Insights from γ-Secretase: Functional Genetics of Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic, relapsing inflammatory disease of the skin characterized by painful acne-like lesions, nodules, abscesses, sinus tracts, and scar formation primarily in intertriginous regions (i.e., axillae, submammary folds, groin). HS is associated with a high comorbidity burden and the lowest QOL among any dermatologic condition, yet it remains underrecognized and poorly understood (Reddy et al., 2019). Global incidence of HS varies by country. In the United States, the incidence of HS is rising. HS is reported in individuals of all age groups, races, and sexes but shows a predilection toward African Americans and women. Registry studies estimate the prevalence of HS at 0.3%, 0.22%, and 0.09% in individuals of African, biracial, and Caucasian descent, respectively. Furthermore, among these groups, the prevalence peaks between 20 and 40 years and declines after 50 years (Garg et al., 2017a, 2017b; Sabat et al., 2020). Despite this demonstrated need, the pathogenesis of HS remains poorly studied.

Studies report that 30–42% of patients with HS report a positive family history of the disease, which points toward a potential genetic etiology. A recent Dutch twin cohort study found a narrow-sense heritability of 77% for HS (van Straalen et al., 2020). Furthermore, a minority of these patients across multiple ethnicities have been found to exhibit a monogenic form of the disease that is associated with heterozygous mutations in the γ-secretase complex (Ingram, 2016). There is an increased incidence of HS in the setting of other genetic inflammatory syndromes, and multiple syndromic forms of HS have been identified, such as pyoderma gangrenosum, acne, and HS (PASH) and Dowling-Degos disease (DDD), many of which have been tracked to specific mutations in a small number of candidate genes (Scheinfeld, 2013). The sporadic form of HS, in contrast to the familial form, appears to encompass the majority of disease burden (60–70%) and is thought to be driven by a polygenic architecture (Jiri et al., 2019). Several unique phenotypes have even been identified in both familial and sporadic HS and in certain endemic populations (such as males of Asian ancestry) with clear evidence of heritability (Pink et al., 2012; Wang et al., 2010; Xu et al., 2016). Studies have found associations between environmental factors and HS, which suggests a multifactorial etiology. Whether specific genetic variations increase susceptibility to developing HS in the presence of specific environmental triggers remains an open question and suggests the existence of previously undescribed genetic risk factors.

The paucity of HS GWASs has made systematic dissection of HS pathophysiology challenging from a genetic level, and because of its inflammatory nature, some have turned to immune profiling for insights (Gudjonsson et al., 2020; Lowe et al., 2020). Furthermore, a more complete understanding of genetic features underlying HS may help to develop a more nuanced classification system with better prognostic value, improve patient management, and identify key candidate therapeutics. To date, no genotype—phenotype correlation has been established, but combined genetic and immunological studies could bridge the gap (Frew et al., 2019). Although a minority of patients with HS exhibit family history, clinical, genetic, and molecular studies in familial cohorts harboring γ-secretase mutations began to define pathological mechanisms involved in the etiology of HS. Subsequent studies in laboratory animals further identify...
molecular mechanisms involved in HS. Together, GWASs and laboratory studies have shown feasibility in dissecting potential mechanisms of HS pathology. In this review, we synthesize the current information on γ-secretase genetics underlying a subpopulation of patients with HS and evaluate its importance in the context of disease pathobiology and future research.

Mutations in γ-secretase demonstrate clinical significance in a subset of patients with HS

The γ-secretase complex is a heterogenous transmembrane protease complex composed of the catalytic PSEN1/PSEN2 and cofactor subunits PSENEN, NCSTN, and APH1A/APH1B. It functions to cleave over 70 type I membrane proteins, such as cadherins, Notch, and APP (Merilahti et al., 2017). Dysfunctional γ-secretase—APP axis is well known in the development of Alzheimer disease; however, epidemiologic studies to date have not identified an increased risk of Alzheimer among patients with HS with γ-secretase complex mutations or overlapping pathogenic variants between the two disease populations (Garg and Strunk, 2017; Theut Riis et al., 2017). Alzheimer- and HS-associated γ-secretase mutations may have distinct functional outcomes with regard to downstream signaling and efficacy in cleaving different substrates. More specifically, one could hypothesize that HS-associated γ-secretase mutations have no effect on the ability of γ-secretase to cleave APP or that these mutations are found in isoforms not expressed in the brain.

In 2006, Gao et al. identified a putative risk locus within 1p21.1-1q25.3, a >900 gene region, in a four-generation Chinese family using genome-wide linkage scan (GWLS) (Gao et al., 2006). This was further narrowed to a >200 gene region within 1q21.3-1q23.2 in a follow-up Chinese case report. In a 2010 GWLS, Wang et al. (2010) identified γ-secretase mutations in a cohort of six Han Chinese families with an autosomal dominant transmission pattern that harbored separate heterogenous rare variants in NCSTN, PSEN1, or PSENEN, which localized to the 1q23.2 locus. Gao et al. (2006) and Wang et al. (2010) represented two of four genetic studies employing a genome-wide approach in HS kindreds to date. The final two identified putative risk loci at 1q23.2 (NCSTN) in an Iranian family and both chromosome 19 and 6q25.1-25.2 in a number of European families, respectively (Faraji Zonooz et al., 2016; Irwin McLean et al., 2006). In addition, a small number of other studies probing African American, Indian, Japanese, British, and French families identified γ-secretase mutations that cosegregated with a disease phenotype (Ratnamala et al., 2016; Takeichi et al., 2020). The remainder of mutations were identified via targeted sequencing; overall, 50 SNPs associated with HS have been identified in Chinese (23), French (3), British (3), Thai (3), and African Americans (1), encompassing the NCSTN, PSEN1, and PSENEN genes (Table 1), 23 of which were determined to be likely pathogenic by American College of Medical Genetics criteria (Frew et al., 2017). The locations of these mutations in γ-secretase protein domains are shown in Figure 1. Current population data indicate that such heterozygous, nonsynonymous γ-secretase mutations are rarely found in healthy controls and demonstrate high penetrance in affected pedigrees (Wang et al., 2010). Linkage disequilibrium was identified in 12 pairs of variants, and two specific mutations, NCSTN-R117X and -Q568X, were each found in families from different races (Frew et al., 2019; Li et al., 2019a).

Most of these γ-secretase mutation—positive patients are identified in families, often with multiple affected family members. Of note, classification terms such as familial, typical, atypical, syndromic, and sporadic are unreconciled and require further validation (Frew et al., 2019). The majority of these patients are found in particular demographics (e.g., male, Asian) and observed to have severe, widespread, treatment-resistant, anatomically atypical, or syndromic disease with superimposed comorbidities, such as acne conglobata, pyoderma gangrenosum, and hyperpigmentation, among others (Pink et al., 2013). Comparisons against existing HS classification systems demonstrate that γ-secretase mutation—positive patients, compared with patients with sporadic HS, fit best with the categories of LC2 or follicle-centered, atypical, nodular, and scarring folliculitis using the Canou, Naasan, Martorell-Calatayud, and van der Zee classification systems, respectively (Canou-Poirine et al., 2013; Ingram and Piguet, 2013; Martorell et al., 2020; Naasan and Affleck, 2015; van der Zee and Jemec, 2015; Xu et al., 2016). However, poor interrater reliability and the lack of validation limit the utility of these classification systems (van Straalen et al., 2018).

In the Alzheimer IDENTITY trial, semagacestat, a γ-secretase inhibitor, was administered but resulted in unspecified skin toxicity in a large portion of patients (Henley et al., 2014). More striking is that, in a subsequent study of patients with desmoid tumor, in which niragacestat, another γ-secretase inhibitor, was administered, 12 of 17 exhibited adverse skin toxicities, and 6 of 7 evaluated by dermatology exhibited new-onset, recurring follicular and cystic lesions with surrounding inflammation in intertriginous areas, strongly resembling the HS phenotype (O’Sullivan Coyne et al., 2018). Biopsies of two patients showed inflamed follicular cysts, confirming pathology localized to the hair follicle (HF). These lesions then resolved on halting of treatment. These patients had no personal or family history of HS or its commonly cited comorbidities, suggesting that targeted γ-secretase inhibition can induce HS-like lesions, which supports the findings from genetic studies identifying loss-of-function mutations in components of the γ-secretase complex.

γ-secretase dysfunction leads to defective terminal HF homeostasis

Occlusion of the follicular infundibulum because of mechanisms including hyperkeratosis and disrupted epithelial differentiation is considered the initiating event in HS pathogenesis (Prens and Deckers, 2015), although some believe subclinical inflammation may precede or even contribute to the occlusion (Frew et al., 2018). Several studies suggest that γ-secretase may play a key role in occlusion.

Developmentally, the absence of γ-secretase in mice is known to convert HFs into epidermal cysts by altering the differential fate of outer root sheath cells (Pan et al., 2004). Several studies have linked impaired functionality of γ-secretase to the formation of HS-like lesions in mice. Conditional knockout of γ-secretase components results in many
Table 1. Identified Mutations in Patients with HS in NCSTN, PSENEN, and PSEN1

<table>
<thead>
<tr>
<th>Gene</th>
<th>DNA Change</th>
<th>Amino Acid Change</th>
<th>Mutation Type</th>
<th>Ethnic Origin (No. of Families)</th>
<th>F/C</th>
<th>Isolated HS or Syndrome/Associated Conditions</th>
<th>Method of Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCSTN</td>
<td>1q23.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>c.223G&gt;A</td>
<td>p.Val73Ile</td>
<td>Missense</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>3</td>
<td>c.210_211delAG</td>
<td>p.Thr706X18</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Whole-exome sequencing</td>
</tr>
<tr>
<td>4</td>
<td>c.218delC Exon 4</td>
<td>p.P73LFs*15</td>
<td>Frameshift</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>5</td>
<td>c.278delC</td>
<td></td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>C</td>
<td>SAPHO</td>
<td>Whole-exome sequencing</td>
</tr>
<tr>
<td>6</td>
<td>c.344_351del</td>
<td>p.Thr115Asn*20</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS (all)</td>
<td>GWLS</td>
</tr>
<tr>
<td>7</td>
<td>c.349C&gt;T</td>
<td>p.Arg117X</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F (all)</td>
<td>Isolated HS (all)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Japanese (1)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>African American (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>c.477 C&gt;A</td>
<td>p.C159X</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>9</td>
<td>c.487delC</td>
<td>p.Gln163Ser66X39</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>10</td>
<td>c.497C&gt;A</td>
<td>p.Ser166X</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>11</td>
<td>c.533G&gt;A</td>
<td>p.Asp185Asn</td>
<td>Missense</td>
<td>Chinese (1)</td>
<td>C</td>
<td>Isolated HS</td>
<td>N/A</td>
</tr>
<tr>
<td>12</td>
<td>c.582+1delG</td>
<td>Splice site</td>
<td>Splice site</td>
<td>Japanese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>13</td>
<td>c.617C&gt;A</td>
<td>p.Ser206X</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>14</td>
<td>c.612C&gt;G</td>
<td>p.Pro211Arg</td>
<td>Missense</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>15</td>
<td>c.647A&gt;C</td>
<td>p.Gln216Pro</td>
<td>Missense</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>16</td>
<td>c.687insCC</td>
<td>p.Cys230Pro66X31</td>
<td>Frameshift</td>
<td>Indian (1)</td>
<td>F</td>
<td>HS + AC</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>17</td>
<td>c.887A&gt;G</td>
<td>p.Pro296Arg</td>
<td>Missense</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>18</td>
<td>c.944C&gt;T</td>
<td>p.Ala315Val</td>
<td>Missense</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>19</td>
<td>c.978delG</td>
<td>p.M226fsX10</td>
<td>Truncating</td>
<td>Singaporean (Haines et al., 2012)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>20</td>
<td>c.996+7G&gt;A</td>
<td>Splice site</td>
<td>Splice site</td>
<td>Mixed European (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>21</td>
<td>c.1101+1G&gt;A</td>
<td>Splice site</td>
<td>Splice site</td>
<td>Mixed European (2)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>22</td>
<td>c.1101+10A&gt;G</td>
<td>Splice site</td>
<td>Splice site</td>
<td>British (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>23</td>
<td>c.1125+1G&gt;A</td>
<td>Splice site</td>
<td>Splice site</td>
<td>British (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>24</td>
<td>c.1180-5C&gt;G</td>
<td>Splice site</td>
<td>Splice site</td>
<td>British (1)</td>
<td>F (1)</td>
<td>Isolated HS (2)</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>25</td>
<td>c.1229C&gt;T</td>
<td>p.A410V</td>
<td>Missense</td>
<td>Caucasian (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>26</td>
<td>c.1258C&gt;T</td>
<td>p.Gln420X</td>
<td>Truncating</td>
<td>Singaporean (Haines et al., 2012)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Japanese (1)</td>
<td>S</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>27</td>
<td>c.1258C&gt;T</td>
<td>p.Arg429X</td>
<td>Truncating</td>
<td>Japanese (1)</td>
<td></td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>28</td>
<td>c.1300C&gt;T</td>
<td>p.Arg434X</td>
<td>Truncating</td>
<td>French (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>29</td>
<td>c.1352+1G&gt;A</td>
<td>Splice site</td>
<td>Splice site</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>30</td>
<td>c.1551+1G&gt;A</td>
<td>Splice site</td>
<td>Splice site</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>GWLS</td>
</tr>
<tr>
<td>31</td>
<td>c.1635C&gt;G</td>
<td>p.Ala486Thr517del</td>
<td>Truncating</td>
<td>Iranian (1)</td>
<td>F</td>
<td>PASH</td>
<td>GWLS</td>
</tr>
<tr>
<td>32</td>
<td>c.1695T&gt;G</td>
<td>p.Tyr565X</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>33</td>
<td>c.1702C&gt;T</td>
<td>p.Gln568X</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F (all)</td>
<td>Isolated HS (all)</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>34</td>
<td>c.1752delG</td>
<td>p.Glu584AspfsX44</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>GWLS</td>
</tr>
<tr>
<td>35</td>
<td>c.1768A&gt;G</td>
<td>p.Ser590AlafsX3</td>
<td>Truncating</td>
<td>Chinese (1)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>36</td>
<td>c.1799delTG</td>
<td>p.Leu600X</td>
<td>Truncating</td>
<td>Indian (1)</td>
<td>F</td>
<td>FS + AC</td>
<td>Targeted sequencing</td>
</tr>
</tbody>
</table>

(continued)
histopathologic features of HS (He et al., 2019; Kamp et al., 2011; Pan et al., 2004).

In vitro, haploinsufficiency of NCSTN in keratinocyte (KC) cell lines upregulated the expression of type I IFN genes (Cao et al., 2019). In molecular studies of familial HS, NCSTN deficiency has been found to impact KC differentiation and proliferation through several candidate pathways (He et al., 2020, 2015). However, the presence of missense mutations in both sporadic (4) and familial (6) cases and conflicting results from translational biology may implicate altered functional enzymatic activity. Loss of a single Psen1 allele in mice does not produce skin disorders and only occurs with more severe reduction in presenilin expression. Wild-type (WT) mice treated with a γ-secretase inhibitor, which maintained levels of γ-secretase but specifically inhibited its enzymatic activity, produced similar epidermal abnormalities to Ncstn−/− mice, including follicular hyperkeratosis and inclusion cyst formation (Li et al., 2007). Another study of Ncstn−/− mice and Ncstn−/− Psen1−/− mice found that both developed follicular inclusion cysts compared with WT, but the double-knockout mice developed these lesions earlier, and this was dependent on the level of γ-secretase (O’Brien and Wong, 2011). In vitro study of human tissue from patients with HS harboring γ-secretase mutations found that membrane expression of γ-secretase was unchanged despite reduction in cellular protein expression (Table 1) (Pink et al., 2016), which may be due to physiologic post-transcriptional selection of <5% of fully assembled complexes that are then localized to the membrane (Yang et al., 2019). It seems likely that patients with only a partial loss of function may still produce enough amounts of functional protein to support normal physiology.

### Table 1. Continued

<table>
<thead>
<tr>
<th>Gene</th>
<th>DNA Change</th>
<th>Amino Acid Change</th>
<th>Mutation Type</th>
<th>Ethnic Origin (No. of Families)</th>
<th>F/C</th>
<th>Isolated HS or Syndrome/Associated Conditions</th>
<th>Method of Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSEN1 − 14q24.2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>c.725delC</td>
<td>p.Phe242LeufsX11</td>
<td>Truncating</td>
<td>Chinese (3) (Wang et al., 2010)</td>
<td>F</td>
<td>Isolated HS</td>
<td>GWLS</td>
</tr>
<tr>
<td>2</td>
<td>c.837+1G&gt;T</td>
<td>p.Glu131Gly</td>
<td>Missense</td>
<td>Chinese (Lazic et al., 2012)</td>
<td>C</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
<tr>
<td>3</td>
<td>c.953A&gt;G</td>
<td>p.Glu131Gly</td>
<td>Missense</td>
<td>British (3) (Ingram et al., 2013)</td>
<td>F</td>
<td>Isolated HS</td>
<td>Targeted sequencing</td>
</tr>
</tbody>
</table>

Abbreviations: AC, acne conglobata; C, case; DDD, Dowling-Degos disease; F, familial; GWLS, genome-wide linkage scan; HS, hidradenitis suppurativa; N/A, not applicable; No., number; PASH, pyoderma gangrenosum, acne, and hidradenitis suppurativa; SAPHO, synovitis, acne, pustulosis, hyperostosis, and osteitis.

1Controls were used.
above a certain threshold. Any reduction in the level of functional protein or \(\gamma\)-secretase activity or increasing the threshold may subsequently elicit a clinical phenotype (Melnik and Plewig, 2013). A recent study identified a new NCSTN mutation causing HS in a Dutch family. The associated immunobiological functions of NCSTN and its coexpressed genes ARNT and PPARD link genetics to the most common environmental and metabolic HS risk factors, smoking and obesity (Vossen et al., 2020). This raises the question, how do environmental factors increase the risk of developing HS in those that harbor \(\gamma\)-secretase mutations? Emerging studies have provided import data supporting this stance. A systematic review and in silico analysis of 34 HS \(\gamma\)-secretase mutations predicted structural alterations in substrate recruitment sites, catalytic domains, and posttranslational modifications, consistent with altered enzymatic activity and substrate processing (Li et al., 2019a). A second, more extensive in silico analysis bolstered these results by showing that 39 pathogenic familial HS-associated \(\gamma\)-secretase mutations underwent significant structural changes in known sites of substrate binding and cleavage, either through nonsense-mediated decay (23) or altered binding affinity (16) (Frew and Navrazhina, 2019). Such changes were found to be distinct from those found in Alzheimer-associated \(\gamma\)-secretase mutations (Frew and Navrazhina, 2019; Li et al., 2019a). One studied HS PSEN1 mutation was found to affect the opposite side of the transmembrane-5 domain from the affected sites of reported Alzheimer mutations (Frew and Navrazhina, 2019). Such mechanistic differences and the myriad of \(\gamma\)-secretase substrates may shed light on the lack of co-occurrence between the familial forms of Alzheimer and HS despite overlapping loci.

\(\gamma\)-secretase may act through multiple secondary pathways, such as Notch, phosphoinositide-3-kinase, and EGFR. Isolation of \(\gamma\)-secretase—dependent pathways specific to HS genesis is complicated by the large number of known \(\gamma\)-secretase substrates, the pleiotropy of its components, and the lack of a reliable animal model for in vivo study. Thus, although the following pathways are the most well described, many likely remain undiscovered.

The Notch pathway has gained attention in HS because of its role in maintaining the HF stem cell pool and functional regulatory T cells (Treg) in the HF and promoting antimicrobial defenses at the epidermis (Sabat et al., 2020). In the skin, Notch normally maintains stemness in the HF stem cells, and disruption of signaling leads to aberrant differentiation and proliferation of KCs and their precursors. Tregs are required for development and maintenance of the HF (Ali et al., 2017) and immunological balance in the skin, both of which Notch

**Figure 1.** Locations of confirmed mutations in \(\gamma\)-secretase protein domains.
signaling supports. Finally, studies have shown an essential role for Notch in supporting T cell–derived IL-22, which maintains the skin microbiome (Sabat et al., 2020). These roles might explain why γ-secretase mutations that influence Notch signaling can elicit the diverse aberrations seen in HS skin lesions (e.g., follicular cystic formation, inflammatory immune cell infiltration, and altered skin microbiota).

Notch 1–4 are well-characterized targets of γ-secretase, and controlled disruption of Notch pathway components in mice results in epidermal and follicular aberrations that resemble histopathological findings in HS (Pink et al., 2012). Although some Notch molecules are abnormally expressed in HS tissue and HaCaT cells with γ-secretase mutations (Li et al., 2019a; Xiao et al., 2016), minimal evidence exists that indicates that Notch aberrations are specific to HS or of sufficient statistical significance to be considered risk-associated loci for disease development (Frew et al., 2019). Functional assessment of four NCSTN missense mutations found that three maintained downstream Notch signaling whereas the fourth did not, casting doubt on the assumption that Notch-dependent pathways drive monogenic HS (Zhang and Sisodia, 2015). In silico and gene expression analyses of identified pathogenic mutations have failed to identify Notch as a specific marker of HS (Blok et al., 2016; Frew and Navrazhina, 2019), and genotype–phenotype correlation revealed no significance between impact on Notch signaling and HS phenotype (Frew et al., 2019). A recent study demonstrated that mRNA levels of NCSTN, Notch, and phosphoinositide-3-kinase (PI3K)/protein kinase B are over-expressed in lesional HS skin versus controls, and there is no association between positive family history and mRNA levels (Hessam et al., 2020). The lack of direct evidence from animal models or human studies makes the role of Notch in HS controversial, suggesting that other pathways play a role in the molecular pathogenesis of HS.

Abnormalities in the PI3K and EGFR pathways have previously been linked to epidermal and follicular dysfunction (Zhang et al., 2007), and emerging studies suggest that these pathways interact with microRNAs to play a role in familial HS pathogenesis. NCSTN knockdown in HaCaT cells led to decreased keratinocyte miR-100-5p, a microRNA that was previously found to be downregulated in patients with familial HS, which then resulted in increased PI3K and KC hyperproliferation (He et al., 2020; Xiao et al., 2016). He et al. (2019) found that NCSTN mutations lead to reduced miR-30a-3p levels, which increases RAB31 expression owing to diminished negative regulation, and this increase in RAB31 accelerates the degradation of activated EGFR on KCs, leading to abnormal differentiation. In silico assessment of pathogenic γ-secretase mutations found that HS-associated ERBB4, SCN1B, and TIE1 were differentially expressed and that this was specific to HS when compared with other inflammatory dermatoses to account for background cutaneous inflammation (Frew and Navrazhina, 2019).

**Questioning the role of γ-secretase: Future work**

Many HS experts cite the poor understanding of disease pathobiology as a significant bottleneck for HS management and a critical area for future work (Hoffman et al., 2017). Despite the myriad of discovered variants, only a minority (≤5%) of patients with HS have been found to harbor the monogenic γ-secretase mutation–associated familial HS phenotype, far fewer than even the 30–40% reporting family history. A recent key study of a predominantly Caucasian cohort of 188 patients with HS found that just 6.4% had mutations in γ-secretase (Duchatelet et al., 2020). Overall, the majority of patients with HS studied to date are found negative for γ-secretase mutations when assessed by targeted sequencing (Frew et al., 2017; Ingram et al., 2013; Pink et al., 2012). Although many pathogenic variants cosegregate with the HS phenotype in familial kindreds, others do not and indicate a benign nature (Al-Ali et al., 2010; Jarvik and Browning, 2016; Nomura et al., 2014). The sole whole-genome expression profiling study done on patients with HS found no difference in whole-blood mRNA expression in NCSTN, PSEN1, or PSEN2 between HS and healthy controls, although a small sample size was studied and no validation was performed (Blok et al., 2016). Most of the disease burden is in sporadic HS (60–70%), yet few studies have been performed in this population robust enough to probe its polygenic architecture and identify low to moderate impact variants and their attributable risks.

The view that HS has a polygenic foundation has subsequently gained traction, supported by strong, well-documented associations with other chronic inflammatory disorders, including inflammatory bowel disease, spondyloarthropathy, lupus, and pyoderma (Deckers et al., 2017; van der Zee et al., 2016; Vekic et al., 2016). Numerous genes besides γ-secretase components have also been identified to associate with HS, including connexin-26, fibroblast GF receptor, and inositol polyphosphate-5-phosphate (Tricarico et al., 2019), albeit with variable phenotypes. The racial predisposition toward African Americans is also important; given that disparate risks in immune-mediated disease development and variable responses to treatment of such conditions can, at least in part, be traced to ancestral heterogeneity (Nédélec et al., 2016), similar assessments in HS, particularly large-scale, hypothesis-free approaches such as GWAS, may be worthwhile.

A small number of studies have employed this approach with promising results. A pharmacogenomics GWAS of the Pioneer I and II trials found a single variant in BCL2 that was associated with response to adalimumab in patients with HS in a TNF-dependent manner localized to the follicular unit (Liu et al., 2020). Sequence investigation of the IL12RB1 receptor subunit gene identified two haplotype groups associated with significant differences in age at disease presentation, stage of disease, and number of skin areas (Giatrakos et al., 2013). Similar analysis of the TNF gene found significant association between SNPs of the promoter region and susceptibility to HS, disease course, and response to TNF antagonists (Savva et al., 2013). A study of two independent cohorts (total N = 261) showcased that high copy number (>6) of the DEF6 cluster was associated with a markedly increased OR (6.72 after meta-analysis, P < 0.0001) for HS development and fewer than six copies was linked with earlier onset, fewer skin localizations, and less frequent purulence (Giamarellos-Bourboulis et al., 2016).
Yet, when approached clinically, the lack of awareness, embarrassment in discussion, low socioeconomic status among patients, lack of follow-up because of increased use of emergency and inpatient care, and dearth of HS specialists in the United States all serve as barriers to obtaining accurate emergency and inpatient care, and dearth of HS specialists in the United States all serve as barriers to obtaining accurate clinical information from patients with HS (Hoffman et al., 2017). At the experimental level, establishing relevant animal models, designing translational studies aimed at distinguishing among the many contributing mechanisms to HS, and performing functional validation of identified variants are key tasks in this process.

In conclusion, here we review the available literature on γ-secretase in HS and evaluate its evidence in the context of clinical, epidemiologic, pathobiologic, and molecular studies. The release of ENCODE 3 and its associated tools poises future studies in HS to uncover important genetic and epigenetic features that may further clarify the etiologies of HS (ENCODE Project Consortium et al., 2020). Studying the γ-secretase complex and the greater genetic architecture of HS will allow for markedly improved and individualized treatment for individuals with this debilitating disease.

Data availability statement
There is no dataset related to this article.

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**CONFLICT OF INTEREST**
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Nonetheless, the several identified HS mutations in NCSTN, PSENEN, and PSEN1, many of which were determined to be causative in familial HS, and their demonstrated relevance at the clinical and pathobiological levels advocate for continued investigation into γ-secretase. The establishment of guidelines for conducting the necessary multi-institutional studies, particularly genotype–phenotype analysis and exome sequencing of affected kindreds representative of the broader HS population, has already been undertaken and is a step in the right direction (Byrd et al., 2019).

**AUTHOR CONTRIBUTIONS**
Conceptualization: QSM, IH, WL; Data Curation: GV, PD, LZ, QSM; Formal Analysis: GV, PD, LZ, QSM; Funding Acquisition: QSM, LZ; Investigation: GV, PD, LZ, QSM; Methodology: GV, PD, LZ, QSM; Project Administration: LZ, QSM; Validation: GV, PD, LZ, QSM; Visualization: GV, PD, LZ, QSM; Writing - Original Draft Preparation: GV, PD, LZ, QSM; Writing - Review and Editing: GV, PD, HWL, DO, WL, IH, LZ, QSM

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