The LCR at the *IKBKG* Locus Is Prone to Recombine

To the Editor: We read with great interest the paper by Vandewalle et al.[1] that appeared in the December 11, 2009, issue of *AJHG*. The manuscript reports on a 0.3 Mb recurrent but variable copy number gain in the Xq28 region that is found to be associated with mental retardation (MR). This paper is of special interest to us as scientists working in the field of Xq28-linked disease. However, we would like to make a few comments, which we feel would contribute to the message of the paper. The authors suggest that the effect of the copy number gain is most likely due to the increased expression of *GDI1*. We think it is important that other genes in the region, and in particular *IKBKG*, be considered for their potential role in MR. In addition, we think that critical previous work on the LCRs L1 and L2 was not included.

As reported, the 0.3 Mb copy number gain in Xq28 contains 18 genes (although 19 genes are listed in Table 1). Vandewalle et al.[1] provided data on the increased expression of *GDI1* (GenBank NM_001493 [MIM 300104]). However, they did not analyze the mRNA expression of the other genes in the duplicated region, and abnormal expression of one or several of these genes might contribute to the mental handicap observed in the affected families. Of particular note, the phenotypic features discussed in the paper are common to inactivation of other Xq28 brain disease genes, such as *IKBKG* (MIM 300248), in which, similar to *GDI1*, loss-of-function mutations are associated with mental retardation.

IKBKG (GenBank NM_003639.3), also called nuclear factor kappaB (NF-κB) essential modulator (NEMO/IKK-gamma), encodes for NEMO/IKKgamma, a protein that is generally recognized to have an essential role in the NF-κB signaling. The NF-κB pathway controls several cellular and developmental processes, and the timely activation and inactivation of this signaling is essential for NF-κB to function in a controlled manner. Indeed, the NEMO/IKKgamma protein, because of its proven crucial role in the pathway, is tightly regulated at posttranslation levels by sequential protein modification,[2] as well as at transcriptional levels by multiple regulatory regions.[3,4] In addition, the *IKBKG* gene is highly expressed in the brain and has a well-documented role in neuronal plasticity and central nervous system (CNS) development.[5,6] It has been reported that an imbalanced function of IKBKG affects the regulation of NF-κB signaling in this tissue,[5] and when NF-κB is hyperactivated or repressed, a defect of neurite growth in a specific subset of neurons is observed.[6] It is noteworthy that such a role of the NF-κB pathway in CNS development was confirmed in three reports appearing in the same issue of *AJHG*, in which Philippe et al.,[7] Mochida et al.,[8] and Mir et al.[9] identified an NF-κB signaling defect as a cause of MR.

The role of IKBKG in the CNS is strongly supported by human genetic data. Loss-of-function mutations of *IKBKG*, although lethal in males, cause incontinentia pigmenti (IP [MIM 308300]) in females, a neurocutaneous disorder often associated with mental retardation, psychomotor delay, seizure, spastic paresis, and microcephaly. IP patients may also present cortical necrosis, several white matter abnormalities, hypoplasia of the corpus callosum, etc.[10,11] A heterozygous exon 4_10 *IKBKG* deletion is the major genetic defect in IP, caused by recombination between two consecutive medium reiterated 67B (MER67B) repeats, located in intron 3 and downstream to exon 10 of the gene, respectively.[12] Nevertheless, missense mutations in IKBKG have also been found in association with severe mental handicap.[13] Thus, the notion that IKBKG is involved in MR is generally recognized, and the gene was added to the list of MR genes in 2005.[14]

In addition, we would like to bring attention to previous work on the two distal LCRs discussed in the manuscript by Vandewalle et al.[1] The authors reported that the LCRs (named L1 and L2 on page 809) recombine to cause the copy-number gain observed in their four MR families. Although the authors correctly reported that the two LCRs share a high sequence homology, citing the paper of Aradhya et al.,[15] they failed to note the presence of *IKBKG* and its truncated pseudogene copy (from exon 3 to 10) within the two repeats. It would be of interest for people working in clinical genetics to know whether any of the putative mechanistic recombination affected the *IKBKG* gene and how many copies of this gene are present in the recombinant alleles.

Furthermore, the notion that the L1 and L2 sequence homology causes recombination was extensively documented in the previous reports. Aradhya et al.,[15] cited by Vandewalle et al.,[1] detected evidence for sequence exchange between the L1 and L2 copies, pointing out that because the two LCR copies are in opposite orientation, inversions might be responsible for their homogeneity. More recently, we reported additional proof of the ability of the LCRs L1 and L2 to recombine.[16] Indeed, we observed in IP families a recombination event produced by non-allelic-homology recombination (NAHR) between two LCRs that repositioned an exon4_10 deletion from the pseudogene (*IKBKGP*, GenBank NG_001576) to the *IKBKG* gene, thereby causing the IP disease (Figure 1).

Vandewalle et al.[1] stated that they were unable to clone the breakpoint junctions because of the very high sequence homology between the LCR subunits and the multiple rearrangements that take place between them. We would like to suggest that the authors review our work in which, by using a PCR-based strategy, we mapped the breakpoint in the IP family carrying IKBKG

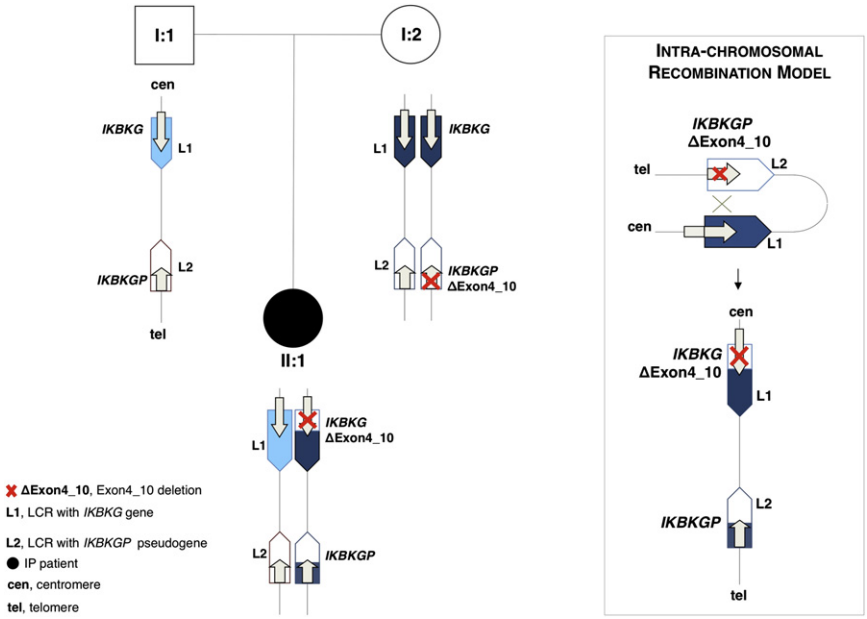

Figure 1. An Example of NAHR between L1 and L2 Causing a Pathological Allele Is Reported: The Case Represents the *IKBKG* Locus in an IP Family

The unaffected mother (I:2) carrying the exon4_10 deletion in the *IKBKGP* pseudogene is reported. The IP patient (II:1) carrying the pathogenic deletion in the gene (exon4_10 deletion) is reported.

The misalignment between the two LCRs, L1 and L2, produces the intrachromosomal recombination transferring the exon4_10 del deletion (ΔExon4_10) from the pseudogene to the gene in the recombinant allele.

recombinant alleles, which occur by NAHR between L1 and L2.[16] We were able to establish that all recombination between LCRs L1 and L2 occur in the MER67B repeats located in intron 3 and downstream exon 10 of the *IKBKG* gene. Notably, other recurrent intrachromosomal recombination, occurring between two MER67B repeats located within the one individual copy of the LCR, may produce microdeletion-microduplication in the *IKBKG* gene and pseudogene.[16,17]

In conclusion, the presence of high-repetitive DNA sequence families, LCRs, and a nonprocessed pseudogene sequence in the Xq28 region is known to enhance homologous recombination. Several previous studies have pointed out the ability of L1 and L2 copies to recombine giving rise to both pathological and nonpathological structural variants of the human genome or copy number variations (CNVs). The recombinant alleles reported by Vandewalle et al.[1] fit very well with these previous findings. In addition, we wonder whether any other gene in the duplicated region may play a role in the MR phenotype described by the authors. In particular, we favor the analysis of *IKBKG*, first of all because it is located exactly in the recombination region, and second, because its nature suggests that any upregulation or decreased expression may cause cellular dysfunction and thus disease in a tissue-specific manner.[18]

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Web Resources

The URLs for data presented herein are as follows:

NCBI Reference Sequence (RefSeq), http://www.ncbi.nlm.nih.gov/RefSeq/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

UCSC Genome Browser, http://genome.ucsc.edu/index.html?org=Human

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Response to Fusco et al.

To the Editor: The authors' comments consist of two parts, to which we will reply separately.

1) The authors state that next to *GDI1*, overexpression of other genes present within the recurrent aberration should be taken into account as well to explain the MR phenotype in our families. In particular, the *IKBKG* gene is a candidate because mutations have been implicated in IP, often associated with neurological abnormalities, and because the NF-κB pathway has been linked to MR. We completely agree with a prominent role for the *IKBKG* gene and its pathway in neurological disorders and we have taken this gene seriously into account for a role in the MR phenotype of our families. However, we identified a 190 kb duplication, which overlaps our recurrent aberration, in a female patient as well as her normal father. This finding is described on page 812 of our paper[1] and the position of this polymorphic duplication is illustrated in Figure 2 (horizontal striped bar). This benign copy number variant includes, among others, *IKBKG*, which demonstrates that at least a duplication of this gene does not cause a pathological condition. Because *IKBKG* is duplicated in affected males of our family 4, we excluded it as a candidate gene. We do mention that we still have to be careful with this "rejection." We agree, however, that in the sentence on page 819 "*Moreover, the role of other genes within the aberration, such as UBL4A and FAM3A, cannot be excluded even though the apparent 190 kb copy-number polymorphism identified in a normal male individual seems to exclude a contribution of a double dosage of both genes in family 4*," the *IKBKG* gene should have been included as well. Of the remaining genes in the nonoverlapping aberrant region, we did check brain expression for all genes. For those with the highest expression (*FLNA*, *RPL10*, *ATP6AP1*, and *GDI1*), we checked their expression levels in patient-derived cell lines. So we did not focus on *GDI1* alone but proposed this gene as the most likely candidate gene, which is clearly discussed.

2) The authors would have liked us to put more emphasis on the recombination events that occur between the two oppositely oriented LCRs, L1 and L2, and the consequences these might have on the *IKBKG* gene. In our study, the aim was not to describe the NAHR events that occur between LCR partners (K1 and K2, or L1 and L2). We clearly point to the occurrence of recombination events (see page 816 " … *multiple possible NAHR-driven inversion events that could have taken place between the subunits of each set*") that probably resulted in homogeneity of the entire LCR. Because *IKBKG* was not regarded as a candidate dosage-sensitive gene for the MR phenotype, and because our male patients as well as their carrier mothers do not show characteristics features of IP, we did not further investigate the *IKBKG*