Articles

Chromosomal Mapping of *HCaRG*, a Novel Hypertension-Related, Calcium-Regulated Gene

(chromosome / localization / gene / comparison / species)

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Abstract. We recently identified a novel gene that is negatively regulated by extracellular calcium concentration with higher levels of transcripts in hypertensive animals (SHR). We named this gene *HCaRG* (Hypertension-related, Calcium-Regulated Gene). In this work we report the chromosomal localization of the *HCaRG* gene among different species. We identified a *Bgl*II RFLP between BN.*lx* and SHR rats. We then analysed the strain distribution pattern of this RFLP in 31 RIS, originating from BN.*lx* and SHR rats, and compared it to the segregation of 475 markers localized in the rat genetic map. *Hcarg* localizes to the rat chromosome 7 between the markers Mit3 and Mit4. This region is homologous to human chromosome 8q21-24. We identified three clones in Genbank that contain the sequence of *HCaRG*. It was therefore possible to narrow down the localization of human *HCaRG* to chromosome 8q24.3. Furthermore, a suggestive localization of mouse *Hcarg* based on conservation of linkage between human and mouse is on chromosome 15. We previously identified a putative calcium-binding motif (EF-Hand) and a nuclear receptor-binding domain (LxxLL) in the rat sequence of the HCaRG protein. Sequence comparison between five different species showed that these domains are highly conserved. Furthermore, a search of ESTs in Genbank for homologous sequences showed that HCaRG is expressed only in eukaryotes, particularly in mammals.

HCaRG, a novel hypertension-related calcium-regulated gene, was recently isolated in a screen of rat parathyroid cells cultured in low-calcium medium. Molecular cloning and analysis revealed that *HCaRG* encodes a nuclear protein of 224 amino acids (a.a) containing 4 overlapping putative 'leucine zipper' consensus motifs and an EF-hand calcium-binding-like motif. A nuclear receptor-binding motif was also identified in its sequence. HCaRG mRNA levels are negatively regulated by extracellular calcium levels and its basal levels are significantly higher in spontaneously hypertensive rats (SHR) when compared to normotensive animals (BN.*lx* or WKY) (Solban et al., 2000).

Human HCaRG is expressed preponderantly in the parathyroid gland, kidney, heart, stomach, jejunum, liver and adrenal glands. Comparison of HCaRG expression between foetal and adult organs revealed that HCaRG mRNA is less expressed in all foetal tissues compared, particularly in the heart, kidney and liver. Its levels are also decreased dramatically in several cancerous cell lines.

We also demonstrated that in renal ischaemia-reperfusion experiments HCaRG mRNA declined rapidly to its lowest levels at 3 h and 6 h of reperfusion. These values then increased steadily to higher than baseline at 48 h of reperfusion (Solban et al., 2000). Human embryonic

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Abbreviations: a.a – amino acid(s), BN – Brown-Norway, EST – expressed sequence tag, RFLP – restriction fragment length polymorphism, RIS – recombinant inbred strain, SDP – strain distribution pattern, SHR – spontaneously hypertensive rat.

kidney cells (HEK293) stably overexpressing HCaRG have a much lower proliferation rate than control cells. Taken together, these results suggest that HCaRG is potentially involved in the regulation of renal cell proliferation.

In this report, we describe the chromosomal localization of the *Hcarg* gene on rat chromosome 7 in a region of conserved synteny with human chromosome 8q21-24 that is associated with several bone diseases, including osteopetrosis and multiple exostosis and several human neoplasms. We identified three clones in Genbank containing the full-length *HCaRG* sequences. These clones were localized on human chromosome 8q24.3, confirming our initial prediction. A suggestive localization of mouse *Hcarg* based on the conservation of linkage between human and mouse is on chromosome 15. Sequence comparison of HCaRG between five different species shows a high degree of homology and the conservation of the identified motifs. Furthermore, a search of translated ESTs showed that *HCaRG* is a highly conserved gene found only in eukaryotes.

Material and Methods

Southern blot analysis

Genomic DNA was extracted from the liver of SHR and Brown-Norway (BN) rats as well as from 33 recombinant inbred strains (RIS) as described earlier (Hamet et al., 1992). Restriction digestions and preparation of Southern blots were performed following standard methods. The probe used corresponds to a 437-bp coding region of rat *HCaRG*, labelled using the random primers DNA labelling system (Life Technologies, Burlington, ON).

Restriction fragment length polymorphism (RFLP) analysis and chromosomal mapping

Southern blot analysis was performed with 10 μg genomic DNA of SHR and BN.*lx* rats after digestion with several restriction enzymes including *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Kpn*I and *Pst*I. Genomic DNA was extracted from 33 RIS and digested with *Bgl*II. The strain distribution pattern (SDP) of *HCaRG* RFLP in these RIS was correlated with those of 475 polymorphic markers mapped previously (Pravenec et al., 1996). The SDP of these markers are available from the Ratmap web site (http://www.ratmap.gen.gu.se). Linkage analysis was performed using the MapManager QT programme of Manly (version 3.0b21) (Manly, 1993). These RIS, originating from reciprocal crosses of normotensive BN.*lx* and hypertensive SHR, are described elsewhere (Pravenec et al., 1989). They are the only set of rat RIS available for the study of the genetics of hypertension and related traits. Human *HCaRG* chromosomal location was first determined by rat–human synteny, and confirmed with the identification of three clones submitted to Genbank (accession numbers AF124523, AF146367, and AF118808). A suggestive chromosomal localization of mouse *Hcarg* was determined by the conservation of linkage between human and mouse available through Genbank.

Protein comparison and taxonomy report

The human HCaRG protein was compared to all available ESTs in Genbank using the tBLASTn programme which translates all ESTs. The taxonomy report was simultaneously generated based on the information in the NCBI taxonomy database. Protein sequences of HCaRG from different organisms were aligned using the ClustalX1.81 programme.

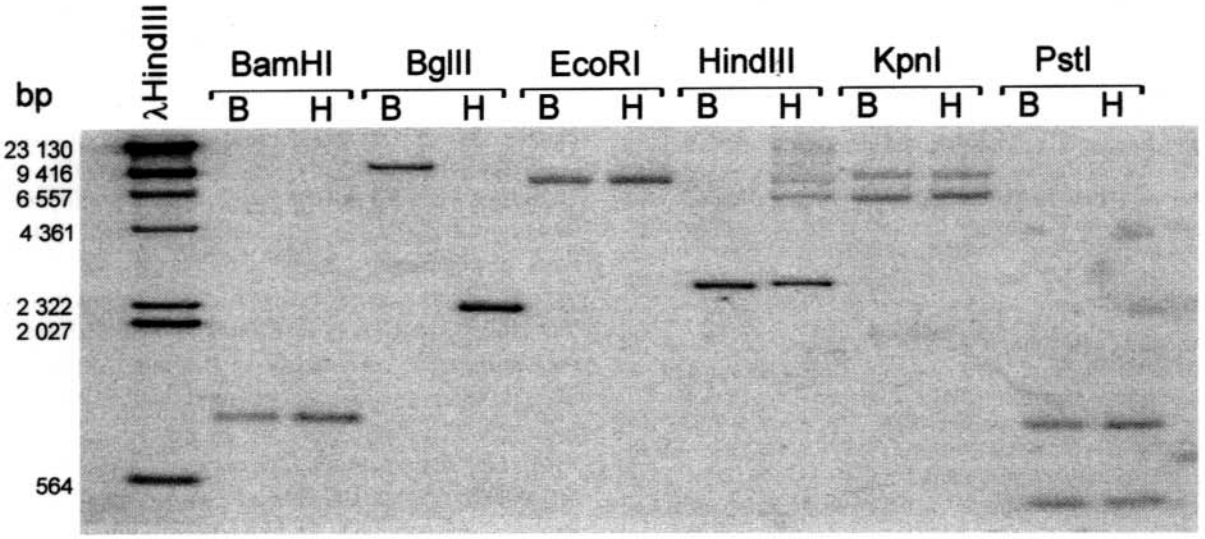

Fig. 1. Determination of a RFLP near the *Hcarg* gene. Genomic DNA from BN.*lx* (B) or SHR (H) rats was extracted and digested with different restriction enzymes. Digestion with *Bgl*II revealed a 12-kb band in BN.*lx* rats and a 2.2-kb band in SHR.