Expression profile and association analysis of the porcine DQB1 gene with peripheral blood T lymphocyte subsets

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Abstract

Major histocompatibility complex (MHC) class II molecules play an important role in immunology by presenting antigens to T lymphocytes. As a key member of the MHC class II gene family, the DQB1 gene is involved in interacting with T cells in immune reactions. This study was designed to screen variations in exon 3 of the DQB1 gene in Large White, Landrace and Songliao Black pigs, and to investigate the association between DQB1 gene polymorphisms and peripheral blood T lymphocyte subsets in Large White samples. In addition, the spatial transcription profile of the DQB1 gene was examined, and the effect of gene polymorphisms on its mRNA level was further analyzed. One missense mutation in exon 3 of the DQB1 gene was first identified by DNA pool sequencing, and samples were genotyped by using the polymerase chain reaction-restriction fragment length polymorphism method. Statistical analysis indicated that the DQB1 genotype was significantly associated with CD4+CD8−, CD4−CD8+ and CD4+/CD8− indexes (P<0.05). Analysis of mRNA distribution showed that DQB1 was predominantly expressed in the spleen. However, no significant differences in splenic DQB1 mRNA levels of pigs with different genotypes were observed (P>0.05). These results suggest that the DQB1 gene may be a promising candidate gene for porcine disease resistance.

Introduction

Improvement in porcine production traits has been achieved through continuous selection in the past decades; however, the resistance of swine has not been substantially enhanced. It is currently not easy to control infectious diseases that continue to cause a high mortality rate in pigs, which in turn lead to huge economic losses in the swine industry. Traditional methods such as vaccination and antibiotic therapy are effective to some extent, but cannot thoroughly resolve the problem of porcine diseases. Therefore, it is essential to take measures to enhance disease resistance in pigs from the perspective of genetics; for example, by incorporating suitable immune traits into its breeding strategy that would facilitate in coping with the spread of pathogens (Henrion et al., 2006; Mach et al., 2013).

T lymphocytes (T cells) are distributed in the peripheral blood and lymphoid organs and play a prominent role in the cellular immunity of animals. T lymphocytes comprise several subsets such as CD4+CD8− and CD4−CD8+ cells, which exert multiple biological functions in immune reactions (Saalmüller et al., 1999). T lymphocyte subsets in blood generally vary in numbers and ratios when pigs are infected by pathogens (Shimizu et al., 1996; Rodríguez-Ropón et al., 2003). Since T lymphocyte subsets in blood are capable of monitoring immune responses, these can be used as a criterion in the evaluation of the health condition of specific organisms. In humans, Hall et al. (2000) reported that the variation in peripheral blood T lymphocyte subset numbers is genetically controlled. The number of blood T lymphocyte subsets in pigs has been reported to be of moderate or high heritability (Clapperton et al., 2009; Fiori et al., 2011), indicating that these are also genetically regulated. Moreover, genomic studies involving blood T lymphocyte subsets in swine have been conducted using quantitative trait loci mapping analysis and genome-wide association studies (Lu et al., 2011, 2012). Accordingly, T lymphocyte subsets in peripheral blood could become favourable immune traits in pigs.

The major histocompatibility complex (MHC), which consists of class I, class II and class III subgroups, plays an essential role in immunology. Investigating genetic variations in various immune parameters may promote studies on host resistance. It is conceivable that some immunological traits are genetically controlled, and even directly regulated by specific immune genes. A quantitative trait locus located in the MHC class II cluster regulates human CD4+ T cell numbers (Ferreira et al., 2010). Furthermore, the MHC class II region is reported to influence blood CD4+ T lymphocyte counts in macaques (Aarmink et al., 2011). Therefore, MHC class II genes might be involved in the regulation of blood T lymphocyte subsets in pigs. Because the β1 domain of MHC class II molecules presents the processed peptide to T cells, polymorphisms in exon 2 that encodes this domain have been detected in pigs (Shia et al., 1995). The β2 domain of MHC class II molecules binds to the CD4 molecule on the surface of T cells (Vignali et al., 1992; Bondinas et al., 2007), which in turn contributes to the proliferation of helper T lymphocytes. Although exon 3 participates in encoding the β2 domain (Scott et al., 1991; Lunney et al., 2009), polymorphisms in this region of MHC class II genes have rarely been detected in pigs. As a main member of MHC class II molecules, DQ is also capable of interacting with T lymphocytes. DQ is a heterodimer consisting of α and β chains, and the β chain is encoded by the DQB1 gene in swine (Ho et al., 2009). Consequently, the aim of this study was to detect single nucleotide polymorphisms (SNPs) in exon 3 of the DQB1 gene in Large White, Landrace and Songliao Black pigs, and further analyze the association...
between the DQBI genotype and peripheral blood T lymphocyte subsets in Large White samples. Moreover, the transcription profile of the DQBI gene was quantified in seven porcine tissues, and mRNA levels were compared among pigs with different DQBI genotypes.

Materials and methods

Experimental samples and trait measurement

Ear tissue samples were gathered from three swine breeds, including Large White (n=382), Landrace (n=84) and Songliao Black (n=90), to detect genetic variations in the DQBI gene. The pigs were reared under the same indoor and feeding conditions at the experimental farm of Chinese Academy of Agricultural Sciences (Beijing, China). The association analysis was investigated only in 63 litters Large White piglets from 16 sires and 63 dams. The 382 21-day-old Large White pigs were only treated once by inoculating with classical swine fever vaccine to trigger an immune response, and then blood samples were immediately immersed in liquid nitrogen and stored at -80°C. The pigs used for gene transcription analysis were slaughtered at the age of 35 days (with an average weight of 10.15±0.63 kg), and tissue samples were adjusted using the Bonferroni correction at a significance level of \( p = 0.05 \).

DQA1 and peripheral T lymphocyte in pigs

Total RNA was extracted from porcine tissues using the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). Afterwards, the quality of total RNA was assessed by 1% agarose gel electrophoresis to ensure that the RNA samples were intact. Total RNA was reverse transcribed into the first-strand cDNA using a 20 μL volume following the instructions of the PrimerScript® RT Reagent Kit (Takara Biotechnology Co., Ltd).

To determine the expression levels of DQBI, quantitative real-time PCR (qPCR) was conducted using the LightCycler® 480 II system (Roche Diagnostics GmbH, Mannheim, Germany). Primer3 was designed to quantify the mRNA level of DQBI (Table 1), and primers for the GAPDH gene were used from Erkens et al. (2006). The 20 μL reaction system contained 10 μL of 2×SYBR green I mixture, 10 pmol of each primer, and 1 μL cDNA. qPCR was amplified at 95°C for 10 min, followed by 40 cycles at 95°C for 10 s, 59°C for 10 s, and 72°C for 10 s. All qPCR reactions were performed in triplicate, and relative mRNA levels were calculated by using the 2^{-ΔΔC_t} method (Schmittgen and Livak, 2008).

Data analysis

The association between the DQBI gene polymorphism and peripheral blood T lymphocyte subsets was analyzed using the procedure MIXED of SAS 9.0 software. The statistical model was as follows:

\[
y = \mu + Xb + K\alpha + Z\alpha + e
\]

where \( y \) is the vector of phenotypic values; \( \mu \) is the overall mean; \( b \) is the vector of fixed effects including genotype, sex and sample batch; \( h \) is the vector of random litter effects; \( a \) is the vector of random additive polygenic effects; \( X \), \( K \) and \( Z \) are incidence matrices for \( b \), \( h \) and \( a \), respectively; and \( e \) is the vector of random residual effects. The variances of \( a \) and \( e \) were defined as \( var(a) = 4\sigma_a^2 \) and \( var(e) = I\sigma_e^2 \), where \( A \) is the numerator relationship matrix constructed from pedigree; \( I \) is the identity matrix; \( \sigma_a^2 \) is the additive polygenic variance; and \( \sigma_e^2 \) is the residual variance. Multiple comparisons were adjusted using the Bonferroni correction at a significance level of \( \alpha = 0.05 \).

![Table 1. Primers for exon 3 of the porcine DQBI gene.](image)

| Name | Primer sequence (5’-3’) | Tm, °C | Product size, bp | Application |
|------|------------------------|--------|-----------------|-------------|
| Primer1 | F:GGGGAGACTTGGCTTGTTGTT R:GTCCGGGGACAAAGAGTGAAG | 58 | 441 | SNP identification |
| Primer2 | F:ATCCAGGACCTGGGAGTTGC | 58 | 231 | SNP genotyping |
| Primer3 | F:AGGGAGGAGGGAGGAGGC | 59 | 136 | mRNA expression |

SNP, single nucleotide polymorphism.
Results and discussion

Detection of polymorphism in exon 3 of the DQB1 gene

Only one mutation was identified in exon 3 of the DQB1 gene by sequencing, and this novel nucleotide substitution (GenBank NM_001113694.1: c.692A>G) leads to an amino acid change (p.Asn214Ser). The variation could be identified using restriction enzyme BanII. Three genotypes, designated as AA (231 bp), AG (231/180/51 bp) and GG (180/51 bp), were detected by the PCR-RFLP method (Figure 1). Various genomic variants in exon 2 of the DQB1 gene have been found in multiple swine breeds (Fang et al., 2005; Park et al., 2010), whereas polymorphisms in the exon 3 region have rarely been detected. This polymorphism in exon 3 was identified in Large White, Landrace and Songliao Black pigs, which would promote to better know variations of the DQB1 gene.

The genotype and allele frequencies of the DQB1 polymorphism in our experimental samples are shown in Table 2. Genotypes AA, AG and GG were detected in Large White and Songliao Black pigs, whereas genotype AA was not found in Landrace pigs. Comparison of the distribution of genotype frequencies indicated that AG was the predominant genotype in Large White and Songliao Black populations, whereas that of the Landrace population was genotype GG. In terms of the allele frequency of the DQB1 gene in each breed, the frequency

Table 2. Genotype and allele frequencies of the DQB1 gene in three pig populations.

| Breed (n)       | Genotype frequency (n) | Allele frequency | P* |
|-----------------|------------------------|------------------|----|
|                 | AA                     | AG               | GG | A   | G   |
| Large White (382)| 0.1571 (60)            | 0.5026 (192)     | 0.3403 (130) | 0.4084 | 0.5916 | 0.4325 |
| Landrace (84)    | –                      | 0.0595 (5)       | 0.9405 (79)  | 0.0298 | 0.9702 | 0.1166 |
| Songliao Black (90)| 0.1778 (16)          | 0.5222 (47)     | 0.3000 (27)  | 0.4389 | 0.5611 | 0.5674 |

*P values from the chi-square test for Hardy-Weinberg equilibrium.

Table 3. Association between the DQB1 gene polymorphism and peripheral blood T lymphocyte subsets in Large White pigs.

| Genotype (n) | CD4+CD8– | CD4–CD8– | CD4+CD8+ | CD4–CD8+ | CD4+ | CD4– | CD4+CD8– |
|--------------|----------|----------|----------|----------|------|------|----------|
| AA (60)      | 36.38±0.99 | 15.69±0.65a | 37.34±1.07b | 10.28±0.51 | 25.81±0.89 | 47.93±1.20 | 0.60±0.03a |
| AG (192)     | 35.30±0.60 | 14.36±0.41ab | 40.11±0.65a | 9.90±0.33 | 24.25±0.60 | 50.16±0.73 | 0.53±0.02b |
| GG (130)     | 37.58±0.70 | 13.90±0.48b | 39.49±0.76b | 9.71±0.38 | 24.01±0.68 | 48.63±0.85 | 0.53±0.02b |

Values are expressed as least squares mean±standard error. **Values with different superscript letters in the same column show statistically significant differences (P<0.05).
of allele G was higher than that of the allele A in the three populations. The mutation existed in all the analyzed swine breeds, and the genotype frequency of each breed conformed to the Hardy-Weinberg equilibrium, as indicated by the results of the chi-square test (P>0.05). Differences in genotype frequency in the three populations may have mainly resulted from the diverse genetic background and sample sizes of each breed.

**Association between the DQB1 gene polymorphism and T lymphocyte subsets**

The relationship between the DQB1 gene polymorphism and peripheral blood T lymphocyte subsets was investigated in 382 Large White pigs. Association analysis revealed that the DQB1 genotype was significantly associated with CD4^{+}CD8^{-}, CD4^{-}CD8^{+} and CD4^{+}/CD8^{+} (P<0.05), but not with CD4^{-}CD8^{+}, CD4^{+}CD8^{-}, CD4^{+} and CD8^{+} (P>0.05) (Table 3). The comparison of least squares means of different genotypes showed that individuals with genotype AA carried the highest CD4^{+}CD8^{-} and CD4^{+}/CD8^{+}, and the lowest CD4^{-}CD8^{+} levels.

MHC molecules directly interact with T cells during antigen presentation, and may be functional candidate genes that are involved in the modulation of T lymphocyte subsets. The association between the DQB1 gene polymorphism and peripheral blood T lymphocyte subsets was observed, which suggests that the DQB1 gene polymorphism probably influenced the generation of blood T lymphocyte subsets. It has been reported that CD4^{-}CD8^{+} cell counts are significantly higher in MHC class II-deficient mice (Marusiagi-Galesic and Walden, 1995). In addition, mutants in the β2 domain of MHC class II molecules could alter the numbers of CD4^{+} and CD8^{+} T cells in mice (Riberdy et al., 1998). Thus, it is possible that the missense mutation in exon 3 of the DQB1 gene influences T lymphocyte subset levels in pigs. As porcine MHC regions are highly polymorphic and in close linkage (Lunney et al., 2009), the DQB1 mutation might be linked with potential causal variants that are related to blood T lymphocyte subset traits. Considering the important biological function of the DQ molecule in immunology, the underlying mechanism for the mutation in exon 3 of DQB1 in relation to T lymphocyte subset parameters is worth further investigation. Nevertheless, the association between the DQB1 gene polymorphism and peripheral blood T lymphocyte subsets still requires confirmation in other pig populations.

The components of peripheral blood T cells vary with the health condition; for example, the number of CD4^{+}CD8^{-}, CD4^{-}CD8^{+} and CD4^{+}CD8^{+} cells in the blood of pigs would change after classical swine fever virus infection (Piriou et al., 2003). Hence, changes in blood T lymphocyte subset counts may be used in the diagnosis of disease and become an indicator of the health status of swine. Investigating differences in immune capacity that are influenced by genetic diversity would improve host resistance to pathogens. Polymorphisms in MHC genomic regions are responsive to the different levels of immunocompetence among various individuals (Traherne, 2008); therefore, pigs carrying different MHC genotypes likely possess diverse immunities. Although pigs with genotype AA have different CD4^{+}CD8^{-}, CD4^{+}/CD8^{+} and CD4^{+}CD8^{+} levels compared to those with genotypes AG and GG, whether differences in immunity exist in pigs with the three DQB1 genotypes still requires confirmation in other pig populations.

![Figure 2. Tissue expression pattern of the DQB1 gene in Large White piglets. Values are expressed as mean±standard error.](image1)

![Figure 3. The expression level of DQB1 in the spleen of Large White piglets with the three genotypes (AA, AG and GG). Values are expressed as mean±standard error.](image2)
genotypes requires further investigation. Pigs with different DQB1 genotypes have been shown to present different hematological indexes, which could provide valuable information in determining the relationship between MHC gene polymorphisms and immune traits.

The mRNA expression of the DQB1 gene

To determine the spatial transcription pattern of the DQB1 gene, its mRNA level in seven porcine tissues was quantified by qPCR. The results showed that the relative expression level of DQB1 varied among swine tissues (Figure 2); the highest mRNA level was observed in the spleen, followed by lung, stomach, liver, kidney, heart, and muscle. The spleen is known as a primary site for immune reactions, so the results of transcription analysis clearly indicated that the DQB1 gene plays a critical role in the porcine disease resistance.

Because the DQB1 gene was predominantly expressed in the spleen, the splenic mRNA levels were further analyzed in pigs with different genotypes. qPCR analysis showed that the mRNA level of the AA genotype was 1.07- and 1.30-fold higher than that observed with the AG and GG genotypes, respectively (Figure 3), whereas no significant differences were detected in pigs with the three DQB1 genotypes (P>0.05). A nonsynonymous variation detected in pigs with the three DQB1 genotypes was identified in exon 3 of the DQB1 gene in Chinese pigs. Biochem. Genet. 43:119-125. 

Conclusions

In conclusion, a new missense mutation was identified in exon 3 of the DQB1 gene in three swine breeds and was significantly associated with peripheral blood CD4+ CD8+ CD4-CD8+ indexes in the Large White population. The DQB1 gene was preferentially expressed in the spleen, but no significant effect of the mutation on splenic DQB1 mRNA levels was observed. The results indicated that the polymorphism in the DQB1 gene might be used as a molecular marker for immune traits in pigs.

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