype in which hypopigmentation is related to local body temperature is associated with a missense substitution in tyrosinase, codon 422 CGG (Arg) → CAG (Gln). This substitution results in a tyrosinase polypeptide that is temperature-sensitive. This form of type I OCA thus is homologous to the temperature-related forms of albinism seen in the Siamese cat and the Himalayan mouse. (*J. Clin. Invest.* 1991. 87:1119–1122.) Key words: albino • pigmentation • pigment • disorders • melanin • PCR

Introduction

Oculocutaneous albinism (OCA)[1] is a group of severe autosomal recessive disorders of pigmentation characterized by reduced or absent melanin in pigment cells of the skin, hair follicle, and eye (reviewed in references 1, 2). Type I OCA results from deficient activity of melanocyte tyrosinase (monophenol, L-dopa:oxygen oxidoreductase, EC 1.14.18.1), a copper-containing enzyme that catalyzes the first two steps in the melanin biosynthetic pathway: the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) and the subsequent oxidation of dopa to dopaquinone. Recently, several mutations of the tyrosinase gene have been identified in patients with typical type I OCA (3–7).

We have described a novel type I OCA phenotype in which the distribution of melanin is correlated with local body temperature. Tyrosinase extracted from scalp and leg hairbulbs of the proband appeared to be thermosensitive, with significant loss of activity at temperatures above 35°C (8). In this report we describe a missense substitution at codon 422 of the tyrosinase gene in this individual, and show that this mutation is specifically associated with the peripheral pigmentation phenotype in her family. Introduction of the codon 422 substitution into normal human tyrosinase cDNA results in thermosensitivity of the tyrosinase enzymatic activity expressed in transfected HeLa cells. These findings demonstrate that the codon 422 mutation is associated with a temperature-sensitive form of tyrosinase. The phenotype of temperature-related albino pigmentation in this family thus appears to be a human homologue to that in other mammals with apparent temperature-sensitive forms of tyrosinase, including the Siamese cat and the Himalayan mouse and rabbit (reviewed in 9, 10).

Methods

Clinical summary. The proband (individual II.1 in Fig. 1), an adult Caucasian female, is a member of an extensive kindred with type I OCA, family 2 in reference 4. The proband is patient XI in that report (4), which describes both genetic linkage between type I OCA and the tyrosinase gene and a frequent tyrosinase gene substitution (codon 81 Pro → Leu) that causes classic type IA (tyrosinase-negative) OCA in several other members of this kindred. The current proband and her two affected brothers (Fig. 1) completely lacked melanin pigment at birth and initially appeared to have classic tyrosinase-negative OCA. After puberty, however, they developed a unique pattern of pigmentation: relatively warm body parts (eyes, skin, hair of the scalp and axilla) remained unpigmented, but less warm parts (facial and pubic hair) developed slight pigmentation and relatively cool parts (arm and leg hair) became well pigmented (8).

Polymerase chain reaction (PCR) and DNA sequence determination. Genomic DNA was isolated from peripheral blood leukocytes (11) of the proband and family members shown in Fig. 1. PCR primers designed to amplify each of the five exons of the tyrosinase gene plus adjacent flanking noncoding sequences were derived from the normal human tyrosinase genomic DNA sequence (12, 13). Each exon was amplified independently from 0.1 μg of genomic DNA of the proband by 40 cycles of PCR (14) using 20-mer oligonucleotide primers P1-P10 (Table I) and Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) exactly as described (7). Each cycle consisted of 30 s at 94°C, 1 min at 50°C, and 2 min at 72°C. The amplified PCR products were purified by electrophoresis in 4% polyacrylamide gels and cloned in bacteriophage vectors M13mp18 or mp19. The nucleotide sequences of at least six independent clones per exon were determined (15).

Restriction enzyme cleavage analysis. Fragments containing a part of the tyrosinase gene exon 1 and the complete exon 4 were amplified by PCR as described above from 0.1 μg of genomic DNA from all family members shown in Fig. 1. Exon 1 fragments were amplifed using primer pair P11-P12, and exon 4 fragments were amplified using primer pair P7-P8 in Table I. Exon 1 PCR amplification products were then digested with Hae III and exon 4 PCR products with Msp I and the

Address reprint requests to Richard A. Spritz, M.D., 309 Laboratory of Genetics, 445 Henry Mall, University of Wisconsin, Madison, WI 53706.

Received for publication 28 September 1990.

1. *Abbreviations used in this paper:* OCA, oculocutaneous albinism; PCR, polymerase chain reaction.

J. Clin. Invest.

0021-9738/91/03/1119/04 $2.00
Volume 87, March 1991, 1119–1122

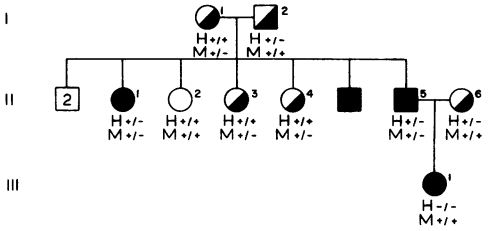

Figure 1. Pedigree of the study family. The proband, individual II.1, is the same as patient XI in family 2 of reference 4. Results of exon 1 HaeIII (H) and exon 4 MspI (M) cleavage analyses of the codon 81 and codon 422 mutations, respectively, are indicated. (+) Site present; (−) site absent.

digests were analyzed by gel electrophoresis for the presence of the codon 81 and codon 422 mutations, respectively.

In vitro mutagenesis. The codon 422 CGG (Arg) → CAG (Gln) mutation was introduced into the human tyrosinase cDNA expression plasmid pcTYR (16) as described (17). Exon 4 of the tyrosinase gene was PCR amplified from DNA of an M13 exon 4 clone containing the codon 422 mutation using oligonucleotide primer pair P13-P14 (Table I), derived from the 5′ and 3′ ends of exon 4, respectively. An exon 2-3-4 product was then amplified from 30 ng of tyrosinase cDNA using as one primer 5 pmol of the purified mutant exon 4 PCR product and as second primer an oligonucleotide P15 (Table I), derived from the 5′ end of exon 2. The resultant mutant exon 2-3-4 PCR product contained the codon 422 mutation and unique Pvu II and Bgl II sites. These sites were used to replace the Pvu II/Bgl II fragment of pcTYR with the corresponding codon 422 mutant fragment. The DNA sequence of the region spanning the Pvu II/Bgl II fragment in the resultant plasmid pcTYR-A422 was confirmed by double-strand DNA sequence analysis (18).

Transfection of HeLa cells and assay of tyrosinase. pcTYR (16) or pcTYR-A422 plus the chloramphenicol acetyltransferase expression plasmid pSV2CAT (19) were transiently transfected into cultured HeLa cells by the Ca-phosphate precipitation procedure (19). Cells were harvested 64 h after transfection, lysed in 20 mM Na-phosphate buffer, pH 6.8, and aliquots of the cell extracts were assayed for tyrosinase (tyrosine hydroxylase; 20, 21) and CAT (19) activities and protein concentrations (22).

Fluorometric assay of tyrosinase activity was as described (20) except that 4 mM ascorbic acid was included in the reaction mixture as reductant for tyrosine hydroxylation (21) and to reduce any dopaquinone formed by tyrosinase back to dopa; measurement of dopa therefore provided a true estimate of the tyrosine hydroxylase activity of tyrosinase. The 55 μl reactions containing 0.1 mM L-tyrosine, 5 μM L-dopa, 4 mM ascorbic acid, 40 mM Na-phosphate buffer, pH 6.8, and 15 μl cell extract were incubated for 4 h. Production of dopa was measured by specific fluorescence at 360 nm excitation and 490 nm emission wavelengths. One unit of tyrosinase activity was defined as 1 pmol dopa formed/min. The tyrosinase specific activities shown in Table II were corrected for slight plate to plate variation in transfection efficiency by dividing by the CAT activity (percent ^{14}C-chloramphenicol converted to the three forms of acetyl-chloramphenicol; reference 19) in each extract.

Tyrosinase enzymatic activities were determined at both 31 and 37°C. Activity of the codon 422 mutant tyrosinase, produced in HeLa cells transfected and cultured at 31°C, was not decreased by assay at 37°C versus assay at 31°C (data not shown). Because the optimal temperature of the tyrosinase assay is 37°C (20), only data for tyrosinase assays at that temperature are shown in Table II.

Results

Identification of the tyrosinase gene codon 422 mutation. To define the molecular basis of the temperature-related type I OCA phenotype, we sequenced PCR fragments amplified from the tyrosinase genes of the proband. The proband was homozygous at two nonpathologic polymorphisms; codon 192 TAT (Tyr; reference 23) and 402 CGA (Arg; reference 24). However, nucleotide sequence analyses also detected two pathologic missense substitutions in her tyrosinase coding regions. One (data not shown) was the previously-described CCT (Pro) → CTT (Leu) substitution at codon 81 (in exon 1) that is a frequent tyrosinase-negative (type IA) OCA allele in this kindred and in the Caucasian population (4). The other (Fig. 2) was a novel substitution within codon 422 (in exon 4), CGG (Arg) → CAG (Gln). The codon 422 mutation is specifically associated with these patients' temperature-related pattern of pigmentation.

The codon 81 and codon 422 substitutions both abolish restriction sites; the codon 81 mutation destroys an Hae III site

Table I. Oligonucleotide Primers used for PCR Amplification of Tyrosinase Gene Exons

Primer	Sequence	Position*	Segment amplified
P1	5′-GCTCTTTAACGTGAGATATC-3′	−40–−21‡	Exon 1 plus adjacent regions and promoter
P2	5′-TTATACCCTGCCTGAAGAAG-3′	1543–1562§	
P3	5′-CTCAGGAGAAGTCTAACAAC-3′	1644–1663	Exon 2 plus adjacent regions
P4	5′-AACTCAGAAATTCTGAATTC-3′	2049–2068§	
P5	5′-GAGTCTCAATACGGAATGAA-3′	2153–2172	Exon 3 plus adjacent regions
P6	5′-TTTAAATCCAATGAGCACGT-3′	2470–2489§	
P7	5′-TTAATATATGCCTTATTTTA-3′	2514–2533	Exon 4 plus adjacent regions
P8	5′-TAAAGTTTTGTGTTATCTCA-3′	2842–2861§	
P9	5′-CTCCAAAGGACTGTGAAAGG-3′	2963–2982	Exon 5 plus adjacent regions
P10	5′-GGAGTCAGTTAATGTAGATT-3′	3661–3680§	
P11	5′-TGCTCCTGGCTGTTTTGTAC-3′	673–692	5′ portion of exon 1
P12	5′-TATGGGGATGACATAGTCTGAGCTG-3′	1106–1130§	
P13	5′-TATTTTTGAGCAGTGGCTCC-3′	2605–2624	Exon 4
P14	5′-CTGAATCTTGTAGATAGCTA-3′	2767–2786§	
P15	5′-ATTGTCTGTAGCCGATTGGA-3′	1750–1769	

* Nucleotide positions refer to the sequence in reference 13. ‡ This primer was derived from the sequence in reference 12; its position is enumerated relative to the sequence in reference 13. § Primer sequence is complementary to that in reference 13.

Table II. Activity of Normal versus Codon 422 Arg → Gln Mutant Tyrosinase in Transfected HeLa Cells

	Tyrosinase specific activity*	
	Temperature of transfection and culture	
	31°C	37°C
pcTYR	5.7±0.6 (100%)	5.6±0.3 (100%)
pcTYR-A422	1.6±0.06 (28%)	0.08±0.01 (1.4%)

HeLa cells were transfected and cultured at either 31°C or 37°C with an expression vector containing either the normal (pcTYR) or codon 422 Arg → Gln mutant (pcTYR-A422) tyrosinase cDNAs plus pSV2CAT. Cells were harvested 64 h after transfection, and cell extracts were assayed for tyrosinase (tyrosine hydroxylase) activity at 37°C.

* Tyrosinase specific activities are expressed as picomoles dopa formed per minute per milligram of protein. The values shown were corrected for slight variation in transfection efficiency by dividing by the CAT activity in each plate, which was not affected by the temperature of transfection and culture. Each experiment was performed in triplicate.

(4) and the codon 422 mutation an Msp I site. To determine whether the codon 81 and codon 422 substitutions are inherited on different tyrosinase alleles, we amplified PCR products containing exon 1 and exon 4 from genomic DNAs of members of the proband's family, digested then with Hae III or Msp I, respectively, and analyzed the products by gel electrophoresis for the presence of the mutations (data not shown). As shown in Fig. 1 the proband's mother (individual I.1) carries the codon 422 mutation and her father (I.2) carries the codon 81 mutation. Both of the sibs with temperature-related type I OCA that we studied (II.1 and II.5) are compound heterozygotes for both the codon 81 and codon 422 mutant alleles. Individual III.1, the daughter of one of the proband's affected brothers, by chance is affected with classic type IA (tyrosinase-negative) OCA. She is homozygous for the common codon 81 mutation, although her two codon 81 mutant alleles are associated with different RFLP haplotypes (see reference 4), consistent with the lack of parental consanguinity.

The codon 422 substitution results in thermosensitivity of the resultant tyrosinase. To specifically define the effect of the codon 422 Arg → Glu substitution on tyrosinase thermostability we introduced this mutation into the normal human tyrosinase cDNA expression vector pcTYR (16) by site-directed mutagenesis (17). The normal, pcTYR, and the mutant, pcTYR-A422, tyrosinase expression plasmids were each transiently cotransfected along with pSV2CAT into cultured HeLa cells, grown for 64 h at either 31 or 37°C, and cell extracts were prepared and assayed for tyrosinase (tyrosine hydroxylase; 20, 21) and chloramphenicol acetyltransferase (CAT; 19) activities. As shown in Table II activity of the normal tyrosinase enzyme was essentially identical in extracts of HeLa cells transfected and cultured for 64 h at either 31 or 37°C. However, activity of the codon 422 mutant tyrosinase was only 28% that of the normal enzyme in cells transfected and cultured at 31°C and this activity was reduced to only 1.4% of the normal in cells transfected and cultured at 37°C. These data demonstrate that the arginine to glutamine substitution at codon 422 results in a temperature-sensitive tyrosinase polypeptide whose enzymatic activity is reduced even at 31°C and is almost abolished at 37°C.

Discussion

Type I oculocutaneous albinism is an heterogeneous group of disorders in which deficient enzymatic activity of tyrosinase, with consequent greatly reduced or absent biosynthesis of melanin pigment, results from homozygosity or compound heterozygosity for a series of mutant alleles of the tyrosinase gene. The temperature-related type I OCA phenotype of the probands described here is caused by compound heterozygosity for a frequent tyrosinase-negative ("null") codon 81 mutant allele and a novel codon 422 mutant allele. Transfection of a codon 422 mutant tyrosinase cDNA into HeLa cells confirms that this substitution indeed results in a functional tyrosinase enzyme that is temperature-sensitive.

Temperature-sensitive mutant tyrosinases most likely account for similar temperature-related albino pigmentation phenotypes in the Siamese cat (reviewed in reference 9) and the Himalayan rabbit and mouse (reviewed in 9, 10). Defective glycosylation (25) or the presence of an abnormal inhibitor (26) of the tyrosinase polypeptide in pigment cells have been proposed as alternative explanations for this phenomenon. However, our demonstration of a thermosensitive tyrosinase in HeLa cells transfected with the codon 422 mutant cDNA indicates that this substitution directly interferes with thermal stability of the tyrosinase polypeptide. Moreover, a tyrosinase gene missense substitution has recently been observed in the Himalayan mouse (27) at codon 420 (His → Arg), only two amino acids away from the human codon 422 substitution described here. These two human and mouse tyrosinase substitutions both occur within a very highly conserved segment of the protein, and it is very likely that they result in analogous defects in structural stability of the corresponding tyrosinase polypeptides.

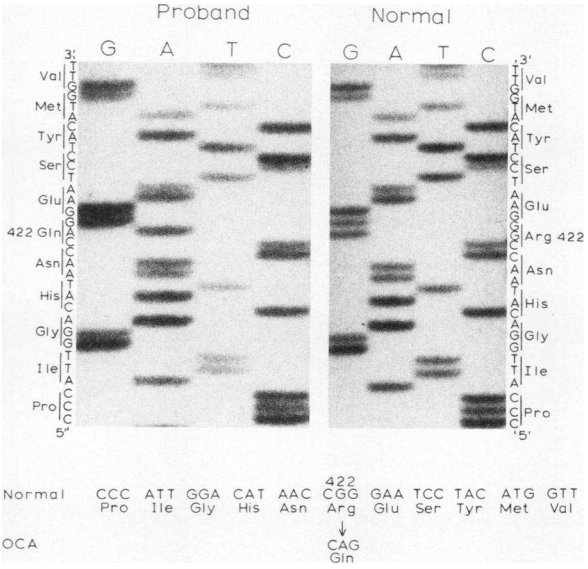

Figure 2. Sequences in the region of the codon 422 mutation in the proband and a normal individual. The sequence of the coding strand is indicated.

Acknowledgments

We thank Dr. B. Bouchard for the tyrosinase expression plasmid pcTYR.

This work was supported by Clinical Research Grant 6-408 of the March of Dimes Birth Defect Foundation and Grant AR-39892 of the National Institutes of Health. This is paper number 3174 from the Laboratory of Genetics, University of Wisconsin.

References

1. King, R. A., and C. G. Summers. 1988. Albinism. *Dermatol. Clin.* 6:217–228.

2. Witkop, C. J., Jr., T. B.. Fitzpatrick, and R. A. King. 1989. Albinism. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Book Co., New York. 2905–2947.

3. Tomita, Y., A. Takeda, S. Okinaga, H. Tagami, and S. Shibahara. 1989. Human oculocutaneous albinism cause by a single base insertion in the tyrosinase gene. *Biochem. Biophys. Res. Commun.* 164:990–996.

4. Giebel, L. B., K. M. Strunk, R. A. King, J. M. Hanifin, and R. A. Spritz. 1990. A frequent tyrosinase gene mutation in classic, tyrosinase-negative (type IA) oculocutaneous albinism. *Proc. Natl. Acad. Sci. USA.* 87:3255–3258.

5. Kikuchi, H., S. Hara, S. Ishiguro, M. Tamai, and M. Watanabe. 1990. Detection of point mutation in the tyrosinase gene of a Japanese albino patient by direct sequencing of amplified DNA. *Hum. Genet.* 85:123–124.

6. Spritz, R. A., K. M. Strunk, L. B. Giebel, and R. A. King. 1990. Detection of mutations in the tyrosinase gene in a patient with type IA oculocutaneous albinism. *N. Engl. J. Med.* 322:1724–1728.

7. Spritz, R. A., K. M. Strunk, C.-L. Hsieh, G. Sekhon, and U. Francke. 1991. Homozygous tyrosinase gene mutation in an American Black with tyrosinase-negative (type IA) oculocutaneous albinsim. *Am. J. Hum. Genet.* 48:318–324.

8. King, R. A., D. W. Townsend, W. Oetting, C. G. Summers, D. P. Olds, J. G. White, and R. A. Spritz. 1991. Temperature sensitive tyrosinase associated with peripheral pigmentation in oculocutaneous albinism. *J. Clin. Invest.* In press.

9. Searle, A. G. 1968. Comparative Genetics of Coat Colour in Mammals. Academic Press, Inc., New York.

10. Silvers, W. K. 1972. The Coat Colors of Mice. Springer-Verlag, New York.

11. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

12. Kikuchi, H., H. Miura, H. Yamamoto, T. Takeuchi, and T. Dei. 1989. Characteristic sequences in the upstream region of the human tyrosinase gene. *Biochim. Biophys. Acta.* 1009:283–286.

13. Giebel, L. B., K. M. Strunk, and R. A. Spritz. 1991. Organization and nucleotide sequences of the human tyrosinase gene and a truncated tyrosinase pseudogene. Genomics. 9:435–445.

14. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Ehrlich. 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC).* 239:487–491.

15. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain termination inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

16. Bouchard, B., B. B. Fuller, S. Vijayasaradhi, and A. N. Houghton. 1989. Induction of pigmentation in mouse fibroblasts by expression of human tyrosinase cDNA. *J. Exp. Med.* 169:2029–2042.

17. Giebel, L. B., and R. A. Spritz. 1990. Site-directed mutagenesis using a double-stranded DNA fragment as a PCR primer. *Nucleic Acids Res.* 18:4947.

18. Zhang, H., R. Scholl, J. Browse, and C. Somerville. 1988. Double stranded DNA sequencing as a choice for DNA sequencing. *Nucleic Acids Res.* 16:1220.

19. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyl-transferase in mammalian cells. *Mol. Cell. Biol.* 2:1044–1051.

20. Husain, I., E. Vijayan, A. Ramaiah, J. S. Pasricha, and N. C. Madan. 1982. Demonstration of tyrosinase in the vitiligo skin of human beings by a sensitive fluorometric method as well as ^{14}C(U)-L-tyrosine incorporation into melanin. *J. Invest. Dermatol.* 78:243–252.

21.Tripathi, R. K., C. Chaya Devi. and A. Ramaiah. 1988. pH-dependent interconversion of two forms of tyrosinase in human skin. *Biochem. J.* 252:481–487.

22. Peterson, G. L. 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Anal. Biochem.* 83:346–356.

23. Giebel, L. B., and R. A. Spritz. 1990. RFLP for MboI in the human tyrosinase (TYR) gene detected by PCR. *Nucleic Acids Res.* 18:3103.

24. Spritz, R. A., L. B. Giebel, R. K. Tripathi, and K. M. Strunk. 1990. Structure and polymorphisms of the human tyrosinase gene and a truncated tyrosinase pseudogene. *Am. J. Hum. Genet.* 47:A117.

25. Halaban, R., G. Moellmann, A. Tamura, B. S. Kwon, E. Kuklinska, S. H. Pomerantz, and A. B. Lerner. 1988. Tyrosinases of murine melanocytes with mutations at the albino locus. *Proc. Natl. Acad. Sci. USA.* 85:7241–7245.

26. Kidson, S. H., and B. C. Fabian. 1981. The effect of temperature on tyrosinase activity in Himalayan mouse skin. *J. Exp. Zool.* 215:91–97.

27. Kwon, B. S., R. Halaban, and C. Chintamaneni. 1989. Molecular basis of mouse Himalayan mutation. *Biochem. Biophys. Res. Commun.* 161:252–260.