A novel human serine-threonine phosphatase related to the Drosophila retinal degeneration C (rdgC) gene is selectively expressed in sensory neurons of neural crest origin

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Through our transcriptional mapping effort in the Xp22 region, we have isolated by exon trapping a new transcript highly homologous to the Drosophila retinal degeneration C (rdgC) gene. rdgC encodes a serine/threonine phosphatase protein and is required in Drosophila to prevent light-induced retinal degeneration. This human gene is the first mammalian member of the serine-threonine phosphatase with EF hand motif gene family, and was thus named PPEF (Protein Phosphatase with EF calcium-binding domain). The expression pattern of the mouse Ppef gene was studied by RNA in situ hybridization on embryonic tissue sections. While rdgC is expressed in the visual system of the fly, as well as in the mushroom bodies of the central brain, we found that Ppef is highly expressed in sensory neurons of the dorsal root ganglia (DRG) and neural crest-derived cranial ganglia. The selective pattern of expression makes PPEF an important marker for sensory neuron differentiation and suggests a role for serine-threonine phosphatases in mammalian development.

INTRODUCTION

Our group is involved in the construction of a transcription map of the human Xp22 region. To achieve this aim, we have constructed a detailed physical map of a 35 Mb region spanning human chromosome Xp22.3–Xp21.3. The backbone of the map is represented by a single contiguous stretch of 585 overlapping yeast artificial chromosome (YAC) clones covering the entire region (1). Several disease loci have been mapped in this region including Retinoschisis (RS), Nance–Horan syndrome (NHS), Coffin–Lowry syndrome (CLS), and Keratosis Follicularis Spinulosa Decalvans (KFSD) (1). As a first step toward building a transcription map of this region, we decided to concentrate our efforts on YAC clone 939H7 which spans the entire critical region for RS and partially spans the critical regions for NHS, CLS and KFSD.

This effort led us to the isolation of a gene highly homologous to the Drosophila retinal degeneration C (rdgC) gene. The rdgC gene encodes a serine/threonine phosphatase protein (2) and is required in Drosophila to prevent light-induced retinal degeneration (3). rdgC is expressed in the visual system of the fly, as well as in the mushroom bodies of the central brain. We named this new gene PPEF, for Protein Phosphatase with EF hand motif.

To test the involvement of PPEF in the pathogenesis of RS, we isolated the full-length cDNA, established the genomic structure, and searched for mutations in RS patients by PTT (protein truncation test) and SSCP (single strand conformation polymorphism) analyses. Identification of the mouse homolog of this gene allowed us to perform RNA in situ hybridization studies on mouse embryo tissue sections, revealing a very specific pattern of expression localized in sensory neurons of cranial and dorsal root ganglia.

RESULTS

Identification and characterization of the PPEF cDNA

One hundred cosmid clones were selected by screening an X-specific cosmid library with Alu PCR products derived from YAC 939H7. Cosmid clones were grouped and used for exon trapping experiments. Several exon trapping products were sequenced and used to search non-redundant DNA and protein databases through the BLAST-X, BLAST-P and TBLAST-N algorithms. One of them (clone 3pn1D2) was found to be identical to EST H18854, which shows significant homology to the Drosophila rdgC protein (accession no. M89628). Clone 3pn1D2 mapped back to YAC939H7 in the RS critical region and to cosmide 44C1 containing marker DXS999 (Fig. 1). The same transcript was subsequently identified in our laboratory, using a bioinformatic approach aimed at the identification of human homologs of Drosophila genes involved in the generation of...
mutant phenotypes, and found to correspond to DRES10 (Drosophila related expressed sequence #10) (4).

The I.M.A.G.E. cDNA clone 51064 corresponding to EST H18854 was used to screen both an infant (5) and a fetal (Clontech) brain cDNA library. Six different cDNA clones were isolated and characterized by end-sequencing, restriction mapping and PCR, using specific primers in combination with vector primers. Subsequently, an additional cDNA (nt19) was isolated by screening a teratocarcinoma/neuron (mature hNT neuron, Stratagene 937233) cDNA library with a different exon trapping product (clone 8bb1a1). Characterization of each of the cDNA clones allowed us to establish a cDNA contig of 2872 bp (base pairs) (accession no. X97867). The authenticity of the 5′ end of the cDNA was validated by sequencing the corresponding genomic region. The putative initiation codon was identified at position 484 and is located within a nucleotide sequence that fulfills Kozak’s criteria for an initiation codon (6). The first in-frame stop codon (TAA) was identified at nucleotide 2443, predicting a protein product of 653 amino acids. Sequence analysis of the predicted protein product revealed the presence of two putative functional domains. The first domain comprises amino acids 150–438 and shares sequence similarity with the catalytic domain of phosphoprotein phosphatases of the serine-threonine kind (Fig. 2A). The second domain is present at the carboxyl end of the predicted protein (amino acids 566–643) and contains potential C_2^++-binding sites as defined by the EF hand motif (7) (Fig. 2A and B). On the basis of these homologies, we therefore named the gene PPEF, for Phosphoprotein Phosphatase with EF hand motif.

Sequence analysis and comparison of PPEF with previously identified phosphoprotein phosphatases revealed conservation of several invariant residues including Asp59, Asp88, His61 and His139. Site-directed-mutagenesis experiments revealed that substitution of these residues results in abolishment of phosphatase activity (8,9). These data suggest that PPEF might be a functional phosphatase.

A BLASTX search with PPEF disclosed the highest homology with the Drosophila rdgC gene, which also encodes for a serine-threonine phosphatase with EF motif and, when mutated, causes light-induced retinal degeneration in the fly. PPEF and rdgC are 60% identical at the nucleotide level, and 62% similar and 42% identical at the protein level. To the best of our knowledge, the rdgC and PPEF proteins are the only two molecules in which a phosphoprotein phosphatase domain coexists with EF hand motifs. Furthermore, within the phosphoprotein phosphatase domain, PPEF displays much higher homology with rdgC than with other members of the phosphoprotein phosphatase gene family (data not shown), indicating that PPEF and rdgC belong to a distinct subfamily of serine-threonine phosphatases.

**PPEF genomic structure and RS patient analysis**

The complete genomic structure of the PPEF gene was determined using the available cosmid clones. Genomic clones corresponding to exon 4 were obtained by screening a total phage genomic library using a specific PCR probe. EcoRI and HindIII
RT-PCR experiments, using nested primers, on RNA isolated from lymphocytes of RS patients and normal controls revealed several different size products (Fig. 3B). Similar results were obtained using RT-PCR on RNA isolated from seven other tissues (data not shown). Sequence analysis of these different products and comparison with the cDNA sequence confirmed that they were the result of alternative splicing. Figure 3A shows a schematic representation of all the splice variants detected. Figure 3B shows the map of the genomic clones and the position of the exons.

Sequence homology and mapping data identified the PPEF gene as a good candidate for retinoschisis and thus, a mutation study in RS patients was undertaken. Thirty-seven unrelated male patients with clinical features of RS, but with no reported chromosomal abnormality involving the Xp22 region, were tested for small rearrangements or point mutations in the PPEF gene by Southern and SSCP analyses (data not shown). Furthermore, PTT analysis was carried out on nine different RS patients (data not shown). No anomalies were found with either technique, thus suggesting that PPEF is not involved in the pathogenesis of RS.

**PPEF is alternatively spliced**

**Figure 2.** Sequence analysis of the PPEF predicted protein. (A) Amino acid sequence comparison between PPEF (top) and *Drosophila rdgC* (bottom) proteins. The region of homology shared with other members of the protein phosphatase family is underlined. The EF calcium-binding domain is indicated by a dashed line. (B) Amino acid identity within the EF hand motifs between PPEF, *rdgC*, Troponin C and Plastin. The positions of the Ca$^{++}$ chelating side chains are labeled X–Z.
Table 1. Splice junction sequences of the PPEF gene

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Expression studies

The expression pattern of the PPEF transcript was determined by both Northern analysis on human tissues and RNA in situ hybridization on embryonic mouse tissue sections. Northern blot analysis on both human adult and fetal tissues detected two transcripts (2.7 and 4.3 kb, respectively) selectively expressed in the brain. These transcripts were found to be strongly up-regulated during fetal life (Fig. 4).

Previous zoo blot experiments indicated evolutionary conservation of the PPEF gene in several species including hamster, rat, mouse, pig, chicken and cow (data not shown). To obtain a probe for RNA in situ hybridization studies on mouse tissue sections, we screened a mouse embryonic E11.5 day cDNA library (Clontech) using probe c14-c22 obtained by PCR with primers c14 and c22. This PCR product corresponds to the region with the highest sequence homology (63.2% at the nucleotide level) between the human PPEF and the Drosophila rdgC genes. This screening led to the isolation of a partial cDNA clone (EM800; accession no. Y08234). Sequence analysis revealed 82.4% identity at the nucleotide level, and 83.1% similarity and 76.4% identity at the protein level between mouse and human (data not shown). The homology between the mouse and Drosophila proteins was revealed to be 65.3% similarity and 43.1% identity.

RNA in situ hybridization was performed on mouse embryonic tissue sections from embryonic day 10.5 (E10.5) to embryonic day 16.5 (E16.5). These experiments revealed an expression pattern overtly different from that displayed by the rdgC gene in the fly. Ppef is almost exclusively expressed in the peripheral nervous system, with sensory neurons of neural crest origin. Ppef expression is up-regulated at E12.5 in dorsal root ganglia (DRG), and in some sensory cranial ganglia (Fig. 5A). Sensory neurons of the vertebrate peripheral nervous system have two distinct embryological origins. Several studies, mainly in the chick, have shown that neurons of the DRG, of the dorso-medial part of the trigeminal ganglion, and of the superior ganglia of cranial nerves IX and X (jugular ganglion) are derived from the neural crest, while neurons of the ventrolateral part of the trigeminal ganglion, the geniculate, vestibuloacoustic, petrosal and nodose ganglia, are derived from ectodermal placodes, i.e., thickening of the surface ectoderm (11). Ppef expression was found to be restricted to neuronal populations of neural crest-derived sensory ganglia. In fact, Ppef is highly expressed in neurons of the DRG (Fig. 5C and D), in distinct neuronal populations of the trigeminal ganglion (Fig. 5B), and in the superior ganglia of the IX and X cranial nerves. No expression was observed in the geniculate, vestibuloacoustic (Fig. 5B), petrosal, and nodose ganglia.

Although sensory ganglia are already formed in the mouse well before E12.5, this stage is believed to correspond with the start of neurogenesis in these structures (12). An enlarged view of Ppef expression within DRG and the trigeminal ganglion (Fig. 5B) clearly demonstrated that most, but not all, neurons express Ppef. A slightly decreased expression could still be detected in sensory ganglia at E16.5. We do not know if Ppef expression in sensory neurons persists in adult life. The discrepancy in the strength of the hybridization signals on Northern blot in adult and fetal brain is probably due to the inclusion of the cranial sensory ganglia in the brain sample of fetal origin. The only sites of Ppef expression, outside sensory neurons, were found in the inner ear (Fig. 5E) and in a small group of neurons located at the midbrain/pons junction (Fig. 5F). We did not detect any expression signals above background level in the embryonic and adult mouse retina (data not shown).

Figure 3. (A) Schematic representation of different splice variants identified by RT-PCR experiments, using nested primers. (B) RT-PCR analysis on RNA isolated from lymphocytes of RS patients and normal controls. Lane 1 corresponds to the products RT-PCR1a and RT-PCR1b. Lane 2 to RT-PCR2, lane 3 to RT-PCR3. Lane 4 corresponds to a product identical to the cDNA described in the text. Lane 5 contains a 100 bp marker.

Figure 4. (A) RT-PCR1a

Figure 5. (A) RT-PCR1b

Figure 6. (A) RT-PCR2

Figure 7. (A) RT-PCR3

Figure 8. (A) Splice site 3

Figure 9. (A) Splice site 5

Figure 10. (A) RT-PCR1

Figure 11. (A) RT-PCR2

Figure 12. (A) RT-PCR3

Figure 13. (A) RT-PCR4

Figure 14. (A) RT-PCR5

Figure 15. (A) RT-PCR6

Figure 16. (A) RT-PCR7

Figure 17. (A) RT-PCR8

Figure 18. (A) RT-PCR9

Figure 19. (A) RT-PCR10

Figure 20. (A) RT-PCR11

Figure 21. (A) RT-PCR12

Figure 22. (A) RT-PCR13

Figure 23. (A) RT-PCR14

Figure 24. (A) RT-PCR15

Figure 25. (A) RT-PCR16

Figure 26. (A) RT-PCR17

Figure 27. (A) RT-PCR18

Figure 28. (A) RT-PCR19

Figure 29. (A) RT-PCR20

Figure 30. (A) RT-PCR21

Figure 31. (A) RT-PCR22

Figure 32. (A) RT-PCR23

Figure 33. (A) RT-PCR24

Figure 34. (A) RT-PCR25

Figure 35. (A) RT-PCR26

Figure 36. (A) RT-PCR27

Figure 37. (A) RT-PCR28

Figure 38. (A) RT-PCR29

Figure 39. (A) RT-PCR30

Figure 40. (A) RT-PCR31

Figure 41. (A) RT-PCR32

Figure 42. (A) RT-PCR33

Figure 43. (A) RT-PCR34

Figure 44. (A) RT-PCR35

Figure 45. (A) RT-PCR36

Figure 46. (A) RT-PCR37

Figure 47. (A) RT-PCR38

Figure 48. (A) RT-PCR39

Figure 49. (A) RT-PCR40

Figure 50. (A) RT-PCR41

Figure 51. (A) RT-PCR42

Figure 52. (A) RT-PCR43

Figure 53. (A) RT-PCR44

Figure 54. (A) RT-PCR45
Linkage mapping of *Ppef* in mouse

The finding in the N2 progeny of the BSS backcross (13) of male individuals carrying only B alleles (hemizygotes) led to the unequivocal assignment of *Ppef* to the murine X chromosome. The analysis of the strain distribution pattern (SDP) observed in the same progeny permitted the localization of *Ppef* to the distal third of the chromosome (Fig. 6). *Ppef* is in linkage with DXXrI132 (Spencer et al., in preparation) (Θ = 1.41; LOD = 19.1), and was mapped using a probe containing dbEST 122118 (accession no. F06456) with homology to the *Saccharomyces cerevisiae* GTR1 gene. In the BSS map, *Ppef* is located telomeric to DXXrI132. *Ppef* maps -1.5 CM centromeric to *Grpr*, the gastrin releasing peptide receptor gene, identified using DXMit20 primers (Korobova and Arnheim, unpublished) and *Piga* (Θ = 1.37; LOD = 19.7). The locus order defined in mouse between the *Anel* (Korobova and Arnheim, unpublished) and *Ppef* loci correlates with the physical map established in human (1). However, the position of the *Oa1* gene with respect to the *Cle4-1-Amel-(Piga-Grpr)-Ppef* linkage group confirms the prior notion of a rearrangement (inversion) within this region of human–mouse synteny (14).

DISCUSSION

**PPEF is the first mammalian member of the protein phosphatase with EF hand motif gene family**

A wide variety of cellular functions, including cell signalling, gene expression, membrane transport and secretion and cell division, are regulated by the reversible phosphorylation of proteins on serine and threonine residues (15). The phosphatases that catalyze the dephosphorylation of these amino acids are a crucial component of this regulation. The serine-threonine phosphatases belong to a rapidly expanding gene family, and six different mammalian members (PP1, PP2A, PP2B, PP2C, PP4 and PP5) have been described so far. They can be distinguished depending on their ability to dephosphorylate either the α- or the β-subunit of phosphorylase either the α- or the β-subunit of phosphorylase kinase, and on their sensitivity to specific inhibitors (16). These phosphatases have been highly conserved during evolution from yeasts to vertebrates (17).

In *Drosophila*, the *rdgC* gene encodes a serine/threonine phosphatase. This phosphatase shares 30% homology with the catalytic domain of PP1, PP2A and PP2B, but is unique due to the presence of five Ca ++ -binding sites, as defined by the EF hand motif in the C-terminus. Owing to its particular features, *rdgC* is likely to be a member of a novel subfamily of protein phosphatases, characterized by the coexistence of the catalytic phosphatase domain and Ca ++ -binding sites. Very little is known about the function of this phosphatase and no evidence for vertebrate homologs has been produced so far. The *rdgC* gene is required in *Drosophila* to prevent light-induced degeneration of the retina. *Drosophila rdgC* mutants show normal retinal morphology and photoreceptor physiology at a young age. The retina of one-day-old *rdgC* mutants has wild type structure, but by three days, the photoreceptors R1–R6 begin degenerating. By five days, degeneration of photoreceptors R1–R6 is complete and photoreceptors R7 and R8 begin showing signs of degeneration (3). The *rdgC* gene is thought to be involved in the regeneration of rhodopsin and is expressed in the retina, ocelli, optic lobes and in the mushroom bodies of the central brain (2).

We report here the isolation of the first mammalian member of the serine-threonine phosphatase with EF hand motif gene family. The new gene was named *PPEF* (Protein Phosphatase with EF calcium-binding domain) and is highly homologous to the *Drosophila rdgC* gene. In order to study the expression pattern of *PPEF* during embryonic development, we isolated a partial murine homologous cDNA. This cDNA shows 82.4% identity at the nucleotide level and 83.1% similarity and 76.4% identity at the protein level with the human *PPEF* gene. Linkage mapping experiments, performed in the mouse, established that this gene is located on the murine X chromosome in a region syntenic with human Xp22 (18). These data strongly indicate that the murine *Ppef* gene may be considered a bona fide ortholog for the human *PPEF*.

**PPEF expression is restricted to sensory neurons of neural crest origin**

In the fly, *rdgC* is highly expressed in the compound eye as well as in the ocelli, the other photoreceptor-containing organ, and is thought to participate in a rhodopsin-initiated pathway that regulates photoreceptor membrane renewal. The tissue
Figure 5. Expression pattern of PPEF, as revealed by in situ hybridization in a 13.5 day old mouse embryo. (A) Autoradiography of a sagittal section shows expression restricted to the trigeminal ganglion and the dorsal root ganglia. Sagittal sections through the head show PPEF expression in the trigeminal ganglion (B). A strong hybridization signal is present in the dorsal root ganglia, as displayed in a sagittal section (C) and in a transverse section (D). Ppef positive signal is also present in the inner ear (E) and in a group of neurons located at the pons/midbrain junction (arrow in F). Abbreviations: tg, trigeminal ganglion; g, geniculate ganglion; va, vestibuloacoustic ganglion; drg, dorsal root ganglia; sc, spinal cord; ie, inner ear.
and TrkA receptor expression begins in sensory ganglia. Although the phosphorylation events mediated by the neurons. NGF exerts its effect by eliciting a phosphorylation ganglia suggest that this gene is expressed by NGF-responsive initiated by NGF in sensory neurons. Consistently, up-regulation and the phosphatases involved. It is an appealing hypothesis that studied, very little is known about dephosphorylation pathways enzymes and specific carbohydrate groups (19,20). So far, populations in sensory ganglia by the presence of peptides, immunocytochemical and histochemical techniques have been specific transcripts within sensory ganglia. In addition, localized of the mouse Ppef gene was studied by RNA in situ hybridization on embryonic tissue sections. In contrast with the expression pattern of the Drosophila rdgC gene, we found that Ppef is selectively expressed in neurons of the DRG, in distinct neuronal populations of the trigeminal ganglion, and in the superior ganglia of the IX and X cranial nerves. No expression was observed in the geniculate, vestibulocuacoustic, petrosal and nodose ganglia. This selective pattern of expression correlates with embryological origin, with Ppef being a marker specific for sensory neurons of neural crest origin. Several genes have been found to be expressed in sensory neurons and RNA in situ hybridization studies have allowed the study of the distribution of specific transcripts within sensory ganglia. In addition, immunocytochemical and histochemical techniques have been used extensively to biochemically define distinct neuronal populations in sensory ganglia by the presence of peptides, enzymes and specific carbohydrate groups (19,20). So far, however, the only other genes that show an expression pattern restricted to sensory neurons are the neurotrophin receptor genes (21).

For their survival, sensory neurons of the developing peripheral nervous system rely upon specific members of the Nerve Growth Factor (NGF) family of neurotrophins, which are secreted by their targets. It is believed that different neuronal populations in DRG are responsive to different neurotrophins and express different neurotrophin receptors (22,23). In vitro studies of neurons from cranial sensory ganglia have shown that there is a difference in the response of placode-derived and neural crest-derived neurons to neurotrophins; neural crest-derived neurons are responsive to NGF, while placode-derived neurons respond to Brain-Derived Neurotrophic Factor (BDNF) (24,25).

The selective expression of Ppef in cranial sensory ganglia of neural crest-origin and the lack of expression in placode-derived ganglia suggest that this gene is expressed by NGF-responsive neurons. NGF exerts its effect by eliciting a phosphorylation cascade through activation of the TrkA tyrosine-kinase receptor (26). Although the phosphorylation events mediated by the binding of NGF to the TrkA receptor have been extensively studied, very little is known about dephosphorylation pathways and the phosphatases involved. It is an appealing hypothesis that PPEF might be involved in the specific signalling pathway initiated by NGF in sensory neurons. Consistently, up-regulation of Ppef expression coincides with the time at which neurogenesis and TrkA receptor expression begins in sensory ganglia.

In conclusion, our study provides evidence for the presence of a mammalian protein phosphatase with EF-hand motifs. Although this phosphatase is highly homologous to the Drosophila rdgC gene, expression studies seem to suggest that it represents a related gene with a different function. We do not exclude the possibility that additional members of this protein phosphatase gene family will be identified in the near future. Alternatively, PPEF might be the evolutionary equivalent of the Drosophila rdgC gene, but we must assume that the two genes have diverged in terms of function. Since mapping data placed PPEF within the critical region for RS, we have excluded its involvement in the pathogenesis of X-linked juvenile RS by searching mutations in RS patients. Future experiments, including the identification of sub-populations of sensory neurons expressing PPEF phosphatase and of the natural substrate of the protein, will greatly contribute to the understanding of the biological role of the PPEF gene in mammalian development and will allow testing for its possible involvement in NGF signal transduction.

**MATERIALS AND METHODS**

**cDNA identification**

YAC clone 939H7 was converted into cosmid clones by hybridization of long range Alu-PCR product obtained with a variety of human-specific Alu primers. PCR amplification was performed according to Gu et al. (27). Cosmid clones were grouped (10 clones per group), digested with BamHI/BgIII, cloned in the pSPL3 vector, and used for the exon amplification experiments as described previously (28) and by Montini et al. (manuscript in preparation). In order to identify the PPEF full-length transcript, three human cDNA libraries were screened: a teratocarcinoma/neuron cDNA library (mature hNT neuron, Stratagene 937233), a fetal brain cDNA library (Clontech HL3003a), and the Bento Soares infant brain 1NIB arrayed cDNA library. For the isolation of a partial murine cDNA clone, an 11.5 day embryo (Clontech ML3003a) mouse cDNA library was used. Plating, hybridization and washing conditions were performed as previously described (29). Primers used to obtain the probe used to screen the mouse cDNA library were as follows: c14, AAGTCTCTGAACTCAATGCG; c22, GCCATACCTCAAGTATC.

**cDNA sequence analysis**

cDNA sequence analysis and nucleotide and protein database searches were performed as previously described (4). Data on similarity/identity were obtained using the Bestfit program of the GCG software package, version 8.1. The multiple alignment analyses were generated using the PileUp program of the Wisconsin GCG software package, ver. 8.1.

**Expression studies**

Commercial Northern blots (Clontech) containing human RNA from fetal and adult tissues were hybridized and washed using the conditions recommended by the manufacturer. Mouse embryo tissue sections were prepared and RNA in situ hybridization experiments were performed as previously described (30). cDNA clone EM800 was linearized with appropriate restriction enzymes to transcribe either sense or antisense 35S-labelled riboprobes. Slides were exposed for 10 days. Micrographs are double
exposures: red represents the in situ hybridization signal, and blue shows the nuclei stained with Hoechst 33258 dye.

RNA isolation and RT-PCR
RNA isolation from blood and RT-PCR experiments were carried out as previously described (31). RT-PCR was carried out with nested primers. Primers 1F+1R were used for the first round, the resulting PCR products were then reamplified using primers 2F+2R. Primers used for RT-PCR:
1F, 5′-TGAAGGGCAGGACACACTATG-3′
1R, 5′-ACTAGTTGCACTACAGTTCCT-3′
2F, 5′-TAACTAGCTACACTAGGACAGCACCACCAT
GGAATATGCTGATAAACAAGGC-3′
2R, 5′-CTTTTCTCTGCTACTGACTA TGAA-3′

Primers for sequencing:
909, 5′-CTTGGAGAAATTTTATGCTTGG-3′
947, 5′-ATTGTCGAGTGAGGCACCA-3′
1528, 5′-CCATAGTATGCCCCCTTTGGC-3′
1501, 5′-CTCTTTCGCAAAAGTGGATA-3′.

Linkage mapping in mouse
Genetic mapping was achieved utilizing a (C57BL/6j × SPRET/Ei)F1 × SPRET/Ei (BSS) backcross generated and distributed by the Jackson Laboratory (Bar Harbor, ME) (13). An Mspl RFLP was identified by hybridization of C57BL/6j and SPRET/Ei parental DNAs cut with each of the six restriction enzymes (EcoRI, EcoRV, KpnI,MspI, TaqI and XbaI). Four Southern panels containing Mspl-cut parental DNAs and N2 progeny (n = 94) DNAs were hybridized with a Ppef cDNA probe. The resulting strain distribution pattern (SDP) was analyzed with the Map Manager 2.6 program (32).

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