

# Array comparative genomic hybridization analysis of olfactory neuroblastoma

Mohamed Guled<sup>1</sup>, Samuel Myllykangas<sup>1</sup>, Henry F Frierson Jr<sup>2</sup>, Stacey E Mills<sup>2</sup>, Sakari Knuutila<sup>1</sup> and Edward B Stelow<sup>2</sup>

<sup>1</sup>Department of Pathology, Haartman Institute and HUSLAB, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland and <sup>2</sup>Department of Pathology, University of Virginia Health System, Charlottesville, VA, USA

Olfactory neuroblastoma is an unusual neuroectodermal malignancy, which is thought to arise at the olfactory membrane of the sinonasal tract. Due to its rarity, little is understood regarding its molecular and cytogenetic abnormalities. The aim of the current study is to identify specific DNA copy number changes in olfactory neuroblastoma. Thirteen dissected tissue samples were analyzed using array comparative genomic hybridization. Our results show that gene copy number profiles of olfactory neuroblastoma samples are complex. The most frequent changes included gains at 7q11.22–q21.11, 9p13.3, 13q, 20p/q, and Xp/q, and losses at 2q31.1, 2q33.3, 2q37.1, 6q16.3, 6q21.33, 6q22.1, 22q11.23, 22q12.1, and Xp/q. Gains were more frequent than losses, and high-stage tumors showed more alterations than low-stage olfactory neuroblastoma. Frequent changes in high-stage tumors were gains at 13q14.2–q14.3, 13q31.1, and 20q11.21–q11.23, and loss of Xp21.1 (in 66% of cases). Gains at 5q35, 13q, and 20q, and losses at 2q31.1, 2q33.3, and 6q16–q22, were present in 50% of cases. The identified regions of gene copy number change have been implicated in a variety of tumors, especially carcinomas. In addition, our results indicate that gains in 20q and 13q may be important in the progression of this cancer, and that these regions possibly harbor genes with functional relevance in olfactory neuroblastoma.

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Olfactory neuroblastoma is rare, with an estimated incidence of 0.4 per million people per year. It accounts for approximately 2–3% of sinonasal tract tumors.<sup>1</sup> Olfactory neuroblastoma is a malignant neuroectodermal tumor, which is believed to originate at the olfactory membrane of the sinonasal tract.<sup>1</sup> Symptoms include unilateral nasal obstruction and epistaxis. The Kadish system classifies cases of olfactory neuroblastoma into three stages. Stage 1 consists of tumors confined to the nasal cavity; stage 2 tumors extend to the paranasal sinuses; and stage 3 neoplasms extend beyond the sinonasal cavities (some have added a stage 4 for those tumors with metastases). Patients with high-

stage tumors have a worse prognosis compared with those with low-stage tumors, as 5-year survival rates are 40 and 80%, respectively.<sup>2</sup>

Cytogenetic data for olfactory neuroblastoma are limited. The most recently published study was carried out by Holland *et al*,<sup>3</sup> who performed cytogenetic characterization of one case using trypsin Giemsa staining (GTG banding), multicolor fluorescence *in situ* hybridization (M-FISH), and single-nucleotide polymorphism karyotyping. They reported numerous chromosomal aberrations predominantly involving chromosomes 2q, 5, 6q, 17, 19, 21q, and 22, as well as trisomy 8. Bockmüehl *et al*<sup>4</sup> applied conventional comparative genomic hybridization (CGH) to 22 olfactory neuroblastomas and reported frequent deletions of 1p, 3p/q, 9p, and 10p/q, and amplifications of 17q, 17p13, 20p, and 22q. They also noted a specific deletion on chromosome 11 and gain on chromosome 1p, which were associated with metastasis and a worse prognosis. Three olfactory neuroblastomas were studied by Riazimand *et al*<sup>5</sup> using conventional CGH, and amplification of whole chromosome 19, partial gains of 1p, 8q, 15q, and 22q, and deletions of 4q and 6p

Correspondence: Dr EB Stelow, MD, Department of Pathology, University of Virginia Health Sciences, Box 800214, Jefferson Park Avenue, Charlottesville, VA 22908, USA.

E-mail: edstelow@yahoo.com and

Dr S Knuutila, PhD, Department of Pathology, Haartman Institute and HUSLAB, PO Box 21 (Haartmaninkatu 3), University of Helsinki, FI-00014 Helsinki, Finland.

E-mail: Sakari.Knuutila@helsinki.fi

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were detected. Szymas *et al*<sup>6</sup> studied a single olfactory neuroblastoma and found gains of whole chromosomes 4, 8, 11, and 14, partial gains of 1q and 17q, partial deletions of 5q and 17q, and whole chromosome losses of 16, 18, 19, and X. In our study, we applied for the first time an oligonucleotide-based array CGH (aCGH) to identify the most frequently occurring DNA copy number changes in 13 cases of olfactory neuroblastoma. We identified novel chromosomal regions that were frequently altered in addition to previously reported abnormal regions.

## Materials and methods

### Tumor Samples

The Surgical Pathology Database (Department of Pathology at the University of Virginia, Charlottesville, VA, USA) was searched for resected cases of olfactory neuroblastoma. Slides from formalin-fixed, paraffin-embedded tissues were reviewed, and 15 cases were selected based on the availability of abundant and well-preserved neoplastic tissue. Only cases with conventional histology, as described by Mills and Frierson,<sup>7</sup> were chosen (Figure 1). All cases exhibited a typical immunophenotype and were immunoreactive with antibodies to synaptophysin and not with antibodies to keratins. Areas representing cancer tissue were dissected from paraffin-embedded tumors using conventional light microscopy and scalpel blades. All 15 samples submitted for analysis were composed of at least 50% tumor cells. Each of six samples was classified as Kadish stage 2 and 3 (Figure 1), whereas information regarding the stage was not available for three tumors. Patients' age ranged from 27 to 62 years. The clinicopathological data for the patients and samples are summarized in Table 1.

### DNA Extraction and Digestion

Total DNA was extracted using QIAamp DNA mini kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany). DNA concentrations of the extracted samples were determined using the NanoDrop ND-1000 spectrophotometer (Nanodrop technologies, Wilmington, DE, USA) at 260/280 nm. The integrity of the extracted DNA was verified by agarose gel electrophoresis (data not shown). A 1.5 µg quantity of genomic DNA from test and reference samples was digested using *Alu1* and *Rsa1* restriction enzymes (Promega, Madison, WI, USA) for 2 h at 37°C. To inactivate the enzymes, the samples were incubated at 65°C for 20 min.

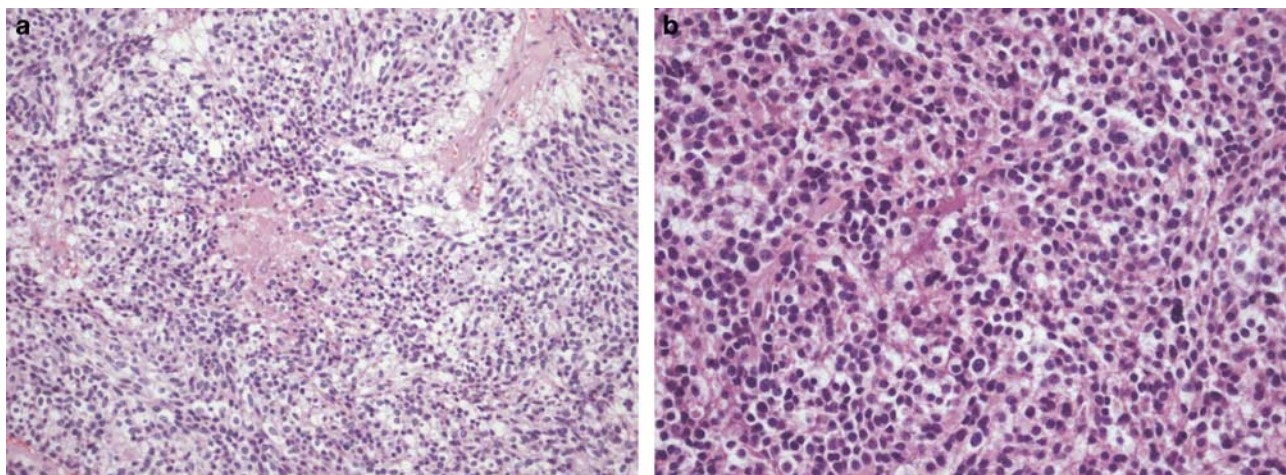
### Array CGH Experiments

Array CGH was performed for 13 samples that had good quality DNA with sufficient yields. For

**Table 1** Clinicopathological data for the samples

Sample	Sex/ age	Dead/ alive	Disease status	Time (months)	Stage (Kadish)
1	m/50	D	WD	156	2
2	m/49	A	NED	192	UNK
3	m/53	D	WD	20	3
4	f/38	A	UNK	136	UNK
5	f/40	A	UNK	149	2
6	f/27	A	NED	119	3
7	f/61	A	NED	106	3
8	m/27	A	NED	59	2
9	m/40	A	NED	57	3
12	f/62	D	WD	35	3
13	m/41	UNK	UNK	UNK	3
14	m/44	A	NED	24	UNK
15	m/60	A	NED	6	2

Abbreviations: A, alive; D, dead; NED, no evidence of disease; UNK, unknown; WD, with disease.



**Figure 1** Standard H&E sections of conventional olfactory neuroblastoma: (a) sample 11 (100 ×), (b) sample 12 (200 ×).

reference, pooled DNA extracted from buffy coat fractions of whole blood obtained from sex-matched, healthy individuals provided by the Finnish Red Cross was used. The Agilent genomic DNA labeling kit PLUS (Agilent Technologies, Santa Clara, CA, USA) was used to label 1.5 µg of digested genomic DNA. The DNA of olfactory neuroblastoma samples and the reference DNA were labeled with cyanine 5-dUTP and cyanine 3-dUTP fluorochromes, respectively, for 2 h at 37°C. For inactivation of the enzymes, the samples were incubated at 65°C for 10 min. Human cot-1 DNA (Invitrogen, Carlsbad, CA, USA) was added to the reactions for 3 min at 95°C and then for 30 min at 37°C. Prior to hybridization experiments, labeled DNA was cleaned according to Agilent's protocol. Labeled sample and reference DNA were hybridized on Agilent's 4 × 44K CGH microarray (product number G4426A and G4426B, Agilent Technologies) for 24 h at 65°C.

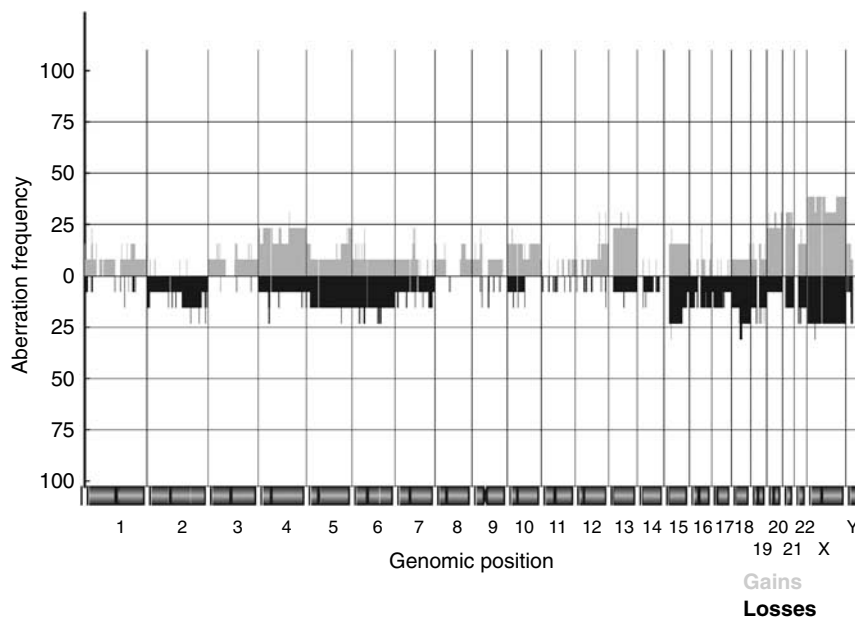
Prior to scanning, microarray slides were washed according to the manufacturer's instructions. The microarray slides were scanned using DNA Microarray Scanner and the scanned images were analyzed using the Feature Extraction Software (Agilent Technologies). Data were imported to the CGH Analytics Software 3.4 for analysis of individual cases. Z-score algorithm with threshold at 3.5 was used in the identification of copy number changes. To obtain a global view of the copy number alteration frequencies in olfactory neuroblastoma, we also analyzed the data using GeneSpring (Agilent Technologies) and CGH Explorer software 3.1.<sup>8</sup> The raw data from the Feature Extraction software were

imported into the GeneSpring software and normalized using the Feature Extraction data import plug-in (Agilent Technologies). Outlier probes marked by Feature Extraction were ignored in normalization. Sample spot intensities were divided by control channel intensities, and microarrays were normalized to the 50th percentile and probes to the median value. Outlier probes were filtered from further analysis. Normalized and filtered data were imported into the CGH Explorer software. Copy number aberrations were identified using Piecewise Constant Fit (PCF) algorithm. Default parameters were applied to detect long copy number aberrations. The significance threshold was set at 0.2.

## Results

### Gene Copy Number Profiling of Olfactory Neuroblastoma

Array CGH was applied to study gene copy number alterations in 13 olfactory neuroblastoma samples. The CGH Explorer software was used to analyze global frequencies of copy number change (Figure 2). A total of 15.74% of the probes were found aberrant when a threshold of 0.2 was used in the PCF analysis. DNA copy number gains (8.37%) were slightly more frequent than losses (7.36%). In addition, the CGH analytics software was used to determine copy number aberrations in individual samples. Copy number aberrations for each case are summarized in Table 2. Changes in the copy number of entire chromosomes (aneuploidy) or chromosome



**Figure 2** Frequency profile of copy number aberrations in 13 olfactory neuroblastoma samples. CGH Explorer software and Piecewise Constant Fit algorithm were used to determine copy number aberrations in olfactory neuroblastoma samples. The chromosomal alterations are shown in each probe position as incidence bar. Gains of genomic material are indicated in gray on the upper side of the middle line (at 0). Losses are indicated in black on the bottom side of the middle line. Genomic positions of the aCGH probes are marked on the x axis.

**Table 2** aCGH results for 13 ONB samples

ONB sample	Stage	Tumor DNA copy number changes
1	2	+1p31.2, +1p31.1, +1p35.3, +1q31.1q31.3, +2p24.3, -2p25.1p25.3, -2q36.3q37.3, +3p13.12p13.31, +4p, +4q, +5q23.1, +8q22.3q24.12, -9p21.1, -10p12.31, -11q14.1q14.3, -12q12, -16q13, -19p, -19q, -22q
2	UNK	+1p21.1p21.2, +1q31.3p32.1, -2q37.1, +3q25.33, +4p16.2p16.3, +4p12p15.31, +4q, +6p21.2, +9p23, +12p11.21p12.1, +12q23, +12q24.31, -12q12, +16q23.2q24.2, +17q11.2, -18p, -18q, -19p, -19q, +22q, +Xp, +Xq12
3	3	-2q37.2, +3q21.32p21.33, +3p21.31, +3q25.33, +4p, +4q12, +4q35.2, -5p, -5q, +6p22.3, -6q21, -7p, -7q11.21q21.2, -7q33q35, +8p23.2p23.3, +8p12, +10p11.21p15.3, +10q11.21q21.1, +10q23.1p26.2, +12q21.33q24.33, +13q31.1, +13q34, -14q32.33, -15q11.2q13.1, +16q24.2, -16p12.3p13.2, -16q22.1q24.2, -17p, -17q11.2q12.31, -18p, -18q, +19q13.12, +20q11.2111.25, +20q13.32q13.33, -22q
4	UNK	+1q44, -1q32.1, +2p25.3, +3q26.2, -3q26.2, -4q35.2, +5q34, -5q31.2, +6p12.3, -8q12.1, -9p13.1, -9q12q13, +11p13, -11p14.1, -11q21, +12q23.1, +14q23.3, -14q11.2, -15q11.2q25.3, +16p13.3, +16p11.2, -16p13.3, -16p12.1, +21q21.1q21.3
5	2	+1p35.2p35.3, +1p36.11, -1p36.11, +3p22.3, +3q26.2, -3p21.31, -3q26.2, +4q27, +9p24.3, +11p15.5, +11q13.1, +12q24.31, -12q14.3, -18q12.2q12.3, +13q34, +19p13.2
6	3	+1p34.1p36.33, +1p31.2p32.1, +1p21.2p22.3, +1q21.1q21.3, +1q22q24, +2q14.1, +2q21.1, -2q31.1, +3p14.1p26.3, +3q13.11q29, +4q22.1 to q21.22, -5p11p15.1, -5q, -6q12.3, -6q16.3, -6q21q22.1, +7q22.1, +8p, +8q22.1q24.3, +9p21.1, +9p15.2p13.3, -10q21.3, -11p15.4, -11p11.12p11.2, -11q11q12.2, -11q13, q23-2, +12p13.31p13.33, +12q12q13.12, +12q13.13q14.1, +12q14.2q23.1, +12q23.3q24.33, +13q14.2q14.3, -14q12q23.3, -14q31.1q31.3, -16p11.2, -16q11.2, +17q21.2, +17q24.2, +17q25.1, -17q24.3, -18q12.2q23, -19p12, -19q12, -20p, -20q, -22q11.21, -Xp, -Xq
7	3	-1q31.2, -6p, -6q, -7q31.1, -10q11.21, +12p13.31, -12q24.23q24.31, -12q24.31, +13q, +14q24.3, -15q, -16q12.1q21, -16q24.2, +17p, +17q, -19q13.32, +21q, -22q11.23, -Xp, -Xq
8	2	-1p36.22p36.33, -4p, -4q, -5p15.33, +6p22.1, +6p12.3, -6p22.1, -7p12.3p13, +9, +9q21.11q32.2, +9q33.3, +10p, +10q11.21q23.1, +10q23.3210q26.3, +11p13, -12q24.31, -13q, -14q12, -21q, -22q12.11, +Xp21.2p22.33, +Xp11.22p11.4, +Xq
9	3	+1p36.33, +1p36.31, +1p33, -1q23.3, -1q25.2, -2p11.2, -2q11.2, -2q22.1q35, -4p13p14, -4q34.1, +5p15.2p15.33, +5q35.1q35.3, -6p, -6q, +7p, +7q11.2q21.2, -7q21.3q36.3, +9p13.1, +9q12q13, +12p13.31p13.33, +12q24.33, -12q23.3, +13q, +14q12, +15q13.3, -15q14q24.1, -15q14q26.3, -16p, -16q, +18p, +18q, +19p, +19q, +20p, +20q, +21q, +22q, -Xp12.1
12	3	-1q23.3, -2p, -2q, -3q13.2, +5p, +5q, +6p, +6q, +7q36.1, +7q36.3, -9p13.1, -9q13, -10p, -10q11.21q21.3, +11q23.3, -12p13.33, +13q, -14q13.2, -14q24.3, +15q11.2q22.31, +15q22.32q26.33, +16q12.1q21, -17p, -17q, 18q25, +19p13.2, +19q12q13.11, +20p, +20q, -21q, -Xp, -Xq
13	3	+1q21.2, -2q33.3, +5q31.2q35.3, +7q11.23, +7q32.1q32.2, -7p21.3, +9p13.3, -9q21.33, -10p12.1p12.2, +11q12.2, +11q23.1q23.2, -11q23.2q23.3, +14q11.2, -17q21.33q22, +19q13.33, +19q13.43, +20p, +20q, +Xp, +20q
14	UNK	+1p36.11, -4q21.21, +2p22.3p23.1, +2q24.1, -12q13.2, -12q14.3q15, +16q12.1, +19p13.2, +22q12.1
15	2	-1p35.2p35.3, -1p11.2p12, -1q21.1, -1q31.3q32.1, -4p12p13, -6p21.33, +7q11.22q21.11, -9q31.3, -13q21.2, -17p13.1, +21q22.12q22.3, +22q, +Xp, +Xq

arms represented 16% of all alterations; interestingly, the most altered whole chromosomes were 20, 21, 22, and X, and almost 70% of the aberrations of these were whole chromosome losses or gains.

To obtain a more detailed overview of the most frequently occurring alterations, only those alterations shared by at least 20% of the cases were recorded. This approach identified an equal number of gained and lost chromosomal regions ( $n=21$ ). Tables 3 and 4 show the size of the altered areas and whether they have been reported in previous copy number change studies for olfactory neuroblastoma. The tables also include suggested target genes that are located at the sites of alteration and that may play a role in tumorigenesis.

**Specific Copy Number Alterations Were Identified in Cases of Olfactory Neuroblastoma of Different Stage**

A stage 3 olfactory neuroblastoma showed the most DNA copy number changes, with 59% of all alterations. Also, most aneuploidy occurred in high-stage tumors; 70% of whole-chromosome aberrations were identified in these tumors. For stage 3 tumors, the mean DNA copy number change per case was 28.5, whereas stage 2 olfactory neuroblastomas had a mean number of 17 changes per tumor. However, only 4 out of 6 stage 2 cases contained sufficient good quality DNA to perform aCGH.

To further characterize the genomic changes that occurred in a later-stage olfactory neuroblastoma,

**Table 3** DNA copy number gains in at least 20% of the cases detected by aCGH

Chromosome location	Start (kb)	Stop (kb)	Size of area (kb)	No. of genes in the area	Suggested target genes	Szymas et al (1997)	Riazimand et al (2002)	Bockmühl et al (2004)	Holland et al (2007)
1p36.31	6220	6385	165	4			1p32-pter		
1p35.3	28196	28354	158	3					
1p35.3	28625	28689	64	1					
4p16.2–p16.3	1	6116	6116	94		4			
4p12–p15.31	19927	48749	28822	132					
4q12	52152	56155	4003	27					
4q21.22–q22.1	82376	90382	8006	71					
4q27–q35.2	119605	188460	68855	349					
4q27–q35.2 <sup>a</sup>	123408	124209	801	38					
5q34	160870	161694	824	6					
5q35.1–q35.3	167872	180708	12836	71					
6p12.3	48645	1446	1446	12					
7q11.23	72440	73209	769	16	<i>LIMK1</i> , <i>FZD9</i>			7q11.2	7q11.21, 7q11.23
7q21.11	76991	79296	2305	6					
9p13.3	35062	35844	782	31					9p13.3
10p12.31	18796	20430	1634	7					
12q23.1	94828	97459	2631	18					
12q24.31	120738	121633	895	18					
13q				Whole q arm					
13q14.2–14.3 <sup>a</sup>	47839	51752	3913	54					13q12.11, 13q33.3
13q31.1 <sup>a</sup>	78642	80092	1450	5					
13q34 <sup>a</sup>	112925	113288	363	8	<i>TFDP1</i> , <i>CUL4A</i>				13q34
15q13.3	29439	30347	908	6			15q-qter		
16q12.1	48851	49785	934	11				16q	
20p/q				Whole chr.				20p/q	
20p12.3–p12.2 <sup>a</sup>	7241	8681	1440	6					20q11.1–q12
20q11.21–q11.23 <sup>a</sup>	30417	36618	6201	126				20q13	20q13.31
20q13.32–q13.33 <sup>a</sup>	56224	62300	6076	110	<i>BRK</i>				21q22.3
21q				Whole q arm					
22q12.1	26643	27535	892	6				22q	
Xp/q				Whole chr.					

The start and end points of the particular aberration were estimated from CGH Explorer software. Number of genes in the area is according to NCBI database. Previously reported results that match those obtained in this study are also indicated.

<sup>a</sup>Gains in at least 30% of the cases.

alterations that were shared in at least half of these cases were recorded (Figure 3). Loss of Xp21.1 and gains of 20q11.21–q11.23, 13q14.2–q14.3, and 13q31.1 were present in two-thirds of stage 3 cancers. Gains at 5q35 and 13q, and losses at 6q16–q22, 2q31.1, and 2q33.3, were present in half of the cases. The long arms of chromosomes 13 and 20 were gained in 50% of these olfactory neuroblastomas, possibly indicating important roles in tumor progression.

## Discussion

The aim of our study was to identify DNA copy number changes in olfactory neuroblastoma by aCGH. Until recently, genome-wide-profiling studies have relied on conventional CGH in chromosome band resolution. In one study, FISH and single-nucleotide polymorphism microarray analysis were used for one case of olfactory neuroblastoma.<sup>3</sup> Moreover, the CGH studies of cytogenetic

aberrations in olfactory neuroblastoma are few.<sup>4–6</sup> We performed an oligonucleotide-based aCGH analysis on 13 olfactory neuroblastoma samples, which, to the best of our knowledge, was the first time that array-based CGH was applied to study the copy number changes in this neoplasm. Several copy number changes reported in previous studies were observed in our study, with identical, overlapping, or slightly different minimal common regions of alteration. Gains at the distal parts of 1p, 4, 9p, 13q, 15q, 22q, and 21q, and deletions at 4p and X, were reported in at least one study. Gains at 7q11 and 20q and deletions at 2q, 5q, 6p, 6q, and 18q were detected in two studies (Tables 3 and 4).

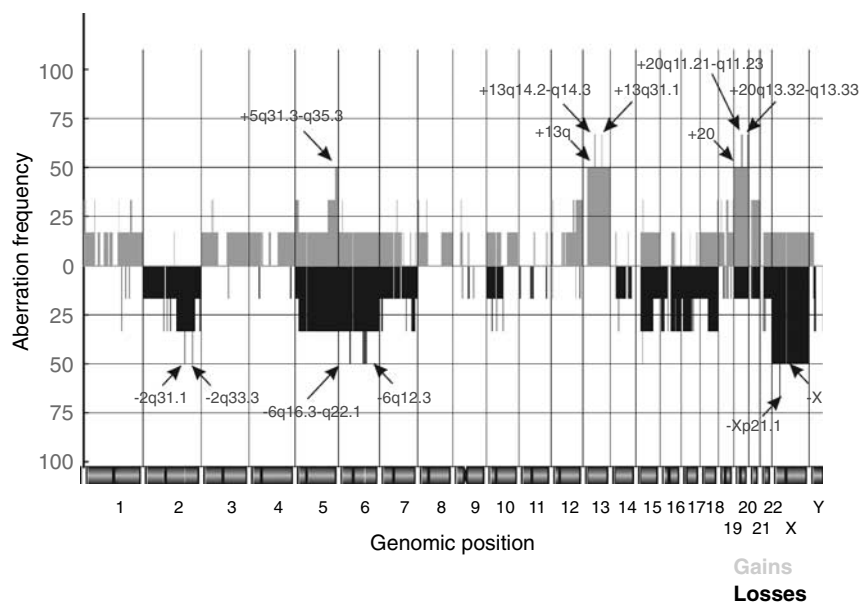
Overall, olfactory neuroblastomas have highly complex copy number changes that occur over the entire genome. All samples analyzed showed genomic imbalances with slightly more gains than losses. As expected, aCGH revealed more copy number changes than previous studies that used conventional CGH. Furthermore, our results showed novel aberrations, which were not described in previous

**Table 4** DNA copy number losses in at least 20% of the cases detected by aCGH

Chromosome location	Start (kb)	Stop (kb)	Size of area (kb)	No. of genes in the area	Suggested target genes	Szymas <i>et al</i> (1997)	Riazimand <i>et al</i> (2002)	Bockmühl <i>et al</i> (2004)	Holland <i>et al</i> (2007)
2q31.1	173492	175245	1753	19				2q22–q32	2q31–q33
2q33.3	206359	207454	1095	18	<i>ADAM23</i>				
2q37.1	231775	233528	1753	38					2q37, 2q37.3
4p13	41143	44546	3403	19				4p13–p15, 4p/q	
5q31.2	138286	138835	549	11		5q		5q	
6p22.1	26803	28249	1446	50			6p-pter		
6p21.33	30853	31865	1012	57				6p21	
6p12.3	47054	50959	3905	27					
6q16.3	101732	105348	3616	2				6q14–q23	
6q21	107952	110411	2459	35	<i>FOXO3</i> , <i>CCNC</i>				
6q22.1	113449	116631	3182	13					6q22–q24
15q11.2–q24.1			Whole q arm						
15q13.1 <sup>a</sup>	27625	27834	209	2					
18q12.2–q12.3	31999	76014	44015	197		18		18q	
18q12.2–q12.3 <sup>a</sup>	33268	41040	7772	9					
19q12	32846	34776	1930	6					
19q13.11	41195	41484	289	11					
19q13.32	53405	53791	386	10					
19q13.43	63057	63539	482	20					
22q11.23	21670	22493	823	21					
22q12.1	26506	26849	343	3					
22q11.1–q11.21	15393	17451	2058	48					
Xp/q			Whole chr.			X			

The start and end points of the particular aberration were estimated from CGH Explorer software. Number of genes in the area is according to NCBI database. Previously reported results that match the results obtained by this study are also indicated.

<sup>a</sup>Losses in at least 30% of the cases.



**Figure 3** Frequency profile of copy number aberrations in six stage 3 olfactory neuroblastoma samples. The Piecewise Constant Fit algorithm of the CGH Explorer software was applied to identify DNA copy number alterations. Frequencies of copy number gains (gray) and losses (black) are indicated on the y axis. Genomic positions of the aCGH probes are indicated on the x axis. Alterations occurring in at least 50% of stage 3 cases are marked in the figure.

reports. In accordance with at least two previous studies, we found gains at 7q11.2 and 20q13, and losses at 2q31–q37, 5q, 6p, 6q, and 18q. In addition

to these previously reported alterations, we identified novel gains in our samples at 5q34–q35, 6p12.3, 10p12.31, 12q23.1–q24.31, and all of chromosome

X. Losses at 15q11.2–q24.1, 15q13.1, 19q12–q13, 22q11.1–q11.21, 22q11.23, and 22q12.1 have not been described previously.

We identified a 770 kb region of chromosomal gain at 7q11.2. This region has been implicated in other cancers, and is overexpressed in prostate carcinomas, adenoid cystic carcinomas, head and neck squamous cell carcinomas, and pancreatic endocrine tumors.<sup>9–12</sup> Candidate genes located within this region include *LIMK1* (NCBI GeneID: 3984), a possible oncogene that contributes to cell cycling and invasion. Another possible candidate is *FZD9* (GeneID: 8326), a member of the ‘frizzled’ gene family that is upregulated in astrocytomas and gastric carcinomas.<sup>13,14</sup>

A 6 Mb region of gain at 20q13.32–q13.33 was also identified. DNA copy number increases at chromosome 20q13 have been observed frequently in a variety of cancers, including breast, ovarian, and squamous cell carcinomas,<sup>15</sup> suggesting that the region harbors one or more oncogenes. In particular, 20q13.2 has been proposed as a hotspot for candidate genes.<sup>15</sup> It should also be noted that overexpression at this locus has been associated with reduced patient survival and higher tumor grade. 20q13.3, frequently overexpressed in ovarian cancers, has been recently shown to localize the *BRK* tyrosine kinase gene (GeneID: 5753), which is thought to have an important role in the development of ovarian cancers.<sup>16</sup>

Losses occurring at chromosome 2q have been described for various carcinomas, including head and neck squamous cell carcinoma,<sup>17–19</sup> breast carcinoma,<sup>20</sup> lung carcinoma,<sup>21</sup> neuroblastoma,<sup>22</sup> cervical cancer,<sup>23</sup> and prostate adenocarcinoma.<sup>24</sup> Studies using different approaches have increasingly shown that the most affected region is 2q32–q37. This region also seems to be implicated in the development of ONB, as it has been reported in three cytogenetic studies including our investigation. Several candidate tumor suppressor genes have been suggested, including *ADAM23* (GeneID: 8745), thought to function as an adhesion molecule, which promotes the attachment of neural cells.<sup>25</sup> This gene at 2q33.3 was lost in 20% of all olfactory neuroblastoma samples analyzed and, more significantly, in 50% of stage 3 tumors. Takada *et al* have suggested an essential role for this candidate tumor suppressor gene in the progression of gastric cancer, and our results indicate that this might be the case for olfactory neuroblastoma as well.

Another area of loss identified in our study, and also reported by Bockmühl *et al*<sup>4</sup> and Holland *et al*,<sup>3</sup> is located at 6q21–22. This region is frequently deleted in a variety of neoplasms, including pancreatic endocrine tumors,<sup>26</sup> prostate carcinoma,<sup>27</sup> breast carcinoma,<sup>28</sup> and central nervous system lymphomas.<sup>29</sup> Three main putative tumor suppressor genes have been proposed, including *FOXO3* (GeneID: 2309), *CCNC* (GeneID: 892), and *PTPRK*

(GeneID: 5796). The first two candidate genes are in the regions found to be deleted in our samples.

Our study also identified two small gains at 9p13.3 (782 kb) and 13q34 (363 kb) that were previously reported by Holland *et al*. The 9p13.3 locus has been shown to be gained in prostate cancer cell lines in two recent studies using aCGH.<sup>30,31</sup> These authors suggested the involvement of three genes, including *UBE2R2* (GeneID: 54926), *DCTN3* (GeneID: 11258), and *IL-11RA* (GeneID: 3590). Kamradt *et al* screened 20 primary prostate cancer samples and found that only *IL-11RA* was gained in 75% of the tumors, whereas only *DCTN3* was not gained in any of the cases; both genes were gained together in 10% of the tumors. This observation led the authors to suggest that *IL-11RA* is the target of gain rather than *DCTN3*. Gains at 13q34 have also been described previously in different cancers, including breast cancer, hepatocellular carcinoma, esophageal squamous cell carcinoma, and lung adenocarcinoma.<sup>32</sup> The *TFDP1* (GeneID: 7027) and *CUL4A* (GeneID: 8451) genes were suggested as target genes that possibly have a role in carcinogenesis. Further studies are required to determine if these genes are involved in olfactory neuroblastoma.

As for most tumors, stage is the most important parameter associated with survival in olfactory neuroblastoma.<sup>2</sup> Our results clearly indicate that alterations in 20q and 13q are important in the progression of olfactory neuroblastoma. Gain of 20q has been widely associated with progression of several tumors, including breast carcinoma,<sup>33</sup> cervical carcinoma,<sup>34</sup> and pancreatic carcinoma.<sup>35</sup> Both losses and gains of chromosome 13q have been noted in many recent studies of various tumors, suggesting the existence of novel oncogenes or tumor suppressor genes or both in this region. Furthermore, this region has been reported to contain microRNAs that could function as tumor suppressor genes or oncogenes.<sup>36</sup> The only known tumor-related gene in this region is the tumor suppressor gene *RB1*. It is associated with loss of function or decreased gene expression in tumor cells and, consequently, cannot be considered further. Lillington *et al*<sup>37</sup> found that gain of 13q is more frequent in older patients, whose tumors display more abnormalities than those in younger patients. These authors have also reported that gain of 13q is seen more frequently in poorly differentiated malignancies, which typically contain more abnormalities than well-differentiated cancers. Gains of both 13q and 20q are seen in colorectal carcinomas and their progression.<sup>38,39</sup>

In conclusion, we have identified novel chromosomal regions, which are frequently altered in olfactory neuroblastoma. When the results of our study are combined with previously published data, several regions are identified that are consistently abnormal in olfactory neuroblastoma. These regions are also frequently associated with tumorigenesis of a variety of cancers, especially carcinomas. A

number of genes located at these sites have been suggested to be important, but further experiments are required to determine their roles in olfactory neuroblastoma.

## Disclosure

The authors state no conflict of interest.

## References

- Barnes L, (ed) Pathology and Genetics of Head and Neck Tumours. International Agency for Research on Cancer (IARC), Lyon, France, 2005, pp 430.
- Morita A, Ebersold MJ, Olsen KD, *et al*. Esthesioneuroblastoma: prognosis and management. *Neurosurgery* 1993;32:706–714, discussion 714–715.
- Holland H, Koschny R, Krupp W, *et al*. Comprehensive cytogenetic characterization of an esthesioneuroblastoma. *Cancer Genet Cytogenet* 2007;173:89–96.
- Bockmuhl U, You X, Pacyna-Gengelbach M, *et al*. CGH pattern of esthesioneuroblastoma and their metastases. *Brain Pathol* 2004;14:158–163.
- Riazimand SH, Brieger J, Jacob R, *et al*. Analysis of cytogenetic aberrations in esthesioneuroblastomas by comparative genomic hybridization. *Cancer Genet Cytogenet* 2002;136:53–57.
- Szymanski J, Wolf G, Kowalczyk D, *et al*. Olfactory neuroblastoma: detection of genomic imbalances by comparative genomic hybridization. *Acta Neurochir (Wien)* 1997;139:839–844.
- Mills SE, Frierson Jr HF. Olfactory neuroblastoma. A clinicopathologic study of 21 cases. *Am J Surg Pathol* 1985;9:317–327.
- Lingjaerde OC, Baumbusch LO, Liestol K, *et al*. CGH-explorer: a program for analysis of array-CGH data. *Bioinformatics* 2005;21:821–822.
- Davila M, Frost AR, Grizzle WE, *et al*. LIM kinase 1 is essential for the invasive growth of prostate epithelial cells: implications in prostate cancer. *J Biol Chem* 2003;278:36868–36875.
- Kasamatsu A, Endo Y, Uzawa K, *et al*. Identification of candidate genes associated with salivary adenoid cystic carcinomas using combined comparative genomic hybridization and oligonucleotide microarray analyses. *Int J Biochem Cell Biol* 2005;37:1869–1880.
- Bockmuhl U, Schluns K, Schmidt S, *et al*. Chromosomal alterations during metastasis formation of head and neck squamous cell carcinoma. *Genes Chromosomes Cancer* 2002;33:29–35.
- Stumpf E, Aalto Y, Hoog A, *et al*. Chromosomal alterations in human pancreatic endocrine tumors. *Genes Chromosomes Cancer* 2000;29:83–87.
- Zhang Z, Schittenhelm J, Guo K, *et al*. Upregulation of frizzled 9 in astrocytomas. *Neuropathol Appl Neurobiol* 2006;32:615–624.
- Kirikoshi H, Sekihara H, Katoh M. Expression profiles of 10 members of frizzled gene family in human gastric cancer. *Int J Oncol* 2001;19:767–771.
- Quinlan KG, Verger A, Yaswen P, *et al*. Amplification of zinc finger gene 217 (ZNF217) and cancer: when good fingers go bad. *Biochim Biophys Acta* 2007;1775:333–340.
- Schmandt RE, Bennett M, Clifford S, *et al*. The BRK tyrosine kinase is expressed in high-grade serous carcinoma of the ovary. *Cancer Biol Ther* 2006;5:1136–1141.
- Beder LB, Gunduz M, Ouchida M, *et al*. Genome-wide analyses on loss of heterozygosity in head and neck squamous cell carcinomas. *Lab Invest* 2003;83:99–105.
- Yamamoto N, Mizoe J, Numasawa H, *et al*. Allelic loss on chromosomes 2q, 3p and 21q: possibly a poor prognostic factor in oral squamous cell carcinoma. *Oral Oncol* 2003;39:796–805.
- Cengiz B, Gunduz M, Nagatsuka H, *et al*. Fine deletion mapping of chromosome 2q21–37 shows three preferentially deleted regions in oral cancer. *Oral Oncol* 2007;43:241–247.
- Piao Z, Lee KS, Kim H, *et al*. Identification of novel deletion regions on chromosome arms 2q and 6p in breast carcinomas by amplotype analysis. *Genes Chromosomes Cancer* 2001;30:113–122.
- Otsuka T, Kohno T, Mori M, *et al*. Deletion mapping of chromosome 2 in human lung carcinoma. *Genes Chromosomes Cancer* 1996;16:113–119.
- Takita J, Yang HW, Chen YY, *et al*. Allelic imbalance on chromosome 2q and alterations of the caspase 8 gene in neuroblastoma. *Oncogene* 2001;20:4424–4432.
- Narayan G, Pulido HA, Koul S, *et al*. Genetic analysis identifies putative tumor suppressor sites at 2q35–q36.1 and 2q36.3–q37.1 involved in cervical cancer progression. *Oncogene* 2003;22:3489–3499.
- Ueda T, Komiya A, Suzuki H, *et al*. Loss of heterozygosity on chromosome 2 in Japanese patients with prostate cancer. *Prostate* 2005;64:265–271.
- Takada H, Imoto I, Tsuda H, *et al*. ADAM23, a possible tumor suppressor gene, is frequently silenced in gastric cancers by homozygous deletion or aberrant promoter hypermethylation. *Oncogene* 2005;24:8051–8060.
- Barghorn A, Speel EJ, Farspour B, *et al*. Putative tumor suppressor loci at 6q22 and 6q23–q24 are involved in the malignant progression of sporadic endocrine pancreatic tumors. *Am J Pathol* 2001;158:1903–1911.
- Kim JH, Dhanasekaran SM, Mehra R, *et al*. Integrative analysis of genomic aberrations associated with prostate cancer progression. *Cancer Res* 2007;67:8229–8239.
- Theile M, Seitz S, Arnold W, *et al*. A defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer. *Oncogene* 1996;13:677–685.
- Nakamura M, Kishi M, Sakaki T, *et al*. Novel tumor suppressor loci on 6q22–23 in primary central nervous system lymphomas. *Cancer Res* 2003;63:737–741.
- Saramaki OR, Porkka KP, Vessella RL, *et al*. Genetic aberrations in prostate cancer by microarray analysis. *Int J Cancer* 2006;119:1322–1329.
- Kamradt J, Jung V, Wahrheit K, *et al*. Detection of novel amplicons in prostate cancer by comprehensive genomic profiling of prostate cancer cell lines using oligonucleotide-based arrayCGH. *PLoS ONE* 2007; 2:e769.
- Abba MC, Fabris VT, Hu Y, *et al*. Identification of novel amplification gene targets in mouse and human breast cancer at a syntenic cluster mapping to mouse ch8A1 and human ch13q34. *Cancer Res* 2007;67:4104–4112.
- Hodgson JG, Chin K, Collins C, *et al*. Genome amplification of chromosome 20 in breast cancer. *Breast Cancer Res Treat* 2003;78:337–345.

- 34 Wilting SM, Snijders PJ, Meijer GA, *et al*. Increased gene copy numbers at chromosome 20q are frequent in both squamous cell carcinomas and adenocarcinomas of the cervix. *J Pathol* 2006;209:220–230.
- 35 Loukopoulos P, Shibata T, Katoh H, *et al*. Genome-wide array-based comparative genomic hybridization analysis of pancreatic adenocarcinoma: identification of genetic indicators that predict patient outcome. *Cancer Sci* 2007;98:392–400.
- 36 Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. *Oncogene* 2006;25:6202–6210.
- 37 Lillington DM, Kingston JE, Coen PG, *et al*. Comparative genomic hybridization of 49 primary retinoblastoma tumors identifies chromosomal regions associated with histopathology, progression, and patient outcome. *Genes Chromosomes Cancer* 2003;36:121–128.
- 38 Lips EH, de Graaf EJ, Tollenaar RA, *et al*. Single nucleotide polymorphism array analysis of chromosomal instability patterns discriminates rectal adenomas from carcinomas. *J Pathol* 2007;212:269–277.
- 39 Hermsen M, Postma C, Baak J, *et al*. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. *Gastroenterology* 2002;123:1109–1119.