Polymorphisms of the IGF1R gene and their genetic effects on chicken early growth and carcass traits

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Abstract

Background: The insulin-like growth factor I receptor (IGF1R) has an important effect on growth, carcass, and meat quality traits in many species. However, few studies on associations of the IGF1R gene with growth and carcass traits have been reported in chickens. The objectives of the present study were to study the associations of the IGF1R gene with chicken early growth and carcass traits using a neutral test, variation scan of the gene, genetic diversity, linkage disequilibrium and association analyses.

Results: The tree generated from the amino acid sequences of 15 species showed that the IGF1R gene was conservative in the whole evolution among the mammalian animals and chickens. In a total of 10,818 bp of sequence, 70 single nucleotide polymorphisms were identified in the chicken IGF1R gene. The allelic and genotypic frequency distribution, genetic diversity and linkage disequilibrium of 18 single nucleotide polymorphisms (SNPs) in the Xinghua and White Recessive Rock chickens showed that six of them were possibly associated with growth traits. Association analyses showed that the A17299834G SNP was significantly associated with chicken carcass body weight, eviscerated weight with giblets, eviscerated weight, body weights at 28, 35, and 56 d of age, leg length at 56 d of age, and daily weight gain at 0–4 weeks. The haplotypes of the A17307750G and A17307494G were associated with early growth traits. The haplotypes of the A17299834G and C17293932T were significantly associated with most of the early growth traits and carcass traits.

Conclusion: There were rich polymorphisms in the chicken IGF1R gene. Several SNPs associated with chicken early growth traits and carcass traits were identified in the IGF1R gene by genetic diversity, linkage disequilibrium, and association analyses in the present study.

Background

The insulin-like growth factor 1 receptor (IGFIR) is a membrane glycoprotein mediating most biological actions of IGF-1 and IGF-2, which have an important effect on chicken growth, carcass, and meat quality traits [1-3]. Two receptors (IGF1R and IGF2R) were found in the mammals but only one (IGF1R) was found in the birds. IGF1R not only regulated the half-life time and
activity of IGFs, but also played important roles on the key developmental stage and adult stage such as the cell life cycle, transplantation, metabolism, subsistence, proliferation, and differentiation.

Many variations in the genome affected gene expression at the transcription and translation levels [4,5]. Variations in the genes of somatotropic axis could function as candidates for the evaluation of their effects on animal growth and development traits. In humans, mutations at important regulatory sites of the IGF1R gene were associated with growth. Such mutations resulted in the failure of processing of proIGF1R to mature IGF1R and caused dysfunction and short stature of IGFR [6-9]. These variations affected partly the expression and physiological functions of the IGF1R gene, and subsequently affected growth. However, few studies on associations of the IGF1R gene with growth and carcass traits were reported in chickens.

In the present study, the objectives were to study the associations of the IGF1R gene polymorphism with chicken early growth and carcass traits. Polymorphisms of the chicken IGF1R gene were scanned in a 10,818 bp of sequence. The single nucleotide polymorphisms (SNPs) used in association analyses were selected based on the genetic diversity and linkage disequilibrium analyses in the Xinghua (XH) and White Recessive Rock (WRR) chickens. The associations of the SNPs or their haplotypes with chicken early growth and carcass traits were analyzed in a F2 resource population generated from a reciprocal cross between XH and WRR chickens.

Results

The molecular evolution of the IGF1R gene

The tree generated from the amino acid sequences of 15 species showed that a positive selection was possible for the IGF1R gene in the early evolution of the Japanese firebelly newt and Africa clawed frog, and in the middle evolution of the zebrafish, turbot, and common carp (ω > 1). But the IGF1R gene was conservative in the whole evolution between the mammals and chickens (Figure 1).

Variations of the chicken IGF1R gene

In the present study, 10,818 bp of sequence in the IGF1R gene, in which exon regions were preferred but segmental introns were also included (Table 1 in Supplementary Materials File 1), was scanned, and 70 SNPs were identified between XH and WRR chickens. Among the 70 SNPs, 7 SNPs were located in the 5' regulated region, 15 in the coding regions, 57 in the intron regions, and 1 in the 3' regulated region. Average density of SNPs was one SNP per 173 bp (70/12,038) in the whole region studied, with one SNP per 273 bp (15/4092 bp) in the coding regions, and one SNP per 140 bp (48/6726 bp) in the intron regions. Among the 70 SNPs, 51 SNPs are transition, 17 transversion, and 1 one base insertion/deletion. Fifteen SNPs were found in the coding regions, but only one SNP was non-synonymous mutation (Asn → Ser in the exon 3).

Nucleotide diversity and neutral test

The nucleotide diversity of the chicken IGF1R gene was (2.87 ± 0.28) × 10⁻³, the nucleotide polymorphism was

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![Figure 1](http://www.biomedcentral.com/1471-2156/9/70)

**Figure 1**

The ω value and UPGMA tree from CODEML arithmetic.
(2.62 ± 1.06) × 10^{-3}, and all parameters of the neutral test were negative but not significant. For the 5' flanking region, the nucleotide diversity of the chicken IGFR1 gene was (3.83 ± 1.62) × 10^{-3}, the nucleotide polymorphism is (3.01 ± 1.0) × 10^{-3}, all parameters of the neutral test were positive, and the values of Fu and Li's D, and F test were significant (P < 0.05). For the intron region, the nucleotide diversity of the chicken IGFR1 gene was (3.52 ± 1.49) × 10^{-3}, the nucleotide polymorphism was (3.98 ± 0.43) × 10^{-3}, all parameters of the neutral test were negative but not significant. For the exon region, the nucleotide diversity of the chicken IGFR1 gene was (1.59 ± 0.57) × 10^{-3}, the nucleotide polymorphism was (1.43 ± 0.32) × 10^{-3}, and all parameters of the neutral test were positive but not significant (Table 2 in Supplementary Materials File 1).

**Allelic frequency and heterozygosity in the XH and WRR chickens**

Between the XH and WRR chickens, the allelic frequencies of C17393427T and A17323673G were significantly different at the P < 0.05 level and the allelic frequencies of the 7 SNPs, C17445985T, A17417734G, C17417042G, T173334342C, A17327275C, A17307494G, and A17299834G, were significantly different at the P < 0.01 level. No significant differences for mean heterozygosity of the 18 SNPs were found, but significant differences of A17313488G, A17307750G, A17307494G, A17299834G, and C17293932T were observed in the XH and WRR chickens (Table 3 in Supplementary Materials File 1).

**TagSNP of the IGFR1 gene in the XH and WRR chickens**

Eight TagSNP, C17293932T, A17299834G, A17307750G, A17313488G, C17393427T, T17416994C, C17445985T, and C17445985T, were identified in the XH chickens by HapBLOCK software. Another eight TagSNP, C17293932T, A17299834G, A17307494G, A17307750G, T17416994C, C17417042G, A17417734G and C17445596A, were also identified as TagSNPs in the WRR chickens.

**Linkage disequilibria of the IGFR1 gene in the XH and WRR chickens**

Average values of r^2 showed that the linkage disequilibria declined with increasing physical distance between SNP pairs in the XH and WRR chickens (Figure 1 in Supplementary Materials File 1). The effective extent of linkage disequilibrium was 27, 441 bp in the WRR chickens, but not found in the XH chickens. Possible regions of strong linkage disequilibrium were found between exon 6 and the 3' untranslated region (between A17327275C and C17293932T) in the WRR chickens (Table 4 in Supplementary Materials File 1).

**Association of the 6 SNPs with chicken early growth and carcass traits**

Associations of the 6 SNP with chicken early growth and carcass traits were analyzed, but only the A17299834G of the chicken IGFR1 gene was significantly associated with some growth and carcass traits. The A17299834G of the chicken IGFR1 gene was significantly associated with chicken carcass weight, eviscerated weight with giblets, eviscerated weight, body weights at 28, 35, and 56 d of age, leg length at 56 d of age, and daily weight gain at 0–4 weeks (P < 0.05). Significantly and suggestively dominant effects of AG genotype were observed for chicken carcass weight, eviscerated weight with giblets, breast muscle weight, eviscerated weight, fat thickness under skin, fat width, body weights at 14, 21, 28, 35, 42, 49, 56, and 77 d of age, and leg length at 42 and 56 d of age (Table 1). In other words, the A17299834G SNP affected the chickens' early growth.

**Haplotype structure within the 6 SNP in the F2 resource population**

For the IGFR1 gene, two haplotype blocks were observed in the F2 resource population. Block 1 comprised A17307750G and A17307494G, located between intron 17 and 18, and block 2 comprised A17299834G and C17293932T, located between exon 20 and the 3' untranslated region (Figure 2 in Supplementary Materials File 1). In block 1, four haplotypes were observed in the F2 individuals of the resource population. Three distinct haplotypes, H1, H2, and H3, accounted for 95.4% of the total number of the four haplotypes. Among the four haplotypes, allele H4 had the lowest allelic frequency of 0.46%, and H1 had the highest allelic frequency of 51.60% (Table 5 in Supplementary Materials File 1). In block 2, four haplotypes were also observed in the F2 individuals of the resource population. Three distinct haplotypes, E1, E2, and E3, accounted for 96.6% of the four observed haplotypes. Among the four haplotypes, the allelic frequency of E4 was the lowest at 0.34%, the highest allelic frequency was 57.88% for haplotype E2.

**Associations of the haplotypes with chicken growth and carcass traits**

Significant associations of the haplotypes of A17307750G and A17307494G with chicken growth and carcass traits were observed. The haplotypes of A17307750G and A17307494G were significantly associated with body weights at 28 and 49 d of age, and with daily weight gain at 0–4 weeks at the P < 0.05 level, and significantly associated with body weight at 35 d of age, and leg length at 42 and 49 d of age at the P < 0.01 level. The haplotypes composed of A17299834G and C17293932T affected the chickens' early growth. Significantly and suggestively
Table 1: Association of the A17299834G with chicken growth and carcass traits

| Traits                        | P     | AA (24)       | AG (87)       | GG (327)       |
|-------------------------------|-------|---------------|---------------|---------------|
| CW                            | 0.0138| 1454 ± 49.61A | 1378 ± 28.9A  | 1311 ± 14.08B |
| Fat thickness under skin      | 0.0811| 4.71 ± 0.29a  | 4.31 ± 0.16ab | 4.05 ± 0.08b  |
| Fat width                     | 0.0556| 13.98 ± 0.84a | 12.36 ± 0.48ab| 11.84 ± 0.24b |
| EWG                           | 0.0135| 1334 ± 45.81A | 1263 ± 26.12A | 1201 ± 13.29B |
| EW                            | 0.0165| 1154 ± 40.71A | 1095 ± 23.21A | 1040 ± 11.81B |
| Breast muscle weight          | 0.0588| 98.35 ± 3.72a | 94.24 ± 2.12ab| 89.86 ± 1.08B |
| BW at 14 days                 | 0.0718| 130.3 ± 3.42a | 126.3 ± 1.95ab| 122.6 ± 0.99b |
| BW at 21 days                 | 0.1364| 221.3 ± 6.51a | 214.3 ± 3.73a | 208.4 ± 1.89a |
| BW at 28 days                 | 0.0313| 320.1 ± 10.39a| 319.5 ± 5.94a | 305.7 ± 3.03B |
| BW at 35 days                 | 0.0143| 471.1 ± 15.87a| 452.9 ± 9.16ab| 428.1 ± 6.46B |
| BW at 42 days                 | 0.2734| 596.3 ± 21.50a| 581.9 ± 12.29a| 563.9 ± 6.24a |
| LL at 42 days                 | 0.0608| 62.45 ± 0.98a | 61.38 ± 0.56a | 60.23 ± 0.28B |
| BW at 49 days                 | 0.1730| 744.4 ± 25.38a| 719.1 ± 14.47a| 697.4 ± 7.38a |
| BW at 56 days                 | 0.0466| 909.2 ± 30.27a| 889.3 ± 17.36a| 845.9 ± 8.78B |
| LL at 56 days                 | 0.0066| 74.58 ± 1.07A | 74.26 ± 1.07A | 71.48 ± 0.31B |
| BW at 77 days                 | 0.0829| 1470 ± 59.32a | 1363 ± 28.95a | 1299 ± 14.66a |
| DGW at 0–4 weeks              | 0.0267| 10.80 ± 0.37a | 10.35 ± 0.21a | 9.84 ± 0.10B  |

CW—carcass weight; EWG—eviscerated weight with giblets; EW—eviscerated weight
BW—body weight; LL—leg length; DGW—daily weight gain
The capital letters indicate that multiple comparison is greatly significant (P < 0.01), and small letters indicate that multiple comparison is significant (P < 0.05).

Table 2: Association of the haplotype composed of A17307750G and A17307494G with chicken early growth and carcass traits

| Traits                        | P     | H1H1 (122) | H1H2 (184) | H1H3 (24) | H2H2 (91) | H2H3 (12) | H2H4 (4) | H3H3 (1) |
|-------------------------------|-------|------------|------------|------------|------------|------------|------------|------------|
| BW at 7 days                  | 0.08  | 59.62 ± 0.69ab | 60.38 ± 0.68ab | 55.71 ± 1.77ab | 59.53 ± 0.9ab | 53.91 ± 2.43b | 55.97 ± 4.04ab | 62.18 ± 7.18a |
| BW at 14 days                 | 0.10  | 123.6 ± 125.5ab | 122.1 ± 123.1 | 109.1 ± 5.20A | 108.8 ± 7.82A | 133.2 ± 15.63B |
| BW at 21 days                 | 0.10  | 211.5 ± 211.9ab | 211.1 ± 209.4 | 179.6 ± 9.86A | 183.1 ± 218.1 | 296.8 ± 24.66B |
| BW at 28 days                 | 0.03  | 310.2 ± 308.8ab | 322.5 ± 309.9 | 256.2 ± 15.9A | 296.8 ± 24.74A | 347.3 ± 47.13B |
| BW at 35 days                 | 0.005 | 435.8 ± 437.1ab | 442.0 ± 436.2 | 237.7 ± 46.52B | 455.1 ± 72.08B |
| BW at 42 days                 | 0.102 | 578.5 ± 567.1ab | 594.1 ± 561.7 | 473.2 ± 543.6 | 571.9 ± 32.45A | 48.65AB | 97.40AB |
| LL at 42 days                 | 0.008 | 60.70 ± 60.32ab | 61.91 ± 60.67 | 55.45 ± 54.14A | 63.75 ± 21.99B | 60.93 ± 43.99B |
| LD at 42 days                 | 0.09  | 7.89 ± 7.86ab | 7.84 ± 7.86 ± 0.16A | 7.19 ± 0.23A | 7.93 ± 0.35AB | 8.73 ± 0.71AB |
| BW at 7 days                  | 0.04  | 712.9 ± 703.7ab | 718.9 ± 697.3 | 606.9 ± 38.94A | 788.8 ± 58.15B | 646.2 ± 115.44B |
| LL at 7 days                  | 0.001 | 67.91 ± 67.71ab | 66.98 ± 67.34A | 59.68 ± 2.23A | 73.18 ± 2.75B | 67.68 ± 4.90AB |
| LD at 7 days                  | 0.09  | 9.28 ± 9.30ab | 9.64 ± 9.48 ± 0.11A | 9.26 ± 0.33A | 9.29 ± 0.43A | 11.53 ± 0.82B |
| DGW at 0–4 weeks              | 0.04  | 10.00 ± 9.96ab | 10.40 ± 9.48 ± 0.20A | 8.12 ± 0.57A | 9.56 ± 0.84AB | 11.23 ± 1.68B |

BW—body weight, LL—leg length, LD—leg diameter, DGW—daily weight gain
Capital letters indicate that multiple comparison is greatly significant (P < 0.01), and small letters indicate that multiple comparison is significant (P < 0.05).
and leg length at 42 and 49 d of age at the P < 0.01 level. In other words, the haplotypes affected the chickens’ early growth. The E1E4 diplotype was dominant for body weights at 7, 14, 21, 28, 35, 42, and 49 d of age, daily weight gain at 0–4 weeks, carcass weight, eviscerated weight with giblets, eviscerated weight, breast muscle weight, brain neck weight, breast angle width, small intestines length at 90 d of age, and leg length at 56 d of age (Table 3). Multiple comparisons showed that the E1E4 diplotype had a dominant effect in all the traits.

**Discussion**

A molecular phylogenetic tree generated from the amino acid sequences of 15 species showed that the IGF1R gene was conservative and favored subsistence in the evolution of these species. At the same time, the ω value in the mammals and chickens proved that the IGF1R gene was conservative in the whole evolution of the mammal animals and chickens. Analysis of evolutionary conservation had provided insights into essential regions of molecules such as IGF-I and their receptors, in which the tyrosine kinase domain is highly conserved [23]. Sequence comparison showed that the primary structures of zebrafish IGF1R are highly preserved in vertebrates [24]. However, the nucleotide diversity of the chicken IGF1R gene seems to be much higher, and chickens have a higher SNP incidence and polymorphisms.

Among the 70 SNPs, 18 SNPs were selected to study the allelic frequencies, heterozygosity, TagSNP, and linkage disequilibria of the XH and WRR chickens based on their location, possible transcriptional site and the distribution density. In 9 of the 18 SNPs, there were significant differences of allelic frequencies between the XH and WRR chickens. These SNPs with obviously different allelic frequencies between slow-growing XH and fast-growing WRR chickens could contribute to their divergent growth performance. Considering some associations might be false positives, allelic frequency differences between XH and WRR may partially provide support to the results of the association analyses.

SNP markers were preferred for disease association studies because of their high abundance along the human genome, the low mutation rate, and accessibility to high-throughput genotyping. Thus, the selection of a maximally informative set of SNPs (tag SNPs) for genome-wide association studies has recently attracted much attention. In those high LD regions, only a small number of SNPs were sufficient to capture most of haplotype structure [12]. In the present study, 8 different TagSNP were found in the XH and WRR chickens.

The purpose of the study was to find functional SNPs by genetic diversity, linkage disequilibrium and association analyses of the SNPs with the economically important traits. There were several successful examples in plants and humans. Yu identified 6 SNPs of the rabl7 gene associated with drought tolerance in maize based on genetic diversity and linkage disequilibrium [13]. Fu et al. reported a systematic search for polymorphisms in the CASQ1 gene on chromosome 1q21 and identified a significant association between the CASQ1 polymorphism and type 2 diabetes by linkage disequilibrium for the first time [14]. Using linkage disequilibrium, some important SNPs or QTL were found [15–17]. Morahan et al. reported that a single base change in the 3’UTR showed a strong linkage disequilibrium with the T1D susceptibility locus and the alleles showed different levels of expression in cell lines [18]. In the present study, a possible strong linkage

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**Table 3: Association of the haplotype composed of SNP A17299834G and C17293932T with chicken growth and carcass traits**

| Traits                        | P       | E1E1 (55) | E1E2 (107) | E1E3 (14) | E1E4 (3) | E2E2 (164) | E2E3 (70) | E3E3 (27) |
|-------------------------------|---------|-----------|------------|-----------|----------|------------|-----------|-----------|
| Breast angle width            | 0.022   | 60.23 ± 0.85 | 60.74 ± 0.54 | 64.38 ± 1.41 | 67.86 ± 2.86 | 59.81 ± 0.42 | 60.32 ± 0.75 | 61.36 ± 1.15 |
| CW                            | 0.061   | 1335 ± 38.55 | 1302 ± 24.46 | 1453 ± 64.03 | 1481 ± 129.10 | 1301 ± 19.36 | 1351 ± 34.23 | 1434 ± 51.93 |
| EW                            | 0.043   | 1245 ± 35.56 | 1245 ± 35.56 | 1333 ± 59.07 | 1373 ± 119.09 | 1193 ± 17.86 | 1238 ± 31.57 | 1316 ± 47.91 |
| EWF                           | 0.062   | 1080 ± 31.62 | 1032 ± 20.07 | 1151 ± 52.53 | 1182 ± 105.90 | 1030 ± 15.88 | 1072 ± 28.08 | 1136 ± 42.60 |
| Brain and neck weight         | 0.034   | 93.48 ± 2.87 | 89.20 ± 1.82 | 104.00 ± 4.77 | 108.23 ± 9.63 | 88.74 ± 1.44 | 90.72 ± 2.55 | 95.79 ± 3.87 |
| Brain weight                  | 0.063   | 128.3 ± 4.40 | 116.4 ± 2.79 | 134.7 ± 7.31 | 141.1 ± 14.73 | 122.9 ± 2.21 | 124.0 ± 3.91 | 129.0 ± 5.92 |
| Small intestine length        | 0.101   | 139.2 ± 2.41 | 136.6 ± 1.53 | 145.0 ± 4.00 | 147.9 ± 8.08 | 139.5 ± 1.21 | 135.1 ± 2.14 | 136.2 ± 3.25 |
| BW at 14 days                 | 0.071   | 127.8 ± 2.65 | 121.6 ± 1.68 | 127.8 ± 4.40 | 138.2 ± 8.88 | 121.6 ± 1.33 | 124.8 ± 2.36 | 129.1 ± 3.57 |
| BW at 21 days                 | 0.134   | 215.1 ± 5.10 | 206.6 ± 3.22 | 219.7 ± 8.38 | 251.0 ± 19.73 | 207.1 ± 2.55 | 211.0 ± 4.49 | 218.9 ± 6.79 |
| BW at 28 days                 | 0.032   | 315.5 ± 8.10 | 303.9 ± 5.09 | 333.7 ± 13.33 | 374.4 ± 26.78 | 302.9 ± 4.05 | 312.0 ± 7.15 | 326.5 ± 10.83 |
| BW at 35 days                 | 0.023   | 439.2 ± 12.34 | 424.8 ± 7.97 | 486.4 ± 20.59 | 521.0 ± 41.10 | 425.0 ± 6.25 | 440.1 ± 11.15 | 462.1 ± 16.60 |
| Leg length at 56 days         | 0.021   | 72.03 ± 0.83 | 71.10 ± 0.53 | 74.97 ± 1.38 | 77.12 ± 2.79 | 71.42 ± 0.41 | 72.77 ± 0.74 | 74.21 ± 1.12 |
| DGW at 0–4 weeks              | 0.024   | 10.24 ± 0.29 | 9.76 ± 0.18 | 10.85 ± 0.47 | 12.30 ± 0.95 | 9.74 ± 0.14 | 10.08 ± 0.25 | 10.60 ± 0.38 |

**Notes:**
- **CW:** carcase weight; **EWG:** eviscerated weight with giblets; **EW:** eviscerated weight; **BW:** body weight.
- **DGW:** daily weight gain.
- Capital letters indicate that multiple comparison is greatly significant (P < 0.01), and small letters indicate that multiple comparison is significant (P < 0.05).
disequilibrium region was found in the WRR chickens. The results showed that some SNPs were linked and were tightly scattered between the C17293932T and A17327275C. Combining the results of the linkage disequilibrium, 6 SNPs, C17293932T, A17299834G, A17307494G, A17307750C, G17445596A, and C17445985T, were selected and used in the association analyses.

The results in the present study showed that the IGF1R gene affected the chickens’ early growth, which is consistent with reports on humans. Previous studies on humans indicated that there were important associations of the IGF1R gene with growth and development [6,7,9]. Kawashima et al. also reported that a heterozygous mutation (R709Q) changing the cleavage site from Arg-Lys-Arg-Arg to Arg-Lys-Gln-Arg was identified in a 6-year-old Japanese girl and the mutation resulted in the failure of processing of the IGF1R proreceptor to mature IGF1R, causing short stature [8]. Mutations or SNPs in the IGF1R gene could partially affect the gene expression, and thus could affect animal physiological metabolism and growth. Other studies proved that the association of haplotype with economic traits was more predominant and reliable [19-21], perhaps due to the multiple-loci interaction of the haplotype.

The chicken IGF1R gene was located on GGA10 with a physical distance around 187 Mb, and a genetic distance close to 100 cM. Recently, some quantitative trait loci (QTLs) associated with growth and carcass traits were found in the GGA10 [22,23]. Rabie et al. showed that a single QTL related to body weight at 5 weeks under ascites conditions was located on 82–101 cM [24]. Zhou et al. reported that 5 QTLs were identified to be associated with abdominal fat weight, body weight, heart weight, liver weight, spleen weight at the 11–120 cM [25]. These studies suggest that the associations of the SNP or haplotype with economic traits in the present study were reliable.

In conclusion, there were rich polymorphisms in the chicken IGF1R gene. Several SNPs associated with chicken early growth traits and carcass traits were identified in the IGF1R gene by genetic diversity, linkage disequilibrium, and association analyses in the present study.

Materials and methods

DNA pools

The initial SNP discovery was carried out on the DNA pools of 7 breeds, XH chickens, Taihe Silkie chickens, Beijing Fatty chickens, Yangshan chickens, Dwarf chickens, White Leghorn chickens, and WRR chickens. Ten individuals for each breed generated one pooled DNA sample. An equal amount of DNA was taken from each individual and was pooled to generate the 7 pooled DNA samples. DNA samples were diluted and reassessed to obtain an equal amount of DNA from each individual.

A F2 resource population for association analyses

A F2 resource population was constructed by reciprocal crossing the XH with WRR chickens [26]. The F2 individuals were raised in floor pens and fed commercial corn-soybean diets that met NRC requirements. The birds from six batches were kept in different pens, and the sizes of all pens were the same. The body weight was measured in grams at hatch, 7, 14, 21, 28, 35, 42, 49, 56, and 90 d of age. The 434 individuals from the F2 generations (221 male and 213 female) were slaughtered at 90 d of age. Shank length (mm), head width (mm), breast width (mm), breast depth (mm), body length (cm), breast angle width (degree), carcass weight (g), fat thickness under skin (mm), fat width (mm), eviscerated weight with giblets (g), eviscerated weight (g), breast muscle weight (g), leg muscle weight (g), wing weight (g), abdominal fat pad weight (g), head and neck weight (g), weights of heart, liver, and gizzard (g), and small intestine length (cm) were recorded.

Chicken populations for genetic diversity study and linkage disequilibrium analyses

Two unrelated populations, consisting of 112 XH individuals and 86 WRR individuals, respectively, were sampled for genetic diversity investigation in the present study. The XH and WRR chickens were parents of the F2 resource population, both from Guangdong Wens Foodstuff Corporation Ltd. (Guangdong, China). The XH chicken is a Chinese native breed with slow growth rate, and the WRR chicken is of fast growth rate. There is significant difference in growth and carcass traits between the XH and WRR chickens.

Primer design for sequencing

Available sequences of the chicken IGF1R gene were used as templates for designing specific primers by the Gene-tool software http://www.biologysoft.com. Twenty-two primers were obtained and an optimal length of the PCR product was set between 450 and 800 bp. Exon regions were preferred, but segmental intron sequences were also included. Details were listed in Table 1 in Supplementary Materials File 1.

Selections of the SNPs used for genetic diversity investigation and linkage disequilibrium analyses

For genetic diversity investigation and linkage disequilibrium analyses, 18 SNPs (Table 3 in Supplementary Materials File 1) were selected based on the following criterions. (1) Positions: SNP in coding regions were selected preferably over those from non-coding regions. (2) Functional domain: SNP in the important structural and conservative functional domains such as extracellular
domain, joint of α subunit and β subunit, and tyrosine kinase domain, were preferred to those in the other domains. (3) Potential regulating units: SNP located at the potential regulatory sites of the un-translating region were preferred to those located at the other sites. (4) Density: An average density of 1 SNP per 8.3 kb was determined, and a total of 18 SNPs were selected in a 150 kb full sequence of the chicken IGFR gene.

Neutral test of the IGFR gene
The ω value was calculated using codeml program of PAML software [27], and formula was followed,

\[ \omega = \frac{dN}{dS} \]

Where dN and dS are the number of non-synonymous substitutions per non-synonymous site, and the number of synonymous substitutions per synonymous site, respectively. In the present study, the selection function of the DNA sequences was analyzed in species using Branch model of codeml program (M0 and M1), and the selection function of the amino acid was analyzed using site model (M7 and M8) in the evolution http://taxonomy.zoology.gla.ac.uk/rod/treeview.html.

Discovery and identification of SNPs
SNPs having different allelic frequencies in the 7 pooled DNA samples were validated by sequencing the PCR product from both ends and by re-sequencing PCR products. SNPs were identified by alignment of sequences using the BioEdit program http://www.biologysoft.com. SNP calls were made on sequences of high quality.

Amplification and Genotyping
The PCR was performed in a final volume of 25 μL containing 1 μL genomic DNA (2.5 ng/μL), 0.25 μL each primer (25 μM), 0.5 μL deoxynucleotide triphosphates (10 μM) mixture, 1.5 μL MgCl2 (25 mM), 0.2 μL DNA polymerase (5 U/μL) (TaKaRa, Japan) and 2.5 μL 10 × reaction buffer on an ABI 2700 thermal cycle1 with the following profile, initial denaturation at 94°C for 4 min; 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and a final elongation at 72°C for 5 min.

Nucleotide diversity of the chicken IGFR gene
We used DNASP4.10 to perform tests of neutrality on the basis of the allelic frequency spectrum [28]. These included Tajima’s D [29], and Fu and Li’s D and F test statistics [30].

Tag SNP Selection
In the dynamic programming algorithm for haplotype block partitioning and tag SNP selection based on haplotype data, Zhang et al. used the following recursive formula [19,31],

\[ S_j = \min \{ S_{i-1} + f(i,..., j) \} \]

if block(i,..., j) = 1 \(1 < j < n\).

Where f(i,..., j) is the number of tag SNPs in this block, block(i,..., j) is a Boolean function, and block(i,..., j) = 1 if and only if SNP (i,..., j) can form a block, Sj is the minimum number of tag SNPs for the optimal haplotype block partition of the first j SNPs, and S0 = 0 http://www.cmb.usc.edu/msms/HapBlock.

Statistical Analyses
The difference of allelic frequencies between the two unrelated chicken populations was tested using Mantel-Haenszel ChiSquare (SAS 8.1 FREQ).

The linkage disequilibrium \(r^2\) value between each pair of SNPs and the haplotype structure of SNP within the gene were estimated by Haploview [32].

Haplotypes were constructed based on haplotype structure of the 18 SNPs in all 434 experimental animals by use of the PHASE 2.0 programme [33], whose function was to reconstruct haplotypes from the population data.

Data were analyzed by the GLM procedure of SAS 8.0 (Statistical Analysis Systems Institute Inc., Cary, NC) and the genetic effects were analyzed by a mixed procedure according to the following model,

\[ Y = \mu + S_i + B_j + g_k + f_s + e_i j k \]

Where Y represented the dependent variable, μ, S_i, B_j, g_k, f_s and e_i j k represented the population mean, fixed effects of sex, fixed effects of hatch, genotype effect, family effect, and random error, respectively. Multiple comparisons were analyzed with least squares means, followed by the multiple comparison procedure, the multiple comparison procedures was followed:

\[ Y_i - \bar{Y}_i = (Y_i - \bar{Y}) + (Y_i - \bar{Y}_i) \]

Where \( \sum (Y_i - \bar{Y}_i)^2 \) was least value, and \( \sum (Y_i - \bar{Y})^2 = 0 \)

Abbreviations
Bp: basepair; IGFR: insulin-like growth factor I receptor; QTL: quantitative trait loci; SNPs: single nucleotide polymorphisms; UTR: un-translating region; WRR: White Recessive Rock chicken; XH: Xinghua chicken.

Authors’ contributions
ML contributed to the genotyping of most of the SNPs, summarized the data and drafted the manuscript. XP contributed to the genotyping of 6 SNPs in XH and WRR chickens. MZ contributed to the genotyping of 5 SNPs in
the XH and WRR chickens. CL contributed to linkage disequilibrium analyses and haplotype construction. QN contributed to the design of the study and the revision of this manuscript. XZ designed the study, supervised the study, edited and made final improvements of this manuscript.

Additional material

Additional file 1
Linkage disequilibrium of the Xinghua chickens and Recessive White Rock chickens. Pairwise LD versus physical distance between all pairwise SNP, average values of r2 show that LD declines with increasing physical distance between SNP pairs.
Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2156-9-70-S1.doc]

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References
1. Amills M, Jiménez N, Villalba D, Tor M, Molina E, Cubiló D, Marcos M: Allelic variation in gene expression is common in the human genome. Genome Res 2003, 13:1855-1862.

2. Wyzyńska-Koko J, Pierzchala M, Fiszkowski K, Kameczuk M, Rożycki M, Kury J: Polymorphisms in coding and regulatory regions of the porcine MYF6 and MYOG genes and expression of the MYF6 gene in m. longissimus dorsi versus productive traits in pigs. J Appl Genet 2006, 47:131-138.

3. Bonafe M, Barbieri M, Marchegiani F, Oливier F, Ragno E, Giampieri C, Muginesi E, Centurelli M, Franceschi C, Paolisso G: Polymorphic Variants of Insulin-Like Growth Factor I (IGF-I) Receptor and Phosphoinositide 3-Kinase Genes Affect IGF-I Plasma Levels and Human Longevity: Cues for an Evolutionarily Conserved Mechanism of Life Span Control. J Clin Endocrinol Metab 2003, 88:3299-3304.

4. Hamer E, Kutsche K, Haag F, Ulrich K, Sudbrak R, Willig RP, Braulke T, Kubler B: Mono-allelic expression of the IGF-I receptor does not affect IGF responses in human fibroblasts. Eur J Endocrinol 2004, 151:521-529.

5. Kawashima Y, Kanazaki S, Yang F, Kinoshita T, Hanaki K, Nagaiishi J, Ohnaka Y, Hitosato I, Ninomoya H, Nanba E, Fukushima T, Takahashi S: Mutation at Cleavage Site of Insulin-Like Growth Factor Receptor in a Short-Stature Child Born with Intrauterine Growth Retardation. J Clin Endocrinol Metab 2005, 90:4679-4687.

6. Inagaki K, Tulipakv A, Rubsov P, Sverdlova P, Peterkova Y, Yakar S, Terekhov S, LeRoith D: A familial IGF-I receptor mutant leads to short stature: Clinical and biochemical characterization. J Clin Endocrinol Metab 2007, 92:1542-1548.

7. Le Roith D, Kavasen VM, Koval AP, Roberts CT Jr: Phytology of the insulin-like growth factors (IGFs) and receptors: a molecular approach. Mol Reprod Dev 1993, 35:322-336.

8. Maures T, Chan SJ, Xu B, Sun H, Ding J, Duan C: Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. Endocrinology 2002, 143:1858-1871.

9. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Loechner A, Faggart M, Liu-Cordero SN, Rotimi C, Audeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. Science 2002, 296:2225-2229.

10. Yu YT: Population Structure of Core Inbred Lines and Allelic Diversity of rab17, A Candidate Gene for Drought Tolerance in Maize. Doctor Thesis of Chinese Academy of Agricultural Sciences 2006.

11. Maures T, Chan SJ, Xu B, Sun H, Ding J, Duan C: Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. Endocrinology 2002, 143:1858-1871.

12. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Loechner A, Faggart M, Liu-Cordero SN, Rotimi C, Audeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. Science 2002, 296:2225-2229.

13. Yu YT: Population Structure of Core Inbred Lines and Allelic Diversity of rab17, A Candidate Gene for Drought Tolerance in Maize. Doctor Thesis of Chinese Academy of Agricultural Sciences 2006.

14. Fu M, Damcott CM, Sabra M, Pollin TI, Sabra M, Pollin TI, Ott SH, Wang J, Garant MJ, O’Malley RB, Shattuck AR: Polymorphisms in the casein gene (CASQ1) on chromosome 1q21 is associated with Type 2 diabetes in the old Order Amish. Diabetes 2004, 53:3292-3299.

15. Fan R, Spinka C, Jin L, Jung J: Pedigree linkage disequilibrium mapping quantitative trait loci. Eur J Hum Genet 2005, 13:216-231.

16. Rinaldo A, Barcan SA, Devin B, Sonpar V, Wasserman L, Roeder K: Characterization of multilocus linkage disequilibrium. Genet Epidemiol 2005, 28:193-206.

17. Ordoni M, Narita A, Watanabe T, Yokouuchi K, Sugimoto Y, Fujita T, Oguni T, Matsumoto M, Sasaki Y: Genome-wide linkage disequilibrium in two Japanese beef cattle breeds. Anim Genet 2006, 37:139-144.

18. Morahan G, Huang D, Yimer S, Cancilla MR, Stephen K, Dabaghpo W, Werther G, Tait BD, Harrison LC, Colman PG: Linkage disequilibrium of a type 1 diabetes locus with a regulatory IL12B allele. Nat Genet 2001, 27(2):218-221.

19. Zhang K, Qin ZS, Liu JS, Chen T, Waterman MS, Sun F: Haploptype Block Partitioning and Tag SNP Selection Using Genotye Data and Their Applications to Association Studies. Genome Res 2004, 14:908-916.

20. Diatchenko L, Anderson AD, Slade GD, Fillingim RB, Shabalina SA, Higgins TJ, Sama S, Belfer I, Goldman D, Max MB, Weir BS, Maxner W: Three major haplotype types of the beta2 adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder. Am J Med Genet B Neuropsychiatr Genet 2006, 141B(5):449-462.

21. Rodriguez SA, Gaunt TR, Dennison E, Chen XH, Syddall HE, Phillips DI, Cooper C, Day IN: Replication of IGF2-INS-TH5 haplotype effect on obesity in older men and study of related phenotypes. Eur J Hum Genet 2006, 14:109-116.

22. McElroy JP, Kim JJ, Harry DE, Brown SR, Dekkers JC, Lamont SJ: Id entification of trait loci affecting white meat percentage and other growth and carcass traits in commercial broiler chickens. Poult Sci 2006, 85:593-605.

23. Park HB, Jacobsson L, Wahlberg P, Siegel PB, Andersson L: QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. J Anim Sci 2006, 84:216-223.

24. Rabie TS, Crooijmans RP, Bovenhuis H, Vereijken AL, Veenendaal T, Poel J van der, Van Arendonk JA, Pakdel A, Groenen MA: Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop pulmonary hypertension syndrome. Anim Genet 2005, 36:468-476.

25. Zhou H, Evock-Clover CM, McMurtry JP, Ashwell CM, Lamont SJ: Genome-wide Linkage Analysis to Identify Chromosomal Regions Affecting Phenotypic Traits in the Chicken. II. Body Composition. Poult Sci 2005, 84:1712-1721.

26. Lei MM, Nie QH, Peng X, Zhang DX, Zhang XQ: Single nucleotide polymorphisms of the chicken insulin-like factor binding protein 2 gene associated with chicken growth and carcass traits. Poult Sci 2005, 84:1191-1198.
bipolar mood disorder on chromosome 18p11.3 in the Costa Rican population. Proc Nat Acad Sci USA 2001, 98:11485-11490.

28. Rozas J, Sánchez-DelBarrio JC, Meseguer X, Rozas R, Dna SP. DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 2003, 19:2496-2497.

29. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 1989, 123:585-595.

30. Fu YY, Li WH. Statistical test of neutrality of mutations. Genet 1993, 133(3):693-709.

31. Zhang K, Calabrese P, Nordborg M, Sun F. Haplotype Block Structure and Its Applications to Association Studies: Power and Study Designs. Am J Hum Genet 2002, 71:1386-1394.

32. Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES. High-resolution haplotype structure in the human genome. Nat Genet 2001, 29:229-232.

33. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001, 68:978-989.