Detection of CNV in the *SH3RF2* gene in chickens and its effects on growth and carcass traits

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**BMC Genetics**  **BMC Series**

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DOI: 10.21203/rs.2.20034/v1

SUBJECT AREAS  Population Genetics  Medical Genetics

KEYWORDS  
SH3RF2 gene, CNV, growth traits, carcass traits, association analysis
Abstract

Background

*SH3RF2* gene was a protein-coding gene located in the quantitative trait locus associated with body weight, and its deletion was positively correlated with body weight in chickens.

Results

In the present study, the *SH3RF2* genotype of the “Gushi ×Anka” F2 resource population, as well as local chickens and commercial layers and broilers in China was detected, and the relationship between the genotype and chicken production traits was further studied by molecular biology technology. The results showed that the local chickens and commercial layers were all homozygous for the wild-type allele. In commercial broiler breeds, deletion mutation individuals were detected in all commercial broiler breeds except Hubbard breeds. In addition, 798 individuals in the F2 resource group were used to analyze the effects of different genotypes (DD\ ID\ II) on chicken production traits. The results showed that the mutation was associated with 2, 6, 10, and 12-week body weight and a significant correlation with 8-week breast bone length (P<0.05); The mutation was significantly correlated with 8-week body weight and 4-week breast bone length (P<0.01). Additionally, evisceration weight, legs muscle weight, carcass weight and other significant correlations (P<0.05) and breast muscle weight and gizzard weight were significantly correlated (P<0.01).

Conclusions

The results of this study indicated that the copy number variation of the *SH3RF2* gene contributed to the growth and weight gain of chickens.
Background

In recent years, with the development of the economy and the improvement of people's living standards, consumers have higher requirements for the quality of poultry products, especially flavor and taste. Chinese local chickens have excellent characteristics, such as tender meat, good taste and unique flavor, which are favored by consumers. Because growth traits and carcass traits are the main economic traits of poultry, there are some shortcomings such as slow growth rate and low feed utilization rate in local chicken breeds in China. Therefore, genetic improvement, such as cultivating new varieties, increasing the growth rate of chickens and increasing the rate of lean meat, has been the focus of researchers[1]. Thus, the use of modern molecular markers to aid in marker selection and molecular breeding of chickens is particularly important.

DNA molecular marker technology uses the gene library of the organism itself without compromising the natural composition of the gene and the expression of the gene itself [2]. It is a kind of genetic marker technology that directly analyzes the nucleic acid level and reflects the individual characteristics. Molecular marker technology is helpful in revealing the difference in the composition and arrangement of the whole genome or the variation of the base within the gene to evaluate DNA variability and polymorphism and to identify individuals containing target genes by genotype analysis of closely linked genetic markers of target genes to improve the selection efficiency, reduce blindness and speed up the breeding process[3]. Compared with traditional genetic markers, molecular marker-assisted selection (MAS) has many marker loci, a large amount of genetic information, and strong repeatability of experiments not susceptible to environmental impact and has no limitation of sex and age. Therefore, it allows early selection, shortens the
generation interval, improves the selection intensity, and thus improves the efficiency and accuracy of selection. Therefore, MAS has a broad application prospects in animal genetic improvement [4]. At present, the application of DNA molecular marker technology in poultry genetic breeding mainly includes genetic diversity analysis, germplasm identification, genetic relationship research, genetic map construction, QTL mapping and molecular marker-assisted breeding [5]. A large number of genetic polymorphisms, including single nucleotide polymorphism (SNPs), insertions/deletions (indels) and copy number variation (CNV), have been revealed through whole-genome sequencing of many species [6,7]. CNV is an important source of genetic variation [8]. CNV is the main form of genome structural variation, which refers to the insertion, deletion, duplication, translocation and derived chromosome structural variation of DNA fragments larger than 1 kb in the genome compared with the reference sequence of the genome [9]. Because many CNVs contain entire genes, they are more difficult to identify and type than SNPs and indel copy number variants. As a result, they affect organisms even more. CNV is an important source of genetic variation complementary to SNP. It is related only to disease and abnormal development of livestock and poultry, but also to physical appearance and many economic traits [10]. For example, Wright [11] found that the first intron CNV of the SOX5 gene is related to the crown type. The bean crown mutation in the chicken is a dominant mutation, which greatly reduces the size of the bean crown, thereby reducing heat loss and preventing frostbite, and is an adaptive characteristic of the chicken in a cold environment [8]. Ben [12] found that the insertion of the EDN3 gene was the main cause of overstaining of the skin of the black chicken. Elferink [13] found that a segment comprising a chicken prolactin receptor in the Z-chromosome is
associated with the growth of the chicken undefineds fast-and-slow feather. Fu [14] found that the CNV of the DGAT2 gene had a significant effect on the growth and carcass traits of chickens when exploring the relationship between the CNV of DGAT2 and the economic characteristics of the F2 resource group. The above results indicate that the CNV in the gene has an important influence on the economic characteristics of the poultry and that the diversity of the organism is enriched. At present, there are few studies on the effect of SH3RF2 gene CNV on poultry growth and development. Rubin[15] re-sequenced the chicken genome and found a selective clearance region in many chicken genomes, including the SH3RF2 gene mutation site, which is located on chromosome 13, except for retention of the first exon. All other exons were deleted, the total deletion length was 18961 bp, and the gene was located in a body-related quantitative train locus (QTL) range. The results of the study showed that the deletion mutation in the gene was positively correlated with the body weight of the chicken and fixed in the high-growth line of the broiler, appearing at lower frequencies in low-growth lines and commercial broilers, indicating that this deletion is closely related to broiler growth and body weight. Zhao[16] studied the frequency distribution of the indel mutation of the SH3RF2 gene in 15 local chicken species in China, and the results showed that there were no local chickens with the deletion mutation. In this study, we tested the genotypes of local chicken breeds, commercial broilers, commercial laying hens and the “Gushi × Anka” chicken F2 resource population to determine which SH3RF2 gene mutations existed in the population and to analyze the gene. The relationship between growth and carcass traits of F2 resources in “Gushi × Anka” chickens showed that the SH3RF2 gene can be used as an effective marker for poultry production traits.
Results

Genotype analysis

After amplification, if only 305 bp product bands were found in all DNA samples, the result was determined to be a nondeletion homozygous type and named the II genotype. If there were 305 bp and 416 bp product bands, the results were determined to be heterozygous and named the ID genotype. If there were only 416 bp product bands, the result was determined to be deletion homozygous type and named the DD genotype (Fig 1).

Analysis of genetic parameters of SH3RF2 gene mutation in different populations

This experiment analyzed the distribution of SH3RF2 gene indel genotypes and gene frequencies in 17 varieties. As shown in Table 2, there is a significant difference in the genotype frequency among the F2 resource population, AA broilers, and 308 and CB broilers. ID genotypes and II genotypes were the main genotypes. I was the primary allele, and the frequency of II genotypes in each population was higher than that of ID genotypes and DD genotypes. Among them, the frequency of the DD genotype was the highest in the AA broiler group. The He and Ne values were 0~0.35 and 1~1.55, respectively. According to the standard polymorphism information and polymorphism information content (PIC), PIC>0.5 represents high polymorphism, PIC 0.25~0.5 represents moderate polymorphism, and PIC<0.25 represents low polymorphism. Only the F2 resource group and AA broiler species showed moderate polymorphism, while other species showed low polymorphism.

Association analysis of SH3RF2 genotypes and growth traits in F2 resource population
As shown in Table 3, the results showed that the growth traits in the F2 generation were significantly correlated with the mutation of the *SH3RF2* gene: the mutation was significantly correlated with BW at 2, 6, 8, 10, 12 weeks and BBL at 8 weeks (P<0.05). The mutation was significantly correlated with 8-week BW and 4-week BBL (P<0.01). The growth trait of the homozygous genotype DD was higher than those of the other two genotypes in each week. Among them, the 8-week-old BW and 4-week BBL of individuals with the DD genotype were 92.3% and 97.2% higher than those of individuals with the II genotype, respectively, indicating that the DD genotype was an economically dominant genotype. Compared with the II genotype and ID genotype, the growth traits of the ID genotype were higher than that of the II genotype each week, indicating that allele D was the dominant allele.

**Association analysis between *SH3RF2* genotypes and carcass traits in the F2 resource population**

The results of the correlation analysis showed that the *SH3RF2* gene mutation was significantly related to SEW, EW, SEP, EP, LMW, LWR, GWR and CW (P < 0.05) and significantly correlated with MW, GW and BMWR (P < 0.01). Table 4 shows that the trend of the slaughter index of each genotype is DD genotype > ID genotype > II genotype, which is consistent with the trend of the analysis results associated with the growth traits.

**Discussion**

**Analysis of *SH3RF2* gene polymorphism and genetic population structure of different breeds of chickens**

The genetic resources of poultry species in China are rich and diverse and contain great genetic variation and selection potential [20]. The vigorous development of
animal husbandry will promote breeding efforts to improve the medium and low performance of chicken production [21]. Therefore, it is necessary to study the genetic variation of different chicken breeds. In this study, the frequencies of DD genotypes and D alleles were lower in the tested population, and few individuals with deletions were found in local and commercial layers. In addition to moderate polymorphism in the F2 resource population and AA broiler species, all other breeds showed low polymorphism. According to the polymorphism information content standard, this means that the F2 resource population and AA broiler species have better selection in breeding and growth development. In general, allele frequency reflects genetic diversity between populations, and we found that allele I was the dominant allele in each species, suggesting that the gene did not undergo the same selection in the evolution of different breeds of chicken. In addition, artificial selection also has a significant impact on the number of genes and distribution of genetic variation in different varieties [22]. We found that the distribution of DD genotypes was higher in commercial broiler populations with fast, large and high yields than that in other breeds, indicating that targeted breeding of commercial broiler chickens promoted the fixation of DD genotypes, which may be related to the rapid growth of commercial broiler chickens. We found no mutant deletion in commercial layers, which may be related to the breeding direction of layers.

**Association between SH3RF2 gene mutation and growth and carcass traits**

Body weight and body size are important indicators reflecting the body development of poultry and are closely related to important economic characteristics [23]. Currently, here are few studies on the SH3RF2 gene in poultry production. Rubin [15] used 400 chickens from F8 generations of high-growth and low-growth lines to analyze the effect of the SH3RF2 gene deletion mutation on chicken growth
traits, and the results showed that the effect of this mutation on chicken growth traits was highly significant (P<0.01). In this study, the F2 resource population of Anka and Gushi chickens was used to analyze the relationship between the *SH3RF2* gene mutation and growth traits and carcass traits of chickens. The results also showed that the mutation had a significant influence on the growth traits and carcass traits of chickens. As poultry carcass muscles are mainly distributed on the chest and legs, the yield of chest and leg muscles is also an important factor affecting the slaughtering performance of poultry and is one of the traits considered in poultry breeding Wang[24]. In this study, the *SH3RF2* gene mutation was significantly correlated with growth traits, such as BW and BBL, and slaughter indicators, such as GW, GWLMW and CW (P<0.05). In addition, individuals with DD genotypes had higher phenotype values than individuals with II genotypes and ID genotypes. The results show that the DD genotype has a significant dominant effect on the F2 resource population.

Conclusion

This study found that *SH3RF2* gene CNV may one of the main genes controlling the growth traits of chickens. The results show that the mutation can be used for MAS breeding in chickens. Molecular breeding of chickens can reduce the breeding cost, shorten the generation gap, and improve the efficiency of breeding to provide technical support for local chicken breeding in China.

Materials and methods

**Experimental animals**

To verify the absence of mutations in different groups, this experiment tested the
Gushi-Anka chicken F2 generation resource population, eight local varieties, five chicken varieties and two layer varieties, for a total of 4079 individuals of DNA samples (Table 1). All blood samples were collected through the wing vein, after which 1:300 multidimensional hormone was used to reduce the stress response. Whole genome DNA was extracted from whole blood using the phenol-chloroform method. All these chickens used in the experiment were healthy animals raised in the same environment with ad libitum access to feed and water. Among them, the F2 generation resource population of Gushi-Anka F2 chickens consists of 7 families and 836 individuals. A total of 836 F2 resource population were euthanised at 84 d. Specific euthanasia methods are listed in additional file 2. From hatch to slaughter, several chicken growth traits including body weight and body size indexes were measured. Each chicken was weighed every 2 weeks; shank length was measured at 0, 4, 8 and 12 weeks; shank girth, chest depth, breast bone length, body slanting length and pelvis breadth were determined at 4, 8 and 12 weeks, respectively. The specific construction method, feeding management and trait determination procedures can be found in previous studies [17].

**Primer and PCR amplification**

The primers used in this study were all designed by Rubin [15] in the research process (Forward:5’-TGCTTCGGGCTGAGCCTTCT-3’, Reverse1:5’-CGCCCAAGCTGTGTCCT-3’, Reverse2:5’-CTGTCGGGCACGTGAGTGAA-3’). Assays were performed by PCR in a total volume of 10 μL containing 5 μL of 2 × Taq Master Mix (Kangwei, Beijing, China), 0.5 μL of forward primer and 0.5 μL of reverse primer, 2.5 μL of ultrapure water and 1 μL of genomic DNA. The PCR amplification was performed as follows: 95°C for 5 min; followed by 30 cycles at 95°C for 30 s, 64°C for 30 s, and 72°C for 10 min; then, samples were chilled at 4°C. To determine the
genotype, aliquots from each reaction (7 μL) were subjected to electrophoresis in a 2% agarose gel.

**Statistical analysis**

Statistical analyses of the associations between the genotypes and the selected traits of the F2 chickens were performed using IBM SPSS (SPSS for Windows, Standard version 24 ; SPSS, USA). Genotype effects were analyzed by a multivariate linear model, and the differences among genotypes were compared by Benferroni's multiple comparison method. The specific analysis method referred to previous studies [18]. All data for each trait obtained by statistical analysis are presented as the mean ± standard error (mean ± SE). P < 0.05 was considered to be statistically significant [19].

**Abbreviations**

F2, AA, 308, HBD, CB, RW, 817, HL, LM, XC, GS, CS, LS, YY, WH, GF, GC indicate F2 resource population, Arbor Acres broiler, Ross308, Hubbard breeders, Cobb broilers, Recessive white chicken, 817 broiler, Hyline brown hen, Lohmann brown laying hen, Xichuan chicken, Gushi chicken, Changshun chicken, Lushi chicken, Yunyang chicken, Wuhei chicken, Guifei chicken, and Henan gamecock, respectively.

BW: body weight, SL: Shank length, CW: Chest width, SG: Shank circumference, BBL: Breastbone length, BSL: Body slanting length, EW: evisceration weight, GW: gizzard weight, CW: carcass weight, MW: breast muscle weight, SEW: semievisceration weight, LMW: leg muscle weight, EP: evisceration percentage, SEP: semievisceration weight rate, GWR: gizzard weight rate, HWP: head weight percentage, LWR: liver weight rate, BMWR: breast muscle weight rate, BWLP:
breast muscle water loss rate.

Declarations

**Ethics approval**

All animal experiments and animal care methods were approved by the Institutional Animal Care and Use Committee (IACUC) of Henan Agricultural University, Zhengzhou, P.R. China (Permit Number: 11-0085) and were performed in accordance with the protocols outlined in the “Guide for Care and Use of Laboratory Animals” (Henan Agricultural University).

**Consent for publication**

Not applicable.

**Availability of data and materials**

All the data supporting the conclusions of the study are included in the manuscript and the additional file 3.

**Competing interests**

Conflict of interest On behalf of all authors, the corresponding author states that there are no conflicts of interest.

**Funding**

National Natural Science Foundation of China-Henan joint grant (U1804107) to Zhuanjian Li. The funder had role in study design the experiments and data analysis. National Natural Science Foundation of China (31872987) to Yadong Tian. The funder had role in study oversaw the data collection process. Earmarked Fund for Modern Agro-Industry Technology Research Systems of China (NO. CARS-40-K04) to Xiangtao Kang. The funder had role in study oversaw the data collection process.
Authors’ contributions

Conceived and designed the experiments: Z.Z.J, X.L.W, Z.J.L

Performed the experiments: Z.Z.J, C.J.W, D.H, T.L, W.Y.L, Y.Y.C

Analyzed the data: Z.Z.J, X.L.W, Z.J.L

Wrote the paper: Z.Z.J

Oversaw the data collection process: R.L.H, H.L, G.R.S, Y.D.T, X.J.L, X.T.K

All authors read and approved the final manuscript.

Acknowledgements

We wish to thank our lab members for their helpful discussions.

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Tables

Table 1. Name of variety and number of samples

| Breed | Sample/N | Breed | Sample/N |
|-------|----------|-------|----------|
| F2    | 798      | XC    | 260      |
| AA    | 636      | GS    | 174      |
| 308   | 238      | CS    | 144      |
| HBD   | 282      | LS    | 202      |
| CB    | 193      | YY    | 44       |
| RW    | 187      | WH    | 216      |
| 817   | 83       | GF    | 18       |
| HB    | 456      | GC    | 88       |
| LB    | 60       |       |          |

Note: F2, AA, 308, HBD, CB, RW, 817, HL, LM, XC, GS, CS, LS, YY, WH, GF, GC indicate F2 resource population, Arbor Acres broiler, Ross308, Hubbard breeder, Cobb broilers, Recessive white chicken, 817 broiler, Hyline brown hen, Lohmann brown laying hen, Xichuan chicken, Gushi chicken, Changshun chicken, Lushi chicken, Yunyang chicken, Wuhei chicken, Guifei chicken, and Henan
Table 2. Genotypic and allelic frequencies and related genetic parameters for the chicken SH3RF2 gene

| Breed /N  | Genotypic and allelic frequencies | He | Ne |
|-----------|----------------------------------|----|----|
|           | DD   | ID   | II  | D   | T   |     |    |
| F2/798    | 0.06 | 0.34 | 0.60 | 0.23 | 0.77 | 0.35 | 1.55 |
| AA/636    | 0.31 | 0.28 | 0.41 | 0.45 | 0.55 | 0.49 | 1.98 |
| 308/238   | 0.01 | 0.33 | 0.32 | 0.13 | 0.87 | 0.22 | 1.40 |
| CB/193    | 0.01 | 0.16 | 0.83 | 0.17 | 0.91 | 0.09 | 1.10 |
| HBD/282   | 0    | 0.08 | 0.92 | 0.04 | 0.96 | 0.08 | 1.08 |
| 817/83    | 0    | 0.30 | 0.70 | 0.15 | 0.85 | 0.26 | 1.34 |
| RW/187    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| XC/260    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| GS/174    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| CS/144    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| GF/18     | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| YY /44    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| LS/202    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| WH/216    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| GC/88     | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| HB/456    | 0    | 0    | 1    | 0    | 1    | 0    | 1   |
| LB/60     | 0    | 0    | 1    | 0    | 1    | 0    | 1   |

Note: F2, AA, 308, HBD, CB, RW, 817, HL, LM, XC, GS, CS, LS, YY, WH, GF, GC indicate F2 resource population, Arbor Acres broiler, Ross308, Hubbard breeders, Cobb broilers, Recessive white chicken, 817 broiler, Hyline brown hen, Lohmann brown laying hen, Xichuan chicken, Gushi chicken, Changshun chicken, Lushi chicken, Yunyang chicken, Wuhei chicken, Guifei chicken, and Henan gamecock, respectively.

Table 3. Association analysis between SH3RF2 gene mutation and growth traits in the F2 resource population

| Traits    | II(n=478) | Mean ± SE | ID(n=272) | DD(n=47) |
|-----------|-----------|-----------|-----------|----------|
| BW2(g)    | 115.985±2.855<sup>ab</sup> | 122.347±1.162<sup>a</sup> | 123.847±0.861<sup>a</sup> |
| BW4(g)    | 308.965±6.814 | 322.282±2.814 | 324.787±2.115 |
| BW6(g)    | 536.205±12.649<sup>ab</sup> | 563.207±5.306<sup>a</sup> | 569.033±3.984<sup>a</sup> |
| BW8(g)    | 762.844±19.211<sup>b</sup> | 817.485±7.894<sup>a</sup> | 826.332±5.989<sup>a</sup> |
| BW10(g)   | 1055.384±23.335<sup>ab</sup> | 1113.946±9.888<sup>a</sup> | 1123.156±7.358<sup>a</sup> |
| BW12(g)   | 1287.379±27.936<sup>ab</sup> | 1358.504±11.755<sup>a</sup> | 1361.616±8.84<sup>a</sup> |
| SL8(cm)   | 7.713±0.094 | 7.923±0.039 | 7.947±0.029 |
| CW8(cm)   | 5.548±0.082 | 5.689±0.034 | 5.698±0.025 |
| SG8(cm)   | 3.377±0.033 | 3.411±0.014 | 3.439±0.01 |
| BBL4(cm)  | 6.061±0.074<sup>ab</sup> | 6.193±0.031<sup>a</sup> | 6.267±0.023<sup>a</sup> |
| BBL8(cm)  | 8.722±0.097<sup>ab</sup> | 8.908±0.04<sup>a</sup> | 8.964±0.03<sup>a</sup> |
| BSL4(cm)  | 11.154±0.118<sup>ab</sup> | 11.347±0.048<sup>a</sup> | 11.475±0.036<sup>a</sup> |
Note: BW0, 2, 4, 6, 8, 10, and 12 at 0 days, 2, 4, 6, 8, 10 and 12 weeks, BW: body weight, SL: Shank length, CW: Chest width, SG: Shank circumference, BBL: Breastbone length, BSL: Body slanting length. The same shoulder letters on the same line indicate no significant difference (P > 0.05); the different letters indicate significant differences (P < 0.05).

Table 4. Association analysis between SH3RF2 gene mutation and carcass traits in the F2 resource population

| Traits   | II(n=478)       | ID(n=272)       | DD(n=47)       | P-v  |
|----------|-----------------|-----------------|----------------|------|
| EW(g)    | 870.526±20.885^a | 922.81±8.717^a | 928.011±6.63^a | 0.0  |
| GW(g)    | 25.955±0.674^b  | 27.568±0.284^b | 28.450±0.214^ab | 0.0  |
| CW(g)    | 1134.15±24.818^ab | 1190.264±10.44^a | 1198.651±7.936^a | 0.0  |
| MW(g)    | 63.095±2.21b    | 71.055±0.918a   | 71.3±0.695a     | 0.0  |
| SEW(g)   | 1046.01±24.113^ab | 1104.431±10.102^a | 1109.572±7.62^a | 0.0  |
| LMW(g)   | 141.966±3.465^ab | 149.74±1.464^a | 151.338±1.103^a | 0.0  |
| EP(%)    | 67.374±0.288^ab | 67.829±0.121^a | 68.109±0.092^a | 0.0  |
| SEP(%)   | 81.075±0.288^a  | 81.109±0.122^ab | 81.54±0.092^a  | 0.0  |
| GWR(%)   | 2.016±0.045^a   | 2.053±0.019^ab  | 2.113±0.015^a  | 0.0  |
| HWP(%)   | 3.311±0.048     | 3.186±0.02      | 3.208±0.016    | 0.0  |
| LWR(%)   | 2.246±0.045^a   | 2.125±0.019^ab  | 2.145±0.015^a  | 0.0  |
| BMWR(%)  | 14.383±0.267^b  | 15.346±0.11^a   | 15.233±0.085^a | 0.0  |
| BWLP(%)  | 22.687±0.745    | 23.738±0.313    | 24.342±0.238   | 0.0  |

Note: EW: evisceration weightGW: gizzard weightCW: carcass weightMW: breast muscle weightSEW: semievisceration weightLMW: leg muscle weightEP: evisceration percentage SEP: semievisceration weight rateGWR: gizzard weight rateHWP: head weight percentage LWR: liver weight rateBMWR: breast muscle weight rate BWLP: breast muscle water loss rate. The same shoulder letters on the same line indicate no significant difference (P > 0.05); the different letters indicate significant differences (P < 0.05).

Figures
Figure 1

Agarose gel electrophoresis of SH3RF2 gene mutation

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Additional file 3 .xls
Additional files 1.doc
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