New polymorphism of the influenza virus resistance *Mx1* gene in Iberian domestic pigs

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Abstract

Mx1 (Myxovirus (Influenza virus) resistance 1, interferon-inducible protein p78) gene has been implicated in the resistance to a wide range of RNA viruses including influenza A in several species such as *Sus scrofa*. In the present study a 28-bp deletion in exon 14 of the *Mx1* gene has been identified in Iberian domestic pigs but not in other domestic breeds neither in wild boars. The mutation produces a frameshift giving a protein with 6 amino acid substitutions and the extension of the C-terminal region with additional 20 amino acids with respect to the wild type MX1 protein. The new allelic polymorphism affects the antiviral domain of the MX1 protein and therefore might impact its anti-influenza virus activity. It has been demonstrated that polymorphisms in the *Mx1* murine locus, affect the survival rate of mice upon experimental infection with influenza virus. It might be possible to improve the innate resistance of pigs to influenza virus infection by determining the porcine *Mx1* alleles with more potent antiviral activity and genetically selecting animals bearing such alleles.
Keywords: *Mx1*, polymorphisms, Iberian pigs, Influenza resistance

Introduction

It has been clearly demonstrated that MX proteins are major effectors molecules preventing influenza-infected animals from severe disease or death (Grimm et al., 2007; Iwasaki and Pillai, 2014). Thus, most of inbred strains of mice carry defective alleles of the *Mx1* gene and are highly susceptible to mouse adapted strains of influenza (Staeheli et al., 1988). Porcine *Mx1* has been mapped in chromosome 13 (Rettenberger et al., 1996). Mx1 is mapped on SSC13 214,988,891-215,011,285, a QTL associated with "spontaneous cell proliferation" immune response trait has been previously detected at 107cM on SSC13 (http://www.animalgenome.org/QTLdb/).

Thus, *Mx1* might be a positional and biological candidate gene for this QTL. The porcine *Mx1* gene has more than 25 kbp and contains 14 exons. The coding sequence has 1989 bp and extends from position 101 of first exon to 219 of exon 14. MX1 protein has 663 amino acids; it accumulates in the cytoplasm (Horisberger and Gunst, 1991) and has anti-influenza activity (Arnheiter et al., 1990). The analysis of the *Mx1* gene sequence has allowed the identification of polymorphisms in most of the species resulting in differential antiviral activity of the distinct isoforms (Haller et al., 1987). In pig, two main deletions have been identified;

3bp in exon 13, which produces the lost of the serine residue at amino acid 565 (AB259856:g.1778_1810del3, p.Ser565del), and 11bp in exon 14, which leads to a frameshift with 8 amino acid substitutions and a 23 amino acid extension at the C-terminal region of the MX1 protein (c.2131_2141del11, referred to GenBank DQ095779; p.Arg656GlnfsX31), where the antiviral effect is located (Morozumi et al., 2001; Nakajima et al., 2007). Mutations in this region in mouse have been related to

increased susceptibility to influenza virus infection (Staeheli et al., 1988).

Iberian pigs constitute an autochthonous Spanish breed with high economic and social impact in Spain. As free-ranged reared animals, these pigs frequently share humid areas with wild aquatic birds which are considered the main reservoir of influenza (Scholtissek et al., 1985). Serological studies have shown an increased prevalence of influenza infections in Iberian pigs (more than 90%) than in other domestic breeds of pigs (author's unpublished results). In addition, occasional transmission of human influenza viruses to pigs and vice verse has been reported (Hay et al., 2001) and the recent outbreak of new influenza A virus illustrates the zoonotic origin of influenza pandemics.

Materials and Methods

A collection of 652 genomic DNA samples from blood was examined. Samples were collected from wild boars from Spain (n=62), Iberian pigs (n=333) corresponding to Torbiscal (n=153), Guadyerbas (n=38) and other Iberian strains (n= 142), and from other breeds of domestic pigs including Landrace (n=70), Duroc (n=39), Large White (n=21), Meishan (n=16) and a synthetic Chinese line (n=80). Total DNA was extracted using a conventional phenol-clorophorm precipitation protocol. As a first step in the characterization of the Iberian *Mx1* gene, we searched for polymorphisms in the exons 13 and 14. Two regions of 458 bp of *Mx*1 exon 13 and 450 bp of exon 14 were amplified by PCR from 40 individuals (Iberian=25, Duroc=7, Landrace=5; Wild Boar=5). Primer pairs used were 5´-ATTTGCTCCCTGGCCACTGTTTT-3´ and 5´-TAGAGGCTTACCGGGGGCTGAA-3´ for exon 13 and 5´-TCGGCAAGCGCATCTCCA-3´ and 5´-GGCGGGGCTCATTCAAGTAAA-3´ for exon 14. The amplification conditions were 94°C for 5min, followed by 40 cycles consisting of 94º for 30sec, 58°C for 45sec, and 72°C for 45sec, and finally 72°C for 10min. DNA fragments were cloned in a pGEM-T plasmid (promega) and sequenced in both directions with the Dye-Terminator Cycle Sequencing3.0 kit in an ABI377 automatic sequencer (Applied Biosystems).

Results

The aim of the present study was to determine in the Iberian pig breed the sequence of the *Mx1* exons 13 and 14 where the main polymorphisms in porcine *Mx1* alleles have been described. We have counted three synonymous SNPs in exon 13 (g.83143C>T; g.82987G>A; g.82999C>T, referred to GenBank AB259856), a 4bp-insertion in the splicing zone before exon 14 (g.86161_86164dupCCTT), and a 28bp-deletion (c.2129_2156del28, referred to GenBank DQ095779), in exon 14 (Figure 1). This new deletion extends 1 nucleotide upstream and 16 downstream the previous 11bp-deletion and results in a frameshift which is expected to encode a protein with 9 amino acid substitutions and a 20 amino acid extension compared to the wild type (p.Arg655GlufsX29). Most of the mutations localized in C-terminal domain of MX proteins appear to abolish antiviral effect (Morozumi et al., 2001; Nakajima et al., 2007). Therefore, it could be expected that in this new isoform the anti-influenza activity could be also affected. A deeper analysis of the 28bp-deletion effect on MX1 function will be performed in future studies.

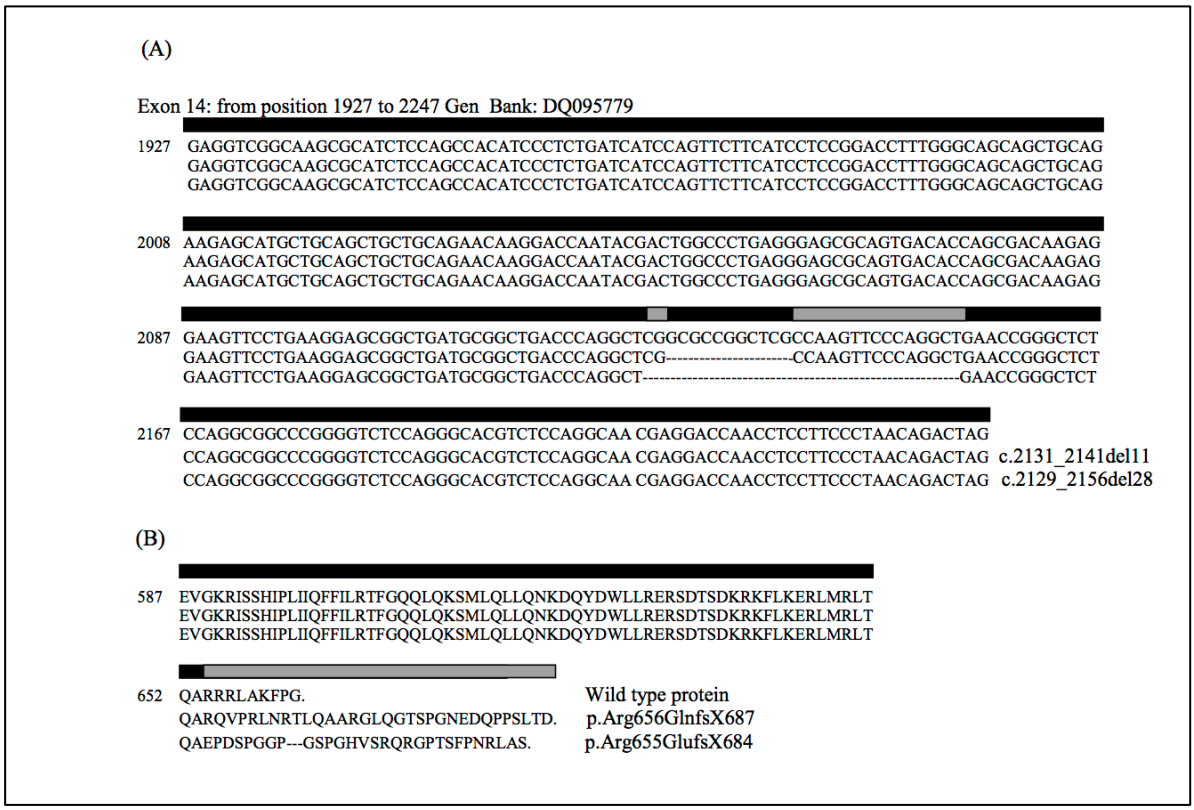

Figure 1: (A) Nucleotide sequence of the exon 14 of *Mx1* wild type, 11bp-deletion and 28bp-deletion. (B) Amino acid sequence corresponding to exon 14 wild type, 11bp-deletion and 28bp-deletion.

A genotyping protocol was implemented for the 11bp and 28bp deletions on exon 14 by capillary electrophoresis. This protocol was applied for the total 652 pig samples. The 28bp-deletion has only been found in two lines of Iberian breed: *Torbiscal and Guadyerbas* (Table 1) with a frequency of 6 and 18%, respectively. Those populations were in Hardy-Weinberg equilibrium (Table 1). Unexpectedly, the 28bp-haplotype has not been found in Iberian pigs belonging to other strains (n=139) in which, however, the 11bp-deletion was present (2%). This could be explained by the coancestry of Guadyerbas and Torbiscal, given that both belong to a conservation program carried out in *"El Deheson del Encinar"* (Spain) back in the 40s (Miguel Toro, 2002) . The 11bp-allele has been identified in Landrace (43%) and Duroc (18%) pigs and in wild boars (11%) but not in Large White, Meishan and synthetic Chinese line pigs. These results do not agree with previous reports where wild boars from Walonia (Belgium) were not found to have the mutated allele and, conversely, a small proportion of Large White pigs had the 11bp-deletion (Palm et al., 2007). Geographic genetic heterogeneity of the animals might be the cause of this discordance (Shin et al., 2015).

		Allelic Frequency			Genotype Frequency					H-W	
Population	**N**	**wt**	**del11**	**del28**	**wt/wt**	**wt/del11**	**wt/del28**	**del11/ del11**	**del28/ del28**	**χ2**	**p***
IBERIAN											
TORBISCAL	153	0.94		0.06	0.88		0.12		0	0.74	>0.1
GUADYERBAS	69	0.82		0.18	0.67		0.33		0.03	3.69	>0.1
OTHERS	142	0.98	0.02		0.98	0.02		0		1.31	>0.1
WILD BOAR	62	0.89	0.11		0.79	0.2		0.01		6.7	0.03
DUROC	39	0.82	0.18		0.67	0.3		0.03		4.4	>0.1
LANDRACE	70	0.57	0.43		0.33	0.49		0.18		6.2	0.03
LARGE WHITE	21	1			1						>0.1
MEISHAN	16	1			1						>0.1
ZUMU	80	1			1						>0.1

Table 1: Frequency of distributions of *Mx1* genotypes and alleles in *Sus scrofa* populations. Wt (wild type), del11 (allele *Mx1* 11bp-deleted) del28 (allele *Mx1* 28bp-deleted)

Conclusion

The existence of polymorphisms in *Mx1* gene suggests that it could be possible to select animals less susceptible to viruses. Consequently, lower rates of influenza virus infection would mitigate economic losses in pig husbandry and reduce the chances of generation and transmission of new potentially pandemic viruses to humans.

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