J Hum Genet (2000) 45:250–253

SHORT COMMUNICATION

Renate Zippert · Andrea Bäßler · Stephan R. Holmer
Christian Hengstenberg · Heribert Schunkert

Eleven single nucleotide polymorphisms and one triple nucleotide insertion of the human TGF-β III receptor gene

Received: December 22, 1999 / Accepted: February 25, 2000

Abstract We found 11 single nucleotide polymorphisms and one triple nucleotide insertion in the cDNA of the human transforming growth factor β (TGF-β) III receptor gene (*TGFBR3*) located on 1p33–p32, encoding betaglycan, a component of the TGF-β receptor system. Inside the 5′ untranslated region (UTR), a G→A polymorphism was identified at position 311. In the open reading frame (ORF), a non-conservative T→C polymorphism was identified at position 392, and three conservative polymorphisms were found at positions 563 (G→A), 1548 (G→A), and 2370 (C→T). A triple nucleotide insertion (GCA) was identified at position 1419. Inside the 3′ UTR, six polymorphisms were identified: four G→A, at positions 2918, 3055, 3098, and 3355; one T→A, at position 3183; and one G→C, at position 3966. In addition to these changes, some divergences from the published sequence were observed in all 12 chromosomes tested. These included, in the ORF, an additional C after position 555, two additional G after position 563, and an additional T after position 1388. No T was found at position 1394. The alterations translate to a changed amino acid sequence. Inside the 3′ UTR, additional discrepancies were identified. The discovered changes and polymorphisms may be useful for further genetic studies of *TGFBR3* receptor deficiencies.

Key words TGF-β III receptor gene · *TGFBR3* · Polymorphism · Chromosome 1p33–p32

Introduction

Transforming growth factor-β (TGF-β) belongs to a family of multifunctional proteins which controls cell growth, differentiation, and morphogenesis in many different cell types (Roberts and Sporn 1990; Massagué 1990). Signal transduction is mediated through a receptor complex, consisting of TGF-β-receptors I and II (*TGFBR1/TGFBR2*), both containing a serine-threonine kinase domain, and the receptor-associated proteins betaglycan (*TGFBR3*) and endoglin (Lopez-Casillas et al. 1991; Buske et al. 1998). The function of *TGFBR3* is not fully clarified; however, evidence suggests that it supports the binding of TGF-β to the *TGFBR1-TGFBR2* complex, albeit that it lacks a recognizable signaling domain (Lopez-Cassilas et al. 1993). Experimentally induced deficiency of *TGFBR3* interfered with endocardial cell transformation in the heart, supporting its essential role in TGF-β signaling (Brown et al. 1999). In this report, we present 12 polymorphisms and some divergences from the published sequence found by sequencing the cDNA of *TGFBR3* ($n = 6$ Caucasian individuals), based on the known sequence of a 4208-bp fragment, consisting of an open reading frame (OFR) of 2547 bp (849 amino acids), flanked by a 5′ untranslated region (UTR) of 348 bp and a 3′ UTR of 1310 bp (Morén et al. 1992; GenBank accession no., L07594).

R. Zippert · A. Bäßler · S.R. Holmer · C. Hengstenberg · H. Schunkert (✉)
Klinik und Poliklinik für Innere Medizin II, University of Regensburg, D-93042 Regensburg, Germany
Tel. +49-941-944-7233; Fax +49-941-944-7235
e-mail: heribert.schunkert@klinik.uni-regensburg.de

Materials and methods

RNA extraction

Total RNA was extracted from a lymphocyte and monocyte fraction of heparinized blood obtained after Ficoll sedimentation and homogenization, using a commercial kit (Qiagen, Hilden, Germany). Written informed consent was obtained from all subjects. The protocol was approved by the institutional ethics committee.

PCR conditions

cDNA was prepared in a Superscript One-Step-RT-PCR-System (GIBCO BRL, Karlsruhe, Germany). About 150 ng of total RNA was incubated with 10 pmol of specific primers (Table 1) in a 50-μl system, with the reaction mix containing

Table 1. Oligonucleotide primers used in sequencing the cDNA and genomic DNA of the TGF β III receptor gene

cDNA	Oligo sequence 5′ to 3′	Region amplified by PCR	Fragment size (bp)
Sense	GATGGTCTGTGCTCCGAGC	cDNA 287 to 798	511 or 514
Antisense	CTTTCTTCTGTTTCTGCTGTCAAG		
Sense	GCATCTGAAGACAGAGAGAC	cDNA 681 to 1061	380
Antisense	TGATTAGCTCGATGATGTG		
Sense	CAAGTGTTCCCTCCAAAGTG	cDNA 916 to 1440	524
Antisense	TTCCTCATCTCCCATCTCC		
Sense	GGATCTTGAAGTGGTCAAAAATCT	cDNA 1128 to 1659	531 or 534
Antisense	CTGAGACCAGGAAACAGTTGTATG		
Sense	AACCCGCCCATCCGGGGA	cDNA 1504 to 1856	352
Antisense	AGAGGAGACTCCAAAACAAAGTGT		
Sense	GATCGTGGCTGTAGAAAAAGATTC	cDNA 1725 to 2208	483
Antisense	TCAACATAAACGTGTCCATTCTCT		
Sense	GCCAGAGAATGGACACGTTTA	cDNA 2181 to 2640	459
Antisense	TGGACCTTTTTCTTTAGATTCTGC		
Sense	CGCTGTGTACGAAGATGG	cDNA 2465 to 2896	431
Antisense	GGCCGTGCTGCTGCTGGA		
Sense	TGGGCATTGCGTTTGCAGCC	cDNA 2705 to 3204	499
Antisense	AACTGGCATGTGTTTCACATAGAA		
Sense	GTGAGAAAGCTAAAATGGTGGTCT	cDNA 3125 to 3772	647 or 646
Antisense	TATATCACTGTGCAAATTCGTCCT		
Sense	GTGCCCATTCCTAATATTTTGTTC	cDNA 3681 to 4208	527 or 529
Antisense	ATCACCTGACTCCAGATC		
Genomic DNA			
Sense	TGCACTGTGTGAACTGTCA	Genomic fragment containing P563 G/A polymorphism	423 to 598
Antisense	GTGTGACCTCTCTCTGTAGC		
Sense	AACCCGCCCATCCGGGGA	Genomic fragment containing P1548 G/A polymorphism	1504 to 1658
Antisense	CTGAGACCAGGAAACAGTTGTATG		

TGF, Transforming growth factor; PCR, polymerase chain reaction

0.4 mM of each dNTP, 2.4 mM $MgSO_4$, and the RT/Taq-Mix. cDNA was synthesized by reverse transcription-polymerase chain reaction (RT-PCR) for 30 min at 50°C and terminated with a 2-min incubation at 94°C. The *TGFBR3* cDNA was amplified by PCR, consisting of 40 cycles of 20 s at 94°, 30 s at 58°C, and 30 s at 72°C, in 11 overlapping fragments, nucleotides (nt.) 287 to 798, 681 to 1061, 916 to 1440, 1128 to 1659, 1504 to 1856, 1725 to 2208, 2181 to 2640, 2465 to 2896, 2705 to 3204, 3125 to 3772, and 3681 to 4208.

Genomic DNA (n = 20 Caucasian individuals) was used for the examination of polymorphisms in two fragments, nt. 423 to 598 and 1504 to 1658. Primers utilized in this study are shown in Table 1. The PCR was performed in a volume of 25 µl, containing 50 ng genomic DNA, 10 mM Tris-HCl (pH 8,3), 1.5 mM $MgCl_2$, 50 mM KCl, 250 µM dNTPs, 25 pmol of each primer, and 1 unit of Taq polymerase. The PCR conditions were: initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C, 58°C, and 72°C, each for 1 min, with a final extension step of 7 min at 72°C.

The amplified PCR products were sequenced with the same primers on an Applied Biosystems (Foster City, CA, USA) automatic sequencer, using the dideoxy termination method.

Results

The cDNA of *TGFBR3* was sequenced from six individuals in 11 fragments from positions 287 to 4208, i.e., the entire ORF, as well as 348 nucleotides of the 5′ UTR and 1309 nucleotides of the 3′ UTR.

A polymorphism in the 5′ UTR

At position 311, the most frequent nucleotide was G (four homozygotes and two heterozygotes) in contrast to the published sequence (A).

Polymorphisms in the ORF

The sequencing of the ORF was realized with eight overlapping cDNA fragments (Table 1). At position 392, four individuals were homozygous and two were heterozygous for a T→C transversion, translating to a deduced amino acid change of phenylalanine to serine at amino acid position 15 (Phe15Ser). A single nucleotide polymorphism was found at position 563 (one individual was homozygous and one heterozygous for a conservative G→A transversion). All individuals had a guanine/cytosine/adenine insertion (1419GCA) after position 1419, translating to an additional alanine. It appears that these three bases were deleted in 20%–40% of the cDNA in all individuals. One individual showed an additional conservative homozygous 1548G→A polymorphism. A further conservative mutation in the ORF was found at position 2370. Here we detected four homozygotes and two heterozygotes for a 2370C→T mutation.

Polymorphisms in the 3′ UTR

The 3′ UTR was sequenced in three overlapping fragments. One individual was heterozygous at positions 2918, 3055, and 3098 for a G→A mutation and at position 3355 for a

A→G mutation. Five individuals were homozygous at position 3183 for a T→A transversion. Moreover, at position 3966, five individuals were homozygous and one individual was heterozygous for a G→C polymorphism.

Genomic sequence

The polymorphisms at positions 563 and 1548 were further analyzed in the genomic DNA of an additional 20 individuals (primers; see Table 1). At position 563, 13 individuals were homozygous for guanine, and 4 for adenine; 9 were heterozygous for this G→A transversion (allele frequency of the G allele, 0.67 and of the A allele, 0.33). At position 1548, 12 individuals were homozygous for guanine and 4 for adenine; 10 were heterozygous for this G→A transversion (allele frequency of the G allele, 0.65 and of the A allele, 0.35).

Divergences from the published sequence data

Throughout the entire sequence, some divergences from the published sequence were observed in all six subjects tested (Fig. 1). In the ORF, divergences were detected after positions 555 (one additional cytosine) and 563 (two additional guanines). Compared with the published sequence, this results in the insertion of one single amino acid and translates to a changed amino acid sequence at positions 69/70/71 (AlaLeuArg to ArgThrAlaGly). Comparisons of the amino acid sequences in rat and pig *TGFBR3* receptors (Morén et al. 1992) with the human amino acid sequence proposed herein showed conserved amino acids (pig: **Arg**/-/**Ala**Asp; rat: **Arg**SerThrAsp). After cytosine at position 1388, we observed an additional thymine in all individuals sequenced. By contrast, none of our sequences showed the thymine at position 1394. Compared with the published sequence, this translates to a changed amino acid sequence at positions 348/349, from IleVal to AsnArg). The amino acid sequences of the pig and rat showed, likewise, AsnArg at these positions. Inside the 3′ UTR, we found no guanine at position 2909, and additional bases after position 2954 (guanine), after position 3586 (thymine), after position 3993 (cytosine), and after position 4060 (adenine). Discrepant bases were found at position 3424 (T→A), at positions 3468 and 3469 (AA→TT), and at position 3525 (G→T).

Discussion

TGFBR3 is a proteoglycan that lacks significant intracellular signaling or enzymatic motifs, but may present the ligand to the *TGFBR1/TGFBR2* signaling complex. Thus, the gene product may be essential for the high-affinity binding of TGF-β2 (Lopez-Casillas 1994; Lin et al. 1995). *TGFBR3* may also facilitate TGF-β binding to other receptors, stabilize multimeric receptor complexes, or segregate growth factor from activating receptors (Ji et al. 1999). In testing genetic defects of the TGF-β III receptor gene, the present characterization of 12 polymorphisms may be useful for further linkage or association studies on potentially related phenotypes, including developmental defects of the heart (Brown et al. 1999).

Acknowledgments Supported by the Deutsche Forschungsgemeinschaft (DFG Schu 672/9-1, 672/10-1, 672/12-1 and Ho 1073/8-1), the Bundesministerium für Forschung und Technologie, the Vaillant Stiftung, the Ernst and Bertha Grimmke Stiftung, and the Deutsche Stiftung für Herzforschung.

References

Brown CB, Boyer AS, Runyan RB, Barnett JV (1999) Requirement of type III TGF-β receptor for endocardial cell transformation in the heart. Science 283:2080–2082

Buske C, Becker D, Feuring-Buske M, Hannig H, Griesinger F, Hiddemann W, Wormann B (1998) TGF-beta and its receptor complex in leukemic B-cell precursors. Exp Hematol 26:1155–1161

Ji C, Chen Y, McCarthy TL, Centrella M (1999) Cloning the promoter for transforming growth factor beta type III receptor. Basal and conditional expression in fetal rat osteoblasts. J Biol Chem 274:30487–30494

Lin HY, Moustakas A, Knaus P, Wells RG, Henis YI, Lodish HF (1995) The soluble exoplasmic domain of the the type II transforming growth factor (TGF)-beta receptor. A heterogeneously glycosylated protein with high affinity and selectivity for TGF-beta ligands. J Biol Chem 270:2747–2754

Lopez-Casillas F, Cheifetz S, Doody J, Andres JL, Lane WS, Massagué J (1991) Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-beta receptor system. Cell 67:785–795

Lopez-Casillas F, Payne HM, Andres JL, Massagué J (1994) Betaglycan can act as a dual modulator of TGF-beta access to signaling receptors: mapping of ligand binding and GAG attachement sites. J Cell Biol 124:557–568

Lopez-Casillas F, Wrana JL, Massagué J (1993) Betaglycan presents ligand to the TGF beta signaling receptor. Cell 73:435–1444

Massagué J (1990) The transforming growth factor-β family. Annu Rev Cell Biol 6:597–641

Morén A, Ichijo H, Miyazono K (1992) Molecular cloning and characterization of the human and porcine transforming growth factor-β type III receptors. Biochem Biophys Res Commun 189:356–362

Roberts AB, Sporn MB (1990) The transforming growth factor-betas. In: Sporn M, Roberts AB (eds) Peptide growth factors and their receptors. Springer, Heidelberg, Berlin Tokyo New York, pp 419–472

Fig. 1. Nucleotide and deduced amino acid sequence of human transforming growth factor (TGF)-beta III receptor cDNA. The newly discovered polymorphisms are shown in *boldface* and further indicated by a *single underline and the position number*. Some divergences from the published sequence are indicated as follows: additional bases are *underlined twice*, bases not found are in *gray boxes*, and exchanged bases are *underlined* with the divergent bases *above*. Change in the amino acid sequence is indicated in *italic letters*