



Association between a point mutation at the -743-bp region of the transthyretin (*TTR*) gene and familial vitreous amyloidosis

X.M. Nie, S.J. Cai, B. Xie, X.W. Chen and M. Jiang

Department of Ophthalmology Affiliated Hospital of Zunyi Medical College,
Zui Yi, China

Corresponding author: S.J. Cai
E-mail: caishanj_l@163.com

Genet. Mol. Res. 15 (1): gmr.15016926
Received August 2, 2015
Accepted November 13, 2015
Published March 31, 2016
DOI <http://dx.doi.org/10.4238/gmr.15016926>

ABSTRACT. The aim of this study was to identify changes in the base sequence of the upstream regulatory region of the transthyretin (*TTR*) gene. Whole-blood DNA was extracted from ten subjects belonging to a family with familial amyloidosis vitreoretinopathy; the upstream regulatory sequence was amplified by polymerase chain reaction, detected by gel electrophoresis, and sequenced. The DNA sequence of the upstream regulatory region of the *TTR* gene was successfully sequenced, and a point mutation (-743A→T) was identified in six of the ten blood samples: four patients and two family members without disease incidence. Therefore, a point mutation was identified in the upstream regulatory region of the *TTR* gene in a Han Chinese family with familial vitreous amyloidosis.

Key words: Vitreous amyloidosis; Transthyretin; Gene; Mutation; Upstream

INTRODUCTION

Many studies have revealed that family amyloidosis is correlated with mutations in the 7616-bp transthyretin (*TTR*) (Westermarck et al., 2002) gene located at the q11.2-q12.1 region of chromosome 18, containing four exons and three introns (Ikeda et al., 2002). So far, approximately 99% of the disease-causing mutations in the *TTR* gene have occurred in the second, third, and fourth exons of this gene. A previous study focusing on a Han family with vitreous amyloidosis discovered a *TTR* mutation site located in the third exon of chromosome 18, inducing a mutation in the 83rd amino acid (Gly83Arg) (Xie et al., 2013), which, however, was not expressed by several patients. Further studies have shown that the relationship between the *TTR* mRNA and proteins among amyloidosis patients and normal subjects was as follows: patients in a family < normal subjects in a family < healthy controls from a different family. Therefore, this disease was not exclusively characterized by the Gly83Arg mutation in exon 3 of the *TTR* gene. This study attempted to identify mutations in the upstream regulatory region of the *TTR* gene, and consequently attempted to determine the possible correlations between these mutations and amyloidosis.

MATERIAL AND METHODS

Sample collection

Whole-blood samples were obtained from 10 subjects belonging to a family with familial vitreous amyloidosis (Table 1) who visited our hospital again around the same time period; whole-blood DNA was extracted to detect the upstream regulatory region.

Table 1. Comprehensive overview of the study subjects.

Patient No.	No.	Gender	Age	Sickness	Mutations in third exon	Operation	Recurrence
1	III-10	Male	42	Yes	Gly 83 Arg	Yes	Yes
2	III-7	Female	47	Yes	Gly 83 Arg	Yes	Yes
3	III-1	Female	57	Yes	No mutation	Yes	Yes
4	IV-9	Male	25	Yes	No mutation	-	-
5	III-3	Female	52	Yes	Gly 83 Arg	Yes	Yes
6	III-24	Male	43	Yes	No mutation	Yes	No
7	III-16	Male	45	New case	No detection	No	-
8	III-19	Male	38	Yes	Gly 83 Arg	Yes	No
9	III-17	Male	43	Yes	Gly 83 Arg	Yes	No
10	IV-39	Female	18	No	No detection	-	-

Reagents and kits

The Blood Genomic DNA Isolation Kit (Tiangen Biotech Co., Ltd., Beijing, China), DNA Marker (Marker V) (Tiangen Biotech Co., Ltd.), polymerase chain reaction (PCR) primers (Sangon Biotech Co., Ltd., Shanghai, China), and Resin PCR and Gel Purification Kits (SBS Genetech Co., Ltd.) were used in this study.

Methods

DNA extraction and amplification by PCR

Blood genomic DNA was extracted from the sample blood using the Blood Genomic

DNA Isolation Kit as per the instructions provided by the manufacturer. The target exon region in the *TTR* gene was amplified by PCR. The upstream regulation region of the *TTR* gene (-1226 bp) was divided into three portions to facilitate detection. The PCR amplification was performed using the following primers (for each portion) synthesized by Invitrogen (Carlsbad, CA, USA): 663-bp, F: 5'-TGGCTTCTGGTTCTCACAGTC-3', R: 5'-TGGCTTCTGGTTCTCACAGTC-3'; 623-bp, F: 5'-GTGGACTTTATCTGGGCAGAAC-3', R: 5'-TCTCCTGAGCTAGGCTGCTTAT-3'; 574-bp, F: 5'-ACGCAGTCACACAGGGAGAA-3', R: 5'-CCCAGCTCAGTAAGCTCAGTG-3'. The 25- μ L PCR system was comprised of a 10X PCR buffer (2.5 μ L), 10 mM dNTP mix (0.5 μ L), 50 mM MgCl₂ (0.75 μ L), 5 μ M forward primer (1 μ L), 5 μ M reverse primer (1 μ L), DNA template (1 μ L), platinum Taq DNA polymerase (0.25 μ L), ddH₂O (13 μ L), and 5*GC buffer (5 μ L). The reaction conditions were as follows: initial denaturation at 94°C for 3 min; 37 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 75°C for 45 s; and a final extension step at 72°C for 5 min.

Identification of PCR products

The PCR products were detected by 2% agarose gel electrophoresis (Figure 1).

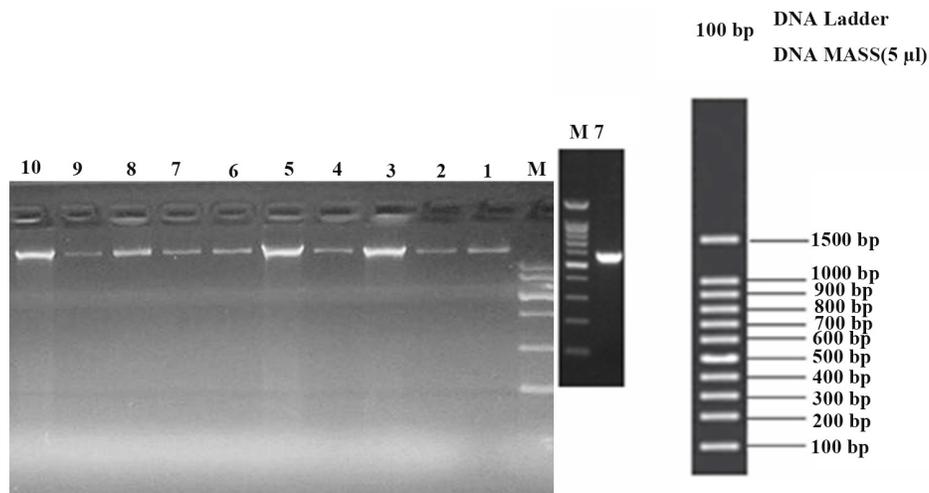


Figure 1. Agarose gel displaying the amplified *TTR* gene extracted from various members of a family with familial vitreous amyloidosis.

Sequencing and analysis

The products were sequenced using a DNA sequencer (3730XL) after purification. The primers were designed and synthesized by Invitrogen. The PCR products were sequenced using the fluorescence marker method, and the sequencing results were analyzed by DNAMAN (for Windows) v.5.2.2.0 and Chromas. The obtained sequence was aligned by comparing with the start codon anterior segment of the *TTR* gene available at the National Center for Biotechnology Information (NCBI).

RESULTS

The results showed that the length of upstream regulation regions in the 10 subjects was in accordance with the length of the reference sequence extracted from GenBank. The sequence alignment revealed the absence of any mutations in 6 of the subjects (4 patients and 2 without disease), which corresponded with the sequencing results. The mutations in the subjects occurred at the same site (-743, A→T). (Figure 2 and 3).

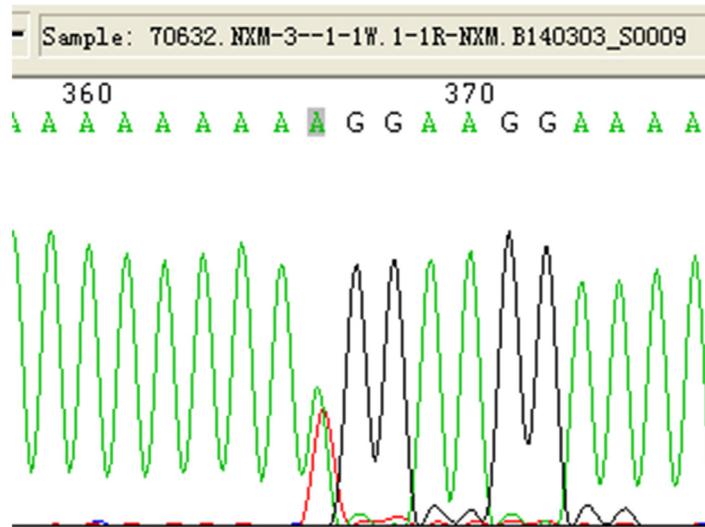


Figure 2. Results of sequencing of the upstream regulatory region of the *TTR* gene in study subjects (-743, A→T).

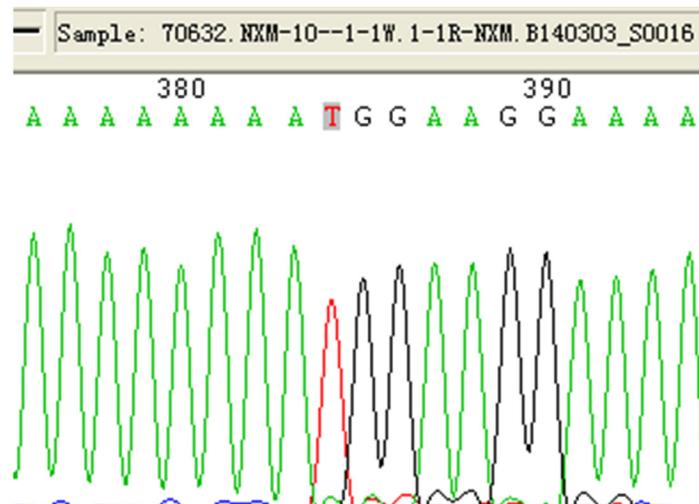


Figure 3. Sequencing results of the upstream regulatory region of the *TTR* gene in controls (-743, AA).

DISCUSSION

Changes to the *TTR* structure have been identified as the main factors inducing familial amyloidosis (Coelho et al., 2012). Domestic reports regarding vitreous amyloidosis in the eye, however, are very few. Familial vitreous amyloidosis was studied in a family from the AnShun region of the Gui Zhou Province, which confirmed that amyloid substances resulted in vitreous opacity. This familial mutation site, located in the third exon of the *TTR* gene, which induced a mutation in the 83rd amino acid (Gly83Arg), was found to be a heterozygous mutation (Xie et al., 2013).

During preparatory analyses, a point mutation (Gly83Arg) of the *TTR* gene was observed in 2 diseased family members. DNA mutation was also not observed in 2 members diagnosed with vitreous amyloidosis. We attempted to validate our theory that the abnormal base sequence or the level of modification in the upstream regulatory region of *TTR* gene could be pathogenic by regulating the non-mutated *TTR* mRNA and protein sequences.

Blood was collected from 10 members of a family with vitreous amyloidosis; the DNA sequencing results showed the presence of a base heterozygous mutation (-743, A→T) in 6 subjects: 4 patients and 2 without disease. Therefore, we believed that vitreous amyloidosis might be associated with a point mutation in the upstream regulatory region of the *TTR* gene.

The gene expression was regulated on multiple levels: the eukaryotic cells were regulated at the DNA and chromosomal, post-transcriptional, translational, post-translational, and mRNA degradation levels; moreover, the cis-acting elements and trans-acting factors, such as the promoter, enhancer, and terminator, were also regulated. Translational regulation was most important in the promoter region (Pedersen et al., 1999). Although promoters also influenced transcription, the site of mutation in the upstream regulatory region of the *TTR* gene was not in the typical TATA-box and GC-box in this study (Zhang and Qi, 2008; Kutach and Kadonaga, 2000).

A promoter prediction software was used to predict the promoter region in the *TTR* upstream regulatory region; the mutation site was found to be located far from the promoter or start codon (-743 bp), and not in promoter region. This basically indicated that changes in the promoter region did not induce corresponding changes in the expression of the regulatory region.

Future studies must attempt to determine the mechanism with which changes in the upstream regulatory region induce changes in the mRNA and protein expression, and identify any direct relationship or causality between these changes and base changes in the upstream region. Moreover, we must attempt to establish if the regulation of gene expression induced changes in the regulatory region or if the opposite holds true.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Science Foundation.

REFERENCES

Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, et al. (2012). Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology* 79: 785-792. <http://dx.doi.org/10.1212/WNL.0b013e3182661eb1>

- Ikeda S, Nakazato M, Ando Y and Sobue G (2002). Familial transthyretin-type amyloid polyneuropathy in Japan: clinical and genetic heterogeneity. *Neurology* 58: 1001-1007. <http://dx.doi.org/10.1212/WNL.58.7.1001>
- Kutach AK and Kadonaga JT (2000). The downstream promoter element DPE appears to be as widely used as the TATA box in Drosophila core promoters. *Mol. Cell. Biol.* 20: 4754-4764. <http://dx.doi.org/10.1128/MCB.20.13.4754-4764.2000>
- Pedersen AG, Baldi P, Chauvin Y and Brunak S (1999). The biology of eukaryotic promoter prediction--a review. *Comput. Chem.* 23: 191-207. [http://dx.doi.org/10.1016/S0097-8485\(99\)00015-7](http://dx.doi.org/10.1016/S0097-8485(99)00015-7)
- Westermarck P, Benson MD, Buxbaum JN, Cohen AS, et al. (2002). Amyloid fibril protein nomenclature -- 2002. *Amyloid* 9: 197-200. <http://dx.doi.org/10.3109/13506120209114823>
- Xie B, Cai SJ and Li H (2013). Gly83Arg mutation of thyroid hormone binding proteins in family vitreous amyloidosis. *Zhonghuayandibingzazhi* 29: 569-571.
- Zhang XH and Qi YX (2008). Analysis of TATA-box, GC-box and CAAT-box in eukaryotic promoter. *J. Anhui Agri. Sci.* 36: 1380-1381.