ELSEVIER

European Journal of Cancer 40 (2004) 1255–1261

European Journal of Cancer

www.ejconline.com

Molecular analysis of the putative tumour-suppressor gene *EXTL1* in neuroblastoma patients and cell lines

D. Mathysen[a], N. Van Roy[b], W. Van Hul[a], G. Laureys[c], P. Ambros[d], F. Speleman[b], W. Wuyts[a,*]

[a]*Department of Medical Genetics, University of Antwerp (UA), Universiteitsplein 1, 2610 Antwerp, Belgium*
[b]*Department of Medical Genetics, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium*
[c]*Pediatric Oncology, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium*
[d]*Department of Tumor Cytogenetics, CCRI, Children's Cancer Research Institute, Kindersitalg 6, 1090 Vienna, Austria*

Received 19 May 2003; received in revised form 12 January 2004; accepted 15 January 2004

Abstract

Although neuroblastoma is the most common extracranial solid tumour of childhood, little is known about its aetiology. Together with *MYCN* amplification and chromosome 17q gain, chromosome 1p deletion is one of the most frequently occurring genetic abnormalities in neuroblastoma. Based upon mapping of deletion breakpoints, putative tumour suppressor gene loci have been assigned to the distal part of the short arm of chromosome 1. Recently, the *EXTL1* gene was suggested as a candidate neuroblastoma-suppressor gene and to evaluate this hypothesis, we performed 1p deletion analysis and mutation screening of the *EXTL1*-coding region on DNA from 22 primary neuroblastomas and 21 neuroblastoma cell lines. Deletions of the chromosome region 1p36.1, including the *EXTL1* gene, were detected in several neuroblastoma cell lines and primary tumours. *EXTL1* mutation screening resulted in the detection of one unclassified variant (Ser28Cys) but could not provide additional evidence of *EXTL1* being involved in the aetiology of neuroblastoma.

Keywords: *EXTL1*; Neuroblastoma; 1p36

1. Introduction

Neuroblastoma, one of the most common malignant diseases in infancy [1], solely accounts for about 10% of all childhood tumours [2]. This solid tumour is the result of malignant transformation of migrating pluripotent sympathetic cells of neural-crest origin, leading to tumour development in the adrenal medulla or sympathetic ganglia. Metastasis of the localised solid tumour to lymph nodes, liver, lung, bone or bone marrow follows the distribution of the sympathetic nervous system [3] and has usually occurred before the time of diagnosis. It is even believed that in most cases neuroblastoma is present at birth and remains undetected until either symptoms arise due to pressure from tumour tissue or from bone metastasis resulting in bone pain. The clinical outcome of neuroblastoma is highly variable, ranging from spontaneous regression to rapid progression associated with poor prognosis [4]. Sometimes the tumour spontaneously differentiates toward a benign ganglioneuroma (maturing neuroblastoma) [5]. Three major genetic subgroups of neuroblastoma have been identified [6]. Near-triploid tumours with few, if any, structural imbalances are mostly observed in infants, with favourable prognosis. In older children, near-diploid or tetraploid tumours are usually observed, and are correlated with a poor probability of survival. Typically these tumours exhibit chromosome 17q gain in association with either *MYCN* amplification and/or chromosome 1p deletion, or alternatively chromosome 11q deletion often in association with chromosome 3p deletion.

Partial monosomy, most often resulting from the deletion of a distal fragment of the short arm of chromosome 1, occurs in 30–35% of primary neuroblastomas, predominantly in high stages of the tumour type. In most tumours these deletions are large, extending

* Corresponding author. Tel.: +32-3-820-26-77; fax: +32-3-820-25-66.
E-mail address: wwuyts@uia.ua.ac.be (W. Wuyts).

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.01.013

to the telomere. Although various loci for putative tumour-suppressor genes have been proposed, most of the loci are clustered to the most distal 1p36 band [7]. The recently refined physical mapping of the putative tumour-suppressor gene *EXTL1* to chromosome region 1p36.1 [8] has led to the hypothesis that inactivation of this member of the *EXT*/*EXTL* gene family could play a part in neuroblastoma development. The *EXT*/*EXTL* gene family comprises five members, i.e. two tumour-suppressor *EXT* genes [9–11] that are mutated in the hereditary multiple exostoses syndrome (EXT) and three *EXTL* genes [12–14]. Functional characterisation of the different members of the *EXT*/*EXTL* gene family of (putative) tumour-suppressor genes revealed their involvement in heparan sulphate biosynthesis. Both *EXT* genes act as a complex responsible for the polymerisation of growing heparan sulphate chains [15,16]. *EXTL2*, by contrast, encodes the critical enzyme distinguishing heparan sulphate from chondroitin sulphate biosynthesis [17], while *EXTL1* and *EXTL3* encode α-1,4-*N*-acetylglucosaminyltransferases [18].

To investigate the potential involvement of *EXTL1* in neuroblastoma development, we performed *EXTL1* deletion analysis and mutation screening in the coding region of *EXTL1* in neuroblastoma cell lines and primary neuroblastoma tumours.

2. Materials and methods

2.1. DNA samples

A total of 43 DNA samples derived from 21 neuroblastoma cell lines and 22 primary neuroblastomas from non-related patients were subjected to *EXTL1*-mutation and -deletion analysis. Details of the tumour cell content and the staging of the neuroblastomas are summarised as part of Table 3 below. For one of the cell lines, STA-NB-3, as well as for 16 of the 22 primary neuroblastomas investigated, constitutional genomic DNA was available. This study was approved by the ethical committee of the University of Antwerp (Belgium).

2.2. EXTL1 deletion analysis

Chromosome 1p deletion status was determined by fluorescence *in situ* hybridisation (FISH) using a probe for the subcentromeric heterochromatic 1q12 region (D1Z1) [19] in combination with a 1p subtelomeric D1Z2 probe [20] as described elsewhere [21] and with an *EXTL1* cosmid probe B2137Q [8]. Heterozygosity at the *EXTL1* locus was determined using intragenic *EXTL1* exon 5 polymorphisms (1135 A < C and 1146 G > A). In addition, the panel of primary tumours was also evaluated for loss of heterozygosity at the *EXTL1* proximal flanking marker D1S2749 (57.2 cM) and the *EXTL1* distal flanking marker D1S2885 (56.6 cM), and at distal loci D1S76 (1p36.3) and D1S80 (1p36.3) as described elsewhere [22]. For 12 of the 21 neuroblastoma cell lines, loss of heterozygosity had been studied by means of southern blotting [23]. A sample was scored as deleted for *EXTL1* if FISH experiments showed only one signal for the *EXTL1* probe in comparison to D1Z1 signals, or if FISH showed fewer *EXTL1* probe signals than D1Z1 signals (allelic imbalance) (e.g. three D1Z1 signals and two *EXTL1* signals) in combination with homo- or hemizygosity for all tested markers. The sample was considered as non-deleted for *EXTL1* if FISH showed no loss of 1p material, or if FISH showed an allelic imbalance in combination with retention of heterozygosity at one of the tested intragenic *EXTL1* markers or *EXTL1* proximal flanking markers. FISH results from fewer than 10% of the analysed cells of a particular sample were considered not significant and are not included in Table 3 below.

MYCN copy number was determined using FISH with an *MYCN*-specific probe (LSI N-*myc*, Vysis).

2.3. Polymerase chain reaction (PCR) amplification and single-stranded conformational polymorphism (SSCP) analysis

Recently, Wuyts and colleagues [8] have described the genomic organisation of *EXTL1*, comprising 11 exons. A total of 12 primer pairs were designed in order to amplify the coding region of *EXTL1* (Table 1). The relatively large first exon contains a 5′ UTR sequence and the first 779-bp coding sequence. The coding sequences of exons 2–11 inclusive do not exceed 180 bp. Because of size limitation in SSCP resolution, two primer pairs were designed to amplify the coding region of the first exon, while only one primer pair was needed for separate amplification of the other coding exons. PCR amplification reactions were performed in a total reaction volume of 50 μl comprising 100 ng of genomic tumour-derived DNA, 5 μl 10× PCR buffer, 0.075 μmoles of $MgSO_4$, 50 pmoles of each primer, 12.5 nanomoles of each dNTP, 0.012 mCi of α-^{32}P-dCTP, 0.012 mCi of α-^{32}P-dATP and 0.25 units of *Taq* DNA polymerase. The PCR programme used to amplify *EXTL1* fragments consisted of an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at the optimal annealing temperature (summarised in Table 1) for 1 min and extension at 72 °C for 1 min. Finally, an extension step at 72 °C for 10 min was included. Amplification products were analysed on MDE polyacrylamide gel (FMC Bioproducts, Rockland, MN). Gel-electrophoresis variables were 0.5 kV, 150 mA, 68 W, with a runtime of 13–16 h. The migration pattern was visualised by exposure to a Kodak radiosensitive photographic film.

Table 1
Individual sequences and optimal annealing temperatures (Tm) for the primer pairs used for single-stranded conformational polymorphism-polymerase chain reaction amplification of the EXTL1 gene

Amplicon	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	Tm (°C)
Exon 1 (1)	tctgtacaatgacagcacagg	atcttctgggagctctccagg	58
Exon 1 (2)	cctgcccccgttccattgc	gctggccccgctggctgg	55
Exon 2	ctggagagtgcagctcaatgc	ccaggagtaaaggagcaggc	55
Exon 3	tgcagaacaaccataacctccc	gaaggcagataggaatgagg	55
Exon 4	gtgcagtgggacgaatatgg	ggttccccaaacactgtggc	58
Exon 5	ggggaggtgtggccatggtc	ggctctccagatgaggctgg	55
Exon 6	ccaagacctttccaagccctg	caaatagtggtcatctttggg	58
Exon 7	gatcactcctgcccctcgc	gctcccagcatgggcccacc	55
Exon 8	ctcctacttattggatgg	agaggctggacctcatgac	55
Exon 9	ctcagcctggctgccctgc	ggccactgtgctccgttcgg	58
Exon 10	ggagaggtgaggtcaggagg	gctcccatccctggcgtgaacc	55
Exon 11	gtcaggagggaggagaatggg	ggctgagacctttgatggggtctcc	55

2.4. Sequence analysis

DNA samples showing an aberrant banding pattern were reamplified and these amplification products were purified with the Concert™ Rapid PCR Purification kit according to the manufacturer's guidelines. Purified fragments were sequenced using BigDye Terminator chemistry (Applied Biosystems) and analysed on an ABI 3100 automatic-sequencing apparatus according to the manufacturer's recommendations.

3. Results

3.1. Chromosome 1p deletion analysis

The combination of FISH with probes D1Z1 (1q12), D1Z2 (1p36.3) and B21317Q (*EXTL1*; 1p36.1) and heterozygosity analysis with intragenic *EXTL1* markers revealed deletion at the *EXTL1* locus in 16 of the 21 neuroblastoma cell lines investigated. Five of the lines had no deletion at the *EXTL1* locus, although three of them showed deletion of the subtelomeric region of chromosome 1, indicating that these lines contained 1p deletions distal to the *EXTL1* locus (Table 2). A similar combination of FISH and heterozygosity analysis revealed deletion at the *EXTL1* locus in 3 of the 22 primary tumours (Table 3). One tumour (patient LS) showed a smaller terminal 1p deletion, and an allelic imbalance with heterozygosity for D1S80 was observed in two tumours (CE and BB).

17 of the 21 cell lines showed *MYCN* amplification, a phenomenon that was also observed in three primary tumours.

3.2. EXTL1 mutation analysis

PCR–SSCP was applied for molecular screening of the coding region of *EXTL1* in tumour-derived genomic DNA from the 21 different neuroblastoma cell lines and 22 primary tumours. For two exons, exons 1 and 5, several samples showed a different migration pattern. Sequence analysis of exon 1 identified an 83 C > G mutation in codon 28, resulting in a Ser28Cys substitution in DNA from cell line STA-NB-3. To investigate whether this might represent a polymorphism, exon 1 of the *EXTL1* gene was screened in 50 control individuals (100 chromosomes) but none of them showed the G allele. An additional 50 individuals from the same geographical region (Austria) as the patient were screened, but none of them showed the G allele. Further analysis revealed that the 83 C > G substitution was also present in the original tumour tissue, but was not found in constitutional genomic DNA obtained from the patient which the STA–NB-3 cell line was derived from, nor was it detected in any of the other cell lines or primary neuroblastoma samples.

Sequence analysis of the first *EXTL1* exon also revealed the presence of a 99 G > A variance compared to the original reported cDNA sequence in all neuroblastoma cell lines and neuroblastoma patients. However, all of the control samples also showed the A allele at this position, suggesting that the G allele represents a sequencing error in the originally reported sequence.

Apart from these two variations in the *EXTL1* exon 1 coding sequence, two other sequence alterations were observed in exon 5. An 1135 A > C polymorphism in codon 379 that results in an amino acid change (Asn379His) was found in the homo(hemi)- or heterozygous state in 11 neuroblastoma cell lines and 11 primary neuroblastomas (Table 3). Allele frequencies in the control population were 0.62 for the A allele and 0.38 for the C allele. Another polymorphism in codon 382 in the 5th exon, 1146 G > A, does not result in an amino acid change (Leu382Leu) and was found in eight neuroblastoma cell lines (Table 2) and nine primary neuroblastomas (Table 3). Allele frequencies for this

Table 2
Molecular analysis of neuroblastoma cell lines

Cell line	Stage*	FISH signal D1Z1/D1Z2	FISH signal D1Z1/*EXTL1*	*MYCN* status	1135A > C	1146G > A	Conclusion for *EXTL1* locus
STA-NB-10	3	2/1	2/1	A	H	H	Deletion
STA-NB-1.2	3	2/1	2/1	A	H	H	Deletion
SJNB-6	4	2/1	2/1	A	H	H	Deletion
GI-LI-N	4	3/1	3/1	A	H	H	Deletion
SMS-KCNR	4	2/1	2/1	A	H	H	Deletion
SMS-KAN	4	2/1	2/1	A	H	H	Deletion
UHG-NP	4	4/2	4/4	A	H	H	No deletion
NBL-S	3	2/2	2/2	N	h	h	No deletion
CHP134	Unknown	2/1	2/2	A	H	H	No deletion
LA-N-1	4	3/1	3/1	A	H	H	Deletion
SJNB-8	4	3/2	3/2	A	H	H	Deletion
SK-N-SH	4	2/2	2/2	N	h	H	No deletion
NMB	4	5/3	5/3	A	H	H	Deletion
NLF	Unknown	4/3	4/3	A	H	H	Deletion
STA-NB-3	2B	4/2	4/2	A	H	H	Deletion
GI-ME-N	4	4/2	4/2	N	H	H	Deletion
N206	4	3/2	3/2	A	H	H	Deletion
STA-NB-8	4	2/1	2/1	A	H	H	Deletion
LA-N-5	Unknown	4/2	4/2	A	H	H	Deletion
CHP901	4	3/2	3/2	A	H	H	Deletion
LA-N-6	4	2/1	2/2	N	H	H	No deletion

*Stage refers to stage of the patient the cell line is derived from. FISH, fluorescence *in situ* hybridisation; H, homo- or hemizygous; h, heterozygous; MYCN status: A, amplified; N, normal.

polymorphism in the control population were 0.78 for the G allele and 0.22 for the A allele.

4. Discussion

Deletions of the short arm of chromosome 1 are one of the most frequent genetic aberrations in high-stage neuroblastomas and also occur in many other more common types of human cancer [24]. These findings suggest an important role in human tumorigenesis for alterations in chromosome 1. Most of the chromosome 1p deletions in neuroblastoma are large and extend to the telomere. Data from experiments on loss of heterozygosity and chromosome transfer suggest that the distal part of chromosome 1p is important in the development of neuroblastoma and harbours one or more tumour-suppressor loci [7,25]. There are several indications that *EXTL1* could be a candidate for a putative neuroblastoma-suppressor gene. First, the *EXTL1* gene is homologous to both *EXT* genes. Since *EXT* genes are known to be tumour-suppressor genes involved in the development of the EXT syndrome, the similarity in sequence between *EXT* and *EXTL* genes might indicate a similar function for EXTL proteins. Secondly, the localisation of *EXTL* genes in chromosome regions is known to play an important part in tumorigenesis further suggests a putative tumour-suppressor capacity for the *EXTL* genes. The recently refined physical mapping of *EXTL1* to chromosome band 1p36.1 in close proximity to the translocation breakpoint observed in the neuroblastoma cell line UHG-NP [8,26,27] points to *EXTL1* as a candidate gene.

To test the hypothesis that *EXTL1* is a candidate neuroblastoma tumour-suppressor gene, we screened 21 neuroblastoma cell lines and 22 primary tumours for the presence of deletions and mutations at the *EXTL1* locus. In keeping with previous findings of rather large 1p deletions in the majority of the neuroblastoma cell lines, most of the cell lines investigated here (16/21) contained an *EXTL1*-comprising, chromosome 1p deletion. Two of the 21 cell lines did not contain a chromosome 1p deletion, while 3 of the 21 contained a deletion that lies distal to the *EXTL1* gene. The proportion of chromosome 1p-deleted primary tumours (3 out of 22) is relatively low in comparison with published data [6]. This finding can partly be explained by the fact that almost half of the primary tumours we investigated are low stage, whereas chromosome 1p deletions are predominantly found in high-stage tumours. The fact that the three primary tumours showing an *EXTL1* deletion were all stage 4 tumours supports this hypothesis. The overall number of primary tumour samples showing *MYCN* amplification was low (3 out of 22) in comparison with the neuroblastoma cell lines (17 out of 21). Two of these three were found to be *EXTL1* deleted. It would therefore be interesting to test a larger number of

Table 3
Determination of chromosome 1p status in primary neuroblastomas[b]

Patient	Stage	Tumour cell content (%)	FISH signal D1Z1/D1Z2	FISH signal D1Z1/*EXTL1*)	% FISH ratio	MYCN status	D1S80	D1S76	*EXTL1* 1135A > C	*EXTL1* 1146G > A	D1S2749	D1S2885	Conclusion for *EXTL1* locus
VT	4	70	2/1	2/1	68	N	f	f	ni	ni	LOH	LOH	Deletion
BS	1	80	2/2	2/2	90	N	H	h	h	H	nd	nd	No deletion
LS	1	80	2/1	2/2	75	N	H	H	h	h	nd	nd	No deletion
VSM	3	90	2/2	2/2	95	N	h	H	h	H	nd	nd	No deletion
WB	1	70	2/2	2/2	80	N	f	f	h	h	nd	nd	No deletion
SO	4	75	2/2	2/2	85	N	H	h	h	h	nd	nd	No deletion
DCM	1	99	2/2	2/2	90	N	H	h	H	H	h	H	No deletion
DWM	4	85	2/2	2/2	82	N	h	h	H	H	h	H	No deletion
QD	2	65	2/2	2/2	70	N	H	h	H	H	H	h	No deletion
CM	4	70	2/2	2/2	90	N	h	h	H	H	H	h	No deletion
DSJ	1	60	2/2	2/2	60	N	h	h	H	H	H	h	No deletion
WJ	3	95	4/4	4/4	54	N	h	h	H	H	H	h	No deletion
			2/2	2/2	30								
VI	4	90	2/2	2/2	95	N	h	h	H	H	H	H	No deletion
JB	4	60	2/2	2/2	90	N	h	h	H	H	H	H	No deletion
PE	4	85	2/1	2/1	80	A	LOH	LOH	H	H	H	H	Deletion
EM[a]	1	90	3/3	3/3	15	N	H	h	H	H	h	H	No deletion
DMS[a]	4	70	2/2	2/2	80	N	h	H	H	H	H	h	No deletion
MG[a]	1	20	3/3	3/3	25	N	H	H	H	H	H	h	No deletion
CE[a]	3	60	3/2	3/2	65	A	h	H	H	H	H	H	No deletion
CC[a]	1	90	3/3	3/3	50	N	h	H	H	H	H	H	No deletion
			2/2	2/2	50								
RM	4	75	4/2	4/2	80	A	LOH	LOH	H	H	H	H	Deletion
BB[a]	4	50	4/2	4/2	30	N	h	H	H	H	H	H	No deletion
			3/2	3/2	54								

FISH, fluorescence *in situ* hybridisation; LOH, loss of heterozygosity; H, homo- or hemizygous; h, heterozygous; nd, not done; f, failure; *MYCN* status: A, amplified; N, normal

[a] No constitutional DNA available.

[b] FISH ratio <10% were not included.

high-stage tumours showing *MYCN* amplification to see whether a true association exists between high stage/*MYCN* amplification and *EXTL1*-deletion status.

In addition to the *EXTL1*-deletion analysis we performed mutation screening of the *EXTL1*-coding region in the same panel of neuroblastoma cell lines and primary tumours. A 83 C > G missense mutation in the sequence of the first *EXTL1* exon was observed in neuroblastoma cell line STA-NB-3, a line containing a chromosome 1p deletion encompassing the *EXTL1* locus. Sequence analysis of the constitutional genomic DNA of STA-NB-3 did not reveal the presence of this sequence variation, nor was it detected in any of the other tumour genomic DNA samples or in the DNA from 100 control individuals. Since the 83 C > G variation results in an amino acid change (a serine residue is replaced by a cysteine residue), it is possible that the function of the EXTL1 protein is altered and thereby contributes to malignant transformation. However, little is known about the function of exon 1 of the *EXTL1* gene, which makes the effect of the 83 C > G missense mutation difficult to predict. Further study is thus needed to evaluate whether this 83 C > G substitution in exon 1 of the *EXTL1* gene is truly a disease-causing mutation.

Detailed analysis of the entire coding region also revealed two frequent *EXTL1* polymorphisms (1135 A > C and 1146 G > A), but failed to identify additional neuroblastoma-causing mutations.

The results of the *EXTL1* mutation analysis and chromosome 1p deletion analysis in our set of neuroblastoma cell lines and primary tumours imply an important role for the *EXTL1* region in (high-stage) neuroblastoma development, but suggest that the *EXTL1* gene itself is not a key player in this process.

Acknowledgements

Nadine Van Roy is a postdoctoral researcher with the FWO. This work was also supported by BOF-grant 011F1200 and 011B4300, GOA-grant 12051203, FWO-grant G.0028.00, VEO-grant 011V1302 and FWO 'Krediet aan navorser' to WW.

References

1. Gurney JG, Ross JA, Wall DA, Bleyer WA, Severson RK, Robison LL. Infant cancer in the U.S.: Histology-specific incidence and trends, 1973 to 1992. *J Pediatr Hematol Oncol* 1997, **19**, 428–432.
2. Brodeur GM, Castleberry RP. Neuroblastoma. In Pizzo PA, Poplack DG, eds. *Principles and Practice in Pediatric Oncology,* 2nd edn. Philadelphia, J. B. Lippincott, 1993, 739–767.
3. Brodeur GM, Fong C. Molecular biology and genetics of human neuroblastoma. *Cancer Genet Cytogenet* 1989, **41**, 153–174.
4. D'Angio GJ, Evans AE, Koop CE. Special pattern of widespread neuroblastoma with a favourable prognosis. *Lancet* 1971, **1**, 1046–1049.
5. Ambros IM, Zellner A, Roald B, *et al*. Role of ploidy, chromosome 1p, and Schwann cells in the maturation of neuroblastoma. *N Engl J Med* 1996, **334**, 1505–1511.
6. Maris JM, Matthay KK. Molecular biology of neuroblastoma. *J Clin Oncol* 1999, **17**, 2264–2279.
7. Versteeg R, Caron H, Cheng NC, *et al*. 1p36: every subband a suppressor? *Eur J Cancer* 1995, **31A**, 538–540.
8. Wuyts W, Spieker N, Van Roy N, *et al*. Refined physical mapping and genomic structure of the EXTL1 gene. *Cytogenet Cell Genet* 1999, **86**, 267–270.
9. Ahn J, Lüdecke H, Lindow S, *et al*. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nature Genet* 1995, **11**, 137–143.
10. Stickens D, Clines G, Burbee D, *et al*. The EXT2 multiple exostoses gene defines a family of putative tumour suppressor genes. *Nature Genet* 1996, **14**, 25–32.
11. Wuyts W, Van Hul W, Wauters J, *et al*. Positional cloning of a gene involved in hereditary multiple exostoses. *Hum Mol Genet* 1996, **5**, 1547–1557.
12. Wise CA, Clines GA, Massa H, Trask BJ, Lovett M. Identification and localization of the gene for EXTL, a third member of the multiple exostoses gene family. *Genome Res* 1997, **7**, 10–16.
13. Wuyts W, Van Hul W, Hendrickx J, *et al*. Identification and characterization of a novel member of the EXT gene family, EXTL2. *Eur J Hum Genet* 1997, **5**, 382–389.
14. Van Hul W, Wuyts W, Hendrickx J, *et al*. Identification of a third EXT-like gene (EXTL3) belonging to the EXT gene family. *Genomics* 1998, **47**, 230–237.
15. Lind T, Tufaro F, McCormick C, Lindahl U, Lidholt K. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J Biol Chem* 1998, **273**, 26265–26268.
16. McCormick C, Duncan G, Goutsos KT, Tufaro F. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi-apparatus and catalyzes the synthesis of heparan sulfate. *PNAS* 2000, **97**, 668–673.
17. Kitagawa H, Shimakawa H, Sugahara K. The tumor suppressor EXT-like gene EXTL2 encodes an alpha 1, 4-N-acetylhexosaminyltransferase that transfers N-acetylgalactosamine and N-acetylglucosamine to the common glycosaminoglycan-protein linkage region- The key enzyme for the chain initiation of heparan sulfate. *Journal of Biological Chemistry* 1999, **274**, 13933–13937.
18. Kim BT, Kitagawa H, Tamura ?, *et al*. Human tumor suppressor EXT gene family members EXTL1 and EXTL3 encode alpha 1,4-N-acetylglucosaminyltransferases that likely are involved in heparan sulfate/heparin biosynthesis. *Proc Natl Acad Sci USA* 1903, **98**, 7176–7181.
19. Buroker N, Bestwick R, Haight G, Magenis RE, Litt M. A hypervariable repeated sequence on human chromosome 1p36. *Hum Genet* 1987, **77**, 175–181.
20. Cooke HJ, Hindley J. Cloning of human satellite III DNA: different components are on different chromosomes. *Nucleic Acids Res* 1979, **6**, 3177–3197.
21. Van Roy N, Laureys G, Ching Cheng N, Willem P, Opdenakker G, Versteeg R, Speleman F. 1;17 translocations and other chromosome 17 rearrangements in human primary neuroblastoma tumor and cell lines. *Genes Chromosom Cancer* 1994, **10**, 103–114.
22. Christiansen H, Delattre O, Fuchs S, *et al*. Loss of the putative tumor suppressor-gene locus 1p36 as investigated by a PCR-assay and N-myc amplification in 48 neuroblastomas: results of the German Neuroblastoma Study Group. *Prog Clin Biol Res* 1994, **385**, 19–25.
23. Cheng NC, Van Roy N, Chan A, *et al*. Deletion mapping in neuroblastoma cell lines suggests two distinct tumor suppressor genes in the 1p35-36 region, only one of which is associated with N-myc amplification. *Oncogene* 1995, **10**, 291–297.

24. Schwab M, Praml C, Amler LC. Genomic instability in 1p and human malignancies. *Genes Chromosom Cancer* 1996, **16**, 211–229.
25. Bader SA, Fasching C, Brodeur GM, Stanbridge EJ. Dissociation of suppression of tumorigenicity and differentiation in vitro effected by transfer of single human chromosomes into human neuroblastoma cells. *Cell Growth Differ* 1991, **2**, 245–255.
26. Spieker N, Beitsma M, Van S, Roobeek I, Den D, Speleman F, Caron H, Versteeg R. An integrated 5-Mb physical, genetic, and radiation hybrid map of a 1p36.1 region implicated in neuroblastoma pathogenesis. *Genes Chromosomes Cancer* 2000, **27**, 143–152.
27. Spieker N, Beitsma M, Van S, Chan A, Caron H, Versteeg R. Three chromosomal rearrangements in neuroblastoma cluster within a 300-kb region on 1p36.1. *Genes Chromosomes Cancer* 2001, **31**, 172–181.