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# Ectopic Transcription and the Possibility of RNA Editing of the Human Arrestin Gene

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**Purpose:** To investigate the ectopic transcription of arrestin in various human organs and tissues.

**Methods:** Reverse transcriptase–polymerase chain reaction was used to investigate the ectopic transcription of the arrestin gene in the retina, anterior capsule of the lens, iris, bulbar conjunctiva, muscle, skin, placenta, intestine, and human blood. In addition, we examined the expression of arrestin mRNA isolated from whole blood cells of patients with Oguchi disease who had the 1147delA mutation in the arrestin gene.

**Results:** Arrestin was expressed at the mRNA level in all our samples. The mRNA isolated from the blood cells of Oguchi patients was not associated with the same 1147delA mutation as was reported earlier in the arrestin gene but had the normal sequence.

**Conclusions:** These results demonstrate the ectopic transcription of the arrestin gene in various tissues and also suggest the possibility of RNA editing in the blood cells of Oguchi patients. **Jpn J Ophthalmol 1999;43:295–299** © 1999 Japanese Ophthalmological Society

**Key Words:** Arrestin gene, 1147delA mutation, ectopic transcription.

## Introduction

Arrestin is a soluble protein with a molecular weight of 45 kDa that is expressed at high levels in the rod photoreceptor cells and pineal gland.[1–3] Recent reports have shown that human arrestin is expressed not only in the photoreceptor cells and pineal gland but also in the iris, anterior capsule of the lens, choroid, and brain.[4–8] Arrestin is believed to inhibit the phototransduction cascade by binding to the photo-activated, phosphorylated rhodopsin by directly interacting with α-transducin.[1,2,9] Arrestin is also known to induce experimental autoimmune uveitis (EAU).[2]

Recently, linkage analysis in an Indian family showed that the locus of Oguchi disease was on chromosome 2q, which is the chromosomal location of the arrestin gene.[10] After this report, mutation screening was performed on Japanese patients with Oguchi disease to search for mutations in the arrestin gene. A homozygous 1147delA mutation in this gene was found to occur frequently in Japanese patients with Oguchi disease.[11]

As the technique of polymerase chain reaction (PCR) developed, some kinds of mRNA that had been considered to be expressed in specific tissues only, have been shown to be expressed in other organs and tissues as well. This phenomenon is known as ectopic transcription. In this study, we investigated the ectopic transcription of the arrestin gene in various human organs and tissues. In addition, we also examined the mRNA isolated from the blood of Oguchi patients with the 1147delA mutation in the arrestin gene to detect the presence of abnormal mRNA in the blood cells.

## Materials and Methods

### *Patients and Materials*

We obtained 12 human samples that consisted of ocular and nonocular tissues and organs. Ocular

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samples, obtained during the course of ophthalmic surgery, included the retina, anterior capsule of the lens, iris, and bulbar conjunctiva. Non-ocular samples, including skeletal muscle, skin, placenta, and intestine, were excised from four unrelated patients without eye diseases during surgery for their diseases. Peripheral blood was taken from three patients with Oguchi disease associated with a homozygous 1147delA mutation in the arrestin gene[12] and one normal control. Informed consent was obtained from all subjects.

### *Extraction of mRNA and Synthesis of cDNA*

mRNAs were isolated from 400 μL of fresh peripheral blood or tissue specimens by using oligo-dt cellulose (Pharmacia Biotech, Uppsala, Sweden). The cells or tissue specimens were pelleted and suspended in extraction buffer (4 mmol/L guanidium thiocyanate and 0.5% N-lauroyl sarcosine). The cleared cell homogenate obtained by centrifugation was mixed with oligo-dt cellulose. This mixture was washed with high salt buffer (10 mmol/L Tris-HCl [pH 7.5] and 1 mmol/L EDTA, 0.5 mmol/L NaCl) five times, followed by low salt buffer (10 mmol/L Tris-HCl [pH 7.5] and 1 mmol/L EDTA, 0.1 M NaCl) three times. Subsequently, the mRNA was eluted by prewarmed elution buffer (10 mmol/L Tris-HCl [pH 7.5] and 1 mmol/L EDTA). First strand cDNA was generated by random hexadeoxynucleotides at 0.2 μg in each reaction that was catalyzed by Moloney murine leukemia virus reverse transcriptase (Phamacia Biotech).

### *Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)*

We set the primers to amplify 175 bp between exons 11 and 12 in the arrestin gene (forward primer, 5′-GCACTTTGACCAAGACGTTG-3′; reverse primer, 5′-TTGATCTGGTAAGACACCAG-3′). The RT-PCR reactions were carried out in a thermocycler (Perkin Elmer, Norwalk, CT, USA). Briefly, the PCR reaction mixture (50 μL) contained 1.0 μL of the cDNA solution, 20 μmol/L of each primer, 200 μmol/L each of DATP, DCTP, DGTP, and TTP, 50 mmol/L of KCl, 10 mmol/L of Tris-HCl (pH 8.3), 1.5 mmol/L of $MgCl_2$, 0.001% gelatin, and 2.5 units of *Taq*-polymerase for 45 cycles. After denaturation of the reaction mixture at 94°C for 2 minutes, PCR was performed as follows: 45 cycles consisting of 94°C for 1 minute for denaturation, 45°C for 2 minutes for annealing, and 72°C for 2 minutes for polymerization. Each PCR product was analyzed in 1.5% agarose gel (Seakem; FMC Bioproducts, Rockland, ME, USA) containing 0.005 mg/mL of ethidium bromide. The DNA was observed with an ultraviolet transilluminator. For routine negative control, distilled water without the mRNA sample was used with the same times for each RT-PCR procedure. Also, a mRNA solution pretreated with 50 μL of ribonuclease A (Pharmacia Biotech) was prepared as another negative control for the mRNA from Oguchi patients. All other solutions and procedures were identical for each RT-PCR.

### *Polymerase Chain Reaction of Genomic DNA Samples*

Genomic DNA samples were isolated from the same blood samples of patients with Oguchi disease using QIA Amb blood kit (Qiagen, Hilden, Germany). Exon 11 of the arrestin gene was amplified from the genomic DNA samples by the method described previously.[11] The amplified DNA fragments were subsequently processed for nucleotide sequencing analysis.

### *Sequence Analysis*

For sequence analysis, the amplified PCR products were subcloned into a plasmid vector (pGEM; Promega, Madison, WI, USA) and sequenced with an automatic DNA sequencer (A.L.F. DNA Sequencer; Pharmacia Biotech) using a dideoxy chain termination protocol.[13]

## Results

After the samples were obtained, the mRNAs were immediately isolated and the cDNAs were subsequently synthesized. RT-PCR was carried out with the primers that were selected to amplify exons 11 and 12 of the arrestin gene. The results of RT-PCR

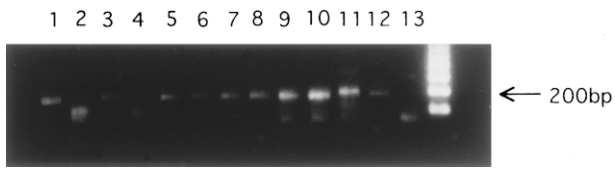


**Figure 1.** Results of RT-PCR of arrestin gene. 1, retina; 2, anterior capsule; 3, iris; 4, bulbar conjunctiva; 5, placenta; 6, muscle; 7, skin; 8, intestine; 9–11, blood from three Oguchi patients; 12, blood from normal human control; 13, negative control; size marker. Although DNA bands are only weakly visible in lanes 2 and 4, same size bands can be detected in all samples.

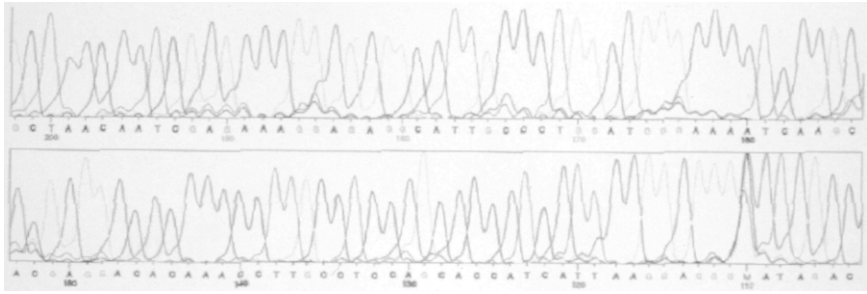

**Figure 2.** Sequence analysis of RT-PCR products from normal human blood using our primer, which amplifies 175 bp between exons 11 and 12. Results indicate that this sequence matches that of α-arrestin gene.

are shown in Figure 1. A single band was amplified in each sample, although the amplified products in lanes 2 (anterior capsule of lens) and 4 (bulbar conjunctiva) of the DNAs are only slightly visible on the photograph. The nucleotide sequencing analyses of these RT-PCR products revealed that they had the same sequence of 175 bases from exons 11 and 12 of the α-arrestin gene, suggesting that α-arrestin is expressed in all our samples (Figures 1 and 2).

Subsequently, the same RT-PCR analysis was performed on the mRNA samples independently purified from three patients with Oguchi disease who had the homozygous 1147delA mutation in the arrestin gene. The results showed that the RT-PCR products had the same size as those from the various tissues. Nucleotide sequencing analysis revealed that the RT-PCR products from all three Oguchi patients did not have the same 1147delA mutation as had been seen in genomic DNA but did have the normal sequence at nucleotide 1147 (Figure 3).

## Discussion

Recently, ectopic transcription has been reported by using the RT-PCR technique, and this technique has been applied to the diagnosis of some kinds of hereditary metabolic disorders without performing liver biopsy.

Until now, it was reported that arrestin is expressed in the retina and pineal gland at high levels, and in the iris, choroid, and lens epithelial cells with attached anterior capsule, and in the brain at low levels.[1–3,8] In this study, we examined the ectopic transcription of the arrestin gene in various human organs and tissues. RT-PCR disclosed that arrestin was clearly expressed in 10 tissues although only weakly in the bulbar conjunctiva and the anterior capsule.

Arrestin has high homology with β-arrestin. Therefore, we had to be certain that these RT-PCR products originated from α-arrestin. To eliminate

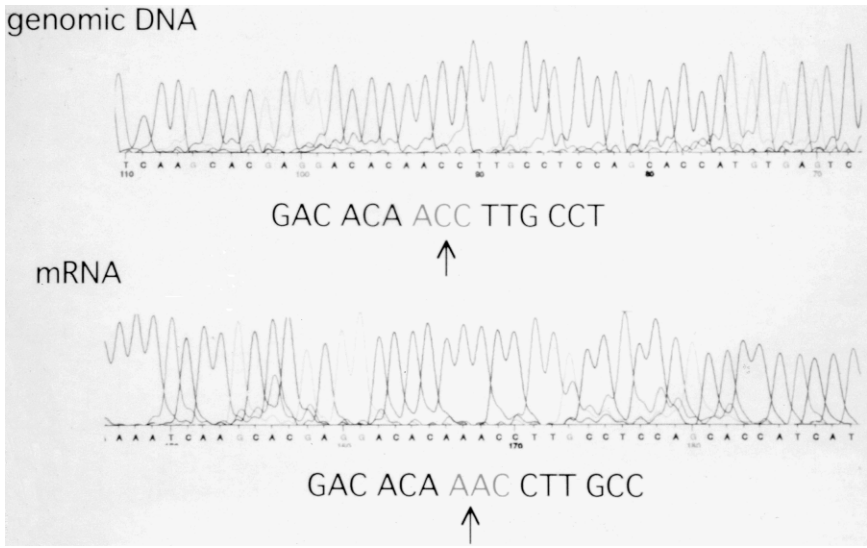


**Figure 3.** Sequence analysis of mRNA and genomic DNA of Oguchi patient with 1147delA mutation in arrestin gene. This sequence shows part of exon 11 that includes sequence at nucleotide 1147. Nucleotide sequence derived from mRNA did not contain 1147delA mutation whereas genomic DNA had mutation. Same pattern was also seen in other two Oguchi patients.

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the possibility of artifacts, we performed nucleotide sequencing analyses and the results revealed that α-arrestin was expressed in all our samples. We discovered that the human α-arrestin gene was ectopically expressed in many kinds of tissues other than the retina and the pineal gland. The biological significance of this is still unknown.

In 1995, it was reported that Japanese patients with Oguchi disease frequently had a 1147delA mutation in the arrestin gene.[10,11] Subsequently, we reported that patients with the 1147delA mutation in the arrestin gene showed clinical heterogeneity, for example, in the fundus appearance, the electroretinogram and the visual acuity.[12]

This study showed that arrestin is also expressed in human blood. Therefore, as the second step, we investigated the mRNA in fresh blood samples of Oguchi patients with the 1147delA mutation in the arrestin gene. Sequence analysis showed that the mRNA from their blood did not have the same 1147delA mutation that was seen in genomic DNA but did have the normal sequence. We carefully eliminated the possibility that this finding was due to contamination. For instance, we used a negative control in which no mRNA sample was included when the RNA samples were processed. No amplified products were found in this negative control. In addition, the RT-PCR for each Oguchi patient was carried out independently at different times to rule out the possibility of contamination.

The nucleotide sequence of the cDNAs obtained in this study showed the same result for each patient. We also prepared mRNA pretreated with ribonuclease A as another control for mRNA from Oguchi patients, and none of the amplified products was observed.

Recently, discrepancies between genomic DNA and mRNA have been found; this phenomenon is called RNA editing. It is known that some nucleotide sequences in mitochondrial or genomic DNAs are changed by transcription. van Leeuwen et al[14] reported the possibility that RNA editing was a means of producing phenotypic variability. Although RNA editing has been reported mainly as base substitutions resulting in amino acid alterations or non-sense changes in mammalian tissues, such as apolipoprotein B[15,16] and glutamate receptor,[17] examples of addition or deletion of nucleotides have also been reported.[18,19] Therefore, one possible explanation for the discrepancy between sequences of mRNA and genomic DNA in the present study is RNA editing. Because the genomic DNA samples from all three Oguchi patients showed homozygous 1147delA and did not show any heterozygosity, as far as we were able to determine, the mechanism by which the 1147delA was corrected is largely unknown.

We could not investigate if RNA editing occurred in the photoreceptor cells of patients with Oguchi disease because we could not obtain mRNA from the retina of these patients at the time of our study. Because each patient with Oguchi disease showed complete cosegregation between 1147delA mutation and clinical findings,[11,12] it is reasonable to think that Oguchi disease is caused by the 1147delA mutation. As a possible explanation, we theorize that the ratio of RNA editing is different in the retina of patients with Oguchi disease. Further investigation using some other tissues from Oguchi patients will help us determine the presence of RNA editing in these patients.

We also theorize that the ratio of RNA editing in the retina of Oguchi patients may vary with the clinical severity. For example, some patients who showed only stationary night blindness may have high frequency RNA editing in the retina. We recently reported three different patients with progressive retinal degeneration who had the same 1147delA mutation in the arrestin gene.[20,21] We hypothesize that these patients most likely did not have RNA editing in their retinas. This is compatible with the explanation that the 1147delA mutation in the arrestin gene causes not only Oguchi disease but also progressive retinal degeneration. Further investigation is necessary to clarify the relationship between the mRNA expression of the arrestin gene and the clinical severity of Oguchi disease.

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