Chromosomal aberrations in head and neck squamous cell carcinomas in Norwegian and Sudanese populations by array comparative genomic hybridization

ERIC ROMAN[1,2], LEONARDO A. MEZA-ZEPEDA[3], STINE H. KRESSE[3], OLA MYKLEBOST[3,4], ENDRE N. VASSTRAND[2] and SALAH O. IBRAHIM[1,2]

[1]Department of Biomedicine, Faculty of Medicine and Dentistry, University of Bergen, Jonas Lies vei 91; [2]Department of Oral Sciences - Periodontology, Faculty of Medicine and Dentistry, University of Bergen, Årstadveien 17, 5009 Bergen; [3]Department of Tumor Biology, Institute for Cancer Research, Rikshospitalet-Radiumhospitalet Medical Center, Montebello, 0310 Oslo; [4]Department of Molecular Biosciences, University of Oslo, Blindernveien 31, 0371 Oslo, Norway

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Abstract. We used microarray-based comparative genomic hybridization to explore genome-wide profiles of chromosomal aberrations in 26 samples of head and neck cancers compared to their pair-wise normal controls. The samples were obtained from Sudanese (n=11) and Norwegian (n=15) patients. The findings were correlated with clinicopathological variables. We identified the amplification of 41 common chromosomal regions (harboring 149 candidate genes) and the deletion of 22 (28 candidate genes). Predominant chromosomal alterations that were observed included high-level amplification at 1q21 (harboring the *S100A* gene family) and 11q22 (including several *MMP* family members). Regions of copy number increase was also identified at 6p21 (*p21*), 7p12 (*EGFR*), 17p13 (*p53*) and 19p13.2 ($p19^{INK4d}$), while regions showing deletion included among others 3p25.2 (*RAF1*) and 9p21 (*p15, p16*). We found genes from four common biological pathways (MAPK signaling, cytokine-cytokine receptor interaction, ECM-receptor interaction and Jak-STAT signaling) to be predominantly over-represented in areas of gain and loss. The current study provides valuable information on chromosomal aberrations likely to be involved in the pathogenesis of head and neck cancers. An increased copy number of the *S100A* and *MMP* gene family members, known to be involved in invasion and metastasis, may play an important role in the development of the tumors. Hierarchical clustering of the chromosomal alterations with clinicopathological parameters showed little correlation, suggesting an occurrence of gains/losses regardless of ethnic differences and clinicopathological status between the patients from the two countries. Our findings indicate the existence of common gene-specific amplifications/deletions in these tumors, regardless of the source of the samples or attributed carcinogenic risk factors.

Correspondence to: Dr Eric Roman, Department of Biomedicine, Faculty of Medicine and Dentistry, University of Bergen, Jonas Lies vei 91, 5009 Bergen, Norway
E-mail: eric.roman@biomed.uib.no

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Introduction

Head and neck squamous cell carcinoma (HNSCC), including the oral squamous cell carcinoma (OSCC) subtype, is an aggressive disfiguring disease characterized by events of heterogeneous chromosomal/genetic alterations influencing growth, proliferation and differentiation of the cells (1). The main etiological factors related to the development of HNSCCs involve the use of tobacco and alcohol consumption (2-5). While the incidence of HNSCCs in industrial countries seems to be related to cigarette smoking and alcohol consumption, the use of smokeless tobacco is suggested to be the main carcinogenic risk factor in developing countries. Although social habits of cigarette smoking and alcohol consumption are common in Norway, the incidence rate of HNSCCs reported in the period 1996-2001 has been rather low, affecting 6.1% of male and 2.5% of female patients (6). Due to cultural and religious constraints, cigarette smoking and alcohol consumption are not common in Sudan and the high incidence of HNSCCs reported in that country (11.6% for males and 6.91% for females) is attributed to the extensive use of the locally produced, highly carcinogenic smokeless tobacco, locally known as *toombak* (7).

Microarray-based comparative genomic hybridization (array CGH) is a powerful method that allows detection and analysis of chromosomal copy number changes (gains/losses) at a high resolution throughout the genome (8,9). Contrary to the conventional CGH that is limited by its resolution, array CGH can be used to capture chromosomal changes at levels of 5-10 kb of the DNA sequence, including the detection of

microamplifications and microdeletions (10,11). CGH studies of head and neck squamous cell carcinoma, including HNSCCs, have previously identified gains of chromosomal regions at 1q21, 3q26.3, 5p15, 7p12, 8q24, 9q34, 11q13 and 20q12, and deletions of 3p, 4q, 5q, 7q22, 8p23, 9p21, 13q12-24, 17p, 18q21, 21q11-21 and 22q (12). The loss of heterozygosity (LOH) and several regions of deletions of the short arm of chromosome 3 and 9, as well as frequent deletions at 8p, 17p, 13q and 9p, represent some of the earlier events of the development and progression of the HNSCCs (9). It has been suggested that chromosomal gains detected at 3q26 and 11q13 and deletions of 8p23 and 22q may be associated with the high aggressiveness status of HNSCCs (13,14).

In the present study, we applied array CGH to investigate genome-wide profiles of chromosomal aberrations in HNSCCs compared to their pair-wise controls from Sudan (n=11) and Norway (n=15), using genomic microarrays with 1 Mb resolution. The findings were correlated with clinicopathological data.

Materials and methods

Patients and tissue specimens. Tissue samples of 26 tumors and their pair-wise normal controls were obtained from Sudanese (n=11) and Norwegian (n=15) patients, clinically diagnosed with HNSCCs. Samples from Sudan were obtained from the Department of Oral and Maxillofacial Surgery at the Khartoum University Dental Teaching Hospital, Khartoum, Sudan. Samples from Norway were obtained from the Department of Otolaryngology/Head and Neck Surgery at the Haukeland University Hospital, Bergen, Norway. For Sudan, the study was evaluated and approved by the Medical Ethics Committee at the University of Khartoum and for Norway the study was approved by the Regional Committee for Medical Ethics at Haukeland University Hospital, Bergen, Norway.

Immediately after surgery, the biopsies were submerged in the tissue storage and RNA stabilization solution, RNA*Later*™ (Ambion, Applied Biosystems, 850 Lincoln Center Dr., Foster City, CA, USA) and stored at -20˚C. Tumors were staged according to the 1987 Unio Internationale Contra Cancrum (UICC) staging system. Using haematoxylin and eosin (H&E) for the staining of fresh-frozen or 10% formalin-fixed, paraffin-embedded tissue sections, a histopathological diagnosis was performed at the Department of Oral Pathology, Dental Faculty Haukeland University Hospital, Bergen, Norway. Tumors were histopathologically graded into high (H), moderate (M) and poorly (P) differentiated according to Cawson and Eveson (15) (Table I). Each tumor sample was confirmed to contain ≥70% of cancer cells and <10% necrotic tissue to avoid stromal cell contamination.

Clinicopathological data, including the anatomical site of the tumor, age, gender, differentiation status, tumor stage in the patients and social habits of cigarette smoking/use of smokeless tobacco and alcohol consumption, are shown in Table I. For the two countries, the use of smokeless tobacco and cigarette smoking was quantified and patients were grouped into non-smokers (NS), light smokers (LS: 1-10 cigarettes/day), moderate smokers (MS: 11-20 cigarettes/day) and heavy smokers (HS: >20 cigarettes/day). The patients were grouped according to their habit of alcohol consumption into non-drinkers (ND), light drinkers (LD: 1-2 drinks/day), moderate drinkers (MD: 3-5 drinks/day) and heavy drinkers (HD: >5 drinks/day) (Table I). The distribution (%) of the clinicopathological characteristics in patients diagnosed with HNSCC from Sudan and Norway is given in Table II.

Array CGH. The genomic microarray that was used contained 4,549 bacterial- and P1 artificial chromosome (BAC and PAC) clones representing the human genome at ~1 Mb resolution, as well as the minimal tiling-path between 1q12-q25. The microarrays were provided by the Norwegian Microarray Consortium (NMC, www.microarray.no).

Tissue samples of the tumor and normal controls were removed from the RNA*Later* storage solution and genomic DNA was extracted using a DNeasy purification kit (Qiagen Inc, Valencia, CA, USA) according to the manufacturer's instructions. Array-CGH was performed as described previously (49). In brief, 500 ng genomic DNA purified from tumor and normal control DNA were digested with DpnII (New England Biolabs, Ipswich, MA, USA). Tumor DNA was labeled with Cy3-dCTP and normal control DNA with Cy5-dCTP (NEN Life Science Products Inc., Boston, MA, USA) by random priming. The labeled DNA samples were combined and mixed with 135 μg human Cot-1 DNA (Roche Diagnostics Corp., Indianapolis, IN, USA). Hybridization was performed using an automated hybridization station, GeneTAC/HybArray (Genomic Solutions, Ann Arbor, MI, USA) with agitation for 42 h at 37˚C. The arrays were scanned using an Agilent G2565B scanner (Agilent Technologies Inc., Santa Clara, CA, USA).

Array CGH data analysis. Images were analyzed using GenePix Pro 3.1 software (Molecular Devices Corp., Sunnyvale, CA, USA). Further data processing, including filtering and normalization, was performed as previously described using M-CGH, a MATLAB toolbox designed for the analysis of array CGH experiments (16,17). Detailed clone information, chromosomal regions, gene content and genes involved in various forms of cancer were accessed using the Ensembl (http://www.ensembl.org), GeneCards® (http://www.genecards.org) and The Cancer Genome Anatomy Project - CGAP (http://cgap.nci.nih.gov/) databases.

For the detection of chromosomal regions showing gains/losses common for the two populations, CGH-Explorer software was used (http://www.ifi.uio.no/forskning/grupper/bioinf/Papers/CGH). Alterations of specific chromosomal regions >0.3 and <-0.3 on $\log_2$ scale found in a minimum of 20% of the Sudanese and Norwegian patients were considered for further investigation.

Furthermore, we investigated chromosomal alterations (gains/losses) for each population separately. Chromosomal regions that were found to be either amplified or deleted in a minimum of 18% of one population were expected not to show any alterations in the other population. We performed an analysis of the panel of all genes located in regions of chromosomal gains/losses in respect to their possible involvement in biological pathways using Kyoto Encyclopedia of Genes and Genomes - KEGG (http://www. kegg.com).

Hierarchical clustering of the chromosomal gains and losses found in the tumors examined from patients from Sudan

Table I. Clinicopathological parameters of the patients diagnosed with the OSCCs.

Tumor no./site	Age	Gender	H.D.	TNM	Tobacco	Alcohol	Snuff
Sudanese cases							
15/Buccal	55	M	H	T3N0M0	NS	MD	Yes
17/Plate	55	M	H	T3N1M0	NS	ND	No
32/Tongue	75	F	M	T3N1M0	NS	ND	No
44/Buccal	65	M	H	T3N1M0	NS	MD	Yes
50/Floor of mouth	70	F	P	T3N1M0	NS	ND	Yes
51/Gingiva	56	M	P	T3N1M0	NS	ND	No
52/Floor of mouth	66	M	M	T2N1M0	NS	ND	No
59/Buccal	60	M	P	T2N1M0	MS	ND	No
63/Tongue	75	F	M	T2N1M0	NS	ND	No
65/Buccal	75	F	M	T2N1M0	NS	ND	No
69/Buccal	34	M	H	T2N1M0	NS	ND	No
Norwegian cases							
17/Gingiva	65	F	H	T3N0M0	MS	ND	nd
21/Larynx	60	M	M	T2N0M0	MS	nd	nd
23/Tongue	31	M	H	T2N0M0	NS	nd	nd
39/Gingiva	86	F	H	T2N1M0	XS	nd	nd
46/Tongue	58	M	M	T1N0M0	nd	nd	nd
47/Tonsil	59	M	P	T3N1M0	nd	ND	No
48/Pharynx	59	M	M	T2N0M0	nd	nd	nd
50/Larynx	75	M	M	T3N0M0	nd	ND	No
51/Larynx	48	M	M	T3N0M0	nd	ND	No
52/Floor of mouth	76	M	P	T3N1M0	nd	ND	No
53/Floor of mouth	81	M	H	T1N0M0	nd	nd	nd
54/Tongue	45	F	M	T2N0M0	nd	ND	No
57/Sinus max.	89	F	H	T3N0M0	nd	nd	nd
58/Tongue	67	M	M	T1N0M0	nd	nd	nd
59/Tongue	67	M	M	T2N0M0	nd	nd	nd

M, male; F, female; H.D., histological differentiation; H, high; M, moderate; P, poor; TNM, (T) tumor; (N), lymph node; (M), metastasis; HS, heavy smoker (>20 cigarettes per day); MS, moderate; smoker (11-20 cigarettes per day); LS, light smoker (1-10 cigarettes per day); NS, non-smoker; XS, ex-smoker; HD, heavy drinker (>5 drinks per day); MD, moderate drinker (3-5 drinks per day); LD, light drinker (1-3 drinks per day); ND, non-drinker and nd, not determined.

and Norway was performed using J-Express Pro software (http://www.molmine.com/software.htm). Chromosomal alterations found in the tumors from the two countries were correlated with clinicopathological data including tumor site, age, gender, histological differentiation, tumor, lymph node, cigarette smoking and use of snuff.

Results

Clinicopathological evaluation. Samples of HNSCCs from Norway (n=15) and Sudan (n=11) were investigated in this study (Table I). There was a relative predominance of males for the two populations (Norway: 73%, Sudan: 64%). Although the tumors were obtained from different sites of the head and neck region in the two populations, the oral cavity was predominant among Sudanese (100%) compared to Norwegian patients [oral cavity (60%), larynx (33%) and maxillary sinus (7%)]. With regard to histological differentiation of the tumors, high differentiation (HD) was found in four (36%) of the Sudanese and five (33%) of the Norwegian patients, moderate (MD) in four (36%) Sudanese and eight (54%) Norwegian and poor (PD) in three (28%) Sudanese and two (13%) Norwegian patients. The tumor stage profile showed advanced stage (III/IV) in six (55%) of the Sudanese and six (40%) of the Norwegian samples. For Sudan, the data on the patients' habits of tobacco use/alcohol consumption showed that there were 10 (91%) non-smokers, nine (82%) non-drinkers and eight (73%) non-users of smokeless tobacco. Among the Norwegian patients, two (13%) were moderate smokers, six (40%) non-drinkers and five (33%) non-users of smokeless tobacco.

Chromosomal gains common in tumors examined from Sudan and Norway. Array CGH was used to investigate

Table II. Distribution (%) of the clinicopathological characteristics of the patients diagnosed with the OSCCs.

Variable	Sudan	Norway
Samples, n (%)	11 (42)	15 (58)
Gender		
Male, n (%)	7 (64)	11 (73)
Female, n (%)	4 (36)	4 (27)
Mean age	62	64
Tumor location		
Oral cavity, n (%)	11 (100)	9 (60)
Larynx, n (%)	0	5 (33)
Sinus, n (%)	0	1 (7)
Tumor differentiation		
Poor, n (%)	3 (28)	2 (13)
Medium, n (%)	4 (36)	8 (54)
High, n (%)	4 (36)	5 (33)
TNM stage		
Stage I-II, n (%)	5 (45)	9 (60)
Stage III-IV, n (%)	6 (55)	6 (40)
Smoking, n (%)	1 (10)	3 (20)
Smokeless tobacco use, n (%)	3 (27)	10 (67)
Alcohol use, n (%)	2 (18)	9 (60)

genome-wide profiles of chromosomal aberrations in HNSCCs compared to their pair-wise controls. Fig. 1 shows a representative ratio plot for one HNSCC from Norway and one from Sudan. Frequency plots of chromosomal aberrations detected in HNSCCs from Norway and Sudan, are shown in Fig. 2A and B, respectively.

A panel of 41 regions of chromosomal gains common in the samples from the two populations were identified. These regions contained 149 candidate genes. Using KEGG analysis of the 149 genes, we detected candidate genes participating in 13 biological pathways; cell communication, MAPK signaling, calcium signaling, cytokine-cytokine receptor interaction, cell cycle, cell apoptosis, Wnt signaling, focal adhesion, ECM-receptor interaction, cell adhesion molecules, adherens junctions, gap junctions and Jak-STAT signaling. Distribution (%) of amplifications/deletions and corresponding biological pathways found in the tumors examined from Sudan and Norway are given in Table III. Four pathways were particularly predominant; MAPK signaling, cytokine-cytokine receptor interaction, ECM-receptor interaction and Jak-STAT signaling.

The distribution of common significant gains in the HNSCCs examined from Sudan and Norway is shown in Table IVA. The amplification of MAPK signaling genes (including *EGFR*, *FGF3*, *FGF4*, *FGF19*, *JUND*, *MYC*, *MAPK12* and *p53*) represented one of the largest groups of candidate genes located at different regions of the genome (17/149 genes). We found an increased copy number of several cytokine-cytokine receptor interaction interleukins (*IL10*, *IL19*, *IL20* and *IL24*) in 6/15 (40%) of Norwegian and 6/11 (54.5%) of Sudanese samples mapped to BAC RP11-534L20 (1q32). All genes were previously found to be associated with the development of HNSCCs. The genes involved in cytokine-cytokine receptor interaction were the largest group of genes (23/149) showing chromosomal gains and in addition to the interleukins at 1q32, there were amplified genes from other regions of the genome, such as *CCL26*, *EGFR*, *IL17R*, *IL28A*, *IL29*, *IL2RB*, *IL12RB1*, *VEGF*, *XCL2*, *TNFSF9* and *CD40* (Table III and IVA).

Genes participating in cell communication pathways were found to be amplified, including *COL1A1*, *COL4A1*, *COL11A2*, *LMNA* and *NES*. Of interest was the observation of *COL4A1* copy number increase in 14/15 (93.3%) of Norwegian and 11/11 (100%) of Sudanese HNSCC samples (Table IVA). We found a copy number increase of genes involved in the cell cycle regulatory pathway *p21*, *p19^{INK4d}*, *EP300*, *CCND1*, *CCND2* and *p53* (Table IVA). Gains were also observed at the chromosomal region containing *CDK10* in 3/15 (20%) of Norwegian and 4/11 (36.4%) of Sudanese samples. The amplification of chromosomal regions containing regulatory genes of apoptosis were found in samples from the two populations, particularly *BAD*, *BCL2L1* and *FADD*. An increased copy number on $\log_2$ >2 was observed in two samples from Norway (N17, N46) and a copy number on $\log_2$ >3 was found in two samples from Norway (N48, N50) and one sample from Sudan (S50) of regions harboring the genes *NTRK1*, *BIRC2* and *BIRC3* were observed. A summary of the common copy number gains in Norway and Sudan are shown in Fig. 3A and B.

We found several altered regions on chromosome 1 in the two populations, with a copy number increase of 1p36, 1q21, 1q23 and 1q32. The *TNFRSF4* gene, mapped to BAC CTB-14E10 at 1p36, was amplified in 5/15 (33.3%) and 3/11 (27.3%) of Norwegian and Sudanese patients, respectively. One of the Norwegian samples (N21) showed a copy number increase higher than two of *TNFRSF4* on the $\log_2$ scale. In the two populations, BAC RP1-148L21 at 1q21 showed a notable amplification of members of the *S100A* gene family. An increased copy number of a regulatory gene of the cell cycle, *Mdmx* and *KISS-1* precursor was detected in 26.7% of the Norwegian and 27.3% of the Sudanese HNSCC samples. It was noteworthy to observe that the gene for metastasis suppressor protein 1 (*MTSS1*), mapped to BAC RP11-532M24 at 8p22, was amplified in 6/15 (40%) of the Norwegian and 8/11 (72.7%) of the Sudanese HNSCC samples and one Sudanese patient (S69) showed a copy number increase higher than three of this region on the $\log_2$ scale.

Co-amplification was detected at 11q13, hosting *EMS1*, *SHANK2* and *CCND1* genes. BAC RP11-750P5 at 11q22 contained several matrix metallopeptidases (*MMPs*) cell-cell interaction genes, including *MMP1*, *MMP3*, *MMP7*, *MMP8*, *MMP10*, *MMP12*, *MMP13* and *MMP20*, which were co-amplified in the two populations.

Chromosomal losses common in tumors examined from Sudan and Norway. We identified 28 candidate genes that were located in deleted regions in the two populations. The distribution of common significant losses in the tumors examined from Norway and Sudan is shown in Table IVB.

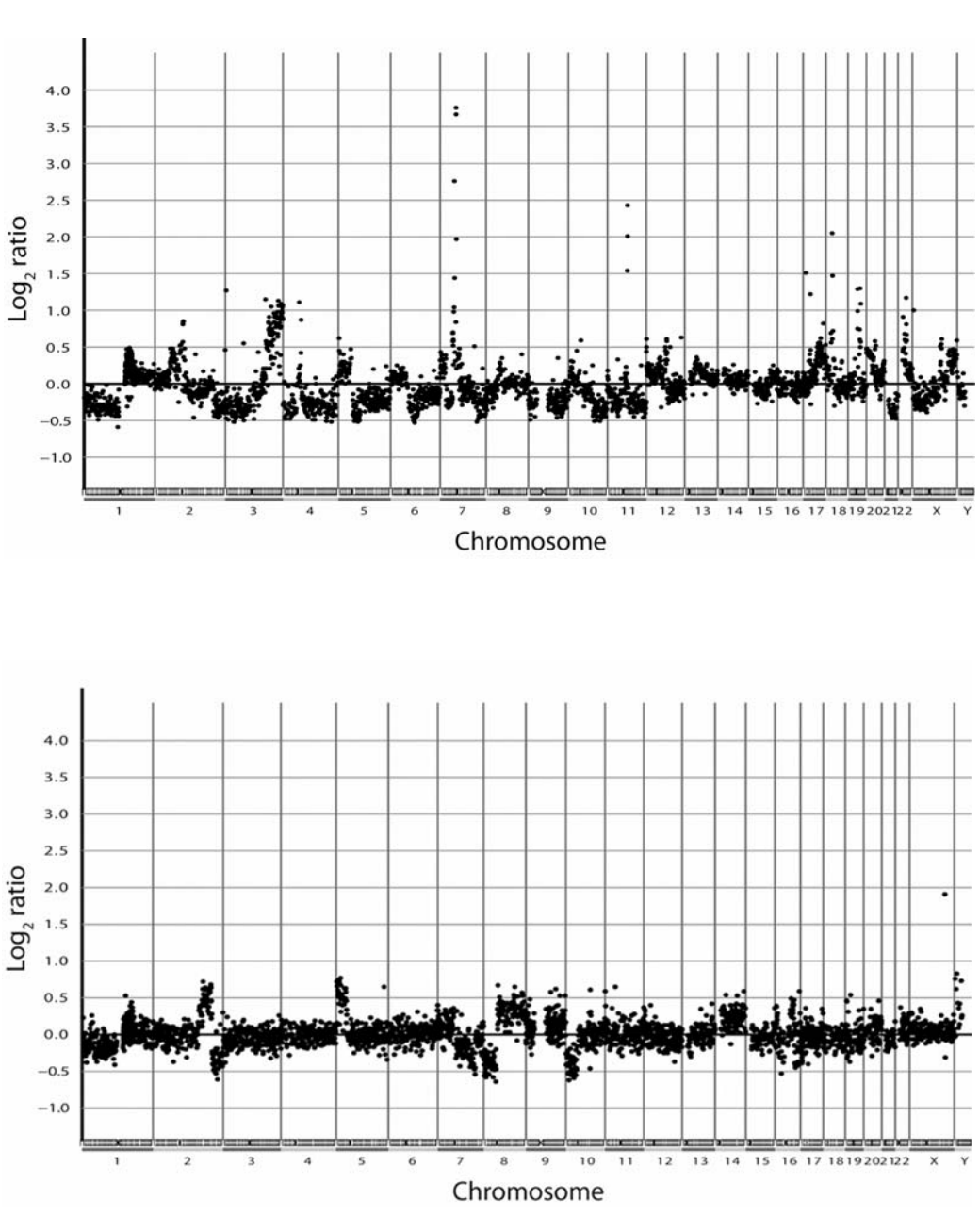


Figure 1. Chromosomal alterations in a case of oral squamous cell carcinoma from Norway (A, case N50) and Sudan (B, case S65).

The deletion of chromosomal regions hosting genes involved in MAPK signaling included *FGF5*, *FGF9* and *RAF1* (involved in the Ras-dependant signaling pathway between the receptors and the nucleus), as well as *TGFBR2*. Common copy number losses in the samples from Norway and Sudan are shown in Fig. 3C. We found three genes participating in cytokine-cytokine receptor interaction were deleted, *IL5RA*, *TGFBR2* and *TNFSF11* (mapped to BAC RP11-10H6, RP11-69K20 and RP11-117I13, respectively). For the two countries, a copy number loss at 3p22 was observed in 5/15 (33.3%) of Norwegian and 3/11 (27.3%) of Sudanese HNSCCs. This region contains a gene involved in DNA repair, *MHL1* (DNA mismatch repair protein). The cell cycle genes $p15^{INK4a}$ and $p16^{INK4b}$ mapped to BAC RP11-149I2 at 9p21, which was deleted in 4/15 (26.7%) and 3/11 (27.3%) of Norwegian and Sudanese patients, respectively.

Among the genes located in common deleted chromosomal regions, MAPK signaling, cytokine-cytokine receptor interaction and the regulation of the actin cytoskeleton represented the predominant biological pathways.

Hierarchical clustering. Hierarchical clustering of the chromosomal gains and losses found in the tumors examined from patients from Sudan and Norway and the clinicopathological data were performed (Fig. 4). Although, there was a tendency for the majority of the tumors from each country to group tightly to each other in one of the two main subgroups, we did not find any specific correlation between chromosomal alterations and clinicopathological parameters such as age, gender, tumor stage, tumor site, differentiation status or social habits of alcohol, tobacco and snuff use.

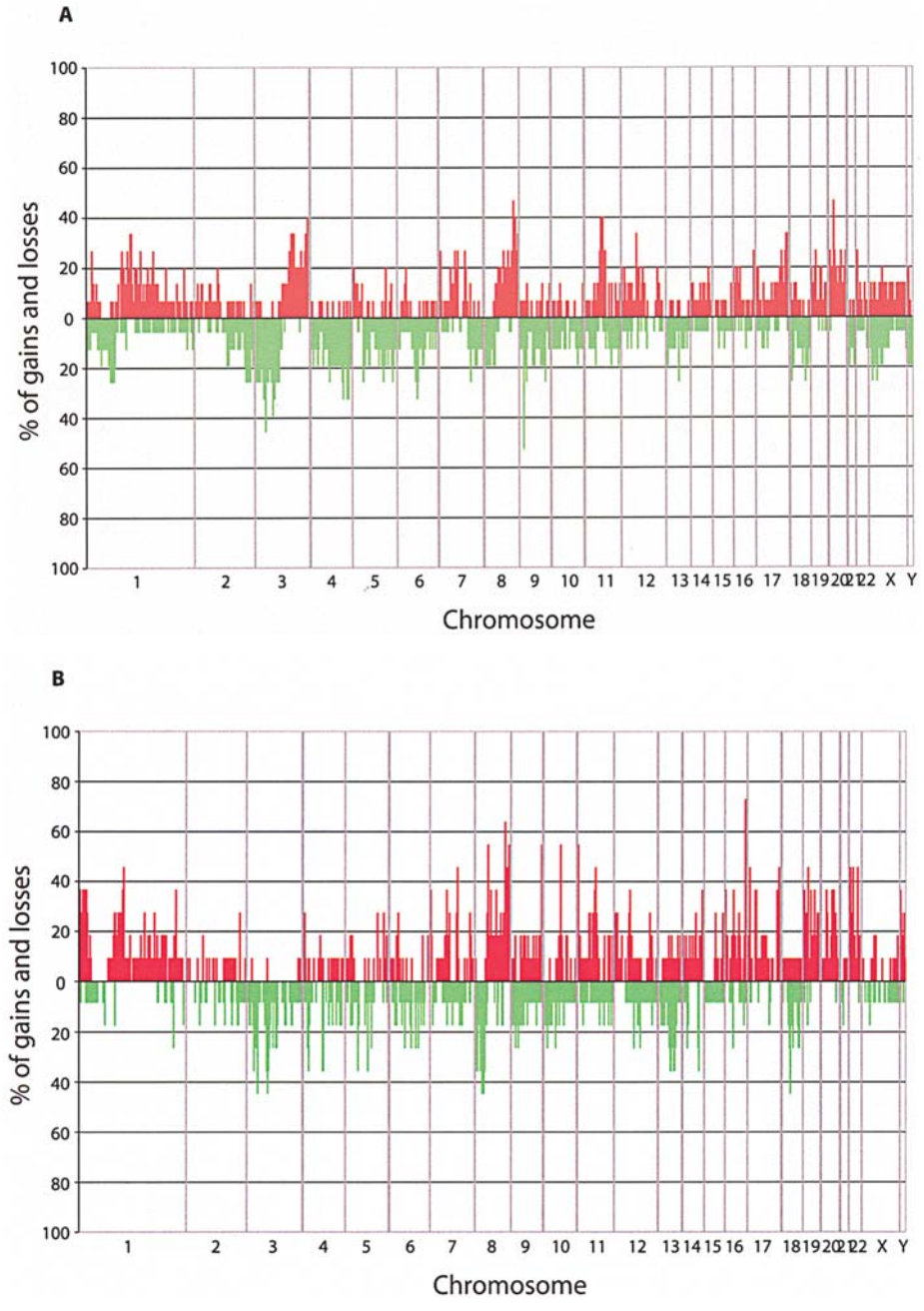


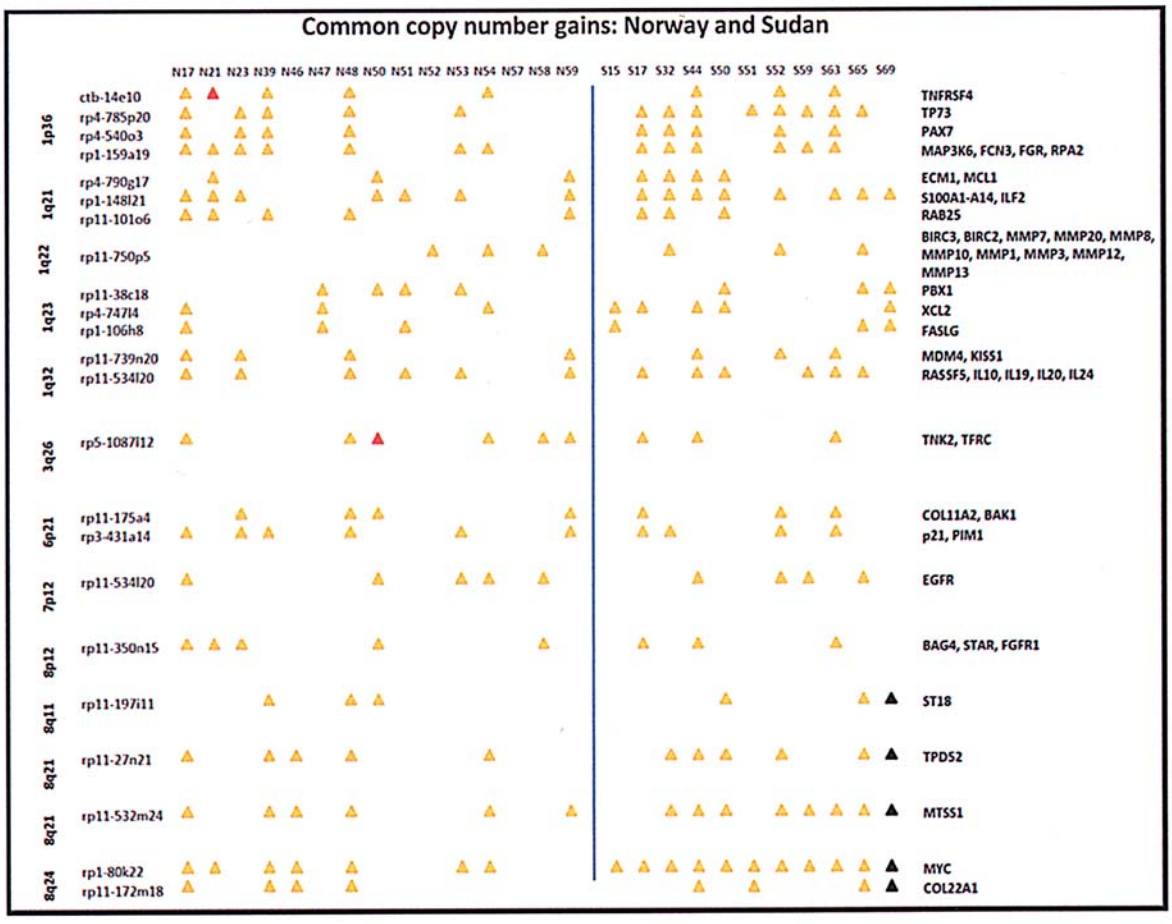
Figure 2. Genomic frequency plot representing amplified and deleted chromosomes in oral squamous cell carcinomas from Norway (A) and Sudan (B).

Chromosomal gains and losses that differ significantly between populations. Furthermore, we analyzed the data in search for regions of copy number gains/losses specific for each population. The results of country-specific chromosomal alterations in the HNSCCs examined from Sudan and Norway are shown in Fig. 2A and B. For Sudan, we found regions hosting 75 genes to be preferentially amplified. The genes included *COL5A1*, *COL2A1*, *RASEF*, *MAPK4* and a large family of keratins (*KRT10*, *KRT12*, *KRT20*, *KRT23*, *KRT24* and *KRT25A-D*). Additionally, we found chromosomal regions that were deleted only in Sudanese samples. These regions hosted 66 genes, among others several interleukins (*IL22RA1*, *IL28RA1*, *IL7R*, *BIRC6*, *COL1A2*, *MMP19* and *CDK2*). For Norway, we detected preferentially amplified regions with 33 genes, of which five (15%) participated in the MAPK signaling pathway, four (12%) in the regulation of actin cytoskeleton and four (12%) as cell adhesion molecules (CAMs). Deletions of chromosomal regions specific for Norway affected 65 genes, including several interleukins (*IL3*, *IL4*, *IL5*, *IL9* and *IL13*) and *CD2*, *CD58*, *COL7A1*, *MAPK10* and *CD44*. Collagens, keratins and interleukins were the predominant genes found to be located in amplified or deleted regions in each population separately.

Discussion

Genome-wide analysis by array CGH was used to identify quantitative chromosomal alterations in HNSCCs from Sudan and Norway. The amplification and deletion of regions harboring in total 149 and 28 candidate genes, respectively,

A


Common copy number gains: Norway and Sudan
N17 N21 N23 N39 N46 N47 N48 N50 N51 N52 N53 N54 N57 N58 N59
S15 S17 S32 S44 S50 S51 S52 S59 S63 S65 S69
1p36
ctb-14e10
rp4-785p20
rp4-540o3
rp1-159a19
TNFRSF4
TP73
PAX7
MAP3K6, FCN3, FGR, RPA2
1q21
rp4-790g17
rp1-148l21
rp11-101o6
ECM1, MCL1
S100A1-A14, ILF2
RAB25
1q22
rp11-750p5
BIRC3, BIRC2, MMP7, MMP20, MMP8, MMP10, MMP1, MMP3, MMP12, MMP13
1q23
rp11-38c18
rp4-747l4
rp1-106h8
PBX1
XCL2
FASLG
1q32
rp11-739n20
rp11-534l20
MDM4, KISS1
RASSF5, IL10, IL19, IL20, IL24
3q26
rp5-1087l12
TNK2, TFRC
6p21
rp11-175a4
rp3-431a14
COL11A2, BAK1
p21, PIM1
7p12
rp11-534l20
EGFR
8p12
rp11-350n15
BAG4, STAR, FGFR1
8q11
rp11-197i11
ST18
8q21
rp11-27n21
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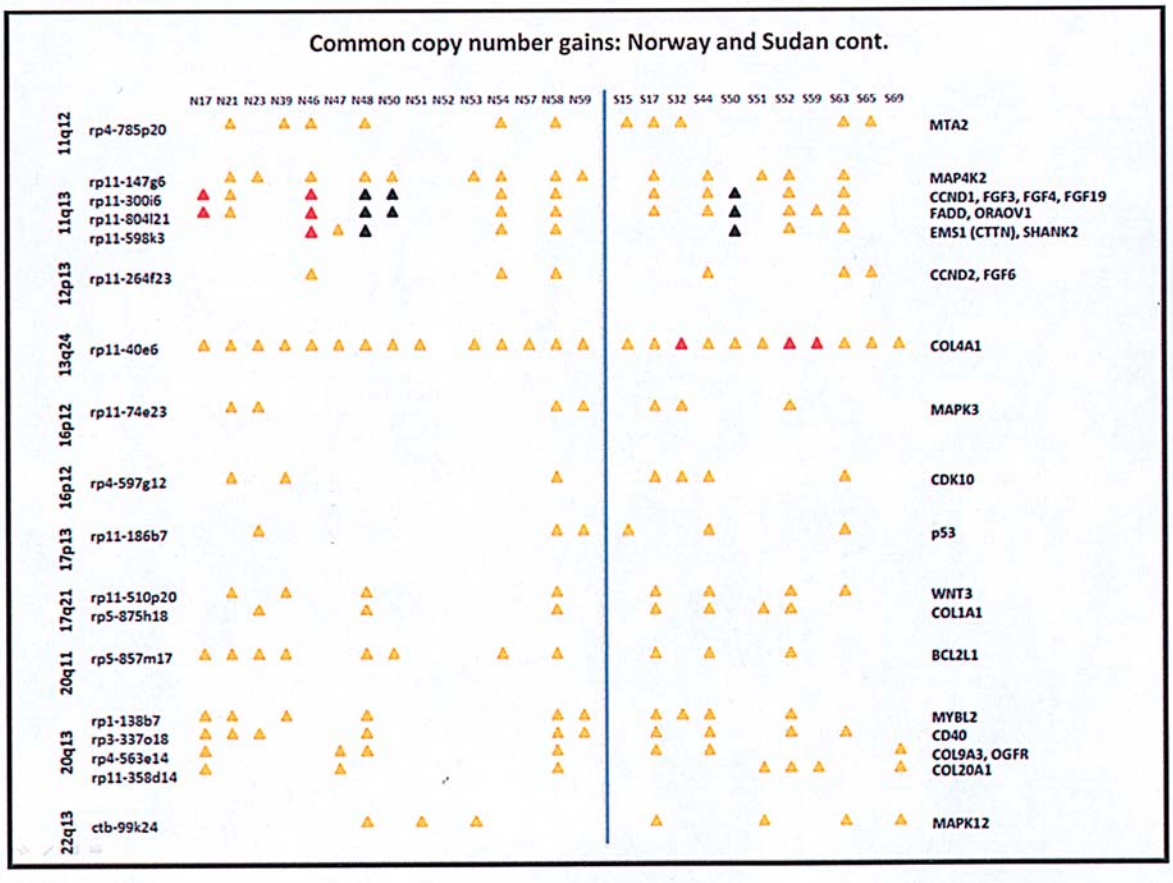
rp1-80k22
rp11-172m18
MYC
COL22A1

B


Common copy number gains: Norway and Sudan cont.
N17 N21 N23 N39 N46 N47 N48 N50 N51 N52 N53 N54 N57 N58 N59
S15 S17 S32 S44 S50 S51 S52 S59 S63 S65 S69
11q12
rp4-785p20
MTA2
11q13
rp11-147g6
rp11-300i6
rp11-804l21
rp11-598k3
MAP4K2
CCND1, FGF3, FGF4, FGF19
FADD, ORAOV1
EMS1 (CTTN), SHANK2
12p13
rp11-264f23
CCND2, FGF6
13q24
rp11-40e6
COL4A1
16p12
rp11-74e23
MAPK3
16p12
rp4-597g12
CDK10
17p13
rp11-186b7
p53
17q21
rp11-510p20
rp5-875h18
WNT3
COL1A1
20q11
rp5-857m17
BCL2L1
20q13
rp1-138b7
rp3-337o18
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COL20A1
22q13
ctb-99k24
MAPK12

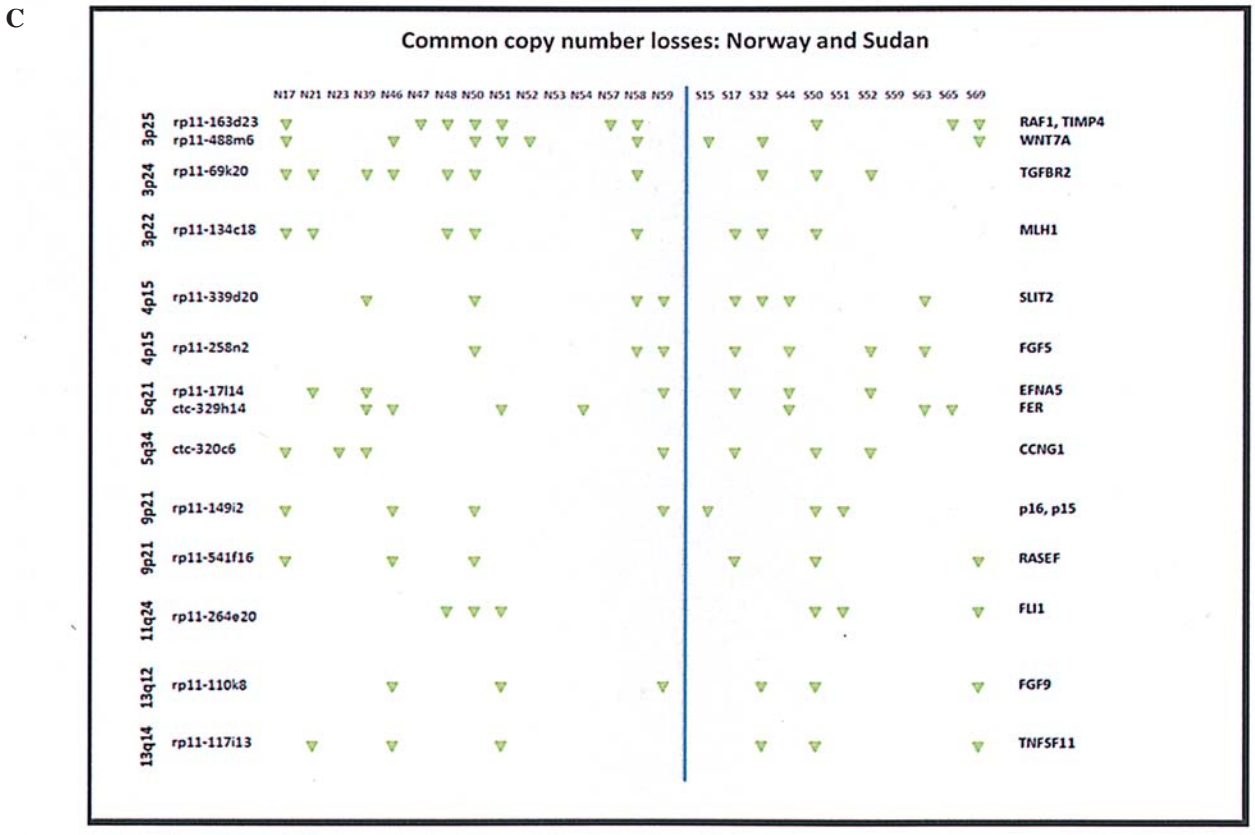

Figure 3. Common copy number alterations in HNSCC samples from Norway and Sudan; (A and B) gains and (C) losses. Triangles: yellow, 1-fold copy number increase; red, 2-fold copy number increases; black, 3-fold copy number increases and green, 1-fold copy number decrease.

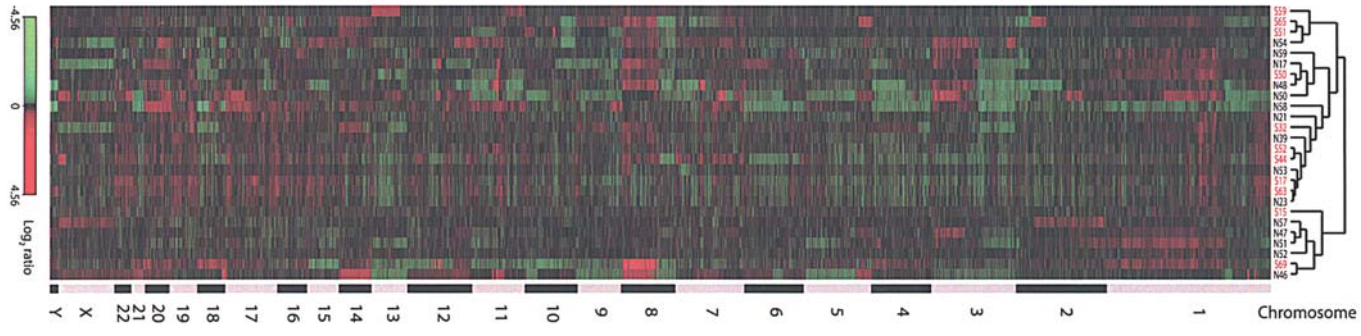

Figure 4. Hierarchical clustering of the chromosomal gains and losses found in the tumors examined from patients from Sudan (red numerals) and Norway (black numerals).

were found as common for the samples examined from the two countries. Although the degree of copy number increase/decrease varied by ±3-4-fold on the $\log_2$ scale between the two populations, the alterations seemed to affect the same chromosomal regions. Affected genes were involved in cell communication, MAPK signaling, cell cycle, apoptosis, Jak-STAT signaling, as well as DNA repair.

Of interest was the detection of amplification of 13q24 in 100% of the Sudanese and 93.3% of the Norwegian samples. This region contains the gene *COL4A1*, which is a basic structural component of all basement membranes and has previously been reported in the HNSCCs (20-23). The resulting amplification of this gene may be one of the important factors contributing to structural disintegration of the basement membrane in cells undergoing a dysplastic formation.

Several previous studies have shown overexpression or mutation of the *EGFR* gene in a significant number of cancers, including different subtypes of HNSCCs. The 7p12 region containing this gene was found amplified in 36.3 and 33.3% of Sudanese and Norwegian samples, respectively. Overexpression of *EGFR* and *MYC* (found to be amplified in 100%

Table III. Distribution (%) of the chromosomal amplifications/deletions and the corresponding biological pathways.

Pathway groups	Amplifications	Deletions	Total no (%) with alterations
Cell communication			
Sudan	4 (4%)	6 (10%)	10 (14%)
Norway	0	1 (2%)	1 (2%)
Total	4 (3%)	0	4 (3%)
MAPK signaling			
Sudan	6 (6%)	0	6 (6%)
Norway	5 (15%)	6 (9%)	11 (24%)
Total	17 (11%)	4 (14%)	21 (25%)
Calcium signaling, cytokine-cytokine receptor interaction			
Sudan	9 (9%)	6 (10%)	15 (19%)
Norway	3 (10%)	10 (15%)	13 (25%)
Total	24 (16%)	3 (11%)	27 (27%)
Cell cycle			
Sudan	2 (2%)	3 (5%)	5 (7%)
Norway	2 (6%)	3 (4%)	5 (10%)
Total	8 (5%)	0	8 (5%)
Wnt signaling			
Sudan	3 (3%)	0	3 (3%)
Norway	0	3 (4%)	3 (4%)
Total	7 (5%)	3 (11%)	10 (16%)
Focal adhesion, ECM-receptor interaction, cell adhesion			
Sudan	6 (6%)	8 (12%)	14 (18%)
Norway	7 (21%)	10 (15%)	17 (36%)
Total	22 (15%)	2 (7%)	24 (22%)
Gap junction, tight junctions, adherens-junction			
Sudan	7 (7%)	2 (3%)	9 (10%)
Norway	1 (3%)	3 (4%)	4 (7%)
Total	5 (3%)	3 (11%)	8 (14%)
Jak-STAT signaling			
Sudan	2 (2%)	3 (5%)	5 (7%)
Norway	2 (6%)	9 (13%)	11 (19%)
Total	17 (11%)	1 (4%)	18 (15%)
Regulation of actin cytoskeleton			
Sudan	2 (2%)	3 (5%)	5 (7%)
Norway	4 (12%)	5 (7%)	9 (19%)
Total	9 (6%)	3 (11%)	12 (17%)

of the Sudanese and 46.7% of the Norwegian samples in our experiment), represent common genetic alterations in oral-esophageal cancers (24,25). The genes regulate multiple biological processes, including cell proliferation, differentiation and growth. The cell cycle gene *p15*^{INK4a}, which interacts with *CDK4*/*CDK6* and negatively regulates the proliferation of normal cells and *p16*^{INK4b}, which potentially affects TGF-ß-induced cell cycle arrest and inhibits *CDK4* and *CDK6*, both mapped to BAC RP11-149I2 at 9p21, which was found deleted in nearly 27% of the samples from the two populations.

Of interest was the finding of the copy number increase of 1q32 harboring the *MDM4* gene, which plays a key role as a

Table IV. Distribution of the common significant gains and losses in the HNSCCs examined from Sudan (n=11) and Norway (n=15).

A, Significant gains

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
CTB-14E10	1p36	1,136,569-1,139,381	TNFRSF4/Tumor necrosis factor receptor superfamily member 4 precursor	5 (33.3)	3 (27.3)	8 (30.8)
RP4-785P20	1p36.3	3,558,944-3,642,625	TP73/Tumor protein p73	5 (33.3)	8 (72.7)	13 (50.0)
RP4-540O3	1p36.2-p36.12	18,830,087-18,935,219	PAX7/Paired box protein	4 (26.7)	5 (45.5)	9 (34.6)
RP1-159A19	1p36.11-1p35	27,321,849-28,321,848	MAP3K6/Mitogen-activated protein 3 kinase 6, FCN3/Ficolin collagen/fibrinogen domain, FGR/Gardner-Rasheed feline sarcoma viral oncogene homolog, RPA2/Replication protein A2	7 (46.7)	6 (54.5)	13 (50.0)
RP4-790G17	1q21	148,747,162-148,818,760	ECM1/Extracellular matrix protein MCL1/Induced myeloid leukemia cell differentiation protein	14 (26.7)	3 (27.3)	7 (26.9)
RP11-235D19	1q21	149,048,813-149,115,837	ARNT/Aryl hydrocarbon receptor nuclear translocator	6 (40.0)	2 (18.2)	8 (30.8)
RP1-20N18	1q21	151,147,645-151,150,986	IVL/Involucrin	2 (13.3)	5 (45.5)	7 (26.9)
RP1-148L21	1q21-q21.3	151,867,026-151,910,148	S100A1-14/Calcium binding protein A1-A14 ILF2/Interleukin enhancer-binding factor 2	7 (46.7)	8 (72.7)	15 (57.7)
RP11-350G8	1q21.1-q21.2	151,190,742-151,413,547	IL6R/Interleukin 6 receptor ADAR/Double-stranded RNA-specific adenosine deaminase	4 (26.7)	3 (27.3)	7 (26.9)
RP11-307C12	1q21.3	151,836,835-151,848,325	ADAM15/A disintegrin and metalloproteinase domain 15	4 (26.7)	5 (45.5)	9 (34.6)
RP11-101O6	1q21.3	152,844,039-152,853,366	RAB25/Ras-related protein	5 (33.3)	3 (27.3)	8 (30.8)
RP11-336K24	1q21.2-q23	152,791,913-152,922,944	LMNA/Lamin A/C SSR2/Signal sequence receptor (ß)	5 (33.3)	5 (45.5)	10 (38.5)
RP11-443G18	1q21-q23.1	153,524,975-153,460,262	HDGF/Hepatoma-derived growth factor NES/Nestin	7 (46.7)	6 (54.5)	13 (50.0)

Table IVA. Continued.

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
RP11-98G7	1q21-q22	153,643,800-153,664,715	NTRK1/Neurotrophic tyrosine kinase, receptor, type 1	8 (53.3)	5 (45.5)	13 (50.0)
RP11-444M10	1q22-q23	155,037,000-155,058,903	CD1A/T-cell surface glycoprotein CD1a precursor	4 (26.7)	4 (36.4)	8 (30.8)
RP11-312J18	1q21.2-q21.3	157,779,057-157,821,807	F11R/Junctional adhesion molecule A precursor, USF1/Upstream stimulatory factor 1	6 (40.0)	6 (54.5)	12 (46.2)
RP11-38C18	1q23	161,260,595-161,551,235	PBX1/Pre-B-cell leukemia transcription factor 1	4 (26.7)	3 (27.3)	7 (26.9)
RP11-104L21	1q24.1	163,753,740-163,791,526	GPA33/Cell surface A33 antigen precursor	5 (33.3)	3 (27.3)	8 (30.8)
RP4-747L4	1q23-q25	165,241,661-165,244,880	XCL2/Cytokine SCM-1 ß precursor	3 (20.0)	5 (45.5)	8 (30.8)
RP1-117P20	1q23-q25	166,391,466-166,412,385	SELL/Leukocyte adhesion molecule 1	8 (53.3)	4 (36.4)	12 (46.2)
RP1-106H8	1q23	169,359,842-169,367,670	FASLG/Tumor necrosis factor ligand superfamily member 6	3 (20.0)	3 (27.3)	6 (23.1)
RP11-739N20	1q32	201,217,223-201,273,492	MDM4/p53-binding protein Mdm4 KISS1/Metastasis-suppressor KiSS-1 Precursor	4 (26.7)	3 (27.3)	7 (26.9)
RP11-534L20	1q32.1	203,069,274-203,151,002	RASSF5/Ras association domain family 5 (isoform B), IL10, IL19, IL20, IL24/ Interleukin 10, 19, 20, 24	6 (40.0)	6 (54.5)	12 (46,2)
RP11-328L16	2p23	29,327,293-30,056,083	ALK/Anaplastic lymphoma kinase	3 (20.0)	4 (36.4)	7 (26.9)
RP5-1087L12	3q26	197,264,466-197,297,256	TNK2/Tyrosine kinase non-receptor protein 2 TRFC/Transferrin receptor protein 1	6 (40.0)	3 (27.3)	9 (34.6)
RP11-175A4	6p21.3	33,044,415-33,057,059	BRD2/Bromodomain-containing protein 2 HLA-DOA/HLA class II histocompatibility antigen COL11A2/Collagen, type XI, α 1 DAXX/Death domain-associated protein 6 BAK1/Bcl-2 homologous antagonist/killer	4 (26.7)	3 (27.3)	7 (26.9)
RP3-431A14	6p21.2	36,754,465-36,763,087	CDKN1A (p21)/Cyclin-dependent kinase inhibitor 1 (p21), PIM1/Proto-oncogene serine/threonine-protein kinase	6 (40.0)	4 (36.4)	10 (38.5)

Table IVA. Continued.

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
RP11-227E22	6p12	43,845,924-43,862,194	VEGF/Vascular endothelial growth factor A precursor, HSPCB/Heat shock protein HSP 90-ß	3 (20.0)	3 (27.3)	6 (23.1)
RP5-1091E12	7p12	54,860,934-55,049,240	EGFR/Epidermal growth factor receptor precursor	5 (33.3)	4 (36.4)	9 (34.6)
RP11-429B10	7q11.23	75,043,493-75,046,225	CCL26/Small inducible cytokine A26 precursor	10 (66.7)	5 (45.5)	15 (57.7)
RP11-350N15	8p12	38,153,263-38,189,968	BAG4/BCL2-associated athanogene 4 STAR/Steroidogenic acute regulatory protein FGFR1/Basic fibroblast growth factor receptor 1 precursor	5 (33.3)	3 (27.3)	8 (30.8)
RP11-197I11	8q11.23	53,185,952-53,484,856	ST18/Suppression of tumorigenicity 18	3 (20.0)	3 (27.3)	6 (23.1)
RP11-446E9	8q13	56,954,926-57,086,493	LYN/v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	3 (20.0)	3 (27.3)	6 (23.1)
RP11-27N21	8q21	81,110,304-81,246,425	TPD52/Tumor protein D52	5 (33.3)	6 (54.5)	11 (42.3)
RP11-419l20	8q23	110,621,105-110,647,393	EBAG9/Cancer associated surface antigen RCAS1	4 (26.7)	3 (27.3)	7 (26.9)
RP11-532M24	8p22	125,632,212-125,809,840	MTSS1/Metastasis suppressor protein 1	6 (40.0)	8 (72.7)	14 (53.8)
RP1-80K22	8q24.12-q24.13	128,817,686-128,822,856	MYC/Myc proto-oncogene protein	7 (46.7)	11 (100.0)	18 (79.2)
RP6-98A24	8q24.1-q24.3	134,272,494-134,310,753	WISP1/WNT1 inducible signaling pathway protein 1	8 (53.3)	8 (72.7)	16 (61.5)
RP11-172M18	8q24.23	139,669,661-139,995,418	COL22A1/Collagen, type XXII, α 1	4 (26.7)	4 (36.4)	8 (30.8)
RP11-381K7	10q24	112,618,107-112,649,754	PDCD4/Programmed cell death 4 isoform 1	12 (80.0)	11 (100.0)	23 (88.5)
RP3-466A11	11q13	61,488,614-61,491,679	FTH1/Ferritin heavy chain	4 (26.7)	4 (36.4)	8 (30.8)
RP11-163K24	11q12-q13.1	62,117,251-62,125,879	MTA2/Metastasis-associated protein	6 (40.0)	5 (45.5)	11 (42.3)
RP11-424O11	11q13	63,758,842-63,762,835	VEGFB/Vascular endothelial growth factor B precursor, BAD/ Bcl2-antagonist of cell death	4 (26.7)	4 (36.4)	8 (30.8)
RP11-147G6	11q13	64,313,186-64,327,289	MAP4K2/Mitogen-activated protein 4 kinase 2	9 (60.0)	5 (45.5)	14 (53.8)

Table IVA. Continued.

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
RP11-300I6	11q13	69,165,054-69,178,423	CCND1/G1/S-specific cyclin D1 FGF3, FGF4, FGF19/Fibroblast growth factor 3, 4, 19	7 (46.7)	5 (45.5)	12 (46.2)
RP11-804L21	11q13.3	69,726,917-69,731,144	FADD/FAS-associating death domain-containing protein, ORAOV1/Oral cancer overexpressed protein 1	13 (50.0)	7 (46.7)	6 (54.5)
RP11-598K3	11q13	69,922,292-69,960,338	EMS1 (CTTN)/Oncogene EMS1 SHANK2/ SH3 and multiple ankyrin repeat domains protein 2	5 (33.3)	3 (27.3)	8 (30.8)
RP11-750P5	11q22	101,693,404-101,713,675	BIRC3, BIRC2/Inhibitor of apoptosis protein 1, MMP7, MMP20, MMP8, MMP10, MMP1, MMP3, MMP12, MMP13/Matrix metallopeptidase 7, 20, 8, 10, 1, 3, 12, 13	3 (20.0)	3 (27.3)	6 (23.1)
RP11-264F23	12p13	4,253,199-4,284,777	CCND2/G1/S-specific cyclin D2 FGF6/Fibroblast growth factor 6 precursor	3 (20.0)	3 (27.3)	6 (23.1)
RP3-467F14	12p13	6,363,589-6,370,995	LTBR/Tumor necrosis factor receptor superfamily member 3 precursor	3 (20.0)	4 (36.4)	7 (26.9)
RP11-437A15	12q13	47,658,503-47,662,746	WNT1/Wnt-1 proto-oncogene protein precursor	7 (46.7)	11 (42.3)	4 (36.4)
RP11-95G6	13q12.3	27,434,279-27,441,317	CDX2/Homeobox protein CDX-2, FLT1/ Fms-related tyrosine kinase 1	8 (53.3)	17 (65.4)	9 (81.8)
RP11-40E6	13q34	109,599,312-109,757,459	COL4A1/Collagen α 1(IV) chain precursor	14 (93.3)	11 (100.0)	22 (84.6)
RP11-74E23	16p12-p11.2	30,032,927-30,042,042	MAPK3/Mitogen-activated protein kinase 3	4 (26.7)	3 (27.3)	7 (26.9)
RP4-597G12	16q24	88,280,579-88,290,263	CDK10/Cell division protein kinase 10	3 (20.0)	4 (36.4)	7 (26.9)
RP11-433M14	17p13.3	1,272,199-1,306,294	CRK/Proto-oncogene C-crk (P38)	4 (26.7)	3 (36.4)	7 (26.9)
RP11-459C13	17p13.2	3,710,358-3,743,086	CAMKK1/Calcium/calmodulin-dependent protein kinase kinase 1	6 (40.0)	6 (54.5)	12 (46.2)
RP11-186B7	17p13.1	7,512,464 - 7,531,642	TP53/Tumor protein p53	3 (20.0)	3 (27.3)	6 (23.1)

Table IVA. Continued.

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
RP11-436J4	17q12-q21	39,233,695-39,266,064	MPP3/Membrane protein, palmitoylated 3	3 (20.0)	3 (27.3)	6 (23.1)
RP11-510P20	17q21	42,196,860-42,251,081	WNT3/Wnt-3 proto-oncogene protein precursor	4 (26.7)	4 (36.4)	8 (30.8)
RP5-875H18	17q21.3-q22.1	45,616,456-45,633,992	COL1A1/Collagen α 1(I) chain precursor	3 (20.0)	4 (36.4)	7 (26.9)
RP11-475F12	17q25	77,629,503-77,649,395	FASN/ Fatty acid synthase CD7/T-cell antigen CD7 precursor (p41)	5 (33.3)	6 (54.5)	11 (42.3)
RP11-330I7	19p13.3	6,482,037-6,486,933	TNFSF9/Tumor necrosis factor ligand superfamily member 9, VAV1/Vav proto-oncogene	4 (26.7)	3 (27.3)	7 (26.9)
RP11-197O4	19p13.2	10,538,139-10,540,655	ICAM1, ICAM3, ICAM4, ICAM5/Intercellular adhesion molecule 1, 3, 4, 5 precursor, CDKN2D/Cyclin-dependent kinase 4 inhibitor D (p19-INK4d)	4 (26.7)	10 (38.5)	6 (54.5)
CTC-539A10	19p13.2	10,625,988-10,664,095	ILF3/Interleukin enhancer-binding factor 3	5 (33.3)	4 (36.4)	9 (34.6)
RP11-285H8	19p13.12	14,003,262-14,025,026	IL27RA/Interleukin-27 receptor α chain precursor	3 (20.0)	3 (27.3)	6 (23.1)
CTC-251H24	19p13.11	18,251,570-18,253,468	IL12RB1/Interleukin-12 receptor ß-1 chain precursor, JUND/Jun D proto-oncogene ELL/RNA polymerase II elongation factor	6 (40.0)	7 (63.6)	13 (50.0)
RP11-38C1	19q13.12	40,511,930-40,530,104	CD22/B-cell receptor CD22 precursor	6 (23.1)	3 (20.0)	3 (27.3)
CTC-246B18	19q13.2	44,478,805-44,481,152	IL28A/Interleukin-28A precursor, IL29/Interleukin-29 precursor	5 (33.3)	9 (34.6)	4 (36.4)
RP11-537N4	19q13.2	45,973,218-45,975,236	MIA/Melanoma derived growth regulatory protein precursor	6 (40.0)	10 (38.5)	4 (36.4)
RP3-324O17	20q11.21	29,656,753-29,657,974	ID1/DNA-binding protein inhibitor ID-1	8 (53.3)	5 (45.5)	13 (50.0)
RP5-857M17	20q11.21	29,715,924-29,774,317	BCL2L1/Apoptosis regulator Bcl-X	8 (53.3)	3 (27.3)	11 (42.3)
RP1-138B7	20q13.12	41,729,123-41,778,536	MYBL2/V-myb myeloblastosis viral oncogene homolog (avian)-like 2	6 (40.0)	4 (36.4)	10 (38.5)

Table IVA. Continued.

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
RP3-337O18	20q13.12	44,180,313-44,191,339	CD40/Tumor necrosis factor receptor superfamily member 5 precursor	6 (40.0)	4 (36.4)	10 (38.5)
RP5-1005L2	20q13.12	44,746,412-44,751,683	MMP9/Matrix metalloproteinase-9 precursor, TP53RK/p53-related protein kinase	4 (26.7)	6 (54.5)	10 (38.5)
RP4-563E14	20q13.33	60,918,832-60,942,955	COL9A3/Collagen α-3(IX) chain precursor, OGFR/Opioid growth factor receptor	4 (26.7)	3 (27.3)	7 (26.9)
RP11-358D14	20q13.33	61,394,983-61,436,593	COL20A1/Collagen α-1(XX) chain precursor	3 (20.0)	4 (36.4)	7 (26.9)
XX-P273A17	22q11.1	15,940,412-15,965,959	IL17R/Interleukin-17 receptor precursor	4 (26.7)	7 (26.9)	3 (27.3)
RP4-539M6	22q12.2	28,960,995-28,967,302	LIF/Leukemia inhibitory factor precursor OSM/Oncostatin M precursor	3 (20.0)	9 (34.6)	6 (54.5)
XXBAC-677F7	22q12.3	31,521,356-31,583,581	TIMP3/Metalloproteinase inhibitor 3 precursor	3 (20.0)	4 (36.4)	7 (26.9)
LL22NC01-132D12	22q13.1	35,846,382-35,870,462	IL2RB/ Interleukin-2 receptor ß chain precursor	4 (26.7)	5 (45.5)	9 (34.6)
CTA-228A9	22q13.1	36,922,498-36,937,014	MAFF/v-maf musculoaponeurotic fibrosarcoma oncogene homolog	3 (20.0)	5 (45.5)	8 (30.8)
RP5-979N1	22q13.2	39,812,290-39,900,578	EP300/E1A-associated protein p300	5 (33.3)	7 (63.6)	12 (46.2)
CTB-99K24	22q13.33	48,993,788-49,002,704	MAPK12/Mitogen-activated protein kinase p38 γ	3 (20.0)	4 (36.4)	7 (26.9)
B, Significant losses						
RP11-272E3	2q24.3	165,174,840-165,303,867	GRB14/Growth factor receptor-bound protein 14	4 (26.7)	8 (30.8)	4 (36.4)
RP11-10H6	3p26.2	3,086,421-3,127,031	IL5RA/Interleukin-5 receptor α chain precursor	6 (40.0)	3 (27.3)	9 (34.6)
RP11-163D23	3p25.2	12,600,108-12,680,614	RAF1/RAF proto-oncogene serine/threonine-protein kinase TIMP4/Metalloproteinase inhibitor 4 precursor	7 (46.7)	3 (27.3)	10 (38.5)
RP11-488M6	3p25.1	13,835,083-13,896,619	WNT7A/Wingless-type MMTV integration site family, member 7A	6 (40.0)	3 (27.3)	9 (34.6)

Table IVB. Continued.

Clone ID	Chromosome region	Basepair position	Gene symbol/name	Norway no. (%)	Sudan no. (%)	Total no. (%)
RP11-69K20	3p24.1	30,622,998-30,710,628	TGFBR2/TGF-ß receptor type II precursor	7 (46.7)	3 (27.3)	10 (38.5)
RP11-134C18	3p22.3	37,009,983-37,067,341	MLH1/DNA mismatch repair protein Mlh1	5 (33.3)	3 (27.3)	8 (30.8)
RP11-80H18	3p14.3	58,465,904-58,497,948	ACOX2/Acyl-coenzyme A oxidase 2	6 (40.0)	3 (27.3)	9 (34.6)
RP11-91B3	3q13.11	106,568,450-106,778,433	ALCAM/Activated leukocyte-cell adhesion molecule	4 (26.7)	5 (45.5)	9 (34.6)
RP11-339D20	4p15.31	19,931,152-20,297,057	SLIT2/Slit homolog 2 protein precursor	4 (26.7)	4 (36.4)	8 (30.8)
RP11-258N2	4q21.21	81,545,008-81,569,338	FGF5/Fibroblast growth factor 5 precursor	3 (20.0)	4 (36.4)	7 (26.9)
RP11-17L14	5q21.3	106,744,250-107,034,495	EFNA5/Ephrin-A5 precursor	3 (20.0)	3 (27.3)	6 (23.1)
CTC-329H14	5q21.3	108,111,422-108,551,272	FER/Proto-oncogene tyrosine-protein kinase	4 (26.7)	3 (27.3)	7 (26.9)
CTC-320C6	5q34	162,797,155-162,804,600	CCNG1/Cyclin-G1	5 (33.3)	3 (27.3)	8 (30.8)
CTB-114A6	7q31.2	116,511,233-116,557,294	WNT2/Wingless-type MMTV integration site family member 2	6 (40.0)	3 (27.3)	9 (34.6)
CTD-2629I16	8p23.1	6,347,601-6,408,172	ANGPT2/Angiopoietin-2 precursor	4 (26.7)	7 (26.9)	3 (27.3)
RP11-113D19	9p21.3	21,430,440-21,431,315	IFNA/Interferon α IFNB/Interferon ß precursor	5 (33.3)	5 (45.5)	10 (38.5)
RP11-149I2	9p21.3	21,957,751-21,984,490	CDKN2A (p16)/Cyclin-dependent kinase inhibitor 2A, CDKN2B (p15)/ Cyclin-dependent kinase inhibitor 2B	7 (26.9)	4 (26.7)	3 (27.3)
RP11-541F16	9q21.32	82,826,877-82,907,646	RASEF/RAS and EF hand domain containing	3 (20.0)	3 (27.3)	6 (23.1)
RP11-556E13	10q21.1	53,744,064-53,747,152	DKK1/Dickkopf-related protein 1 precursor	3 (20.0)	4 (36.4)	7 (26.9)
RP11-309J20	11p15.4	4,966,000-4,970,235	MMP26/Matrix metalloproteinase-26 precursor	3 (20.0)	5 (45.5)	8 (30.8)
RP11-264E20	11q24.3	128,069,199-128,187,521	FLI1/Fli-1 proto-oncogene	3 (20.0)	6 (23.1)	3 (27.3)
RP11-110K8	13q12.11	21,143,875-21,174,187	FGF9/Fibroblast growth factor 9	3 (20.0)	3 (27.3)	6 (23.1)
RP11-117I13	13q14.11	42,034,872-42,080,148	TNFSF11/Tumor necrosis factor ligand superfamily member 11	6 (23.1)	3 (20.0)	3 (27.3)
RP11-564N10	13q33.1	100,902,967-101,166,795	ITGBL1/Integrin, ß-like 1	3 (20.0)	3 (27.3)	6 (23.1)
RP11-397A16	18q21.2	51,045,967-51,406,858	TCF4/Transcription factor 4	3 (20.0)	3 (27.3)	6 (23.1)

negative regulator of *p53* (43). Although a number of various human cancers show amplification and overexpression of *MDM4*, the exact mechanism of the negative regulation of *p53* by the mdm4 protein and the contribution of this to tumor formation, remains uncertain (44). We found a predominant copy number increase of the chromosomal region hosting *p53*, which has been previously reported as mutated and over-expressed in HNSCCs examined from Sudan and Norway (18,19).

We detected the amplification of 1q21, harboring the calcium-binding protein genes *S100A1-A14*, in 72% of Sudanese and 46% of Norwegian samples. These findings are in agreement with previous studies showing potential involvement of the *S100A* gene family in OSCCs examined from Sudan and Norway (31,32). An interaction between *S100A1* and *-A4* has previously been studied with the suggestion that they may be involved in cancer invasion and metastasis (33,34). The *S100A*2 gene, has previously been reported as a potential marker in early-stage patients with oral cancer and was shown to interact with *p53*, thereby modulating its activity (35,36).

Loss of heterozygosity (LOH) and frequent chromosomal rearrangements at 11q in HNSCC have been previously described (37,38). Of interest, is the finding that the amplification of 11q13, containing among others *CCND1*, registered on average in 30.3% of all samples. This chromosomal region was found to be of key importance in the early events of tumor development and progression by proto-oncogene activation, and was recently reported to be gained in another array CGH study of HNSCCs (26). Amplification of *CCND1* has been reported in bladder, gastric, breast and pancreatic cancers, suggesting its role as a predictive biomarker (27-30).

In head and neck cancers, high-level amplification and/or overexpression of *CCND1* has been suggested to enhance tumor growth, aggressive development and poor patient prognosis (13,39,40). The 11q13 amplicon hosts in addition to *CCND1*, *EMS1* and *SHANK2* two important growth factors, *FGF3* and *FGF4*. These genes seem to play an important role in driving the 11q13 amplification and show good correlation between the amplification status and over-expression (41).

Amplification of 11q22 were found in 27% of the Sudanese and 20% of the Norwegian samples and this finding seems to be consistent with previous reports in a genome-wide analysis of HNSCCs by array CGH (42). The region hosts two genes that function as inhibitors of apoptosis, *BIRC2* and *BIRC3*, as well as eight members of a rich family of matrix metallo-peptidases (MMPs) that play an important role in cell-cell interaction, remodeling and degradation of extra-cellular matrix (*MMP1*, *MMP3*, *MMP7*, *MMP8*, *MMP10*, *MMP12*, *MMP13* and *MMP20*). Previous studies have shown that protelytic/enzymatic properties of *MMPs* are essential for a tumor's ability to initiate the degradation of ECM and to access and establish new blood and lymphatic pathways in order to invade new regions and metastasize to remote organs (45). The status of the copy number increase of the MMPs found in our study, along with amplification of chromosomal regions hosting genes involved in cell apoptosis, such as *BAD*, *BCL2L1*, *FADD*, *NTRK1*, *p53*, *BIRC2*, *BIRC3* and *PDCD4*, may have the potential as a predictor of invasiveness and metastatic ability of HNSCCs and thereby determining patients survival prognosis (23,46-48). Since *MMPs* are related to ECM degradation and genes for several collagens, which are important constituents of ECM, were found to be amplified in this study the possible interaction between *MMPs* and collagens warrants further studies.

To conclude, the results presented here provide valuable information on common chromosomal alterations possibly involved in the carcinogenesis of oral cancers. The profile of common chromosomal aberrations in the HNSCCs investigated, of which 149 genes were found located in 41 amplified regions and 28 genes in 22 deleted regions, suggests the existence of similar gene-specific alterations in the patients examined from Sudan and Norway, regardless of differences related to ethnicity and social-cultural risk factors. Hierarchical clustering of the chromosomal gains and losses found in the tumors against clinicopathological parameters showed little correlation, suggesting the occurrence of these tumors regardless of ethnic differences and clinicopathological status between the patients from the two countries.

Furthermore, cytokine-cytokine receptor interaction (calcium signaling), MAPK signaling and ECM-receptor interaction/focal adhesion/cell adhesion were found as important biological pathways involved in the pathogenesis of the cancers examined. High-level amplification of collagens, *MMPs* and *S100A* gene family members observed in the HNSCCs investigated, may provide valuable information that can be used for understanding the pathogenesis of the disease. Further studies are therefore necessary to elaborate on the role(s) of these gene family markers in HNSCCs.

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