

## LOH-profiling by SNP-mapping in a case of multifocal head and neck cancer

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### Abstract

**AIM:** To introduce an approach for the detection of putative genetic host factors that predispose patients to develop head and neck squamous cell carcinomas (HNSCC).

**METHODS:** HNSCC most often result from the accumulation of somatic gene alterations found in tumor cells. A cancer-predisposing genetic background must be expected in individuals who develop multiple cancers, starting at an unexpectedly young age or with little carcinogen exposure. Genome-wide loss of heterozygosity (LOH) profiling by single nucleotide polymorphism microarray mapping was performed in a patient with a remarkable history of multifocal HNSCC.

**RESULTS:** Regions of genomic deletions in germline DNA were identified on several chromosomes with a remarkable size between 1.6 Mb and 8.1 Mb (mega base-pair). No LOH was detected at the genomic location of the tumor suppressor gene *P53*.

**CONCLUSION:** Specific patterns of germline DNA deletions may be responsible for susceptibility to HNSCC

and should be further analyzed.

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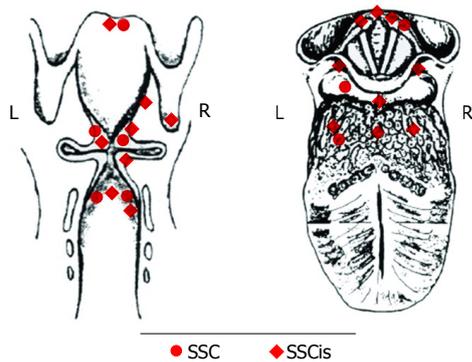
**Key words:** Genome-wide analysis; Head and neck cancer; Loss of heterozygosity; Multifocal cancer; Single nucleotide polymorphism microarray; Squamous cell carcinoma

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### INTRODUCTION

Head and neck squamous cell carcinomas (HNSCC) account for approximately 5% of all carcinomas in industrialized countries and represent the sixth most common human neoplasm with an estimated annual worldwide incidence of 500 000 new cases. It is primarily a disease of older age, occurring most frequently in the 6th and 7th decades of life<sup>[1]</sup>. Although significant advances in radiation therapy, chemotherapy and surgical techniques have improved organ preservation and the overall quality-of-life of patients with HNSCC, the long-term survival rate for this disease has not improved significantly during the past 20 years and remains unchanged at approximately 50%<sup>[2,3]</sup>. Tobacco-and alcohol consumption are highly significant etiological risk factors associated with the development of HNSCC, with more than 90% of these tumors occurring in individuals who smoke or drink<sup>[4]</sup>. However, beside these established risk factors individual variations in genetic susceptibility must contribute sig-



**Figure 1** Overview of the locations with biopsy-proven squamous cell carcinoma and squamous cell carcinoma *in situ* in the upper aerodigestive tract of the patient (profile of the hypopharynx, larynx and trachea and top view of the tongue, hypopharynx and larynx). L: Left; R: Right; SSC: Squamous cell carcinoma; SCCis: Squamous cell carcinoma *in situ*.

nificantly to the development of HNSCC, because some patients develop this cancer in the absence of identifiable life-style or environmental factors, at an exceptionally young age or at multiple locations. About 5% of patients with HNSCC develop synchronous or metachronous second primary cancers of the aerodigestivetract<sup>[5]</sup>. The concept of “field cancerization” hypothesizes that regions of the mucosal epithelium, although normal in appearance, are “preconditioned” by exposure to carcinogenic agents, thus priming them for the subsequent development of invasive lesions<sup>[5]</sup>. While the development of most human malignancies is caused by the accumulation of somatic gene alterations in the tumor cell, a genetic host factor for the development of HNSCC in the form of an additional predisposition on the genomic level can be supposed in patients with multiple primary head and neck tumors<sup>[4]</sup>. Loss of heterozygosity (LOH) is an indicator of genomic instability and has traditionally been characterized by the additional mutation of an intact allele at chromosomal locations of a pre-existing allelic heterozygosity, that is the somatic conversion of heterozygous germline alleles to homozygosity<sup>[1]</sup>. Instead of analyzing somatic LOH in DNA isolated from tumor tissue, the intention of this report is to introduce an approach for the detection of potential host factors that predispose patients to develop HNSCC. Therefore, the remarkable results of genome-wide LOH profiling in a patient with multifocal head and neck cancer are presented.

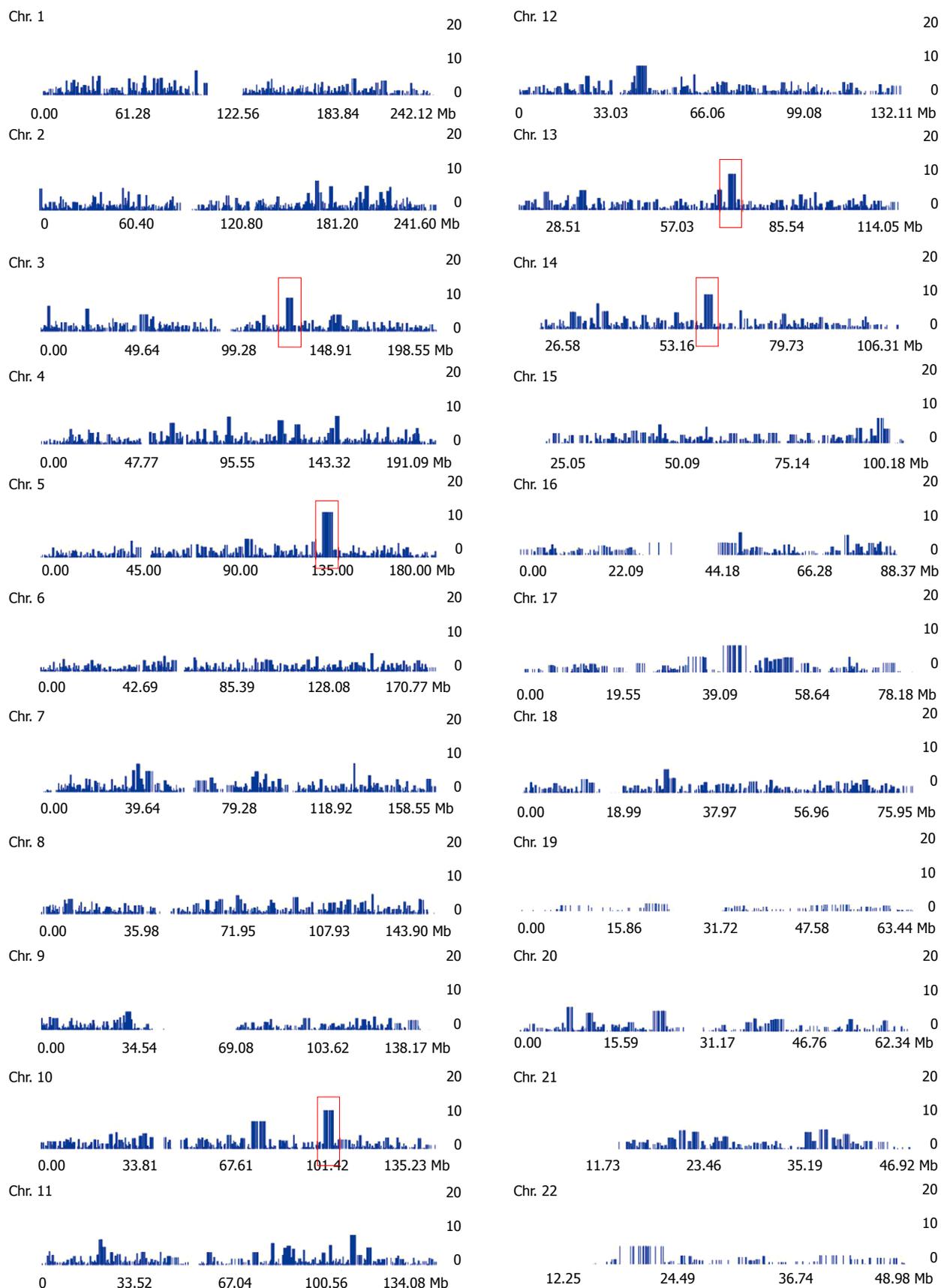
## MATERIALS AND METHODS

The study was performed in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 1983 and the local institutional review board approved this project. In 2000, a 49-year-old man was admitted to our department for further management of multifocal invasive and microinvasive SCC as well as multifocal carcinoma *in situ* (SCCis) of the upper aerodigestive tract. He had stopped smoking cigarettes after 15 pack-years in 1988 at the age of 37. He had no history of alcohol

abuse. In 1997, at the age of 46, excision biopsy of an enlarged cervical lymph node had revealed a metastasis of a poorly-differentiated SCC of unknown primary. Despite radiological investigations, panendoscopy with directed biopsies of Waldeyer’s ring, and bilateral tonsillectomy, a primary tumor site was not detected at that time. After 3 cycles of chemotherapy with cisplatin, 5-fluorouracil and folinic acid, radiation therapy was administered to the neck with a cumulative radiation dose of 50 Gy. As the primary tumor site remained unclear and at the request of the patient, the larynx and the pharynx were not irradiated. Between July 1998 and March 2007 control investigations including panendoscopy performed at regular 3 to 6 mo intervals revealed multifocal invasive or microinvasive SCC and SCCis. An overview of their locations is presented in Figure 1. The patient was repeatedly treated by local tumor resection, redifferentiation therapy with interferon alpha, isotretinoin and alpha-tocopherol as well as photodynamic therapy requiring temporary tracheostomy. Long-term improvement has not been attained up to now. No distant metastases have been found. Genome-wide LOH profiling by high-density single nucleotide polymorphism (SNP) microarray mapping was performed from peripheral blood using the Affymetrix 50K XbaI SNP Mapping Array. Genomic DNA was extracted from lymphocyte serum samples using the QIAGEN Blood and Cell Culture DNA kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). The Affymetrix 50K Xba I SNP mapping array includes around 58 000 SNPs. The chips and reagents were obtained from Affymetrix and the assays were carried out according to the manufacturer’s instructions. Briefly, 250 ng of genomic DNA were digested with *Xba*I and then ligated to adapters. A generic primer that recognizes the adapter sequence was used to amplify adapter-ligated DNA fragments with polymerase chain reaction (PCR) conditions optimized to preferentially amplify fragments in the 250-2000 bp size range in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, United States). After purification with the QIAquick purification system (Qiagen), a total of 40 µg of PCR product was fragmented and a sample was visualized on 4% TBE agarose gel to confirm the right average size. The fragmented DNA was then labeled with biotin and hybridized to the GeneChip Mapping 50K Xba I Set for 20 h. The arrays were washed and stained using the Affymetrix fluidics Station 450 and scanned with the GeneChip Scanner 3000 G7 (Affymetrix, Santa Clara, CA, United States). The GeneChip Genotyping analysis software was used to analyze feature intensity data stored in the GCOS Database, and provided high-throughput and accurate genotyping analysis.

## RESULTS

Results are summarized in Figure 2 and Table 1. Regions of genomic deletions were identified on chromosomes 3,



**Figure 2** Single nucleotide polymorphism 50K XbaI genome-wide loss of heterozygosity profiling using the AffymetrixGeneChip Assay System. Regions of genomic deletions between 1.6 Mb and 8.1 Mb in size were identified on 5 chromosomes (red labeling). The x-axis shows physical position along the respective chromosome. The y-axis indicates  $-\log_{10}P$  values where homozygous stretches are due to loss of heterozygosity.

**Table 1 Summary of loss of heterozygosity regions on five chromosomes**

Chromosome	Genomic location	Size
Chromosome 3	123.2-126.4 Mb	3.2 Mb
Chromosome 5	128.3-132.8 Mb	4.5 Mb
Chromosome 10	97.1-105.2 Mb	8.1 Mb
Chromosome 13	71.3-72.9 Mb	1.6 Mb
Chromosome 14	59.2-61 Mb	1.8 Mb

5, 10, 13 and 14 ranging in size from 1.6 to 8.1 Mb (mega base-pair). It can be hypothesized that the identified regions of genomic deletions harbor candidate genes that might be associated with cancer susceptibility or a function in tumor suppression. No LOH was detected at the genomic location of the tumor suppression gene *P53*, which is located on chromosome 17 (17p13.1).

## DISCUSSION

Most human cancers are characterized by genetic instabilities. Cancer arises when gene mutations lead to an abnormal cell resulting in clonal proliferation, aggressive spread, or prevention of apoptosis. As with other solid tumors, HNSCC is believed to originate *via* a multistep process which involves defects in proto-oncogenes, suppressor genes, and several other functionally essential genes. Mathematical models estimate that approximately seven to ten individual genetic alterations must accumulate in the epithelia of the upper aerodigestive tract for the development of cancer<sup>[4,6-8]</sup>. The vast majority of these mutations occur after birth and are found only in the cancer cells themselves. Inactivation of the *P53* tumor suppressor gene is the most common genetic alteration in all cancer types and is extremely common in squamous cell carcinoma, with approximately 50% of the lesions expressing a mutant form of the protein<sup>[4]</sup>. Most studies on genetic instability in HNSCC analyze somatic mutations in the cancer cell themselves using DNA from microdissected tumor tissue<sup>[9,10]</sup>. LOH is a frequent mechanism of inactivation of tumor suppressor genes where one allele is already altered. LOH profiling is a powerful molecular genetic approach for high-resolution screening of genomic alterations. The Affymetrix 50K SNP mapping array provides a high throughput tool for genotyping and genome-wide LOH profiling<sup>[9-12]</sup>. One advantage of the use of DNA microarray technology is the ability to screen the entire genome with small amounts of DNA<sup>[13]</sup>. The most common method is to directly compare genotypes between tumor and paired germline DNA, in which only SNPs that are heterozygous in the germline are informative as to whether LOH has taken place<sup>[11]</sup>. About 5% of cancers are thought to arise in the context of a cancer-preconditioning genetic background which predisposes for the development of more than one tumor<sup>[5]</sup>. A cancer-predisposing genetic background can be expected in individuals who develop multiple cancers during their life-

time, starting at an unexpectedly young age or develop HNSCC with little carcinogen exposure, e.g., smoking and drinking. We present here a novel approach to identify predisposing host factors for the development of HNSCC. Genome-wide LOH profiling by high-density SNP microarray mapping at the genomic level in a patient with an exceptional history of multifocal head and neck cancer localized a number of genomic deletions in the germline DNA with remarkable sizes. The advantage of SNP mapping arrays is that they provide marker densities that enable the identification of “LOH regions” without the use of paired “normal” DNA. Statistical software analysis are applied to identify strings of consecutive homozygous SNPs that are longer than would be expected to appear by chance alone and, as such, every SNP is informative<sup>[10]</sup>. Frequent allelic loss at specific loci in the germline DNA of patients with multiple primary HNSCC may therefore indicate the location of putative genes, whose malfunction can be evaluated as a host factor predisposing the patient for the development of invasive carcinomas. Based on our observation we hypothesize that patterns of germline deletions at multiple sites may favor the accumulation of genetic defects in the epithelia of the aerodigestive tract and finally in the cancer cell and predispose for the development of HNSCC. To validate the replication of our findings with an increased number of patients, this observation should be systematically studied using the presented approach in a selected group of patients who clinically appear particularly susceptible for the development of HNSCC.

## COMMENTS

### Background

Head and neck squamous cell carcinomas (HNSCC) account for approximately 5% of all carcinomas in industrialized countries and are primarily a disease of older age. More than 90% of HNSCC occur after tobacco or alcohol abuse. However, individual variations in genetic susceptibility must contribute significantly to the development of HNSCC because some patients develop this disease at an exceptionally young age, without the established risk factors of alcohol and tobacco consumption or at multiple primary sites in the head and neck.

### Research frontiers

Loss of heterozygosity (LOH) is an indicator of genomic instability and has traditionally been analyzed at the somatic level in DNA isolated from tumor tissue. The authors analyzed LOH at the genomic level in a patient with multiple primary HNSCC to identify genetic host factors that predisposed the patient to develop this disease.

### Innovations and breakthroughs

The authors present a novel approach to identify predisposing host factors for the development of HNSCC in patients that clinically appear susceptible to this disease. They localized a number of genomic deletions with remarkable sizes in the germline DNA of a patient with an exceptional history of multifocal HNSCC by genome-wide LOH profiling.

### Applications

The study results suggest that this approach should be systematically applied and the observation studied in a larger group of patients who clinically appear predisposed for the development of HNSCC. This approach might help to identify patients susceptible to head and neck cancer particularly in the follow-up of this disease.

### Terminology

Single nucleotide polymorphisms (SNPs) are short polymorphism in the human DNA. For each SNP the genomic location and frequency distribution in the aver-

age human population is known. The statistical analysis of the hybridization pattern on SNP arrays can indicate LOH regions with high probability. The technique was used for genome-wide LOH profiling to identify regions of genomic deletions.

#### Peer review

The authors present an interesting approach and an interesting finding in a single case study. Future research should try to validate the findings with an increased number of patients.

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