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Does Recessive EKV Exist?

To the Editor:

We read the article by Terrinoni *et al* in the March issue of the *JID* with much interest. In it, they described their finding of a putative recessive *GJB3* (connexin 31) mutation associated with erythrokeratodermia variabilis (EKV). The existence of recessive skin disease-associated connexin mutations would contribute significantly to our understanding of gap junction biology. But we have a number of concerns with the current report, that in our view, need to be addressed before the existence of recessive EKV can be accepted.

All data that are currently available with regard to gap junction gene mutations associated with skin disease suggest that the skin symptoms are caused by (trans-)dominant effects of the mutations. Almost all skin disease-associated mutations interfere with the transport of the mutant protein. The mutant protein will, however, be incorporated into a gap junction in the presence of a wild-type homotypic or heterotypic gap junction protein (Thomas *et al*, 2004). For *GJB2*, mutations such as D66H, which is associated with Vohwinkel's syndrome, have been shown to interfere in a trans-dominant manner with the expression of *GJA1* (Rouan *et al*, 2001; Thomas *et al*, 2004) and can lead to increased sensitivity to apoptosis of keratinocytes (Bakirtzis *et al*, 2003). The dominant mutations in *GJB3* that cause EKV have been shown to be gain-of-function mutants that lead to increased sensitivity to apoptosis of keratinocytes or interfere with gap junction conductance (Di *et al*, 2002; Common *et al*, 2003; Rouan *et al*, 2003). Dominant mutations in *GJB6* can interfere with transport of the protein and impair the assembly of heteromeric GJB6-containing gap junctions (Common *et al*, 2002). At least one of the mutations associated with KID syndrome, D50N, leads to a gain of function of the gap junction (D. Gonzalez, personal communication, 2003). Dominant mutations associated with hearing loss seem to traffic to the cell membrane but impair channel function once inserted (Common *et al*, 2002; Marziano *et al*, 2003). They do not modify channel composition or lead to a gain-of-function in contrast to the mutations associated with skin disease. Recessive mutations associated with hearing loss have been found mostly in *GJB2*, suggesting that recessive missense or nonsense mutations in other connexins that are expressed in the ear need not have functional significance there with the possible exception of *GJA1* in which recessive mutations have been found in deaf patients (Liu *et al*, 2001). These findings, however, have so far not been replicated. The same observation may very well apply to the skin. There is a previous report that describes a recessive mutation in *GJB3*, L34P, and claims that it causes EKV (Gottfried *et al*, 2002). The clinical data supplied are scarce and, in our view, do not support the diagnosis of EKV. Moreover, the paper shows that the mutant protein is sequestered in the cytoplasm and does not contribute to gap junction assembly. Functional studies showing alteration of gap junction function were not performed. As stated above, incorporation into a gap junction and alteration of its function or, alternatively, interference with the composition of the gap junction seem to be required for causation of a skin phenotype. Also, the absence of a gap junction protein does not necessarily have functional consequences. We have shown that connexin 30.3 (GJB4) is not required for skin function at all. Dominant point mutations in *GJB4* cause EKV but homozygosity for a 4 bp-deletion in *GJB4* appears to be a polymorphism, at least in the Dutch population (Macari *et al*, 2000; Van Geel *et al*, 2002). The polymorphism is rare, underscoring the need for screening of a sufficient number of controls. We note that Terrinoni *et al* do not mention the number of controls used to exclude E100K as a polymorphism. Finally, conservation of residues in gap junctions is not a guarantee for their functional importance. The conserved arginine at position 32 in GJB4 can apparently be substituted by a tryptophan without adverse consequences, as this change has been demonstrated to be a polymorphism in Spanish and Dutch populations (Lopez-Bigas *et al*, 2001; Van Geel *et al*, 2002). It was also demonstrated that this polymorphism does not lead to functional impairment of gap junctions (Rouan *et al*, 2003). In conclusion, the available functional data suggest that gap junction mutants that cause skin disease seem to be causing symptoms through a gain-of-function mechanism that requires incorporation of the mutant into a gap junction.

Another concern is with the correctness of the clinical diagnosis. The photographs accompanying the paper show abnormalities that in our view could also be consistent with the diagnosis of cyclic hyperkeratosis with ichthyosis as described by Sybert *et al* (1999). A hallmark feature of this particular disorder is an epidermolytic hyperkeratosis. Looking at the histology depicted in Fig 1*c* of the paper, there is a definite indication of epidermolysis there, particularly in the suprabasal layer, where cytolysis of keratinocytes also seems to be present. This may also be an artifact, but other features of EKV such as elongation of rete ridges seem to be absent. There seems to be some hypergranularity of the granular layer but at the magnification and quality provided it is not possible to discern whether actual clumping of tonofilaments is present. Electron microscopy would be helpful in this regard. The clinical appearance of cyclic hyperkeratosis is also quite similar to that of EKV as evident from Fig 3 in the Sybert *et al* paper. This keratin

Abbreviation: EKV, erythrokeratodermia variabilis

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disease leads to migrating, erythematous, and sharply demarcated plaques that mimic EKV. It would be important to know whether Terrinoni's patient had any blistering shortly after birth as this symptom can distinguish the two disorders. As it is, we are not convinced that the diagnosis EKV would be the correct one in the patient described by Terrinoni *et al*. The diagnosis of cyclic ichthyosis with epidermolytic hyperkeratosis seems more likely. We suggest that the present case of EKV be tested for *KRT1* mutations. It is conceivable that testing of cases of apparent EKV without mutations in *GJB3* or 4 will reveal several *KRT1* mutations.

A recessive mutation associated with a skin disease would be a significant finding. We feel that the present case report raises sufficient concerns to suggest that the further confirmatory studies are required. First of all, we would suggest that a *KRT1* mutation be ruled out. Second, if the diagnosis of EKV can be maintained, the E100K mutation needs to be confirmed as such by testing an adequate number of controls and with functional studies. It would be of interest to examine whether the mutation influences the expression or transport of other skin-expressed connexins in any way and impairs gap junction functionality if inserted into the membrane. Until these concerns are addressed, we feel that there is insufficient evidence to accept the existence of recessive EKV caused by a homozygous E100K substitution.

Maurice A. M. van Steensel and Michel van Geel
Department of Dermatology, University Hospital Maastricht, Maastricht, The Netherlands

DOI: 10.1111/j.0022-202X.2004.23517.x

Manuscript received April 23, 2004; revised July 12, 2004; accepted for publication July 20, 2004

Address correspondence to: Maurice A. M. van Steensel, MD, Dermatology Department, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands. Email: mvst@sder.azm.nl

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