Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia

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Autosomal dominant hypercholesterolemia (ADH; OMIM144400), a risk factor for coronary heart disease, is characterized by an increase in low-density lipoprotein cholesterol levels that is associated with mutations in the genes *LDLR* (encoding low-density lipoprotein receptor) or *APOB* (encoding apolipoprotein B). We mapped a third locus associated with ADH, *HCHOLA3* at 1p32, and now report two mutations in the gene *PCSK9* (encoding proprotein convertase subtilisin/kexin type 9) that cause ADH. *PCSK9* encodes NARC-1 (neural apoptosis regulated convertase), a newly identified human subtilase that is highly expressed in the liver and contributes to cholesterol homeostasis.

To identify the third locus associated with ADH (called *HCHOLA3* or *FH3*), which we previously mapped[1] to 1p34.1–p32 (OMIM 603776) and was confirmed in a large Utah kindred[2], we carried out positional cloning using the family in whom linkage was originally identified and 23 French families in whom the implication of *LDLR* and *APOB* had been excluded (**Supplementary Note** online). Family HC92 was identified through the proband (HC92-II-7), who belongs to a multiplex pedigree affected with ADH. We obtained samples from 29 members of this family and tested them using parametric linkage analyses. We excluded linkage to *LDLR* and *APOB* (lod scores of –14.05 and –10.01 (θ = 0.0), respectively). We genotyped family members for eight Genethon markers in the 1p34–p32 region (**Fig. 1**) and obtained highly significant lod scores with a maximum of 4.26 (θ = 0.0) at *D1S2742* that reached 4.80 in the multipoint analyses (**Supplementary Table 1** online and **Fig. 2a**). Haplotype analysis identified a critical interval of 5.9 Mb between *D1S231* and *D1S2890*.

The critical interval that our team had previously reported in family HC2 (ref. 1) was more distally located between markers *D1S472* and *D1S211*. Reexamination of haplotype data (**Supplementary Fig. 1** online) showed that all affected members of family HC2 also shared the same haplotype between markers *D1S2722* and *D1S2890*, except for individual HC2-II-5. This affected individual had a recombinational event at *D1S211*, which formed the centromeric boundary of the region that we originally described[1]. Therefore, all family members were reinvestigated. Individual HC2-II-5 (who refuses treatment) was the only one who showed a substantial variation (a marked elevation of triglycerides) and thus no longer conforms with the inclusion criteria.

The region between *D1S197* and *D1S2890* contains 41 genes, including *PCSK9*. It encodes NARC-1, a novel putative proprotein convertase belonging to the subtilase subfamily[3]. A related protein is the subtilisin kexin isoenzyme-1 (SKI-1)/site-1-protease (S1P), which has a key role in cholesterol homeostasis through processing the sterol regulatory element–binding proteins[4,5]. The cDNA spans 3,617 bp and encodes a protein of 692 amino acids. By sequencing the 12 exons of *PCSK9*, we identified in family HC92 a T→A substitution in exon 2 at nucleotide 625 predicting a substitution at codon 127 of arginine for the conserved serine (S127R), thereby creating a *Mnl*I cleavage site (**Fig. 2b** and **Supplementary Fig. 2** online). We tested the members of family HC92 and 100 control individuals for this substitution. It was absent in the 200 control chromosomes, indicating that it is not a polymorphism. We found it in the 12 affected family members and in individual HC92-IV-3, whose total cholesterol level is in the 90th percentile when compared with other French individuals matched by age and sex. Thus, the penetrance in the family is estimated at 0.94.

Notably, the 625T→A mutation was also found in the proband of family HC2 and cosegregated with the disease except in individual HC2-II-5, confirming that he had been misclassified in the linkage analyses previously reported[1]. To assess the possible recurrence of this mutation, we tested flanking and intragenic polymorphic markers in both families. The same haplotype segregated with the 625T→A mutation in families HC2 and HC92 (**Supplementary Table 2** online), indicating that despite different geographical origins, the families share a common ancestor. The possibility of a French founder effect was ruled out, as the mutation was not found in 22 other French probands with ADH.

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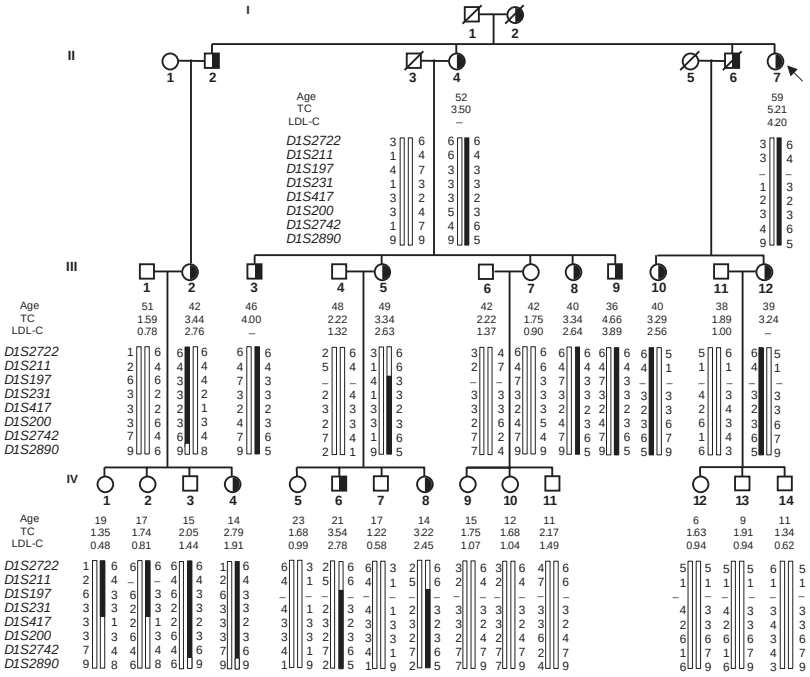

Figure 1 Pedigree of family HC92 and genetic analysis with markers spanning the 1p34.1–p32 region. Affected individuals present with a history of tendon xanthomas (individuals HC92-II-7 and HC92-III-3), coronary heart disease, early myocardial infarction (individuals HC92-II-2 and HC92-II-6) and stroke (individual HC92-II-4). Filled bars indicate the mutated allele. Age (in years) at lipid measurement, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C; in g per liter; untreated values for affected individuals) are given.

Through systematic bidirectional sequencing of the 12 exons of *PCSK9* in 22 probands with ADH, we identified a second mutation (890T→C, resulting in the amino-acid substitution F216L) in the proband of family HC60 (**Fig. 2c**), who died from myocardial infarction at 49 years of age (**Fig. 2d** and **Supplementary Fig. 2** online). This mutation segregated with the ADH phenotype in the family and was not found in 200 control chromosomes. No large rearrangement was found in any of the probands by Southern blotting (data not shown). Thus, mutations in *PCSK9* have been found in 12.5% of the families with ADH that we tested. We also identified 25 polymorphisms present in different probands and on control chromosomes, none of which gives rise to new donor or acceptor splice sites (**Supplementary Table 2** online).

NARC-1 is a novel proprotein convertase[3]. It is synthesized as a soluble zymogen that undergoes autocatalytic intramolecular processing in the endoplasmic reticulum at the primary cleavage site YVVVL↓–KEE[85], indicative of the enzymatic specificity[3] of NARC-1. Prosegment cleavage is necessary for NARC-1 to exit from the endoplasmic reticulum. The S127R mutation resides between the primary and putative secondary zymogen processing sites of the NARC-1 propeptide, and F216L is located close to the active site (which is at His226). Notably, the S127R mutation creates an RGD site that may

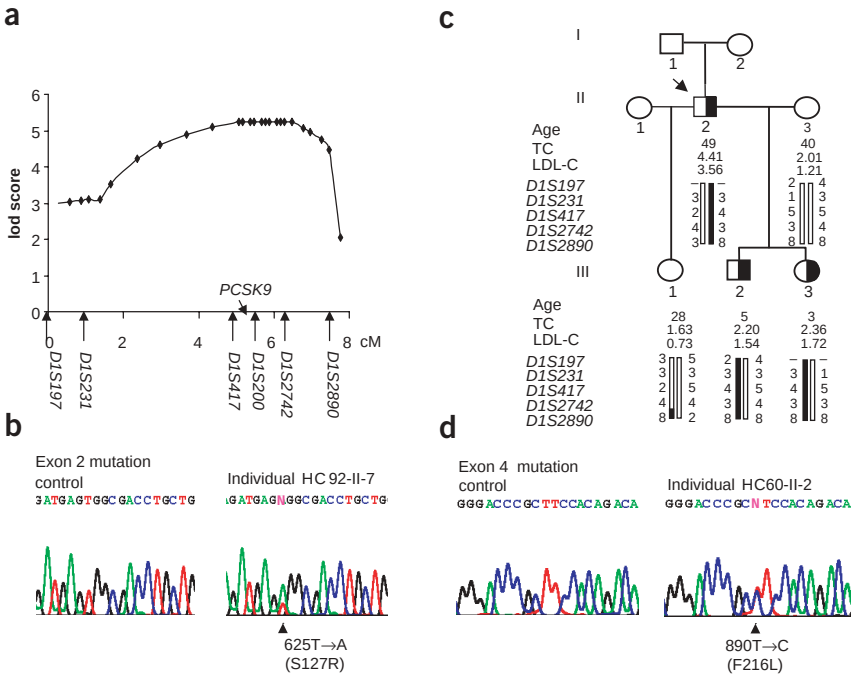

Figure 2 Genetic analysis and mutation detection in families HC92 and HC60. (**a**) Results of LINKMAP analyses in family HC92 indicating a maximum lod score for *D1S2742* at θ = 0. *PCSK9* maps 1.2 Mb away from this marker. (**b**) Mutation in family HC92. The proband (HC92-II-7, indicated by an arrow in **Fig. 1**) is heterozygous with respect to the 625T→A substitution in exon 2 (resulting in the amino-acid substitution S127R). (**c**) Pedigree and genetic analysis of family HC60. Age (in years) at lipid measurement, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C; in g/L; untreated values for affected individuals) are given. (**d**) Sequence analysis in family HC60. The proband (HC60-II-2, indicated by an arrow) is heterozygous with respect to the 890T→C substitution in exon 4, predicting the amino-acid substitution F216L.

be involved in integrin binding[6]. Although proprotein convertases activate a wide variety of proteins, none of them has yet been linked to a dominant human disease. NARC-1 is thus unique in this respect, and its identification may lead to the discovery of others.

At present, the molecular mechanisms that underlie the dominance of the trait are unknown. At this time, only missense mutations have been identified in *PCSK9*, favoring a gain-of-function or a dominant-negative mechanism. Loss of function cannot be ruled out at this stage, however, as documented in acute intermittent porphyria (OMIM 176000), a dominant trait sometimes associated with reduction of porphobilinogen deaminase activity in the liver.

The related convertase SKI-1/S1P has a key role in regulating cholesterol and fatty acid homeostasis[4,5], but the precise implication of NARC-1 in cholesterol homeostasis is unknown. Notably, NARC-1 is expressed mainly in the liver and small intestine[3], both of which are important in cholesterol synthesis and regulation. The crucial role of NARC-1 is indicated by the hypercholesterolemia that occurs when the gene is mutated. The identification of NARC-1 substrates may elucidate novel disease mechanisms and constitute targets for new intervention strategies to limit elevation of low-density lipoprotein particles and prevent morbidity and mortality from premature atherosclerosis.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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