A new discovery of genetic polymorphisms of important genes in WNT pathway (LPR5 and AXIN1) associated with osteoporosis susceptibility in Chinese Han population

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Abstract

Background: genetic factors play a critical role in the pathogenesis of osteoporosis. The imbalance of WNT/β-catenin will cause the occurrence of osteoporosis. LPR5 and AXIN1 play an important role in the classical Wnt/β-catenin signaling pathway. Our study was aimed to determine the association between 5 candidate single nucleotide polymorphisms (SNPs) of LPR5 or AXIN1 and osteoporosis susceptibility in Chinese Han population.

Methods: the association analysis was conducted between 5 candidate SNPs and osteoporosis susceptibility among 1198 participants. Agena MassARRAY was used to genotype SNPs. The association between SNPs and osteoporosis susceptibility in different genetic models was analyzed by logistic regression analysis. Multi-factor dimension reduction (MDR) was used to analyze the interaction of SNP-SNP in the osteoporosis risk. The difference of clinical indicators under different genotypes was completed by one-way analysis of variance.

Results: we found that LPR5 rs11228240, AXIN1 rs2301522 and rs9921222 were significantly associated with the osteoporosis susceptibility. The results of subgroup analysis showed that LPR5 rs11228240 (protective factor) and AXIN1 rs2301522 (risk factor) were significantly associated with the susceptibility of osteoporosis among participants who were age > 60 years, female or BMI≤24; AXIN1 rs9921222 significantly increased the risk of osteoporosis among participants with BMI≤24. The results of Haplotype analysis showed that $A_{rs2301522}C_{rs9921222}$ could increase the susceptibility of osteoporosis. We also found that LRP5 rs11228219, AXIN1 rs2301522 and rs9921222 showed a potential association with some clinical indicators of osteoporosis.

Conclusion: the SNPs of LPR5 and AXIN1 which are important genes in WNT classical pathway, have a potential association with osteoporosis susceptibility in Chinese Han population.

Introduction

Osteoporosis reduces the production and function of osteoblasts, reduces bone mass, and increases the proportion of bone marrow adipocytes (1). The serious consequences will lead to increased bone fragility and prone to osteoporotic fractures. Among the many pathogenic conditions of osteoporosis (2-4), genetics has always been an important factor that has attracted the attention of experts worldwide (5). At the same time, some research results suggest and confirm that genetic factors, namely gene polymorphism or gene mutation, play an important role in the complex pathological mechanism of primary osteoporosis (6, 7).

WNT signaling pathway plays an irreplaceable role in embryonic development, and plays an important role in controlling bone development, bone mass and osteoblast differentiation (8-10). The classical WNT signaling pathway (WNT / β-catenin signaling pathway) can guide bone marrow mesenchymal stem cells into differentiation (11). In osteoporosis cases, the number of common bone marrow mesenchymal stem cells is small, and the ability of bone differentiation is reduced, and the formation of osteoblasts is

less (12). Therefore, more and more scholars are interested in the molecular mechanism between WNT signaling pathway related genes and osteoporosis. Up to now, many studies have been reported on the relationship between genetic polymorphism and susceptibility to osteoporosis (13-16), but the mechanism of WNT signaling pathway in the development of osteoporosis is still unclear .

Low density lipoprotein receptor-associated protein 5 (LPR5) is involved in the WNT classical signaling pathway, which can affect the activation of WNT/β-catenin signaling pathway by changing the distribution of β-catenin in cells and regulating the transcription activity of TCF/LEF. The imbalance of WNT/β-catenin signaling pathway will reduce bone mineral density (BMD) and the occurrence of osteoporosis (17). Yavropoulou, M. P. et al. found that the interaction among the three single nucleotide polymorphisms in VDR, COL1A1 and LPR5 genes jointly affected osteoporosis in a young man (18). However, there are few studies on the association between LPR5 gene polymorphism and the occurrence and development of osteoporosis in Chinese Han population (19, 20). In order to further understand the occurrence / development mechanism of osteoporosis, this study will analyze the association between LPR5 gene polymorphism and susceptibility to osteoporosis.

In addition, WNT/β-catenin signaling pathway is regarded as one of the main ways to control bone mass (17). The interaction between AXIN1 and β-catenin in cytoplasm is conducive to continuous phosphorylation (21). Studies have found that AXIN1 gene polymorphisms can be used as a new bone mineral genetic signal, and BMD is an important indicator of osteoporosis (17). Therefore, it is necessary to explore more AXIN1 genetic polymorphism associated with osteoporosis, which will help to clarify the mechanism of AXIN1 affecting the occurrence and development of osteoporosis.

In summary, our study conducted an association study between 5 candidate genetic polymorphisms (LPR5 rs11228219, rs4988321, rs11228240 and AXIN1 rs2301522, rs9921222) and osteoporosis susceptibility in 1198 Chinese Han population. This study will help to better understand the mechanism of key genes (LPR5 and AXIN1) in WNT signaling pathway in the occurrence and development of osteoporosis in Chinese Han population. Our research will lay a certain theoretical foundation for the individualized treatment and diagnosis of osteoporosis in clinic.

Materials And Methods

Study subjects

The subjects of our study were consistent with 1198 Chinese Han people from the Second Affiliated Hospital of Xi'an Jiaotong University at the same period. These participants were diagnosed by 599 patients with osteoporosis (no other diseases; No family history of hereditary diseases) and 599 healthy individuals (healthy individuals from a health check-up center in the Second Affiliated Hospital of Xi'an Jiaotong University). All the participants' demographic information and epidemiological information are obtained by questionnaire and access to the participants' medical records. This study followed the Helsinki Declaration of the World Medical Association and approved by the Ethics Committee of the

Second Affiliated Hospital of Xi'an Jiaotong University. After obtaining the written informed consent of all participants, we collected their peripheral blood samples for DNA extraction.

Selection of SNPs

After consulting the relevant information of LPR5 and AXIN1 gene polymorphisms in the dbSNP database, we selected the genetic loci with the smallest allele frequency ≥ 5% in the study population. Finally, 3 LPR5 SNPs (rs11228219 T/C, rs4988321 A/G, rs11228240 T/C) and two AXIN1 SNPs (rs2301522 G/A, rs9921222 T/C) were selected as candidate SNPs for this study.

DNA extraction and genotyping

When we extract and purify DNA from peripheral blood samples, the specific steps are based on the instructions of the kit (GoldMag Co. Ltd. Xi'an, China). The purified DNA is stored in the refrigerator. All primers in this study were designed by MassARRAY Assay Design software (Supplemental table 1). Genotyping was performed by the MassARRAY system (Agena, San Diego, CA, USA).

Quality control: We randomly select 3% of DNA samples for repeated tests. The repetition rate of the experimental results is >99%, indicating that the results have reached the statistical standard, and the reliability is high.

Statistical analysis

The differences in demographic characteristics (age, gender, etc.) in this study were tested with SPSS 21.0 version. Whether the candidate SNPs in this study meet the Hardy-weinberg equilibrium (HWE) is completed by Fisher's exact test with SPSS 21.0 software. The logistic regression model was used to analyze the calculated odds ratio (OR) and 95% confidence interval (CI) to evaluate the association between candidate SNPs and the osteoporosis susceptibility in Chinese Han population (The susceptibility to osteoporosis is evaluated based on the OR value; OR = 1: this factor has no effect on the susceptibility to osteoporosis; OR <1: this factor can reduce the susceptibility to osteoporosis; OR> 1: this factor can increase the susceptibility to osteoporosis). Using wild-type alleles as a reference, the SNPstats online tool software is used to predict multiple genetic models (codominant, dominant, recessive and log-additive). In this study, haplotype analysis was conducted by PLINK 1.07 and Haploview software and linkage disequilibrium (LD) was calculated. All statistical results are adjusted by age and gender. Finally, we use multi-factor dimensionality reduction (MDR) to evaluate the impact of the interaction between candidate SNPs on the susceptibility of osteoporosis. All statistical analyses in this study are two-sided tests, and $p < 0.05$ was considered statistically significant.

Results

Sample overview and collection

There is no genetic relationship between all participants in this study. The case group composed of osteoporosis patients includes 300 males (50%) and 299 females (50%), their average age is 57.46 ± 13.40 years. The control group composed of healthy individuals included 299 males (50%) and 300 females (50%), their average age was 58.09 ± 13.25 years. The statistical results (Table 1) of our study showed that there was no statistical difference between the case group and the control group in age (p = 0.408) and gender (p = 0.954) (Table 1). In addition, Table 1 summarized the differences in clinical indicators between the case group and the control group. These indicators include total bilirubin, indirect bilirubin, aspartate aminotransferase, eosinophil percentage, eosinophils count, platelets, platelet distribution width, mean platelet volume, red blood cell count, hemoglobin, red blood cell distribution width. The statistical results showed that these clinical indicators were significantly different between the case group and the control group (p <0.05).

Genotyping and information about candidate SNPs

The 3 candidate SNPs of LRP5 (rs11228219 T/C, rs4988321 A/G, rs11228240 T/C) and the 2 candidate SNPs of AXIN1 (rs2301522 G/A, rs9921222 T/C) were successfully genotyped. The results of Hardy-weinberg balance test showed that all candidate SNPs all meet HWE (p > 5%). The results of HaploReg v4.1 showed (Table 2) that the candidate genetic loci may be regulated by a variety of factors, such as Enhancer histone marks; DNAse; Motifs changed; NHGRI/EBIGWAS hits; GRASP QTL Hits, et al.

Analysis of association between candidate SNPs and susceptibility risk (overall analysis)

The overall analysis results showed (Table 3): LPR5 rs11228240 has a significant association with the susceptibility of osteoporosis under the homozygote (TT Vs. CC: OR = 0.38, CI = 0.19-0.75, p = 0.005) and recessive genetic models (TT Vs.TC-CC: OR = 0.37, CI = 0.19-0.72, p = 0.003). And rs11228240 can reduce the susceptibility of osteoporosis by more than half. We found no evidence that the remaining two candidate SNPs of LPR5 were associated with susceptibility of osteoporosis. AXIN1 rs2301522 was significantly associated with increasing the susceptibility of osteoporosis under the allele (G Vs. A: OR = 1.23, CI = 1.05-1.45, p = 0.011), homozygote (GG Vs. AA: OR = 1.49, CI = 1.08-2.08, p = 0.017), dominant (GG-GA Vs. AA: OR = 1.32, CI = 1.04-1.69, p = 0.023) and log-additive models (OR =1.23, CI = 1.05-1.44, p = 0.013); AXIN1 rs9921222 was significantly associated with increasing susceptibility of osteoporosis under homozygote (TT Vs. CC: OR = 2.34, CI = 1.23-4.46, p = 0.010) and recessive genetic models (TT Vs. TC-CC: OR = 2.39, CI = 1.26-4.54, p = 0.008), and the susceptibility of osteoporosis can be increased more than twice.

Analysis of association between candidate SNPs and susceptibility of osteoporosis (subgroup analysis)

The results (Table 4) of the age subgroup analysis showed that all candidate genetic loci were not significantly associated with osteoporosis among participants aged ≤ 60 years. Among participants > 60 years old, LPR5 rs11228240 was potentially significantly associated with reducing susceptibility of osteoporosis under homozygote (TT Vs. CC: OR = 0.28, CI = 0.09-0.87, p = 0.028) and recessive genetic models (TT Vs.TC-CC: OR = 0.29, CI = 0.09-0.88, p = 0.029). AXIN1 rs2301522 was significantly

associated with increasing susceptibility of osteoporosis under allele (G Vs. A: OR = 1.27, CI = 1.02-1.58, *p* = 0.035), homozygote (GG Vs. AA: OR = 1.65, CI = 1.06-2.57, *p* = 0.026), recessive (GG Vs. GA-AA: OR = 1.49, CI = 1.01-2.21, *p* = 0.047)and log-additive genetic models (OR =1.27, CI = 1.02-1.28, *p* = 0.029).

The results (Table 4) of gender subgroup analysis showed that all candidate genetic loci were not significantly associated with the susceptibility of osteoporosis among male participants. Among female participants, LPR5 rs11228240 was significantly associated with a significant reduction in osteoporosis susceptibility under homozygote (TT Vs. CC: OR = 0.19, CI = 0.05-0.65, p = 0.008) and recessive genetic models (TT Vs.TC-CC: OR = 0.19, CI = 0.05-0.65, p = 0.008), and the osteoporosis susceptibility can be reduced more than half. AXIN1 rs2301522 were significantly associated with the increasing susceptibility of osteoporosis under allele (G Vs. A: OR = 1.29, CI = 1.03-1.63, *p* = 0.029), homozygote (GG Vs. AA: OR = 1.69, CI = 1.06-2.69, *p* = 0.026), dominant (GG-GA Vs. AA: OR = 1.43, CI = 1.01-2.00, *p* = 0.042), recessive (GG Vs. GA-AA: OR = 1.51, CI = 1.55-2.93, *p* = 0.003) and log-additive genetic models(OR =1.30, CI = 1.04-1.64, *p* = 0.022) among female participants.

Body mass index subgroup analysis results (Table 5) showed that all candidate SNPs were not associated with the susceptibility of osteoporosis in participants with BMI ≤ 24. However, among participants with BMI > 24, LPR5 rs11228240 was significantly associated with the reduction in susceptibility of osteoporosis under homozygote (TT Vs. CC: OR = 0.21, CI = 0.06-0.70, p = 0.011) and recessive genetic models (TT Vs.TC-CC: OR = 0.20, CI = 0.06-0.65, p = 0.008); AXIN1 rs2301522 was significantly associated with increasing susceptibility of osteoporosis under allele (G Vs. A: OR = 1.32, CI = 1.03-1.69, *p* = 0.029), homozygote (GG Vs. AA: OR = 1.73, CI = 1.04-2.88, *p* = 0.035) and log-additive genetic models (OR =1.32, CI = 1.03-1.68, *p* = 0.029). AXIN1 rs9921222 was significantly associated with increasing the susceptibility of osteoporosis under the homozygote (TT Vs. CC: OR =1.97, CI = 1.23-3.69, *p* = 0.021) and recessive genetic models (TT Vs. TC-CC: OR = 1.54, CI = 1.21-3.37, *p* = 0.021).

Haplotype analysis

In this study, haplotype analysis was performed on the genetic polymorphism of AXIN1 and LPR5. The result was shown in Figure 1. We observed 1 block (rs2301522 and rs9921222) in AXIN1 SNPs. Table 6 summarized the frequency of haplotypes formed by AXIN1 genetic polymorphisms in the case and control groups. We also found that the haplotype '$A_{rs2301522}C_{rs9921222}$' can increase the susceptibility of osteoporosis (crude analysis: OR = 1.23, CI = 1.04-1.44, *p* = 0.013; with adjustment: OR = 1.23, CI = 1.05-1.44, *p* = 0.013). However, the results showed that the LPR5 candidate genetic polymorphisms could not form a haplotype (Supplemental Figure 1).

Analysis of MDR

MDR was used to evaluate the interaction of candidate SNPs in the susceptibility of osteoporosis. As shown in Figure 2, a dendrogram of SNP-SNP interaction is shown (blue lines indicate that candidate SNPs have redundant effects in regulating liver cancer susceptibility, and yellow lines indicate synergistic

effects). MDR analysis results showed (Table 7 and Supplemental table 2) that the best unit point model for predicting the risk of osteoporosis is: $rs230152_{AXIN1}$ (testing accuracy = 0.523, CVC = 9/10, p = 0.0127); the two-site model is: $rs11228219_{LPR5}$, $rs2301522_{AXIN1}$ (testing accuracy = 0.527, CVC = 6/10, p = 0.0002); the three-site model is: $rs11228219_{LPR5}$, $rs11228240_{LPR5}$, $rs2301522_{AXIN1}$ (testing accuracy = 0.511, CVC = 3/10, $p < 0.0001$); the four-site model is: $rs11228219_{LPR5}$, $rs11228240_{LPR5}$, $rs2301522_{AXIN1}$, $rs9921222_{AXIN1}$ (testing accuracy = 0.546, CVC = 10/10, $p < 0.0001$).

Differences in clinical indicators under different genotypes

In this study, when analyzing the association between candidate SNPs and susceptibility of osteoporosis, it was found that there were many missing data for LPR5 rs4988321. Therefore, we finally evaluated and analyzed the association between the other 4 candidate SNPs polymorphisms (rs10934270, rs9288999, rs9841504, rs73230612) and clinical indicators of osteoporosis patients. The results (Table 8) showed that LRP5 rs11228219 had a significant association with the levels of total bilirubin, indirect bilirubin and erythrocyte sedimentation rate under different genotypes; AXIN1 rs2301522 was significantly associated with the levels of eosinophil percentage and eosinophils count under different genotypes. Under different genotypes, AXIN1 rs9921222 was significantly associated with the levels of clinical indicators L4, rheumatoid factor and alkaline phosphatase.

Discussion

As a complex disease, the pathogenesis of osteoporosis is affected by both genetic and environmental factors, among which genetic factors play a decisive role in the disease process (5). The extensive development of omics research at different biological levels, such as genomics, transcriptomics, proteomics, and metabolomics, respectively, play a huge role in the field of bone research. So far, genomics research has successfully identified some genetic loci that are significantly associated with osteoporosis and its alternative phenotypes (22). However, most of these studies are limited by the research subjects, genetic background and other factors, and the pathogenesis of osteoporosis cannot be fully elucidated. In this study, we selected the key genes (LPR5 and AXIN1) in the classical WNT/β-catenin signaling pathway which plays an important role in bone mass in patients with osteoporosis (12). Finally, the association between five candidate SNPs and susceptibility of osteoporosis in Chinese Han population was evaluated.

Overall, we found that LPR5 rs11228240 and AXIN1 rs2301522, rs9921222 may play important roles in the susceptibility of osteoporosis in Chinese Han population. LPR5 rs11228240 may be a protective factor for osteoporosis in Chinese Han population (OR <1), while AXIN1 rs2301522 and rs9921222 are risk factors (OR> 1). Up to now, AXIN1 rs9921222 has never been reported in the field of orthopedics, but it has been reported in other diseases. And similar to our study, it has been reported as a risk factor. The AXIN1 rs9921222 is only found in previous studies to be significantly associated with the risk of colon cancer (23). In addition, the AXIN1 haplotype ($A_{rs2301522}C_{rs9921222}$) showed a significant impact on the susceptibility of osteoporosis. As far as we know, our study is the first to find that LPR5

rs11228240 and AXIN1 rs2301522 have a potential association with osteoporosis, and they have never been reported in other disease-related studies. Our study will provide a new theoretical basis for the diagnosis, prevention and treatment of osteoporosis.

Osteoporosis is a typical complex disease controlled by genetic and environmental factors. For example, with the increase of age, the risk of osteoporosis will increases (24). Elderly, weight loss may lead to a general increase in the incidence of fractures (25). Affected by estrogen levels, women, especially postmenopausal women, have a high risk of osteoporosis (26). According to the epidemiological characteristics of osteoporosis and the difference of the risk of osteoporosis, we divided the subjects into subgroups (age, gender, BMI) in order to provide valuable reference for the prediction of osteoporosis risk in specific populations. Our study found that AXIN1 rs2301522 will increase the risk of osteoporosis in participants who are older than 60 years old, female and BMI ≤ 24. rs9921222 only increased the risk of osteoporosis in participants with BMI ≤ 24. It is well known that older people, female and weight loss are all susceptible to osteoporosis (25-27). Combined with the results of this study, we speculate that this may be due to the role of AXIN1 rs2301522 and rs9921222, but the specific mechanism needs further experiments to confirm. In addition, LPR5 rs11228240 was found that can significantly reduce the osteoporosis risk more than twice among participants who were female, > 60 years old and with BMI ≤ 24. And we found that although the association between LPR5 rs11228240 and the risk of osteoporosis was not statistically significant in participants who were younger than 60 years old or male. But the overall trend was to reduce the risk of osteoporosis (OR < 1). Our results suggested that LPR5 rs11228240 is a protective factor for osteoporosis, especially in those participants who were older than 60 years, female, and with BMI ≤ 24. Our study will provide a valuable reference for the risk assessment, individualized treatment or diagnosis of osteoporosis in specific populations.

The WNT signaling pathway plays an irreplaceable role in embryonic development, controlling bone hair and affecting bone mass and osteoblast differentiation (8-10). A number of studies have confirmed that both LPR5 and AXIN1 are involved in the conduction of the canonical WNT signaling pathway (21, 28, 29). In the classical WNT signaling pathway (WNT/β-catenin), the transcriptional regulator β-catenin is the 'central axis' factor in the pathway. When the classical WNT ligand is absent , in the cytoplasm , the combined reaction of β-catenin with Axin1 and APC promotes the degradation of β-catenin (30). When the classical WNT ligand binds to the Frizzled (FZD) family receptor and low density lipoprotein receptor associated protein (LRP5/6) auxiliary receptor, the phosphorylation and degradation of β-catenin in the cytoplasm are inhibited (31, 32). Combined with our results, we can speculate that AXIN1 may play a role in promoting WNT pathway, and the genetic loci rs2301522 or rs9921222 may play a certain role. Similarly, in the inhibitory effect of LPR5 on WNT pathway, the genetic locus rs11228240 played a certain role. But the specific mechanism needs further experimental verification.

It was found that LRP5 rs11228219 was significantly associated with the levels of T-BIL, I-BIL and ESR in participants with osteoporosis under different genotypes. AXIN1 rs2301522 was significantly associated with the levels of clinical indicators of erythrocyte percentage and erythrocyte count in different genotypes. AXIN1 rs9921222 was significantly associated with L4, RF and ALP levels under

different genotypes. Previous studies have confirmed that L4, RF, ALP, T-BIL, I-BIL and ESR levels are associated with osteoporosis, bone mineral density or bone metabolism (33-36). Our results showed that AXIN1 rs2301522 and rs9921222 were significantly associated with the risk of osteoporosis may be due to the two genetic loci can affect the level of clinical indicators related to osteoporosis. But this is only a speculation, the specific mechanism of action needs further study to confirm. Nevertheless, our research still provides a lot of clues and directions for the follow-up study on the functional genomics of LPR5/AXIN1 in osteoporosis.

It is worth noting that there are still some inevitable limitations in this study, in order to ensure the reliability and repeatability of the results, it is necessary to expand the sample size for the verification research. Nevertheless, our study will provide a new theoretical basis for the study of the mechanism of action of LPR5 and AXIN1 in the pathogenesis of osteoporosis. It also provides valuable reference for the risk assessment of osteoporosis in Chinese Han population.

Conclusion

In summary, our study found that the genetic polymorphisms of the important genes LPR5 and AXIN1 in the WNT classic pathway had a potential association with the susceptibility to osteoporosis in Chinese Han population. And these SNPs are new discoveries of osteoporosis susceptibility loci.

Declarations

Ethics approval and consent to participate

This study was conducted under the standard approved by the the Second Affiliated Hospital of Xi'an Jiaotong University, and conformed to the ethical principles for medical research involving humans of the World Medical Association Declaration of Helsinki. All participants signed informed consent forms before participating in this study.

Consent to publication

All the authors agreed to publish the manuscript.

Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Conceptualization, Kunzheng Wang and Chen Zhang; methodology, Yongsheng Cui; software, Yongsheng Cui and Xinglv Hu; data curation, Yongsheng Cui and Xinglv Hu; writing, review and editing, Yongsheng Cui.

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Tables

Due to technical limitations, the tables are only available as a download in the supplemental files section.

Figures

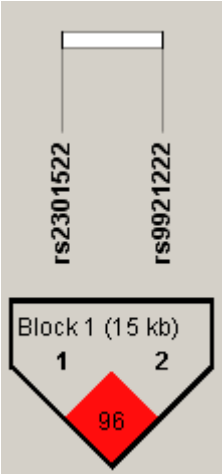

Figure 1

Linkage disequilibrium (LD) plots containing two polymorphisms from AXIN 1. Block 1 includes Rs2301522 and rs9921222. The numbers inside the diamonds indicate the D' for pairwise analyses.

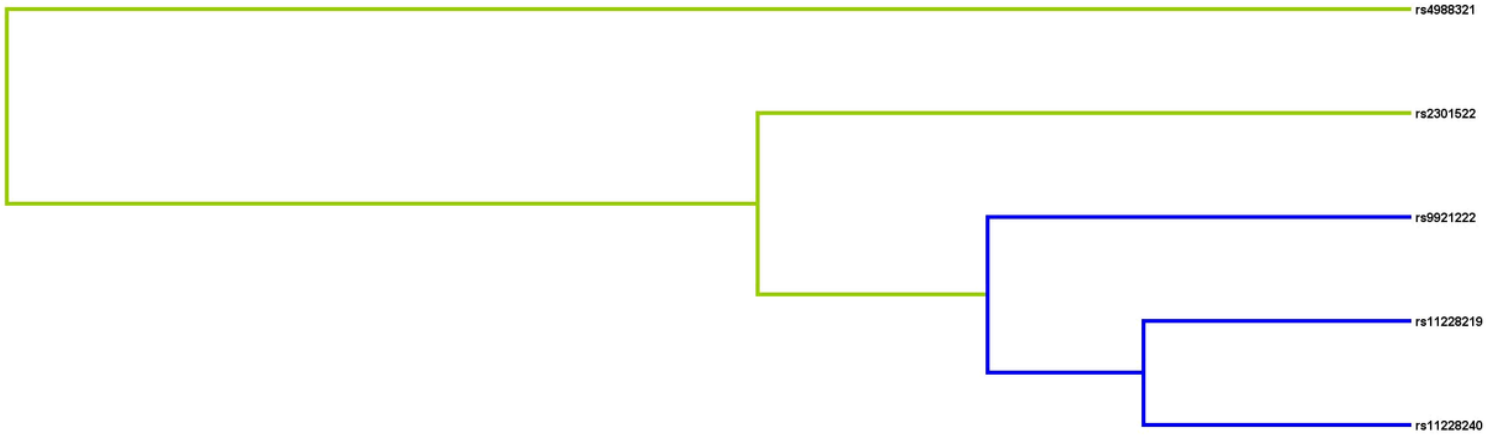

Figure 2

Dendrogram analysis of SNP-SNP interaction. The colors in the tree diagram represent synergy (yellow) or redundancy (blue).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure1.tif
- Supplementaltables.docx
- Tables.docx