

	Score
Eukaryota (eukaryotes)	
... Coelomata (animals)	
..... Euteleostomi (vertebrates)	
..... Tetrapoda (vertebrates)	
..... Amniota (vertebrates)	
..... Eutheria (mammals)	
..... Homo sapiens (human)-----	211 hits 367
..... Sus scrofa (pig)-----	10 hits 263
..... Mus musculus (house mouse)-----	81 hits 263
..... Bos taurus (cow)-----	36 hits 260
..... Rattus norvegicus (norway rat)-----	12 hits 250
..... Rattus sp. (rats)-----	8 hits 178
..... Gallus gallus (chicken)-----	1 hit 37
..... Xenopus laevis (african clawed frog)-----	5 hits 97
..... Danio rerio (zebrafish)-----	2 hits 38
..... Bombyx mori (domestic silkworm)-----	2 hits 36
..... Glycine max (soybean)-----	1 hit 55

Fig. 5. Taxonomy report. The taxonomy report was generated by comparing the human HCaRG protein sequence to translated ESTs using the tBLASTn programme. The report shows the number of BLAST hits in different species. The highest numbers of hits are in eutheria (placental mammals) with the highest level of significance. HCaRG is not found in prokaryotes but only in eukaryotes, particularly in mammals.

8q21-24. In a recent search of *HCaRG* homologous sequences in Genbank, homologies were found with three clones from chromosome 8 containing the zinc-finger protein 7 (*ZFP7*). It was therefore possible to localize *HCaRG* on chromosome 8q24.3, confirming our initial prediction. Interestingly, this region contains loci involved in several bone diseases, including osteopetrosis, multiple exostosis, and early-onset osteoarthritis (McKusick et al., 1994). Osteopetrosis represents a group of genetically and clinically heterogeneous disorders characterized by increased bone density and abnormalities of skeletal modelling. One form of osteopetrosis associated with renal tubular acidosis and characterized by deficiency of carbonic anhydrase II has been localized at position 8q22. Multiple exostosis, an abnormal dominant-inherited disease characterized by displacement of areas of the growth plate on bone metaphysis, has been localized at 8q24.11-8q24.13 (Mertens et al., 1994). Calcium pyrophosphate-deposition disease (CPDD), also called "chondrocalcinosis" or "pseudogout," is a disorder characterized by deposition of calcium-containing crystals in the joint tissue, which leads to arthritis-like symptoms. A locus for early-onset CPDD and severe degenerative osteoarthritis was found on chromosome 8q. The high level of expression of HCaRG in the parathyroid glands and its co-regulation with parathormone by extracellular calcium suggests a role for HCaRG in calcium metabolism (Solban et al., 2006).

Furthermore, this region is implicated in the 8;14 translocation associated with Burkitt's lymphoma (Adams et al., 1983), and is often abnormal in cancers. Chromosome 8 abnormalities can be detected in 15% of patients with acute myeloid leukaemia (AML), and recent work showed that the region 8q22 to 8qter might be of particular pathogenic importance (Batanian et al., 2001). Gains of the 8q region were also reported in

prostate cancer (El Gedaily et al., 2001); among other regions, 8q21 and 8q24 were found to be amplified. Amplifications of *MYC* have also been found in a large fraction of hormone-refractory prostate cancer (Nupponen and Visakorpi, 2000). Gains and high-level amplification at 8q were more frequent in metastatic gastrointestinal stromal tumour (GIST) (El-Rifai et al., 2000). We observed that HCaRG levels were decreased in all cancerous cell lines studied, and also decreased in a glioblastoma, a partly differentiated renal cell carcinoma and a moderately differentiated hepatocellular tumour, when compared to the same amount of normal RNA of adjacent tissues excised from the same operational site (Solban et al., 2000). Chromosome 8 abnormalities in certain cancers could disrupt the *HCaRG* gene, leading to uncontrolled proliferation.

We previously evaluated *Hcarg* expression after unilateral renal ischaemia/reperfusion in uninephrectomized rats (Solban et al., 2000). This process of kidney injury and repair recapitulates many aspects of development. It involves de-differentiation and regeneration of epithelial cells, followed by differentiation (Molitoris et al., 1988; Bacallao and Fine, 1989; Wallin et al., 1992). We observed that *Hcarg* mRNA declined rapidly to its lowest levels at 3 h and 6 h of reperfusion, then increased steadily to higher than baseline at 48 h of reperfusion. In contrast, the levels of the proto-oncogene *c-myc*, which is correlated with hyperplastic response in mammalian cells (Nakajima et al., 1996), was rapidly increased following renal ischaemia and reperfusion. In the same study we showed that overexpression of HCaRG in a human embryonic kidney cell line (HEK293) inhibited proliferation, as demonstrated by cell counting and ³H-thymidine incorporation. Furthermore, these cells exhibit characteristic features of differentiation: lower proliferation rate, higher protein content, increased production of ANP, and desmosomes (Devlin et al., in preparation). Taken together, this suggests that *Hcarg* contributes to kidney cell differentiation.

In conclusion, we have localized rat *Hcarg* on chromosome 7, and the human gene on chromosome 8q24.3. This region contains loci involved in several bone diseases and is associated with Burkitt's lymphoma. Amino acid comparison with translated ESTs showed that HCaRG is only expressed in eukaryotes, mostly in mammals. Two putative domains of HCaRG including an EF-hand domain and a nuclear receptor interaction domain are conserved among species.

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