SUPPLEMENTARY NOTE

SUPPLEMENTARY RESULTS

Clinical features

Computed tomography scans of 8 affected subjects in family 312 did not show any inner ear malformations. In all affected members of families 312 and 705, vestibular functions are normal and there are no complaints of tinnitus (an auditory sensation that cannot be attributed to external sound).

Pejvakin expression in the organ of Corti

Within the organ of Corti, pejvakin expression was restricted to hair cells and pillar cells (**Supplementary Fig. 4**). Notably, strong pejvakin expression was detected in OHCs at all stages we tested; in contrast, pejvakin labeling in IHCs was weaker and transient, with no labeling apparent in adult IHCs. In vestibular sensory epithelia, pejvakin labeling was associated with the hair cell kinocilium at all the developmental stages we tested (data not shown). In *Dfnb59* $^{tm1Ugds/tm1Ugds}$ mice, no difference was apparent in the distribution pattern of pejvakin labeling either in the kinocilia of hair cells (both cochlear and vestibular) or in pillar cells (data not shown). However, we consistently observed a weaker pejvakin staining in the cuticular plate of OHCs (both in whole-mount cochlea and in isolated hair cells), that was always combined with an enhanced punctate signal in their cell bodies (**Supplementary Fig. 4f,g**). This result indicates that mutant pejvakin is partly mislocalized in OHCs.

SUPPLEMENTARY DISCUSSION

Sequence analysis revealed a significant similarity between pejvakin and the nonsyndromic deafness protein DFNA5. Extensive characterization of DFNA5 in different model systems (yeast[41], zebrafish[42], mammalian culture cells[43], and mouse[18]) has unfortunately failed to shed light on the cellular function of DFNA5 to date. In view of the very specific type of mutations that cause DFNA5 hearing impairment, and of the dominant nature of the disorder, a gain-of-function pathogenic mechanism was invoked[18,41,43]. Based on the present results it is worth considering the possibility of auditory neuropathy in DFNA5 patients. We consider it unlikely, though, because their speech recognition scores remain relatively good throughout life[44,45], which contrasts with the very poor speech discrimination typical of auditory neuropathy subjects, regardless of their audiometric thresholds[5]. We did not observe any difference in the auditory phenotypes of *Dfnb59* $^{tm1Ugds/tm1Ugds}$ *Dfna5*$^{+/+}$ animals and *Dfnb59* $^{tm1Ugds/tm1Ugds}$ *Dfna5*$^{-/-}$ double mutants, which argues against any functional association between both proteins, although it must be taken into account that the original *Dfna5*$^{-/-}$ strains, which mimic the human DFNA5 mutation, have themselves no hearing anomalies[18].

Pejvakin and DFNA5 are the most closely related members of a novel family of proteins from vertebrates which also includes the gasdermins and MLZE[13]. Members of this family share two characteristics. Firstly, as shown here for pejvakin, all of them contain

putative nuclear localization signals and DNA-interaction domains[41,46,47] whose functional significance is unknown. In fact, pejvakin was never detected in the nucleus in our immunolabeling experiments. Secondly, the expression levels of some members of the family are significantly modified in specific classes of carcinomas (either increased: MLZE[47] and DFNA5[48]; or reduced: DFNA5[48,49] and gasdermin[46]), which has led to suggesting that those alterations play a role in the acquisition of metastatic potential[46,47] or in the loss of susceptibility to programmed cell death[49]. It remains to be tested whether this is also the case for pejvakin.

SUPPLEMENTARY NOTE REFERENCES

41. Gregan, J., Van Laer, L., Lieto, L.D., Van Camp, G. & Kearsey, S.D. A yeast model for the study of human *DFNA5*, a gene mutated in nonsyndromic hearing impairment. *Biochim. Biophys. Acta* **1638**, 179-186 (2003).
42. Busch-Nentwich, E., Söllner, C., Roehl, H. & Nicolson, T. The deafness gene *dfna5* is crucial for *ugdh* expression and HA production in the developing ear in zebrafish. *Development* **131**, 943-951 (2004).
43. Van Laer, L. *et al*. DFNA5: hearing impairment exon instead of hearing impairment gene? *J. Med. Genet.* **41**, 401–406 (2004).
44. De Leenheer, E.M. *et al*. Clinical features of DFNA5. *Adv. Otorhinolaryngol*. **61**, 53-59 (2002).
45. De Leenheer, E.M. *et al.* Further delineation of the DFNA5 phenotype: results of speech recognition tests. *Ann. Otol. Rhinol. Laryngol*. **111**, 639-641 (2002).
46. Saeki, N., Kuwahara, Y., Sasaki, H., Satoh, H. & Shiroishi, T. Gasdermin (Gsdm) localizing to mouse chromosome 11 is predominantly expressed in upper gastrointestinal tract but significantly suppressed in human gastric cancer cells. *Mamm. Genome* **11**, 718-724 (2000).
47. Watabe, K. *et al*. Structure, expression and chromosome mapping of MLZE, a novel gene which is preferentially expressed in metastatic melanoma cells. *Jpn. J. Cancer Res*. **92**, 140-151 (2001).
48. Thompson, D.A. & Weigel, R.J. Characterization of a gene that is inversely correlated with estrogen receptor expression (ICERE-1) in breast carcinomas. *Eur. J. Biochem.* **252**, 169-177 (1998).
49. Lage, H., Helmbach, H., Grottke, C., Dietel, M. & Schadendorf, D. DFNA5 (ICERE-1) contributes to acquired etoposide resistance in melanoma cells. *FEBS Lett.* **494**, 54-59 (2001).