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Association of E-Cadherin Gene 3′-UTR C/T Polymorphism with Primary Open Angle Glaucoma

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Key Words

E-cadherin · Primary open angle glaucoma · Metalloproteinases · Apoptosis · Polymerase chain reaction

Abstract

Purpose: E-cadherin (E-CDH) is one of the most important cell surface glycoproteins involved in cell morphogenesis. In primary open angle glaucoma (POAG), the extracellular matrixes of trabecular meshwork and lamina cribrosa in the optic nerve head are out of balance. We suspected that E-CDH by way of metalloproteinases is closely related to POAG. We therefore investigated the relationship between CDH-1 gene 3′ untranslated region (3′-UTR) polymorphism and POAG patients in order to support this hypothesis. ***Patients and Methods:*** We enrolled 60 POAG patients and 103 healthy volunteers from the Department of Ophthalmology at the China Medical University Hospital, Taichung, Taiwain, ROC. None of the control subjects had a history of eye disease and all underwent the same examination as the POAG patients. PCR-based analysis of the restriction fragment length polymorphism was used to test the CDH-1 gene 3′-UTR polymorphism. All statistical analyses were performed by the χ^2 test. ***Result:*** There was a significant difference in the distribution of the CDH-1 gene 3′-UTR C/T polymorphism between POAG patients and the normal controls ($p < 0.000$). The odds ratio of the 'C' allele was also significantly different between both groups (odds ratio = 5.510, 95% confidence interval = 3.171–9.574). ***Conclusion:*** CDH-1 is closely related to metalloproteinase and plays an important but not well-understood role in the onset and progression of POAG. The exact role of CDH-1 in POAG could be resolved by the posttranslated products of the gene and the protein-protein interaction of the gene products in the future.

Introduction

The most mentioned genes related to primary open angle glaucoma (POAG) are myocilin (MYOC) gene (chromosome position 1q23–q24) and optineurin gene (chromosome position 10p13). The pathophysiology of POAG is not precisely known. POAG is a multifactorial disease [1, 2], and the assumption that a single gene is involved is not reasonable. POAG may be the result of multiple and interactive genetic and environmental ef-

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fects. There is a recognized increased risk of developing the disease in family members of patients with POAG. Currently, there is a lack of information regarding the genetics of the disease and, hence, the molecular biology of glaucoma is currently under close investigation. In our laboratory, we investigated the relations of many polymorphisms with apoptosis, immune and cell morphogenesis. We have already found some single nucleotide polymorphisms (SNPs) associated with POAG [3, 4], and we tried to map POAG with genetic polymorphisms. E-cadherin (E-CDH) is closely related to matrix metalloproteinases (MMPs) involved in the outflow of aqueous humor in trabecular meshwok. Recently, MMPs have also attracted great attention in treatments and response to prostaglandin analog eye drugs in POAG [5]. Prostaglandin analog eye drops are believed to lower intraocular pressure (IOP) by increasing the uveoscleral outflow of aqueous humor [6]. The effects of these drugs have been proved by eliciting prostaglandin $F_{2\alpha}$ receptor, and produce the maximum level of MMP enzymes, thus clearing out the extracellular matrix. The reduction of collagens within the uveoscleral outflow pathway reduces hydraulic resistance and facilitates outflow [7]. The disorganization of E-CDH complexes and the expression of MMPs are frequently involved in the capacity of tissues. The MMP/E-CDH ratio is a significant independent prognostic factor of many diseases [8]. Therefore, we chose E-CDH as a candidate gene.

Morphogenesis is the process by which cells become organized into distinctive structures and histological patents in the tissues and organs of the body. In seeking to understand the cellular basis of many diseases, it has been postulated that aberrant morphogenetic processes play fundamental roles in the etiology of many diseases.

Cell adhesion is a fundamental determinant of tissue morphogenesis [9–12]. Cadherins (CDHs) play important roles in tissue morphogenesis, and they comprise a large family of Ca^{2+}-dependent, homophilic cell-cell adhesion molecules essential for morphogenic movements and tissue formation during development and for the maintenance of tissue integrity in the adult organism [13–15]. The CDH family comprises a large superfamily of cell surface glyoproteins which have in common a repeated 110-amino-acid subdomain (called the 'cadherin' domain) in their extracellular region [9]. CDH-based adhesion is responsible for physiological regulation of CDHs by aberrant function of the signaling pathways.

E-CDH is found in many tissues and is among the best understood of the CDHs. E-CDH fragments include the extracellular and transmembrane domains. Shedding the E-CDH extracellular domains, which are mediated by metalloproteinases, eliminates the residual adhesive activity and results in a complete disruption of CDH-mediated cell-cell adhesion. Metalloproteinase is a well-known enzyme in the human trabecular meshwork, and is closely involved in the pathogenesis of glaucoma [16–19]. The extracellular matrixes of trabecular meshwork and lamina cribrosa in the optic nerve are in a constant state of turnover, and several studies have suggested that this homeostasis is out of balance in patients with POAG. An increase in collagen synthesis and a decrease in collagen degradation may contribute to the excessive deposit of collagen with loss of the function of trabecular cells during the development of POAG. Recent evidence suggests that MMPs, which are the enzymes primarily responsible for degradation, play a role in many modern glaucoma therapeutic drugs [17, 18]. Direct and indirect regulation of this system increases aqueous humor outflow facility. Besides the function of MMPs in the trabecular meshwork, MMPs can activate microglia and phagocyte which cause the production of cytokines and enzymes that can alter the extracellular matrix of the lamina cribrosa in the glaucomatous optic nerve heads [19]. On the other hand, glaucoma is a complex disease, which may involve mechanical or vascular mechanisms. Regardless of the mechanism, the ganglion cells ultimately die by apoptosis, a process which involves the cleavage of E-CDH. E-CDH helps the extruding of apoptotic cells from cell layers [10]. Glaucomatous optic neuropathy may also indirectly relate to E-CDH in the basis of apoptosis. Thus, we suspected a relationship between E-CDH and POAG.

The human E-CDH gene has been mapped to the long arm of chromosome 16q22.1, which is a site with many stories about genetic events [20, 21]. E-CDH mutations in particular diseases may reflect not only the probability of acquiring an E-CDH mutation but also the extent to which subsequent events preferentially affect the remaining wild-type allele [9]. The C/T polymorphism on the CDH-1 gene 3′ untranslated region (3′-UTR) is one of the most important polymorphisms noted. Due to its relatively high frequency, it may be helpful for further genetic studies [22]. The C/T polymorphism is at sequence nucleotide 2797 of E-CDH cDNA, located 54 nucleotides downstream from the TAG stop codon. The E-CDH cDNA sequence can be found in the EMBL/GenBank database libraries (accession No. Z13009). The attractive relationship between E-CDH and POAG is worthy to be investigated and we tried to analyze this aspect in the molecular field.

Materials and Methods

From May 2000 to July 2000, we enrolled POAG patients from the Department of Ophthalmology at the China Medical University Hospital. All patients in this study received serial ophthalmic examinations which included IOP, visual acuity, automated perimetry, gonioscopy, optic disc examination and retinal examinations. The volunteers in the control group were selected from the patients who received routine physical examination and were examined by the same ophthalmologist. Volunteers were all free of any systemic diseases including cardiovascular, reproductive and urologic diseases. Volunteers suspected of having glaucoma were excluded from the study. Patients with ocular diseases other than POAG were also excluded from our study. All patients included in this study had POAG and met at least one criterion from each of the following categories.

(1) Visual field criteria: (a) at least two abnormal visual field tests by Humphrey automated perimetry, as defined by computer-based objective criteria, and (b) the presence of one or more absolute defects in the central visual field 30°, with ophthalmologic interpretation as glaucomatous visual field loss.

(2) Optic disc criteria (optic disc damage present in fundus photographs): (a) either a horizontal or vertical cup-to-disc ratio of 0.6 or more, and (b) narrowest remaining neuroretinal rim was $\leq 20\%$ of disc diameters.

Patients with other possible causes of disc and field changes than POAG were excluded.

We investigated the CDH-1 gene 3′-UTR in all subjects. The prevalence of the polymorphism was compared between the control group and patient group. Odds ratio (OR) was used to calculate the frequencies of the different alleles. This study was carried out with approval from the Human Study Committee of the China Medical University Hospital. Informed consent was obtained from all patients who participated in this study.

The POAG group consisted of 60 patients and the control group was made up of 103 healthy volunteers in this study. The volunteers ranged in age from 52 to 71 years (mean: 50 years), and were free of any ophthalmic diseases. The volunteers were all Chinese and unrelated. There were 51 females and 52 males. The POAG patients ranged in age from 20 to 70 years (mean: 55 years) and were unrelated. There were 30 females and 30 males.

The genomic DNA was prepared from peripheral blood by a DNA Extractor WB kit (Wako, Japan). Polymerase chain reactions (PCRs) were carried out to a total volume of 50 μl, containing genomic DNA, 2–6 pmol of each primer, 1 × Taq polymerase buffer (1.5 m*M* $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, Calif., USA). The primer for the E-CDH gene 3′-UTR polymorphism was designed by amplifying the created restriction site: forward (5′-CAGACAAAGACCAGGACTAT-3′) and reverse (5′-AAGGGAGCTGAAAAACCACCAGCCAC-3′). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer). The cycling condition for CDH-1 was set as follows: one cycle at 94°C for 5 min, 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, and one final cycle of extension at 72°C for 7 min.

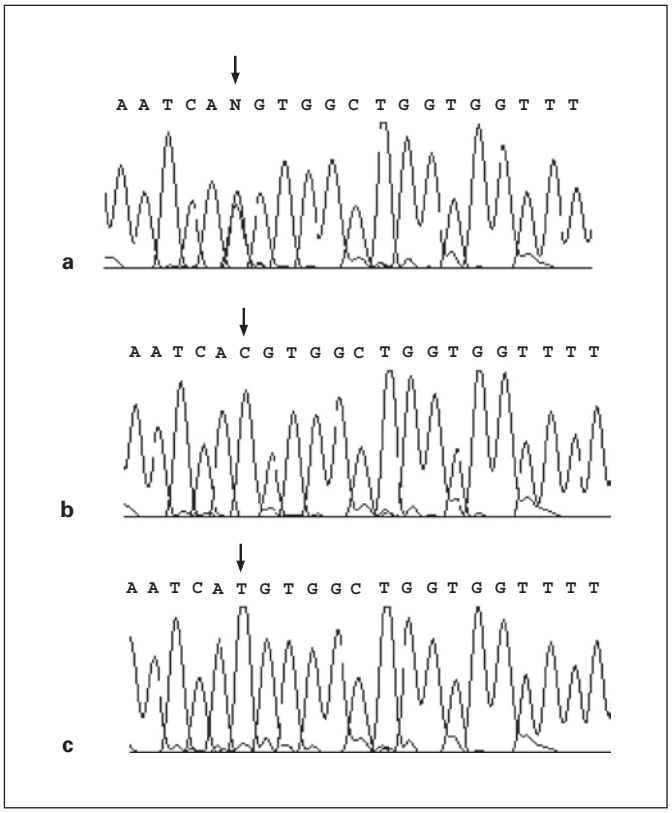
The PCR product of 172 bp was mixed with 2 units *Pml* I (New England Biolabs, Beverly, Mass., USA) and the reaction buffer according to the manufacturer's instructions. For future reference, two fragments of 146 bp and 26 bp will be present if the product is digestible. The reaction buffer and PCR product were incubated at 37°C for 3 h. Then, 10 μl of the product were loaded onto 3% agarose gel containing ethidium bromide for electrophoresis. The resulting products were classified as digestible (CC homozygote), undigestible (TT homozygote) and combined C/T heterozygote. The frequency of allele distribution in this polymorphism between the control and POAG patient groups was compared with the χ^2 test. The software used for the calculation was the SPSS® system with sample power analysis. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($p < 0.05$). ORs with 95% confidence intervals (CIs) were calculated in order to estimate the risk of suffering POAG between genotypes.

Results

All patients were followed up for 2–8 years (average: 5 years). Ten of the patients received trabeculectomy and 2 of the 10 patients underwent trabeculectomy twice at different sites. Fifty patients in the POAG group controlled IOP by topical drugs. Each patient used an average of 1.3 types of antiglaucomatous drugs. Nine patients did not require drugs to control IOP following trabeculectomy.

The bands on the gel revealed digested (CC) homozygotes, undigested (TT) homozygotes and (C/T) heterozygotes (fig. 1d). For quality control, we sequenced the PCR products in order to define the polymorphism (fig. 1a–c). The frequencies of the genotypes in the POAG group and the control group are shown in table 1. The allelic frequency of 'C':'T' was 47.6:52.4% in the control group and 83.3:16.7% in the POAG group (table 1). The distribution of the E-CDH gene 3′-UTR C/T polymorphism showed statistical differences in the distribution of genotype frequencies between POAG patients and normal controls ($p < 0.000$).

The OR was significantly different between the normal control and POAG patient groups in C allele frequency (OR = 5.510, 95% CI = 3.171–9.574). The frequency distribution of the C allele was significantly higher in the POAG group than in the normal control group ($p < 0.000$). The OR was also significantly different between the two groups in the frequency of the CC and CT genotype (OR = 40.089, 95% CI = 13.919–115.466), as well as of the CC and TT genotype (OR = 82, 95% CI = 8.594–782.371). The frequency distribution of the CC homozygote was significantly higher in the POAG group than in the normal control group ($p < 0.000$). OR was still significant when analyzed by methods of regression according to age. We also calculated 'power' to test of the null hypothesis by SPSS. Under the consideration of the genotype CC



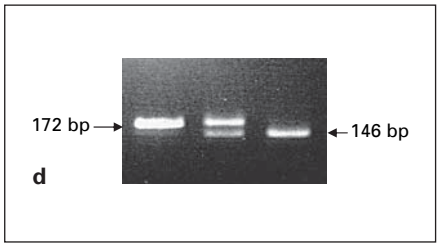


Fig. 1. a–c The sequence of the E-CDH 3′-UTR C/T polymorphism. **d** The PCR products of the E-CDH gene 3′-UTR C/T polymorphism on 3% agarose gel.

Table 1. Distribution of the E-CDH gene 3′-UTR C/T polymorphism between healthy control subjects and POAG patients

	CC	CT	TT	C, %	T, %
Control group	5	88	10	47.6	52.4
POAG patients	41	18	1	83.3	16.7
Odds ratio					
Unadjusted	82.0*	2.04	1	29.4 (11.1–78.1)*	
Age-adjusted	88.2*	1.7	1	36.1 (12.3–105.3)*	

Figures in parentheses indicate 95% CIs. * $p < 0.05$.

homozygote, there is a power of 100% to yield a statistically significant result in this sample size (table 1).

Discussion

Cell-cell adhesion plays an essential role in organogenesis, physical transport, signal transmission and immunological function in multicellular organisms. CDHs are single-pass transmembrane glycoproteins that associate as cis-dimers on the cell surface and then combine to form a linear zipper-like structure which promotes homophilic intercellular adhesion. MMP is required for the efficient cleavage of E-CDH during apoptosis.

In this study, the 'C' allele (C allele frequency was significantly higher in the POAG group than in the normal control group: $p < 0.000$) and 'CC' genotype of the E-CDH gene 3′-UTR gene polymorphism (CC homozygote frequency was significantly higher in the POAG group than in the normal control group: $p < 0.000$) are useful markers for Chinese POAG. We suspect this allele disturbs the effect of E-CDH in the trabecular meshwork and even the lamina cribrosa of the optic nerve head. Moreover, the disturbed effects of E-CDH may change the balance of E-CDH and MMPs, causing unbalance of aqueous humor outflow in the trabecular meshwork and changing the resistance of the lamina cribrosa of the glaucomatous optic nerve. Besides, E-CDH itself has a close relationship with apoptosis, which also plays an important role in the onset and progression of POAG.

The roles of the genetic polymorphism may include direct causation, linkage disequilibrium, natural selection and population stratification. Therefore, we are not going to conclude that the 'C' allele of E-CDH gene 3′-UTR polymorphism is the direct cause of POAG; we can just indicate that there is an association. As we know, 3′-UTR is not the translated area of the protein, the exact role E-CDH plays in the development of POAG is unknown and other factors may be added to explain the effects of the 'C' allele gene polymorphism on POAG. The understanding of E-CDH gene 3′-UTR is limited. Keirsebilck et al. [23] proposed that the E-CDH 3′-UTR sequence may trigger mRNA instability and downregulation of the expression of E-CDH in mesenchymal tumor cells. However, there are many examples that 3′-UTR may change the expression of genes, such as p53 3′-UTR that contains an Alu-like repetitive element and a deletion of the distal end of the p53 3′-UTR which increased the efficiency of translation [24]. 3′-UTR is also critical for the cAMP-mediated Na^+-coupled glucose cotrans-

porter SGLT1 message stabilization [25]. The exact role of the 'C' allele of the E-CDH gene 3′-UTR polymorphism needs to be resolved by advanced studies such as 'proteomics', that is, the posttranslated products of the gene and the protein-protein interaction of the gene-related proteins may be needed to solve this question in the future.

SNPs have important implications in human genetic studies. The screening for such alleles helps in the detection of a genetic predisposition to a disease. The presence of a specific SNP allele can be implicated as a causative factor of a genetic disorder. Besides, understanding the associated polymorphism is expected to increase the understanding of the course of a disease. Knowing the genetic pathway of POAG is important for designing new treatments for glaucoma.

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