Hereditary cataract of the Nakano mouse: Involvement of a hypomorphic mutation in the coproporphyrinogen oxidase gene

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ABSTRACT

The Nakano cataract (NCT) is a recessive disorder in the mouse linked to the *nct* locus on chromosome 16. In this study, we positionally cloned the critical gene in the *nct* locus. Herein, we report that cataracts in the BALB/c-*nct*/*nct* mouse are caused by a hypomorphic mutation in the coproporphyrin oxidase gene (*Cpox*), encoding the enzyme responsible for catalyzing oxidative decarboxylation of the heme precursor, coproporphyrinogen III, in the heme biosynthetic pathway. BALB/c-*nct*/*nct* mice are homozygous for a G to T nucleotide substitution in the *Cpox* gene, which results in a p.R380L amino acid substitution in the CPOX protein. The CPOX isoform with the p.R380L substitution retained only 15% of the activity of the wild type isoform. BALB/c-*nct*/*nct* mice had excessive accumulation of coproporphyrin III in the lens. The NCT phenotype was normalized by the introduction of a wild type *Cpox* transgene. The mechanisms by which impairment of CPOX leads to lens opacity in the NCT are elusive. However, our data illuminate a hitherto unanticipated involvement of the heme biosynthesis pathway in lens physiology.

Key Words: *cataract; mouse model; gene cloning; mutation; coproporphyrin*

1. Introduction

The Nakano cataract (NCT) is a mutant mouse model for hereditary cataract, first described by Nakano et al. (1960). Mutant mice develop cataracts at around 30 days of age as an autosomal recessive trait governed by a single locus, referred to as *nct* (Nakano et al., 1960; Takehana et al., 1990). A congenic strain in which the *nct* locus was introduced into the background of an inbred BALB/c strain (BALB/c-*nct*/*nct*) was bred to fix the genetic background and establish an authentic control (Matsuzawa and Wada, 1988; Wada et al., 1991; Koyama-Ito and Wada, 1992). Compared to mice from the original Nakano model, BALB/c-*nct*/*nct* congenic mice exhibit differences in cataract development. For example, Nakano mice develop intense nuclear cataracts, whereas congenic mice have a milder cortical form of cataract. In addition, the age at cataract onset is considerably delayed in congenic mice compared to Nakano mice.

The initial morphological change observed in Nakano mice is delayed denucleation of lens cells (Hamai et al., 1974). This is followed by the degeneration of lens epithelial cells, subsequent lens fiber swelling, and posterior sutural separation, which are thought to induce opacity in the perinuclear zone (Hara et al., 1999). An extension of these changes into the surrounding cortex finally leads to the establishment of a mature cataract. Early biochemical studies of the opaque lens of Nakano mice revealed decreases in glutathione levels, deficiencies in Na^+,K^+-ATPase, changes in ion cation contents, and increases in insoluble proteins (Takehana, 1990).

Despite what is known about cataract-associated morphological and biochemical changes, the culprit genetic defect underlying cataractogenesis in Nakano mice has not yet been determined. Elucidation of the *nct* gene would help establish the sequence of biochemical, cellular, developmental, and physiological events that lead from lens epithelial cell damage to

cataract formation. In the present study, we attempted positional cloning of the *nct* gene based on the prior mapping results of the *nct* on chromosome 16 (Hiai et al., 1998; Narita et al., 2002).

2. Materials and Methods

2.1. Mice

Breeding pairs of a congenic BALB/c-*nct*/*nct* mouse strain, in which the mutant *nct* locus for cataract was introduced onto the background of a BALB/c strain (Matsuzawa and Wada, 1988), were obtained from RIKEN BRC through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan (RBRC00422). The congenic mice were subsequently bred by brother × sister mating at the Institute of Experimental Animals, Shinshu University. The BALB/c mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and used as controls. These mice were maintained under clean conventional conditions and had free access to a commercial diet (MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water. Under these conditions, BALB/c-*nct*/*nct* mice reproducibly developed cataract by the age of 10 weeks. All experimental procedures involving mice were carried out in accordance with the Regulations for Animal Experimentation of Shinshu University.

2.2. Mutation survey of the nct *candidate genes*

Messenger RNA (mRNA) was extracted from the lenses of mice using the QuickPrep Micro mRNA Purification Kit (Amersham Biosciences, Buckinghamshire, England). First-strand complementary DNA (cDNA) was synthesized using a First-Strand cDNA Synthesis Kit (Amersham Biosciences) and was then subjected to PCR amplification. Primers

for PCR amplification of cDNA of 9 target genes were designed on the basis of nucleotide sequences retrieved from the GenBank database (Table 1). PCR amplification was performed using TaKaRa LA *Taq* DNA polymerase (TaKaRa BIO, Otsu, Japan) in 25-μl reactions following the manufacturer's instructions with minor modifications. The PCR reactions were carried out under the following conditions: initial denaturation at 94˚C for 1 min, followed by 35 cycles at 94˚C for 20 s, 50˚C for 15 s, and 72˚C for 2 min. PCR products were purified using the UltraClean PCR Clean Up Kit (MO BIO Laboratories, Carlsbad, USA) and sequenced using the BigDye Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems Japan, Tokyo, Japan) on an ABI310 PRISM DNA Analyzer (Applied Biosystems Japan). Complete nucleotide sequences of the PCR products were determined by sequence walking.

Nucleotide alterations in *Cpox* exon 5 and *Crybg3* exon 7 were corroborated by PCR-amplification and direct sequencing of the target exons by using the following primer pairs: Cpox-exon5F (5′-AAAGTCTGGCTAGAGTCCTC-3′) and Cpox-exon5R (5′-GAGCTGTGTAGACTGTTCAC-3′), and Crybg3-exon7F (5′-CCATCTATACCGGTGTAGATCAG-3′) and Crybg3-exon7R (5′-ATCGGATTCACTTGGGTACC-3′), respectively, and direct sequencing.

2.3. Transgenic rescue

Genomic DNA (approximately 9.6 kb in size), containing the entire mouse coproporphyrinogen oxidase (*Cpox*) gene, was generated by PCR from genomic DNA of a BALB/c mouse by using the primer pair Cpox-1 (5′-GGCTTATTCTTGGCTAGCTTCACATTAGCAGAGCAG-3′) and Cpox-2 (5′-TGGCGGCCGCATCAGTGCACCCAGTCCTTTGGATGGCGCAA-3′). PCR products were digested with *Nhe*I and *Not*I and inserted into the *Nhe*I/*Not*I site of the pCI-neo plasmid

vector (Promega, Madison, USA). Nucleotide sequences of the recombinant plasmids were verified. The expression unit was separated from the plasmid backbone by digestion with *Nhe*I and *Kpn*I; it was then injected into fertilized eggs from (C57BL/6 × DBA/2)F_1 mice. Transgenic founder mice and their descendants were screened for the presence of the transgene by PCR analysis of the genomic DNA isolated from tail tips using the primer pair Cpox-ex7F (5′-CTACCTGTGTACTACCCAAG-3′) and pCI-B (5′-TCTTATCATGTCTGCTCGAAGC-3′). Mice that yielded PCR products were considered to have the transgene. Three transgene-positive founder mice were identified. These mice were crossed with BALB/c-*nct*/*nct* mice to obtain F_1 progeny. All founder mice transmitted the transgene to the F_1 progeny. Transcription of transgene mRNA in the lens of transgene-positive F_1 mice was confirmed by RT-PCR amplification and direct sequencing using the primer pair Cpox-3 (5′-CAAGCTGTGGCAGCAGCTGA-3′) and pCI-B. To obtain mice that were homozygous for the mutant *Cpox* gene and either transgene-negative ($Cpox^{nct/nct}$, -/-) or positive ($Cpox^{nct/nct}$, TG/-), transgene-positive F_1 mice ($Cpox^{nct/+}$, TG/-) were crossed with BALB/c-*nct*/*nct* mice ($Cpox^{nct/nct}$, -/-). Mice with the genotype $Cpox^{nct/nct}$, TG/- were selected and crossed with BALB/c-*nct*/*nct* mice. At subsequent generations, transgene-positive mice were selected and crossed with BALB/c-*nct*/*nct* mice that allowed segregation of mice with the ($Cpox^{nct/nct}$, -/-) and ($Cpox^{nct/nct}$, TG/-) genotypes at each generation. These mice were examined for cataract development and coproporphyrin III contents in the lens as described below.

2.4. Measurement of CPOX activity

Liver samples were collected from 4-week-old mice after euthanasia by cervical dislocation. Enzymatic activity of the liver homogenate was determined as previously described (Rossi et al., 1993).

The bacterial expression construct was generated using the pET-21a(+) plasmid (Novagen/Merck Millipore, Darmstadt, Germany) such that a truncated CPOX (starting with the 36th amino acid residue) was fused to a histidine tag. PCR was used to amplify *Cpox* cDNA fragments from mice, which were subsequently inserted into the *Nde*I/*Xho*I site of the pET-21a(+) plasmid. Bacterial expression and purification of recombinant mouse CPOX were performed as previously described for human CPOX (Akagi et al., 2005) with modifications. The protein concentration was determined by the Lowry method (Lowry et al., 1951). Enzymatic activity of the purified CPOX was determined as described previously (Taketani et al., 1994). One unit of the enzyme activity was defined as the formation of 1 nmol of protoporphyrinogen per hour at 37°C.

2.5. Measurement of coproporphyrin III

Blood samples were collected from the heart of 4- and 12-week-old mice under ether anesthesia. After euthanasia by cervical dislocation, the lens and liver were collected. Serum and tissue coproporphyrin III content was measured as previously described (Taketani et al., 1985). Briefly, serum (0.1 ml) and tissues (0.1–0.5 g) were extracted with ethylacetate/acetate (3:1, v/v). After centrifugation, the supernatant was washed once with distilled water, and coproporphyrin III was extracted 3 times with 0.1 M HCl. The amount of coproporphyrin III was determined by scanning the fluorescence emission from 580 to 670 nm by using a fluorescence spectrophotometer at an excitation wavelength of 400 nm.

3. Results

3.1. BALB/c-nct/nct *has a missense mutation in the coproporphyrinogen oxidase gene*

We previously performed chromosomal mapping of the *nct* locus by breeding BALB/c-*nct*/*nct* × (BALB/c-*nct*/*nct* × MSM/Ms) backcross mouse progeny, and demonstrated that the locus is located in a region between 2 microsatellite marker loci, *D16Mit5* and *D16Mit185*, on chromosome 16 (Hiai et al., 1998; Narita et al., 2002). BLAT search of the marker sequences on the mouse genome (GRCm38/mm10; http://genome.ucsc.edu/cgi-bin/hgBlat) revealed that *D16Mit5* and *D16Mit185* are located at the coordinates 57773564-57773713 and 60476984-60477070 on chromosome 16, respectively. It was also revealed that 38 genes are located in the region between the 2 marker loci (Table 1). Twenty-six of these are olfactory receptor genes, and are therefore unlikely to be the *nct* gene. In addition, 3 other genes can be excluded as candidates because their transcriptional expression is restricted to the ovary (Gene model 813 and E330017A01Rik), or gonad and brain (Eph receptor A6) in mice according to UniGene database (http://www.ncbi.nlm.nih.gov/unigene/). The remaining 9 genes (*Dcbld2, St3gal6, Cpox, Gpr15, Cldn25, Gabrr3, Mina, Crybg3,* and *Ar16*) are expressed in a broad range of tissues, including the eye.

RT-PCR analysis was performed to examine mRNA transcripts for the 9 *nct* candidate genes in the lens of BALB/c-*nct*/*nct* mice. PCR products from BALB/c-*nct*/*nct* mice were run on an agarose gel, and band intensities similar to those generated from BALB/c mice were observed, suggesting comparable transcription levels between BALB/c-*nct*/*nct* and BALB/c control mice. Of the 9 genes screened by RT-PCR and sequencing, only 2 genes (coproporphyrinogen oxidase (*Cpox*) and beta-gamma crystalline domain containing 3

(*Crybg3*)) contained BALB/c-*nct*/*nct* strain-specific nucleotide differences: the lack of mutations in the remaining 7 genes in *nct* interval suggested that these genes were unlikely to have critical roles in the development of cataracts in BALB/c-*nct/nct* mice.

Missense nucleotide substitutions were detected in the RT-PCR products for *Cpox* and *Crybg3* of BALB/c-*nct*/*nct* mice. As for the *Cpox* gene, a c.1139G > T nucleotide substitution was identified. This nucleotide substitution led to a substitution of a leucine residue for an arginine residue at codon 380 (p.R380L; Fig. 1). In the *Crybg3* gene, a c.2230A > G nucleotide substitution was identified, which resulted in a substitution of an aspartic acid residue for an asparagine residue at codon 744 (p.N744D; Fig. 1) in BALB/c-*nct*/*nct* mice. Subsequent examination of genomic DNA from a BALB/c-*nct*/*nct* mouse verified the presence of these nucleotide substitutions in *Cpox* exon 5 and *Crybg3* exon 7. These genomic mutations were not found in other 7 mouse strains with clear lenses examined (C57BL/6J, 129/SvJ, NZB/N, AKR/J, C3H/He, DBA/2, and SJL/J), indicating that they are unique to BALB/c-*nct*/*nct* mice.

*3.2. CPOX activity is markedly reduced in BALB/c-*nct/nct *mice*

Coproporphyrinogen oxidase (EC 1.3.3.3) is the sixth enzyme of the heme biosynthetic pathway and catalyzes the oxidative decarboxylation of the heme precursor coproporphyrinogen III to protoporphyrinogen IX via an intermediate. The amino acid residues 377–386 of mouse CPOX are perfectly conserved among species (Lamoril et al., 2001; Fig. 1), suggesting that arginine at the 380th position is required for proper catalytic activity. Analysis of the effects of the p.R380L substitution by the SIFT algorithm (Kumar et al., 2009) predicted it to affect protein function with a score of 0.00 (threshold for intolerance is 0.05.). Then, we investigated the influence of the p.R380L amino acid substitution on

CPOX activity. CPOX activity in liver homogenates of BALB/c-*nct*/*nct* mice was less than 15% of that of BALB/c mice (Fig. 2). Moreover, the recombinant CPOX isoform with the p.R380L substitution of the BALB/c-*nct*/*nct* strain-type exhibited a similar reduction in activity (~15%) compared to equal amounts of the BALB/c-type isoform. These results indicate that *Cpox*^{nct} was a hypomorphic allele.

Every mammalian cell requires porphyrin and its main final metabolite heme for its proper functioning. The liver and bone marrow in particular are the organs mainly involved in heme synthesis. Thus, we evaluated the accumulation of coproporphyrin III, an autonomously oxidized form of the substrate for CPOX, in the sera, liver, and lenses of BALB/c-*nct*/*nct* mice. All BALB/c-*nct*/*nct* mice examined had apparently transparent lenses at 4 weeks of age, whereas all lenses of 12-week-old BALB/c-*nct*/*nct* mice were opaque. These mice had excessive accumulation of coproporphyrin III in the serum, liver, and most importantly, in the lens at both 4 and 12 weeks of age (Fig. 3).

3.3. Introduction of the wild type Cpox *transgene could prevent cataract of the Nakano mouse*

Next, transgenic rescue was attempted to demonstrate a direct etiological link between the *Cpox* mutation and cataract. Three transgenic founder mice (No. 04-8, 06-2, and 10-3) were obtained, and 3 transgenic lines were bred from the founder mice. Wild type *Cpox* transcripts were corroborated by RT-PCR analyses of the lenses of transgenic F_1 mice bred from the founder mice (data not shown). Subsequent backcross progeny mice homozygous for the mutant *Cpox* gene were either negative (*Cpox*$^{nct/nct}$, -/-) or positive (*Cpox*$^{nct/nct}$, TG/-) for the wild type *Cpox* transgene (Fig. 4). Transgene-negative progeny mice manifested cataract by the age of 3 months for all 3 lines. In contrast, no transgene-positive progeny mice developed cataract until they were terminated at 6 months of age. Examination of these mice

indicated that transgene-negative animals had higher coproporphyrin III levels in their lenses than that in transgene-positive animals.

4. Discussion

This study demonstrates that a hypomorphic mutation in the *Cpox* gene is a primary cause of hereditary cataract in the NCT mouse. Our data strongly support a role for the porphyrin biosynthesis pathway in lens physiology. At this point, a major question remains to be answered: what mechanism associated with a *Cpox* mutation lead to the development of cataract in the Nakano cataract mouse? In connection with this, another puzzling point is that cataracts in the BALB/c-*nct*/*nct* mice do not manifest until around 10 weeks of age, despite the fact that coproporphyrin is already accumulated to high levels in lenses at 4 weeks of age. The NCT is an osmotic type caused by sodium ions accumulation (Iwata and Kinoshita, 1971), and indeed, deficiencies in the Na^+/K^+-ATPase activity are considered to be the basis for defects in cation balance. Furthermore, it has also been shown that Na^+/K^+-ATPase deficiency is caused by a small polypeptide inhibitor (molecular weight of 6300) specific to the lenses of Nakano mice (Kinoshita, 1974; Fukui et al., 1978). However, the identity of the polypeptide is unknown, as is its relevance to compromised CPOX activity.

We hypothesized that cataracts are caused by cellular phototoxicity of accumulated coproporphyrin in the lenses of Nakano mice. Porphyrin precursors, including coproporphyrin, absorb photons and are converted from their ground state to the excited state. Highly reactive, excited state porphyrins could interact with oxygen to produce reactive oxygen species, resulting in tissue damage (phototoxicity) such as cutaneous damage in porphyria patients (Lim and Cohen, 1999). To explore the phototoxicity hypothesis, we kept BALB/c-*nct*/*nct* mice in constant darkness from the time they were weaned at 3 weeks of age, expecting that it

would prevent cataract onset. Contrary to our expectation, these mice developed cataracts at similar ages as mice raised under a daily 12-h light/dark cycle, precluding the possibility of phototoxicity as a cause of cataracts in these mice. Over accumulation of coproporphyrin in the lens might exert cytotoxicity by a different mechanism. Accumulation of another porphyrin precursor, protoporphyrin IX, has been shown to cause mitochondrial damage and cell death (Antolín et al., 1994). Indeed, an abundant amount of small mitochondria in lens fibers (Hamai et al., 1974) and epithelial cell degeneration (Hara et al., 1999) are hallmarks of the NCT lens. Furthermore, accumulation of protoporphyrin IX under hypoxic conditions could be detrimental to cells (Krishnamurthy et al., 2004). Because the lens environment is hypoxic, it is conceivable that it is constantly exposed to the cytotoxicity of accumulated coproporphyrin. Thus, it is noteworthy that the NCT is manifested after the fetal lens vasculature regresses and the lens environment becomes hypoxic (Ito and Yoshioka, 1999).

In humans, defects in the *CPOX* gene cause hereditary coproporphyria (Martásek et al 1994; Lamoril et al., 2001; Schmitt et al., 2005; http://omim.org/entery/121300). Unlike Nakano mice, human hereditary coproporphyria patients do not develop cataracts, possibly reflecting species differences. Human hereditary coproporphyria patients are usually asymptomatic, although acute attacks may be precipitated by certain drugs, alcohol, infections, or low caloric intake (Martásek et al., 1998). In contrast, we found that Nakano mice always had extremely elevated levels of coproporphyrin systemically in the absence of external stimuli. Importantly, accumulation of coproporphyrin was also observed in the lenses of Nakano mice before they developed cataracts. The reason for the continuous occurrence of coproporphyria in Nakano mice, even in the absence of external stimuli is not clear. However, this could account for the differences in the cataract development between Nakano mice and human coproporphyria patients.

Another less plausible possibility for the absence of cataract in human coproporphyria patients is related to the observation that BALB/c-*nct*/*nct* mice possess missense mutations in both *Cpox* and *Crybg3* genes. Hence, a mutation in the *Crybg3* gene in addition to that in *Cpox* could be obligatory for cataractogenesis in BALB/c-*nct*/*nct* mice. In contrast, it is unlikely that hereditary coproporphyria patients have mutations in the *CRYBG3* gene. These genes are so tightly linked (~820 kb) on mouse chromosome 16 that the NCT segregates as a trait governed by a single recessive gene. Because the BALB/c-*nct*/*nct* mouse we used in this study is a congenic strain established from the original NCT mouse 25 years ago (Matsuzawa et al., 1988), we assessed the possibility that the *Crybg3* allele of the BALB/c-*nct*/*nct* mouse resulted from a *de novo* mutation that occurred during or after the establishment of the strain. Although we could not obtain and examine any original Nakano mice, we obtained a lens epithelial cell line established from the original Nakano mouse (Russell et al., 1977). Mutations were found in both *Cpox* and *Crybg3* genes of the cell line, suggesting that the original Nakano mouse carried both mutant genes. Given the specificity of our transgenic rescue test, we cannot exclude the possibility that the mutant *Crybg3* is involved in cataract development in BALB/c-*nct*/*nct* mice. Transgenic rescue using a wild type *Crybg3* gene should help resolve this issue. If the cataract is rescued by the wild type *Crybg3* gene, it would prove that double compound mutations are necessary for cataractogenesis in Nakano mice. We confirmed by RT-PCR analysis that *Crybg3* transcripts were expressed in the lenses of BALB/c-*nct*/*nct* mice (not shown). The amino acid sequence around the 744th position of mouse CRYBG3 are identical to that in humans (Fig. 1), suggesting that asparagine at the position is important for its proper function. We could not experimentally examine the influence of the p.N744D amino acid substitution, because the function of CRYBG3 is not known. Analysis of the effects of the p.N744D substitution by the SIFT algorithm, however,

predicted it to be tolerated with a score of 0.17, suggesting that it is not pathogenic.

The present data indicate that the NCT mouse serves as an unprecedented model for human hereditary coproporphyria. Our preliminary study revealed that Nakano mice show mild phototoxic lesions in the skin, which are also occasionally observed in hereditary coproporphyria patients. Further characterization of the Nakano mouse as a coproporphyria model is underway in our laboratory.

In conclusion, we elucidated the primary genetic defect underlying the Nakano cataract. Further studies of cataract in Nakano mice will provide additional insight into the mechanisms mediating lens development and optical quality.

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Figure legends

Fig. 1. Alignment of partial nucleotide and deduced amino acid sequences (numbered) for *Cpox* (A) and *Crybg3* cDNA (B) derived from BALB/c and BALB/c-*nct*/*nct* mouse strains. Nucleotide and amino acid substitutions are indicated in red. Corresponding amino acid sequences from representative species are also shown. The amino acid sequence data are based on the Protein database (http://www.ncbi.nlm.nih.gov/protein); CPOX of human (accession NP_000088), *Drosophila melanogaster* (AAD46837), *Saccharomyces cerevisiae* (CAA89966), *Escherichia coli* (BAA16325), and human CRYBG3 (NP_705833). Chromatograms for nucleotide sequencing of the RT-PCR products are shown at the top. Nucleotide substitutions are indicated by arrows.

Fig. 2. (**A**) Comparison of CPOX activity in the liver of mice (mean ± SD; n = 3). (**B**) Comparison of activity of the wild type and mutant recombinant CPOX isoforms (mean ± SD; n = 3). The top panel shows an SDS-PAGE and Coomassie Blue staining image of recombinant mouse CPOX. Statistical differences between strains and isoforms were evaluated using the Student's *t*-test.

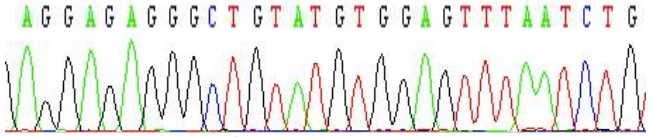
Fig. 3. Comparison of coproporphyrin III contents in the serum, liver, and lens (mean ± SD; n = 3). Statistical differences between strains were evaluated using the Student's *t*-test.

Fig. 4. Phenotype of *Cpox*-transgenic mice. (**A**) Genotyping of a litter of backcross mice for the presence of a transgene. (**B**) Gross appearance of eyeballs of the mice and coproporphyrin

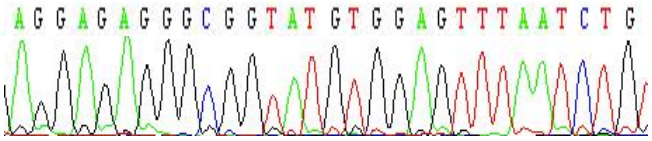
III contents of their lenses at 3 months of age. This figure represents data from N4 mice of the 04-8 transgenic line.

A

BALB/c-*nct*/*nct*



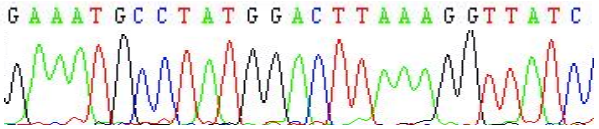
BALB/c



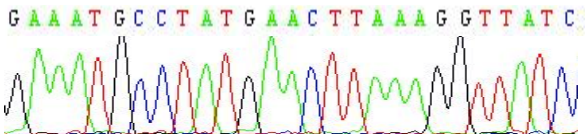
BALB/c-*nct*/*nct*	AGG	AGA	GGG	CTG	TAT	GTG	GAG	TTT	AAT	CTG
	^{377}R	R	G	**L**	Y	V	E	F	N	L^{386}
BALB/c	AGG	AGA	GGG	CGG	TAT	GTG	GAG	TTT	AAT	CTG
	^{377}R	R	G	R	Y	V	E	F	N	L^{386}
Human	^{388}R	R	G	R	Y	V	E	F	N	L^{397}
D. melanogaster	^{325}R	R	G	R	Y	V	E	F	N	L^{334}
S. cerevisiae	^{262}R	R	G	R	Y	V	E	F	N	L^{271}
E. coli	^{236}R	R	G	R	Y	V	E	F	N	L^{245}

B

BALB/c-*nct*/*nct*



BALB/c



BALB/c-*nct*/*nct*	GAA	ATG	CCT	ATG	GAC	TTA	AAG	GTT	ATC	
	^{740}E	M	P	M	**D**	L	K	V	I^{748}	
BALB/c	GAA	ATG	CCT	ATG	AAC	TTA	AAG	GTT	ATC	
	^{740}E	M	P	M	N	L	K	V	I^{748}	
Human	^{2474}E	M	P	M	N	L	K	V	I^{2482}	

Fig. 1

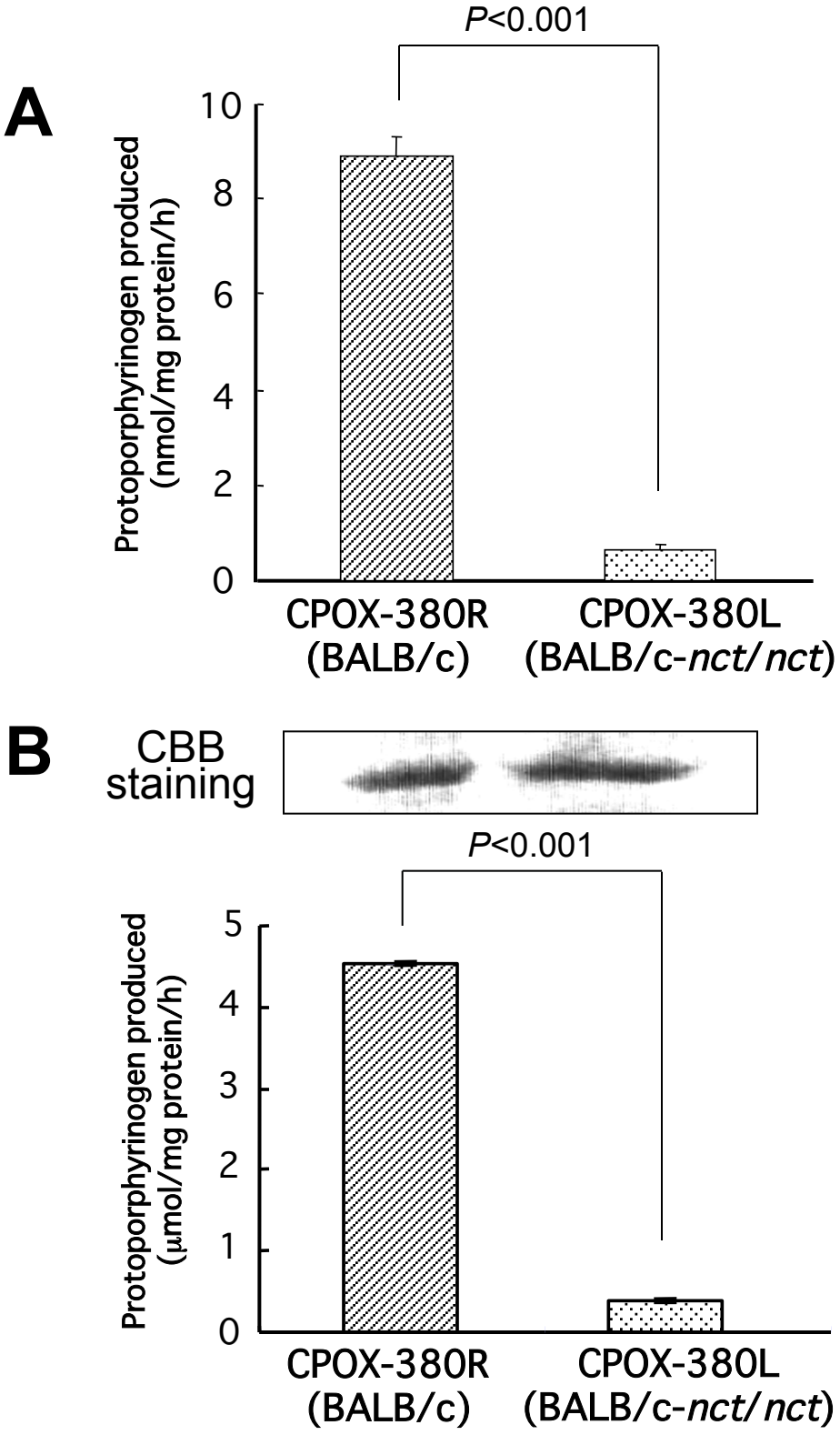

A
P<0.001
Protoporphyrinogen produced
(nmol/mg protein/h)
10
8
6
4
2
0
CPOX-380R
(BALB/c)
CPOX-380L
(BALB/c-nct/nct)
B
CBB
staining
P<0.001
Protoporphyrinogen produced
(μmol/mg protein/h)
5
4
3
2
1
0
CPOX-380R
(BALB/c)
CPOX-380L
(BALB/c-nct/nct)

Fig. 2.

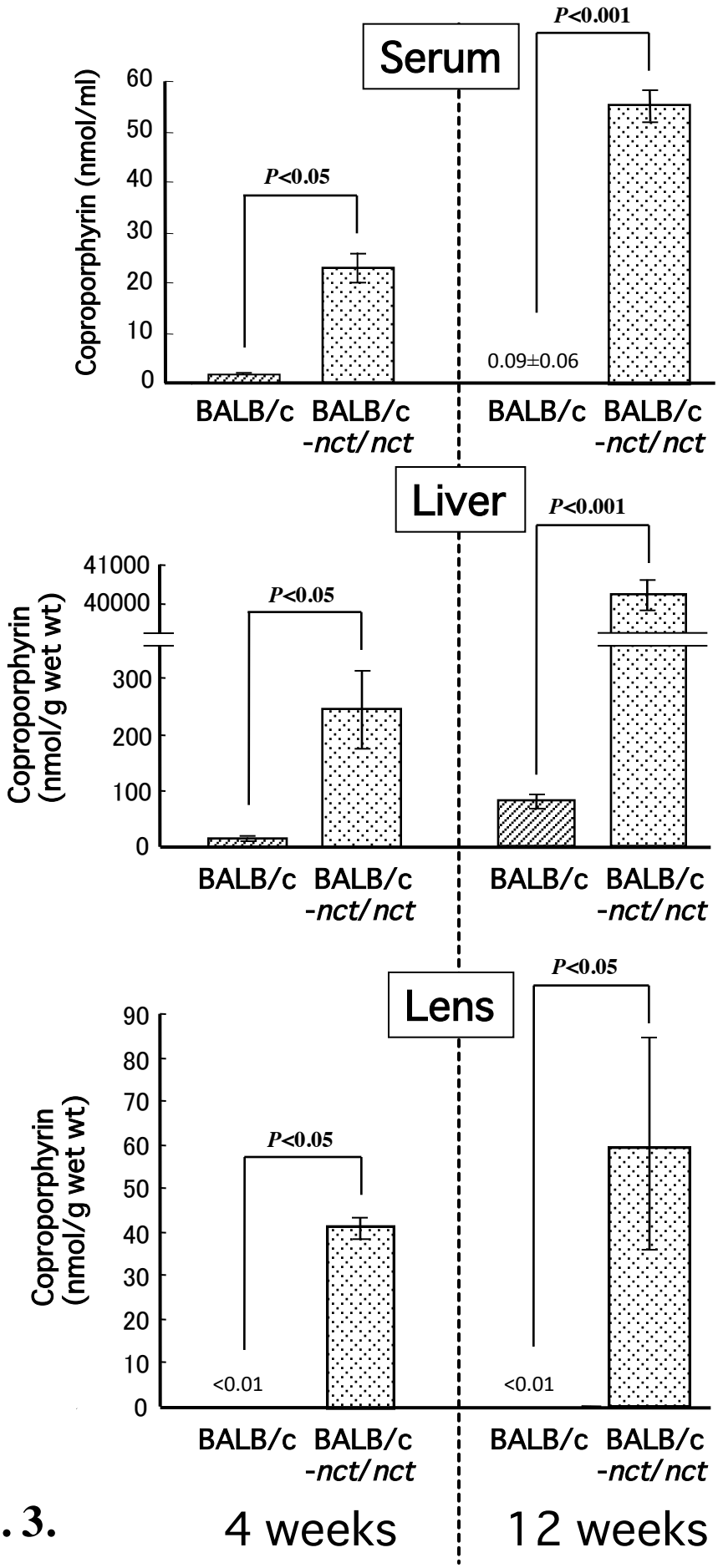

Serum
Coproporphyrin (nmol/ml)
60
50
40
30
20
10
0
P<0.05
P<0.001
0.09±0.06
BALB/c
BALB/c -nct/nct
BALB/c
BALB/c -nct/nct
Liver
Coproporphyrin (nmol/g wet wt)
41000
40000
300
200
100
0
P<0.05
P<0.001
BALB/c
BALB/c -nct/nct
BALB/c
BALB/c -nct/nct
Lens
Coproporphyrin (nmol/g wet wt)
90
80
70
60
50
40
30
20
10
0
P<0.05
P<0.05
<0.01
<0.01
BALB/c
BALB/c -nct/nct
BALB/c
BALB/c -nct/nct
4 weeks
12 weeks

Fig. 3.

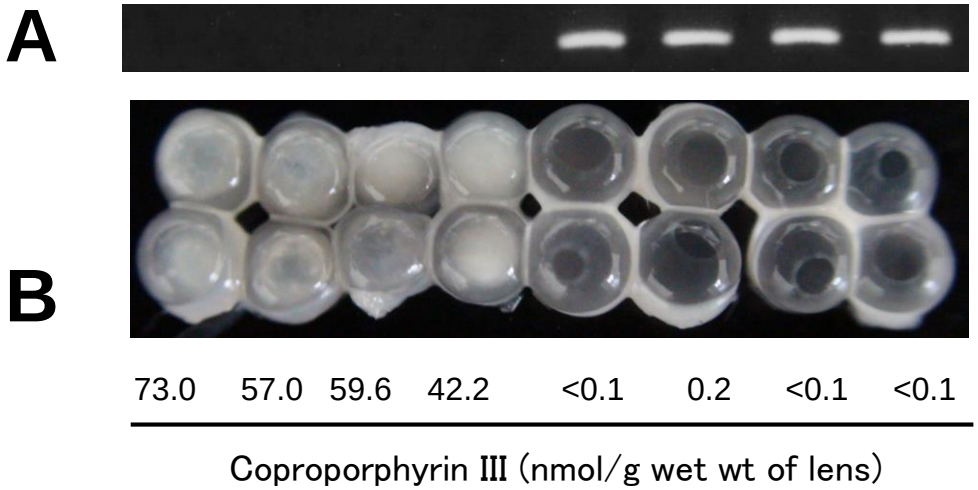

A
B
73.0
57.0
59.6
42.2
<0.1
0.2
<0.1
<0.1
Coproporphyrin III (nmol/g wet wt of lens)

Fig. 4.

Table 1. List of candidate genes for *nct*

Gene name	Gene symbol	Accession No.	Expression in the eye*	Forward primer	Reverse primer
Discoidin, CUB and LCCL domain containing 2	*Dcbld2*	NM_028523	Yes	5′-aggacgctggaggccagca-3′	5′-cctaaccacttcagtgacag-3′
ST3 beta-galactoside alpha-2,3-sialyltransferase	*St3gal6*	NM_018784	Yes	5′-tttcaggcaggccagccatg-3′	5′-tcatcttttggatccatagggtc-3′
Predicted gene 813			No		
E330017A01Rik			No		
Coproporphyrinogen oxidase	*Cpox*	NM_007757	Yes	5′-aggatcggatttgcccagtc-3′	5′-gagtttcctggtacctatcatag-3′
G protein-coupled receptor 15	*Gpr15*	NM_001162955	Yes	5′-atggaaccagcaacagccct-3′	5′-cagagcttgacatgtcacag-3′
Claudin 25	*Cldn25*	NM_171826	Yes	5′-gcagtctgaatgccagaatg-3′	5′-tctgaacctagatcagtgtgg-3′
Olfactory receptor 172, 173, 177, 178, 180, 181, 183, 186, 187, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 201, 202, 203, 204, 205, 206, 207	*Olfr172*		No		
GABA receptor, rho 3	*Gabrr3*	NM_001081190	Yes	5′-atggtcctg ctgtttggtt g-3′	5′-tgtaacaccctgagtactgc-3′
Myc induced nuclear antigen	*Mina*	NM_025910	Yes	5′-gcatttcctccctactcgtga-3′	5′-tccacaaggacataaaccagc-3′
Beta-gamma crystallin domain containing 3	*Crybg3*	NM_174848	Yes	5′-tagacatggtacctgccgaac-3′	5′-ttagcttccccatggagcag-3′
ADP-ribosylation factor-like 6	*Arl6*	NM_019665	Yes	5′-tgctctttaaatatttaagccgtgatatg-3′	5′-aaccagtatgccttacgcag-3′
Eph receptor A6	*Epha6*		No		

*Based on UniGene database (http://www.ncbi.nlm.nih.gov/unigene/).