[CANCER RESEARCH 64, 1972–1974, March 15, 2004]

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

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ABSTRACT

Nasopharyngeal carcinoma (NPC) poses one of the serious health problems in southern Chinese, with an incidence rate ranging from 15 to 50/100,000. Chromosome translocation t(1;3) and frequent loss of heterogeneity 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 evidence for NPC linkage to chromosomes 4 and 9 was observed.

INTRODUCTION

Nasopharyngeal carcinoma (NPC), one of the most common malignant tumors in southern China, shows familial clustering as other human cancers. Epidemiological studies suggest that most of this familial aggregation derives form inherited susceptibility (1). However, the molecular genetic basis of NPC remains unknown. Xia *et al.* reported a giant marker chromosome t (1;3)(q44;p11) in lymphoblastoid cells from two unrelated NPC patients that was also found in both peripheral blood and biopsy cells in another patient with poorly differentiated squamous NPC (2, 3). We and other groups (4–10) have detected frequent loss of heterozygosity on chromosome 3p21-26 and 9p21-22 in NPC, and the most frequent loss of heterozygosity region was found on short arm of chromosome 3. These findings suggest that potential susceptibility loci linked to NPC are located on these chromosome regions.

To find possible susceptibility genes for NPC, Feng *et al.* (11) performed a genome-wide scan in 20 Cantonese-speaking families and identified a locus for NPC on chromosome 4p15.1-q12.

In this study, we performed a linkage analysis to search for possible loci contributing to risk for NPC in 18 pedigrees from Hunan province, China. Each pedigree had at least two available genetically linked individuals affected by NPC. Totally, 46 affected and 96 unaffected individuals with an average age of 48.36 ± 15.27 years (20−84 years) were genotyped. Twenty polymorphic microsatellite markers were scanned, including five markers on chromosome 4 that showed highest heterozygosity and logarithm of odd (Lod) scores in Feng *et al.*'s study (11), eight markers on short arm of chromosome 3 and 7 markers on short arm of chromosome 9. Seven additional markers flanking 3p21 were used for fine mapping. LINKAGE (12, 13) for two-point parametric analysis and GENEHUNTER (14) for the parametric and model-independent nonparametric linkage analysis were performed. Meanwhile, multipoint linkage analysis was carried out using all markers on a chromosome as a group.

MATERIALS AND METHODS

Families. The subjects are from 18 high-risk NPC families from Hunan Province, southern China. Most of these families were collected from the Xiangya Hospital of Central South University and the Hunan Tumor Hospital, Changsha, Hunan, China. All patients were diagnosed by pathological examination, and the age at diagnosis of NPC was confirmed from medical records or other independent sources. Totally 46 affected and 96 unaffected individuals were used in this study. Written informed consent was obtained from all studied participants. The study was approved by the ethical review committees of the appropriate institutions. Five-to-10 ml peripheral blood samples were taken from each individual.

Genotyping Analysis. Genomic DNA was prepared from lymphoblastoid transformed cell lines for 4 families and whole blood for the other 14 families. Overall, samples from 142 individuals were genotyped (46 affected and 96 unaffected individuals). A total of 225 unrelated individuals recruited from Changsha area of Hunan Province was also typed to provide allele frequency estimate for this population (15, 16). High-throughput, semiautomated genotyping was accomplished using 377 DNA sequencer. The sequences of primers were obtained from the Genome Database.[7] The average heterozygosity of the markers selected for the study was 0.73. Multiplex PCR and microsatellite allele analysis were performed as described previously (11).

Linkage Analysis. We calculated pairwise Lod scores using the MLINK option of the LINKAGE program package (12, 13).[8] Multipoint analysis has the advantage of using data from multiple linked markers to maximize the information in a given pedigree. For multipoint analysis, we used all of the markers in a chromosome region for computing with GENEHUNTER (14). Nonparametric multipoint analysis, which is robust even when the mode of inheritance is not known, was also performed, with GENEHUNTER (14) to calculate normalized *Z* scores and associated *P* values. Autosomal dominant inheritance was assumed for parametric analysis, with a disease-allele frequency of 0.0089 and a penetrance of 73% (11). Haplotypes were constructed using the program GENEHUNTER (14). The admixture test as implemented in HOMOG (17) was used to test for genetic heterogeneity in the context of the two-point parametric analysis.

RESULTS

A plot of Lod and nonparametric linkage Lod (NPL) scores for chromosome 4 was shown in Table 1. The highest two-point 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

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).

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[7] Internet address: http://www.gdb.org.
[8] Internet address: http://linkage.rockefeller.edu.

Table 1 *The two-point and multipoint Lod,[a] HLod, and NPL scores of five loci on chromosome 4 were calculated with GENEHUNTER for the 18 families*

		Two-point linkage analysis			Multipoint linkage analysis		
cM	Loci	Lod	HLod	NPL	Lod	HLod	NPL
0.00	D4S405	−4.665	0.235	0.085	−11.629	0.006	−0.170
1.60	D4S3045	−6.672	0.000	0.293	−11.281	0.018	0.141
4.47	D4S3002	−3.270	0.221	−0.039	−11.086	0.036	−0.129
6.63	D4S2996	−10.644	0.000	−0.719	−15.771	0.002	−0.346
7.29	D4S428	−11.858	0.000	−0.945	−13.553	0.000	0.012

[a] Lod, logarithm of odd; HLod, heterogeneity-adjusted Lod; NPL, nonparametric linkage Lod.

Table 2 *The two-point and multipoint Lod,[a] HLod, and NPL scores of eight loci on chromosome 3p were calculated with GENEHUNTER*

		Two-point linkage analysis			Multipoint linkage analysis		
cM to 3pter	Loci	Lod	HLod	NPL	Lod	HLod	NPL
2.5	D3S1297	−1.557	0.248	−0.527	−1.826	0.221	−0.457
21.4	D3S1489	1.807	1.829	0.724	1.389	1.514	0.592
46.9	D3S1266	−0.135	0.531	0.779	1.097	1.302	0.683
67.8	D3S1568	3.765	3.765	2.958	3.572	3.571	2.877
69.1	D3S1289	1.629	1.667	1.603	2.039	2.037	2.264
79.0	D3S1300	0.240	0.845	1.336	−0.209	0.571	1.276
91.0	D3S1285	−0.897	0.100	−0.221	−1.289	0.074	0.043
111.0	D3S3681	−3.441	−0.001	−0.318	−3.337	−0.001	−0.317

[a] Lod, logarithm of odd; HLod, heterogeneity-adjusted Lod; NPL, nonparametric linkage Lod.

only 0.141 and 0.036, respectively. Therefore, linkage of NPC to chromosome 4 was excluded in these 18 families.

To investigate the linkage of NPC to short arm of chromosome 3, eight markers with high frequency of loss of heterozygosity in NPC were analyzed (4–8). The maximum multipoint Lod scores of 3.572 ($P = 1.91 \times 10^{-4}$) 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 ($P = 1.197 \times 10^{-4}$). 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 ($P = 4.06 \times 10^{-4}$). 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 HLod 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 from southern Chinese, suggesting that the genetic abnormality appear to be causative for 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

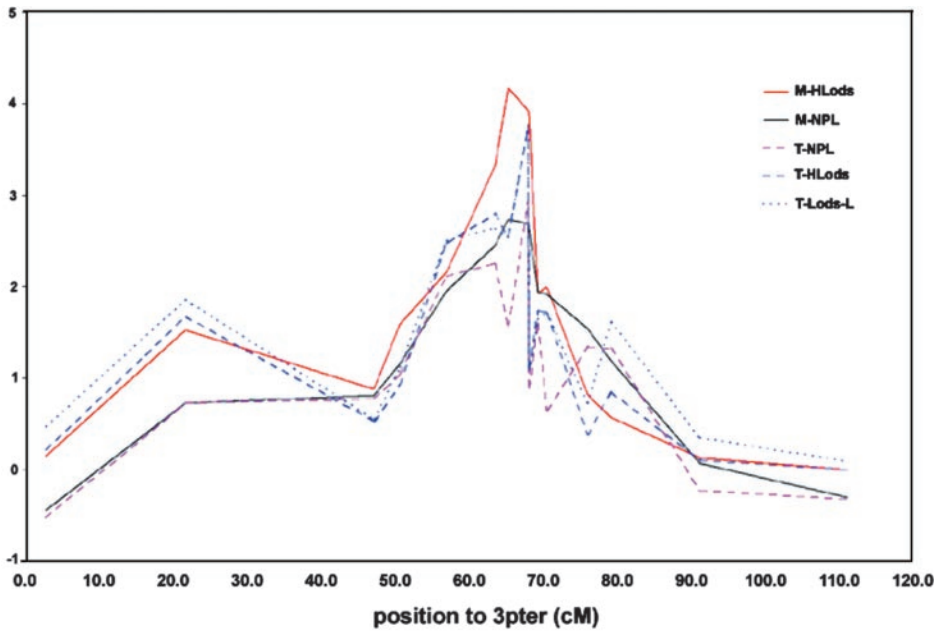

Fig. 1. The Lod scores of multipoint and two-point analysis from fine mapping on chromosome 3. M-HLods and M-NPL are multipoint parametric and nonparametric linkage scores calculated with GENEHUNTER. T-NPL and T-HLods are two-point parametric and nonparametric linkage scores calculated with GENEHUNTER. T-Lods-L is two-point parametric linkage score calculated with LINKAGE.

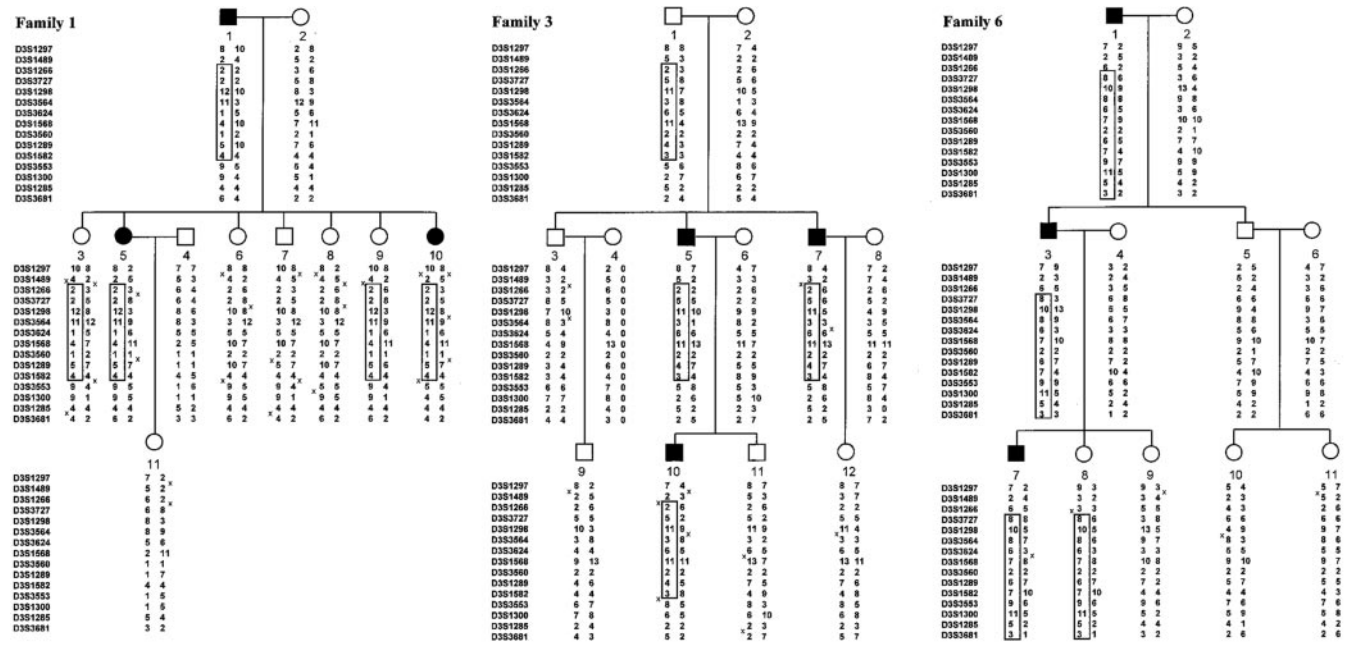

Fig. 2. Representative pedigree structure and haplotypes of family 1, 3, and 6. Haplotypes were inferred by GENEHUNTER with minimum recombination between markers. *Filled symbols* represent affected individuals. Inferred genotypes of individuals are shown in *italics*; other experimentally typed genotypes are in *bold*. Crosses represent recombination. *Boxes* indicate the chromosome region shared by affected members of the pedigree. The markers used for genotyping are listed beside each pedigree.

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.

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