LETTERS TO THE EDITOR

PSENEN and *NCSTN* Mutations in Familial Hidradenitis Suppurativa (Acne Inversa)

TO THE EDITOR

Hidradenitis suppurativa (HS; OMIM 142690) is an aggressive, chronic inflammatory skin disease that presents with painful boils, cysts, and abscesses in flexural areas, commonly resulting in the formation of sinus tracts and scars. It is a clinically heterogeneous, post-pubertal condition that affects ∼1% of Europeans (Revuz *et al.*, 2008) and it is associated with smoking (Konig *et al.*, 2009) and obesity (Rompel and Petres, 2000). In some families, the condition has been shown to follow an autosomal dominant inheritance pattern (Fitzsimmons and Guilbert, 1985; Von der Werth *et al.*, 2000). Mutations have recently been reported in *Presenilin-1* (*PSEN1*), *Presenilin Enhancer-2* (*PSENEN*), and *Nicastrin* (*NCSTN*) (Wang *et al.*, 2010), encoding three of four (the other being *Anterior Pharynx Defective-1, APH1*) proteins integral to γ-secretase. This is an endoprotease complex that catalyses intramembrane cleavage of membrane proteins, such as amyloid precursor protein and Notch receptors (De Strooper *et al.*, 1999; De Strooper *et al.*, 1998). In this report, we describe mutations in *PSENEN* and *NCSTN,* which to our knowledge are previously unreported, in two from seven pedigrees highlighting further locus heterogeneity in HS.

We recruited 53 individuals from seven multigenerational pedigrees, in whom HS was inherited as an autosomal dominant trait. All individuals were of British origin. A history of five or more painful or discharging nodules, cysts, or abscesses in areas frequently affected by HS (axilla, chest, groin, buttocks, and thighs) was required for diagnosis (Von der Werth *et al.*, 2000). Twenty-seven of the 53 individuals

recruited met this criteria. All subjects provided full written consent, and the study was conducted in accordance with the Declaration of Helsinki principles and approved by the East London Research Ethics Committee 2 (reference 09/H0704/50).

DNA was extracted from saliva or venous blood. All coding regions of *NCSTN* (17 exons), *PSEN1* (10 exons), and *PSENEN* (3 exons) were amplified by PCR using exon flanking intronic primers and Sanger sequenced in 19 affected individuals, one or more from each of the seven pedigrees. Sequencing revealed a heterozygous single-nucleotide insertion in *PSENEN* (c.66_67insG) and a single-nucleotide substitution in the *NCSTN* exon 9/intron 9 donor splice site (c.1101 + 1 G > A) in each of two pedigrees. Individuals II:2 and III:1 from pedigree 1 (Figure 1) were both heterozygous for the c.66_67insG insertion in *PSENEN* (Figure 1). The insertion is predicted to lead to a frameshift and an altered protein product (p.Phe23ValfsX98). The mutation was absent in unaffected family members (Figure 1, II:1, III:2) and 200 control chromosomes of European ancestry. Sequencing of *PSENEN* cDNA, generated from lymphoblast mRNA (Ribopure extraction and cDNA Reverse transcription kits, Applied Biosystems, Carlsbad, CA) from venous blood, confirmed the presence of the c.66_67insG variant; however, mRNA expression, analyzed via reverse transcription-PCR, revealed a marked reduction in *PSENEN* expression compared with control samples (Figure 1) suggesting that the mutant transcript is subject to nonsense-mediated decay.
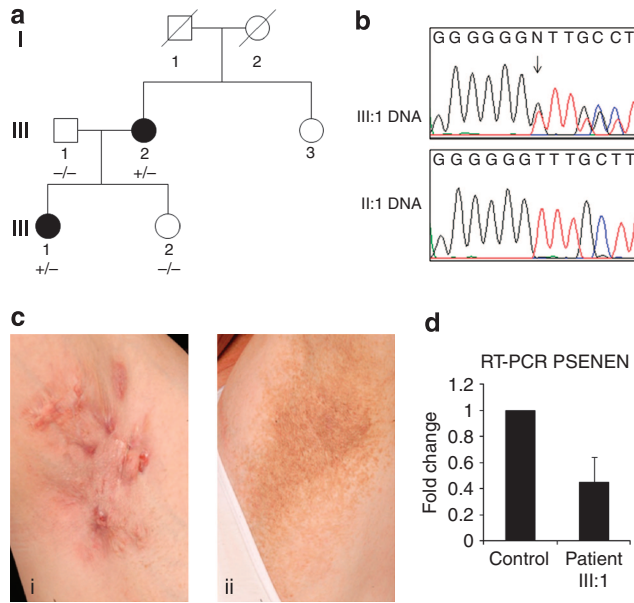
Both affected individuals from pedigree 1 developed boils and abscesses in

their axillae, under their breasts, and in their groins from the age of 15 years. Individual III:1 was more severely affected, having additional lesions over her buttocks with resultant scarring and sinus tract formation. They had a body mass index of 33 (II:2) and 23 (III:1) and had never smoked. Individual II:2 felt that her symptoms worsened around menstruation, whereas her mother, III:2, noticed no cyclical change. On examination, III:2 had light macular hyperpigmentation in her axillae and groins, and II:2 had very mild hyperpigmentation in the groin.

The proband from Pedigree 2 (Figure 2, III:1) was heterozygous for a single-nucleotide substitution in *NCSTN* (c.1101 + 1 G > A; Figure 2). The substitution is of the first base of intron 9 in the highly conserved donor splice site. However, no variant transcript was detected, and expression levels of NCSTN in III:1 were observed to be significantly lower than wild-type controls (Figure 2), again suggesting that this HS allele is subject to nonsense-mediated decay. The mutation was absent in his unaffected mother (Figure 2, II:4), but no other affected individuals were available for analysis. The variant was not observed in 200 control chromosomes of European ancestry. Individual III:1 from pedigree 2 (Figure 2) reported boils and abscesses in his axillae, suprapubic area, groin, buttocks, thighs, and neck, all associated with scarring and sinus tract formation, from the age of 16 years. He had a body mass index of 30 and a 15 pack-year smoking history.

Our results provide further evidence that mutations resulting in haploinsufficiency of the γ-secretase genes, *NCSTN* and *PSENEN*, are involved in the pathogenesis of some familial cases of HS. To our knowledge, these findings have not

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Abbreviation: HS, hidradenitis suppurativa

Figure 1. Clinical and genetic findings in pedigree 1. (**a**) Pedigree 1, showing affected individuals II:2 and III:1; (**b**) sequence from *Presenilin Enhancer-2* (*PSENEN*) exon 3, showing c.66_67insG (arrow) in genomic DNA from III:1 and wild-type sequence in unaffected II:1; (**c**) (i) individual III:1, inflamed nodules, abscesses, fistulae, and scarring; (ii) individual II:2, multiple open comedones and an inflammatory nodule on the background of patchy, macular hyperpigmentation; (**d**) reverse transcription-PCR (RT-PCR), showing reduced expression of mRNA in affected individual III:1 compared with unaffected control (error bar: mean ± SD).
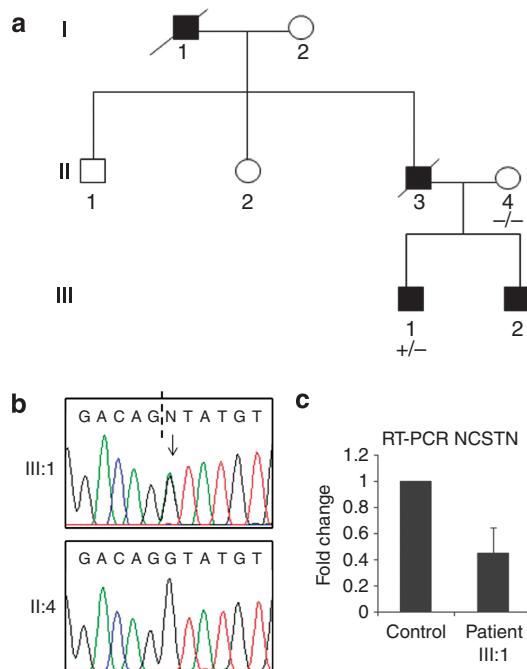
Figure 2. Clinical and genetic findings in pedigree 2. (**a**) Pedigree 2, showing affected individuals III:1 and III:2; (**b**) sequence from the end of *Nicastrin* (*NCSTN*) exon 9 showing a single-nucleotide substitution (adenine for a guanine, see arrow) (c.1101 + 1 G > A) at the first base of the donor splice site (line marks the end of the exon) in affected individual III:1. No substitution can be seen in the unaffected individual (II:4); (**c**) reverse transcription-PCR (RT-PCR), showing reduced expression of mRNA in affected individual III:1 compared with control (error bar: mean ± SD).

previously been reported in Caucasian individuals. These results indicate the involvement of γ-secretase in the development of HS, which is further supported by the development of cysts in γ-secretase-deficient mice (Pan *et al.*, 2004). γ-Secretase is involved in the cleavage of a wide array of transmembrane proteins; however, it is noteworthy that the hair follicle and sebaceous glands of mice deficient in γ-secretase are phenotypically identical to those of mice deficient in Notch 1 and 2 (Pan *et al.*, 2004), potentially indicating the involvement of the Notch cell signalling pathway in HS. The binding of ligands to the extracellular domain of Notch receptors induces γ-secretase-driven cleavage of the intracellular domain which, in the nucleus, affects gene expression (Oswald *et al.*, 2001). Our investigations highlight the γ-secretase–Notch pathway as a potential therapeutic target in HS. However, it is notable that screening of *NCSTN*, *PSEN1*, and *PSENEN* did not reveal mutations in five of the seven pedigrees. These HS pedigrees may therefore represent HS alleles comprising non-coding or undetected coding variants, represent phenocopies of HS, or be explained by further locus heterogeneity in HS. Further loci may include genes encoding other proteins involved in the γ-secretase–Notch pathway. Investigations of additional familial and sporadic cases are required to establish the contributions of γ-secretase alleles in HS and identify further genes underlying this debilitating disease. The pathophysiological mechanisms underlying HS may also provide insight into the basis of more common conditions such as acne vulgaris.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Confirmation by Exome Sequencing of the Pathogenic Role of NCSTN Mutations in Acne Inversa (Hidradenitis Suppurativa)

TO THE EDITOR

Acne inversa (AI; also known as hidradenitis suppurativa; OMIM #142690) is an autosomal dominantly inherited skin disorder characterized by recurrent draining sinuses and abscesses with subsequent scarring and chronic seepage. It mainly affects the scalp, neck, the axillae, perineum, and inframammary regions, which may lead to social embarrassment and have a profound impact on the quality of life. Its prevalence has been estimated to be 1–4% (Danby and Margesson, 2010). Our previous studies have already discovered that the genetic locus responsible for AI is located at chromosome 1p21.1–1q25.3 by genome-wide linkage scan in a four-generation Chinese family (Gao et al., 2006).

Recently, exome sequencing has been demonstrated to be a powerful and cost-effective strategy for identifying the responsible genes of rare monogenic disorders (Ng et al., 2009, 2010;

Hoischen et al., 2010). Here, we subjected the exomes of two affected (II4 and III8) and an unaffected (III7) individual in our previously mapped kindred (Family 1) to exome sequencing. Informed consent was obtained from all sequenced individuals. This study was approved by the ethics committee of Anhui Medical University and was conducted according to Declaration of Helsinki principles. Exome capture was carried out using Agilent SureSelect Human All Exon Kit (in solution; Santa Clara, CA) guided by the manufacturer's protocols. Each captured library was then loaded on Hiseq2000 platform (Illumina, San Diego, CA) and paired-end sequencing was performed, with read lengths of 90 bp, providing at least 50 average depths for each sample. Raw image files were processed by Illumina Basecalling Software 1.7 (San Diego, CA) for base-calling with default parameters. Sequence reads in each individual were aligned to

human reference genome builds hg18 using SOAPaligner 2.20 (Li et al., 2009b). The consensus genotypes in target regions were called by using SOAPsnp(v1.03) (Li et al., 2009a).

On average, 10.36 Gb of sequence was generated per individual as paired-end, 90-bp reads. After discarding the reads that had duplicated start sites, we achieved ~50-fold coverage of the 2.2-Gb mappable, targeted exome defined by RefSeq genes (Pruitt et al., 2007). On average, 87.4% of the exome was covered at least 10-fold, and 42,678 genetic variants were identified per individual, including 34,570 nonsynonymous changes.

To distinguish potentially pathogenic mutations from other variants, we focused on nonsynonymous variants and splice acceptor/donor site mutations (SS), anticipating that synonymous variants would be far less likely to be pathogenic. We also presumed that the variants responsible for AI would be rare and therefore likely to be absent in general population. A novel variant was

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Abbreviation: AI, acne inversa