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A Small, Variable, and Irregular Killer Cell Ig-Like Receptor Locus Accompanies the Absence of MHC-C and MHC-G in Gibbons

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The killer cell Ig-like receptors (KIRs) of NK cells recognize MHC class I ligands and function in placentation reproduction and immune defense against pathogens. During the evolution of monkeys, great apes, and humans, an ancestral KIR3DL gene expanded to become a diverse and rapidly evolving gene family of four KIR lineages. Characterizing the KIR locus are three framework regions, defining two intervals of variable gene content. By analysis of four KIR haplotypes from two species of gibbon, we find that the smaller apes do not conform to these rules. Although diverse and irregular in structure, the gibbon haplotypes are unusually small, containing only two to five functional genes. Comparison with the predicted ancestral hominoid KIR haplotype indicates that modern gibbon KIR haplotypes were formed by a series of deletion events, which created new hybrid genes as well as eliminating ancestral genes. Of the three framework regions, only KIR3DL3 (lineage V), defining the 5' end of the KIR locus, is present and intact on all gibbon KIR haplotypes. KIR2DL4 (lineage I) defining the central framework region has been a major target for elimination or inactivation, correlating with the absence of its putative ligand, MHC-G, in gibbons. Similarly, the MHC-C-driven expansion of lineage III KIR genes in great apes has not occurred in gibbons because they lack MHC-C. Our results indicate that the selective forces shaping the size and organization of the gibbon KIR locus differed from those acting upon the KIR of other hominoid species. The Journal of Immunology, 2010, 184: 1379–1391.

Killer cell Ig-like receptors (KIRs) recognize MHC class I ligands and are expressed on NK cells and subsets of T cells. The human KIR locus, in the leukocyte receptor complex on chromosome 19, exhibits extensive variation in gene copy number and in allelic polymorphism of the individual genes (1, 2). Various aspects of KIR gene variation, either alone or in combination with HLA class I, have been associated with infectious (3–7), autoimmune (8), and malignant (9) diseases, as well as the outcome of transplantation (10) and success in reproduction (11, 12).

So far, comparison of mammalian species shows that only catarrhine (Old World monkeys and hominoids) (13) and platyrhine primates (New World monkeys) (14), as well as cattle (15), have a variable family of KIR genes. In other species, the KIR locus can consist of a single functional gene (e.g., wild seals) (16), be completely absent from the genome (e.g., domestic dog) (16), or have been translocated from the leukocyte receptor complex to the X chromosome and not expressed by NK cells (e.g., laboratory mice) (17). Certain species, notably mouse, rat, and horse, have evolved by convergence an equally diverse family of structurally divergent Ly49 receptors with remarkably similar functions to KIRs (18). Ancient duplication of the primordial KIR3D gene gave rise to two distinct KIR lineages: KIR3DX and KIR3DL (15). Modern cattle have an expanded family of KIR3DX genes and retain KIR3DL as a single-copy gene, whereas modern catarrhine primates retained KIR3DX as a single-copy gene while expanding the family of KIR3DL genes. Thus, the complex KIR gene families present in cattle and catarrhine primates are the consequence of independent expansions driven by forces of natural selection on different orders of placental mammals.

Variable KIR gene families have been studied in one species of New World monkey: owl monkey (14); three Old World monkeys: rhesus macaque (19, 20), cynomolgus macaque (21), and green monkey (22); four great apes: orangutan (23), gorilla (24), bonobo (25), and chimpanzee (26); as well as the human species (27). The majority of KIR genes are species-specific, consistent with their rapid evolution through extensive gene duplication, deletion, and hybridization by nonhomologous recombination (14, 22, 24, 28, 29). Phylogenetic analyses show there are four lineages of KIR in
these species (23, 24). Of the known ligand specificities, MHC-C recognition is associated with lineage I, MHC-A and -B recognition with lineage II, MHC-B and MHC-C recognition with lineage III, and for lineage V, represented by KIR3DL3, neither ligand nor function has yet to be identified (1, 30).

Whereas lineage II KIR makes up the major lineage in macaques (20, 21), it is represented by only one or two genes in hominids (great apes and humans). Conversely, lineage III KIR is the major hominoid lineage but is represented by a single gene in macaques. Coevolution with rapidly evolving MHC class I ligands is thought to have contributed to such KIR diversification, and in great apes, expansion of lineage III KIRs coincides with formation of MHC-C (23), whereas in Old World monkeys, the expansion of lineage II KIRs correlates with the expansion of the MHC-A and MHC-B genes (19–21, 31–33).

Uniquely missing from our picture of hominoid KIRs are the smaller apes, as represented by diverse species of gibbon. There are at least 14 extant species of smaller apes, or hylobatids, who are subdivided into four genera: Nomascus, Hylabates, Hoolock (formerly named Bunopithecus), and Symphalangus (34–37). Gibbons are arboreal apes inhabiting Southeast Asia and small regions of south and east Asia. Although they share habitat and geographical range with orangutans, gibbons are equally related to all the great apes and humans (38). Compared with great apes and humans, hylobatids have smaller bodies and are social animals that generally live in monogamous family groups engaging in relatively few contacts between groups (39). The gibbon genome is characterized by an accelerated chromosomal evolution, recently evaluated to be 10–20 times higher than is typical for mammalian evolution rates (40). A preliminary study of gibbon MHC class I identified only MHC-A and MHC-B (41), raising the possibility that gibbons lack the MHC-C gene present in hominids, which in humans and chimpanzees is the predominant source of KIR ligands (42). If that were the case, then the gibbon KIR system is predicted to be significantly different from what has been described for other hominoid species. To investigate this intriguing possibility, we characterized KIR haplotypes from two diverse genera of gibbons (Nomascus and Hoolock).

Materials and Methods
KIR nomenclature
KIR genes and alleles were named by the KIR Nomenclature Committee (45), formed from the World Health Organization Nomenclature Committee for factors of the HLA system and The Human Genome Organization Genome Nomenclature Committee. A curated database is available at www.ebi.ac.uk/ipd/kir/ (44).

Bacterial artificial chromosome library screening
High-density filters of bacterial artificial chromosome (BAC) libraries derived from northern white-cheeked gibbon (Nomascus leucogenys leucogenys, CHORI-271) and eastern hoolock gibbon (Hoolock hoolock leuconedys, CHORI-278) were obtained through the BACPAC resources of the Children’s Hospital Oakland Research Institute (http://bacpac.chori.org/home.htm). Both libraries were screened with a rhesus macaque KIR cDNA probe, and 11 clones were selected for exploratory analysis. Clones CH271-127M10, -109N22, -44K12, -49H24, -423M19, and -182G20 are derived from N. leucogenys; clones CH278-86P8, -86L16, -1164J2, -141F13, and -116G5 are derived from H. hoolock.

Preliminary characterization by Southern blotting and end sequencing showed that hoolock gibbon clones 86P8 and 86L16 represent one KIR haplotype (eastern hoolock gibbon primate species [Hoho-H1]), whereas clones 116G5, 141F13, and 1164J2 represent the second KIR haplotype (Hoho-H2). Clone CH278-86P8 from Hoho-H1 and clone CH278-116G5 from Hoho-H2 were selected for complete sequence determination.

As part of the ENCODE project (45), clones CH271-127M10 and CH271-423M19 from white-cheeked gibbon were sequenced to comparative grade standard (46) by the National Institutes of Health Intramural Sequencing Center (www.nisc.nih.gov). Our analysis of these sequences showed that clone 127M10 contained a complete KIR haplotype (northern white-cheeked gibbon primate species [Nole-H1]) and that clone 423M19 contained much of the second KIR haplotype (Nole-H2) but lacked the 3′ region. Further sequence analysis of both haplotypes was undertaken.

Phylogenetic analysis of gibbon KIR haplotypes was facilitated by the sequence of a KIR haplotype from a Sumatran orangutan (Pongo abelii). The BAC clone containing this haplotype (CH276-252G20) was sequenced by the Washington University Genome Sequencing Center, as part of the orangutan genome project, and is deposited in GenBank (www.ncbi.nlm.nih.gov/Genbank/) under accession number AC200148.

BAC DNA sequencing
BAC clone DNA was purified by CsCl density centrifugation and then sheared by sonication. DNA fragments were separated by agarose gel electrophoresis, and fragments of 1.3–3.5 kb were cloned into plasmid pUC19. Clones were sequenced using M13 forward and reverse primers. The sequence data were processed by Phred (www.phrap.org) and assembled into a contiguous sequence by Phrap (www.phrap.org). Both the Phred and Phrap computer programs can be obtained from P. Green (Genome Sciences Department, University of Washington, Seattle, WA) (47).

For the eastern hoolock gibbon, BAC clones CH278-86P8 and CH278-116G5 were selected for sequencing because they cover the same genomic region and contain the Hoho-H1 and Hoho-H2 KIR haplotypes, respectively. Complete sequences for these clones were determined to high-quality standard (error rate for each base pair of the consensus = 1 in 100,000; Phred score ≥ 50). White-cheeked gibbon BAC clone CH271-423M19, containing the incomplete N. leucogenys haplotype Nole-H2, was completely sequenced using the same approach, but the resulting sequence did not adequately cover the Nole-KIR2DL1 gene. To determine the sequence of Nole-KIR2DL1 to high accuracy, we first performed a new assembly of the ENCODE project sequence reads (from the National Institutes of Health Intramural Sequencing Center) using the STADEN package (48). This allowed precise identification of the regions of the Nole-KIR2DL1 gene that required further sequence analysis. These regions were amplified by PCR and cloned using the TOPO-TA system (Invitrogen, Carlsbad, CA). Sequencing was performed using the M13 forward and reverse primers, and each nucleotide in the final sequence was minimally covered by three sequences representing at least two different templates and had a quality Phred score (1 putative mistake/100,000 bases).

Unlike the other three BAC clones studied here, BAC clone CH271-127M10 containing the complete Nole-H1 KIR haplotype was not completely sequenced to high precision. Instead, an accurate sequence of the Nole-H1 haplotype was obtained by combining and refining the sequence data obtained by the ENCODE project and the N. leucogenys whole-genome shotgun sequencing project. To do this, a region of ∼105 kb containing the KIR region was targeted for analysis. A draft assembly of this region was obtained by assembling the ENCODE project sequence reads (from the National Institutes of Health Intramural Sequencing Center) using the STADEN package (48). This initial assembly was then complemented by sequence reads generated at Washington University Genome Sequencing Center and Baylor College of Medicine in the course of the ongoing N. leucogenys whole-genome shotgun project. These sequence reads were obtained by basic local alignment search tool (BLAST) searches, using as probes the genomic segments with low coverage in the initial assembly. Those sequence reads that displayed a well-supported match (i.e., a difference at a position with a good quality in both sequence reads) with those sequence reads generated at Washington University Genome Sequencing Center and Baylor College of Medicine in the course of the ongoing N. leucogenys whole-genome shotgun project. These sequence reads were obtained by basic local alignment search tool (BLAST) searches, using as probes the genomic segments with low coverage in the initial assembly. Those sequence reads that displayed a well-supported match (i.e., a difference at a position with a good quality in both sequence reads) with sequence reads in the initial assembly likely represented the other KIR haplotype and were discarded. The final assembly had no gaps, and each base pair of the final assembly had a quality ≥ 50 (1 putative mistake/100,000 bases), except for two intronic regions of Nole-KIR3DL3 (∼500 bp in intron 3 and ∼1 kb in intron 4).

Sequencing of the four BAC clones was performed using BigDye terminator chemistry and either an ABI 3730xl or an ABI 377 sequencer (Applied Biosystems, Foster City, CA). Exons and introns of genes were either identified manually or by using the BLAST (www.ncbi.nlm.nih.gov/BLAST) and FGENESH-2 (softberry.com/softberries) algorithms.

Phylogenetic analysis of KIR
KIR gene sequences were aligned using MAFFT (49) and manual correction of the resulting alignments. The aligned sequences were then divided into 12 segments as described previously (23). Each segment was analyzed by three methods: maximum-likelihood (ML), neighbor-joining (NJ), and maximum-parsimony (MP). NJ analyses were performed with MEGA4 (50) using the Tamura-Nei method with 500 replicates. PAUP* 4.0b10 (51) and the tree bisection-reconnection branch swapping algorithm were used for MP analyses with 500 replicates and a heuristic search. ML analyses were performed with RAxML 7.2 (under the GTR+CAT model with 500 replicates (rapid bootstrapping). The results of these analyses are in Supplemental Fig. 1. The
analysis of KIR intergenic regions was similarly performed on an alignment of sequences that started ∼600 bp after the end of exon 9 of the first KIR gene and ended ∼400 bp before exon 1 of the second KIR gene.

Analysis of the deletions forming gibbon KIR haplotypes

To identify deletion events leading to modern gibbon KIR haplotypes, we first aligned the intergenic and intragenic segments of the four sequenced KIR haplotypes based on the results of the phylogenetic analysis; the segments used in this analysis are those described above, under “Phylogenetic analysis of KIR.” The structure of the predicted progenitor for all hominoid KIR haplotypes was then used to identify deletion and duplication events and the KIR gene segments lost or gained as a consequence of these events (the structure of the progenitor for all hominoid KIR haplotypes used here is a refinement of previous evolutionary models (23), as detailed in Results).

KIR haplotype comparisons in Old World monkey and hominoids pointed to the lineage Ia, II, III, and V KIRs as having been present on all ancestral KIR haplotypes (23), so absence of any of these from gibbon KIR haplotypes was considered a gibbon-specific loss. The lineage Ib KIR2DL5 is typically present on only a fraction of haplotypes in hominoid species, so it is possible that it was not present on all ancestral haplotypes. As a result, absence of KIR2DL5 was only considered a gibbon-specific loss when KIR2DL5 segments could be found on the haplotype; otherwise, the lineage Ib deletion was only considered “possible” and labeled accordingly.

Characterization of MHC class I, Nole-H2 from N. leucogenys

To assess the MHC class I gene content of the gibbon genome, we searched the database from the ongoing N. leucogenys whole-genome shotgun-sequencing project generated by the Washington University Genome Sequencing Center and the Baylor College of Medicine. First, BLAST searches were performed on sequences corresponding to the six functional human HLA class I genes: HLA-A, -B, -C, -E, -F, and -G. More than 200 sequences were gathered for each of the six independent BLAST searches. The resulting sequences were pooled and assembled using the STADEN package (48). The contiguous sequences thus generated were aligned with human and macaque MHC class I genes and pseudogenes using MAFFT (49), and the resulting alignment was corrected manually. The alignment was then divided into two halves, and phylogenetic analyses were performed on each half as described above for the KIR genes. The results of these analyses are in Supplemental Fig. 2. Several gene fragments had insufficient sequence for analysis by this approach. For each of these gene fragments, a customized dataset was made, which was limited to the particular genomic region covered by the gene fragment under test. Phylogenetic analyses were performed using the NJ method, as described above for the KIR.

To determine whether the Nole-H2 haplotype contained KIR genes additional to those in BAC423M19, we investigated the N. leucogenys whole-genome shotgun-sequencing project through BLAST searches with KIR gene sequences from the three complete haplotypes as queries. This initial analysis revealed three additional KIR genes related to 2DL4, 2DH1, and 3DL1. Sequence reads for these three genes were then obtained by BLAST searches using the Hoho-2DL4, Nole-2DH1, and Nole-3DL1 gene sequences as queries. For each of these three genes, the sequence reads gathered were assembled using the STADEN package (48).

For Nole-KIR2DL4, the analysis was facilitated by the absence of 2DL4 on Nole-H1, the final assembly containing only one gap of ∼100 bp in intron 6 (assembly represents ∼99% of the Hoho-2DL4 gene sequence). For KIR2DH1 and 3DL1, the final assemblies had four and five gaps, respectively, covering ∼70% of the sequence of their respective equivalent on Nole-H1. This coverage likely represents an underestimation of the coverage from the whole-genome shotgun sequences, because only the sequences that unambiguously represented the Nole-H2 haplotype (i.e., that contained at least one unique polymorphism) were included.

The positions of these additional three genes on the Nole-H2 haplotype (following 2DP2) were determined using read-pair information and are as follows: 2DL4 - 2DH1 - 3DL1, with 3DL1 being the 3′-most KIR of the haplotype, as indicated by the presence of a G3 intergenic segment in its 5′ end. The sequences of these three genes were not included in the phylogenetic analyses, because they are incomplete and contain segments of draft quality.

Results

Gibbons have small KIR haplotypes with diverse gene content

We investigated KIR haplotype structures in the eastern hoolock (Hoolock hoolock leucomedus) and northern white-cheeked (Nomascus leucogenys leucomedus) gibbons, two species that diverged several million years ago and represent the most divergent gibbon generaa, b (34, 53, 54). Three BAC clones contained complete gibbon KIR haplotypes: two from H. hoolock (Hoho-H1 and Hoho-H2) and one from N. leucogenys (Nole-H1) (Fig. 1A). In these BAC clones, the gibbon KIR genes are flanked on the 5′ side by the LIIR gene cluster and on the 3′ side by the FCAR gene, an arrangement identical to that observed in all other primates studied (23) as well as in most other mammalian species (16).

BAC clone analysis also defined the 5′ part of a second KIR haplotype from N. leucogenys (Nole-H2), which extended from the flanking LIIR gene through 3DL3, 2DL1, and 2DP2 to end ∼300 bp before the first exon of 2DL4. To characterize this haplotype further, we took advantage of the fact that genomic DNA from the same individual gibbon was used to make the BAC library and to conduct the N. leucogenys whole-genome shotgun-sequencing project. By extracting and assembling all the sequences generated in the latter project, we showed that the Nole-H2 haplotype contains three additional KIR linked upstream from 2DP2 in the following order: 2DL4, 2DH1, and 3DL1, with 3DL1 being the last KIR of the haplotype (Fig. 1A). Two of these three genes, 2DH1 and 3DL1 represent alleles of Nole-H1 genes and minimally differ by 33 (2DH1) and 55 (3DL1) nucleotide substitutions distributed along the length of the gene. Although unambiguous complete sequences were not obtained (contrasting with the other three haplotypes), there was in this extensive dataset no evidence for any deletion, insertion, or substitution that would corrupt the functions of 2DH1 and 3DL1. All the evidence is thus consistent with Nole-H2 having functional 2DH1 and 3DL1 genes. This complementary analysis of shotgun sequences allowed the KIR gene content of the Nole-H2 haplotype to be described completely (Fig. 1A).

FIGURE 1. Gibbons have small, irregular, and diverse KIR haplotypes. A, Schematic representation of the four gibbon KIR haplotypes. Haplotypes Hoho-H1 and Hoho-H2 are from Hoolock hoolock leucomedus, and haplotypes Nole-H1 and Nole-H2 are from Nomascus leucogenys leucomedus. Double arrows indicate genes that are either equivalent within species (alleles) or between species (orthologs). Black boxes represent genes encoding functional KIR, whereas gray boxes represent KIR pseudogenes, and white boxes represent genes flanking the KIR locus. *Activating KIR. For Nole-H2, KIR3DL3, 2DL1, and 2DP2 were fully characterized through sequencing of a BAC; 2DL4, 2DH1, and 3DL1 were characterized by analysis of whole-genome shotgun sequences. B, Shown are the numbers of KIR genes, KIR pseudogenes, and the average number of KIR genes and pseudogenes; the size of the KIR locus in the KIR haplotypes of several primate and nonprimate species is also given. For gibbons, the size of each of the four haplotypes is given.
Three of the four gibbon KIR haplotypes are much smaller than their counterparts in other hominoids and rhesus macaque, containing between two to four genes and pseudogenes and being 30–62 kb in length (Fig. 1B). The largest gibbon haplotype (Nole-H2) is also smaller than the haplotypes of other hominoids but has the same number of KIR genes as the one rhesus macaque haplotype defined (Fig. 1B). The mean number of genes per gibbon KIR haplotype (3.75) is thus lower than that in other hominoids and rhesus macaque (6–11). Despite their size, the four gibbon KIR haplotypes all differ in gene content, with a total of six different genes and two pseudogenes being represented. Of these, 2DL4, 3DL3, and 3DL1 are common to the two gibbon species and represent orthologous genes. KIR3DL3 is polymorphic in both gibbon species and the two allotypes differ by either four or five amino acid substitutions (Fig. 2). Such allelic variability is small compared with the divergence between the gibbon species, which constitutes 25–27 aa differences for 3DL3. The species divergence for 3DL1 is even higher, 48 residues (Fig. 2), suggesting 3DL1 has accumulated substitutions at a higher rate than 3DL3. In addition to their variability in gene content, gibbon KIR haplotypes also encode a variety of KIR structures: together the four haplotypes encode 10 functional KIRs, 8 inhibitory, and 2 activating (Fig. 1A). Six of the eight inhibitory KIRs have three Ig domains, whereas both activating receptors and the remaining two inhibitory KIRs have two Ig domains (Fig. 2).

All four hominid KIR lineages are represented in gibbons

To assess the relationships of gibbon KIR with the four primate KIR lineages (I, II, III, and V) defined in previous analyses (23, 24), we divided the gene sequences into 12 segments on which phylogenetic analysis was independently performed. The results are summarized in Fig. 3A, and two examples of the phylogenetic trees are given in Fig. 3B and 3C. The other trees are in Supplemental Fig. 1. The results demonstrate that all four KIR lineages present in great apes and humans are also present in gibbons: Hoho-KIR2DL4 from lineage I, Hoho- and Nole-KIR3DL1 from lineage II, Nole-KIR2DL1 from lineage III, and two alleles each of Hoho- and Nole-KIR3DL3 from lineage V (Fig. 3A). The other four gibbon KIRs are either recombinants, having segments derived from at least two lineages (Nole-KIR2DH1 and Hoho-KIR2DL2), or pseudogenes (Nole-KIR2DP2 and Hoho-KIR2DP1) lacking the last four exons of complete KIR genes (Fig. 3A–D). Whereas a majority of haplotypes in other hominoid species contain genes from all four KIR lineages, three of the four gibbon haplotypes do not carry intact genes from all four lineages (Fig. 3D).

KIR haplotypes lacking the central framework region are common in gibbons

The basic organization of the gibbon KIR locus is distinguished from that seen in other species. Characteristic of KIR haplotypes in other hominoids, and the rhesus macaque, are three conserved regions common to all haplotypes (23). These framework regions make up the 5′ part of a lineage V KIR at the 5′ end, 2DL4 and the 3′ part of a pseudogene in the central part, and the 3′ part of a lineage II KIR at the 3′ end. This arrangement is preserved in one haplotype from each gibbon species: Hoho-H1 and Nole-H2, but not in the other. Notably, the only genes in Hoho-H1 are the framework genes. The Hoho-H2 and Nole-H1 haplotypes both lack the central framework region. In addition, Hoho-H2 lacks part of the 3′ framework region. The only complete gene common to the four haplotypes is lineage V KIR3DL3, which forms the 5′ framework region. Three haplotypes (Hoho-H1, Nole-H1, and Nole-H2) have a complete lineage II 3DL1 gene as the most 3′ gene, whereas Hoho-H2 has a recombinant gene, 2DL2, in which the 3′ part derives from a lineage II gene and the 5′ part is from lineage III (Fig. 3D). The lineage III pseudogene KIR2DP and the lineage I

FIGURE 2. Structural diversity of gibbon KIR. Shown is an alignment of the deduced amino acid sequences of the 10 functional gibbon KIR encoded by genes in the four KIR haplotypes of Fig. 1. The alignment is organized according to the seven functional domains of the protein: D0, D1, and D2 are the three Ig-like domains, the Stem separates the ligand-binding Ig-like domains from the transmembrane (TM) region and the cytoplasmic tail (CYT). Sequence identity with Nole-KIR3DL1 is indicated by a dot, and an asterisk (*) denotes a stop codon. ITIMs are boxed. Other functionally significant residues are gray shaded. The characteristic arginine residue at position 325 in the transmembrane region of activating KIR is also indicated by an arrowhead. Nole-KIR are from Nomascus leucogenys leucogenys; Hoho-KIR are from Hoolock hoolock leuconedys. Nole-H2 KIR2DL4, 2DH1, and 3DL1 genes characterized by analysis of whole-genome shotgun sequences were not included in this alignment.
KIR2DL4, which provide the central framework region, are present in both Hoho-H1 and Nole-H2. Both KIR2DP and KIR2DL4 are absent from haplotypes Hoho-H2 and Nole-H1.

Among humans, great apes, and macaques, KIR2DL4 is the most conserved KIR gene. In addition to the absence of KIR2DL4 from two of the gibbon KIR haplotypes, the Hoho-2DL4 and Nole-2DL4 genes have a single nucleotide insertion near the 3' end of exon 6 that causes frameshift and premature termination (Fig. 4A). Consequently, these genes encode truncated KIR2DL4 proteins that lack ITIMs in the cytoplasmic tail and thereby lack inhibitory signaling function (Fig. 2). Several KIR2DL4 alleles in other hominoids are similarly affected by single base pair deletions or point substitutions in the same region of exon 6 or in exon 8 (24–26, 55, 56). On the basis of the characteristics of these sequences and the relationships between them, we estimate at least six independent events have inactivated ITIMs in hominoid KIR2DL4 allotypes (Fig. 4B). The situation in gibbons is extreme, because none of four haplotypes we characterized has a KIR2DL4 gene encoding a 2DL4 protein with an ITIM. The recurrent selection against 2DL4 inhibitory function in hominoids stands in dramatic contrast to Old World monkeys, where the KIR2DL4 ITIMs are intact (Fig. 4B).

The contrasting fate of the KIR2DL4 and KIR3DL3 framework genes in gibbons is striking. Whereas all forms of 2DL4 have either been deleted or lost inhibitory function, 3DL3 is not only fixed in

**FIGURE 3.** All four primate KIR lineages are represented in gibbons. To assess the lineage affinities of gibbon KIR with other hominoid KIR, the genes were divided into 12 segments that were independently subjected to phylogenetic analyses. A summarizes the results of the phylogenetic analyses. Lineage assignment for each segment is shown, and the boxes are color-coded according to lineage: Ia (dark purple), Ib (light purple), II (pink), III (green), and V (blue). Segments for which lineage affinity could not be narrowed down to a single lineage are colored in light brown: exon 1 (lineages Ib, II, and III), exon 5 (lineages II and III), and intron 6c to exon 9 (lineages III and V). Light gray boxes indicate gene segments absent in some gibbon KIR. B and C, Representative phylogenetic trees for two of the 12 KIR gene segments are shown in B, for intron 3, and in C, for the segment from intron 6c to exon 9; analyses for the other 10 segments are in Supplemental Fig. 1. Phylogenetic reconstruction was performed using NJ, ML, and MP approaches. The NJ tree is shown (midpoint rooting), with support from all three methods being indicated at the nodes where the bootstrap proportion (BP) ≥ 50 for two of the three methods (from top to bottom: NJ, MP, and ML). At the nodes, ◦ denotes strong phylogenetic support (BP ≥ 80 with the three methods), and gray circles denote moderate support (BP ≥ 60). P. abelii; P. pygmaeus; P. troglodytes. D, Relationships between the four gibbon KIR haplotypes. Regions that are equivalent are shaded in gray. Black arrows indicate equivalent genes. A–D, The KIR2DL4, 2DH1, and 3DL1 genes of Nole-H2 were not included in the phylogenetic analysis because they are incomplete and contain segments of draft quality.
a form that preserves both ITIMs, but it also displays polymorphism in both gibbon species (with 4–5 aa substitutions), as also observed in humans (30, 57–59) (Fig. 2). These properties strongly suggest that KIR3DL3 has been of persistent value to gibbons.

Nonfunctional forms of gibbon KIR2DL4 correlate with genomic deletion of MHC-G

Because HLA-G is the ligand for human KIR2DL4, we examined gibbon MHC class I genes for the presence of MHC-G. This was achieved by using HLA-A, B, C, E, F, and G sequences in BLAST searches to extract all MHC class I sequences from the database generated by the N. leucogenys whole-genome shotgun-sequencing project. Over 300 unique sequence reads were obtained and then assembled into 28 gibbon MHC class I contigs. Phylogenetic comparison of gibbon, human, and macaque MHC class I sequences (Supplemental Fig. 2) allowed us to identify functional gibbon genes corresponding to the expressed HLA-A, B, E, and F genes and gibbon pseudogenes corresponding to the HLA-J, L, S, W, and X pseudogenes. However, no gibbon counterpart of HLA-G emerged from this analysis (Fig. 5). Our failure to find gibbon MHC-G suggests that some gibbon MHC haplotypes lack MHC-G, but the fact that both MHC haplotypes in the animal we studied lack the HLA gene indicates that MHC haplotypes lacking MHC-G are common. In summary, deletion and loss of function of gibbon KIR2DL4 correlates with the absence of MHC-G.

Minimal representation of lineage III KIR in gibbons correlates with absence of MHC-C

Lineage III is the most highly represented KIR lineage in great apes and humans (23). In the latter species, for example, lineage III makes up 8 of the 14 KIRs and >50% of the genes per KIR haplotype. As a group, the four gibbon haplotypes contain only one intact lineage III KIR (Nole-KIR2DL1). In addition, two recombinant KIR contain lineage III segments (Nole-KIR2DH1 and Hoho-KIR2DL2) (Fig. 3A). Phylogenetic analysis shows that the
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**gibbon lineage III** sequences are orthologous to all the great ape and human lineage III KIR but not to any particular KIR (Fig. 4D). This shows that the difference in lineage III KIR gene content was not caused by gene loss in the gibbons, because in that circumstance the residual gibbon KIR would exhibit greater similarity to particular KIR in great apes and humans. Consequently, the difference in gene content is due to expansion of the lineage III KIR genes in great apes and humans.

**FIGURE 5.** Gibbon MHC haplotypes lack MHC-C and MHC-G. The MHC class I gene sequences generated by the whole-genome shotgun-sequencing project for *N. leucogenys* were assembled into 28 contiguous sequences (contigs) and compared with human HLA class I by phylogenetic analysis (Supplemental Fig. 2). Although the majority of gibbon *HLA class I* have orthologs in the *HLA class I* region, notably absent are gibbon counterparts to *HLA-G*, and closely linked pseudogenes and *HLA-C*. In this summary diagram, the inferred *HLA class I* gene content of the gibbon *MHC* is compared with the established map of the human *HLA class I* genomic region. Functional genes are indicated by ■ and pseudogenes by □. The terms centromeric and telomeric refer to the orientation of the *HLA* complex on the short arm of human chromosome 6. Results from a study that combined paired-end sequence analysis and molecular cytogenetics suggest that in *N. leucogenys* the *MHC* is on chromosome 1b, with an opposite centromeric/telomeric orientation compared to human (73). B. This shows the characteristics of the 28 gibbon MHC class I-containing contigs and their relationships to human *HLA class I* genes and pseudogenes. The first column on the left lists the six *HLA class I* genes and the 11 largest *HLA class I* pseudogenes; the second column indicates presence or absence of orthologs in the gibbon genome. Under “Phylogeny,” gray boxes indicate whether phylogenetic analysis was performed on the 5' untranslated region (UTR)-E4 part of the *class I* gene and/or the I4-3 UTR part, and they also show the part of the MHC-class I gene region covered by each of the 28 gibbon *MHC class I* contigs. Parentheses indicate segments that were analyzed separately (see Materials and Methods), and “xx” indicates a sequence that cannot be aligned with the other MHC class I sequences, suggestive of a class I gene fragment. Under “Notes,” the sequence motif at positions 76 and 80 is given for MHC-B, because valine 76, asparagine 80 defines the C1 epitope, a ligand for lineage III KIR; also given is the diagnosis of certain sequences as pseudogenes or null genes. To demonstrate the coverage of analysis, the single nucleotide polymorphism (SNP) content of each contig is given, excluding those representing the duplicated MHC-B and MHC-S, for which the allelic relationships are uncertain.

**Lineage III KIRs** are receptors for MHC-C, and expansion of the lineage III KIR genes is associated with presence of MHC-C in humans and great apes but not in Old World monkeys (23). A previous study of cDNA characterized expressed MHC-A and -B genes in gibbons but failed to detect any MHC-C transcripts (41). Our analysis of 28 *N. leucogenys* MHC class I sequences from one individual also failed to identify any gene or pseudogene that was orthologous to the *MHC-C* locus shared by orangutan, chimpanzee,
bonobo, and human (60). Two copies of a pseudogene with some similarities to MHC-B and -C were defined (contig number 276081453; Fig. 5B). Thus, the paucity of gibbon lineage III KIR correlates with the absence of a functional MHC-C gene in gibbons.

In humans and great apes, the specificities of lineage III KIRs for the C1 and C2 epitopes of MHC-C are determined by substitutions at position 44 in the D1 domain (61). Ancestral reconstructions and species comparison have shown that the C1 epitope and its complementary KIR (containing lysine 44) were the first to emerge (42). Consistent with this model, Nole-KIR2DL1, Nole-KIR2DH1, and Hoho-KIR2DL2 all have lysine 44, which corresponds to position 139 in the alignment in Fig. 2. The C1 epitope is dependent upon valine 76 and asparagine 80 in the α1 domain of MHC-C. Certain MHC-B allotypes also have both valine 76 and asparagine 80 and can function as ligands for C1-specific KIRs (42). One of the four gibbon MHC-B sequences (number 282426381) has both valine 76 and asparagine 80 and represents a candidate ligand for the gibbon lineage III KIR (Fig. 5B).

The ancestral hominoid haplotype contained five KIR genes and a pseudogene

As a foundation upon which to investigate how the short gibbon KIR haplotypes arose, we reconstructed ancestral hominoid haplotypes using intragenic and intergenic sequence comparisons. To refine previous evolutionary models (23), information from the four gibbon KIR haplotypes was incorporated, as well as data from a KIR haplotype from a Sumatran orangutan (Pongo abelii). This haplotype differs from the previously reported orangutan haplotype (23) by presence of KIR2DL5 and an activating lineage II KIR3DS and absence of activating lineage III KIR2DS (Fig. 6A).

Paralleling the four human and great ape KIR lineages defined by intragenic sequences (23), the intergenic sequences also fall into four groups (G1, G2a, G2b, and G3). Each of these groups is represented in gibbons (Fig. 6B). For three groups, the genes flanking the intergenic sequence are similar in all hominoids: these are composed of G3, between the most 3’ KIR and FcAR; G2b, between KIR2DL4 and a lineage II KIR; and G2a, between one lineage III KIR and either KIR2DL5 or a second lineage III KIR (one exception is the orangutan KIR2DL5-3DS segment). The fourth group of intergenic sequences (G1) contains the two gibbon sequences located 5’ of hybrid genes whose 5’-most segments are related to KIR2DL5 (Fig. 6B). The segment 5’ of orangutan P. abelii-KIR2DL5 also belongs to the G1 group, whereas the intergenic sequences 5’ of human KIR2DL5 belong to the G2a group (Fig. 6B). Discovery of a complete KIR2DL5 gene in orangutan (Fig. 6A) and of KIR2DL5 segments in gibbon shows KIR2DL5 was a component of early hominoid haplotypes. Our analysis also indicates that KIR2DL5 in early hominoids was 3’ of a G1 intergenic region, later to be replaced by the G2a segment present in modern humans. Taken together with phylogenetic analysis of the intragenic sequences, these observations allowed us to refine previous models of KIR haplotype evolution. Ancestral hominoids are now seen to have had KIR haplotypes with five complete genes, each corresponding to one of the five human KIR lineages, and a lineage III pseudogene (Fig. 6A).

The irregular structures of gibbon KIR haplotypes were shaped by successive deletions

To discern how modern gibbon KIR haplotypes evolved from their common hominoid ancestor, we aligned their intergenic and intragenic segments on the basis of the results of phylogenetic analysis (Fig. 6A). This comparison showed that a minimum of five deletions and one duplication were necessary to evolve the four gibbon haplotypes from the ancestral KIR haplotype. That three deletions are specific for N. leucogenys and two for H. hoolock suggests partial deletion of KIR haplotypes has been a widespread phenomenon during the history of gibbons. Whereas partial or complete gene duplications are the main mechanism for diversifying KIR haplotypes in great apes, in smaller apes KIR haplotype diversity has been generated mostly through successive events of deletion.

Deletion is a mechanism that can create new genes by hybridizing parts originating from two parental genes. Of the five genomic deletions in the gibbon KIR haplotypes, three caused only gene loss but the other two combined gene loss with the creation of a new gene (Fig. 6C). Assessing the effect of deletion on the presence and integrity of exons encoding the Ig domains showed that lineage I (KIR2DL4-5) was a major target, accounting for at least four of the seven losses of an Ig domain. Of the other three Ig domain losses, two targeted lineage III KIR and one targeted lineage II KIR. Three genes were more than once a target for deletion: KIR2DL1, KIR2DL4, and KIR2DL5 (Fig. 6D).

Among the genomic deletions that created new hybrid KIR genes, one produced Hoho-KIR2DL2 in H. hoolock, the other KIR2DH1 in N. leucogenys (Fig. 6D). Hoho-KIR2DL2 represents a fusion between a lineage III inhibitory KIR and a lineage II inhibitory KIR, where a fragment of D2, the stem, transmembrane and cytoplasmic tail of the former were replaced by those of the latter KIR (Fig. 3A). Because the coding sequences of lineage II and III KIR are closely related in the signaling domains (Supplemental Fig. 1) and because they share the same ITIM sequences (Fig. 2), the function of the new hybrid gene is likely to be similar to that of the original 5’-donor gene (lineage III KIR). In contrast, the gene fusion in N. leucogenys had more dramatic effect. Nole-KIR2DH1 combines the Ig domains of an inhibitory lineage III KIR with a KIR2DL4 transmembrane and cytoplasmic tail, which lacks an ITIM and has only activating potential (Figs. 2, 3A). As an activating KIR with lineage III Ig domains, Nole-KIR2DH1 represents a functional, and independently evolved, equivalent to the great ape activating KIR2DS of lineage III. Nole-KIR2DH1 is located between 2DL4 and 3DL1 in the Nole-H2 haplotype, representing the first example of a nonhuman primate gene in the 3’ part of the KIR locus that encodes lineage III Ig domains.

In summary, lineage I and III KIRs have been the main targets of the deletions diversifying gibbon KIR haplotypes. For lineage I, these events caused loss of Ig domain function, whereas for lineage III KIR, two events caused loss of Ig domain function, and two events involved swapping of the signaling domains with either preservation of function (Hoho-KIR2DL2) or functional shift from an inhibitory to an activating receptor (Nole-KIR2DH1). These deletions are correlated with absence of MHC-C and MHC-G from some, if not all, gibbon MHC haplotypes.

Acquisition of novel signaling function accompanied lineage III KIR expansion

In humans and great apes, the activating KIR of lineages II and III have a lysine residue in the transmembrane region that mediates interaction with the DAP12 signaling adaptor (62). In contrast, the gibbon-activating KIR2DH1 has an arginine residue in the transmembrane region that is shared with Mm-KIR3DH, a macaque-activating KIR of lineage II. KIRs having arginine in the transmembrane region are predicted to interact with the FcRy signaling adaptor and not with DAP12 (63). Because KIR signaling domains with lysine evolved from inhibitory lineage III KIR (28) and gibbon lineage III KIR are equally related to all great ape and human lineage III KIR (Fig. 4D), activating KIRs with lysine-mediated signaling via DAP12 are seen to be specific to great apes and humans. Thus, the emergence of MHC-C and lineage III KIR specific for MHC-C in a great ape ancestor was also accompanied by acquisition of a lysine-containing transmembrane by the KIR and recruitment of DAP12.
FIGURE 6. Successive deletions diversified gibbon KIR haplotypes. A. Relationships between the genes and intergenic segments of the four gibbon KIR haplotypes. Vertical black arrows indicate equivalent genes or gene segments. Red arrows indicate deletion (Del) or duplication (Dup) events. Dotted arrows denote hybrid genes with segments originating from different ancestral genes. The KIR intergenic regions are color-coded based on the phylogenetic analysis presented in B. At the top is shown the predicted ancestral hominoid KIR haplotype, with framework genes in bold. At the bottom are shown two orangutan KIR haplotypes. KIR genes are color-coded according to lineage: Ia (dark purple), Ib (light purple), II (pink), III (green), and V (blue). B. Phylogenetic analysis of KIR intergenic segments. Analysis and display are as described for Fig. 3B and 3C. Sequences characterized in this study are colored yellow (gibbon) or pink (orangutan). C and D. Summary of the functional effects of the five deletions shown in A. All five deletions led to the loss of Ig-like domains; two of them also formed new hybrid genes (C). Both species of gibbon experienced deletions that caused loss of Ig-like domains and formation of new genes (D). Because KIR2DL5 is not a framework gene, its Ig domains were only considered “lost” when a KIR2DL5 remnant was present on the haplotype; other potential KIR2DL5 “losses” were indicated by parentheses. Deletions were numbered as in A. **Two independent events. A–D, The KIR2DL4, 2DH1, and 3DL1 genes of Nole-H2 were not included in the analysis because they are incomplete and contain segments of draft quality.
Discussion

The expansion of the KIR locus, which now constitutes a large, plastic gene family in humans, occurred in primates during the past ∼85 million years (64). Four ancient KIR lineages predate the separation of hominoids from Old World monkeys 24–34 million years ago (65), and they have been retained to the present day. Additional species-specific gene expansions have, however, led to markedly different repertoires of KIRs in modern primate species. In great apes and humans, the lineage III KIRs form the largest and most diverse group of KIR (23), whereas this role has been taken by the lineage II KIR in Old World monkeys (macaques and green monkeys) (19–22). Functional interaction with rapidly evolving and highly polymorphic MHC class I is considered to drive the dynamic evolution of KIRs. For example, in great apes, the expansion of lineage III KIR correlates with emergence of MHC-C (23). Notably absent from the current picture of hominoid KIR evolution have been the smaller apes, the gibbons, equally close relatives of great apes and humans. Filling this gap are the results from this study of gibbon KIR and MHC class I.

Previously, gibbon cDNA sequences corresponding to polymorphic MHC-A and MHC-B genes were described, but no cDNA corresponding to MHC-C (41). From a systematic search through the genome of an individual gibbon, we identified 28 MHC class I sequences, including two alleles for MHC-A, -E, and -F genes and MHC-J, -L, -W, and -X pseudogenes that are orthologous to the corresponding HLA class I. Contrasting with such similarities was the presence of three MHC-B like genes in the gibbon and the absence of MHC-C and MHC-G. Also absent from the gibbon were several MHC-G–linked pseudogenes conserved by human and rhesus macaque, consistent with deletion of an extended genomic segment containing MHC-G from the gibbon genome. Multiple MHC-B and absence of MHC-C are features the gibbon shares with rhesus macaque and other Old World monkeys. Absence of MHC-C could reflect the fact that gibbons, like Old World monkeys, diverged from great apes and humans before MHC-C arose. Alternatively, gibbon ancestors may have had MHC-C, but it was subsequently lost, as clearly happened for gibbon MHC-G. A third possibility is that MHC-C is not fixed in gibbon genomes, as is the case for the orangutan MHC-C (66), and by chance the individuals studied here and in the previous investigation (41) had two MHC-C negative MHC haplotypes.

Further perspective on the presence or absence of gibbon MHC-C came from the study of gibbon KIRs. Comparison of four gibbon KIR haplotypes showed that they have fewer genes on average than KIR haplotypes in other hominoids and rhesus macaque. They are also highly variable, each haplotype having a different KIR gene content and irregular organization. This state of affairs arose by a series of independent deletions that targeted different parts of a common ancestral haplotype and differentially affected individual KIR genes. As a consequence of these deletions, the generally conserved organization of the KIR locus into two variable regions bounded by three constant framework regions (23) has been eroded in gibbons. That these unusual features are common to species representing the most divergent genera of gibbon (separated by ∼11 million years of evolution) (34, 53, 54) points to their generality in this taxonomic group.

All known MHC-C–specific KIRs are lineage III KIR. In humans and chimpanzees, these make up the most numerous KIRs, and several lineage III KIR genes are represented on each haplotype (2, 19). In contrast, the rhesus macaque, which lacks MHC-C, has a single lineage III gene (19). Expansion of lineage III KIR has been correlated with the presence of MHC-C and is clearly evident in the orangutan, even though MHC-C is present only on ∼50% of orangutan MHC haplotypes and the MHC-C allotypes only carry one (C1) of the two epitopes (C1 and C2) elaborated in human and chimpanzee (55, 60, 66). In this context, the structures of the gibbon haplotypes are both striking and informative. Three of the haplotypes have no intact lineage III KIR gene, and the fourth (Nole-H2) has only one (KIR2DL1). Thus, we have no evidence for expansion of lineage III KIR genes in gibbons, but in fact the exact opposite. For most KIR haplotypes, lineage III has been extinguished as a distinct entity (although segments can be preserved in recombinant forms). This feature of gibbon KIR and its contrast with the situation in the orangutan (23, 55) suggest that absence of MHC-C is likely to be a general property of gibbon MHC haplotypes (Fig. 7).

Before this study, the lineage I KIR2DL4 in the central framework region was seen as the most conserved and phylogenetically stable KIR gene (23). But in the gibbon, KIR2DL4 has been eliminated from two of the haplotypes and the 2DL4 genes of the other two haplotypes encode proteins that lack the ITIMs necessary for inhibitory function. Whereas 2DL4 in other species has the potential for both activating and inhibitory function, gibbon 2DL4 has only the potential for activation. The defined ligand for human KIR2DL4 is HLA-G, which is physiologically expressed during pregnancy by fetal extravillous trophoblast (67). Interactions between trophoblast HLA-G and KIR2DL4 expressed by maternal uterine NK cells are implicated in the invasive remodeling of maternal blood vessels that accompanies implantation (68). The observed deletion of gibbon MHC-G, combined with deletion of 2DL4 from some KIR haplotypes and functional incapacitation on others, suggests that cognate interactions between MHC-G and KIR2DL4 have been selected against in gibbons. Trophoblast also expresses HLA-C, but not HLA-A and -B (67), and polymorphic interactions between trophoblast HLA-C and cognate lineage III KIR on uterine NK cells are associated with human pregnancy syndromes (11, 12). Our results suggest that gibbons lack MHC-C and have few MHC-C–specific KIRs, implying that neither of the two types of HLA-KIR interaction that are important for human reproduction contribute to gibbon reproduction. Whereas human placentaion is associated with a deep trophoblast invasion of the uterine wall, pregnancy in gibbons involves a shallow invasion comparable to that seen for Old World monkeys (69), species that also lack MHC-C and functional MHC-G (33).

KIR3DL3, the 5′ framework gene, stands out as the only gene in the gibbon KIR locus that has withstood the effects of widespread deletion and has done so for ∼11 million years, since gibbon genera diverged (53). KIR3DL3 is the only gene common to the four haplotypes. All four KIR3DL3 variants have two intact ITIMs, the ones found in the genome of an individual gibbon, we identified 28 MHC class I sequences, including two alleles for MHC-A, -E, and -F genes and MHC-J, -L, -W, and -X pseudogenes that are orthologous to the corresponding HLA class I. Contrasting with such similarities was the presence of three MHC-B like genes in the gibbon and the absence of MHC-C and MHC-G. Also absent from the gibbon were several MHC-G–linked pseudogenes conserved by human and rhesus macaque, consistent with deletion of an extended genomic segment containing MHC-G from the gibbon genome. Multiple MHC-B and absence of MHC-C are features the gibbon shares with rhesus macaque and other Old World monkeys. Absence of MHC-C could reflect the fact that gibbons, like Old World monkeys, diverged from great apes and humans before MHC-C arose. Alternatively, gibbon ancestors may have had MHC-C, but it was subsequently lost, as clearly happened for gibbon MHC-G. A third possibility is that MHC-C is not fixed in gibbon genomes, as is the case for the orangutan MHC-C (66), and by chance the individuals studied here and in the previous investigation (41) had two MHC-C negative MHC haplotypes.

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From our analysis, it is clear that the KIR locus in gibbons does not conform to the principles previously established from comparison of an Old World monkey (rhesus macaque), two great apes (orangutan and chimpanzee), and the human species. In these species, one can see a progression of increasing complexity, which roughly correlates with the emergence, fixation, and diversification of MHC-C (23). During gibbon evolution, we see that three of the four KIR haplotypes have been reduced in size to two to three functional genes, through the action of gene deletion, but in an irregular manner in which the only constant feature appears to have been the preservation of KIR3DL3. Two gibbon KIR haplotypes (Hoho-H1 and Nole-H2) do conform to precedent and make up all the framework genes, but two other haplotypes have lost the central framework, and one of them has also lost part of the 3' framework (Hoho-H2). Correlating with these differences is the absence of MHC-C and MHC-G genes that are present in other hominoid species (60) and implicated, in humans, in regulating NK cell functions during reproduction (68). At some point in the history of gibbons, there appears to have been a shift in the action of natural selection to eliminate or diminish the functions of all KIRs except KIR3DL3. Because the two gibbon species studied here are distantly related (34, 53, 54), it is unclear whether this selection represented an episode in early gibbon evolution or is a persistent phenomenon. Because of the diversity of NK cell functions, the pressure for such change could have come from epidemic infectious disease, from reproductive competition, or some combination of the two. This change in selection did not lead to the expansion of Ly49, which is represented by a single gene in the gibbon genome (74).

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Disclosures
The authors have no financial conflicts of interest.

References

FIGURE 7. Model for the diversification of KIR in hominoids and macaques. KIR genes are color-coded according to lineage: Ia (dark purple), Ib (light purple), II (pink), III (green), and V (blue). In great apes and humans, the lineage III genomic regions are highlighted by green boxes; in macaques, the lineage II region is highlighted by a pink box. To simplify the display, KIR pseudogenes were not included in the model, except for gibbons.


**FIGURE S1.** Phylogenetic analysis of the 12 genomic segments used to compare *KIR* haplotypes. Analysis and figure legend are as described for Fig. 3B-C. Because several recombination events took place within the intron 6C - exon 9B genomic region, this region was split into three segments (intron 6C, exon 7 to exon 9A and exon 9B) in a previous analysis of *KIR* gene sequences (23). These three segments were also analyzed separately here (panels 12b, 12d, and 12e) but the summary of Fig. 3A presents the results obtained with the complete region (panel 12a). While we had to discard several (non-gibbon) sequences to perform an analysis of the complete region, this analysis has a better resolution for the gibbon sequences comparing to individual analyses of the three sub-regions.

**FIGURE S2.** Phylogenetic analysis of the two genomic segments used to compare *MHC-class I* content in human and gibbon. Analysis and figure legend are as described for Fig. 3B-C. *, human *HLA-P* and macaque *Mamu-P-like* sequences are weakly related in the I4-3'UTR analysis but are paraphyletic in the 5'UTR-E4 analysis.
Supplementary Figure 1
Phylogenetic analysis of the genomic segments used to compare KIR genes (1/4)
Supplementary Figure 1
Phylogenetic analysis of the genomic segments used to compare KIR genes (2/4)
Supplementary Figure 1
Phylogenetic analysis of the genomic segments used to compare KIR genes (3/4)
Supplementary Figure 1
Phylogenetic analysis of the genomic segments used to compare KIR genes (4/4)
Supplementary Figure 2

A

5’UTR to E4

Mamu B15
Mamu B13

291213818
282426381
287195460
286330801
Popy B
B*070201
Popy C
C*010201

Mamu V
Mamu E
Mamu S
Mamu L
Mamu T

MHC-B/C
MHC-V
MHC-E
MHC-L
MHC-F
MHC-P*
MHC-W

B

I4 to 3’UTR

Popy B
B*070201
Mamu B15
Mamu B13
276081453

Mamu F

MHC-B/C
MHC-S
MHC-E
MHC-L
MHC-W
MHC-P*
MHC-F
MHC-J
MHC-G

MHC-A/A-related

Support shown if:
≥50 with at least 2 methods

MHC-A/A-related

Support 280
Support ≥60

Maximum-Likelihood
Neighbor-Joining
Parsimony

0.02