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Diversity of the FcR- and KIR-related genes in an amphibian *Xenopus*

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1. ABSTRACT

Receptors subdivided into inhibitory and activating forms play important roles in the regulation of leukocyte development and effector functions. Two prototypic examples of paired receptors are Fc-receptors (FcR) and Killer cell Immunoglobulin-like receptors (KIR). FcRs are cell surface proteins that bind to the constant regions of IgG and IgE. Classical KIRs recognize MHC class I molecules and regulate natural killer (NK) cell cytotoxic functions. The evolution of these proteins and the time of their origin remain enigmatic. So far, molecules unequivocally related to mammalian FcRs and KIRs have been identified in chicken and an amphibian *Xenopus*. The lineage-specific evolution of the FcR and KIR families apparently led to the generation of unique sets of receptors in all species studied. Members of both families show extraordinary diversity of domain architectures. This structural diversity makes elusive the functional relationships between the highly specialized mammalian FcR and KIR genes and their homologs in nonmammalian species.

2. THE FCR- AND KIR-RELATED MOLECULES

It is commonly recognized that the initiation, proliferation and termination of the immune responses in jawed vertebrates are determined by a complex balance of signals delivered from paired receptors on the leukocyte surface. The majority of these receptors belong to the Immunoglobulin Superfamily. Paired receptors can be divided into two groups according to their signaling properties: inhibitory (negative signals) and activating (positive). The first group includes type I transmembrane (TM) receptors with an ectodomain composed of variable number of the Immunoglobulin (Ig)-like extracellular (EC) domains, a TM and a cytoplasmic tail containing one or few immunoreceptor tyrosine-based inhibitory motifs (ITIM). Activating receptors usually lack signaling motifs in their cytoplasmic tails but associate with auxiliary transmembrane signaling subunits bearing immunoreceptor tyrosine-based activating motifs (ITAM). Upon ligand-receptor interaction ITAMs are able to recruit intracellular kinases and trigger intracellular phosphorylation cascades resulting in transcription factors activation and alteration of

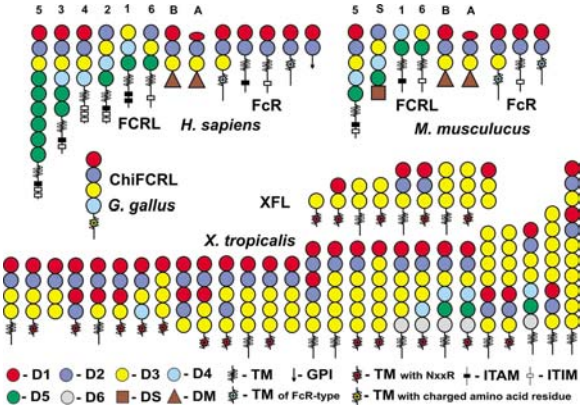

Figure 1. Schematic representation of domain architecture of human, mouse, chicken and *Xenopus tropicalis* FcR-related proteins. The structure of *X. tropicalis* molecules is deduced from genomic and cDNA sequences. The Ig-like domains belonging to the D1-D6 subtypes are shown by circles filled with appropriate color, Scavenger domains are depicted by square and Mucin-like by triangles. Zigzag lines designate TM regions, zigzag line with (+) label designates TMs interacting with signal subunits and an arrow designates Glycosylphosphatidylinositol-anchor. Straight lines and rectangles designate cytoplasmic tails and ITAM/ITIM motifs, respectively.

gene (s) expression. In turn, ITIMs recruit phosphatases opposing the effect from ITAM-mediated phosphorylation. Another common theme is the existence of pairs of receptors with almost identical extracellular portions but opposite signaling properties. Such pairs may function on the same cell in such a way that the outcome of an immune response will be determined by the sum of their signals (1, 2).

Two prototypic examples of paired receptors are Fc-receptors (FcR) and Killer cell Immunoglobulin-like receptors (KIR). FcRs, or classical leukocyte Fc-receptors, were discovered several decades ago and underwent extensive study by many scientists around the world. As named, FcRs are cell surface proteins that bind to the constant (or Fc-) regions of IgG and IgE. The structural and functional heterogeneity of FcRs exerts pleiotropic effects on the expressing cells. These proteins participate in antibody-dependent cell-mediated cytotoxity, phagocytosis and antigen presentation, various inflammatory reactions and feedback regulation of antibody production (3, 4).

Classical KIRs regulate natural killer (NK) cell cytotoxic functions and are responsible for "missing self" recognition in humans. These receptors recognize HLA-A, B or C class I molecules. The inhibitory forms mediate their action via ITIMs in the cytoplasmic tails and prevent lysis of cells expressing self MHC I; the activating versions have truncated cytoplasmic domains but associate with the ITAM-bearing DAP12 signal subunits (5-7).

Despite a key role of FcRs and KIRs in regulation of the immune responses in mammals, the evolution of these proteins and a phylogenetic period of their origin remain enigmatic. There is little doubt that the functions of Ig and MHC class I recognition are as ancient as Igs and MHC themselves. However, striking species-specific diversity of the FcR- and KIR-related molecules that has been uncovered during the past decade makes it unclear which molecules perform these functions in different vertebrate lineages.

Here we review the available data on *Xenopus* FcR- and KIR-related receptors and assess the structural and possible functional relationships of these molecules with their mammalian and avian counterparts.

2.1. The FcR-related molecules in human, mouse and chicken

The FcR family has been originally described as a group of molecules relatively homogeneous in structure and function. Four main FcR classes, FcgRI, FcgRII, FcgRIII and FceRI, differ from each other in binding affinity to Ig subclasses, expression pattern and/or signaling potential. The extracelluar regions of FcRs are composed of two or three Ig-like domains that have been subdivided into three structural subtypes, D1, D2 and D3. The D1 and D2 domains are mostly important for FcR interaction with their ligands. FcgRII is the only inhibitory classical FcR described so far. The other FcRs are activating receptors either associating with FcRgamma signal subunit or bearing an ITAM in the cytoplasmic tails (3).

Recent studies revealed that classical FcRs belong to a larger family composed of structurally diverse proteins. The novel members of the FcR family according to a new nomenclature (8) designated as FcR-like proteins (FCRLs) are subdivided into intracellular FCRLA, FCRLB (9-14) and cell surface receptors FCRL1-FCRL6 (15-19). FCRLA and FCRLB are composed of three Ig-like domains and a unique C-terminal proline-rich, mucin-like domain. These two proteins are strongly conserved among mammals. The other FCRLs are typical transmembrane receptors with extracellular portions composed of two to nine Ig-like domains. Their cytoplasmic tails show different patterns of ITIM/ITAM-like sequences and show either inhibitory, activating or combined functions (20-22). The mouse and dog families include also a secreted protein, FCRLS, which is composed of four Ig-like domains and a C-terminal domain belonging to the scavenger receptor cysteine-rich (SRCR) superfamily (23). Sequence comparisons revealed five types of homologous Ig-like domains (designated D1 through D5) in the FCRL proteins (Figure 1). Only three of these domains are present in classical FcRs (D1, D2 and D3). Interestingly, each of ten human and mouse proteins has its own composition of domains in the extracellular region. The functional significance of FCRLs remains unknown. However, their expression patterns suggest a participation in regulation of either lymphocyte differentiation or function (reviewed by Davis (24)).

Studies of the chicken (*Gallus gallus*) genome demonstrated that this species has a single FcR-related gene (23, 25). The chicken FCRL protein (chiFCRL) is a transmembrane receptor with four extracellular Ig-like domains and short cytoplasmic tail lacking signal motifs.

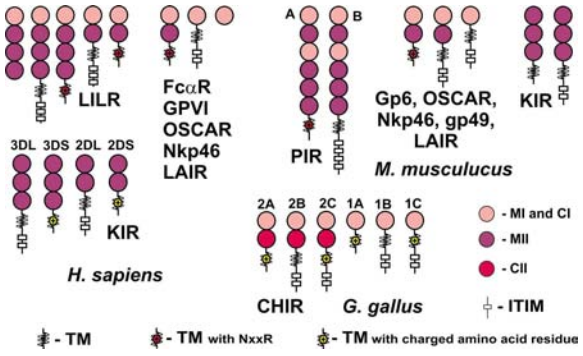

Figure 2. Schematic representation of domain architecture of human, mouse and chicken KIR-related proteins. The Ig-like domains belonging to MI, MII, CI and CII subtypes are shown by circles filled with appropriate color. Zigzag lines designate TM regions, zigzag line with (+) label designates TMs interacting with signal subunits. Straight lines and rectangles designate cytoplasmic tails and ITIM motifs, respectively.

The extracellular Ig-like domains have up to 48% amino acid identity with Ig-like domains of the mammalian FcR-related molecules. ChiFCRL requires FcRgamma subunit for surface expression (25).

2.2. The KIR-related molecules in human, mouse and chicken

The current nomenclature divides human KIRs into four groups according to the number of the Ig-like domains in the extracellular region (2D or 3D) and the length of the cytoplasmic tail (Long or Short): KIR2DS, KIR2DL, KIR3DS and KIR3DL. KIRs with long cytoplasmic tails contain 1 or 2 ITIMs and perform inhibitory functions; KIRs with short ones are DAP12-associating activating receptors. This association is promoted by the presence of a lysine residue in KIR TMs. KIR2DL4 has both ITIM and charged residue in the TM. The human KIR genes are located on chromosome 19 at a locus known as LRC (leukocyte receptor complex). In humans, the KIR family includes also the LILR (ILT/LIR/MIR) subfamily, LAIR-1 and LAIR-2, FcaR, NKP46, OSCAR and GPVI receptors (26-28). The LILR family consists of both inhibitory and activating forms, LAIR1 is an inhibitory receptor, LAIR2 is soluble, and the others are activating. Unlike KIRs, activating LILR family members, as well as FcaR, OSCAR, NKP46, and GPVI, associate with the FcRgamma subunit (29-33). These receptors differ by their ligands and expression patterns on cells of the hemopoietic lineage. LAIR, FcaR, and GPVI are not expressed by NK cells; LILRs are expressed by a broad range of cells and only some of them were found on subpopulations of NK cells. LILRB1 and LILRB2, inhibitory members of LILR family, are capable of binding both classical and nonclassical MHC class I molecules (34, 35). MHC molecules are not the only ligands of KIR-related receptors: FcaR binds to IgA, LAIRs and GP-VI interact with collagen (36-38).

Instead of classical KIRs, a large family of C-type lectins, Ly-49, are implicated in the MHC class-I recognition by NK cells in rodents (39). Two mouse genes showing the highest similarity (~40% identical residues) to human KIRs code for receptors with unknown function: the first with ITIMs and the second lacking both ITIMs and charged residues in the TM regions (40). Mouse possesses orthologs of the LAIR-1, GPVI, Nkp46, and OSCAR genes, but lacks FcaR. In contrast, gp49, a mouse member of the KIR family is absent in humans. The mouse counterpart of human LILRs has been described as the PIR family (27). As LILRs, PIRs may be divided into activating (PIR-A) and inhibitory (PIR-B) forms with similar extracellular part but different signaling properties (Figure 2). Recently, it has been shown that the extracellular portion of PIR can bind to murine MHC class I molecules (41) and, surprisingly, to *S. aureus* proteins (42). Moreover, last experiments (43) revealed that *cis*-interaction between PIR-B and MHC class I on mast cells leads to the increased cytokine release, thus PIR-B molecule is involved in regulation of allergic responses.

The only reliable nonmammalian homologs of the NK cell MHC class I-specific receptors identified so far are chicken CHIRs (44). Although composed of only two domains, CHIRs are most similar to the human LILR and mouse PIR molecules. Like their mammalian counterparts, CHIRs appear to be divided into inhibitory and activating forms. Recent data showed that chicken has more than 100 CHIR genes (45-47). CHIR genes encode transmembrane receptors with one or two extracellular Ig-like domains. Further CHIR proteins form three functional classes: CHIRs with short cytoplasmic tail and charged Arg residue in their TM are presumably activating; CHIRs with two ITIMs in the cytoplasmic tail are inhibitory; Finally, CHIRs with both Arg residue and ITIMs are referred as bifunctional receptors (Figure 2). Unexpectedly, the recent studies revealed that one of the bifunctional CHIRs binds to the Fc-portion of IgY with high affinity (48).

2.3. FcR- and KIR-related molecules in channel catfish

Recently a family of leukocyte immune-type receptors (LITR) has been identified in channel catfish (Ictalurus punctatus) (49, 50). LITRs are typical class I transmembrane receptors with 2 to 7 extracellular Ig-like domains. As classical paired receptors LITRs can be divided into activating receptors with short cytoplasmic tail and in their TMs, and inhibitory – with ITIM motifs in the intracellular parts. Moreover, as mammalian FCRLs, some LITRs possess in their cytoplasmic tails both ITAM and ITIM motifs. Ig-like domains of LITRs belong to several subtypes, two resemble FcR-type (D1 and D2) some others appear to be structurally similar to the domains of KIR-related receptors. Phylogenetic analysis shows that FcR-related D1 and D2 domains of LITRs form separate branches and do not intersect with clades of mammalian and avian D1 and D2. No clear phylogenetic relationship can be drawn between various LITR molecules and mammalian or avian representatives of the FcR and KIR family. It has been suggested that the FcR and KIR-families derived from LITR-like ancestral molecules.

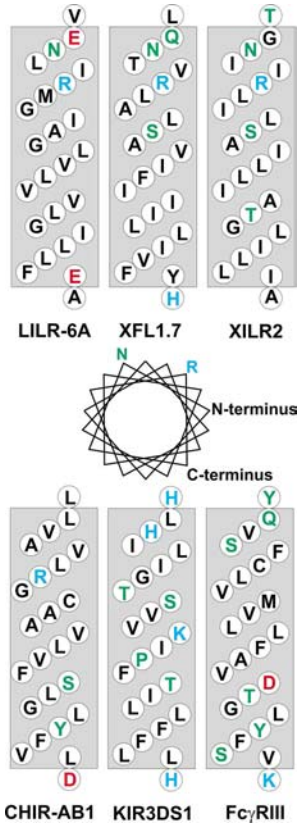

Figure 3. TM alpha-helices of some FcR- and KIR-related receptors. TM helices were predicted using SOSUI WWW Server (http://bp.nuap.nagoya-u.ac.jp/sosui/) and based on LILR6A TM. The top-view of first three helices is shown in the middle of the picture. Non-polar amino acid residues are designated by black letters, polar residues by green, positively charged by blue and negatively charged by red letters.

2.4. FcR- and KIR-related molecules in *Xenopus*

2.4.1. FcR-related molecules in *Xenopus*

Comparisons of the FcR- and KIR-related proteins in different mammalian species and, in particular between mammals and chicken, have demonstrated a strikingly high rate of evolution in both families. Phylogenetic analysis clearly showed that, despite their diversity, mammalian FcR-related domains form monophyletic groups distinct from their chicken homologs (23). This topology suggests two scenarios. The first is that the entire repertoires of the mammalian FcR-related proteins have emerged after the mammalian-avian split. The second is that birds have lost the genes most close to that of mammals. Taking into account clear multiplicity of functions of the mammalian proteins it would be important to determine which of these scenario is correct.

The completion of the *Xenopus tropicalis* genome sequencing project provided an opportunity to analyze the structure and organization of the FcR- and KIR-like genes in amphibians and their evolution in tetrapods. Our bioinformatics search of the *X. tropicalis* genome at the JGI web site (http://genome.jgi-psf.org) has revealed that this species has at least 75 FcR-related genes we designated XFL. The attribution of the XFL genes to the FcR family was unequivocal and was strongly supported not only by reciprocal sequence comparisons, but by conserved synteny too. The genomic data were supported by *Xenopus tropicalis* and *Xenopus laevis* EST cDNA analysis and screening of *X. laevis* cDNA libraries (51). The XFL genes code for class I transmembrane receptors with extracellular parts composed of one to eleven Ig-like domains. In addition to five Ig-domain subtypes recognized in the mammalian FcR-related proteins, their *Xenopus* counterparts may include a novel subtype designated D6. Another distinctive feature of *Xenopus* FcR-related proteins is that they may contain from one to six domains of the D3 subtype. This is in contrast to mammalian FCRL proteins possessing up to six D5 domains (Figure 1).

XFL receptors may be divided into two groups according to their presumable functions: activating or inhibitory. Inhibitory XFLs are represented by receptors with long cytoplasmic regions with ITIM motifs. The cytoplasmic and TM regions are encoded by separate exons in such receptors. The presumed activating receptors lack prolonged intracellular regions. Similar to majority of known activating receptors, their intracellular parts are encoded by exon for the TM region. Their TMs contain an arginine residue and are able to associate with the FcRgamma subunit (51). Interestingly, TMs of activating XFLs have little in common with TMs of classical activating FcRs, which possess characteristic LFAVD (N)TGL motifs (52). Sequence comparisons demonstrated that the XFL TMs are structurally homologous to TMs of mammalian members of the KIR family (NKP46, OSCAR, PIR-A, FcaR, figs 1, 2) which also associate with the FcRgamma subunit (29-33). In both groups of receptors, the arginine residue is embedded into NxxR motif at the NH2-end of TM. This TM subtype differs from TMs of DAP12-associating KIRs and from FcRgamma-interacting CHIRs (45) (Figure 3).

Comparison of the mammalian and *Xenopus* FcR-related molecules shows extraordinary structural plasticity of this family. Extracellular architectures of the receptors from the two lineages are strikingly different. The only EC domain composition shared by mammals and *Xenopus* is D1-D2-D3 characteristic of FcgRI and certain XFLs. However, when TM region subtypes are taken into consideration, the receptor architectures appear completely different in the two lineages. XFL receptors represent the most variable FcR-related group: overall, 75 *X. tropicalis* XFL genes give rise to 27 different extracellular architectures (Table 1). The only avian FCRL has a unique structure too. Human FCRL4 protein possesses similar EC domain composition (D1-D2-D3-D4), but signaling functions of these two receptors are opposing: FCRL4 is an inhibitory receptor with ITIMs and chicken FCRL is rather activating receptor associating with FcRgamma (25). The diversity of domain architectures in the FcR-related protein suggests linage-specific evolution of this family. Phylogenetic analysis supports this suggestion showing that

Table 1. The FcR- and KIR-related molecules in human, mouse, chicken and *Xenopus tropicalis*

		# of genes	# of exons encoding Ig-like domains	# of EC architectures	Attachment to the cell membrane	Intracellular Tyr-based motifs	EC-TM-IC combinations	Signaling properties
Human	FcR	9	20	2	S, TMc, TMh, GPI	Absent or ITAM or ITIM	6	I, A
	FCRL	8	35	7	S, TMh	ITAM or ITIM or ITAM and ITIM	7	I, A, B
	total	**17**	**55**	**9**			**13**	
	KIR	7-11	18-26	2	TMc, TMh	Absent or ITIM	4	I, A, B
	LILR	13	48	2	S, TMc, TMh	Absent or ITIM	5	I, A
	other	6	10	2	S, TMc, TMh	Absent or ITIM	3	I, A
	total	**>25**	**>74**	**6**			**12**	
Mouse	FcR	5	11	2	TMc, TMh	Absent or ITIM	3	I, A
	FCRL	6	19	5	S, TMh	Absent or ITAM or ITAM and ITIM	5	A, B
	total	**11**	**30**	**7**			**8**	
	KIR	2	6	1	TMh	Absent or ITIM	2	I, A?
	PIR	13	78	1	TMc, TMh	Absent or ITIM	2	I, A
	other	5	9	2	TMc, TMh	Absent or ITIM	3	I, A
	total	**20**	**93**	**3**			**5**	
Chicken	chiFCRL	**1**	**4**	**1**	TMc	Absent	**1**	A
	CHIR	**>70**	**>127**	**2**	TMc, TMh	Absent or ITIM	**6**	I, A, B
Xenopus tropicalis	XFL	**>75**	**>313**	**27**	S, TMh, TMc	Absent or ITIM	**>36**	I, A
	XKL	**4**	**13**	**2**	TMc	Absent or ITAM	**2**	A

EC - extracellular, TM - transmembrane, IC - intracellular, S - secreting, GPI - Glycosylphosphatidylinositol-anchored, TMh - hydrophobic TM, TMc - TM with charged amino acid residue, A - activating, I - Inhibitory, B - Bifunctional. References: (9, 12, 19, 23, 46, 51).

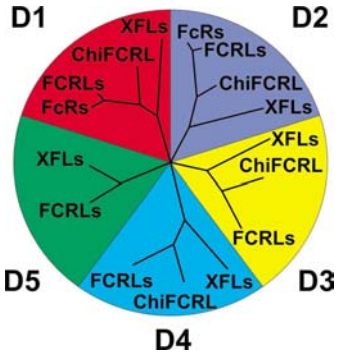

Figure 4. Schematic phylogenetic tree of the mammalian FcR and FCRL, avian chiFCRL and amphibian XFL domains. The tree is based on the NJ trees for mammalian and avian domains (23), for mammalian and amphibian domains (51) and on a complete NJ tree including all known FcR-related domains (our unpublished data). All trees were constructed using MEGA3 software with p-distances for nucleotide sequence sites and pair-wise deletion option.

amphibian and mammalian sequences form monophyletic groups. The trees generated have similar topology for all structural subtypes (D1-D5) of the Ig-like domains shared by mammals, birds and frogs (23, 51) (Figure 4). It should be noted that distances between most divergent Xenopus sequences are comparable with those between amphibian and mammalian sequences (Figure 5). This suggests that the diversification of the amphibian FcR-related molecules started long ago.

2.4.2. KIR-related molecules in *Xenopus*

The *Xenopus tropicalis* genome contains at least four KIR-related genes. BLAST search through scaffolds 1175 and 1418 (assembly version 4.0) resulted in the prediction of three genes encoding three transmembrane receptors we designated XILR1, XIRL2 and XIRL3 (*Xenopus* Immunoglobulin-Like Receptors 1-3). Each has three Ig-like domains in the EC part, a TM and a short cytoplasmic tail lacking ITIMs or ITAMs. Both scaffolds contain additional exons for structurally related Ig-like domains. We suggested that these exons are parts of one more gene designated XILR4. Indeed, we were able to join scaffolds 1175 and 1418 and retrieve a missing part of XILR4, the fourth exon encoding an EC Ig-like domain, by cloning of the corresponding cDNA. XILR4 has organization similar to that of XILR1-3, except for additional membrane-proximal Ig-like domain and two ITAM tandems in the cytoplasmic part (Figure 6). *X. tropicalis* and *X. laevis* EST database analysis are in agreement with our predictions. Although XILR proteins show low level of identity (~40% for XILR2 and XILR3, and ~30% for the rest), they possess a common module - TM region with the NxxR motif. The presence of such TM subtype join XILR receptors with activating XFLs and mammalian KIR-related receptors (figs 1, 2, 3 and 6) and suggests their activating function. This assumption is additionally supported by the presence of ITAMs in the intracellular part of XILR4 and by the fact that each XILR gene has a single exon for the TM region and cytoplasmic tail. The structural data indicates that amphibian KIR-related receptors lack inhibitory forms. For this reason, it is unlikely XILRs may be functionally similar to classical KIRs which are responsible for inhibiting the NK cells ability to lyse MHC class I-deficient cell targets.

Several features allow us to refer XILR genes as true members of KIR family: the greatest similarity of XILR proteins and mammalian KIRs and LILRs (up to 35% identity, Expect value = 1e-12) in BLASTP searches;

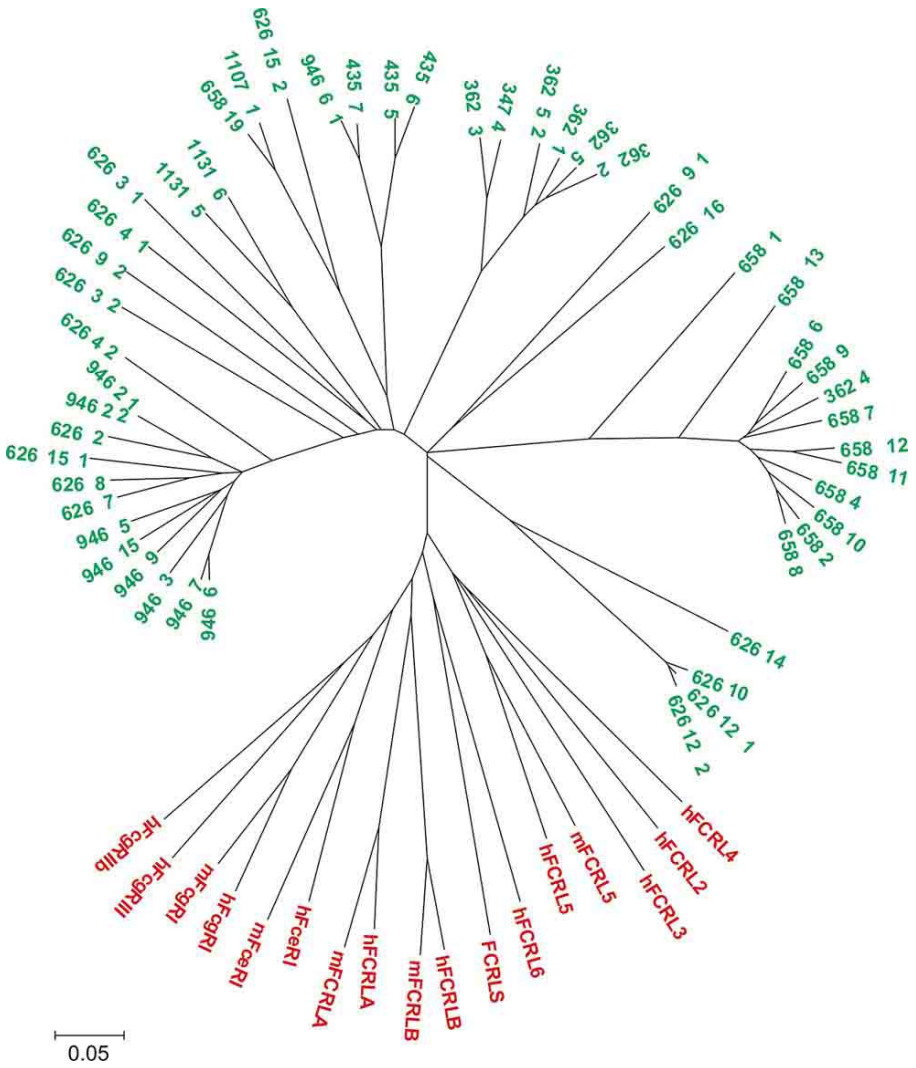

Figure 5. Phylogenetic tree of D2 domains of the mammalian (red color) FcR, FCRL and amphibian (green color) XFL receptors. The tree was constructed using MEGA3 software with p-distances for nucleotide sequence sites and pair-wise deletion option. *Xenopus* sequences designated according to scaffold number, gene number and D2 exon number (53). Thus, 362_5_2 means the second D2 exon of the fifth XFL gene on the scaffold 362 (v. 4.1).

Mammalian KIR, LILR, PIR and singleton KIR family proteins contain from one to six related Ig-like domains. Subdivision of the Ig-like domains in the KIR family is not so evident as in the case of the FcR-related proteins. Some authors discriminate five related domain subtypes (27), others - only two (53). According to the second classification all domains of mammalian receptors fall into two subtypes, named MI and MII. KIR molecules consist of only MII domains, whereas N-terminal domains of LILRs and other KIR-related receptors belong to MI type and the rest of domains to MII type. PIR receptors also contain both types of domains: two domains belong to MI and four to MII type (Figure 2). As mammalian receptors, avian CHIRs have domains of two types: CI and CII. CII domains form independent group, and CI cluster with mammalian MI domains (53). Our phylogenetic analysis shows that amphibian KIR-related domains cluster independently from known KIR-related domains of birds or mammals (Figure 7). Taking into account structural resemblance of the domain sequences from tetrapods, it may be suggested that all of them derived from a single ancestor domain.

2.5. Structural and functional evolution of FcR- and KIR-related molecules

Although all tetrapod species studied so far have FcR- and KIR-related genes, the available data do not allow discriminate orthologous genes in different lineages. Thus, no structural continuity can be tracked to infer the evolution of these receptor families. Both FcR and KIR families expanded significantly in different species (>70 XFL genes in *X. laevis* and CHIR genes in chicken). This expansion was lineage-specific and was accompanied with contraction that results in what appears to be evolutionary related but structurally distinct molecules (i.e. mouse FCRL6 and FcgRII or KIR and LAIR).

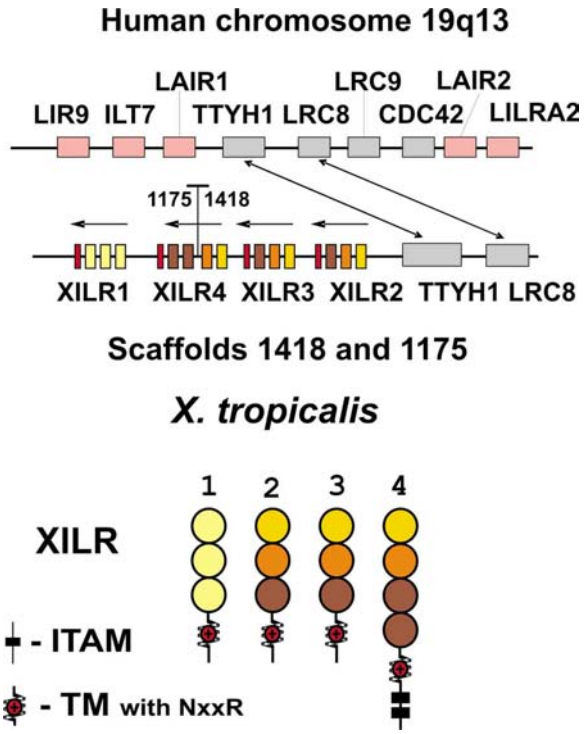

Figure 6. Synteny between human LRC and *X. tropicalis* genomic scaffolds 1175 and 1418 (v. 4.0). The genomic organization of the predicted *X. tropicalis* KIR-related genes (XILRs) and a schematic representation of domain architecture of XILR receptors are shown. Ig-like domains of a particular subtype and the exons encoding these domains are marked by the same color. Exons for TMs with the NxxR motif are designated by red rectangles and TMs themselves - by zigzag line with (+) label. Straight lines and black rectangles designate cytoplasmic tails and ITAM motifs, respectively.

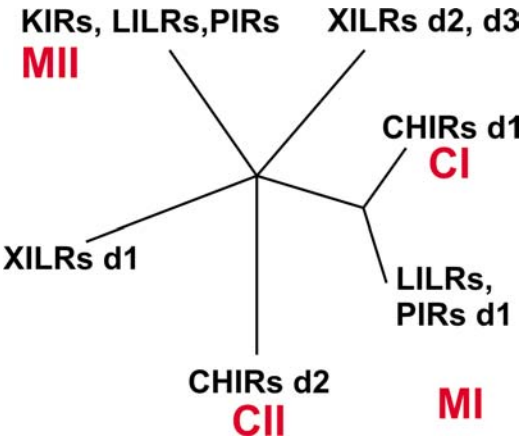

Figure 7. Schematic representation of a phylogenetic tree of the mammalian KIR, LILR, PIR, avian CHIR and amphibian XILR domains. The tree is based on the phylogenetic trees for mammalian and avian domains (53, 63), and on a complete tree including all known KIR-related domains (our unpublished data). The complete tree was constructed using MEGA3 software with p-distances for nucleotide sequence sites and pair-wise deletion option.

the presence of NxxR-containing TMs both in XILR and mammalian KIR-related receptors; and importantly, close linkage of the XILR genes to the TTYH1 and LRC8 gene homologs showing conserved synteny with mammalian LRC (Figure 6).

One fundamental question arising from this analysis is whether there is functional continuity between the mammalian and other vertebrate FcR/KIR-related receptors. For instance, was the Ig/MHC binding function of FcRs/KIRs gained only in mammals, or was it primordial for FcR-/KIR-related receptors? MHC class I molecules are present in all extant jawed vertebrates including sharks and skates. In contrast, IgG and IgE, the ligands of classical FcRs, are mammalian-specific Ig isotypes and have emerged relatively recently. Instead of IgG and IgE, birds and frogs have IgY isotype that is regarded as either the ancestor of IgG and IgE or a modern derivative of the ancient ancestor of IgG, IgE and IgY. In both species IgY-binding receptors have been described. Intriguingly, the recent evidence demonstrates that KIR-related protein (CHIRAB1) is a IgY receptor in chickens (48). This fact together with the absence of structural orthologs of KIRs/LILRs/PIRs and FcRs in birds and amphibians (i. e. the absence of structural continuity) is more consistent with the scenario that the known functions of the mammalian proteins are relatively recent acquisitions. On the other hand, an FcR-related soluble protein able to bind IgM has been described in a teleostean fish *Ictalurus punctatus* (54).

Increasing evidences suggest a common origin for FcR- and KIR-related molecules. Such relationship has been proposed after identification of CHIRs (44) and was supported by structure of teleostean LITRs. Our data also supports a common ancestry of the two families: XFLs, as well as *Xenopus* and mammalian KIR-related molecules retained similar TM module harboring a characteristic NxxR motif and enabling association with the FcRgamma subunit. Assuming a common origin of both receptor families and a species-specific shift in the gene number of either family (Table 1), it is tempting to speculate that the structural discontinuity in FcR or KIR family may be compensated by a functional continuity, i.e. function switching from FcR- to KIR-related receptor and vice-versa. For instance, it cannot be excluded that, in *Xenopus,* a few activating XILRs function as Fc receptors, whereas dozens of activating and inhibitory XFLs interact with polymorphic MHCs. A similar situation can be envisioned in chicken, which displays a very limited MHC repertoire (55) and too many CHIRs to recognize only MHC molecules. Moreover, as mentioned above, it has recently been shown that one of the KIR-related CHIR molecules binds IgY (48).

3. CONCLUSIONS AND PERSPECTIVES

The FcR- and KIR-related molecules have been to date identified in amphibian *Xenopus*, channel catfish, chicken and in all studied mammalian species. The phylogenetic relationships among the molecules from species belonging to different lineages are vague. Although

similar modules were maintained throughout vertebrate evolution, it is apparent that the lineage-specific events have led to the emergence of unique sets of FcR- and KIR-related receptors in each lineage. To obtain more information about for the evolutionary history and the origins of the FcR and KIR families it will be necessary to identify and characterize members of these families in reptiles, various bony fishes and more importantly in cartilaginous fishes.

To compare structural and functional changes in both FcR- and KIR-related families it would be essential to determine the functions of mammalian and avian FCRLs, CHIRs, as well as amphibian XFLs and XILRs. It would be also helpful to identify the structure of Ig and MHC binding proteins in chicken and frog. The study of *Xenopus* XFL and XILR molecules is important in one more respect. Adult frogs express major histocompatibility complex (MHC) class I and class II cell surface molecules, whereas immunocompetent *Xenopus* larvae are naturally MHC class I-deficient (56). During metamorphosis the immune system of tadpole tolerates *de novo* synthesized adult-type antigens. This tolerance was shown to involve regulation rather than deletion process and can be transferred from one frog to another (57). Our data indicate that during metamorphosis the expression patterns of XFL genes change and several genes that are silent at other developmental stages are expressed. Taking in account this differential expression pattern, signaling properties (activating or inhibitory) of XFL receptors and that XFL transcripts are mainly expressed in lymphoid organs, it is possible that at least some of XFL receptors are involved in tolerance induction or/and regulation during the metamorphosis of *Xenopus*. To evaluate this possibility, XFL ligands should be characterized and, if possible, specifically identified or at least we should find out the nature of these ligands. We envisage two main possibilities to explain the existence of so large family of receptors. First XFLs recognize a few highly polymorphic molecules, for example, MHC class II molecules. Second, like certain mammalian members of the KIR family (for instance LILR), XFLs may recognize non-classical MHC class Ib molecules. The *Xenopu*s possesses a large family of the MHC Ib genes (at least 20 genes, (58)). The distinction between these two possibilities will require the development of a comparative functional system. In this regard, *Xenopus* is likely to represent the most suitable model, since its immune system is extensively characterized and many reagents are available. In addition, trangenesis has recently made considerable progress in *Xenopus* by the use phi31 integrase (59), transposase (60, 61), and meganuclease (62). It has therefore become possible to investigate the role of XFLs and XILR by developing knock in (e.g., transgenic frog with an inducible transgene) and knock down (e.g, transgenic frog expressing interfering RNA) strategies.

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5. REFERENCES

1. J. I. Healy and C. C. Goodnow: Positive versus negative signaling by lymphocyte antigen receptors. *Annu Rev Immunol* 16, 645-670 (1998)

2. J. V. Ravetch and L. L. Lanier: Immune inhibitory receptors. *Science* 290 (5489), 84-89 (2000)

3. M. Daeron: Fc receptor biology. *Annu Rev Immunol* 15, 203-234 (1997)

4. F. Nimmerjahn and J. V. Ravetch: Fcgamma receptors: old friends and new family members. *Immunity* 24 (1), 19-28 (2006)

5. K. S. Campbell and M. Colonna: Human natural killer cell receptors and signal transduction. *Int Rev Immunol* 20 (3-4), 333-370 (2001)

6. L. Moretta and A. Moretta: Killer immunoglobulin-like receptors. *Curr Opin Immunol* 16 (5), 626-633 (2004)

7. L. L. Lanier: NK cell recognition. *Annu Rev Immunol* 23, 225-274 (2005)

8. L. J. Maltais, R. C. Lovering, A. V. Taranin, M. Colonna, J. V. Ravetch, R. Dalla-Favera, P. D. Burrows, M. D. Cooper and R. S. Davis: New nomenclature for Fc receptor-like molecules. *Nat Immunol* 7 (5), 431-432 (2006)

9. L. V. Mechetina, A. M. Najakshin, O. Y. Volkova, S. V. Guselnikov, R. Z. Faizulin, B. Y. Alabyev, N. A. Chikaev, M. S. Vinogradova and A. V. Taranin: FCRL, a novel member of the leukocyte Fc receptor family possesses unique structural features. *Eur J Immunol* 32 (1), 87-96 (2002)

10. F. Facchetti, M. Cella, S. Festa, D. H. Fremont and M. Colonna: An unusual Fc receptor-related protein expressed in human centroblasts. *Proc Natl Acad Sci U S A* 99 (6), 3776-3781 (2002)

11. R. S. Davis, H. Li, C. C. Chen, Y. H. Wan, M. D. Cooper and P. D. Burrows: Definition of an Fc receptor-related gene (FcRX) expressed in human and mouse B cells. *Int Immunol* 14 (9), 1075-1083 (2002)

12. N. A. Chikaev, E. A. Bykova, A. M. Najakshin, L. V. Mechetina, O. Y. Volkova, M. M. Peklo, A. Y. Shevelev, T. N. Vlasik, A. Roesch, T. Vogt and A. V. Taranin: Cloning and characterization of the human FCRL2 gene. *Genomics* 85 (2), 264-272 (2005)

13. T. J. Wilson and M. Colonna: A new Fc receptor homolog, FREB2, found in germinal center B cells. *Genes Immun* 6 (4), 341-346 (2005)

14. K. Masuda, R. S. Davis, T. Maruyama, J. Zhang, T. He, M. D. Cooper, J. O-Wang and P. D. Burrows: FcRY, an Fc receptor related gene differentially expressed during B

lymphocyte development and activation. *Gene* 363, 32-40 (2005)

15. G. Hatzivassiliou, I. Miller, J. Takizawa, N. Palanisamy, P. H. Rao, S. Iida, S. Tagawa, M. Taniwaki, J. Russo, A. Neri, G. Cattoretti, R. Clynes, C. Mendelsohn, R. S. Chaganti and R. Dalla-Favera: IRTA1 and IRTA2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy. *Immunity* 14 (3), 277-289 (2004)

16. M. J. Xu, R. Zhao and Z. J. Zhao: Molecular cloning and characterization of SPAP1, an inhibitory receptor. *Biochem Biophys Res Commun* 280 (3), 768-775 (2001)

17. R. S. Davis, Y. H. Wang, H. Kubagawa and M. D. Cooper: Identification of a family of Fc receptor homologs with preferential B cell expression. *Proc Natl Acad Sci U S A* 98 (17), 9772-9777 (2001)

18. Y. Nakayama, S. M. Weissman and A. L. Bothwell: BXMAS1 identifies a cluster of homologous genes differentially expressed in B cells. *Biochem Biophys Res Commun* 285 (3), 830-837 (2001)

19. S. V. Guselnikov, S. A. Ershova, L. V. Mechetina, A. M. Najakshin, O. Y. Volkova, B. Y. Alabyev and A. V. Taranin: A family of highly diverse human and mouse genes structurally links leukocyte FcR, gp42 and PECAM-1. *Immunogenetics* 54 (2), 87-95 (2002)

20. M. J. Xu, R. Zhao, H. Cao and Z. J. Zhao: SPAP2, an Ig family receptor containing both ITIMs and ITAMs. *Biochem Biophys Res Commun* 293 (3), 1037-1046 (2002)

21. G. R. Ehrhardt, J. T. Hsu, L. Gartland, C. M. Leu, S. Zhang, R. S. Davis and M. D. Cooper: Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. *J Exp Med* 202 (6), 783-791 (2005)

22. C. M. Leu, R. S. Davis, L. A. Gartland, W. D. Fine and M. D. Cooper: FcRH1: an activation coreceptor on human B cells. *Blood* 105 (3), 1121-1126 (2005)

23. S. A. Fayngerts, A. M. Najakshin and A. V. Taranin: Species-specific evolution of the FcR family in endothermic vertebrates. *Immunogenetics* 59 (6), 493-506 (2007)

24. R. S. Davis: Fc receptor-like molecules. *Annu Rev Immunol* 25, 525-560 (2007)

25. A. I. Taylor, H. J. Gould, B. J. Sutton and R. A. Calvert: The first avian Ig-like Fc receptor family member combines features of mammalian FcR and FCRL. *Immunogenetics* 59 (4), 323-328 (2007)

26. J. Trowsdale, R. Barten, A. Haude, C. A. Stewart, S. Beck and M. J. Wilson: The genomic context of natural killer receptor extended gene families. *Immunol Rev* 181, 20-38 (2001)

27. A. M. Martin, J. K. Kulski, C. Witt, P. Pontarotti and F. T. Christiansen: Leukocyte Ig-like receptor complex (LRC) in mice and men. *Trends Immunol* 23 (2), 81-88 (2002)

28. J. Kelley, L. Walter and J. Trowsdale: Comparative genomics of natural killer cell receptor gene clusters. *PLoS Genet* 1 (2), 129-139 (2005)

29. H. C. Morton, I. E. van den Herik-Oudijk, P. Vossebeld, A. Snijders, A. J. Verhoeven, P. J. Capel and J. G. van de Winkel: Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR gamma chain. Molecular basis for CD89/FcR gamma chain association. *J Biol Chem* 270 (50), 29781-29787 (1995)

30. L. S. Taylor and D. W. McVicar: Functional association of FcepsilonRIgamma with arginine (632) of paired immunoglobulin-like receptor (PIR)-A3 in murine macrophages. *Blood* 94 (5), 1790-1796 (1999)

31. H. Nakajima, J. Samaridis, L. Angman and M. Colonna: Human myeloid cells express an activating ILT receptor (ILT1) that associates with Fc receptor gamma-chain. *J Immunol* 162 (1), 5-8 (1999)

32. O. Berlanga, D. Tulasne, T. Bori, D. C. Snell, Y. Miura, S. Jung, M. Moroi, J. Frampton and S. P. Watson: The Fc receptor gamma-chain is necessary and sufficient to initiate signalling through glycoprotein VI in transfected cells by the snake C-type lectin, convulxin. *Eur J Biochem* 269 (12), 2951-2960 (2002)

33. E. Merck, C. Gaillard, D. M. Gorman, F. Montero-Julian, I. Durand, S. M. Zurawski, C. Menetrier-Caux, G. Carra, S. Lebecque, G. Trinchieri and E. E. Bates: OSCAR is an FcRgamma-associated receptor that is expressed by myeloid cells and is involved in antigen presentation and activation of human dendritic cells. *Blood* 104 (5), 1386-1395 (2004)

34. J. S. Manavalan, P. C. Rossi, G. Vlad, F. Piazza, A. Yarilina, R. Cortesini, D. Mancini and N. Suciu-Foca: High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. *Transpl Immunol* 11 (3-4), 245-258 (2003)

35. D. Brown, J. Trowsdale and R. Allen: The LILR family: modulators of innate and adaptive immune pathways in health and disease. *Tissue Antigens* 64 (3), 215-225 (2004)

36. B. D. Wines, M. D. Hulett, G. P. Jamieson, H. M. Trist, J. M. Spratt and P. M. Hogarth: Identification of residues in the first domain of human Fc alpha receptor essential for interaction with IgA. *J Immunol* 162 (4), 2146-2153 (1999)

37. R. J. Lebbink, T. de Ruiter, J. Adelmeijer, A. B. Brenkman, J. M. van Helvoort, M. Koch, R. W. Farndale, T. Lisman, A. Sonnenberg, P. J. Lenting and L. Meyaard: Collagens are functional, high affinity ligands for

the inhibitory immune receptor LAIR-1. *J Exp Med* 203 (6), 1419-1425 (2006)

38. J. M. Clemetson, J. Polgar, E. Magnenat, T. N. Wells and K. J. Clemetson KJ: The platelet collagen receptor glycoprotein VI is a member of the immunoglobulin superfamily closely related to FcalphaR and the natural killer receptors. *J Biol Chem* 274 (41), 29019-29024 (1999)

39. W. M. Yokoyama and B. F. Plougastel: Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol* 3 (4), 304-316 (2003)

40. S. E. Hoelsbrekken, O. Nylenna, P. C. Saether, I. O. Slettedal, J. C. Ryan, S. Fossum and E. Dissen: Cutting edge: molecular cloning of a killer cell Ig-like receptor in the mouse and rat. *J Immunol* 170, 2259-2263 (2003)

41. A. Nakamura, E. Kobayashi and T. Takai: Exacerbated graft-versus-host disease in Pirb-/- mice. *Nat Immunol* 5 (6), 623-629 (2004)

42. M. Nakayama, D. M. Underhill, T. W. Petersen, B. Li, T. Kitamura, T. Takai and A. Aderem: Paired Ig-like receptors bind to bacteria and shape TLR-mediated cytokine production. *J Immunol* 178 (7), 4250-4259 (2007)

43. A. Masuda, A. Nakamura, T. Maeda, Y. Sakamoto and T. Takai: Cis binding between inhibitory receptors and MHC class I can regulate mast cell activation. *J Exp Med* 204 (4), 907-920 (2007)

44. G. Dennis Jr., H. Kubagawa and M. D. Cooper: Paired Ig-like receptor homologs in birds and mammals share a common ancestor with mammalian Fc receptors. *Proc Natl Acad Sci U S A* 97 (24), 13245-13250 (2000)

45. B. C. Viertlboeck, F. A. Habermann, R. Schmitt, M. A. Groenen, L. Du Pasquier and T. W. Gobel: The chicken leukocyte receptor complex: a highly diverse multigene family encoding at least six structurally distinct receptor types. *J Immunol* 175 (1), 385-393 (2005)

46. N. Nikolaidis, I. Makalowska, D. Chalkia, W. Makalowski , J. Klein and M. Nei: Origin and evolution of the chicken leukocyte receptor complex. *Proc Natl Acad Sci U S A* 102 (11), 4057-4062 (2005)

47. K. Laun, P. Coggill, S. Palmer, S. Sims, Z. Ning, J. Ragoussis, E. Volpi, N. Wilson, S. Beck, A. Ziegler and A. Volz: The leukocyte receptor complex in chicken is characterized by massive expansion and diversification of immunoglobulin-like Loci. *PLoS Genet* 2 (5), e73 (2006)

48. B. C. Viertlboeck, S. Schweinsberg, M. A. Hanczaruk, R. Schmitt, L. Du Pasquier, F. W. Herberg and T. W. Gobel: The chicken leukocyte receptor complex encodes a primordial, activating, high-affinity IgY Fc receptor. *Proc Natl Acad Sci U S A* 104 (28), 11718-11723 (2007)

49. J. L. Stafford, E. Bengten, L. Du Pasquier, R. D. McIntosh, S. M. Quiniou, L. W. Clem, N. W. Miller and M. Wilson: A novel family of diversified immunoregulatory receptors in teleosts is homologous to both mammalian Fc receptors and molecules encoded within the leukocyte receptor complex. *Immunogenetics* 58 (9), 758-773 (2006)

50. J. L. Stafford, E. Bengten, L. Du Pasquier, N. W. Miller and M. Wilson: Channel catfish leukocyte immune-type receptors contain a putative MHC class I binding site. *Immunogenetics* 59 (1), 77-91 (2007)

51. S. V. Guselnikov, T. Ramanayake, A. Y. Erilova, L. V. Mechetina, A. Najakshin, J. Robert and A. V. Taranin: The Xenopus FcR-family demonstrates continually high diversification of paired receptors in vertebrate evolution. *Manuscript submitted to BMC Evol Biol*

52. M. K. Kim, Z. Y. Huang, P. H. Hwang, B. A. Jones, N. Sato, S. Hunter, T. H. Kim-Han, R. G. Worth, Z. K. Indik and A. D. Schreiber: Fcgamma receptor transmembrane domains: role in cell surface expression, gamma chain interaction, and phagocytosis. *Blood* 101 (11), 4479-4484 (2003)

53. N. Nikolaidis, J. Klein and M. Nei: Origin and evolution of the Ig-like domains present in mammalian leukocyte receptors: insights from chicken, frog, and fish homologues. *Immunogenetics* 57 (1-2), 151-157 (2005)

54. J. L. Stafford, M. Wilson, D. Nayak, S. M. Quiniou, L. W. Clem, N. W. Miller and E. Bengten: Identification and characterization of a Fc-receptor homolog in an ectothermic vertebrate, the channel catfish (Ictalurus punctatus). *J Immunol* 177 (4), 2505–2517 (2006)

55. J. Kaufman, S. Milne, T. W. Gobel, B. A. Walker, J. P. Jacob, C. Auffray, R. Zoorob and S. Beck: The chicken B locus is a minimal essential major histocompatibility complex. *Nature* 401 (6756), 923-925 (1999)

56. L. Du Pasquier, J. Schwager and M. F. Flajnik: The immune system of Xenopus. *Annu Rev Immunol* 7, 251-275 (1989)

57. T. Nakamura, M. Maeno, S. Tochinai and C. Katagiri: Tolerance induced by grafting semi-allogeneic adult skin to larval Xenopus laevis: possible involvement of specific suppressor cell activity. *Differentiation* 35 (2), 108-114 (1987)

58. M. F. Flajnik, M. Kasahara, B. P. Shum, L. Salter-Cid, E. Taylor and L. Du Pasquier: A novel type of class I gene organization in vertebrates: a large family of non-MHC-linked class I genes is expressed at the RNA level in the amphibian Xenopus. *EMBO J* 12 (11), 4385-4396 (1993)

59. B. G. Allen and D. L. Weeks: Transgenic Xenopus laevis embryos can be generated using phiC31 integrase. *Nat Methods* 2 (12), 975-979 (2005)

60. T. Shibano, M. Takeda, I. Suetake, K. Kawakami, M. Asashima, S. Tajima and M. Taira: Recombinant Tol2 transposase with activity in Xenopus embryos. *FEBS Lett* 581 (22), 4333-4336 (2007)

61. L. Sinzelle, J. Vallin, L. Coen, A. Chesneau, D. Du Pasquier, N. Pollet, B. Demeneix and A. Mazabraud: Generation of trangenic Xenopus laevis using the Sleeping Beauty transposon system. *Transgenic Res* 15 (6), 751-760 (2006)

62. H. Ogino, W. B. McConnell and R. M. Grainger: High-throughput transgenesis in Xenopus using I-SceI meganuclease. *Nat Protoc* 1 (4), 1703-1710 (2006)

63. A. L. Hughes: Evolution of the human killer cell inhibitory receptor family. *Mol Phylogenet Evol* 25 (2), 330-340 (2002)

Abbreviations: TM - transmembrane, Ig - Immunoglobulin, EC - Extracellular, ITIM - Immunoreceptor Tyrosine-based Inhibitory Motif, ITAM - Immunoreceptor Tyrosine-based Activating Motif, FcR - classical leukocyte Fc-recepor, KIR - Killer cell Immunoglobulin-like Receptor, MHC - Major Histocompatibility Complex, FCRL - FcR-like, LILR - Leukocyte Immunoglobulin-like Receptor, LAIR - Leukocyte-Associated Immunoglobulin-like Receptor, OSCAR - Osteoclast Associated, Immunoglobulin-like Receptor, LRC - Leukocyte Receptor Complex, PIR - Paired Immunoglobulin-like Receptor, CHIR - Chicken Immunoglobulin-like Receptor, LITR - Leukocyte Immune-Type Receptors, XFL - Xenopus FcR-like, EST - Expressed Sequence Tag, BLAST - Basic Local Alignment Search Tool, XILR - *Xenopus* Immunoglobulin-like Receptor

Key Words: FcR, KIR, evolution, *Xenopus*, Leukocyte, Ig, Immunoglobulin, Review

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