approximately 50% of cases of malignancy.

In conclusion, to more completely understand the sensitivity and specificity of an algorithm, knowledge of the comparative performance of dermatologists would be helpful. Instruction for the usage of algorithms is important, and a misusage will deteriorate their performances. To clarify the performances of algorithms, prospective randomized controlled studies need to be conducted in the future with various ethnicities in various environments.

Data availability statement
Datasets related to this article can be found at https://doi.org/10.6084/m9.figshare.12478238

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NCSTN Deficiency and Depigmentation: All About Tyrosinase?


TO THE EDITOR
In their recent publication in the Journal of Investigative Dermatology, Hsu et al. (2020) employ studies on a zebrafish (ZF) mutant to support their hypothesis that NCSTN deficiency induces tyrosinase-dependent depigmentation. Although we acknowledge the large data set, we are not entirely convinced by their presentation and/or interpretation of the data and certain aspects of their experimental approach.

NCSTN is a type 1 integral membrane protein and a member of the γ-secretase endoprotease complex. Among others, this complex is responsible for the intracellular cleavage of Notch receptors, a process required for the activation of the Notch signaling pathway. Besides NCSTN, γ-secretase comprises three further transmembrane protein subunits: PSEN1/PSEN2, PEN-2, and APH-1a/APH-1b (Wolfe, 2020). NCSTN acts as a substrate receptor and is encoded by the NCSTN gene, which is located on chromosome 1q23.2. Two previous reports have described an association between NCSTN deficiency secondary to NCSTN mutations and pyoderma gangrenosum, acne, and supplicative hidradenitis syndrome. However, this association remains uncertain owing to the low number of cases reported and an overlap of the clinical phenotype with that of other nonsyndromic and syndromic forms of

Abbreviations: AI, acne inversa; ZF, zebrafish
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acne (Duchatelet et al., 2015; Li et al., 2018). In contrast, heterozygous NCSTN mutations underlie autosomal dominant familial acne inversa (AI), a chronic and relapsing inflammatory disorder that affects the hair follicle as well as the sebaceous and sweat glands (Ralser et al., 2017; Wang et al., 2010). Disturbed Notch signaling has been implicated in familial AI secondary to NCSTN deficiency. First, in 1996, the NCSTN-knockdown ZF model was demonstrated that curled-up tails could result from several different genetic alterations. Second, we observed no curled-up tails in ZF mutants from our own NCSTN-knockdown experiments (MAH, unpublished data). Third, in our experiments, we continued to detect ZF morphants with curled-up tails and reduced viability even after morpholino-mediated knockdown of the γ-secretase subunit APH-1b (Figure 1a). We therefore question whether the authors are justified in excluding the possibility that this phenotype represents a nonspecific experimental outcome and concluding that it is a true NCSTN deficiency-associated phenotype.

The second observation in their NCSTN-knockdown ZF model was hypopigmentation and/or depigmentation. Although Hsu et al. (2020) state that the nicastrinhi1384 ZF mutant employed in their studies would be a potential animal model for the investigation of vitiligo. However, the human depigmentation disorder vitiligo is (i) multifactorial in origin; (ii) associated with autoimmune processes; (iii) not associated to date with either disturbed γ-secretase function or Notch signaling; and (iv) not associated with NCSTN mutations and/or NCSTN deficiency in available data sets, even in very rare familial subtypes of the disease (Liu and Huang, 2018; Quan et al., 2010; Xu et al., 2010). Therefore, we disagree with the conclusion of Hsu et al. (2020) and are of the opinion that proposing the nicastrinhi1384 ZF mutant as a vitiligo model is not sufficiently substantiated. The question therefore arises as to why this model should be used to test the putative therapeutic effects of tyrosinase inhibitors, such as propylthiouracil, in patients with vitiligo. Of note, oral propylthiouracil can cause severe adverse effects, such as agranulocytosis, aplastic anemia, fulminant liver failure, and birth defects (Yu et al., 2020). Even if applied topically to avert the development of vitiligo, its efficacy remains speculative.

With regard to cutaneous symptoms, to our knowledge, human NCSTN mutation carriers do not present with disorders of pigmentation. Instead, these individuals present with familial AI, which is an inflammatory disorder of the hair follicles (Frank et al., 2018; Wang et al., 2010). Because fish do not have hair follicles, we wonder how the authors would explain this phenotypic discrepancy, which they do not address in their publication. In this regard, the authors attempt to (i) link phenonona such as the necrosis of melanophores to an activation of inflammation without providing any appropriate experimental evidence, for example, changes in the expression of inflammatory markers, and (ii) deduce from this that NCSTN would contribute to...
the inflammation observed in AI. On the basis of the data presented, we must consider these attempts far-fetched. We are not aware of any sound biological reason why necrosis of melanocytes would be implicated in the pathogenesis of human AI.

With regard to the effects of NCSTN deficiency on tyrosinase expression, Hsu et al. (2020) state that they could not demonstrate the localization of tyrosinase in their ZF mutants owing to the lack of ZF tyrosinase antibodies. However, antibodies often display cross reactivity among species. In whole fish, we were able to detect both tyrosinase expression and localization by conducting immunohistochemistry experiments and western blot using two human antibodies (Figure 2a and b).

In summary, we are of the opinion that the study by Hsu et al. (2020) raises two major concerns. First, several of the hypotheses, suggestions, and conclusions presented by the authors are not sufficiently substantiated and require further clarification and investigation. Second, the nicastrin\textsuperscript{1384} ZF mutant does not appear to represent a suitable model for the investigation of the etiology and treatment of vitiligo or any other specific hypopigmentation disorder.

Data availability statement
Data sets related to this article can be found at https://doi.org/10.17632/jvykf9ff77.1, hosted at Mendeley data under repository name “JID-Nicastrin-Letter-JF,” Mendeley Data, V1, doi: 10.17632/jvykf9ff77.1

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Figure 2. Tyrosinase expression and localization in ZF. (a) Tyrosinase expression and localization in ZF 120 hours after fertilization, visualized by immunohistochemistry with antityrosinase antibody GTX16389 (Genetex, Irvine, CA), dilution of 1:6,000. Tyrosinase expression colocalizes in particular with melanophores, indicated by arrows. Bar = 50 \( \mu \)m. (b) Tyrosinase expression in pooled ZF lysates (24-120 hours after fertilization) and human MEL-derived cell extracts, visualized by western blotting with antityrosinase antibody BS1484 (Bioworld Technology, Bloomington, MN), dilution of 1:1,000. Note the higher molecular weight of the human MEL band that is due to differentially glycosylated variants of human tyrosinase in melanocytic cells, with a range in molecular weight from about 70 kDa to 150 kDa (Rieber and Rieber, 1993). MEL, melanocyte; ZF, zebrafish.

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TO THE EDITOR

We read with interest the letter to the Editor by Hermasch et al. (2021) in this journal. The authors comment on our recent findings on a zebrafish inser-tional nicastrin mutant (Hsu et al., 2020). We showed that zebrafish nicastrin deficiency leads to melanosome defects, Tyrosinase-dependent mitochondrial swelling, and melanophore cell death. On the basis of several clinical observations and animal model studies, we proposed that this animal model may be used for studying depigmentation-related skin diseases and for screening potential drugs (Hsu et al., 2020). Nevertheless, Hermasch et al. (2021) raise some questions and concerns, to which we reply hereafter.

The following facts strongly support that the curled-up tail (CT) is a genuine trait of zebrafish homozygous nicastrin \textsuperscript{hi1348;} mutants. First, CT is associated with Notch signaling. It is also reported in mutants of mib\textsuperscript{hi526} /mib\textsuperscript{991} and mib\textsuperscript{hi332} (Zhang et al., 2007) and embryos treated with \( \gamma \)-secretase inhibitors, including N-(N-[3,5-difluorophenacetyl]-L-alanyl)-S-phenylglycine t-butyl ester (unpublished data; Arslanova et al., 2010). Around 16\% of nicastrin\textsuperscript{hi1348;}\textsuperscript{−/−} offspring showed CT (from 6\% to 29\%) (Table 1). Second, CT is observed only in mib and nicastrin mutants but not in their morphants (Hsu et al., 2020; Zhang et al., 2007). The difference in the CT penetrance of mutants and morphants may arise from the divergence in maternal contribution and/or the variation in knockdown efficiency. Third, CT coexisted only with hypopigmentation in nicastrin\textsuperscript{hi1348;} homozygotes phenotypically and genotypically (Table 1, Figure 1a, and Supplementary Figure S2 in Hsu et al. [2020]). Fourth, the CT can be rescued by overexpressing nicastrin mRNA in the nicastrin\textsuperscript{hi1348;} mutants (Figure 1 and Supplementary Figure S4 in Hsu et al. [2020]). Last but not least, Hermasch et al. (2021) demonstrated that the morphants of aph-1b and aph-1a, two paralogs of another \( \gamma \)-secretase component, exhibit CT and hypopigmentation, respectively. This may be a result of subfunctionalization because gene duplication occurs during teleost evolution, which is exemplified by mitf genes (Altschmied et al., 2002). In contrast, Danio rerio has no nicastrin paralog found.

Owing to one unidentified boundary of the insertion, we cannot completely exclude the existence of a mutation responsible for CT in a gene close to nicastrin. If such a mutation does exist, the corresponding gene will be less than 0.1 cM (centiMorgan) from the nicastrin locus (Supplementary Table S1, calculated by the data from Table 1) and is very likely regulated by Nicastrin because CT can be rescued as mentioned earlier. A genome and literature search found that no CT has been reported in the mutants of nearby genes.

Abundant genetic pigment disorders have been reported, and many of the corresponding genes are shared in processing Notch, Amyloid-\( \beta \), and Pmel (Table 2) (Bedja et al., 2018; Bergam et al., 2018; Cai et al., 2012; Cebers et al., 2016; Fleisher et al., 2008; Hamada et al., 2014; Kawaguchi et al., 2015; Kondo et al., 2008; Kono and Akiyama, 2019; Kono et al., 2013; Kumano et al., 2008; Li et al., 2017, 2013; Lin et al., 2011; Malek et al., 2005; Okamura et al., 2016; Ralser et al., 2017; Ren and Zeng, 2020; Rochin et al., 2013; Shimshek et al., 2016; van Niel et al., 2015; Wassels et al., 2020; Zhang et al., 2013) with severe phenotypes.