Our study confirms increased concentrations of CXCL9 and CXCL10 in morphea serum and correlation of CXCL9 to mLoSSI, a validated measure for disease activity. In addition to that, we also observe correlation between CXCL10 and mLoSSI. Our analysis demonstrates no relations between the presence of disease damage, captured by the Localized Scleroderma Damage Index score, and both CXCL9 and CXCL10. Torok et al. (2015) reported a similar correlation ($r_s = 0.34$) between CXCL10 and mLoSSI and no significant correlation between CXCL10 and Localized Scleroderma Damage Index scores in cohort of 69 pediatric morphea patients (Torok et al., 2015). The strong correlation between CXCL9 and CXCL10 serum concentrations combined with the similar trends in correlations between clinical measures and both chemokines, suggest equal biomarker capabilities for both CXCL9 and CXCL10.

Furthermore, we demonstrate increased CXCL9 and CXCL10 gene expression at both the inflammatory border and the sclerotic center of affected morphea tissue, with normal gene expression at unaffected tissue of morphea patients. A strong correlation between CD68 and CXCL9 and CXCL10 gene expression, together with the lack of an association between circulating monocytes, and these chemokines not only suggests a relationship between the presence of local macrophages and CXCL9/CXCL10 production, it also underscores that morphea is a disease confined to the skin where inflammatory markers are measured in the circulation as the result from “leakage” from the inflammatory sites.

In conclusion, we confirm the potential of CXCL9 as biomarker for disease activity in morphea. Interestingly, CXCL10 serum concentrations showed similar biomarker capabilities. Lastly, increased CXCL9 and CXCL10 gene expression in morphea skin, and absence of increased gene expression in monocytes, supports the hypothesis, postulated by O’Brien et al. (2017), that morphea may result from skin-directed immune dysregulation rather than the systemic presence of inflammation on contrast to that observed in systemic scleroderma.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.11.032.

REFERENCES


Betz et al., 2006; POFUT1, encoding protein O-fucosyltransferase 1 (Basmanav et al., 2015; Li et al., 2013); and POGLUT1, encoding protein O-glucosyltransferase 1 (Basmanav et al., 2014). Available data suggest that mutations in KRT5, POFUT1, and POGLUT1 cause reticulate hyperpigmentation of the skin only, and are not associated with any further cutaneous or extracutaneous signs (Basmanav et al., 2014; Basmanav et al., 2015; Betz et al., 2006; Li et al., 2013). Thus, we understand that our latest molecular data on the genetically heterogeneous hereditary reticulate pigmentation disorder DDD, which indicate that PSENEN mutations can give rise to both DDD and

Table 1. Disease status and symptom constellations in confirmed PSENEN mutation carriers in the Ralser et al. (2017) cohort

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Mutation</th>
<th>Sex</th>
<th>DDD</th>
<th>Comorbid AI</th>
<th>Obesity</th>
<th>Nicotine Abuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>German</td>
<td>c.35T&gt;A</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>German</td>
<td>c.115C&gt;T</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indian</td>
<td>c.61-1G&gt;C</td>
<td>F</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<td>F</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thai</td>
<td>c.84_85insT</td>
<td>M</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<tr>
<td></td>
<td></td>
<td>F</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>German</td>
<td>c.216delC</td>
<td>M</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>French</td>
<td>g.1412T&gt;C</td>
<td>F</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: +, present; –, absent; AI, acne inversa; F, female; M, male; NA, clinical information was not available.

1Derived from Table 1 in Ralser et al. (2017).

Figure 1. Illustration of Notch signaling for genes involved in Dowling-Degos disease (DDD). Proteins involved in the pathogenesis of DDD are highlighted in red. (1) Notch receptor proteins undergo post-translational modification in the endoplasmatic reticulum (ER) via POFUT1 and POGLUT1, respectively. (2) Signaling is activated when Notch ligands (Delta, Jagged), expressed by the signal-sending cell, bind to Notch receptors. This receptor–ligand interaction elicits two proteolytic cleavage steps. (3) ADAM10 catalyzes the shedding of the extracellular juxtamembrane element of the Notch receptor proteins, thus releasing their Notch extracellular domain (NECD). (4) The γ-secretase complex catalyzes intramembranous cleavage, thus releasing the Notch intracellular domain (NICD) into the cytoplasm. (5) The NICD subsequently translocates into the nucleus, where it interacts with the transcription factor CSL to regulate target gene expression.
AI (Ralser et al., 2017), may come as a surprise. 

**PSENEN** encodes γ-secretase subunit PEN-2. This endoprotease complex catalyzes the intramembrane cleavage of integral membrane proteins, such as Notch receptors and β-amyloid precursor protein. To date, **PSENEN** mutations have mainly been reported in familial AI and predominantly consist of null allele mutations leading to haploinsufficiency (Pink et al., 2011; Wang et al., 2010).

The manifestation of DDD with AI has been suggested in earlier clinical case reports (Bedlow and Mortimer, 1996). Therefore, Li et al.’s (2017) call for caution in the interpretation of our data seems to have been prompted by the finding that **PSENEN** mutations can cause both DDD and DDD/AI in susceptible individuals (Ralser et al., 2017). Because caution is always advisable when phenotypic variability is associated with mutations in a single gene, we agree entirely. However, we have several comments on the Li et al. (2017) report.

First, column 7 of Table 1 in the Li et al. (2017) report suggests that DDD was not present in their cohort or those of previous authors (Table 1 in Li et al., 2017). However, in column 8, Li et al. (2017) state that most of the 12 patients investigated by Zhou et al. (2016) exhibited a “comanifestation” (Table 1 in Li et al., 2017). Presumably, this refers to a co-manifestation of DDD and AI, as is stated in the main article text (Li et al., 2017). Zhou et al. (2016) did indeed report the typical lesions of DDD and multiple comedones as the dominant clinical manifestation. The contradictory data and statements presented in the Li et al. (2017) report might cause confusion, and generate the impression that AI was the only phenotype observed in **PSENEN** mutation carriers prior to our study. Similarly, Li et al. (2017) did not appear to consider the mucosal hyperpigmentation reported by Pink et al. (2011) as a possible phenotypic expression of DDD.

We have now reviewed the clinical data on **PSENEN** mutation carriers in Table 1 of our own report (Ralser et al., 2017). The designation “NA” (not applicable; no clinical information) may have generated some misunderstanding. However, the main article text clearly stated that all patients in the cohort presented with DDD. To clarify, we have revised Table 1 of our own report in order to highlight whether the **PSENEN** mutation carriers had DDD only or comorbid DDD/AI (Table 1).

Second, Li et al. (2017) suggest that **PSENEN** mutations are associated with three cutaneous phenotypes: (i) DDD, (ii) AI, and (iii) comorbid DDD/AI. The authors fail to mention familial comedones syndrome, which is also associated with **PSENEN** mutations (Dereure, 2016; Panmontha et al., 2015). Familial comedones syndrome is clinically and histopathologically distinct from AI, and is thus considered its own disease entity (Panmontha et al., 2015).

Third, Li et al. (2017) suggest that DDD is a clinical sub-phenotype, rather than the leading clinical feature, in **PSENEN** mutation carriers with combined DDD/AI. However, our 2017 study generated evidence that **PSENEN** mutations can give rise to either (i) DDD alone or (ii) combined DDD/AI, in susceptible individuals with common risk factors for AI, that is, smoking and obesity. Furthermore, our study showed that this difference can occur within the same family (Ralser et al., 2017). Thus, available data preclude definitive conclusions concerning which **PSENEN**—mutation—associated cutaneous sign is predominant.

Fourth, mutations in Notch pathway genes undoubtedly cause disturbed Notch signaling, which is implicated in the pathogenesis of both DDD and AI (Melnik and Plewig, 2013; Ralser et al., 2017; Wang et al., 2010). As to that, Li et al. (2017) speculate that the pigmentation abnormalities observed in **PSENEN** mutation carriers may be caused by Notch genes located between genes that are associated with DDD and AI. Unfortunately, the authors fail to provide references or their own data to substantiate their hypothesis. In a review of next-generation sequencing data from our **PSENEN** mutation carriers, we found no mutation in any other Notch pathway gene, including NCSTN and PSEN1.

Current understanding of Notch signaling suggests—at least with regard to reticulate hyperpigmentation—that the phenotype in **POFUT1**, **POGLUT1**, and **PSENEN** mutation carriers is attributable to a common pathomechanism and downstream target (Figure 1) (Bray, 2016; Fernandez-Valdivia et al., 2011; Osawa and Fisher, 2008). A plausible hypothesis is that the manifestation of different sub-phenotypes is due to crosstalk between Notch and other signaling pathways, and that this crosstalk varies between individuals, depending on which particular Notch genes and environmental factors are involved. Therefore, elucidation of the precise pathogenetic mechanisms through which Notch signaling orchestrates pigmentation processes is of key importance. Moreover, the issue of why mutations in NCSTN or PSEN1 do not lead to pigmentation abnormalities remains unclear.

In summary, detailed phenotyping and molecular characterization of additional families with DDD, AI, and **PSENEN** mutations are required in order to determine whether the occurrence of AI in heavy smokers and overweight individuals is a sub-phenotype of DDD or vice versa. Irrespective of the result, emphasis should be placed on elucidating the biology of aberrant pigmentation secondary to dysfunctional Notch signaling because this will facilitate the development of causal therapeutic strategies.
Melanoma Tumor Characteristics: An Analysis of Mutational Burden and Copy Number Alterations by Patient Age and Stage


TO THE EDITOR

Malignant melanoma is the fifth most common cancer in the United States across all ages; however, it ranks third among patients aged 15–39 years, those also known as adolescents and young adults (AYAs) (National Cancer Institute, 2015; Weir et al., 2011). There are intrinsic differences in AYAs and older patients with melanomas, with AYAs having female predominance, increased likelihood of developing superficial spreading melanoma, and presence of somatic BRAF and NRAS mutations and germline MC1R mutations (Weir et al., 2011). In contrast, older patients have a male predominance, are more likely to develop lentigo maligna melanoma, develop mutations in NF1 and KIT, and overexpress p53.

Despite its potential importance in elucidating different pathways in melanogenesis, no comparison of genomic alterations between AYAs and older patients exists (Chalmers et al., 2017). Furthermore, melanoma stage is the key prognostic factor in outcomes of melanoma, and the relationship between stage and mutational burden and copy number alterations (CNAs) has not been well studied. We investigated differences in genomic alterations in melanoma tumors from AYAs and older patients and explored the association between genomic characteristics and melanoma staging using publicly available datasets.

Results

Across all studies, 94 patients were AYAs (The Cancer Genome Atlas [TCGA]; n = 50; Hodis et al., 2012; n = 32; Krauthammer et al., 2012; n = 5; Gartner et al., 2013: n = 7), and 507 were older (TCGA: n = 311; Hodis et al., 2012; n = 88; Krauthammer et al., 2012: n = 86; Gartner et al., 2013: n = 222) (see Supplementary Table S1 online). Overall age range was 15–39 years in AYAs and 40–94 years in older patients (see Supplementary Figure S1 online). Females made up 50% of AYAs and 37.5% of older patients. The extremities were the most commonly affected site in both groups.

There were no differences in mutational burden between AYAs and older patients in the combined studies (P = 0.56) (Table 1). More advanced stages of melanoma were not correlated with a higher mutational burden across all ages (P = 0.75) or in younger (P = 0.52) and older (P = 0.65) age when analyzed separately (Figure 1a–c).

Mutations in AHNAK2 (P = 0.01) (see Supplementary Table S2 online) and NF1 (P = 0.001) were significantly more likely to occur among older patients, whereas BRAF mutations were more...