Brief Genetics Report

Isolation, Characterization, and Chromosomal Localization of the Human *ENSA* Gene That Encodes α-Endosulfine, a Regulator of β-Cell K_{ATP} Channels

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Human α-endosulfine is an endogenous regulator of the β-cell K_{ATP} channels. The recombinant α-endosulfine inhibits sulfonylurea binding to β-cell membranes, reduces cloned K_{ATP} channel currents, and stimulates insulin secretion from β-cells. These properties led us to study the human *ENSA* gene that encodes α-endosulfine. Here, we describe the isolation, the partial characterization, and the chromosomal localization of the *ENSA* gene. The *ENSA* gene appears to be a 1.8-kb-long sequence that contains the transcription initiation site located 528 bp upstream of the initiation codon. The *ENSA* gene is intronless, and a single copy gene seems to be present in the genome. Finally, the *ENSA* gene colocalizes on human chromosome 14 (14q24.3–q31) with a locus for susceptibility to type 1 diabetes called *IDDM11*; thus, the *ENSA* gene represents an *IDDM11* candidate. *Diabetes* 48:1873–1876, 1999

Sulfonylureas, which are used in the treatment of type 2 diabetes (1), exert their hypoglycemic action primarily through inhibition of ATP-sensitive potassium (K_{ATP}) channels, which lead to insulin secretion (2). The pancreatic K_{ATP} channel is an octameric complex composed of two subunits: Kir6.2, the channel pore, and SUR1, the sulfonylurea receptor (3–6). The existence of SUR1, which binds sulfonylureas with a high affinity, and its interaction with Kir6.2 suggested the existence of an endogenous regulator of K_{ATP} channel activity. Consistent with this hypothesis, α-endosulfine was characterized (7,8), isolated, and partially sequenced (9). Recently, we cloned a human partial α-endosulfine cDNA that contained the entire open reading frame (10). The corresponding recombinant protein inhibited sulfonylurea binding to β-cell membranes, reduced cloned K_{ATP} channel currents, and thereby stimulated insulin secretion from β-cells in vitro. Such important properties made it possible that changes in α-endosulfine production related to perturbation in its gene structure and function may occur in pathophysiological states such as diabetes. Investigating this possibility required the isolation and characterization of the α-endosulfine gene. In this study, we describe the cloning of this human gene called *ENSA*, its organization, and its chromosomal localization.

We first isolated part of the 5′ sequence of the *ENSA* gene (Fig. 1) by screening a human genomic library with the α-endosulfine cDNA probe that corresponded to the longest available α-endosulfine cDNA (749 bp). To determine whether the 5′ end of the α-endosulfine cDNA was present in this 5′ flanking region, we performed a 5′rapid amplification of cDNA ends (RACE)–polymerase chain reaction (PCR) assay. (Further information can be found in an on-line appendix at www.diabetes.org/diabetes/appendix.asp.) A single band was obtained that led us to locate the beginning of the cDNA 528 bp upstream from the translational initiation codon (Fig. 1). The 5′ *ENSA* sequence does not contain any canonical binding sites for transcription factors, but reveals the presence at positions –113 to –109 of a CCAAT motif that resembles that of a CAT box. Then, using a 3′RACE-PCR method, we were able to extend the α-endosulfine cDNA sequence toward its 3′ end by ~670 bp downstream of the translational stop codon (Fig. 1). This sequence, also found in the genomic DNA, corresponded to a 3′ untranslated mRNA region in which we could identify a consensus polyadenylation signal (AATAAA), giving us the sequence and the precise size of the shortest α-endosulfine transcript (1.5 kb) previously described with Northern blot analysis.

To determine the intron-exon organization of the *ENSA* gene, we performed PCR amplifications (of genomic and cDNA templates) with specific sets of overlapping primers. The amplicons generated by using the same sets of primers (Table 1) with both genomic and cDNA templates are shown in Fig. 2. All fragments (but one) generated by PCR were common. Only a set of primers (GSP2-G5) did not yield any

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K_{ATP}, ATP-sensitive potassium; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends.

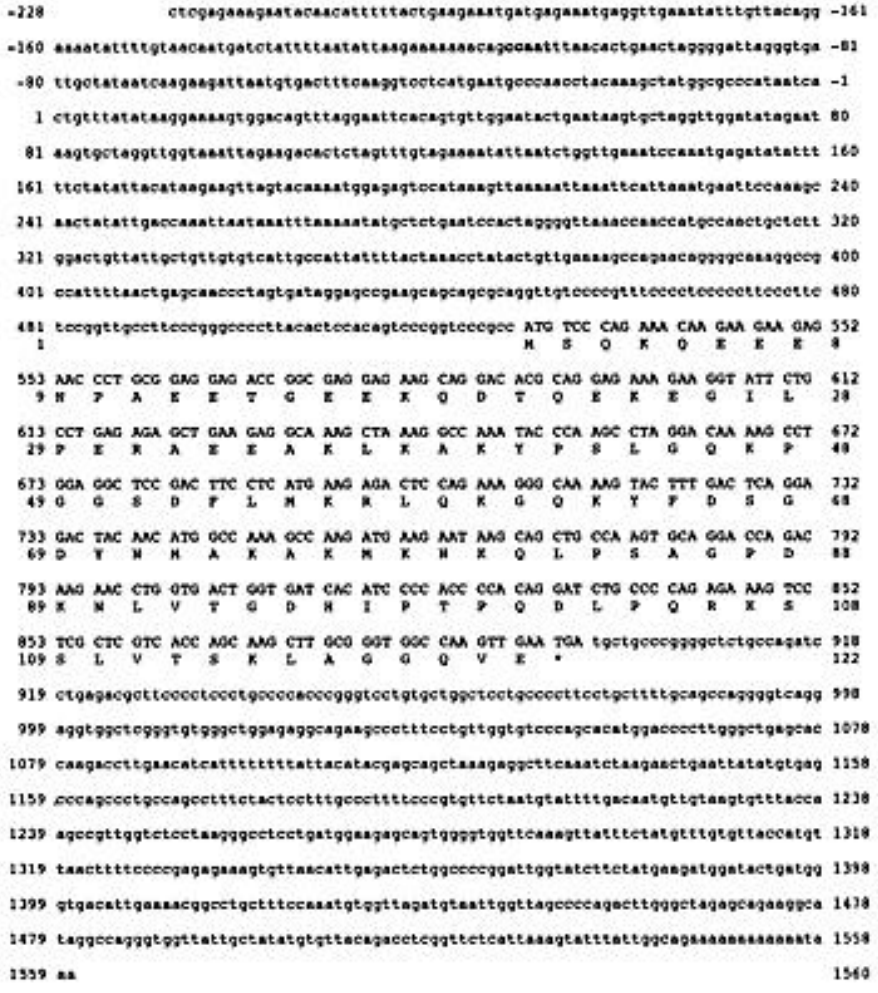

FIG. 1. Nucleotide sequence of the *ENSA* gene and predicted amino acid sequence of human α-endosulfine. Both nucleotide and amino acid numbers are indicated. The transcription initiation site is assigned the position +1, the ATG start codon is at position +529, and the TGA stop codon is marked by an asterisk. A putative CCAAT box is written in bold characters. The sequence of *ENSA* was deposited with the European Molecular Biology Laboratory (EMBL)/GenBank database accession no. AJ010966.

amplification product with a cDNA template, but gave a fragment of the expected size (~650 bp) from genomic DNA. This further confirmed the presence of a transcription initiation site previously mapped within this fragment. The complete identity between sizes and sequences of the other genomic and cDNA-derived amplicons established the lack of introns at least within the part of the *ENSA* gene encompassing the transcription initiation site, the 5′ flanking region, the coding sequence, and the 3′ untranslated *ENSA* gene region described in this study. This appears to rule out the possibility that the two transcripts previously identified (10) could arise from a common alternately spliced precursor. Since we could detect a single transcription initiation site in our study, the use of two different polyadenylation signals might explain the existence of these two different mRNA species. The 3′ untranslated region that we have sequenced contains a polyadenylation site sequence and led us to determine the sequence of the short transcript found in human brain. Concerning the longest transcript, a consensus site would be present in the extreme 3′ region that we did not obtain in our study, even performing 3′RACE-PCR from human muscle RNA. The *ENSA* gene seems to be transcribed at rather low levels in many tissues (10); this is a typical feature of so-called housekeeping genes, which generally lack a consensus TATA box (11,12). This also seems to be the case for the *ENSA* gene, since the CCAAT motif described is located 113 bp from the transcription initiation site (generally CCAAT boxes are found at positions –80 to –100). Although rather unusual, the *ENSA* proximal 5′ flanking region is not unique; it resembles the rat T-kininogen gene promoter, that also lacks all those classical transcriptional elements (13).

To estimate whether the *ENSA* gene is present as several copies in the human genome, Southern blotting analysis was

TABLE 1
Location of primers used for PCR in the *ENSA* sequence

Primer/direction	Position*
16/sense	+529 to +547
17/antisense	+891 to +876
20/sense	+401 to +423
21/antisense	+1151 to +1130
GSP1/antisense	+552 to +525
GSP2/antisense	+451 to +430
GSP3/sense	+972 to +995
GSP4/sense	+1067 to +1091
22/sense	+1160 to +1180
23/antisense	+1543 to +1512
G+1/sense	+338 to +364
G5/sense	–194 to –167

*Nucleotide position +1 represents the transcription initiation site shown in Fig. 1.

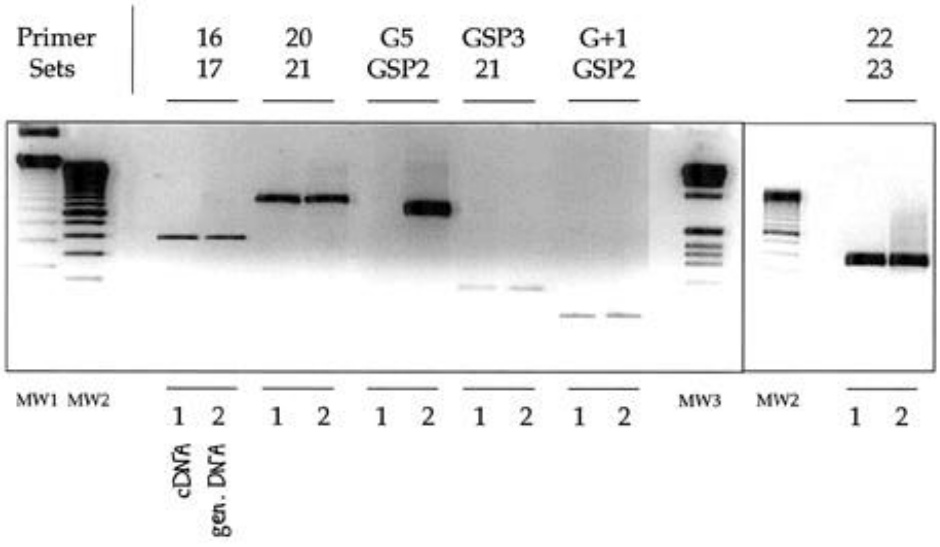

FIG. 2. α-Endosulfine PCR amplified products generated with cDNA (1) and genomic DNA (2) templates. MW1, MW2, and MW3 represent 123-bp, 100-bp, and 1-kb ladders, respectively (Life Technologies molecular weight markers). Location of the primer sets used in PCR is indicated in Table 1.

carried out. Hybridization of the full-length α-endosulfine cDNA probe (position +401 to +1151 with regard to Fig. 1) to restriction fragments generated by digesting human genomic DNA with four different enzymes revealed a rather simple pattern. (Further information can be found in an on-line appendix at www.diabetes.org/diabetes/appendix.asp.) Indeed, single fragments were observed with *Bam*HI, *Hind*III, and *Sac*I, and three hybridizing fragments were obtained with *Eco*RI digestion. These results suggest that a single copy of the *ENSA* gene is present in the human genome.

Fluorescence in situ hybridization (Fig. 3), performed with the α-endosulfine probe, revealed recurrent simple and double spots on human chromosome 14 (band 14q24.3–q31). Of 30 metaphases, 60% exhibited at least one spot on this position (38% of the spots were on band 14q24.3, 62% were on either the distal part of band 14q24.3 or band 14q31). It is of particular interest to note that the *ENSA* gene and a locus for susceptibility to type 1 diabetes referred to as *IDDM11* (14) have the same chromosomal localization. Thus, the *ENSA* gene is a possible *IDDM11* candidate. In addition, it should be recalled that the logarithm of odds score for *IDDM11* represents the strongest reported evidence for the linkage of type 1 diabetes susceptibility to a non-HLA region of the genome (14). Since the *ENSA* gene product α-endosulfine is considered (10) to be a regulator of the β-cell K_{ATP} channels that control insulin secretion (2), it is tempting to speculate that mutations in the *ENSA* gene in some type 1 diabetic patients may render the K_{ATP} channel inoperative, thereby preventing insulin

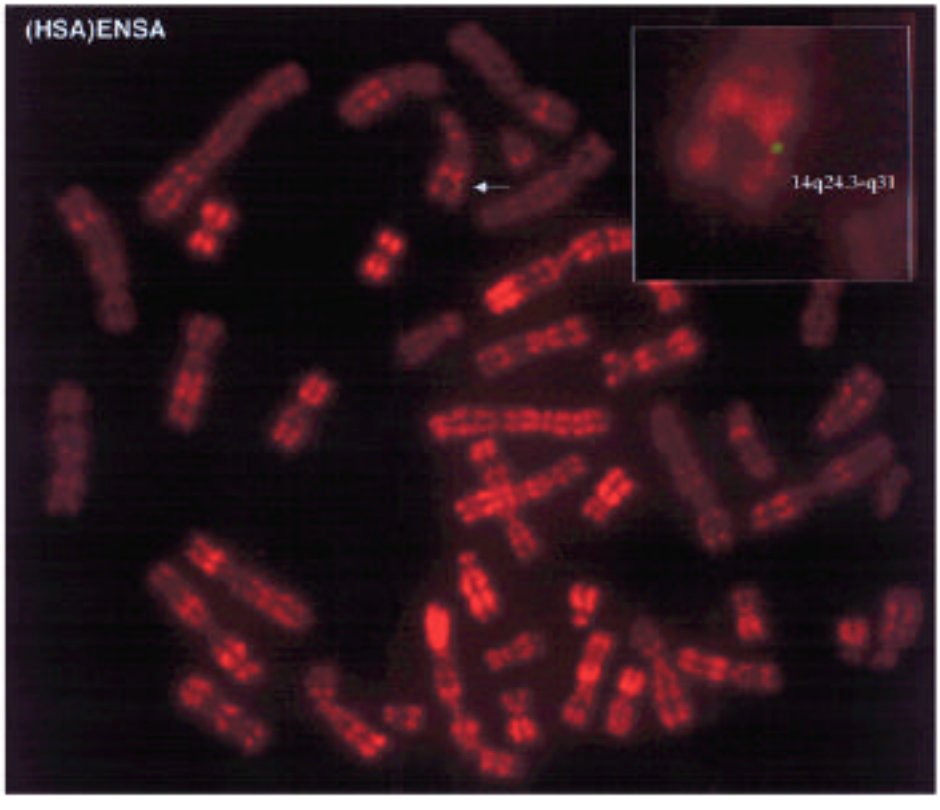

FIG. 3. Human metaphase chromosomes showing the specific hybridization of the α-endosulfine probe to chromosome 14q24.3–q31 (indicated by the arrow).

release and ultimately leading to the occurrence of type 1 diabetes. Further studies on DNA from diabetic patients will now be undertaken to address this important issue.

RESEARCH DESIGN AND METHODS

Cloning of the α-endosulfine gene (*ENSA* gene). A human genomic library (Clontech, Palo Alto, CA) was screened with the α-endosulfine cDNA probe (749 bp) according to the manufacturer's instructions. Three positive clones were isolated and found to be identical by both restriction and Southern blot analysis. The hybridizing region could further be narrowed from 6 kb to a 1-kb–long fragment that was subcloned and sequenced. Because the coding region of α-endosulfine was interrupted in these genomic clones, we had to rely on another method to characterize the 3′ part of the *ENSA* gene. The Human Genome Walker Kit (HGW kit; Clontech) is intended to walk on genomic DNA, starting from a known sequence. A first PCR step was performed using GSP3 primer and AP1 primer of the HGW adaptor. The second step was carried out with GSP4 primer and AP2 primer (Table 1). The 400-bp DNA fragment obtained containing part of the already known cDNA sequence was subcloned and sequenced. 3′RACE-PCR (Boehringer, Mannheim, Germany) was used to determine the polyadenylation site(s) representing the end of the endosulfine message. cDNA was reverse-transcribed from 2.5 μg of human brain total RNA and a nested PCR amplification was performed using GSP3 and GSP4 primers, respectively, with oligo d(T)-anchor primer and PCR anchor primer (according to the manufacturer's protocol). The 490-bp PCR fragment generated was subcloned and sequenced.

Genomic organization. To look for the presence of intron(s) within the *ENSA* gene, we compared the products obtained by PCR amplification from genomic DNA with human muscle cDNA. The latter was obtained by reverse transcription of total muscle RNA (5 μg) (Invitrogen, Carlsbad, CA), using Moloney murine leukemia virus (Stratagene, La Jolla, CA) reverse transcriptase (1 h, 37°C) and oligo d(T) as an annealing primer (5 min, 65°C). Refer to Table 1 for the locations of primers used in PCR.

Fluorescence in situ hybridization analysis. Human chromosomes were prepared from human peripheral blood lymphocyte cultures after BrdU incorporation during the last 7 h before harvesting. The 749-bp α-endosulfine cDNA was used as a probe, labeled with biotin-11-dUTP, and hybridized to human chromosomes (30 ng/μl), as previously described (15). Detection of hybridization was performed by using goat antibiotin antibodies (Vector Laboratories, Burlingame, CA) and rabbit fluorescein isothiocyanate–conjugated antibodies (Biosys, Karben, Germany). Direct binding of 5-bromodeoxyuridine–substituted chromosomes stained with propidium iodide was obtained by incubation in an alkaline solution of p-phenylenediamine (PPD11) (16). Metaphases were observed under a fluorescent microscope (DMRB; Leica, Heerbrugg, Switzerland).

ACCESSION NUMBERS

Database accession numbers are as follows: *Homo sapiens* mRNA α-endosulfine: EMBL X99906; *Homo sapiens* α-endosulfine, genomic DNA/(HSA)ENSA: AJ010966.

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