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Fine mapping of a QTL for ear size on porcine chromosome 5 and identification of high mobility group AT-hook 2 (*HMGA2*) as a positional candidate gene

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Abstract

Background: Ear size and shape are distinct conformation characteristics of pig breeds. Previously, we identified a significant quantitative trait locus (QTL) influencing ear surface on pig chromosome 5 in a White Duroc × Erhualian F_2 resource population. This QTL explained more than 17% of the phenotypic variance.

Methods: Four new markers on pig chromosome 5 were genotyped across this F_2 population. RT-PCR was performed to obtain expression profiles of different candidate genes in ear tissue. Standard association test, marker-assisted association test and F-drop test were applied to determine the effects of single nucleotide polymorphisms (SNP) on ear size. Three synthetic commercial lines were also used for the association test.

Results: We refined the QTL to an 8.7-cM interval and identified three positional candidate genes i.e. *HMGA2*, *SOX5* and *PTHLH* that are expressed in ear tissue. Seven SNP within these three candidate genes were selected and genotyped in the F_2 population. Of the seven SNP, *HMGA2* SNP (JF748727: g.2836 A > G) showed the strongest association with ear size in the standard association test and marker-assisted association test. With the F-drop test, F value decreased by more than 97% only when the genotypes of *HMGA2* g.2836 A > G were included as a fixed effect. Furthermore, the significant association between g.2836 A > G and ear size was also demonstrated in the synthetic commercial Sutai pig line. The haplotype-based association test showed that the phenotypic variance explained by *HMGA2* was similar to that explained by the QTL and at a much higher level than by *SOX5*. More interestingly, *HMGA2* is also located within the dog orthologous chromosome region, which has been shown to be associated with ear type and size.

Conclusions: *HMGA2* was the closest gene with a potential functional effect to the QTL or marker for ear size on chromosome 5. This study will contribute to identify the causative gene and mutation underlying this QTL.

Background

Ear size and erectness are important conformation characteristics of pig breeds. Moreover, ear defects have been observed in different species [1,2]. For example, in humans, congenital underdevelopment of the external ear or microtia, affects about 1 in 6000 children in

Europe and the USA, and 1 in 4000 in Japan and China [3]. Pig can be used as an animal model to investigate the underlying mechanisms responsible for the development of the external ear. However, few genetic analyses have been carried out to study ear development in pigs and to our knowledge, only three studies have reported data on the mapping of QTL (Quantitative Trait Loci) for pig ear traits. In 2004, Guo *et al.* (in Chinese) [4] showed the existence of a QTL for ear erectness on pig chromosome 6 or SSC6 (SSC for *Sus scrofa*) in a commercial population. In 2007, Wei *et al.*[5] identified two

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significant QTL with a major effect on ear size on SSC5 and SSC7 in a Large White × Meishan F_2 resource population. Another study involving a White Duroc × Erhualian F_2 intercross also detected two major QTL for ear size on the same chromosomes, which explained more than 45% and 17% of the phenotypic variance, respectively [6]. Although the genetic positions of the QTL were different (51 cM vs. 70 cM on SSC5 and 70 cM vs. 58 cM on SSC7), the 95% confidence interval of both QTL overlapped partly in the two studies. More recently, a missense mutation on PPARD was identified as the causative mutation for the QTL on SSC7, which influences ear size by mediating down-regulation of β-catenin and its target gene expression [7]. Concerning the QTL on SSC5, the confidence interval was too large (11 cM) for positional cloning analysis and the genetic basis of the QTL remains unexplored.

Thus, the study reported here, aimed at investigating the causative gene for ear size on SSC5 by refining the QTL region using higher-density markers on this chromosome and at analyzing in further detail, the association between the three candidate genes located in the refined region with ear size in the White Duroc × Erhualian F_2 resource population and three synthetic commercial lines. The results will contribute to the final characterization of the causative gene or mutation responsible for this QTL.

Methods
Animals and phenotypic measurements
The White Duroc × Erhualian F_2 resource population and phenotypic measurements have been described in detail in Ma et al.[6]. Briefly, the F_2 resource population was obtained by mating two White Duroc sires with small and erect ears to 17 Erhualian dams with large and floppy ears to produce F_1 animals and then by random crossing between nine F1 boars and 59 F1 sows (avoiding sister-brother mating) to produce a total of 1912 F_2 animals. Among the F2 individuals, 1029 were phenotyped by measuring separately the size of both left and right ears after slaughter at 240 days of age as described in our previous report [6].

Three synthetic commercial lines including Sutai (Duroc × Erhualian, n = 173), Sujiang (Duroc × Jiangquhai, n = 105) and Suzhong (Landrace × Erhualian, n = 78) were used in association tests to confirm the association between candidate genes and ear size. Both Erhualian and Jiangquhai indigenous breeds have large ears. Measurement of ear size was done according to the method described by Ren et al.[7], which differs from that of Ma et al.[6]. Briefly, the ears of each animal were fixed and photographed with a ruler placed on the surface of the ear as an internal size reference. The surface of each ear was calculated using the Leica Qwin

software. All experimental animal procedures were conducted according to the guidelines for the care and use of experimental animals established by the Ministry of Agriculture of China.

Microsatellite genotyping
Genomic DNA was extracted from ear and spleen tissues according to the routine phenol/chloroform extraction method and diluted to a final concentration of 20 ng/μL. To increase marker density in the QTL candidate region, microsatellite markers were selected from the SWR453-SW1987 interval on the USDA-MARC SSC5 linkage map [8] or by mining genomic sequence data of the QTL region (Sscrofa 9.2) using the SSRHunter program [9]. Four informative microsatellite markers, namely SSC5P3, SSC5P13, SSC5P16 and SW2003, were genotyped in all F_0, F_1 and 1029 F_2 animals as described previously [10]. All the primers were designed using the online software Primer3 [11] and primer sequences, annealing temperatures, amplicon lengths and number of alleles are shown in Additional file 1: Table S1.

Selection of candidate genes and RT-PCR
Orthologous regions between the human genome and the refined QTL region were detected using the pig-human comparative map in the pig QTL database [12]. Annotated genes present in the orthologous regions were identified with the UCSC genome browser [9] and their functions obtained from the NCBI database [13] or GeneCards [14].

SOX5, HMGA2 and PTHLH were selected from the refined QTL region as genes with related functions. SOX5 encodes a member of the SOX (SRY-related HMG-box) transcription factors family, and a previous study has indicated that it plays a role in chondrogenesis [15,16]. Moreover, mutations in the genes encoding homeobox transcription factors are responsible for the defective development of outer ears [17]. HMGA2 encodes a protein that belongs to the non-histone chromosomal high mobility group (HMG) protein family. HMGA2-LPP fusion protein promotes chondrogenesis by up-regulating cartilage-specific collagen gene expression through the N-terminal DNA binding domains [18]. The presence of HMGA proteins increases the proliferative activity of chondrocytes cultured in vitro [19]. More interestingly, HMGA2-deficient mice develop smaller ears [20] and in dogs, HMGA2 may be involved in differences in the size and type of ears [21]. PTHLH is a member of the parathyroid hormone family which regulates endochondral bone development through regulation of chondrocyte proliferation and differentiation during early bone growth [22]. The Wnt/beta-catenin pathway interacts differentially with the parathyroid hormone-related protein (PTHRP), which acts as a control

of chondrocyte hypertrophy and final maturation [23]. Cartilage is the major component of ear tissue and genes influencing chondrocyte proliferation have been shown to affect ear size [24]. Thus, based on both the physiological roles of *SOX5*, *HMGA2* and *PTHLH* in chondrogenesis and cartilage formation and their expression in ear tissues, we consider that they are important candidate genes for ear size.

RT-PCR was performed to check expression profiles of *HMGA2*, *SOX5* and *PTHLH* in ear tissue with the β-actin gene as an internal control. Ear tissues were sampled from two Erhualian piglets at two days of age, immediately frozen in liquid nitrogen and finally conserved at –80°C. Total RNA was extracted with the Nucleospin RNA kit (QIAGEN, Hilden, Germany) following instructions in the manual. The first-strand cDNA was synthesized from 2 μg of total RNA according to the protocol of Omniscript reverse transcriptase kit (QIAGEN, Hilden, Germany). Primers were designed to amplify regions across the intron/exon boundaries to avoid genomic DNA contamination. Details on primers are in Additional file 1: Table S2. RT-PCR was performed in a 15 μL reaction volume with a touchdown PCR protocol.

SNP identification and genotyping

Genomic DNA of four randomly selected F_1 boars was used to identify SNP in the candidate genes. All the exons and part of the introns were amplified and sequenced with the primers listed in Additional file 1: Table S3. PCR amplification was carried out with a PE9700 thermal cycler (Applied Biosystem Inc., Drive Foster City, USA). After purification with the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany), PCR products were bidirectionally sequenced on an ABI 3130 XL Genetic Analyzer (ABI, Foster City, USA). The sequences of each amplicon were analyzed with SeqMan in the DNAStar software package.

Informative SNP (i.e. with an allele frequency ≥ 25% in four boars) were chosen for further genotyping using the SNaPshot kit (Applied Biosystem Inc., Drive Foster City, USA) according to the manufacturer's protocol. Primers for PCR and SNaPshot extension assays are listed in Additional file 1: Table S4. After purification and denaturation at 95°C for 5 min, the SNaPshot reaction mixture was separated on an ABI 3130XL genetic analyzer for data collection. The SNP genotypes were recorded and analyzed using the GeneScan version 4.0.

Statistical analysis

The linkage map of SSC5 was re-constructed by CRIMAP 2.4 [25]. The physical positions of the seven SNP on SSC5 were determined by the SOAP2 tool based on the reference genome assembly of Sscrofa 10.2 [26]. QTL analyses were carried out with QTL Express [27]. The statistical model was similar to the initial QTL mapping model [6]. Briefly, batch and sex were treated as fixed effects, and carcass weight as a covariate. The 95% confidence interval (CI) was determined using a bootstrap method through 2000 iterations [28]. A multiple QTL model was used to exclude the effects of previously detected QTL on other chromosomes by treating them as fixed effects. The significance threshold was determined with 1000 permutation tests [29].

Because of the extensive between-breed linkage disequilibrium (LD) in the F_2 resource populations, standard association test, marker-assisted association test and F-drop test were performed to analyze the association between the candidate genes and the ear size in the current F_2 resource population as described in Zhao *et al.* [30]. Briefly, the following models were used:

$$\text{Model} \qquad\qquad\qquad\qquad\qquad\qquad 1:$$
$$y_{ijkl} = \mu + batch_i + gender_j + PPARD_k + \beta cw_{ijkl} + e_{ijkl};$$
$$\text{Model} \qquad\qquad\qquad\qquad\qquad\qquad 2:$$
$$y_{ijkl} = \mu + batch_i + gender_j + PPARD_k + \beta cw_{ijkl} + C_a a + C_d d + e_{ijkl};$$
$$\text{Model} \qquad\qquad\qquad\qquad\qquad\qquad 3:$$
$$y_{ijkl} = \mu + batch_i + gender_j + PPARD_k + \beta cw_{ijkl} + CGL_g + e_{ijkl};$$
$$\text{Model} \qquad\qquad\qquad\qquad\qquad\qquad 4:$$
$$y_{ijklg} = \mu + batch_i + gender_j + PPARD_k + \beta cw_{ijkl} + CGL_g + C_a a + C_d d + e_{ijkl};$$

where y_{ijkl} is the observation for ear size; μ is the overall mean for ear size; $batch_i$ is the fixed effect of the ith batch (i = 1, 2, 3, 4, 5, 6); $gender_j$ is the fixed effect of the jth sex (j = 1, 2); $PPARD_k$ is the fixed effect of the kth genotype of *PPARD* (k = 1, 2, 3); CGL_g is the gth genotype effect of the candidate gene (g = 1, 2, 3); β is the regression coefficient for carcass weight; cw_{ijkl} is the covariate of the carcass weight; a and d are unknown additive and dominance effects of average QTL alleles by breed origin from the two F_0 breeds, respectively, and C_a and C_d are the additive and dominance coefficients, as computed from marker information [31]; and e_{ijkl} is the residual. The standard association test was based on an F ratio of residual sums of squares (RSS) for models 1 and 3. An F ratio reaching or exceeding the significant threshold means that the candidate gene is in LD with the QTL or is the QTL. The marker-assisted association test for candidate genes was based on an F ratio of RSS for models 4 and 2. In this case, if the F ratio reaches or exceeds the significant threshold, then the candidate gene locus can account for most of the phenotype variance effect and it is in LD with the quantitative trait nucleotide (QTN) or is the QTN [30]. The marker-assisted association test removed the impact of between-breed LD. The F-drop test was based on

fitting the models 4 and 3. If a candidate gene that is in LD with the QTL is included as a fixed effect, it is expected to absorb part of the between-breed QTL effect, and the F-ratio for the QTL is expected to drop. All the tests were carried out with GridQTL version 2.0.0 [32].

The haplotype phases were inferred using AlphaPhase [33]. The phenotypes were corrected for sex, batch, carcass weight and additive infinitesimal effects using Qxpak 5 [34]. The residuals were regressed on the number of copies of each haplotype allele separately across all the individuals through the GLM function in R. The P values were corrected for the number of haplotypes (Bonferroni correction). The phenotypic variance explained by all the haplotypes was estimated by the ANOVA function in R. The association of *HMGA2* JF748727: g.2836 A > G with ear size in three synthetic commercial lines was evaluated by the GLM procedure of SAS 9.0 (SAS® Institute Inc. Cary, NC). The stratification effect of the population was excluded in the analysis.

Results

Fine-mapping of the QTL

To refine the linkage map of the region previously reported on SSC5, four additional microsatellite markers (*SSC5P3*, *SW2003*, *SSC5P13* and *SSC5P16*) were identified and genotyped on F_0, F_1 and 1029 F_2 pigs. Thus, the complete linkage map with distances between markers in cM is the following: *Acr - 3.0 - SW413 - 40.0 - SWR453 - 5.4 - SSC5P3 - 12.3 - SW1904 - 10.3 - SW2003 - 2.7 - SSC5P13 - 1.6 - SSC5P16 - 10.9 - SW1987 - 21.1 - SW986 - 7.9 - SW995*. This new round of QTL mapping reduced the confidence interval of the initial 11 cM QTL region between markers *SWR453* and *SW1987* to an 8.7 cM interval between markers *SSC5P3* and *SW2003*. One thousand permutation tests were carried out and the bootstrap histograms are shown in Figure 1. The most likely position of the QTL is located in a 8.7 cM region (between positions 55.6 cM and 64.3 cM). Three genes with related functions i.e. *HMGA2*, *SOX5* and *PTHLH* are located within this refined QTL interval. RT-PCR showed that the three genes are expressed in ear tissue (Additional file 2: Figure S1).

Polymorphisms in candidate genes

We detected 19 SNP and four indels in the *HMGA2* gene (accession number: JF748727, Additional file 1: Table S3). None of these polymorphisms were located in the coding region. We found 28 polymorphisms including 27 SNP and one indel in the *SOX5* gene (accession number: JF748729, Additional file 1: Table S3) and among all these polymorphisms, only one synonymous SNP was detected in exon 2. A total of 20
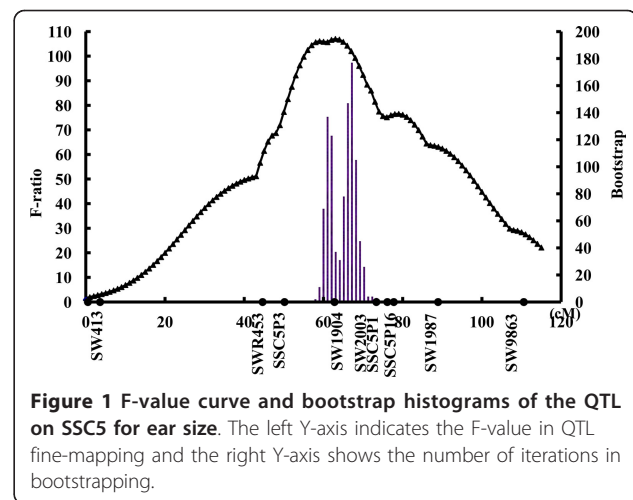

Figure 1 F-value curve and bootstrap histograms of the QTL on SSC5 for ear size. The left Y-axis indicates the F-value in QTL fine-mapping and the right Y-axis shows the number of iterations in bootstrapping.

SNP and one indel were identified in the *PTHLH* gene including one missense mutation in exon 2 (accession number: JF748728, Additional file 1: Table S3). Although a number of mutations were identified in the three candidate genes, most of them were identified in only one animal and thus were not informative and not useful for association tests on the F_2 resource population. Finally, only seven SNP for which the minor allele frequency was > 25% were chosen for further genotyping across the entire White Duroc × Erhualian intercross.

Association of candidate genes with ear size in the F_2 resource population

The physical positions of the seven SNP on the reference genome assembly 10.2 were 32.483 Mb, 32.486 Mb, 32.497 Mb, 46.968 Mb, 51.430 Mb, 51.431 Mb and 51.480 Mb, respectively and were all situated within the interval between SSC5P3 (18.299 Mb) and SW2003 (54.594 Mb). Among the seven SNP, three included within the *HMGA2* gene are located near the SW1904 marker (33.850 Mb), which is in the middle of the QTL. Standard association test, marker-assisted association test and F-drop test were carried out to evaluate the association between the seven informative SNP and ear size in the F_2 resource population. In the standard association test, all the SNP were in strong association with ear size ($P_{-sa} < 0.01$, Table 1). *HMGA2* g.2836 A > G showed the strongest association with ear surface. Allele [G] present in Erhualian pigs is associated with an increase in ear size, while allele [A] is responsible for a smaller ear surface i.e. 32.84 ± 5.46 cm^2 (Table 1).

In the marker-assisted association test, apart from the SNP *SOX5* g.12328 T > G that did not achieve a $P < 0.05$ significance level, the SNP analyzed for the three candidate genes were significantly associated with ear size ($P_{-ma} < 0.05$, Table 1). Similar to the standard

Table 1 *P* values obtained in standard association test and marker-assisted association test and additive effects of the seven SNP

Gene	Polymorphism	P_{-sa}	P_{-ma}	Allele	Additive effect ± s.e. (cm^2)
HMGA2 (JF748727)	g.2836 A > G	9.19E-46	1.92E-05	A	-32.84 ± 5.46
	g.215 C > T	5.04E-26	1.23E-03	T	27.40 ± 5.37
	g.1799 G > A	1.60E-19	0.04	G	22.54 ± 4.76
SOX5 (JF748729)	g.9084 T > C	3.51E-07	0.03	C	32.56 ± 7.25
	g.9908 T > C	4.54E-25	0.02	C	15.72 ± 5.00
	g.12328 T > G	8.12E-07	0.11	G	-15.28 ± 5.02
PTHLH (JF748728)	g.9440 T > C	5.12E-07	0.03	C	11.74 ± 5.15

P_{-sa}: *P* values in standard association analysis; P_{-ma}: *P* values in marker-assisted association analysis; s.e.: standard error; the negative values indicate decrease in ear size

association test, *HMGA2* g.2836 A > G was most strongly associated with ear size (P_{-ma} = 1.92E-05). The results of the F-drop test are shown in Figure 2. When the genotypes of *HMGA2* g.2836 A > G were included as a fixed effect in the QTL analysis, the F value dropped from 134.14 to 3.19. F values of the other SNP were below 90%.

HMGA2 and *SOX5* haplotypes were constructed and their associations with ear size were also analyzed in this study. Overall, the phenotypic variance explained by *HMGA2* haplotypes reached 19.47% (*P* = 1.31E-31, Table 2). Haplotypes [GCA] and [GTG] were associated with an increase in ear size. Five *SOX5* haplotypes showed a significant association with ear size. Animals with haplotype [TCT], [TCG] or [CCT] showed larger phenotypic values than those with haplotype [TTT] or [TTG] (Table 2). However, the phenotypic variance explained by *SOX5* haplotypes was lower

Figure 2 F-value curves of seven SNP in the F-drop test when each SNP was included as a fixed effect. The Y-axis shows F-values and the X-axis indicates the SNP order; F (QTL): F value in QTL mapping (F-values of the model 3), F (drop): F value when the candidate SNP was included as a fixed effect in QTL mapping (values of the F-ratios between models 4 and 3)

than that of the QTL or *HMGA2* haplotypes (12.82%, *P* = 2.48E-22).

Association of *HMGA2* g.2836 A > G with ear size in outbred populations

Given that the strongest association between *HMGA2* g.2836 A > G and ear size was identified in the White Duroc × Erhualian F$_2$ resource population, this SNP was further genotyped in 356 adult pigs from the synthetic commercial lines of Sutai, Sujiang and Suzhong. The results showed that it was significantly associated with ear size in Sutai pigs (*P* < 0.05), but not in Sujiang and Suzhong pigs (Table 3) and allele [G] was responsible for an increase in ear size.

Discussion

Identification of causative genes underlying QTL in domestic animals is still a major challenge because confidence intervals of QTL are typically too large for positional cloning of the underlying gene [35]. In this study, we refined the interval containing the QTL responsible for ear size on SSC5 from an 11 cM interval to an 8.7 cM region. However, the confidence interval of the refined QTL is still large. The linkage disequilibrium-based haplotype-sharing approach has been shown to be effective in fine mapping of QTL in domestic animals [35,36]. Unfortunately, in our study QTL genotypes could be deduced for only three of the nine F$_1$ boars by the marker-assisted segregation analysis (data not shown) and no obvious shared haplotype was observed, which hampered further mapping of the QTL.

In this study, the refined QTL region corresponds to two orthologous regions on human chromosome 12 (65.70-75.07 Mb and 19.80-33.72 Mb), in which three candidate genes with related functions i.e. *SOX5*, *HMGA2* and *PTHLH* were identified. The standard association test, marker-assisted association test and F-drop test were used to test the association between these candidate genes and ear size in the F$_2$ resource
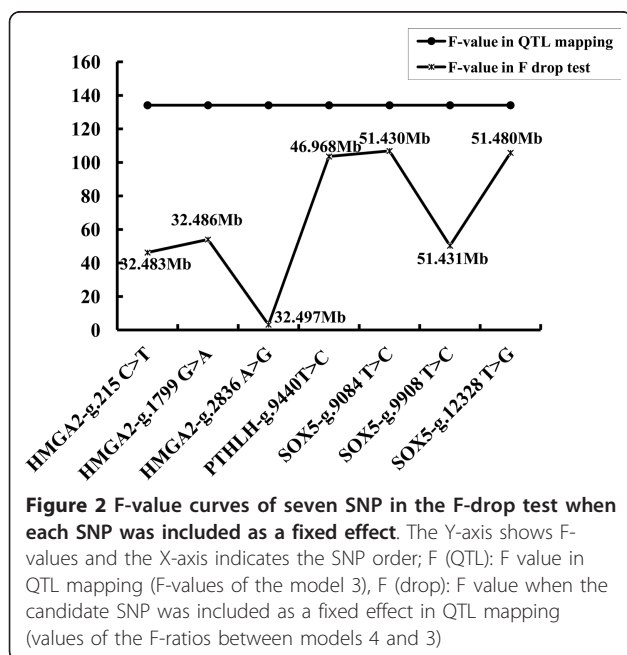
Table 2 Associations of *HMGA2* and *SOX5* haplotypes with ear size in the F$_2$ resource population

Gene	Haplotype	Frequency	Effect ± s.e.* (cm^2)	Corrected *P*-value
HMGA2	A/C/G	0.002	-41.43 ± 28.64	1
	A/C/A	0.012	-5.09 ± 9.66	1
	A/T/G	0.484	-20.96 ± 1.70	4.34E-31
	A/T/A	0.004	-36.21 ± 15.33	0.15
	G/C/G	0.001	8.73 ± 28.68	1
	G/C/A	0.255	22.71 ± 2.22	3.87E-22
	G/T/G	0.213	16.66 ± 2.71	9.71E-09
	G/T/A	0.029	3.72 ± 6.66	1
	phenotypic variance explained by all haplotypes		19.47%	1.31E-31
SOX5	T/T/T	0.170	-7.99 ± 2.69	0.03
	T/C/T	0.234	11.09 ± 2.33	1.83E-05
	T/T/G	0.359	-17.75 ± 1.96	6.45E-18
	T/C/G	0.101	11.85 ± 2.97	5.66E-04
	C/T/T	0.002	45.88 ± 19.90	0.17
	C/C/T	0.114	15.19 ± 2.96	2.71E-06
	C/T/G	0.008	3.87 ± 10.37	1
	C/C/G	0.012	17.88 ± 8.99	0.38
	phenotypic variance explained by all haplotypes		12.82%	2.48E-22

Effect ± s.e. represent the effect of haplotypes and its standard error, the negative values indicate decrease in ear size

population in which extensive linkage disequilibrium exists [30]. *HMGA2* g.2836 A > G showed the strongest association with ear size both in standard association test and marker-assisted association test. Allele [G] originating from Erhualian pigs was responsible for the increase in ear size, which is consistent with the breed's characteristic large and floppy ears. With the F-drop test, F value dropped by more than 97% only if the *HMGA2* g.2836 A > G genotype was included as a fixed effect. The phenotypic variance explained by the *HMGA2* haplotypes was a little higher than that by the QTL (19.47% vs.17.14%). This could be due to the elimination in the association tests of the effect of the causative mutation in *PPARD* involved in the other major QTL for ear size on SSC7. However, the phenotypic

Table 3 Association of *HMGA2* g.2836 A > G with ear size in synthetic commercial lines

Population	Genotype	N	Mean ± s.d.* (cm^2)
Sutai	[AA]	90	258.57 ± 40.29[a]
	[AG]	76	292.60 ±46.28[b]
	[GG]	7	323.50 ±37.96[c]
Sujiang	[AA]	57	252.73 ±45.90
	[AG]	41	265.14 ±41.23
	[GG]	7	280.23 ±45.87
Suzhong	[AA]	47	216.61 ±38.34
	[AG]	28	224.55 ±59.25
	[GG]	3	226.68 ±80.23

*phenotypes were corrected for sex, batch and farm; the mean values marked with different superscript letter a, b and c indicate significant phenotype differences between each genotype (*P* < 0.05)

variance explained by *SOX5* haplotypes is much lower than that by the QTL (12.82% vs. 17.14%). Furthermore, the statistical models used in this study can be applied to determine which candidate gene is closest to the QTL. If *HMGA2* is more strongly associated with the QTL than *SOX5* and *PTHLH*, the residual sum of the squares of *HMGA2* (RSS (*HMGA2*)) in model 3 is expected to be smaller than that of RSS (*SOX5*) and RSS (*PTHLH*) [30]. We found that the ratios of RSS (*HMGA2*)/RSS (*SOX5*) and RSS (*HMGA2*)/RSS (*PTHLH*) were < 1 (0.79 and 0.78, respectively). These results suggest that *HMGA2* is the gene closest to the QTL.

The significant association between *HMGA2* g.2836 A > G and ear size was confirmed in the synthetic commercial line examined in this study. The founder animals of this line and the White Duroc × Erhualian F$_2$ resource population originate from the same pig breeds, i.e. Duroc and Erhualian pigs. However, no significant association was detected in the Sujiang and Suzhong pig populations, which is most likely due to: 1) the different genetic background of the populations; 2) this SNP is only in LD with the causative mutation. Because recombination exists between the SNP and the causative mutation in the Suzhong and Sujiang populations, no significant association can be detected.

More interestingly, an across-breed genome-wide association study (GWAS) in dogs showed that the SNP most strongly associated with dropping ears and ear size is located 98 kb apart from each other between the *MSRB3* and *HMGA2* genes [37]. In addition, an

association between *HMGA2* and ear type has been described in dogs [21] and *HMGA2*-deficient mice are known to have small ears [20]. Combined with the results obtained in this study, we suggest that *HMGA2* is the closest gene to the QTL or the marker for ear size on SSC5 in pigs. More refined mapping of the QTL is needed to demonstrate that *HMGA2* is responsible for ear size in pig.

Conclusions

We have fine-mapped a QTL affecting ear size on SSC5 to an 8.7-cM region. Candidate gene analyses suggest that *HMGA2* is the closest gene with a potential functional effect to the QTL or marker for ear size on SSC5. These results will contribute to the identification of the causative mutation for this QTL.

Additional material

Additional_file_ 1: Primers for QTL fine-mapping, gene expression analyses, and SNP identification and genotyping. **Table S1** contains primer sequences, annealing temperatures and amplicons of the microsatellite markers, **Table S2** primers for gene expression analyses, **Table S3** sequences and assay conditions of the primers for SNP identification and **Table S4** sequences and assay conditions of the primers for SNP genotyping.

Additional_file_ 2: The expression profiles of three candidate genes in ear tissue by RTPCR. **Figure S1** shows the expression level of *HMGA2*, *SOX5* and *PTHLH* in ear tissue obtained by RT-PCR.

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Authors' contributions
PHL performed the experiments and wrote the manuscript; SJX collected the samples and performed the experiments; NW performed the experiments; ZYZ analyzed the data; RHH and YQG collected the samples; YMG analyzed the data; JR, provided comments and revisions for the manuscript; LSH conceived and designed the experiments and revised the manuscript; CYC performed the experiments, analyzed the data, wrote and revised the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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