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A targeted deletion/insertion in the mouse *Pcsk1* locus is associated with homozygous embryo preimplantation lethality, mutant allele preferential transmission and heterozygous female susceptibility to dietary fat

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Abstract

Proprotein convertase 1 (PC1) is a neuroendocrine proteinase involved in the proteolytic activation of precursors to hormones and neuropeptides. To determine the physiological importance of PC1, we produced a mutant mouse from embryonic stem cells in which its locus (*Pcsk1*) had been inactivated by homologous recombination. The inactivating mutation consisted of a 32.7-kb internal deletion and a 1.8 kb insertion of the bacterial neomycin resistance gene (*neo*) under the mouse phosphoglycerate kinase 1 protein (PGKneo). Intercross of $Pcsk1^{+/-}$ mice produced no $Pcsk1^{-/-}$ offspring or blastocysts; in addition, more than 80% of the offspring were $Pcsk1^{+/-}$. These observations suggested that the mutation caused preimplantation lethality of homozygous embryos and preferential transmission of the mutant allele. Interestingly, RT–PCR analysis on RNA from endocrine tissues from $Pcsk1^{+/-}$ mice revealed the presence of aberrant transcripts specifying the N-terminal half of the PC1 propeptide fused to *neo* gene product. Mass spectrometric profiles of proopiomelanocortin-derived peptides in the anterior pituitary were similar between $Pcsk1^{+/-}$ and $Pcsk1^{+/+}$ mice, but significantly different between male and female mice of the same genotype. Relative to their wild-type counterparts, female mutant mice exhibited stunted growth under a low fat diet, and catch-up growth under a high-fat diet. The complex phenotype exhibited by this *Pcsk1* mutant mouse model may be due to PC1 deficiency aggravated by expression of aberrant gene products from the mutant allele.

Keywords: Proprotein convertase; PC1; *Pcsk1*; Genetic deficiency; Preimplantation embryonic lethality; Transmission ratio distortion

Introduction

PC1, also known as PC3 (Smeekens et al., 1991) or SPC3 (Steiner, 1998), belongs to a family of serine proteinases that activate secretory precursor proteins by cleavages after selected pairs of basic residues (Seidah and Chretien, 1999; Steiner, 1998). In mouse, its *Pcsk1* locus maps to chromosome 13 (Seidah et al., 1991). The gene has 15 exons and 14 introns (Ftouhi et al., 1994). It is transcribed into two major mRNA isoforms of 2.8 and 4.4 kb differing in their 3′ untranslated regions (UTR) (Seidah et al., 1991) (Ftouhi et al., 1994). These transcripts are primarily found in neuroendocrine cells (Schäfer et al., 1993; Seidah et al., 1991, 1994; Zheng et al., 1994). They are translated in the endoplasmic reticulum (ER) into a 88-kDa proPC1; the zymogen gets converted in the ER and the Golgi to a 74-kDa form by autocatalytic removal of the prodomain; the mature enzyme is then sorted into secretory granules where it is further processed to its fully active form of 66-kDa by excision of a C-terminal fragment (Benjannet et al., 1993). The pro-domain may promote the proper folding of proPC1 and its exit from the ER, as demonstrated for other PCs (Anderson et al.,

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2002; Muller et al., 2000; Rehemtulla et al., 1992); it has also been shown to inhibit PC1 enzymatic activity (Boudreault et al., 1998; Lee et al., 2004). The P domain stabilizes the catalytic domain of the enzyme (Ueda et al., 2003). The C-terminal domain has been implicated in partial inhibition of PC1 activity as well as in its sorting into secretory granules (Jutras et al., 1997, 2000).

The related proteinase PC2 is often found in the same granules (Kurabuchi and Tanaka, 2002; Malide et al., 1995; Marcinkiewicz et al., 1994; Takumi et al., 1998). The activities of the two convertases are influenced by resident granin-like proteins: proSAAS, a competitive PC1-specific inhibitor (Basak et al., 2001; Fricker et al., 2000; Qian et al., 2000) and pro7B2, a PC2-specific chaperone and inhibitor (Benjannet et al., 1995; Braks and Martens, 1994; Martens et al., 1994; Mbikay et al., 2001; Muller et al., 1997).

PC1 and PC2 often process the same substrates, but with cleavage site preferences. Their substrates are mostly prohormones and proneuropeptides (Seidah and Chretien, 1999). Proopiomelanocortin (POMC) and proinsulin are the best studied of these substrates. In the anterior lobe (AL) of the pituitary, POMC is processed primarily into adrenocorticotropic hormone (ACTH) and β-lipotropic hormones (βLPH); in the neurointermediate lobe (NIL), ACTH is further processed to α-melanocyte-stimulating hormone (α-MSH) and CLIP; and βLPH to βMSH and β-endorphin (βEND) (Chrétien and Seidah, 1981). PC1 is most abundant in the AL; and PC2 most abundant in the NIL (Marcinkiewicz et al., 1993; Seidah et al., 1990, 1991). Accordingly, using co-transfected cells, it was shown that PC1 converts POMC to peptides found in the AL and PC2 to those found in the NIL (Benjannet et al., 1991; Seidah et al., 1990; Zhou et al., 1993). PC1 cleaves proinsulin after KR^{31-32} pair joining the B and C peptides, while PC2 does it after the KR^{61-62} pair joining the C and A peptides (Smeekens et al., 1992).

Two human cases of genetic PC1 deficiency have been reported (Jackson et al., 1997, 2003). The first patient suffered from massive obesity at birth and, as an adult, exhibited hypogonadotropic hypogonadism. After treatment with gonadotropin-releasing hormone (GnRH), she conceived but developed gestational diabetes mellitus associated with increased circulating levels of des-61, 62 proinsulin, suggesting that proinsulin was cleaved by PC2 after the KR^{60-61} pair, but not by PC1 after the RR^{30-31} pair. Genetic analyses revealed that the patient was a *PCSK1* compound heterozygote carrying, on one allele, a splicing mutation that leads to the production of a truncated PC1 and, on the other allele, a missense mutation that causes a G483R substitution in the P domain of the enzyme and retention of the latter in the ER. POMC-derived peptides resulting from incomplete processing were detected in the patient's serum (Jackson et al., 1997). The second patient, an infant affected by obesity at birth and postnatal gastrointestinal dysfunctions, was posthumously identified to be a compound heterozygous for Ala213del and Glu250stop PC1 mutations (Jackson et al., 2003).

A mouse model of heritable PC1 deficiency was generated by Zhu et al. (2002b) by targeted ablation of the promoter and the first exon of the *PC1* gene. Intercross of $Pcsk1^{+/-}$ mice produced offspring of all three genotypes, but homozygous nulls were underrepresented, suggesting partial prenatal mortality. Neonatal mortality of nulls was also observed. Surviving $Pcsk1^{-/-}$ mice exhibited smaller birth weight and stunted growth; they also suffered from gastrointestinal dysfunctions as manifested by a moist texture of their stools. Growth retardation was due to impaired processing of hypothalamic growth hormone-releasing hormone, resulting in less plasma growth hormone and insulin-like growth factor (IGF-1). POMC and proinsulin processing was also impaired in these mice (Dey et al., 2004; Marzban et al., 2004; Zhu et al., 2002a,b). A chemically induced N222D mutation in the catalytic domain of PC1 was recently described in mouse: homozygous mutants develop obesity and glucose intolerance associated with impaired processing of central and peripheral prohormones and proneuropeptides (Lloyd et al., 2006).

Here, we describe another model of PC1 heritable deficiency, generated by substituting a 32.7-kb internal region of its gene with a 1.8 kb heterologous gene. Homozygotes for this mutation died during preimplantation embryonic development; the mutant allele was preferentially transmitted by heterozygous parents and, in female heterozygotes, weight gain with age was influenced by dietary fat content.

Materials and methods

Pcsk1 disruption vector

Genomic DNA was extracted from the R1 embryonic stem (ES) cells established from 129Sv mice. Fragments used as *Pcsk1* homology regions in the disruption vector were amplified by PCR from this DNA and cloned into a vector previously used to disrupt the *Pcsk4* locus (Mbikay et al., 1997). The 5′ homology amplicon was amplified as a 3.2-kb fragment using the primer pair # 1 (Supplementary Table 1); it extended from nucleotide (nt) 6 of exon 1 to nt 77 of exon 2 (numbering based on Ensembl *Pcsk1* locus ID # ENSMUSG00000021587). It was digested at an *Xho*I site, located at nt 252 in the first intron, and at a 3′-most *Bam*HI site created with the PCR primer; the resulting 2.7-kb fragment was cloned into corresponding sites upstream to the *neo* gene for bacterial neomycin phosphotransferase II (NPTII) under the mouse phosphoglycerate kinase 1 (PGK) promoter. The 3′ homology amplicons was obtained using primer pair # 2 (see Table 1). It was 6.6 kb long and extended from nt 34 in exon 10 to nt 460 of exon 13. It was digested at the 5′-most *Sal*I site created with the PCR primer and at a *Kpn*I site located 0.56 kb upstream of intron 12 acceptor splice site. The resulting 3.6-kb 5′ fragment was inserted in the corresponding sites downstream to the *neo* gene and upstream of the Herpes Simplex virus thymidine kinase (*tk*) gene driven by a PGK promoter. The final vector was named pPC1KO.

Table 1
Distorted genotype distribution among offspring of heterozygote intercrosses[a]

Pcsk1 genotype[b]	♀	♂	Total	%[c]
+/+	47	41	88	20
+/−	180	175	355	80
−/−	0	0	0	0

Symbols: ♀, female; ♂, male.
[a] Number of litter: 76; total number of mice: 443.
[b] Genotyping was performed by PCR at weaning.
[c] Expected distribution: 33% +/+ and 67% +/−; $p<0.00001$, χ^2 test.