Genotyping of the VNTR polymorphism in the exon 4 of the L-SIGN gene and its negative association with the risk of SARS coronavirus infection

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Method Article

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Abstract

Introduction

A convincing association study includes at least appropriate sampling, accurate genotyping, and appropriate statistical analysis \(Hattersley _et al._ 2005). Additionally, association studies require replication in independent populations \(Lohmueller _et al._ 2003). We have followed these rules in a recent study to assess the association between the risk of severe acute respiratory syndrome \(SARS) coronavirus infection and the variable number tandem repeat \(VNTR) polymorphism in the exon 4 of the L-SIGN gene \(for liver/lymph node-specific ICAM-3 grabbing nonintegrin, encoded by CLEC4M) \(Zhi _et al._ 2007). In this study, the individuals of three independent case-control populations have been rigorously selected and all of them are unrelated adults \(Zhang _et al._ 2005). The genotyping of the VNTR polymorphism was performed by separating PCR products on agarose gel. As for the statistical analysis, we have used widely accepted methods to evaluate Hardy-Weinberg equilibrium, compare allele or genotype frequencies, and assess the genetic association. By use of this protocol for an association study, we found that there is lack of support for a genetic association between the L-SIGN polymorphism and risk of SARS coronavirus infection, which was previously reported by Chan et al. \(Chan _et al._ 2006; Zhi _et al._ 2007).

Reagents

1. EDTANa2 \(analytical-pure, 10mM) 2. Tris-saturated phenol \(pH 8.0) 3. Chloroform \(analytical-pure) 4. Isoamyl alcohol \(analytical-pure) 5. Proteinase K 6. Ex Taq DNA polymerase \[Code: DRR01AM, supplied with EX Taq, 10×Ex Taq Buffer \(Mg^{2+} Free), dNTP Mixture and $MgCl_2$. TaKaRa Bio Inc.] 7. Regular agarose \(LOT NO. 101645. Shanghai Yito, China)

Equipment

1. PCR System \(GeneAmp® PCR system 9700, 96-well, Applied Biosystems) 2. Centrifuge \(Eppendorf Centrifuge 5810R, Eppendorf) 3. Gel Documentation system \(Gel DocTM XR 170-8170, Bio-Rad) 4. Spectrophotometer \(SmartSpecTM Plus spectrophotometer, Bio-Rad) 5. Water system \(PURELAB Ultra Genetic, ELGA LabWater) 6. Refrigerator \(-20~4ºC, BCD-257WD, Panasonic) 7. Ultra-low temperature freezer \(-80ºC, MDF-U32V, SANYO)

Procedure

A. Blood collection and DNA preparation 1. Rigorously select case-control samples. The case-control population contain unrelated adults recruited from outpatient clinics and hospitals. 2. Collect 5ml blood by standard venipuncture into a tube with 40 µl EDTANa2 \(10 mM) for each participant. 3. Extract genomic DNA from 5 ml whole blood using standard phenol/chloroform method. 4. Determine DNA concentration using a spectrophotometer. 5. Dilute DNA samples to 10 ng/µl and distribute them to 96-well plates. **B. Polymorphism genotyping** 6. Analyze the VNTR polymorphism by PCR in the case-control populations in a blind manner. 7. The PCR reaction was carried out at 94ºC for 2 min, 32 cycles of denaturing at 94ºC for 30 s, annealing at 56.5ºC for 30 s and extension at 72ºC for 40 s, and final extension at 72ºC for 10 min, using a

forward primer 5’-CTTTTCTTCTTGGCCCAGTGT-3’ and a reverse primer 5’-CTCAGGCAGGGTTGGTAACTC-3’ in a reaction mixture of 15 µl containing 10~20 ng genomic DNA, 0.25 mM dNTPs, 1.5 mM MgCl2, 200 pM of each primer and 1 unit \(U) Ex Taq DNA polymerase \(TaKaRa, Otsu, Shiga). 8. Separate the amplified products by 2% agarose gel electrophoresis. 9. Determine the length of alleles using marker standards and express genotypes as the number-of-repeat combinations. 10. Validate the accuracy of genotyping data for the VNTR polymorphism by resequencing 15% masked, random samples of patients and controls. **C. Statistical analysis** 11. The allele and genotype frequencies for the polymorphism can be determined by direct gene counting. The fitness to Hardy-Weinberg equilibrium can be tested by using the Markov chain method implemented in the GENEPOP software package \(available at: "http://wbiomed.curtin.edu.au/genepop/":http://wbiomed.curtin.edu.au/genepop/). Comparisons of sex distribution between patient and control groups can be performed by use of the Chi-square test. Differences of mean age between the groups can be analyzed by use of an unpaired t test. Comparison of allele or genotype frequencies can be done using the CLUMP program \(available at: "http://www.mds.qmw.ac.uk/statgen/dcurtis/software":http://www.mds.qmw.ac.uk/statgen/dcurtis/software). Genetic association for heterozygote and homozygote genotype with the SARS risk can be assessed by use of logistic regression using SPSS software \(version 9.0; SPSS Inc., Chicago, IL), and the odds ratio \(OR) and 95% confidential interval \(c.i.) can be calculated and adjusted for age and gender, where appropriate. An association can be considered significant at a P value of less than 0.05, and all statistical tests should be two-sided.

Timing

Steps 1 to 5, 1 month Steps 6 to 10, 2 days Steps 11, 2 days

References

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