Genetic analysis of NCSTN for potential association with hidradenitis suppurativa in familial and nonfamilial patients

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Dear Editor, Hidradenitis suppurativa (HS; also known as acne inversa) is an autosomal chronic inflammatory inherited disease. Several risk factors are associated with HS, such as smoking, obesity, infection and family history. Based on a genome-wide scan in a four-generation HS family, recent genetic advances in the understanding of HS have led to the conclusion that genes that encode enzymes within the γ-secretase family play a key role in HS. To find genetic variants that are associated with HS susceptibility, research has focused on sequencing the three essential subunits of γ-secretase in kindreds of patients with HS. To date, 20 mutations in NCSTN (NCSTN being the most commonly observed subunit) have been reported in African American, Chinese, Japanese, South Welsh and French multiplex kindreds, with NCSTN being the most commonly observed subunit. Mutations in NCSTN include four deletions (c.210_211delAG, c.487delIC, c.1752delG and c.582+1delG) that result in a frame shift. In addition, 10 nonsense mutations that prematurely stop the translation of the mRNA sequence and induce loss of function (p.R117X, p.R434X, p.Y565X, p.S590AfsX1, p.V75I, p.D185N, p.P211R, p.Q216P, p.Q568X and p.Q420X) have been identified, which could potentially disrupt the structure of NCSTN, leading to impaired activity.

To further explore the potential genetic association of NCSTN to HS susceptibility, we applied next-generation sequencing (NGS) technology to interrogate the entire NCSTN gene [15 679 base pairs (GRCh37/hg19)] from DNA samples extracted from the whole blood of 443 subjects with HS enrolled in two large phase III clinical trials, Pioneer I (NCT01468207) and Pioneer II (NCT01468233) (http://www.multivu.com/players/English/7298751-abbvie-phase-3-hidradenitis-suppurativa-trial-results/). Sequencing was initially completed in 95 subjects (57 with family history, 38 with no family history). Select variants within NCSTN that were identified in the 95 patients were further interrogated in all 443 subjects (Table 1). The allelic odds ratio (OR) was computed, as well as other models of genetic association (dominant, recessive, co-dominant and overdominant) stratified by ethnicity. Control group data were formed using data from the 1000 Genomes (Phase I) and Perlegen (HapMap) European (EUR) and African ancestry (AFR) populations, as our participants were either white or black. ORs were reported with the control group as the reference category. Fisher’s exact method was used to compute P-values and confidence intervals using R version 3.1.0 (http://www.R-project.org). Additional models of association were calculated using the SNPPassoc package (http://CRAN.R-project.org/package=SNPPassoc). Model selection utilizing Akaike’s information criterion was used to determine the most appropriate genetic model of association.

We identified one nonsense mutation, p.R117X (rs387906896), in a white subject with a family history of HS. This mutation has been previously identified in Chinese and African American patients with HS; this is the first study to report this mutation in a white subject. This nonsense mutation has been previously identified in Chinese and African American patients with HS; this is the first study to report this mutation in a white subject. This nonsense mutation has been previously identified in Chinese and African American patients with HS; this is the first study to report this mutation in a white subject.

Table 1. Demographic and clinical characteristics of patients with hidradenitis suppurativa (HS)

| Characteristic                        | Total (n = 595) | DNA genotyped (n = 443) |
|---------------------------------------|----------------|------------------------|
| Sex                                   |                |                        |
| Male                                  | 204 (34-3)     | 165 (37-2)             |
| Female                                | 391 (66-7)     | 278 (62-7)             |
| Age (years)                           |                |                        |
| Mean (SD)                             | 36.5 (11-2)    | 36.4 (11-7)            |
| Median (range)                        | 35 (18-69)     | 35 (18-69)             |
| Ethnicity                             |                |                        |
| White                                 | 481 (80-8)     | 365 (82-4)             |
| Black                                 | 81 (13-6)      | 57 (12-9)              |
| Asian                                 | 14 (2-3)       | 9 (2-0)                |
| American Indian                       | 2 (0-3)        | 3 (0-7)                |
| Other                                 | 17 (2-9)       | 9 (2-0)                |
| Family history                        |                |                        |
| Yes                                   | 141 (23-7)     | 115 (26-0)             |
| No                                    | 453 (76-1)     | 327 (73-8)             |
| Unknown                               | 1 (0-2)        | 0                      |
| BMI                                   |                |                        |
| Mean (SD)                             | 33 (7-8)       | 32.5 (7-5)             |
| Median (range)                        | 32 (16-69-8)   | 31.7 (16-61-6)         |
| Smoker                                |                |                        |
| Yes                                   | 408 (65-6)     | 296 (66-8)             |
| No                                    | 187 (31-4)     | 124 (28-0)             |
| Duration of HS (years)                |                |                        |
| Mean (SD)                             | 11.5 (8-7)     | 11.4 (8-7)             |
| Median (range)                        | 9-3 (1-0-43-5) | 9-2 (1-0-43-5)         |
| Hurley stage at baseline              |                |                        |
| II                                    | 319 (53-6)     | 234 (52-8)             |
| III                                   | 276 (46-4)     | 209 (47-2)             |

Data are n (%) unless otherwise indicated. BMI, body mass index. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
One missense mutation was also identified, P.A410V (rs147225198), in NCSTN exon 11. To our knowledge, this mutation has not been previously reported in any patient with HS. P.A410V was present in a white patient with Hurley stage III and without a family history of HS. P.A410V showed a higher minor allele frequency compared with the control groups (AFR and EUR, depending on ethnicity). The MAF of rs16831883 was higher in white subjects with HS relative to the control database. In contrast, rs6427515 showed a higher significant change in MAF in the black subjects relative to controls (0.47 vs. 0.33, OR 1.45; P < 0.01). Similarly, rs1004607 also showed a higher MAF in black subjects with HS relative to controls (0.020 vs. 0.0 in the control group, OR 0.91; P = 0.03) (Tables 2 and 3). Standard genome browser tools such as UCSC Genome Browser, identified that rs16831883 lies in a DNA splice region and rs1004607 lies in a DNase I hypersensitivity cluster. Using the noncoding variant functional prediction tool GWAVA, none of these SNPs predictive scores reached ‘functional’ threshold. In addition, a recent publication functionally examined rs6427515 as part of a haplotype with five other linked disequilibrium SNPs; in vitro analysis showed no significant effect on NCSTN function. Further in vitro validation work needs to be performed in order to identify if these SNPs have functional significance.

In summary, this is the largest study to date on potential genetic associations underlying HS. After deep sequencing of NCSTN for single nucleotide variant in subjects with HS, we identified two nonsynonymous mutations, one of which had previously been described in African American and Chinese patients with HS. In addition, several intronic polymorphisms within the NCSTN were identified that were present at a significantly higher frequency in the HS population relative to

### Table 2 Clinical data for the two individuals who carry the two mutations

| rsSNP       | Location     | Function      | Previously identified in HS | Hurley stage | Age (years) | Sex  | Ethnicity | Family history | BMI  | Smoker | Alcohol use | Duration of HS (years) |
|-------------|--------------|---------------|-----------------------------|--------------|-------------|------|-----------|----------------|------|--------|------------|-----------------------|
| rs387906896 | chr1: 160319373 | stop_gained Chinese, African American | Novel          | II           | 44          | Female | White    | Yes            | 29.4 | Yes    | No         | 9.27                   |
| rs147225198 | chr1: 16032395 | missense      | Novel                      | III          | 41          | Female | White    | No             | 28.1 | No     | No         | 8.82                   |

rsSNP, nonsynonymous single nucleotide polymorphism; HS, hidradenitis suppurativa; BMI, body mass index.

### Table 3 Frequency, allelic odds ratios (OR) and P-values of carriage of single nucleotide polymorphisms (SNPs) of NCSTN that are associated with hidradenitis suppurativa susceptibility in different ethnic groups

| rsID       | Ethnicity | Minor allele | MAF (control) | MAF (case) | OR   | 95% CI | P-value |
|------------|-----------|--------------|---------------|------------|------|--------|---------|
| rs16831883 | Black     | C            | 0.067         | 0.120      | 1.90 | 0.86–3.91 | 0.10    |
|            | White     | C            | 0             | 0.009      | –    | 1.38–∞  | 0.01    |
| rs6427515  | Black     | C            | 0.331         | 0.471      | 2.27 | 1.45–3.57 | < 0.01  |
|            | White     | T            | 0.065         | 0.092      | 1.48 | 0.99–2.21 | 0.05    |
| rs1004607  | Black     | G            | 0             | 0.020      | –    | 0.91–∞  | 0.01    |
|            | White     | G            | 0.017         | 0.022      | 1.27 | 0.55–2.91 | 0.57    |

The OR is reported as case/control, and calculations are based on Fisher’s exact test. All SNPs shown were in Hardy–Weinberg equilibrium with P > 0.60 in controls. MAF, minor allele frequency; CI, confidence interval.
healthy controls. Whether these SNPs have functional significance is not known. A clear conclusion from this analysis is that the vast majority of subjects enrolled in the HS studies did not display genetic variants in NCSTN. Targeted NGS of NCSTN in all patients may have identified some additional variants in this gene; however, the low number identified in the first 95 patients suggests that additional and as-yet unknown genes also predispose to the development of this disorder. Clearly, further studies are warranted to identify additional factors that contribute to the etiology and mechanism of this disease.

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1Pharmacogenetics and Pharmacogenomics, 2Research and Development, and 3Department of Immunology, AbbVie Inc., One N. Waukegan Road, North Chicago, IL 60064, U.S.A.

E-mail: mohan.liu@abbvie.com

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Fig S1. The structure of nicastrin (NCSTN) showing the locations of the variants Ala410Val and Arg117Ter.

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