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An Investigation of the a_{2A}-adrenergic receptor gene and seasickness in Chinese naval men

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Abstract

Background

The cause of seasickness remains unclear. Both genet-ic and environmental factors are suspected playing an important role in seasickness development. The a„-adrenergic receptor has been thought very important in stress-coping behaviours and may be associated with motion sickness. So we investigate the association between the a_{2A}-adrenergic receptor gene *(ADRA2A)* and seasickness in Chinese navel men.

Methods

A total of 110 new recruited Chinese navy men were examined. The 110 subjects were divided into three groups as Light (42), Medium (33) and Severe (35) by a seasickness questionnaire. Three tag SNPs of *ADRA2A* as -1569 C/A (rs11195419), -1780 G/A (rs553668), -1902 C/A (rs3750625) were selected and genotyped by direct sequencing. Then the genotype frequency, allele frequency and haplotype frequency were compared between the three groups.

Results

The genotypes of the three SNPs were in Hardy-Weinberg equilibrium. The major allele frequencies between the three groups (light vs medium vs severe)
were assessed as 0.143 vs 0.091 vs 0.100, 0.536 vs 0.576 vs o.514, 0.143 vs 0.076 vs 0.100, respectively. No significant difference was found in genotype frequency, allele frequency and haplotype frequency between the three groups.

Conclusion

The negative results of this study indicate ADRA2A is not involved in the susceptibility of seasickness in Chinese Navy men. Considering the limited sample size, further study in large samples and with more SNPs of ADRA2A will be needed to elucidate its role in seasickness/motion sickness. Nevertheless, our investigation is a pilot study to elu-cidating the genetic relationships between ADRA2A and seasickness.

Introduction

Some investigations suggested neurotransmitters such as norepinephrine, acetylcholine and 5-hydroxytryptamine are involved in motion sickness.[1] The α_{2A}-adrenergic receptor (α_{2A}AR) is an inhibition type G protein which could mediate the exocytosis of epinephrine from the adrenal medulla and the release of norepinephrine from presynaptic nerve terminals of peripheral sympathetic nervous system. Previous research found α_{2A}AR is very important in the stress-coping behaviors.[2] α_{2A}-adrenergic receptor gene *(ADRA2A)* is located in 10q23-25 and has a length of 3613 bp. Finley et al. reported rs553668 AA or GA genotype of *ADRA2A* had greater signs and symp-toms of motion sickness than GG genotype.[3] By far there is rare association study between *ADRA2A* and motion sickness or seasickness.

So we genotyped three tag SNPs of ADRA2A as -1569 C/A (rs11195419), -1780 G/A (rs553668), -1902 C/A (rs3750625) and analyzed whether they are associated with seasickness in Chinese navy men.

Methods ***Subjects***

We included 110 new recruited Chinese navy men who had experienced one month voyage (average age 19, all in good health status). They are all Chinese Han nationality and their parents and grandparents are also Chinese Han nationality. All of them have not adapted to sailing life before their recruitment. We used a seasickness questionnaire of Wiker et al. concerning actual seasickness severity during sailing to determine their susceptibility to seasickness.[4] The validity and reliability of this questionnaire has been proved to be high in a seasickness survey conducted by the United States Coast Guard,[5] and it was employed by Gordon et al. in many studies.[6-8] Based on self-reported symptoms, seasickness is rated on a scale from 0 to 7. We selected those subjects (35 per-sons) scoring 6 to 7 points on the Wiker scale (the highest degree of susceptibility denoting frank vomit-ing) as severe group. Those subjects (42 persons) scoring from 0 to 3 points on the categorization scale (subjects with no signs or symptoms of seasickness during sailing, or sometimes suffering only slightly from minor symptoms) were regarded as light group. The rest (33 persons) were medium group. Blood sam-ples of the 110 subjects were obtained with informed consent. The study protocol was reviewed and approved by the Chinese Ethics Committee of Human Genetic Resources.

SNP genotyping

Venous blood samples of the 110 navy men were drawn and the genomic DNA was extracted from the blood samples according to standard protocol. The following PCR primers were designed based on the α_{2A} -adrenergic receptor gene sequence in Genebank and used for genotyping the three SNPs. The primer sequences were 5'-CTG CTC TGC GTT TGC TCG-3' (forward) and 5'-ACG GGA CAG TCA CCA GTT GC-3' (reverse). PCR reaction system was a 15 fil mixture containing 10 ng of DNA, 10 pmol of each primer, 2.5 mM MgCl2, each dNTP at 0.2 mM and 0.25 U of Taq DNA polymerase. Then PCR was performed under the following conditions: initial denaturation at 95°C for 9min; 35 cycles at 95°C for 30s, 61°C for 30s, 72°C for 30s; followed by a final extension at 72°C for 7min in a PCR system 9700 (Applied Biosystems, Foster City, CA). The PCR products were sequenced using ABI Prism BigDye Terminator Cycle Sequencing Kit of version 3.1 (Applied Biosystems, Foster city, CA) on an ABI Prism 3100 sequencer.

Statistical analysis

To test for Hardy-Weinberg equilibrium, $\%^2$test was used and performed on SHEsis program.[9] The geno-type frequencies and allele frequencies of the three SNPs were calculated in SPSS software version 16.0 (SPSS Inc., Chicago, IL), and the odds ratios (OR) were calculated too. Haplotype frequencies were estimated using an accelerated expectation-maximization algorithm.[10] P value < 0.05 was considered statistically significant.

Results

Allele frequencies of the three SNPs in cases and controls are listed in Table 1. To rs11195419 and rs3750625, they had a similar major allele frequency in the three groups; while major allele frequencies of rs553668 were 0.536 in Light group, 0.576 in Medium group, and 0.514 in Severe group. There was no sig-nificant difference (P > 0.05) between those three groups.

Genotype frequencies (Table 1) of the three SNPs were in Hardy-Weinberg equilibrium. There was no significant difference (P > 0.05) in genotype frequency between the three groups.

Haplotypes and their frequencies are shown in Table 2 (frequency less than 0.02 are not listed). P val-ues are calculated using theχ^2test 2X2 contingency table. Frequencies of three major haplotypes AGA, CAC, CGC were similar in Light vs Medium compar-ison and Light vs Severe comparison, consistent with the allele and genotype frequency results.

Discussion

The similar allele and genotype frequencies of rs11195419 and rs3750625 in the three groups suggest a strong linkage between them, which is consistent with the previous study.[11] The similar haplotype frequencies of Light vs Medium comparison and Light vs Severe comparison indicate the divergence of samples. The Medium and Severe group are more likely with the same genotype and allele frequency.

In another way, our results are not consistent with Finley's results.[3] This may rely on the diverse genetic backgrounds of different ethnics or low correlations between different types of motion sickness. Just as Lentz[12] reported the different sensitivities in individu-als to various types of motion and Golding[13] reported the correlation between susceptibility to translational motion and susceptibility to cross-coupled motion sometimes was very low. Women were reported with greater incidence in motion sickness than men[14] and maybe we should include women subjects in future studies.

Conclusions

Overall, the results of this study indicate that the polymorphisms of ADRA2A may not be associated with seasickness in Chinese navy men.

Motion sickness is an expression of a physiological response to unfamiliar motion patterns, whether real or apparent.[15] ***http://en.wikipedia.org/wiki/Motion_*** *sickness***.** It can be caused by many forms of trans-portation, such as cars, buses, trains, ships, planes etc. The most dramatic form of motion sickness is seasick-ness. The development of seasickness signs and symp-toms follows an orderly sequence, which is stomach awareness, pallor, cold sweating, nausea, and recur-rent vomiting.[16] The symptoms may vary with the sus-ceptibility of the individual. Reavley et al. reported that genetic factors play important roles in determin-ing an individual's underlying propensity to motion sickness.[17] It was justifiable to investigate genes which may influence susceptibility.

Declaration of Interests

The authors declare that they have no conflicts of interest.

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References

1 Takeda N, Morita M, Horii A, et al. Neural mechanisms of motion sickness. *J Med Invest* 2001; 48:44-59.

2 Laarakker MC, Raai JR, van Lith HA, Ohl F. The role of the alpha 2A-adrenoceptor in mouse stress-coping behaviour. Psychoneuroendocrinology 2010; 35:490-502.
3 Finley JC, Jr., O'Leary M, Wester D, et al. A genetic polymorphism of the alpha2-adrenergic receptor increases autonomic responses to stress. *J Appl Physiol* 2004; 96(6):2231-2239.
4 Wiker SF, Kennedy RS, McCauley ME, Pepper RL. Susceptibility to seasickness: influence of hull design and steaming direction. Aviat Space Environ Med 1979; 50:1046-1051.
5 Wiker SF, Kennedy RS, McCauley ME, Pepper RL. Reliability, validity and application of an improved scale for assessment of motion sickness severity. Washington, DC: US Department of Transportation, US Coast Guard, Office of Research and Development, 1979; Report CG-D-29-79.
6 Gordon CR, Jackman Y, Ben-Aryeh H, et al. Salivary secretion and seasickness usceptibility. Aviat Space Environ Med 1992; 63:356-359.
7 Shahal B, Nachum Z, Spitzer O, et al. Computerized dynamic posturography and seasickness susceptibility. Laryngoscope 1999; 109(12):1996-2000.
8 Tal D, Hershkovitz D, Kaminski G, Bar R. Vestibular evoked myogenic potential threshold and seasickness susceptibility. *J Vestib Res* 2006; 16(6):273-278.
9 Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15(2):97-98.
10 Qin ZS, Niu T, Liu JS. Partition-ligation-expectation;ic receptor gene maximization algorithm for haplotype inference with single-nucleotide polymorphisms. *Am J Hum Genet* 2002; 71(5):1242-1247.
11 Small KM, Brown KM, Seman CA, et al. Complex haplotypes derived from noncoding polymorphisms of the intronless alpha 2A-adrenergic gene diversify receptor expression. Proc Natl Acad Sci U S A 2006; 103(14):5472-5477.
12 Lentz, JM. Laboratory tests of motion sickness susceptibility. Motion sickness, mechanisms, prediction, prevention and treatment. AGARD Conference Proceedings 1984; 372, 29-1-29-9.
13 Golding JF. Motion sickness susceptibility. Auton Neurosci. 2006; 129(1-2):67-76.
14 Park AH, Hu S. Gender differences in motion sickness history and susceptibility to optokinetic rotation-induced motion sickness. Aviat Space Environ Med 1999; 70(11):1077-1080.
15 Shupak A, Gordon CR. Motion sickness: advances in pathogenesis, prediction, prevention, and treatment. Aviat Space Environ Med 2006; 77(12):1213-1223.
16 Shupak A, Gordon C, Melamed Y. Seasickness: a review of etiology, pathophysiology, evaluation and treatment. Harefuah 1990; 118:153-157.
17 Reavley CM, Golding JF, Cherkas LF, et al. Genetic influences on motion sickness susceptibility in adult women: a classical twin study. Aviat Space Environ Med 2006; 77:1148-1152.