

# Porcine IgG: structure, genetics, and evolution

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**Abstract** Eleven genomic porcine C $\gamma$  gene sequences are described that represent six putative subclasses that appear to have originated by gene duplication and exon shuffle. The genes previously described as encoding porcine IgG1 and IgG3 were shown to be the IgG1<sup>a</sup> and IgG1<sup>b</sup> allelic variants of the IGHG1 gene, IgG2a and IgG2b are allelic variants of the IGHG2 gene, while “new” IgG3 is monomorphic, has an extended hinge, is structurally unique, and appears to encode the most evolutionarily conserved porcine IgG. IgG5<sup>b</sup> differs most from its putative allele, and its C<sub>H1</sub> domain shares sequence homology with the C<sub>H1</sub> of IgG3. Four animals were identified that lacked either IgG4 or IgG6. Alternative splice variants were also recovered, some lacking the C<sub>H1</sub> domain and potentially encoding heavy chain only antibodies. Potentially, swine can transcribe >20 different C $\gamma$  chains. A comparison of

mammalian C $\gamma$  gene sequences revealed that IgG diversified into subclasses after speciation. Thus, the effector functions for the IgG subclasses of each species should not be extrapolated from “same name subclasses” in other species. Sequence analysis identified motifs likely to interact with Fc $\gamma$  receptors, FcRn, protein A, protein G, and C1q. These revealed IgG3 to be most likely to activate complement and bind Fc $\gamma$ Rs. All except IgG5<sup>a</sup> and IgG6<sup>a</sup> should bind to Fc $\gamma$ Rs, while all except IgG6<sup>a</sup> and the putative IgG5 subclass proteins should bind well to porcine FcRn, protein A, and protein G.

**Keywords** IgG subclasses · Evolution · Genetics · Fc receptors · Comparative immunology

## Introduction

IgG can be considered the “flagship” antibody of mammals, since it is unique to this vertebrate class. Its origin is obscure although amphibian IgF is a candidate, since it is phylogenetically the first Ig with a hinge exon like IgG (Zhao et al. 2006). Mammalian IgG is characterized by a diversity of subclasses ranging from 1 to 7 (Butler 1997, 2006) and a range of functions mediated through Fc receptors (Ravetch and Kinet 1991). Subisotype diversification is not restricted to IgG in mammals; a similar process may explain the 13 subclasses of IgA in rabbits (Burnett et al. 1989) and the two subclasses of IgA in humans (Mestecky et al. 2004). Different subclasses possess different biological characteristics. Human IgG1 (IGHG1) and IgG3 (IGHG3) decorate phagocytic cells because of their high affinity for Fc $\gamma$ R1 (Ravetch and Kinet 1991). Therefore, IgG1 and IgG3 are especially important in: (a) the removal of small IgG–Ag complexes and (b) positively

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or negatively stimulating B cell development and antibody production. Human IgG3 is a long-hinged antibody and a potent activator of complement (Lefranc and Lefranc 2001). IgG1 comprises two thirds of total human serum IgG, whereas IgG3 and IgG4 together comprise only about 10%. As in humans, there are differences in relative IgG subclass concentrations in the serum of mice and in the affinity of various mouse IgG subclasses for Fc $\gamma$ Rs (Ravetch and Kinet 1991). In cattle, IgG1 is the predominant IgG in all exocrine fluids and, because it is preferentially transported across the acinar epithelial cells of the mammary glands, comprises 90% of the IgG in colostrum but only 50% of serum IgG (Butler 1969, 1974). Physiological differences are also seen among IgA subclasses; human IgA1 (IGHA1) is relatively more prevalent in blood than secretions (Mestecky et al. 2004), but IgA1 is more susceptible to bacterial IgA proteases than IgA2 (Plaut et al. 1975; Kilian and Russell 2004; IGHA2). The 13 rabbit IgA subclasses also differ in their distribution among tissue (Spieker-Polet et al. 1993) and porcine IgG3 comprises >60% of IgG transcripts in the ileal Peyer's patches (IPP) and mesenteric lymph nodes of fetal piglets (Butler and Wertz 2006).

The studies described were undertaken because of the emerging role of swine in understanding the: (a) role of commensal flora in driving development of adaptive immunity (Butler et al. 2002, 2005), (b) immune response to influenza which infects both humans and pigs (Vincent et al. 2006), (c) action of immunoregulatory viruses on the development of immune homeostasis (Lemke et al. 2004; Butler et al. 2007, 2008a), and (d) causes of cystic fibrosis in a CFTR knockout piglet model (Rogers et al. 2008). Swine are also important in xenotransplantation (Auchincloss and Sachs 1998) and in generating humanized antibodies (Waltz 2006; Butler et al. 2008a; Mendicino et al. 2008). Unlike the major ruminant and guinea pig IgG subclass proteins, those from swine cannot be purified biochemically (Butler 1969, 1983), and there are no naturally occurring or induced plasmacytomas. However, C $\gamma$  diversity in swine was suggested from ion exchange and immunoelectrophoretic (Metzger and Fougereau 1967; Kaltreider and Johnson 1972) differential monoclonal antibody specificity (Bianchi et al. 1990) allotypic studies (Rapacz and Hasler-Rapacz 1982) and from cDNA sequences and genomic blots (Kacskovics et al. 1994) although the extent of diversity was unknown. Here we report the sequences of 11 porcine genomic C $\gamma$  sequences that appear to comprise six subclasses. A comparison with IgG sequences from various mammals agrees with the early prediction that subclass evolution occurred after major mammalian speciation (Kehoe and Capra 1974). Because of this, subclass function for "same name subclasses" cannot be extrapolated among mammals. Therefore, we analyzed the porcine C $\gamma$  gene sequences for binding motifs for C1q, Fc $\gamma$ R, FcRn, as well

as protein A (SpA) and G (SpG). Further characterization of the porcine IgG complex and confirmation of our predictions will require gene mapping, designed mating studies, *in vitro* expression of purified subclass proteins, and preparation of subclass-specific mAbs.

## Materials and methods

**Source of tissue** Animal tissues were by-products of studies on gut colonization in isolator piglet (Butler et al. 2000, 2002, 2005) studies on the effect of neonatal viral infection (Lemke et al. 2004; Butler et al. 2007, 2008a) and studies on IgA allotype distribution (Navarro et al. 2000a). Solid tissues were immediately frozen in liquid nitrogen, and blood samples were collected in EDTA and processed for the recovery of PBLs as described previously (Lemke et al. 2004; Kacskovics et al. 1994; Butler et al. 2000; Navarro et al. 2000b). Additional DNA samples from NIH minipigs were kindly provided by Dr. Joan Lunney, ARS, Beltsville, MD, USA from a variety of swine of different breeds and species by Dr. Max Rothschild, Department of Animal Science, Iowa State University, Ames, IA, USA and from purebred Meishan pigs (Dr. Larry Shook, University of Illinois; Champaign-Urbana, IL, USA).

**Preparation of DNA, RNA, and cDNA** DNA, total RNA, and first strain cDNA were prepared as previously described (McAleer et al. 2005; Butler et al. 2001; Sun and Butler 1996) except that a primer for the 3' UTR shared by all known C $\gamma$  genes and an antisense C $\alpha$ 3 primer (Navarro et al. 2000b), as well as random hexamers, were added (Table 1).

**Identification of C $\gamma$  diversity among transcripts** C $\gamma$ -containing gene products were recovered using a pan-specific C $H$ 1–C $H$ 3 primer set common to all known swine IgG genes (Table 1; Fig. 1). The product was evaluated by electrophoresis in 1.5% agarose gel, the appropriate-sized C $\gamma$ -containing polynucleotide band removed and used as the target for a second round polymerase chain reaction (PCR) that utilized a heminested primer set containing an antisense primer that recognized a conserved sequence in the C $H$ 2 domain (Fig. 1; Table 1). The product of the second round PCR was cloned into PCR TOPO 2.1, grown in TOP TEN cells overnight at 37°C and plated on Luria–Bertani agar containing 50  $\mu$ g/ml kanamycin (LB–Kan). The KAN-resistant clones were transferred to wells of round bottom 96-well microtiter plates (Costar 3799, Corning, NY, USA) and grown overnight at 37°C in LB–Kan. Plasmid DNA, harvested by alkaline lysis and centrifugation, was transferred and cross-linked to nylon membranes (Schleicher & Schull, Keene, NH) as previous-

**Table 1** PCR primers and oligonucleotide probes

cDNA					
Antisense 3' UTR (ttatttcacgtgcccggg)					
Antisense C <sub>α</sub> 3 (agccatgcaccagcacat)					
Routine recovery of C <sub>γ</sub> genes from cDNA					
First round PCR					
C <sub>H1</sub> sense (ggtgacaagcgtgt) and antisense C <sub>H3</sub> (tctgggtgtagtggtgtgca)					
Second round PCR					
C <sub>H1</sub> sense and antisense C <sub>H2</sub> (ccgtccacgtaccaggagaa)					
Recovery of 5' C <sub>H1</sub> from cDNA					
First round PCR					
V <sub>H</sub> gene FR1 5'(gaggagaagctggtggagt) and anti-sense C <sub>H2</sub>					
Second round PCR					
V <sub>H</sub> gene FR1 3'(tctctgtgttgctctgg) and anti-sense C <sub>H2</sub>					
Recovery of 3' C <sub>H3</sub> from DNA					
C <sub>H1</sub> sense and antisense 3' UTR					
C <sub>H1</sub> sense and antisense C <sub>H3</sub>					
C <sub>γ</sub> gene hybridization probes					
C <sub>γ</sub> genes detected					
1a	tacaccagccgcaaa	IgG1 <sup>a</sup>	IgG1 <sup>b</sup>	IgG5 <sup>a</sup>	IgG5 <sup>b</sup>
1b	gctgtgaagtggcc	IgG1 <sup>b</sup>	IgG4 <sup>b</sup>		
2a	cctgtgaatcaccag	IgG2 <sup>a</sup>			
2b	cctgtgaatcgccat	IgG2 <sup>b</sup>			
3	acatgaacccccaca		IgG3		
4	gcctgtgaagggccc	IgG4 <sup>a</sup>	IgG4 <sup>b</sup>		
5a	ggacgacctgtccca	IgG5 <sup>a</sup>	IgG6 <sup>a</sup>	IgG6 <sup>b</sup>	
5b	agcctcgatgtccca	IgG5 <sup>b</sup>			
Pan	ttccctcaaaaccaag				All
Probe combinations required to identify individual C <sub>γ</sub> genes					
IgG1 <sup>a</sup>	1a				
IgG1 <sup>b</sup>	1a, 1b				
IgG2 <sup>a</sup>	2a				
IgG2 <sup>b</sup>	2b				
IgG3	3				
IgG4 <sup>a</sup>	4				
IgG4 <sup>b</sup>	4, 1b				
IgG5 <sup>a</sup>	5a, 1a				
IgG5 <sup>b</sup>	5b				
IgG6 <sup>a</sup>	4, 5a				
IgG6 <sup>b</sup>	5a				
Primers for allotyping IgA transcripts					
C <sub>H1</sub> (gtgaacgtccctgcaa) and anti C <sub>H2</sub> (caggacgaggtctgcaa)					

ly described (McAlear et al. 2005; Butler et al. 2001; Sun and Butler 1996; Sun et al. 1998). Membranes were hybridized with C<sub>γ</sub> probes specific for the originally described C<sub>γ</sub> genes (Kacskovics et al. 1994) and a pan-specific C<sub>γ</sub> probe (Butler and Wertz 2006; Table 1). cDNA clones that hybridized with the pan-specific probe but with none of the original subclass-specific probes, were grown out and sequenced. This process led to the discovery of six additional C<sub>γ</sub> sequences which necessitated that additional C<sub>γ</sub> probes be prepared (Table 1).

**Characterization of genomic C<sub>γ</sub> genes** The same primer set used for first round PCR on cDNA was used to generate

near full-length genomic C<sub>γ</sub> genes, ca. 1 kb. The genomic PCR product was then cloned and sequenced to allow coding and intron sequences to be obtained (Fig. 1). Sequence analysis identified 11 genomic C<sub>γ</sub> gene sequences, the exons of which corresponded to those recovered from cDNA. The extreme 5' portion of the C<sub>H1</sub> domain (78 nt) was obtained from cDNA sequences recovered using a V<sub>H</sub> gene FR 1 primer and an antisense C<sub>H2</sub> primer (Table 1). The extreme 3' region of C<sub>H3</sub> was recovered from DNA using the common C<sub>H1</sub> sense and an anti-sense 3' UTR primer set (Table 1).

**Identification of allotypic variants** Evidence suggesting the existence of allotypic variants was obtained in expression studies in which certain animals were found to lack clones hybridizing with the gene originally described as IgG1 by Kacskovics et al. (1994). This initiated a series of genomic dot-blots using gene- and pan-specific probes to test for the allelic nature of the various C<sub>γ</sub> genes recovered. Dot blots were further verified by restriction fragment length analysis (RFLP) followed by Southern blots using appropriate probes. This form of analysis was then extended to other putative allelic variants.

**Identification and frequency of alternatively spliced IgG transcripts** Sequence analysis of 184 transcripts revealed various alternatively spliced transcripts. While splice variants were seen for all C<sub>γ</sub> genes, they were especially prevalent in IgG5<sup>b</sup>. To estimate the frequency of occurrence in IgG5<sup>b</sup>, we sequenced 25 randomly selected IgG5<sup>b</sup> clones recovered from four different pigs.

**Allotyping of porcine IgA** The IgA<sup>b</sup> allotype of porcine IgA differs from IgA<sup>a</sup> by a 12-nt deletion in the hinge region due to mutation of the splice acceptor site (Brown et al. 1995). This allows for the development of a typing system based on IgA transcripts as previously described by Navarro et al. (2000b). In the case of genomic DNA, the presence or absence of a Dde I site also allows the allotypes to be distinguished by RFLP. In the case of animals for which only DNA was available, this method was used (Navarro et al. 2000a).

**Immunoglobulin nomenclature** The original and familiar system used for immunoglobulins (e.g., IgM) immunoglobulin subclass (e.g., IgG1) or allotypic variants (e.g., IgG1<sup>a</sup>) is well established in the literature including the one for swine. In addition to this nomenclature, we also designated the C<sub>γ</sub> genes according to the IMGT system (Lefranc and Lefranc 2001; Table 2).

**Sequence analysis** Genomic and cDNA clones were grown up overnight, and restriction digested to confirm the insert

size. All insert-containing clones with appropriate-sized inserts were sequenced in the DNA Core Facility of the University of Iowa using the four-color ABI PRISM DNA Analyzer (Applied Biosystems). Amino acid sequences were deduced and analyzed in the Omega program (Accelrys) and added to Fig. 1.

**Cladogram and dendrogram construction** Alignments were calculated with CLUSTAL X (version 1.8) using the GONNET series protein matrices. The gap opening penalties for the pairwise and the multiple alignments were set to 10.00. The gap extension penalties were set to 0.1 and 0.2 for the pairwise and multiple alignments, respectively. From this alignment, rooted phylogenetic trees were constructed by using the neighbor-joining (NJ) method of MEGA 4.0 and Poisson distance correction (gaps ignored). Bootstrapping of the NJ tree was performed with 1,000 replicates (Tamura et al. 2007). The sequence data for species C $\gamma$  genes are available under the following accession numbers: Bovine C $\gamma$ 1=X16701; bovine C $\gamma$ 2=X16702; bovine C $\gamma$ 3=U63638; canine C $\gamma$ 1=AF354264; canine C $\gamma$ 2=AF354265; canine C $\gamma$ 3=AF354266; canine C $\gamma$ 4=AF 354267; Equine C $\gamma$ 1; 2; 3; 4; 5; 6 and 7=AJ1302055; AJ1302056; AJ1312379; AJ1302057; AJ1312380; AJ1312381; AJ1302058, respectively. Human C $\gamma$ 1; 2; 3 and 4=J00228; J00230; X03604; K01316; respectively. Mouse C $\gamma$ 1; 2a; 3; 2c and 2b=J00453; V00825; X00915; J00479; V00763; respectively. Sheep C $\gamma$ 1=X69797; Sheep C $\gamma$ 2=X70983; Rabbit C $\gamma$ =L29172; Camel 1a=AJ421266; Camelid 1b=unpublished; Opossum=AF035195; Possum=AF157619.

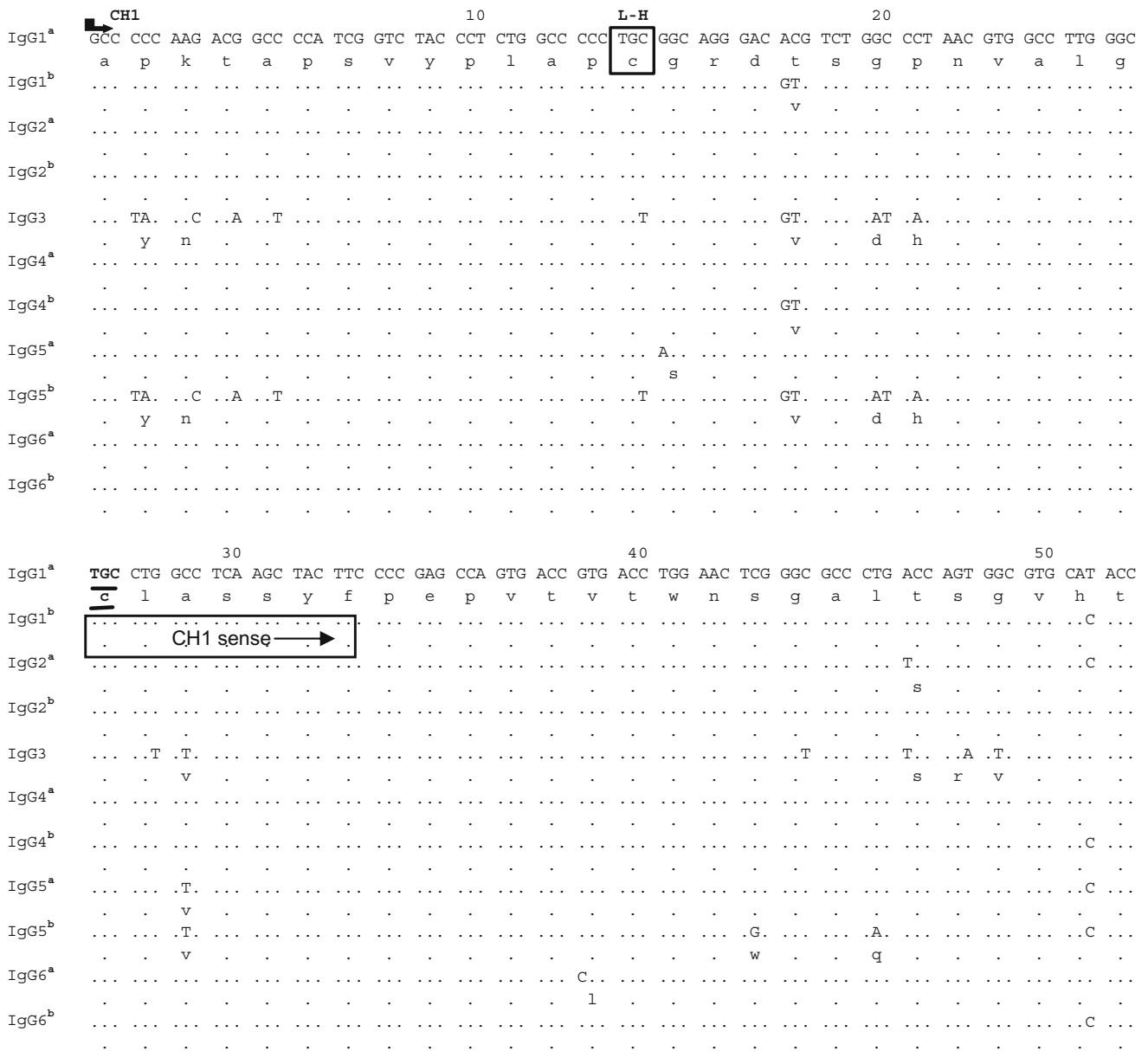
**Binding motifs for C1q, Fc $\gamma$ Rs, FcRn, SpA, and SpG** The analysis of potential interaction sites with C1q was based on the interaction of human IgG1 and the B subunit of the human C1q (Gaboriaud et al. 2003) and the sequence of the B subunit of porcine C1q (GenBank accession code: AY349420) and the mouse orthologue (NP\_033907). Human Fc $\gamma$ Rs (GenBank accession codes: P12314, AAD00641, and AAH33678, respectively) were compared to the sequences of porcine Fc $\gamma$ RI, Fc $\gamma$ RIIB, and Fc $\gamma$ RIII (GenBank accession codes: AAZ20792; NP\_001028185 and Q28942, respectively). Putative Fc $\gamma$ R interaction motifs in porcine C $\gamma$  sequences were identified according to those present in human IgG (Radaev et al. 2001). IgG-FcRn interactions were analyzed using the model described for rat IgG2a and the rat FcRn crystal structure (Martin et al. 2001). The comparison of porcine FcRn to its orthologues has been previously published (Kacskovics et al. 2006). Motifs identified in the porcine C $\gamma$  sequences predicted to bind SpA and SpG were evaluated according to previously published models (Deisenhofer 1981; Sauer-Eriksson et al. 1995).

## Results

**Genomic sequences of porcine C $\gamma$  genes group into six pairs** The complete genomic sequences of the porcine C $\gamma$  genes recovered from DNA (Fig. 1) segregate into six groups (Fig. 2) of which allelic variants were identified for five (see below). Data for Fig. 2 are based on 88 cloned genomic sequences (Table 2).

**The allelic nature and occurrence of porcine C $\gamma$  genes** The examination of >9,000 cDNA clones from outbred animals revealed that IgG1 (now called IgG1<sup>b</sup>) and “old” IgG3 (Kacskovics et al. 1994; now called IgG1<sup>a</sup>) were the most prominently expressed C $\gamma$  genes in all newborn piglets and were expressed in statistically equal amounts as early as 40 days of gestation (Table 2; Butler and Wertz 2006). Since fetal piglet no. 5 and adult pig 8266 failed to express the IgG1 described by Kacskovics et al. (1994; now designated IgG1<sup>b</sup>), we examined their DNA. Neither animal possessed a C $\gamma$  gene that hybridized with a probe specific for the IgG1 of Kacskovics et al. (1994; now designated IgG1<sup>b</sup>) while hybridizing with the probe for the gene designated as IgG3 by Kacskovics et al. (1994; now designated IgG1<sup>a</sup>; Table 2; Fig. 3b,c). Furthermore, DNA from piglet no. 5 digested with Pci I did not yield the products of 560 and 291 nt predicted from the sequence of IgG1<sup>b</sup> (Fig. 3a). The digested DNA of piglet no. 5 did not hybridize with the IgG1<sup>b</sup> probe (Fig. 3b). This suggested that piglet no. 5 and swine 8266 were either deficient in the gene encoding IgG1 or that the original IgG1 and IgG3 were allelic variants or that piglet nos. 5 and 8266 were homozygous for IgG1<sup>a</sup>. Previous immunogenetic studies on IgA had shown that all of >40 NIH minipigs tested were homozygous for IgA<sup>a</sup> (Navarro et al. 2000a). Since the IGHC genes in other species are closely linked, we hypothesized that IGHG and IGHA genes of these minipigs were part of a common haplotype. When minipigs were examined for their transcripts or the possession of hybridizing C $\gamma$  genes, animals that were homozygous IgA<sup>a/a</sup> lacked the C $\gamma$  gene originally designated IgG1 (now designated IgG1<sup>b</sup>) and expressed only the subclass described by Kacskovics et al. (1994) as IgG3 (now called IgG1<sup>a</sup>; Fig. 3C). Because IgA<sup>a/a</sup> and “old IgG3” appear to be part of the same haplotype, we designated the originally described IgG1 as IgG1<sup>b</sup> (IGHG1\*02) and the originally described IgG3 as IgG1<sup>a</sup> (IGHG1\*01; Fig. 1; Table 2). A similar pattern of equal expression (320/275) was seen with the genes initially designated IgG2a and IgG2b (Kacskovics et al. 1994; Table 2). We have therefore renamed them as the IgG2<sup>a</sup> and IgG2<sup>b</sup> alleles of the IGHG2 gene.

In the same manner as described for the putative IgG1 alleles, animals which failed to transcribe certain C $\gamma$



**Fig. 1** The complete genomic sequence of 11 porcine C $\gamma$  genes using the sequence of porcine IgG1<sup>a</sup> as a reference and numbering from the first codon of C<sub>H</sub>1. Intron nucleotides are in *lower case* and the deduced amino acid sequences for the exons are in *lower case*. The *upper* (99–105), *middle* or *core hinge* (106–114), and *lower hinges* (115–121) are separated by *vertical lines*. *Dots* indicate that the reference nucleotide sequence (IgG1<sup>a</sup>) is shared, whereas *solid bold horizontal lines* indicate the absence of the corresponding nucleotide. Cysteine codons involved in intrachain loops are *under-*

*lined* and in *boldface*. Cysteines believed to be involved in H–H covalent bonds are circled. Cysteine 14 in C<sub>H</sub>1 is believed to be involved in L–H bonds. Potential N-linked glycosylation motifs are boxed. Motifs considered to be important in interactions with C1q, SpA, SpG, FcRn, and Fc $\gamma$ R are enclosed in *vertical rectangles* and their importance indicated at the base of the rectangles. Annealing sites for the PCR primers used to recover cDNA and DNA sequences are indicated in *horizontal rectangles*

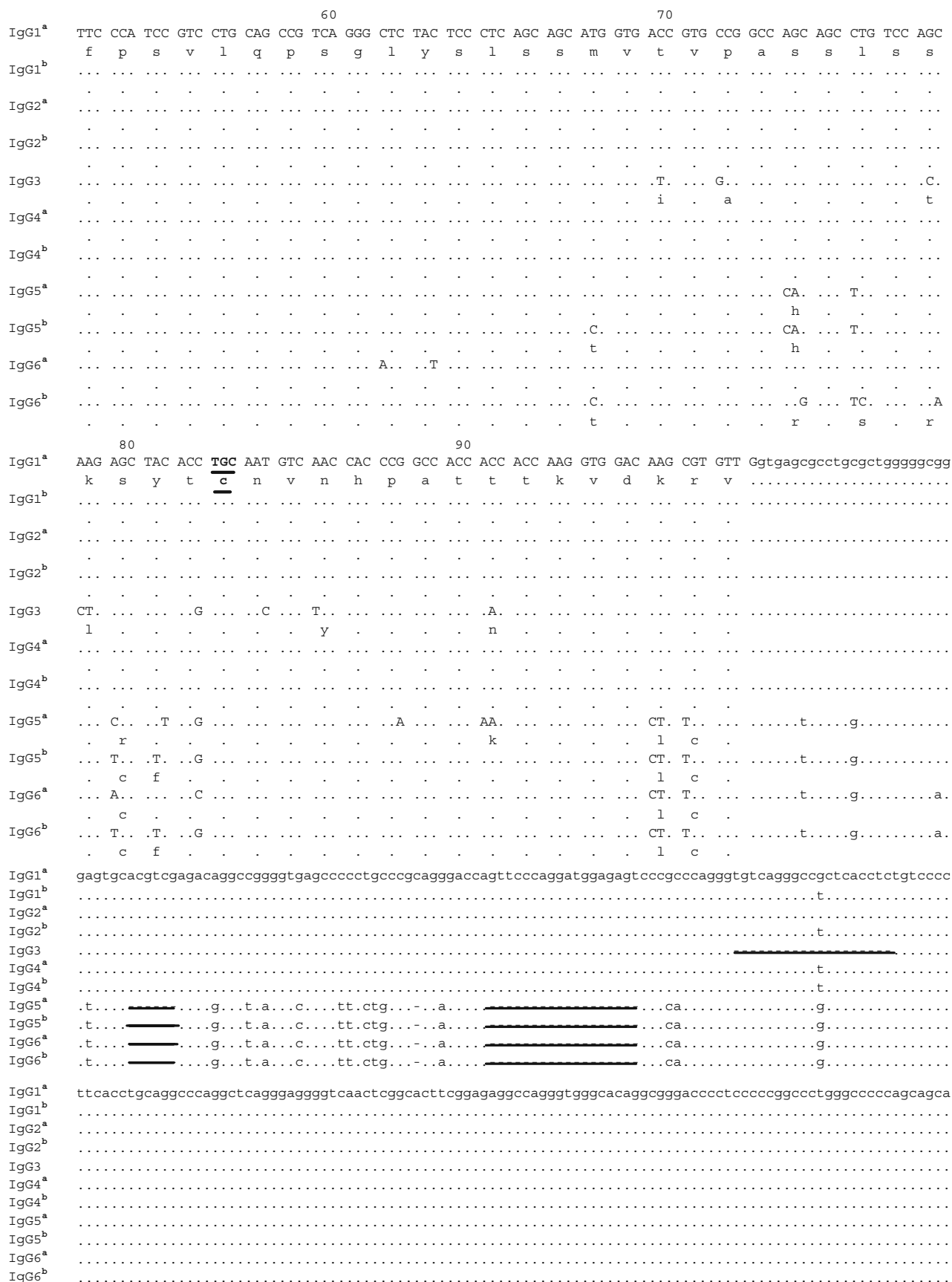


Fig. 1 (continued)

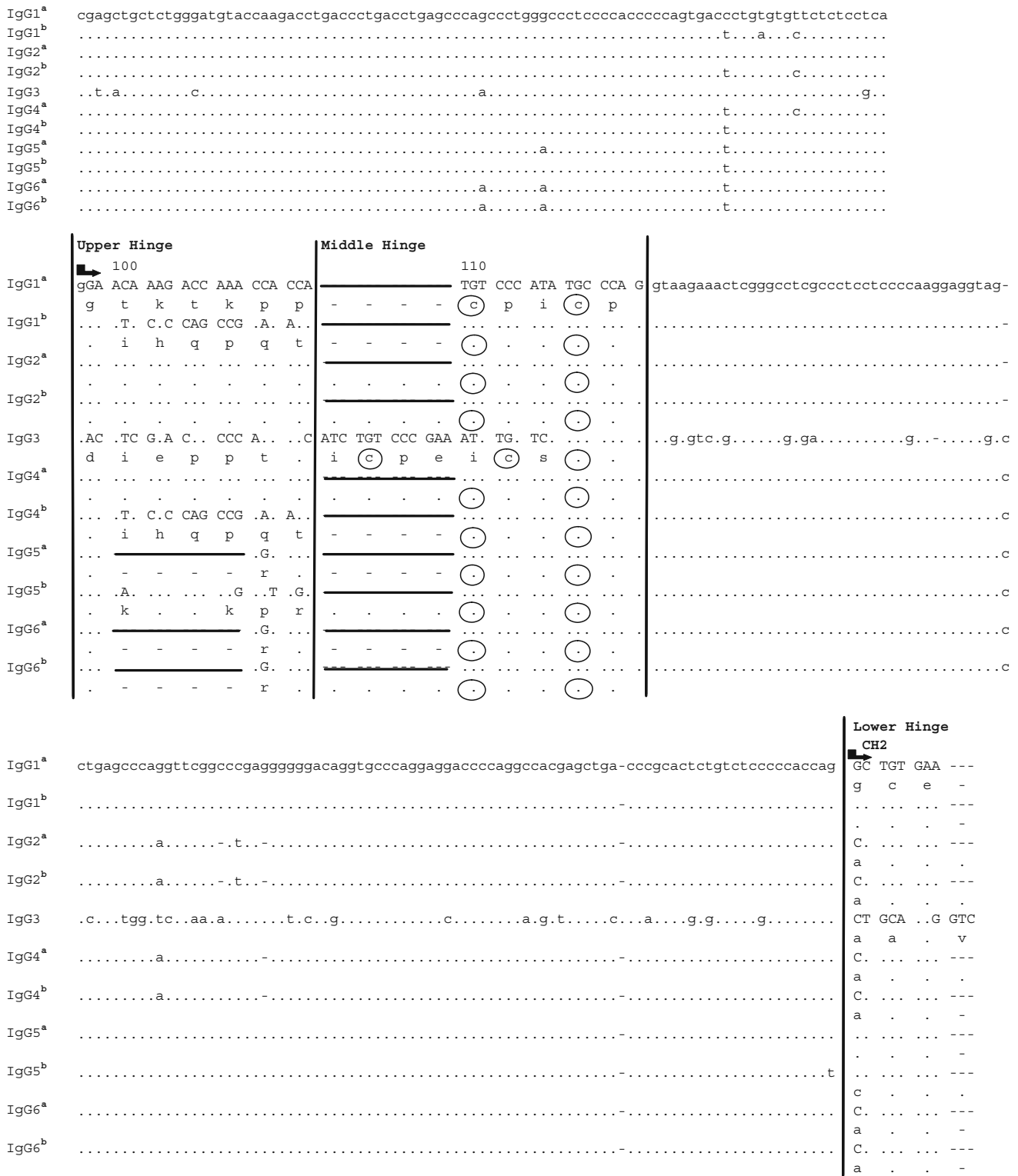


Fig. 1 (continued)

genes were selected for analysis of their DNA. The PCR products were first tested for their ability to hybridize with subclass-specific pan-specific probes. The products were then cloned and the individual clones identified by

hybridization using the putative subclass probes described in Table 1. Table 3 shows recovery data for a selected panel of animals that lacked clones expressing particular putative alleles. Results are based on 29–36 clones for each



	120										130										140									
IgG1 <sup>a</sup>	GTG	GCC	GGG	CCC	TCG	GTC	TTC	ATC	TTC	CCT	CCA	AAA	CCC	AAG	GAC	ACC	CTC	ATG	ATC	TCC	CAG	ACC	CCC	GAG	GTC	ACG				
IgG1 <sup>b</sup>	v	a	g	p	s	v	f	i	f	p	p	k	p	k	d	t	l	m	i	s	q	t	p	e	v	t				
IgG2 <sup>a</sup>	TCA	C.A																			.G.	.A		C.						
IgG2 <sup>b</sup>	s	p																			r			q						
IgG3	C.	.GA	.CA	.G					C.								T.				.G.	.A		A.						
IgG4 <sup>a</sup>	.G.	C.				.C.															r			k						
IgG4 <sup>b</sup>	g	p			a																r			k						
IgG5 <sup>a</sup>																	T.				.G.									
IgG5 <sup>b</sup>																	i				r									
IgG6 <sup>a</sup>	.G.	C.				.C.															.G.	.A		C.						
IgG6 <sup>b</sup>	g	p			a																r			q						
	g	n																			.G.									
	FcγR															SpG & FcRn														

	150										154										160										162										170									
IgG1 <sup>a</sup>	<u>TGC</u>	GTG	GTG	GTG	GAC	GTC	AGC	AAG	GAG	CAC	GCC	GAG	GTC	CAG	TTC	TCC	TGG	TAC	GTG	GAC	GGC	GTA	GAG	GTG	CAC	ACG																								
IgG1 <sup>b</sup>	c	v	v	v	d	v	s	k	e	h	a	e	v	q	f	s	w	y	v	d	g	v	e	v	h	t																								
IgG2 <sup>a</sup>					T	T	G		C.		A.	C.G																																						
IgG2 <sup>b</sup>				A	T	T	G		C.	A	A.	C.G																																						
IgG3							G		C.		G.G	.T	.A										C.	T.	T.																									
IgG4 <sup>a</sup>					T	T	G		C.		A.	C.G												q	l	y																								
IgG4 <sup>b</sup>					T	T	G		C.		A.	C.G																																						
IgG5 <sup>a</sup>									q		n	p																																						
IgG5 <sup>b</sup>																																																		
IgG6 <sup>a</sup>					T	T	G		C.		A.	C.G						T					T																											
IgG6 <sup>b</sup>					T	T	G		C.	A	A.	C.G											g																											
									q		n	p											C	GA																										
										q	n	p											e																											
											Clq							Clq																																

Fig. 1 (continued)

animal. Data reveal animals homozygous and heterozygous for the putative alleles of IgG1, IgG2, and IgG5. In the examples shown, no animal homozygous for either allele of IgG6 was identified. Surprisingly, neither variant of IgG2 was recovered from animal nos. 5 nor 755, and neither variant of IgG6 was recovered from animal nos. 476 nor 481. Two of these “deficient” animals were Meishans, one an inbred NIH minipig and the other a mixed-breed farm pig.

*Nomenclature revision for porcine Cγ genes* Since the original IgG3 has now been renamed IgG1<sup>a</sup>, we have proposed that the recently described long-hinged Cγ gene (Fig. 1; Table 2) be redesignated as IgG3. IgG4, IgG5, and IgG6 and their putative alleles, complete the list of IgG subclass genes in swine. We have proposed designations for these Cγ genes that are consistent with both the familiar and the IMGT nomenclature systems (Lefranc and Lefranc 2001; Table 2).



	180													190												
IgG1 <sup>a</sup>	GCC	GAG	ACG	AGA	CCA	AAG	GAG	GAG	CAG	TTC	AAC	AGC	ACC	TAC	CGT	GTG	GCC	AGC	GTC	CTG	CCC	ATC	CAG	CAC	CAG	GAC
IgG1 <sup>b</sup>	a	e	t	r	p	k	e	e	q	f	n	s	t	y	r	v	v	s	v	l	p	i	q	h	q	d
IgG2 <sup>a</sup>	...	C	...	...	G	...	...	...	...	...	...	...	...	...	C	...	T	...	...	...	...	...	...	...	...	...
IgG2 <sup>b</sup>	...	q	...	...	...	...	...	...	...	...	...	...	...	...	...	...	a	...	...	...	...	...	...	...	...	...
IgG3	...	C	...	...	G	...	T	...	...	...	...	...	...	...	C	...	T	...	...	...	...	...	...	...	...	...
IgG4 <sup>a</sup>	...	C	...	...	G	...	m	...	...	...	...	...	...	...	C	...	a	...	...	...	...	...	...	...	...	...
IgG4 <sup>b</sup>	...	q	...	...	...	...	...	...	...	...	...	...	...	...	...	...	a	...	...	...	...	...	...	...	...	...
IgG5 <sup>a</sup>	...	...	...	...	G	...	...	...	...	...	...	...	...	...	C	...	T	...	...	...	...	...	...	...	G	...
IgG5 <sup>b</sup>	...	...	...	...	...	...	...	...	...	...	...	...	...	T	...	C	...	T	...	...	...	...	...	...	G	...
IgG6 <sup>a</sup>	...	C	...	...	G	...	a	...	C	...	...	...	...	...	...	...	a	...	...	...	...	...	...	...	G	...
IgG6 <sup>b</sup>	...	q	...	...	...	...	...	...	...	...	...	...	...	...	...	...	a	...	...	...	...	...	...	...	G	...

SpG & FcRn

	200						206				210			213		215		217		220						
IgG1 <sup>a</sup>	TGG	CTG	AAG	GGG	AAG	GAG	TTC	AAG	TGC	AAG	GTC	AAC	AAC	GTA	GAC	CTC	CCA	GCC	CCC	ATC	ACG	AGG	ACC	ATC	TCC	AAG
IgG1 <sup>b</sup>	w	l	k	g	k	e	f	k	c	k	v	n	n	v	d	l	p	a	p	i	t	r	t	i	s	k
IgG2 <sup>a</sup>	...	...	C	...	...	...	...	...	...	...	...	...	AA	...	...	...	...	...	...	...	AA	...	T	...	...	...
IgG2 <sup>b</sup>	...	...	n	...	...	...	...	...	...	...	...	...	AA	...	...	...	...	...	...	...	AA	...	T	...	...	...
IgG3	...	...	...	...	...	...	...	...	...	...	...	...	AA	...	...	TT	T	...	...	...	...	...	...	...	...	...
IgG4 <sup>a</sup>	...	...	C	...	...	...	...	...	...	...	...	...	AA	...	...	l	s	...	...	...	...	AA	...	T	...	...
IgG4 <sup>b</sup>	...	...	n	...	...	...	...	...	...	...	...	...	AA	...	...	...	...	...	...	...	...	AA	...	T	...	...
IgG5 <sup>a</sup>	...	...	...	...	...	...	G	...	...	...	...	...	AA	...	...	...	G	...	...	...	...	...	...	...	...	...
IgG5 <sup>b</sup>	...	...	...	...	...	...	e	...	...	...	...	...	e	...	...	g	...	...	...	...	...	...	...	...	...	...
IgG6 <sup>a</sup>	...	...	...	...	...	...	e	...	...	...	...	...	e	...	...	g	...	...	...	...	...	...	...	...	...	...
IgG6 <sup>b</sup>	...	...	...	A	...	...	G	...	...	...	...	...	k	...	...	...	...	...	...	...	AA	...	T	...	...	...
	...	...	g	...	...	...	e	...	...	...	...	k	...	...	...	...	...	...	...	...	AA	...	T	...	...	...

SpA                      Clq                      Clq                      Clq                      Clq                      Clq

Fig. 1 (continued)

Analysis of porcine IgG sequences Figure 1 shows the genomic nucleotide sequence for the various porcine C $\gamma$  genes and the deduced amino acid sequence of the domain exons, while Fig. 2 compares the complete genomic sequences, showing that the putative allelic variants pair together. The deduced amino acid sequences of the individual domains are compared in Fig. 4 and their relationships discussed below.

*C<sub>H1</sub>* domain All C<sub>H1</sub> domains share a potential 56 amino acid intradomain disulfide loop (Cys 27 to Cys 83).

Position 14 is believed to provide the cysteine for the L–H disulfide bond as in human IgG2, IgG3 and IgG4. The C<sub>H1</sub> domain is the most conserved among the different IgGs (Fig. 4A; note genetic distance scale). IgG5<sup>b</sup> and IgG3 are most divergent in C<sub>H1</sub> from other IgG genes and also divergent from each other. IgG1, IgG2, and IgG4 have nearly identical C<sub>H1</sub> domains (Figs. 1, 4A). The last 19 positions are generally shared by the IgG5 and the IgG6 alleles which differ in this region from the IgG1–IgG2–IgG4 group by three to four nonconservative changes. The putative IgG1 alleles differ at only one position, the Thr/Val

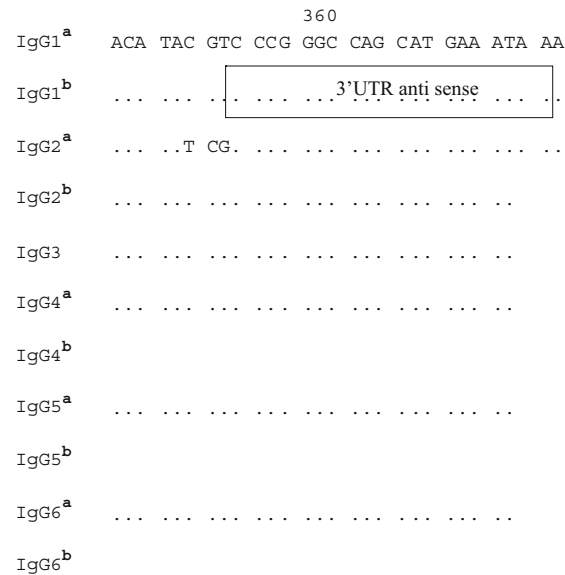
IgG1 <sup>a</sup>	GCT ATA Ggtgggcaaggggggcaaggtgggcaggcggagaggggtcccgtggggccgcctggggggaccatcatgctaactgaggtgctggcctcacag GG
	a i g
IgG1 <sup>b</sup>	.....t.....
IgG2 <sup>a</sup>	...A.....g.....a.t.....a.....a.....A.
	. k
IgG2 <sup>b</sup>	..C.A.....g.....a.t.....a.....a.....
	. k
IgG3	...C. <u>                    </u> tg.gt.ag...a.g.....a.....a.....
	. T
IgG4 <sup>a</sup>	..C.A. <u>                    </u> .....g.....a.t.....a.....a.....
	. k
IgG4 <sup>b</sup>	..C.A. <u>                    </u> .....g.....a.t.....a.....a.....
	. k
IgG5 <sup>a</sup>	..C.A. <u>                    </u> .....c.....a.....t.....
	. k
IgG5 <sup>b</sup>	..C.A. <u>                    </u> .....c.....a.....t.....
	. k
IgG6 <sup>a</sup>	..C.A.....g.....a.t.....a.....a.....a.....t.....
	. k
IgG6 <sup>b</sup>	..C.A.....g.....a.t.....a.....a.....a.....t.....
	. k
IgG1 <sup>a</sup>	CAG AGC CGG GAG CCG CAG GTG TAC ACC CTG CCC CCA CCC GCC GAG GAG CTG TCC AGG AGC AAA GTC ACG CTA ACC <u>TGC</u>
	q s r e p q v y t l p p p a e e l s r s k v t l t <u>c</u>
IgG1 <sup>b</sup>	.....
IgG2 <sup>a</sup>	...C.....A.....GC A.....
	. t . . . . . h . . . . . s i . . .
IgG2 <sup>b</sup>	...C.....A.....GC A.....
	. t . . . . . h . . . . . s i . . .
IgG3	.C. . . . .T. . . . .G.. TGG ..A . . . . .A. . . . .GC A. . . . .
	p . . . . .v . . . . .a w . . . . .k . . . . .s i . . .
IgG4 <sup>a</sup>	...C.....A.....
	. t . . . . . T . . . . .
IgG4 <sup>b</sup>	...C.....A.....
	. t . . . . . T . . . . .
IgG5 <sup>a</sup>	GT. GTA ... AGC ... G.. . . . .A. . . . .T. . . . .
	v v . s . E . . . . .k . i . . . . .
IgG5 <sup>b</sup>	GT. GTA ... AGC ... G.. . . . .A. . . . .T. . . . .
	v v . s . E . . . . .k . i . . . . .
IgG6 <sup>a</sup>	.C. . . . .T. . . . .GC A. . . . .
	p . . . . .s . . . . .s i . . . . .
IgG6 <sup>b</sup>	.C. . . . .T. . . . .GC A. . . . .
	p . . . . .s . . . . .s i . . . . .
IgG1 <sup>a</sup>	CTG GTC ATT GGC TTC TAC CCA CCT <u>260</u> GAC ATC CAT GTT GAG TGG AAG AGC AAC <u>GGA CAG CCG</u> GAG CCA GAG <u>270</u> GGC AAT TAC
	l v i g f y p p d i h v e w k s n <u>g q p</u> e p e g n y
IgG1 <sup>b</sup>	.....AA. CA . . . . .
	. . . . . n t . . . . .
IgG2 <sup>a</sup>	.....G.. C . . . . .C.. A . . . . .
	. . . . . d . . . . . q r . . . . .
IgG2 <sup>b</sup>	.....G.. C . . . . .C.. A . . . . .
	. . . . . d . . . . . q r . . . . .
IgG3	...C.....G.. C . . . . .C.. A . . . . .AA
	. . . . . t . . . . . d . . . . . q . . . . . q . . . . .
IgG4 <sup>a</sup>	...C.....G.. C . . . . .C.A ..A . . . . .
	. . . . . t . . . . . d . . . . . q r . . . . .
IgG4 <sup>b</sup>	...C.....G.. C . . . . .C.A ..A . . . . .
	. . . . . t . . . . . d . . . . . q r . . . . .
IgG5 <sup>a</sup>	..AA A.. A.. T. .G .TT. . . . .A.T. . . . .A.A ..A . . . . .AA . GCA . .T
	. . k s i f . f . . . . .t . . . . .k . . . . . n t y
IgG5 <sup>b</sup>	..AA A.. .T. .G .TT. . . . .A.T. . . . .A.A ..A . . . . .AA . GCA . .
	. . k s . f . f . . . . .t . . . . .k . . . . . n t .
IgG6 <sup>a</sup>	...C.....G.. C . . . . .
	. . t . . . . . d . . . . .
IgG6 <sup>b</sup>	...C.....G.. C . . . . .
	. . t . . . . . d . . . . .

SpG

Fig. 1 (continued)

	280												290												300											
IgG1 <sup>a</sup>	CGC	ACC	ACC	CCG	CCC	CAG	CAG	GAC	GTG	GAC	GGG	ACC	TTC	TTC	CTG	TAC	AGC	AAG	CTC	GCG	GTG	GAC	AAG	GCA	AGA	TGG										
IgG1 <sup>b</sup>	r	t	t	p	p	q	q	d	v	d	g	t	f	f	l	y	s	k	l	a	v	d	k	a	r	w										
IgG2 <sup>a</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.									
IgG2 <sup>b</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	.									
IgG3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG4 <sup>a</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG4 <sup>b</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG5 <sup>a</sup>	.	.	.	.	.	T	G	.	.	A	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG5 <sup>b</sup>	.	.	.	.	.	.	e	.	e	.	r	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG6 <sup>a</sup>	.	.	.	.	.	.	e	.	e	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG6 <sup>b</sup>	.	T	.	.	.	.	G	.	A	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG1 <sup>a</sup>	GAC	CAT	GGA	GAA	ACA	TTT	GAG	<u>TGT</u>	GCG	GTG	ATG	CAC	GAG	GCT	CTG	CAC	AAC	CAC	TAC	ACC	CAG	AAG	TCC	ATC	TCC	AAG										
IgG1 <sup>b</sup>	d	h	g	e	t	f	e	<u>c</u>	a	v	m	h	e	a	l	h	n	h	y	t	q	k	s	i	s	k										
IgG2 <sup>a</sup>	C.G	GG.	.	.GC	.T.	.C	C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG2 <sup>b</sup>	C.G	GG.	.	.GC	.T.	.C	C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG3	C.G	.G.	.	.	CT.	.C	C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG4 <sup>a</sup>	C.G	.G.	.	.C	GC.	.C	C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG4 <sup>b</sup>	C.G	.G.	.	.C	.C.	.C	C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG5 <sup>a</sup>	.	.	.	.A	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG5 <sup>b</sup>	C.	AG.	.	.GC	.C	-	C.C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG6 <sup>a</sup>	C.G	.G.	.	.C	CC.	.C	C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG6 <sup>b</sup>	C.G	AG.	.	.GC	-	A.C	C.C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
											SpG																									
											340																									
IgG1 <sup>a</sup>	ACT	CAG	GGT	AAA	TGA	GCC	ACC	CGC	TGC	ACC	CCA	CGT	GCT	CTC	GGG	TCC	CAA	GAG	CTC	GCC	TAA	GCC	CCA	GCG	CTG	TGT										
IgG1 <sup>b</sup>	t	q	g	k	stop	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG2 <sup>a</sup>	.	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG2 <sup>b</sup>	.	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG3	.	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG4 <sup>a</sup>	.	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG4 <sup>b</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG5 <sup>a</sup>	.	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG5 <sup>b</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG6 <sup>a</sup>	.	.C.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									
IgG6 <sup>b</sup>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.									

Fig. 1 (continued)



**Fig. 1** (continued)

at position 18; this point substitution is also seen in IgG3, IgG4<sup>b</sup>, and IgG5<sup>b</sup>. There is only one difference between the putative IgG4<sup>a</sup>/IgG4<sup>b</sup> allelic variants and the IgG2<sup>a</sup>/IgG2<sup>b</sup> allelic variants. The latter involves the Gly/Ser at position 15. However, the putative IgG5 alleles differ from each other at ten positions, and the IgG6 alleles differ from each other at six positions.

IgG5<sup>a</sup> differs from IgG1<sup>a</sup> at seven positions, all of which are nonconservative substitutions. Only four of these are shared by IgG5<sup>b</sup>. IgG3 differs from IgG1<sup>a</sup> at 15 positions in C<sub>H</sub>1 of which only one (Ala/Val at position 29) is shared with both IgG5 variants but an additional

five are shared with IgG5<sup>b</sup> including the Thr/Val substitution at position 18. Perhaps the C<sub>H</sub>1 domain of IgG5<sup>b</sup> and IgG3 may have arisen from a recent ancestral C<sub>H</sub>1 domain (Fig. 4A).

*The upper hinge* We have divided the genetic hinge, defined as a separate exon, into an upper hinge (residues 99–105) and a middle hinge or “core” hinge from 106–114 (Nezlin 1994). The lower hinge, encoded at the beginning of the C<sub>H</sub>2 domain, encompasses residues 115–121. These divisions are indicated in Fig. 1. The upper genetic hinge of IgG2<sup>a</sup>, IgG2<sup>b</sup>, IgG1<sup>a</sup>, and IgG4<sup>a</sup> are identical. This means

**Table 2** Nomenclature and number of clones examined

Original <sup>a</sup>	Revised <sup>b</sup>	IMGT <sup>c</sup>	GenBank nr.	Hybridizing clones		Recovered and sequenced <sup>d</sup>	
				cDNA	DNA	cDNA	DNA
IgG1	IgG1 <sup>b</sup>	IGHG1.02	U03778	3,081	228	19	8
IgG2a	IgG2 <sup>a</sup>	IGHG2.01	U03779	320	132	27	5
IgG2b	IgG2 <sup>b</sup>	IGHG2.02	U03780	275	161	4	5
IgG3	IgG1 <sup>a</sup>	IGHG1.01	U03781	2,909	521	28	13
IgG4	IgG4 <sup>a</sup>	IGHG4.01	U03782	71	127	4	4
	IgG4 <sup>b</sup>	IGHG4.02	EU372654	0	15	0	5
	IgG3	IGHG3.01	EU372658	1,793	314	27	3
	IgG5 <sup>a</sup>	IGHG5.01	EU372657	526	345	26	12
	IgG5 <sup>b</sup>	IGHG5.02	EU372656	94	312	25	4
	IgG6 <sup>a</sup>	IGHG6.01	EU372655	65	299	16	17
	IgG6 <sup>b</sup>	IGHG6.02	EU372653	147	295	8	12
				9,281	2,749	184	88

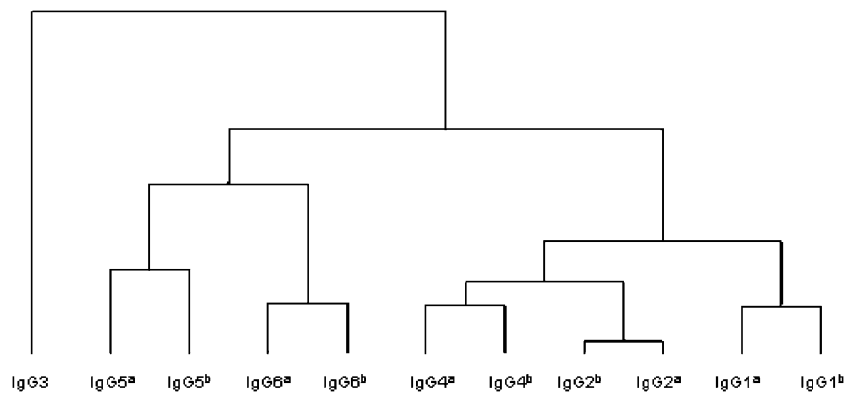
<sup>a</sup>Nomenclature from Kacsokovics et al. (1994)

<sup>b</sup>Proposed nomenclature; superscripts denote putative allotypes

<sup>c</sup>Proposed nomenclature according to the IMGT system

<sup>d</sup>Sequence analysis of hybridizing clones for some C<sub>γ</sub> genes was not continued since all clones recovered had the same sequence

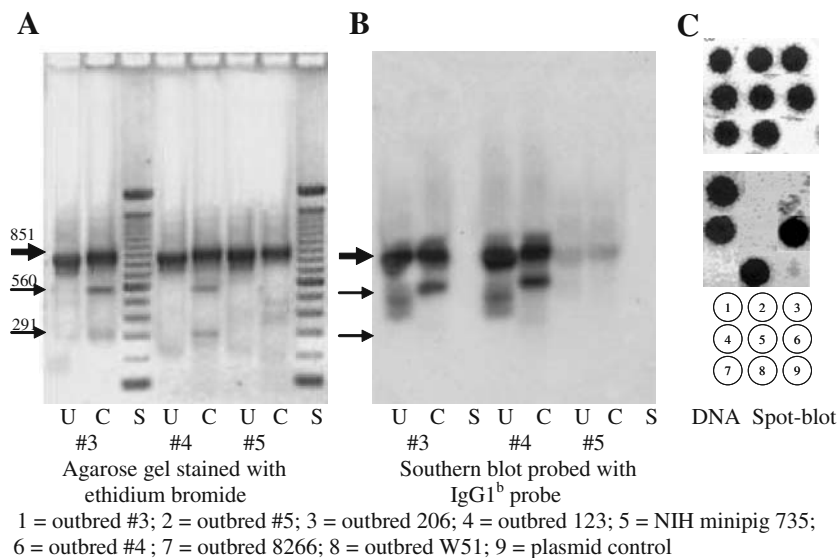
**Fig. 2** Dendrogram comparing the complete genomic sequence of 11 C $\gamma$  genes



that IgG1 and IgG4, which are nearly sequence identical in C<sub>H</sub>1, differ allelically in their upper hinge by four out of five amino acids. IgG4<sup>a</sup> shares the upper hinge of IgG1<sup>a</sup> while IgG4<sup>b</sup> shares the upper hinge of IgG1<sup>b</sup> (Fig. 4B). Thus, IgG4<sup>b</sup> and IgG1<sup>b</sup> lose the two potential O-linked glycosylation sites at positions 100 and 102 but gain a proline at position 103. IgG3 differs from all other porcine IgGs in the upper hinge and adds a double proline, thus expressing something like a middle hinge motif that may have some of the same properties. Paired prolines are also found in the upper hinge of human IgG3, mouse IgG3 and rat IgG2c (Nezlin 1994). Interestingly, IgG5<sup>a</sup>, IgG6<sup>a</sup>, and IgG6<sup>b</sup> all share the short upper hinge, while IgG5<sup>b</sup> has a unique upper hinge. No cysteines are encoded in the upper hinge of any porcine C $\gamma$  genes that could contribute to

interchain disulfide bridges or could be involved in L–H disulfide bonds as in human IgG1. As indicated above, cysteine 14 in C<sub>H</sub>1 must serve that function (Fig. 1). The Lys-Thr motif that defines a trypsin cleavage site, is present in all porcine C $\gamma$  genes except IgG1<sup>b</sup>, IgG4<sup>b</sup>, and IgG3 and of course those C $\gamma$  genes (IgG5<sup>a</sup> and IgG6<sup>a</sup>) with a short upper hinge. This could be predictive of the effect of proteases on IgGs of these putative subclasses/allelic variants (Table 4).

*The middle hinge* All porcine C $\gamma$  genes carry variations of the C-P-P-C motif that characterizes the “hinge core” (Nezlin 1994). All porcine C $\gamma$  genes contain at least two cysteines presumed to be involved in H–H disulfide bonds, while IgG3 has three (Fig. 1). Recent engineering studies



**Fig. 3** Southern blot of restriction digested and intact C $\gamma$  DNA. **A** C $\gamma$  DNA of three outbred piglets (nos. 3, 4, and 5) digested with Pci I (C) or undigested (U) and stained with ethidium bromide after separation in a 1.5% agarose gel. The predicted size of the IgG1<sup>b</sup> polynucleotides after Pci I digestion are depicted by *light arrows* while the *heavy arrows* indicate the size of the undigested C $\gamma$  DNA. *S* nt length standards. **B** Southern blot analysis of agarose gel in (A)

after hybridization with an IgG1<sup>b</sup>-specific probe. *Arrows* indicate the same polynucleotides as in (A). Piglet no. 5 did not express IgG1<sup>b</sup> and yields no product after Pci I digestion. **C** Southern dot-blot of C $\gamma$ -containing DNA. *Top* hybridization with the probe for IgG1<sup>a</sup>, *middle* hybridization with the probe for IgG1<sup>b</sup>, *bottom* code for animal identification (given below the diagram)

**Table 3** Recovery of C $\gamma$  genes form DNA of selected animals<sup>a</sup>

Animal Nr.	Clones Recovered	IgG1 <sup>b</sup>		IgG2		IgG3	IgG4		IgG5		IgG6		IgA	
		a	b	a	b		a	b	a	b	a	b	a	b
#3 <sup>c</sup>	31	X	X	X	X	X	X	O	X	X	X	X	X	X
#5	36	X	O	O	O	X	O	X	X	X	X	X	X	O
#755	29	X	O	O	O	X	O	X	X	X	X	X	X	O
#476	25	X	O	X	O	X	O	O	O	X	O	O	X	O
#481	32	X	O	X	O	X	O	O	O	X	O	O	X	O
#600	33	X	X	X	O	X	O	X	X	X	X	X	X	O
#601	34	X	X	X	O	X	O	X	X	X	X	X	X	O

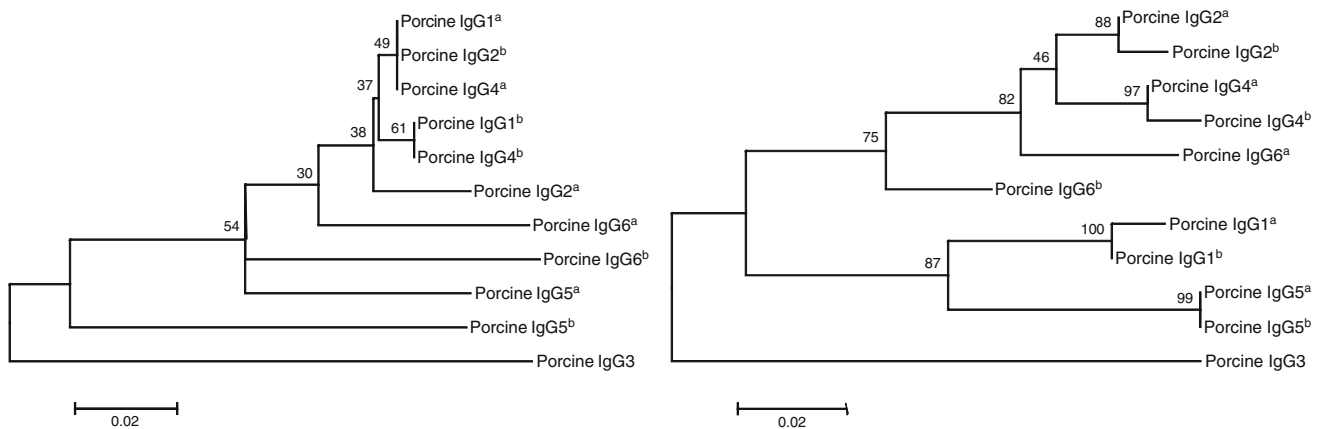
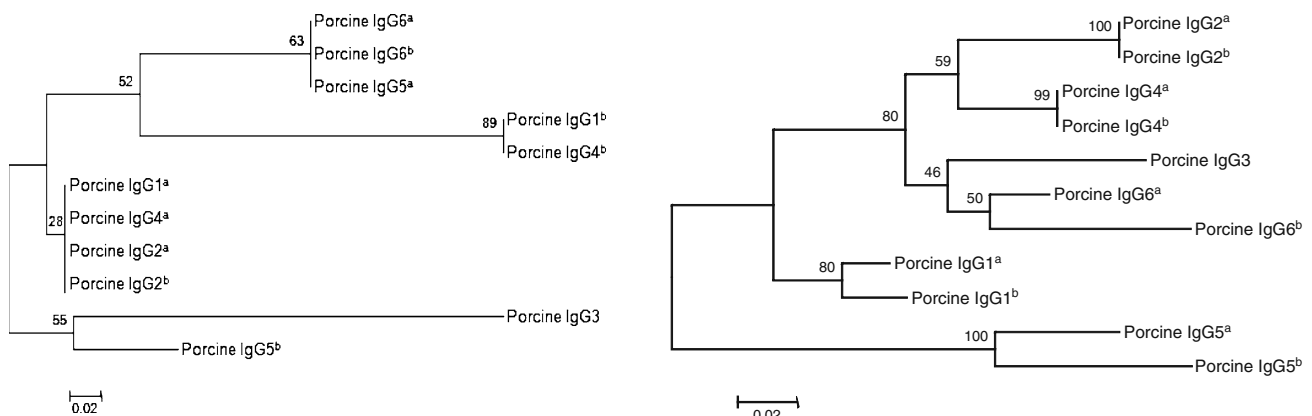
<sup>a</sup> Animals were selected to demonstrate a principle; this is not a population study of all 118 pigs used in the study

<sup>b</sup> Allotype is indicated below without superscript. X= present; O= absent. Boxed symbols emphasize those pigs with a gene deficiency

<sup>c</sup> #3 & #5 are mixed breed farm pigs; 755 is an NIH minipig; 476 & 481 are strain A Meishans and 600 & 601 are strain B Meishans

on human IgG1 show that middle hinge stability resulting from polyproline structures and disulfide bridges are very important in C1q binding (Dall'Acqua et al. 2006). While human IgG3 can use alternate hinge exons, all porcine IgG3 sequences we have recovered have a single hinge exon. Except for IgG3, all porcine C $\gamma$  immunoglobulins have identical core hinge sequences.

*The lower hinge* The first seven amino acids of the C $\gamma$ 2 domain (115–121) constitute the lower hinge. This is identical for both alleles of IgG1 and IgG5<sup>a</sup> which is also true for the middle but not the upper hinge of these C $\gamma$  genes. The putative alleles of IgG2 and IgG4 share with IgG3 and IgG6<sup>b</sup> the Ala at position 115, but the IgG2 alleles have a Ser at position 119, while this is a Gly for

**a** Phylogeny of Amino Acid sequence of porcine CH1 gamma immunoglobulin domain.**c** Phylogeny of Amino Acid sequence of porcine CH2 gamma immunoglobulin domain.**b** Phylogeny of Amino Acid sequence of porcine Hinge gamma immunoglobulin domain.**d** Phylogeny of Amino Acid sequence of porcine CH3 gamma immunoglobulin domain.

**Fig. 4** Cladograms comparing the deduced amino acid sequences of individual domains of the porcine C $\gamma$  genes. **a** C $\gamma$ 1 domain; **b** genetic hinge; **c** C $\gamma$ 2 domain; **d** C $\gamma$ 3 domain. The genetic distances obtained by the Poisson correction are given in the scale bar below the trees

**Table 4** Predicted functional properties of various porcine C $\gamma$  genes

	IgG Subclass										
	1 <sup>a</sup>	1 <sup>b</sup>	2 <sup>a</sup>	2 <sup>b</sup>	3	4 <sup>a</sup>	4 <sup>b</sup>	5 <sup>a</sup>	5 <sup>b</sup>	6 <sup>a</sup>	6 <sup>b</sup>
Clq binding	–	–	–	–	+++	–	–	–	–	–	–
Fc $\gamma$ R	+	+	+	+	+++	+	+	±	+	±	+
FcRn	+	+	+	+	+	+	+	±	±	±	+
Protein A	+	+	+	+	+	+	+	±	±	±	+
Protein G	+	+	+	+	+	+	+	±	±	±	+
Peptic F(ab) <sup>2</sup>	±	±	±	±	±	±	±	±	±	±	±
Tryptic Fc/Fab	+	–	+	+	–	+	–	–	+	–	+

IgG4 alleles and IgG6<sup>a</sup> and IgG6<sup>b</sup>. Both IgG2 and IgG4 have a Pro at position 120, which is also shared by IgG6<sup>a</sup> but not IgG6<sup>b</sup>. IgG6<sup>b</sup> lacks this proline, and the lower hinge terminates in a hydrophilic dipeptide (Glu+ Asn). IgG3 is again most divergent with three very conserved substitutions in the lower hinge; Val → Leu, Ala → Gly, Gly → Ala. The paired leucines that form the pepsin cleavage site in human IgG1 and in lower hinge of various species is absent in all swine C $\gamma$  genes.

Figure 4b shows that when the complete genetic hinge is considered, two features stand out. First, different C $\gamma$  genes share nearly identical hinges even though they are from different putative subclasses. Second, except for those with identical genetic hinges, the porcine C $\gamma$  genes show their lowest sequence similarity (note genetic distance scale in Fig. 4b). Based on motifs in IgG of other species, the sequence differences we have described are unlikely to yield homogeneous Fab/Fc or F(Ab')<sub>2</sub> fragments by proteolytic digestion (Table 4). Furthermore, differences in rigidity or flexibility that are dependent on disulfide bonds and proline residues could effect complement activation (see below).

**The C<sub>H2</sub> domain** The C<sub>H2</sub> domain extends from 115 to 224 and contains a potential 60 amino acid intradomain disulfide loop (Cys 145 and 205) in all sequences. While the sequences of the putative subclasses differ, there are nearly no allelic differences. The allelic variant of IgG1 differs by two amino acids at the 3' end (223, 224), and the alleles of IgG5 are identical except for the conservative Thr to Ser change at 183. All porcine IgG genes differ from IgG1<sup>a</sup> by the Gln → Arg change at position 139, and most others have a Glu → Asn or Glu → Lys change at position 142. The alleles of IgG2 differ only by the nonconservative Glu to Gly change at 178, and the IgG4 alleles are identical. The putative alleles of IgG6 stand out because of seven amino acid differences. Most unique among subclass genes is IgG3 with ten mostly nonconserved unique amino acids changes. Several (Hist → Glu at 154; Glu to Lys at 168, and Hist → Tyr at 169) are quite radical but only

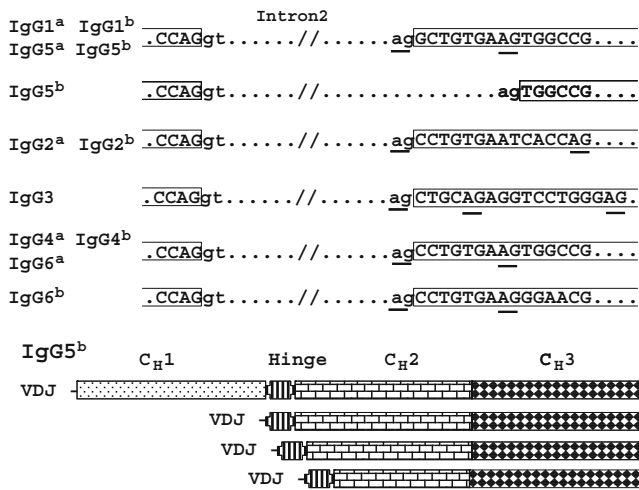
one (Ala → Ser at 214) involves a glycosylation site. The C<sub>H2</sub> domain contains either two or three potential AG splice acceptors sites; this will be discussed in more detail below. The overall sequence similarity of the different C $\gamma$  sequences in C<sub>H2</sub> (Fig. 4C) groups allelic variants as does Fig. 2, unlike when only the C<sub>H1</sub> and the hinge domains are compared.

**The C<sub>H3</sub> domain** The domain (225–334) contains a potential 60 amino acid intradomain disulfide loop (Cys 251–311) and apart from the hinge: (a) the most subclass diagnostic domain and (b) allelic variants of putative subclasses group together in the same manner as is seen for the C<sub>H2</sub> domain (Fig. 4D). IgG1 differs at up to 17 positions from the IgG2, IgG4, IgG6 group. This group shares many of these position changes; nine of the 15 are shared between IgG2 and IgG4, and six are also found in IgG6. Allelic differences in C<sub>H3</sub> among the subclass C $\gamma$  genes are modest. IgG1<sup>a</sup> and IgG1<sup>b</sup> differ at five positions, IgG2 and IgG4 alleles and IgG6 alleles differ at seven positions and IgG5 alleles differ at six positions. The C<sub>H3</sub> domains of IgG3 and IgG5 are most divergent from all other porcine C $\gamma$  genes but neither share tight sequence similarity with any of the C $\gamma$  genes reported here.

**Introns are conserved and are subclass-specific** Further evidence supporting the identification of the six putative porcine IgG subclasses is based on differences in intron sequences; putative alleles of a subclass have nearly identical introns. IgG5 and IgG6 share a similar IVS1 which is shorter than the IVS1 of IgG1, IgG2, IgG3, and IgG4, while IgG3 has a unique IVS1 deletion (Fig. 1). IVS2 is almost the same length in all genomic sequences and for all but IgG3 differs by only 1–3 nt. IgG3 differs at 23 positions, i.e., 26% of the length of IVS2. IVS3 differs in length among C $\gamma$  genes; IgG3, IgG4, and IgG5 have an IVS3 that is 9 nt shorter than for IgG1, IgG2, and IgG6.

In summary, the overall sequence comparison (Figs. 1 and 2), the cladogram comparing the individual domains





**Fig. 5** Porcine IgG lower hinge splice variants and those missing C<sub>H1</sub> domains. The potential splice acceptor sites (ag) in the C<sub>H2</sub> domain are underlined

(Fig. 4), and the detailed analysis reveal several features of the porcine C<sub>γ</sub> genes. First, they appear to belong to three groups; IgG1, IgG2, and IgG4 comprise one group, IgG5 and IgG6 another, and the ancestral-like IgG3 is in its own group. Second, the C<sub>H1</sub> domain is the most conserved and very similar for IgG1, IgG2, IgG4, and IgG6 suggesting they may have duplicated from a common ancestral CH1 domain. The genetic hinge of IgG2, IgG4<sup>a</sup>, and IgG1<sup>a</sup> are identical, whereas IgG1<sup>b</sup> and IgG4<sup>b</sup> share a common hinge suggesting a type of genomic exon shuffle during diversification of porcine C<sub>γ</sub> genes. Finally, the overall sequence of the C<sub>H2</sub>–C<sub>H3</sub> domain complex, i.e., the Fc region, is most diagnostic of the six putative subclasses.

IgG3 is the most divergent C<sub>γ</sub> gene of swine sharing little sequence homology with any other C<sub>γ</sub> gene in any domain except with the C<sub>H1</sub> of IgG5<sup>b</sup> (Fig. 4A). Most obvious is the extended genetic hinge of IgG3, the upper portion showing some similarity to that of IgG1<sup>b</sup>. Cloning of genomic DNA encoding IgG3 failed to provide evidence that the extended hinge of porcine IgG3 was due to the use of multiple hinge exons as is the case for long-hinged, human and bovine IgG3 (Lefranc and Lefranc 2001; Rabbani et al 1997). Like human IgG3, the middle hinge of IgG3 allows for additional inter-heavy chain disulfide bonding.

*Alternative splicing may increase the number of expressed IgG variants* Figure 5 shows that in splicing the hinge exon to the C<sub>H2</sub> domain, there are either two or three splice acceptor sites in the 5' region of C<sub>H2</sub> (ag; dependent on the putative subclass) that are situated to yield in-frame products although the alternative sites are noncanonical by

their lack of a polypyrimidine tract (ppt). Nevertheless, transcripts using these sites were frequently found. It is recognized that if alternative sites are in close proximity to a canonical ppt, they can also be used (C.M. Stolfus, personal communication). Alternatively spliced transcripts were most common in IgG5<sup>b</sup> in which the consensus splice acceptor site was frequently mutated. When 25 were sequenced, 12 spliced the hinge to an acceptor site with a canonical ppt 50 nt downstream (a position not shown in Fig. 5), but that results in a sterile transcript. Only five of the 25 transcripts used the first C<sub>H2</sub> acceptor site, while eight used the second 12 nt downstream (Fig. 5). Both IgG5<sup>b</sup> and IgG6 transcripts have also been recovered that totally lack the C<sub>H1</sub> domain (Fig. 5).

*Porcine IgG3 is most likely to activate complement* The most attractive model describing the ionic C1q–IgG interaction involves Asp<sup>270</sup> and Lys<sup>322</sup> of all human IgGs (154 and 206 in swine; Fig. 1) that form salt bridges with Arg<sup>129</sup> and Glu<sup>162</sup> of the globular human B subunit of C1q, respectively (McCall and Easterbrook-Smith 1989; Emanuel et al. 1982; Burton et al. 1980). In addition, Tyr<sup>278</sup>, Lys<sup>326</sup>, Pro<sup>329</sup>, Pro<sup>331</sup>, Glu<sup>333</sup> of human IgG1 (162, 210, 213, 215, 217, respectively, for swine; Fig. 1) are also involved in the interaction (Gaboriaud et al. 2003). Based on these motifs, only IgG3 is conserved at position 270 (154 in swine) where Glu represents a conserved replacement for Asp in human IgG1. Other pig IgG isotypes show major changes (His or Asn) at this critical site. While in swine the amino acid residues at 162, 206, and 215 (Tyr, Lys, and Pro) are conserved, radical changes were observed at position 210 in IgG1<sup>a</sup>, IgG1<sup>b</sup> (Val), and IgG5 (Glu) and at position 213 in IgG3 (Leu instead of Pro). There is also a radical change at position 217 in all the pig IgGs (Thr instead of Glu; Fig. 1). Our analysis also considered the hinge “spacer” effect in binding to C1q (Gaboriaud et al. 2003; Dall’Acqua et al. 2006). IgG3 has the potential for three inter-heavy chain disulfide bonds, while others have only had two cysteine residues that may form inter-heavy chain disulfide bonds (Fig. 1). Two may be insufficient to provide the rigidity and space to allow effective complement activation.

*Specificity for Fc receptors predicted from sequence motifs* The overall structure of the human Fc<sub>γ</sub>R is remarkably similar, and the critical residues involved in binding to the IgGs are highly conserved (Radaev and Sun 2001). When the pig Fc<sub>γ</sub>R were compared to their human orthologues, most critical residues in Fc<sub>γ</sub>R that interact with human IgG1 were conserved or replaced by nonradical substitutions (amino acid residues 90, 113, 120, 129, 131–132, 155, 158; data not shown). However, some non-conserved replacements were seen (amino acid residues

134, and case of Fc $\gamma$ RIII, 160 and 162). The interface between Fc $\gamma$ RIII and the  $\alpha$ -chain of Fc is dominated by hydrogen-bonding interactions, whereas hydrophobic interactions occur primarily between the Fc $\gamma$ RIII and the  $\beta$ -chain of Fc.

When the lower hinge and C<sub>H</sub>2 domain of the porcine IgGs were compared to their human orthologues, we observed that all the interaction sites of the  $\alpha$ -chain were fully conserved, although the critical interaction site for the  $\beta$ -chain in the lower hinge differed remarkably among the putative porcine subclasses. Only the sequence in IgG3 (positions 119, 120) is conserved (Fig. 1). While some subspecies have nonradical substitutions that should not alter binding (IgG1 and IgG5 have Val-Ala instead of Leu-Gly), the Gly-Pro change in IgG4 and IgG6<sup>a</sup> and the Ser-Pro change in IgG2 and Gly-Asn in IgG6<sup>b</sup>, may prevent hydrophobic interaction with Fc $\gamma$ Rs and lower the affinity for the  $\beta$ -chain (Fig. 1). All pig IgG subspecies except IgG3 have Glu instead of Leu at position 118 which may prevent hydrophobic interaction in this “hot spot”. The other interaction sites for the Fc $\gamma$ R  $\beta$ -chain (213,216; Fig. 1) are conserved or have nonradical substitutions. For example, IgG3 has Leu instead of Pro at site 213 (Fig. 1). Based on differences in the hinge length of porcine IgG subspecies, we speculate that IgG5<sup>a</sup>, IgG5<sup>b</sup>, and IgG6<sup>a</sup> may bind poorly to Fc $\gamma$ Rs compared to the other porcine subspecies because their lower hinges are shorter by one to three amino acids.

A comparison of FcRn  $\alpha$ -chains from several species, among them the pig, has been performed (Kacskovics et al. 2006) using the crystallographic analysis of the rat FcRn–heterodimeric Fc complex as a guide (Martin et al. 2001). The study found Glu117, Glu118, Glu132, Trp133, Glu137 of the rat FcRn to be highly conserved and that mutation at these sites resulted in a significant loss of binding affinity. These important residues are conserved in pig FcRn. Fig. 1 shows that all the important residues in the Fc region (Ile 137, His 194–Gln 195, His 321) are fully conserved in all porcine IgGs except IgG5<sup>a</sup>, IgG5<sup>b</sup>, and IgG6<sup>a</sup> (Fig. 1). Radical replacement at position 195 (an acidic Glu instead of the conserved uncharged polar Gln) in IgG5 and IgG6<sup>a</sup> could prevent association since this residue (Glu) faces Glu 118 in FcRn. At the slightly acidic condition at which FcRn and IgG/Fc interact, both Glu residues would be positively charged.

The bacterial-binding protein SpA establishes contact with residues in the Fc region of human IgG that are located in three separate loops of the C $\gamma$ 2–C $\gamma$ 3 interface (135–138, 195–196, and 319–324) (Deisenhofer 1981) (Fig. 1). These motifs also interact with FcRn. These sites are generally conserved among the putative porcine subclasses except in IgG5<sup>a</sup>, IgG5<sup>b</sup>, and IgG6<sup>a</sup> that show a radical replacement at position 195 where the acidic Glu replaces the neutral Gln

(Fig. 1). This may modify interaction with SpA of these porcine isotypes. Predictions about SpG affinity proved difficult. Gln 195 in C $\gamma$  genes is believed to form a polar interaction with Glu 42 of protein G, which means that IgG5 and IgG6<sup>a</sup> that have Glu at 195 should bind with lower affinity. Glu 380 and Glu 382 (272–274 in swine, Fig. 1) are also considered “hot spots” for protein G binding, and this motif (Glu-Pro-Glu) is conserved in all porcine IgGs.

Predictions concerning specificity of porcine IgG subclass proteins for various Fc receptors and susceptibility to proteases are summarized in Table 4.

## Discussion

Data presented advance our knowledge about C $\gamma$  diversity in a mammal with an emerging role in xenotransplantation (Auchincloss and Sachs 1998), cystic fibrosis (Rogers et al. 2008), the generation of humanized antibodies (Waltz 2006; Mendicino et al. 2008), an established role in developmental immunology (Butler et al. 2002, 2005, 2008b) and that is important to the world food supply.

Our initial studies on the C $\gamma$  genes of swine utilized genomic blots and cDNA sequences to reveal a diverse C $\gamma$  repertoire (Kacskovics et al 1994). Here we show that many of the porcine C $\gamma$  genes share similar or identical exons. IgG1, IgG2, IgG4, and perhaps IgG6 appear to have obtained their C<sub>H</sub>1 exon by recent duplication of an ancestral C<sub>H</sub>1 exon that is genomically rearranged to the same hinge as seen in IgG1<sup>a</sup>, the IgG2 alleles and IgG4<sup>a</sup> whereas IgG1<sup>b</sup> and IgG6 were rearranged to two different hinge exons. The hinge used by IgG1<sup>b</sup> is identical to that found in IgG4<sup>b</sup> while the IgG6 hinge is shared with IgG5<sup>a</sup> (Fig. 4B) so that IgG4<sup>a</sup> is essentially “an IgG6 with an IgG1<sup>a</sup> hinge,” whereas IgG4<sup>b</sup> is an “IgG6 with an IgG1<sup>b</sup> hinge.” Given the alternative splicing observed in this study and discussed below, we initially entertained the idea that the shared exons found among the 11 C $\gamma$  genes were “exon chimeras” resulting from transcriptional/splicing events or PCR artifacts. Since we recovered all 11 from genomic DNA together with their introns, the former is unlikely (Fig. 1). However, heavy chain “hybrids” are known, especially after the advent of hybridoma technology (Mihaesco et al. 1988; Kunkel et al. 1969; Birshstein et al. 1980; Colle et al. 1987). The argument that the C $\gamma$  genes described are PCR cloning artifacts is inconsistent with our previous study on PCR-generated VDJ chimeras in the same species (Sinkora et al. 2000). PCR chimeras can be generated in vitro when only two to five different templates are present and are rarely recovered when large numbers of different templates are present as in a normal cDNA

(Sinkora et al. 2000). Most important is that two such chimeras seldom have the same sequence, i.e., the polymerase does not “jump” at the same point. Considering the >9,000 cDNA and ~ 3,000 DNA clones recovered and that the >270 that were sequenced from different variants had identical sequences (Table 2), it is likely that the IgG subclass/allotypic diversity we have described is genuine. The possibility that the sharing of genomic exons represents some form of AID-dependent templated *somatic mutation*, i.e. “gene conversion” is inconsistent with what is known about this process in chicken (Ratcliffe 2006) in which: (a) each mosaic product has a unique sequence, whereas the 184 cDNA and 88 DNA C $\gamma$  genes that were sequenced (Table 2) yielded the same seven sequences that we ascribed to subclasses and allelic variants, (b) the mosaic is confined to a 10- to 300-bp region of a single exon not mixing of exons and (c) templated *somatic mutation* among Ig genes has never been described for constant region genes. Authentic gene conversion is a *genomic event* in yeast and distinct from the vernacular use of the term in immunology textbooks (Janeway et al 2005). Therefore, it is conceivable that some form of this process combined with gene duplication over the 50 million years of evolution in family Suidae could explain the origin of the porcine subclass genes, specifically the “exon shuffle” aspect. Without further information about the IgG subclasses of other contemporary members of family Suidae and/or DNA sequences recovered from fossils, we are more comfortable with attributing the origin of the porcine C $\gamma$  genes to gene duplication that is well documented among the hundreds of members of the Ig supergene family (Hunkapillar and Hood 1989; Williams 1987) with the added feature of “exon shuffle” (Fig. 4A,B) without assigning the latter to a particular mechanism. Differences between exons of actual or putative allelic variants are very small, and we believe are explained by single point mutations in the genome that occurred during or after duplication and exon shuffle.

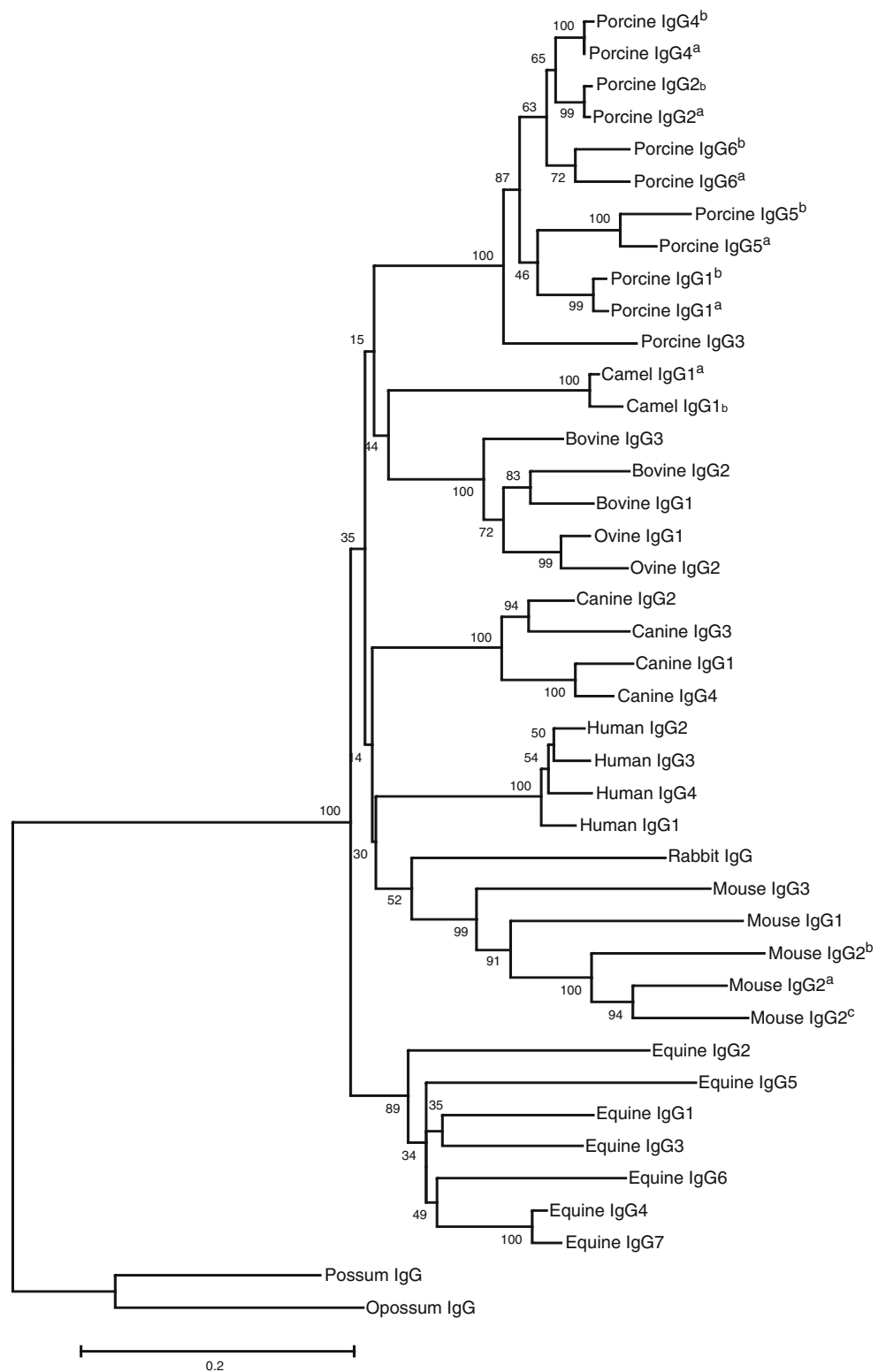
Our analysis of the C $\gamma$  genes of swine and other mammals is consistent with earlier studies suggesting that diversification of IgG occurred after speciation (Kehoe and Capra 1974). Figure 6 shows that the various C $\gamma$  genes of swine group together and are not intermixed with those of other mammals. This pattern is seen throughout the cladogram for the IgGs of other species. The overall pattern in Fig. 6 is generally consistent with accepted taxonomic schemes in that the Artiodactyls (swine, cattle, camel) group together while rabbits are grouped with rodents. Noteworthy is that porcine IgG3 appears to be most similar in sequence to the stem IgG of all mammals. Data of Fig. 6 also show that even when intron sequences are not included as in Fig. 2, the putative alleles of the putative swine IgG subclasses group together. Figure 6 also shows that “name homologs” of IgG subclasses in other mammals do not

share an evolutionary homolog indicating the danger of extrapolating function from a subclass in one species to one of the same name in another.

Despite their apparent species-specific divergence, IgG subclass proteins in different mammals appear to perform similar functions, and certain functions are associated with different subclasses in a number of these mammals (Burnett et al. 1989; Lefranc and Lefranc 2001; Butler 1983; Clark 1997). As a first step in determining which porcine IgG subclasses have analogous behavior to those in other mammals, we analyzed the sequences of the various porcine C $\gamma$  genes to identify motifs that might predict their effector activities. Since our predications are based only on sequence motifs, they await confirmation when purified subclass proteins become available for this species. In any case, our predictions show that all porcine IgGs except IgG5 and IgG6<sup>a</sup> should be able to bind Fc $\gamma$  receptors, but some degree of affinity differences are likely (Table 4). FcRn protects IgG from degradation and is responsible for the long serum half-life of certain subclasses of IgG as well as mediating transepithelial transport (Roopenian and Akilesh 2007). Based on sequence motifs, we predict that IgG5 and IgG6<sup>a</sup> would have the shortest plasma half-life and would be least likely to be transported through epithelial cells such as the acinar epithelial cells of the mammary gland (Butler 1974; Brambell 1971). Since SpA and SpG bind IgG to the same or similar motifs as FcRn (Deisenhofer 1981; Nezlin and Ghetie 2004), all porcine IgG isotypes should bind to SpA and SpG although with different affinity.

C1q shows marked differences in its binding to the CH2 domain of different human IgG subclasses (McCall and Easterbrook-Smith 1989; Duncan and Winter 1988; Idusogie et al. 2000). Furthermore, the Fab/Fc orientation, controlled at least partly by the hinge region, is a critical factor in recognition of C1q (Gaboriaud et al. 2003) as is the importance of the hinge as a spacer (Dall’Acqua et al. 2006). Therefore we predict that pig IgG3 has all the necessary features to activate complement (Table 4). While differences in the ability of the porcine IgG subclasses in complement activation have been suggested, previous investigators identified the IgG subclasses involved using commercial mAbs of untested specificity (Crawley and Wilkie 2003). Our predictions concerning the function of IgG3 in complement activation, affinity for Fc $\gamma$ Rs (Table 4), and its ancestral nature (Fig. 6) is interesting because IgG3 comprises >60% of total IgG transcripts in the ileal Peyer patches (IPP) and mesenteric lymph nodes of late term and newborn fetuses but expression of IgG3 in the IPP precipitously drops in the first 4–6 weeks postpartum (Butler and Wertz 2006). We therefore speculated that IgG3, like those from marginal zone B cells (Lopez-

**Fig. 6** Phylogenetic tree constructed from the deduced amino acid sequences of the C $\gamma$  genes of various placental mammals and those of two marsupials mammals



Carvalho et al 2005) may be an important part of the antigen-independent preadaptive response in newborn piglets and thus important in the innate and pre-adaptive immune response of swine.

Splice variants within the Ig supergene family are well known including swine. The allotypic variants of porcine IgA differ because of a mutated splice acceptor site in C $\mu$ 2 resulting in a four amino acid hinge deletion (Brown et al.



1995) and in *in vitro* studies,  $C_{\mu 1}$  can be spliced to either  $C_{\delta 1}$  or  $H_{\delta}$  (Zhao et al. 2003). Alternative porcine  $C_{\gamma}$  transcripts are not unusual since all but five of the 25  $IgG5^b$  transcripts we sequenced were alternatively spliced. Although it remains to be shown whether any are expressed, there are examples of truncated heavy chain proteins such as in human heavy chain disease in which the  $C_{\gamma}$  chain lacks the  $C_{H1}$  domain (Franklin et al. 1964; Osserman and Takatsuki 1964). The 5 S protein described 40 years ago from newborn piglets (Franek and Riha 1964) may be the product of an  $IgG5^b$  alternative transcription (Fig. 5). Because the putative cysteine involved in the L–H disulfide bond is found at position 14 in the  $C_{H1}$  domain, porcine splice variants lacking a  $C_{H1}$  domain may encode “heavy chain only antibodies” as in camelids (Hamers-Castermann et al. 1993) unless the light chain is noncovalently associated as in the case of human  $IgA2$  (Grey et al. 1968; Marquart and Deisenhofer 1982) and some heavy chain disease proteins (Franklin et al. 1964; Osserman and Takatsuki 1964).

Conclusions regarding allotypic variants are based on: (a) relative occurrence (Tables 2 and 3), (b) genomic sequences in which introns sequences are included (Fig. 2), (c) the absence of some variants in genomic DNA, and (d) the sequence similarity of their  $C_{H2}$ – $C_{H3}$  domain complex, i.e., Fc. Confirmation of our conclusions awaits locus maps and breeding studies.

The complexity of the  $IgG$  system of swine and the inability to physico-chemically purify porcine  $IgG$  subclass proteins, challenges the process of preparing subclass-specific mAbs. The use of vectors that allow *in vitro* expression of each variant will be needed to provide purified subclass proteins to: (a) test the specificity of existing mAb, (b) prepare the next generation of mAbs and, (c) test the functional predictions of Table 4. Since the Fc regions ( $C_{H2}$ – $C_{H3}$ ) of the porcine  $C_{\gamma}$  genes are subclass-specific regardless of putative allotype differences (Fig. 4C,D), they offer the best targets for the preparation of subclass-specific mAbs.

Domesticated farm animals have been randomly inbred and in the case of NIH minipigs, selectively inbred to establish class I homozygous lines suitable for transplantation research (Auchincloss and Sachs 1998). This process has also impacted  $Ig$  genetics. We have previously shown that NIH minipigs and Meishans from a single herd were homozygous for  $IgA^a$  (Navarro et al. 2000a). This now appears only to be true for one strain of Meishans, i.e., it is founder related (Table 3). Most outbred swine are  $IgG1^a/IgG1^b$  and  $IgA^a/IgA^b$  heterozygotes (Butler and Wertz 2006; Navarro et al. 2000a), and the gene deficiencies we observed were only observed in swine homozygous for  $IgG1^a/IgA^a$  (Table 3). Considering the >270  $C_{\gamma}$  gene sequences recovered from DNA that were examined, it is

unlikely that random sampling would have missed any variant of  $IgG2$  or  $IgG4$  in the animals selected. However, data presented in Table 3 are only an example of  $C_{\gamma}$  gene deletions; extensive population studies are needed to determine if this phenomenon is widespread. Deletion of complete  $C_H$  genes in humans is known albeit uncommon, and individuals often present with recurrent infections or other abnormalities (Migone et al. 1984; Terada et al. 2001; Pan and Hammarstrom 2000). However,  $C_H$  gene and haplotype deletions in healthy individuals have also been reported (Lefranc et al. 1982; Olsson et al. 1993). Since our predictive analysis of complement,  $Fc\gamma R$  and  $FcRn$  binding did not identify any unique function for porcine  $IgG2$  and  $IgG4$ , their loss may confer no selective disadvantage.

The data presented represent a major advance in the characterization of  $IgG$  diversity in swine, a model to explain the evolutionary diversification of  $IgG$  subclasses in all mammals and the predictive role of different  $IgG$  subclasses in the porcine immune system. The studies also highlight the potential difficulties in the preparation of subclass-specific mAbs and the danger of extrapolating subclass function from one species to another. We hope this study will encourage further immunogenetic, breeding and functional studies to test the predictions arising from this work.

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