A Susceptibility Locus at Chromosome 3p21 Linked to Familial Nasopharyngeal Carcinoma

Wei Xiaoming,1 Zhao Yang Zeng,1 Jia Hui Xia,2 Kun Xia,2 Shou Rong Shen,3 Xiao Ling Li,1 Dong Xu Hu,1,2 Chen Tan,1 Juan Juan Xiang,1 Jie Zhou,1 Hao Deng,2 Song Qing Fan,3 Wei Fang Li,1 Rong Wang,1 Ming Zhou,1 Shi Guo Zhu,1 Hong Bin Li,1 Jun Qian,1 Bi Cheng Zhang,2 Jie Ru Wang,1 Jian Ma,1 Bing Yi Xiao,1 He Huang,1 Qiu Hong Zhang,1 Yan Hong Zhou,1 Xiao Min Luo,1 Hou De Zhou,1 Yi Yin Yang,1 He Ping Dai,2 Guo Yin Feng,4 Qian Pan,2 Ling Qian Wu,2 Lin He,5,6 and Gui Yuan Li1,2

1 Cancer Research Institute, 2 National Laboratory of Medical Genetics, and 3 The Third Xiangya Hospital, Central South University, Changsha, Hunan; 4 Shanghai Mental Health Center and 5 Bio-X Life Science Research Center, Shanghai Jiao Tong University, Shanghai; and 6 Shanghai Institute for Biological Science, Chinese Academy of Science, Shanghai, China.

Received 10/16/03; revised 1/2/04; accepted 1/12/04.

Grant support: National Natural Sciences Foundation of China (Nos. 30330560, 30300201, and 30100027), the Special Funds for Major State Basic Research of China, and the State 863 High Technology R&D Project of China (Nos. 2002BA711A08 and 2002BA711A03).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Gui Yuan Li, Cancer Research Institute, Central South University, Changsha, Hunan 410078, China. Phone: 86-731-4805446; Fax: 86-731-4805383; E-mail: lgy@xysm.net.

© 2004 American Association for Cancer Research.

Nasopharyngeal carcinoma (NPC), one of the most common malignant tumors in southern Chinese, shows familial clustering as other human cancers. Epidemiological studies suggest that most of this familial aggregation derives from inherited susceptibility (1). However, the molecular evidence for NPC linkage to chromosomes 4 and 9 was observed.

We and other groups (4–10) have detected frequent loss of heterozygosity on short arms of chromosome 3 and 9 have been reported to be associated with NPC, and a genome-wide scan identified an NPC susceptibility locus on chromosome 4p15.1-q12 recently. In our study, we collected samples from 18 families at high risk of NPC from the Hunan province in southern China, genotyped with a panel of polymorphic markers on short arms of chromosomes 3, 9, and 4p15.1-q12. A locus on 3p21 was identified to link to NPC with a maximum logarithm of odds for linkage score of 4.18. Fine mapping located the locus to a 13.6-cM region on 3p21.31-21.2, where a tumor suppressor gene cluster resided. Our findings identified a novel locus for NPC and provided a map location for susceptibility genes candidates. In contrast to a recent study, no significant findings identified a novel locus for NPC and provided a map location for susceptibility genes candidates. In contrast to a recent study, no significant evidence for NPC linkage to chromosomes 4 and 9 was observed.

RESULTS

A plot of Lod and nonparametric linkage (NPL) scores for chromosome 4 was shown in Figure 1. The highest two-locus Lod score for D4S3002 marker was 3.270 with even lower multipoint parametric Lod score. Nonparametric analysis and heterogeneity-adjusted Lod (HLod) scores did not show evidence for linkage of NPC to chromosome 4. The highest multipoint NPL and Lod score being

Internet address: http://linkage.rockefeller.edu.

Internet address: http://www.gdb.org.

Internet address: http://linkage.rockefeller.edu.
eight markers with high frequency of loss of heterozygosity in NPC chromosome 4 was excluded in these 18 families. Therefore, linkage of NPC to linkage Lod.

The two-point and multipoint Lod, a HLod, and NPL scores of five loci on chromosome 3p were calculated with GENEHUNTER (14), was obtained for D3S1568. In fine mapping study, 7 additional markers around D3S1568 that span a 25.4-cM region from D3S3727 to D3S3553 at 3p22.3-p21.1 were studied. Highly significant Lod and NPL scores were obtained for multiple markers (Fig. 1). The maximum two-point Lod score of 3.764, (P = 1.91 \times 10^{-5}) and multipoint NPL scores of 2.877 (P = 0.005) were obtained for D3S1568 at 3p21.31 (Table 2).

In fine mapping study, 7 additional markers around D3S1568 that span a 25.4-cM region from D3S3727 to D3S3553 at 3p22.3-p21.1 were studied. Highly significant Lod and NPL scores were obtained for multiple markers (Fig. 1). The maximum two-point Lod score of 3.764, calculated with the GENEHUNTER (14), was obtained for D3S1568. In multipoint parametric linkage analysis, D3S3624 gave the maximum Lod score of 4.177 (P = 6.653 \times 10^{-5}), D3S1568 produced a Lod score of 3.922 (P = 1.197 \times 10^{-5}). The distance between the two markers was ~2.7 cM. In nonparametric linkage analysis, the highest multipoint NPL score of 2.735 (P = 0.001) for D3S3624 and 2.689 for D3S1568 (P = 0.0012) was produced. For D3S1568, two-point NPL score reached 2.952 (P = 4.06 \times 10^{-6}). These results provided additional evidence that NPC was linked to 3p21 in these 18 pedigrees.

On the basis of genotyping analysis, the most likely haplotype of the pedigrees was constructed to additionally verify the mapping. Three representative haplotypes were shown in Fig. 2. HOMOG (17) program analysis indicated that >90% of families studied were linked to the 3p21 (data not shown).

Two-point linkage analysis for chromosome 9p was also carried out using GENEHUNTER (14). The highest Lod and NPL scores for D9S288 were only 0.683 and 0.536, respectively. Similar results were obtained in multipoint linkage analysis with other markers. Thus, the probability of linkage to chromosome 9 is low.

### DISCUSSION

Our findings provide evidence for the linkage of NPC to chromosome 3p and fine map NPC susceptibility locus to a 13.6-cM region on 3p21.31-21.2. These results are in agreement with several previous studies that suggest deletion of chromosomes 3p is common genetic event in NPC (2–8). Chromosome 3p21 is associated with most human epithelial malignancies, including small cell lung cancer (18, 19), breast cancer (20), uterine cervical carcinoma (21), renal cell adenoma (22) and head and neck cancers (23). Many tumor suppressor candidate genes such as CACNA2D2, DLC1, FUS1, H37, HYAL1, RASSF1A, SEMA3B, and SEMA3F (24–29) and tumor susceptibility genes such as hMLH1 (30, 31) have been isolated from the region. Overexpression of some tumor suppressor candidate genes at 3p21 resulted in inhibition of cell proliferation and induction of apoptosis of lung cancer cell lines as well as suppression of tumor growth and metastasis in lung cancer mouse models (26). This study suggests that genes in the 3p21 may play a critical role in tumorigenesis of familial nasopharyngeal carcinoma. Consistent with this notion, a study detected high frequency of loss of heterozygosity on 3p in histologically normal nasopharyngeal epithelia and dysplastic lesions that suggest deletion of chromosomes 3p is common genetic event in NPC (32). Isolation and identification of susceptibility genes for NPC from the 3p21 may greatly advance understanding of the development formation of NPC.

This study fails to detect an obvious NPC susceptibility locus on

![Fig. 1. The Lod scores of multipoint and two-point analysis from fine mapping on chromosome 3. M-HLods and M-NPL are multipoint parametric and nonparametric linkage scores calculated with GENEHUNTER. T-NPL and T-HLods are two-point parametric and nonparametric linkage scores calculated with GENEHUNTER. T-Lods-L is two-point parametric linkage score calculated with LINKAGE.](cancerres.aacrjournals.org)
chromosome 4p15.1-q12 reported recently by another group (11). One possible explanation is that each locus is linked to NPC susceptibility in certain patient population under the certain environmental factors. Nevertheless, the discrepancy remains to be additionally elucidated.

ACKNOWLEDGMENTS

We thank Yong-Jia Yang and Wei-Min Fan for their expert technical assistance and Li Cao, Ying Yu, and Ke Tang for their help in the early phases of this work. We also thank Gang Chen, Zheng Tan, and Jian-Dong Yang, Jin-Bo Fan at the Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences for helpful advice. We also thank Dr. Zhuohua Zhang for help in preparing this manuscript and all of the physicians at the Xiangya Hospital of Central South University and Hunan Tumor Hospital, who referred families for this study.

REFERENCES

A Susceptibility Locus at Chromosome 3p21 Linked to Familial Nasopharyngeal Carcinoma

Wei Xiong, Zhao Yang Zeng, Jia Hui Xia, et al.