Transcription Factors Zic1 and Zic2 Bind and Transactivate the Apolipoprotein E Gene Promoter*

Enrique Salero†, Raquel Pérez-Sen‡, Jun Aruga§, Cecilio Giménez‡, and Francisco Zafra‡

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Apolipoprotein E (apoE) is a major component of various classes of plasma lipoproteins. It is a single-chain polypeptide synthesized in the liver and also produced in extrahepatic tissues such as adrenals and nervous system. In the rat brain, the synthesis takes place in astrocytic cells (2, 3), whereas in peripheral nervous system apoE is synthesized by nonmyelinating glial cells and resident macrophages (2). In the human brain, the synthesis occurs both in glial cells and in subpopulations of neurons in the cortex and hippocampus (4). A number of previous in vitro and in vivo studies as well as recent experiments with apoE-deficient mice and human APOE transgenic mice reveal that apoE plays an important role in neuronal maintenance and repair (5–12). Genetic studies have identified the apoE4 allele as a major risk factor for developing Alzheimer’s disease (AD), both in sporadic and in familial late onset forms of the disease (13, 14). This allele is also responsible for poor outcome after acute brain injury (15, 16), stroke (17), or neurotoxic damage (12).

ApoE synthesis is regulated in hepatic and steroidogenic cells by complex interaction of developmental, hormonal, and dietary factors (18–22). The regulatory complexity emerges from interactions of a number of proteins which bind to proximal regions of the APOE gene promoter, as well as to far downstream elements involved in its tissue-specific expression (23–30). In brain, the regulation of this gene remains largely unexplored, despite its importance in processes of degeneration and regeneration of the nervous system. The importance of the regulatory region of the APOE gene in the determination of the apoE levels in the brain is emphasized by the recent identification of a number of polymorphisms within the promoter (31, 32). Some of these polymorphic sites are associated with functional changes in the activity of the promoter and with increased risk of AD (33–35). We have initiated a search of possible regulatory proteins that bind to proximal regions of the APOE gene promoter. Previously, we identified the transcription factor AP-2 as a mediator of the cAMP stimulation of apoE synthesis in glial cells (36). In the present report, we analyze the regulatory region that lies between −163 and −124, a region that contains positive regulatory elements in HELA, HepG2, COS, and U87 cells (23, 24, 36, 37). We identify transcription factors Zic1 and Zic2 as positive transcriptional regulatory elements for the APOE gene.

Experimental Procedures

Reporter Constructs for Library Screen—The following oligonucleotides TCGGGGCTCTATGCCCGCCTTCCCTCTCCCTGCCCCTGCTGGTGC and CCCAGGCAACAGAGGGAGGAAGGGAGGTGGGAGCATAGGC, containing the described previously URE1 region (24) of the APOE promoter were synthesized and annealed. The annealed oligonucleotide displayed overhanging ends (underlined) to promote oriented oligomerization upon ligation in the plasmid pAE4 (38). A fragment containing five tandem repeats was subcloned into the yeast reporter plasmids, pHSI-1 and pLaZi (CLONTECH, Palo Alto, CA), yielding plasmids APOE1-pHS-1 and APOE1-pLaZi, respectively.

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† The abbreviations used are: apoE, apolipoprotein E; APOE, human apolipoprotein E gene; EMSA, electrophoretic mobility shift assay; AD, Alzheimer’s disease; Zic, zinc-binding site; PAGE, polyacrylamide gel electrophoresis; GADPH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; RT, reverse transcription; bp, base pairs; BMP, bone morphogenetic protein.
The reporter constructs were subsequently linearized and sequentially integrated into the yeast strain YM4271. Yeast were transformed first with APOE1-pLacZi, followed by APOE1-pPHIS-1. Transformants selected on leu- ura- minimal medium were used as a dual reporter host yeast strain for the library screen.

**Screening of the cDNA Library**—The host yeast strain was transformed with a MATCHMAKER human brain cDNA library constructed in the pGAD10 vector (CLONTECH) by the LiAc/polyethylene glycol method. Approximately 5 × 10⁶ transformants were plated per 150-mm dish containing his- leu- minimal selective medium supplemented with 15 μg 3-aminotriazole. Approximately 2.5 × 10⁶ cDNA plasmids were screened using the filter replica method. Ten clones showed strong positive clones were selected. These clones were tested for β-galactosidase activities using the filter replica method. Ten clones showed strong blue color compared with the host strain. Plasmids were recovered from His“−/LacZ” colonies by transformation into DH15a cells. The plasmids were partially sequenced and the nucleotide sequences were compared with sequences in the GenBank/EMBL data bases using the Fasta program.

**Purification of Recombinant His-Zic1 and His-Zic2**—To produce histidine-tagged Zic1 and Zic2 proteins (His-Zic1 and His-Zic2), we prepared bacterial expression constructs for both proteins. First, the Zic-coding cDNA inserts from the pGAD10 cloning vector, isolated after the one-hybrid procedure, were transferred into the BamHI/BglII sites of the prokaryotic expression vector pTrcHisA (Invitrogen Inc, San Diego, CA). Then, *Escherichia coli* BL21 strain was transformed with the expression constructs and grown in LB culture medium containing 50 μg/ml ampicillin and 70 μg/ml chloramphenicol. Expression was induced by addition of 1 mM isopropyl-β-thiogalactoside for 5 h. Bacteria were collected and lysed by sonication in 50 mM NaHPO₄ 10 mM imidazole, 1 mM phenylmethylsulfon fluoride, 0.5 mg/ml lysozyme. Fusion proteins were purified with Ni-NTA resins (Qiagen Inc, Valencia, CA) using a batch protocol as recommended by the manufacturer.

**Electrophoretic Mobility Shift Assay—Oligonucleotides** were 5′-end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. Recombinant transcription factors (0.5–1 μg) were incubated for 15 min at room temperature in 20 μl of binding buffer 5 mM (Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM MgCl₂, 0.5 mM EDTA, 10 mM ZnCl₂, 1 mM diethiothreitol, supplemented with 2 μg/assay poly(dC-dC-poly(dI-dC)). Where indicated, competitor oligonucleotides were included during the preincubation period. Labeled oligonucleotide (1 ng/binding reaction; 100,000–200,000 cpm) was then added, and the mixture was incubated for 30 min at room temperature. The incubation mixture was electrophoresed on 4% polyacrylamide gels containing 0.35 × Tris borate-EDTA buffer at constant voltage (100 V) for 3–4 h. Gels were dried and autoradiographed.

**Plasmid Constructions**—The luciferase reporter plasmid pXP2 (39) was used to harbor different fragments of the APOE promoter. The fragments were generated by PCR using oligonucleotides from the desired regions as primers and the APOE-pCRII construct (36) as a template. Amplified fragments were ligated to the pCRII vector (Invitrogen, San Diego, CA), and the identity was confirmed by sequencing. Fragments were subcloned in the MCS of pXP2, in front of the luciferase reporter gene. Mutation were introduced by PCR using mutant oligonucleotides as described (40). Mouse Zic1 and Zic2 expression vectors were prepared by cloning the full-length genes into the pEBOS vector as described.³

**Cell Culture and Transfections**—U87 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. The day before transfection, confluent cells were subcultured by trypsinization and 1–3 × 10⁶ cells/well were plated in 24-well tissue culture plates. For transient transfections, cells were transfected with 0.5 μg of DNA/well by the calcium phosphate method using the CalPhos™ mammalian transfection kit (CLONTECH) according to the instructions of the manufacturer.

For stable transfection U87 cells were transfected by electroporation with Zic cDNAs cloned into the expression vector pcDNA3 that carries the neomycin resistance gene for selection. After selection with 0.2 mg/ml ampicillin and 70 μg/ml chloramphenicol. Oligo(dT)16 was used for the first strand cDNA syntheses. The following oligonucleotides were used in the PCR amplification steps: APOE F, GCCAGCTCCTGGACGCCGGAC; APOE R, GGAGCCTGCAGCACTGAAGGGCTG; Zic1 F, GCACCTCCTCTGGACGGGGGGC; Zic2 R, CTCGTACAGGA-GACAGTTGG; GADPH F, CCCACATGGAAATTTCCATGGCA; GADPH R, TTAGAAGCACTGAGTGCTGCCAC. The resultant cDNA fragments were resolved by electrophoresis on a 2% agarose gel and visualized under UV illumination.

**Analysis of Protein Expression**—Cells grown to confluence were lysed in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfon fluoride. The protein concentrations were determined with the Bio-Rad protein assay reagent, and SDS-PAGE was performed in the presence of 2-mercaptoethanol. Samples were transfected by electroporating into a nitrocellulose membrane in a semidyry electroblootting system (Life Technologies, Inc.). Nonspecific protein binding to the blot was blocked by incubation of the filter with 3% nonfat milk in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 7H₂O, 1.4 mM KH₂PO₄, pH 7.3) containing 0.1% Triton X-100. The blot was probed with anti-apoE antibody (Calbiochem, Bad Soden, Germany) diluted 1:5000 for 1 h at room temperature. After washing, the blot was probed with a peroxidase-linked anti-goat IgG. Bands were visualized with the ECL detection method (Amersham Pharmacia Biotech).

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FIG. 1. Binding of recombinant His-Zic1 and His-Zic2 to the bisulfotodeotide. Gel mobility shift assays were performed with 1 ng of 32P-labeled −163−124 DNA fragment incubated with 500 ng of bacterial control extract (Control Extract), purified His-tagged Zic1 (His-Zic1), or purified His-tagged Zic2 (His-Zic2).
RESULTS

Isolation of cDNA Clones Encoding APOE Promoter-binding Proteins—We are interested in identifying transcription factors that could regulate the expression of the APOE gene in the nervous system. The yeast one-hybrid system was used to screen for human brain cDNAs encoding for proteins that bind to sequences of the APOE promoter located upstream of the TATA box, a region of the promoter that has been previously shown to contain a number of functional promoter elements (23, 24, 36, 37). Five tandem copies of the sequence spanning 2163 to 2124 were cloned upstream of a minimal GAL4 promoter either in pHIS1 or in pLacZi reporter plasmids and integrated into the yeast genome of YM4271 (his3, leu2). A hybrid expression library (MATCHMAKER) consisting of human brain cDNAs fused to the GAL4 activation domain was then screened to identify proteins that bound to the APOE promoter and activated HIS3 transcription from the reporter construct. Transformants growing on selective medium were assayed for β-galactosidase expression. After screening 2.5 million independent colonies, 10 transformants produced strong blue color on filter assay after 1 h of incubation in the presence of 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside. Plasmids of these transformants were then recovered and sequenced. Sequence analysis indicated that five clones corresponded to different portions of the transcription factor Zic1 and five to the closely related Zic2. The longest Zic1 clone lacked regions encoding the 72 amino acids of the amino terminus as well as 10 amino acids in the carboxyl end of the open reading frame of the human gene. The longest Zic2 clone spans from amino acid 182 to 530. Both clones contained the respective zinc finger domains and were in frame with the activation domain of GAL4.

The Bait Oligonucleotide Forms Specific Complexes with Recombinant Zic Proteins—To confirm the presence of a Zic-binding site in the APOE gene promoter, we first produced His-tagged Zic1 and Zic2 proteins by transferring the Zic-coding inserts from the yeast vector pGAD10 to the prokaryotic expression vector pTrcHisA. The recombinant proteins were then purified with nickel-containing resins. The binding activity of the recombinant proteins was assayed by EMSA with the 32P-labeled, double-stranded bait oligonucleotide (bp 2163/2124). We detected the formation of stable complexes of this DNA fragment with both His-Zic1 and His-Zic2, but not with control bacterial extracts (Fig. 1). The binding of His-Zic1 was specifically competed by increasing amounts of the unlabeled bait oligonucleotide (Fig. 2, lanes 5–7). Interestingly, the binding was also competed by double-stranded oligonucleotides derived from adjacent regions of the APOE promoter spanning bp −70 to −40 and bp −188 to −169 (Fig. 2, lanes 2–4 and 8–10), suggesting the existence of additional Zic1 binding elements in the promoter. The binding was not displaced by an excess of unlabeled oligonucleotides −39/+1 and −113/−80 (data not shown) or by an unrelated oligonucleotide (Fig. 2, lane 11). Identical results were obtained by using His-
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Mutational analysis of −143/−124 region of the APOE promoter—To determine more precisely the nucleotide sequence required for binding of Zic proteins, a number of double-stranded 20-mers included within the displacing oligonucleotides were synthesized and assayed in EMSA experiments. DNA-protein complexes were only observed when we used the DNA fragments labeled with an asterisk in Fig. 2, and these were selected for further mutational analyses. Several substitution mutations were introduced within these oligonucleotides, and then used in EMSA experiments. Fig. 3 shows results of mutagenesis in DNA fragment −143/−124. Mutation in the 5′ end of this fragment produced a marked decrease in His-Zic1 binding with respect to control, although the retarded DNA-protein complex was clearly detectable (Fig. 3, lane 2). However, when bases from −136 to −125 were exchanged in groups of three for adenine, binding of His-Zic1 was severely impaired and the retarded complexes were undetectable (lanes 3–6). Similar results were obtained when His-Zic2 was used in the assays (data not shown). These experiments define the binding site for Zic proteins within the initial bait. Binding sequence was also investigated within the DNA fragment spanning −69 to −50. As shown in Fig. 4, binding of both His-Zic1 and His-Zic2 was disrupted by mutations in bases located between −65 to −54 (lanes 3–6 and 10–13). Mutations in bases −69 to −66 and −50 to −53 had a weaker effect on binding of both proteins (lanes 2, 7, 9, and 14) as compared with the respective controls (lanes 1 and 8). Finally, another site was defined for both recombinant proteins within the DNA fragment −188/−169. Mutations in positions between −185 and −174 completely abolished the formation of retarded complexes (Fig. 5, lanes 3–6 and 10–13), whereas mutations in bases −173 to −171 produced a weaker reduction of binding (Fig. 5, lanes 7 and 14). In summary, the three binding sites described above were termed Zic-BS1 (−65/−54), Zic-BS2 (−136/−125), and Zic-BS3 (−188/−174), according to their positions with respect to the transcriptional starting point.

Transcriptional Activation of the APOE Promoter by Zic Proteins—To analyze the ability of Zic proteins to stimulate transcription from the APOE promoter, and to assess the relative contribution of each binding site to the measured activities, several APOE promoter-luciferase constructs were prepared and cotransfected with an expression vector containing the full-length coding region of the mouse Zic1 or Zic2 genes into the glioblastoma cell line U87. Along with the test constructs, each plate was cotransfected with a β-galactosidase expression vector that served as an internal reference for transfection efficiency. Luciferase and β-galactosidase activities were determined 48 h later. The luciferase activity was strongly stimulated by both Zic1 and Zic2 in a construct containing the Zic-BS1 (−70/+1) as compared with controls (6 and 27.9 times, respectively) (Fig. 6). The stimulatory effect was greatly reduced after mutation of this site (construct −70/+1 m(−62/−57)), although not completely abolished (the activity for Zic1 and Zic2 was 4 and 8 times over controls, respectively) (Fig. 6). However, the promoter activity was more severely impaired in the deletion mutant −60/+1 (−60/+1 in Fig. 6) (1.4 and 2.7 over controls, respectively), suggesting that the mutant −70/+1 m(−62/−57) still conserved some binding capability. Both transcription factors also stimulated the luciferase activity in a longer construct including also Zic-BS2 (bp −143 to −1) (Fig. 6, construct −143/+1), although to a lower extent than construct −70/+1 (4.9 and 19.9 times over controls, respectively). Mutation in the Zic-BS2 decreased the stimulation mediated by Zic1 and Zic2 by more than half (2.3 and 6.5 times stimulation over controls respectively) (Fig. 6, construct −143/+1 m(−133/−129)). The contribution of the Zic-BS3 was analyzed in construct −189/+1. Both factors stimulated the luciferase activity by 5.2 and 12.6 times, respectively, in the wild form of this construct (Fig. 7, construct −189/+1). Mutations in Zic-BS3 decreased drastically the activity of the promoter (to 2.3 and 2.5 times over controls, respectively) (Fig. 7, construct −189/+1 m(−182/−177)), indicating that in the context of this construct the Zic-BS3 seems to be preponderant over Zic-BS2 and Zic-BS1. Nevertheless, these sites also contributed to the observed activity as the stimulation of the promoter activity was between 60 and 75% lower in mutants of Zic-BS2 or Zic-BS1 in the context of construct −189/+1 (Fig. 7, constructs −189/+1 m(−133/−130) and −189/+1 m(−55/−50)), when compared with the stimulation observed in the wild type version of this construct (construct −189/+1). Moreover, the Zic1- and Zic2-mediated stimulation of the promoter activity was completely abolished in a mutant in which the three binding sites were eliminated (Fig. 7, construct −189/+1 m(−133/−130)).

Zic Proteins Also Stimulate the Endogenous APOE Gene Expression—We also investigated the effect of Zic proteins on the expression of the endogenous APOE gene in the glioblastoma cell line U87. First we generated stably transfected U87 cell lines that constitutively expressed either Zic1 or Zic2. The expression of mRNA for Zic1 or Zic2 was analyzed by RT-PCR (Fig. 8A, upper panel). Only stably transfected U87 cells (Zic-1U87 and Zic2-U87 cell lines) expressed the corresponding Zic mRNA. The expression of apoE mRNA was also assayed by RT-PCR in mock-, Zic1-, and Zic2-transfected U87 cells and compared with that of two other cell lines, the glioblastoma SW1088 and the hepatoma HepG2. ApoE mRNA was detected in all cell lines except in the parental mock-transfected U87
These observations were paralleled by protein expression profile obtained by Western blot analysis with a specific anti-apoE antibody (Fig. 8B). ApoE appeared in blots as a band with the same electrophoretic mobility as the recombinant apoE (34 kDa) (Fig. 8B, rApoE). Whereas the expression in mock-transfected cells was undetectable, the expression in the Zic1-U87 and Zic2-U87 cell lines was greater (Fig. 8, Zic1-U87) or similar (Fig. 8, Zic2-U87) to the endogenous expression levels observed in the glioblastoma SW1088, and slightly lower than that measured in HepG2 cells.

Fig. 4. Mutational analysis of -69/-50 region of the APOE promoter. A, diagram of the mutations introduced into the -69/-50 double-stranded oligonucleotide. B, electrophoretic mobility shift assays were performed with 1 ng of the indicated 32P-labeled DNA fragments incubated with 500 ng of recombinant His-Zic1 or His-Zic2.

Fig. 5. Mutational analysis of -188/-169 region of the APOE promoter. A, diagram of the mutations introduced into the -188/-169 double-stranded oligonucleotide. B, electrophoretic mobility shift assays were performed with 1 ng of the indicated 32P-labeled DNA fragments incubated with 500 ng of recombinant His-Zic1 or His-Zic2.
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FIG. 6. Promoter activity of Zic-BS1 and Zic-BS2. U87 cells were transiently transfected with the indicated apoE-luciferase constructs, a β-galactosidase expression vector, and the empty pEBOS vector (■), or pEBOS-Zic1 (□), or pEBOS-Zic2 (□). Promoter regions are represented as boxes. Mutations in the Zic-binding sites are represented as discontinuities in boxes. Luciferase activities were determined after 48 h and normalized for transfection efficiency as measured by β-galactosidase. For each construct the results are relative to values measured in pEBOS-transfected cells. Values represent means ± S.E. of at least two triplicate determinations.

FIG. 7. Promoter activity of Zic-BS3. U87 cells were transiently transfected with the indicated apoE-luciferase constructs, a β-galactosidase expression vector, and the empty pEBOS vector (■), or pEBOS-Zic1 (□), or pEBOS-Zic2 (□). Promoter regions are represented as boxes. Mutations in the Zic-binding sites are represented as discontinuities in boxes. Luciferase activities were determined after 48 h and normalized for transfection efficiency as measured by β-galactosidase. For each construct the results are relative to values measured in pEBOS-transfected cells. Values represent means ± S.E. of at least two triplicate determinations.

DISCUSSION

In the present report, we identified transcription factors Zic1 and Zic2 as potent transcriptional activators of the human APOE gene. This regulatory activity is mediated by three Zic-binding sites located in the proximal region of the APOE promoter. These sites were identified by screening a human brain library, by the yeast one-hybrid technique, by band mobility shift experiments, and by functional assay of luciferase reporter gene activity in various constructs of the APOE promoter. The Zic-mediated stimulation not only was observed on artificial APOE promoter constructs, but also on the endogenous gene in a human glioblastoma cell line. The determination of the molecular mechanisms involved in the regulation of apoE synthesis in brain is a matter of importance, as this protein seems to be involved in processes of brain repair after traumatic injury or in the pathogenesis of AD (13, 14, 42).

Zic1 and Zic2 belong to the Cys2-His2 family of zinc finger transcription factors and are the orthologues of the Drosophila gene odd-paired (44). They seem to be important genes in the embryonic development. The expression of both proteins is observed in the gastrula stage. Later, the expression becomes restricted to the neural tube, where Zic2 seems to play an essential role in neurulation (45). Indeed, mouse lacking this gene show incomplete closure of the neural tube, producing holoprosencephaly and spina bifida as well as alterations in digits and vertebrae (46). In the human, mutations in ZIC2 gene are associated with holoprosencephaly (47). Later, the expression in adults is mainly observed in the cerebellum, although Zic1 is observed in other areas of the brain, and Zic2 has also been reported in testis (48). Zic proteins are also detected in a number of tumor cells (48, 49). Previous studies have shown that Zic1 is able to bind to the consensus sequence of the closely related Gli proteins (50). The present report indicates that the sequence for Zic binding is rather ambiguous, although there are changes of a few nucleotides that are deletori for the binding. The triplet CTG, followed by a GC-rich region occurs in the three Zic-binding sites of the APOE promoter. Both proteins bind similarly to these sites, although, in functional studies, Zic2 behaved as a more potent activator of the promoter than Zic1. Despite the similarity for the target sequence for both proteins that would suggest a functional redundancy, the phenotype of the Zic1 mutant mice differs markedly from that of Zic2 mutant (45, 51). Further clarification of the target genes would be necessary.

The region of the promoter used in this study had been previously identified as the binding site for a number of proteins in several cell types, although never in nervous cells. For instance, the region from −161 to −141, included in our original bait and upstream to the Zic-BS2 (bp −136 to −125), was protected in DNase I footprinting experiments by nuclear extracts of HepG-2, HeLa, and Chinese hamster ovary cells, defining the regulatory element URE1 (24). This sequence was able to bind two transcription factors in a competitive fashion, one of which was identified as Sp1, the other remaining unidentified (37). Additionally, the Zic-BS1 is flanked by a Sp1-binding site and the Zic-BS3 overlaps with a third Sp1-binding site (37). In extracts of HeLa and HepG2 cells, the protein BEF-1 has been identified as a negative regulator that binds to the −94/−84 sequence (27), and an unidentified protein was reported to bind to URE3 between −89 and −101 (28). Finally, a binding site for AP2 overlaps with Zic-BS1 and is responsible...
for the cAMP-mediated induction of apoE in glial cells (36). These results indicate that this region proximal to the APOE gene promoter must play a critical role in controlling the expression of apoE. Whether Zic proteins interact with some of these factors remains to be determined.

We do not know whether Zic proteins will play a functional role in the expression of apoE in the mature brain. In the adult, both proteins do not share the expression pattern. However, the situation could be different in the developing embryo. ApoE is highly expressed in several developing organs, including brain, eye, or bone (52). ApoE is expressed during osteogenic and chondrogenic differentiation of murine mesenchymal progenitor cells, where it seems to be induced by bone morphogenetic protein-2 (BMP2) (52). Interestingly, a BMP2/4-mediated regulation has been suggested for Zic2 in the differentiating bone of the limb buds (45), and thus, the inductive action of BMP2 on the APOE gene could be mediated by Zic2.

Another relevant relationship between Zic2 and APOE gene could be found in holoprosencephaly. It is known that some alterations in the metabolism of cholesterol such as inhibition of the 7-dehydrocholesterol reductase (53, 54), or alterations in the apoE receptor megalin (55) result in holoprosencephaly. Although it is thought that some of these alteration in the metabolism of cholesterol result in alterations in the posttranscriptional processing of sonic hedgehog and consequently in holoprosencephaly (56), we suggest that alterations in apoE could also play a role in those cases of holoprosencephaly that are mediated by mutations in the Zic2 gene.

ApoE plays a crucial role in a number of physiological processes, including cholesterol transport in peripheral circulation (57) and central nervous system (43). ApoE is also involved in the response to neural injury (43, 58, 59), maintenance of dendritic arborizations (60), and neuronal remodeling in vitro (61, 62) and in AD (63). The recent association of different polymorphisms in the promoter region with AD (32–35) strongly suggests that transcriptional regulation of APOE gene may play an important role in the development of this deleterious disease. Thus, identification and characterization of the transcriptional machinery involved in the regulation of apoE expression may be relevant to devise a possible pharmacological modulation of apoE levels. For instance, recent observations suggest that the protective effect of estrogens, which transcriptionally activates of this gene.

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