Düsseldorf, Germany; [12]*Skin Cancer Center, University Hospital Düsseldorf, Düsseldorf, Germany and* [13]*Department of Dermatology and Allergy Centre, Odense University Hospital, Odense, Denmark*
E-mail: regina.betz@uni-bonn.de
[14]*These authors contributed equally to this work.*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Acne Inversa Caused by Missense Mutations in *NCSTN* Is Not Fully Compatible with Impairments in Notch Signaling

Journal of Investigative Dermatology (2015) **135,** 618–620; doi:10.1038/jid.2014.399; published online 23 October 2014

TO THE EDITOR

Acne inversa (AI; also known as hidradenitis suppurativa; OMIM 142690) is a chronic recurrent follicular occlusion disorder. About 30–40% of patients with AI exhibit a highly penetrant, autosomal dominant mode of inheritance (Alikhan *et al.*, 2009). In many cases, AI patients harbor heterozygous mutations in genes encoding components of the γ-secretase complex, composed of presenilin (PS1 and 2), nicastrin (NCT), anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2) (Wang *et al.*, 2010; Jurisch-Yaksi *et al.*, 2013). PS is the catalytic center of γ-secretase that promotes intramembranous proteolysis of a number of membrane proteins, including the amyloid precursor protein (APP) and Notch 1–4, signaling receptors essential for cell lineage determination, cell proliferation and survival. Activation of Notch signaling occurs upon binding to the Delta/Serrate/Lag-2 family of ligands on the cell surface, leading to exposure of a sequence near the transmembrane domain that is a substrate of a metalloprotease, ADAM 10. This ''shedding'' event generates the membrane-tethered Notch extracellular truncation (NEXT) that is then subject to intramembranous processing by γ-secretase, to generate the Notch intracellular domain (NICD). NICD translocates to the nucleus to form a complex with a transcription factor, C-promoter binding factor-1 (CBF-1), that binds to CBF1-specific cognate DNA sequences to regulate gene expression (Fortini, 2009).

To date, 24 AI-specific mutations have been identified in genes encoding γ-secretase components, and 19 of these are in *NCSTN*, encoding nicastrin (Supplementary Table S1 online). Most mutations in *NCSTN* cause frameshift and premature translation termination as well as nonsense-mediated mRNA decay, leading to significantly reduced levels of NCT, findings which have led to the proposal that AI is caused by genetic haploinsufficiency because of reduced γ-secretase-mediated processing of Notch and signaling in the skin (Pink *et al.*, 2013). Interestingly, four missense mutations, V75I, D185N, P211R, and Q216P, have been identified in the large ectodomain of NCT (Li *et al.*, 2011; Pink *et al.*, 2012; Zhang *et al.*, 2013). These missense mutations could potentially disrupt the structure of this region and result in failed assembly of the γ-secretase complex, leading to impaired activity. To test this notion, we examined the activity of these NCT variants in mediating Notch processing and signaling.

We first coexpressed cDNAs encoding the NCT missense variants together with a constitutively activated membrane-bound Notch 1 derivative (mNΔE; Schroeter *et al.*, 1998) that is similar to NEXT, in *NCSTN*-deficient (*NCSTN*$^{-/-}$) fibroblasts. mNΔE is not subject to intramembranous processing in the absence of NCT (Figure 1a, lane 1), but coexpression of wild-type NCT rescues the generation of NICD

Abbreviation: AI, acne inversa

Accepted article preview online 11 September 2014; published online 23 October 2014

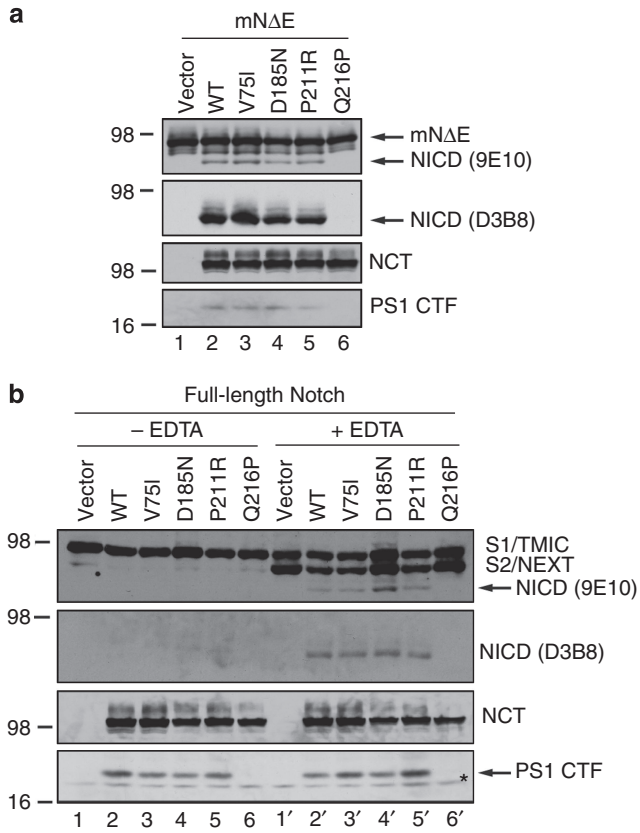

Figure 1. V75I, D185N, and P211R mutant nicastrin rescues Notch processing in *NCSTN*$^{-/-}$ cells. (**a**) V75I, D185N, and P211R mutant nicastrin rescues membrane-bound Notch 1 derivative processing and PS1 endoproteolysis in *NCSTN*$^{-/-}$ cells. (**b**) V75I, D185N, and P211R mutant nicastrin rescues full-length Notch processing and PS1 endoproteolysis in *NCSTN*$^{-/-}$ cells. Notch intracellular domain fragments are detected both by myc-tag and by Notch intracellular domain-specific antibody D3B8. *Indicates a nonspecific band.

(Figure 1a, lane 2). Surprisingly, expression of the V75I, D185N, or P211R variant also rescues γ-secretase activity efficiently (Figure 1a, lanes 3–5, respectively), whereas the Q216P variant is functionally inactive (Figure 1a, lane 6); the identity of NICD was confirmed with the Notch1744V-specific antibody, D3B8 (Figure 1a). In addition, expression of wild-type, V75I, D185N, or P211R NCT variant rescues the endoproteolysis of PS1 that would otherwise accumulate as a full-length molecule in *NCSTN*$^{-/-}$ cells (Figure 1a), suggesting that these variants assemble into active γ-secretase complexes (Zhang *et al.*, 2005). To confirm these results, we have examined the processing of full-length Notch 1 that is activated by the addition of EDTA, leading to exposure of an ADAM10 cleavage site to generate NEXT (Rand *et al.*, 2000). Similar to the results obtained using mNΔE, expression of all but the Q216P variant rescues Notch processing (Figure 1b).

To further validate these results, we employ a dual luciferase reporter assay to examine NICD-mediated activation of nuclear signaling. We coexpress cDNAs encoding mNΔE and NCT variants together with cDNA encoding firefly luciferase downstream of four wild-type CBF1 binding sites (4xWTCBF1Luc) (Shawber *et al.*, 1996) and cDNA encoding *Renilla* luciferase, a transfection control, in *NCSTN*$^{-/-}$ cells. Similar to wild-type NCT, expression of the V75I, D185N, or P211R mutant leads to a significant transactivation of firefly luciferase (Figure 2), whereas the Q216P variant fails to do so. These results indicate that expression of the V75I, D185N, and P211R mutants can support Notch signaling *in vivo*. Moreover, expression of the V75I, D185N, or P211R mutant is also functional in the generation of the APP intracellular domain fragment derived by γ-secretase-mediated processing of APP carboxyl-terminal fragments, which accumulate in *NCSTN*$^{-/-}$ cells (Supplementary Figure S1 online; compare lane 1 with lanes 3–5). Finally, coexpression of mNΔE, wild-type NCT, and each NCT mutant leads to the production of NICD, findings arguing against the possibility that the mutant NCT variants act in a dominant-negative manner (Supplementary Figure S2 online).

The failure of Q216P to rescue Notch processing and nuclear signaling supports the idea that impaired Notch signaling underlies AI in these patients. However, the V75I, D185N, and P211R variants are active in promoting Notch processing and signaling, and hence we would argue that deficits in Notch signaling are unlikely to be the cause of disease in AI patients harboring these mutations. These results strongly suggest that V75I, D185N, and P211R variants have a significant role in the pathogenesis of the disease but through mechanism(s) other than impaired signaling by Notch 1.

In summary, the preponderance of evidence would support the notion that mutations in genes encoding γ-secretase components cause AI because of haploinsufficiency, and it has been proposed that impaired Notch signaling is central to the disease. Although plausible, we would argue that expression of the remaining wild-type alleles encoding γ-secretase components in these patients would be sufficient to promote Notch signaling (Supplementary Figure S2 online). On the other hand, it is conceivable that γ-secretase-mediated proteolysis of any number of cell surface receptors that also engage intracellular signaling pathways is sensitive to the levels or subcellular distribution of the enzyme. Furthermore, local or extrinsic factors involved in homeostasis in hair follicles may have a significant effect on disease pathogenesis (for review, see Pink *et al.*, 2013). Nevertheless, our demonstration that a number of AI disease–causing NCT missense variants are fully active and capable of promoting Notch signaling would argue against

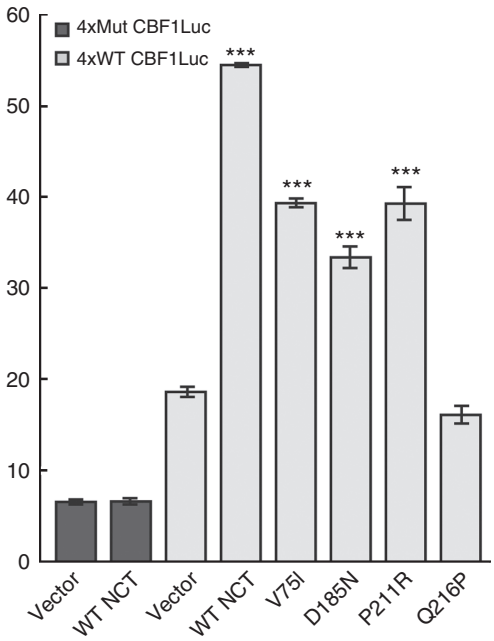

Figure 2. V75I, D185N, and P211R mutant nicastrintransactivate C-promoter binding factor-1-luciferase reporter construct in *NCSTN*$^{-/-}$ cells. Transient expression of wild-type and mutant NCT in *NCSTN*$^{-/-}$ cells is performed to determine whether missense nicastrin variants can rescue Notch processing and activate endogenous C-promoter binding factor-1 to transactivate luciferase reporter construct carrying four copies of wild-type C-promoter binding factor-1 binding sites. Luciferase reporter construct carrying four copies of mutant C-promoter binding factor-1 binding sites is used as a specificity control. The data represent the mean ± SEM ($n = 3$). ***Indicates a significant difference from the vector control ($P < 0.005$).

the view that mutations in genes encoding γ-secretase components cause disease simply as a result of haploinsufficiency that leads to impaired Notch signaling.

CONFLICT OF INTEREST

SSS discloses that he is a paid consultant of Eisai Research Labs, AZ Therapies, and Jannsen Pharmaceutica NV but is not a shareholder in any company that is a maker or owner of an FDA-regulated drug or device. The author states no conflict of interest.

ACKNOWLEDGMENTS

We are grateful for the generous support by Alzheimer's Association, BrightFocus Foundation, and the Edward H. Levi Fund.

***Xulun Zhang*[1] *and Sangram S. Sisodia*[1]**

[1]*Department of Neurobiology, The University of Chicago, Chicago, Illinois, USA*
E-mail: ssisodia@bsd.uchicago.edu

SUPPLEMENTARY MATERIAL

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Gene Expression Differences between Psoriasis Patients with and without Inflammatory Arthritis

Journal of Investigative Dermatology (2015) **135,** 620–623; doi:10.1038/jid.2014.414; published online 16 October 2014

TO THE EDITOR

Genetic and cellular evidence implicate both the innate and adaptive immune systems in the pathogenesis of cutaneous psoriasis (PsC) and psoriatic arthritis (PsA), an inflammatory arthritis that develops in approximately 30% of PsC patients (Gladman, 2005; O'Rielly and Rahman, 2010; Lories and de Vlam, 2012). However, little is known about the specific pathologic differences between PsA and PsC. Comparison of the gene expression profiles of PsC and

Abbreviations: PsA, psoriatic arthritis; PsC, cutaneous psoriasis without arthritis

Accepted article preview online 22 September 2014; published online 16 October 2014