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## ONLINE MUTATION REPORT

## A 17p11.2 germline deletion in a patient with Smith-Magenis syndrome and neuroblastoma

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*J Med Genet* 2005;42:e3 (<http://www.jmedgenet.com/cgi/content/full/42/1/e3>). doi: 10.1136/jmg.2004.022814

**N**euroblastoma is the most frequently occurring extra-cranial tumour type in children. It arises from the undifferentiated neural crest derived cells destined to become the sympathetic nervous system, and primary tumours typically occur in the adrenal medulla and paraspinal location in the abdomen or chest. At diagnosis, most children of >1 year of age have metastases, commonly in lymph nodes, bones, or bone marrow. However, in addition to potential lethal progression, neuroblastomas occasionally mature into benign ganglioneuromas or spontaneously regress, even without treatment.<sup>1 2</sup>

Several acquired genetic changes have been described in neuroblastoma, the most frequent being *MYCN* oncogene amplification, 1p deletion, and 17q amplification.<sup>3</sup> These alterations have been associated with aggressive forms of the disease; *MYCN* amplification in particular is used as a prognostic marker. In addition, chromosome number changes, translocations, and deletions in several other parts of the genome have been identified, but the significance of these changes needs further clarification.

A small subset of neuroblastoma cases have a family history of the disease and are diagnosed at a younger age with multifocal primary tumours.<sup>4</sup> Hence Knudson and Strong postulated that the two hit model of cancer initiation could be applied to neuroblastoma, and that the mode of inheritance was consistent with autosomal dominant Mendelian pattern with incomplete penetrance.

Some linkage studies have been performed on familial neuroblastomas, and linkage to 4p16 and 16p12-13 has been suggested in families from Europe and North America, respectively.<sup>5 6</sup> However, the chromosome 16p region was tested for linkage with negative results in Italian and British families.<sup>5-7</sup> In addition, the known candidate neuroblastoma loci that are frequently altered sporadically have been excluded in some neuroblastoma families.<sup>5 7</sup> Thus, despite extensive studies into neuroblastoma susceptibility, the putative predisposing genetic changes have remained unidentified, and additional analyses are required to further elucidate the mechanisms behind hereditary neuroblastoma development.

We report the genetic analyses of a Finnish girl who was diagnosed with neuroblastoma at the age of 2 years and 10 months. At diagnosis, she had advanced stage neuroblastoma, but with extensive therapy, she was cured of the disease. She was subsequently diagnosed with a psychomotor retardation/multiple congenital anomaly syndrome, Smith-Magenis syndrome (SMS), which was confirmed by karyotyping; the patient displayed a 17p11.2 germline deletion, characteristic of this syndrome. Because of the possible association of the neuroblastoma and the germline deletion, 17p11.2 was further evaluated in the patient's normal tissue DNA by performing microsatellite marker analyses and array comparative genomic hybridisation (CGH). In addition, data from serial analysis of gene expression (SAGE) of

## Key points

- Neuroblastoma is the most common solid tumour type in children, and is characterised by diverse clinical behaviour and multiple genetic alterations. Despite numerous studies on the molecular background of neuroblastoma, the causative changes have not been fully characterised, making additional analyses necessary.
- We report the genetic analyses of a Finnish girl who was diagnosed with an advanced stage neuroblastoma at the age of 34 months. The patient was also diagnosed with Smith-Magenis syndrome at 9 years of age, and a germline deletion in 17p11.2 was detected as the underlying cause. Because of the possible association of the neuroblastoma and the germline deletion, 17p11.2 was further evaluated in the patient's normal tissue DNA, and in sporadic neuroblastomas and neuroblastoma cell lines.
- Using microsatellite marker analysis in the patient's normal tissue DNA, the 17p11.2 deletion was found to span 4.2 Mb between markers D17S1857 and D17S842. Altogether, 11 sporadic neuroblastomas were analysed for loss of heterozygosity in the deleted region, but no allelic imbalance was detected. No other copy number changes were detected in the patient's normal tissue DNA by array comparative genomic hybridization.
- Data from serial analysis of gene expression on neuroblastoma cell lines revealed that five genes in the deleted 17p11.2 region might have a role in neuroblastoma development. Of the identified genes, *Ubb* and *Usp22* are involved in protein degradation, while *Tom1l2*, *Mfap4*, and *Prpsap2* have functions related to protein trafficking, cell adhesion, and synthesis of nucleotides, respectively.
- Studying a more extensive series of neuroblastomas can help assess the importance of the affected region and the candidate genes.

neuroblastoma cell lines was analysed to identify those 17p11.2 genes that could have a role in neuroblastoma differentiation pathways. We also analysed sporadic neuroblastomas for loss of heterozygosity (LOH) in the 17p11.2 region.

## METHODS

The primary tumour (stage IV) was in the left adrenal medulla and dense metastasis in the bone marrow was detected. Amplification at the *MYCN* locus was detected by

fluorescence in situ hybridisation (FISH). With extensive treatment including surgery, chemotherapy, radiotherapy, and stem cell transfusion, she was cured and has remained disease free since, being 11 years of age at present. At the age of 9 years, the patient was diagnosed with SMS, characterised by psychomotor retardation, craniofacial dysmorphism, behavioural abnormalities such as aggressive and self-destructive behaviour, and multiple organ anomalies, including cardiovascular, genitourinary, and ophthalmic anomalies.<sup>8-10</sup> She had normal early development, but strabismus was noted at 6 months of age and speech delay at the time of neuroblastoma diagnosis. Brain MRI at the age of 3 years showed bilateral subependymal nodular heterotopia, and at 5 years of age mild psychomotor retardation was obvious. The patient has major behavioural and sleep disturbances, and shows typical dysmorphic features of SMS, including brachycephaly, flat mid-face, prominent jaw, and large mouth. Her parents and brother are healthy, but her sister has been diagnosed with a heart malformation (atrioventricular septal defect).

To confirm the SMS diagnosis, the patient's lymphocytes were screened for chromosomal aberrations with 550 band accuracy. The karyotype was normal apart from a deletion in chromosome 17p11.2. To complete the SMS diagnostic procedures, the result was confirmed by FISH.

To fine map the deleted region, microsatellite markers spanning 17p11.2 were chosen based on Ensembl database (<http://www.ensembl.org/>) or designed based on the sequence data. We performed microsatellite marker analysis on the normal tissue DNA of the patient and the parents. PCR conditions and primer sequences are available upon request. PCR products were run on an ABI Prism 377 DNA sequencer (Applied Biosystems, Branchburg, NJ, USA) according to the manufacturer's instructions, and the collected data were analysed by GeneScan 3.1 and Genotyper 2.5 software (Applied Biosystems).

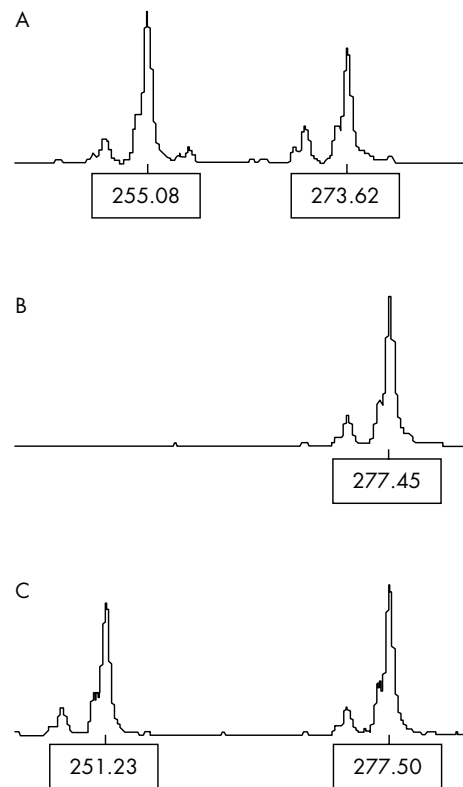
To further assess the deleted region we analysed the patient's normal tissue DNA with array CGH. The hybridisations (using an Agilent Human 1 cDNA Microarray kit) were performed by the Biomedicum Biochip Center, Helsinki, Finland, according to the procedures of Monni *et al.*<sup>11</sup>

To identify those 17p11.2 genes that could have a role in known neuroblastoma differentiation pathways, neuroblastoma SAGE data was scrutinised. More specifically, we studied the pathways of *MYCN* and *Meis1*, which are amplified in a subset of neuroblastomas,<sup>12,13</sup> and of *TrkA*, the expression of which is associated with neuroblastomas with good prognosis.<sup>14</sup> The SAGE data was acquired by transfecting neuroblastoma cell lines SH-EP, SJ-NB8, and SH-SY5Y with *MYCN*, *Meis1*, or *TrkA*, respectively. The downstream pathways of the transfected genes were analysed by high throughput mRNA expression profiling. A set of SAGE libraries was constructed from the transfected and untransfected cells, and the libraries were analysed for differentially expressed genes.<sup>15,16</sup>

In addition, we analysed 11 sporadic neuroblastomas and corresponding normal tissue samples for LOH using microsatellite markers in the deleted region.

## RESULTS

Chromosomal analyses on the patient's lymphocytes performed in a diagnostic setting showed a deletion in 17p11.2 (46,XX,del(17)(p11.2p11.2)). The finding was confirmed by FISH with an *FLII* probe (del(17)(p11.2p11.2) (MDCR+,*FLII*-)). We fine mapped the affected region with microsatellite marker analysis, and the deletion was shown to lie between markers D17S1857 and D17S842. These markers delineate the deletion, as they displayed heterozygosity in the patient's normal tissue DNA.



**Figure 1** Normal tissue DNA of the mother (A), patient (B), and father (C) analysed with marker D17S976. The maternal allele is absent from the patient's DNA.

Altogether, 12 markers were examined between these two markers, five of which were informative and showed a deletion of the other allele (fig 1). The deleted interval corresponds to a 4.2 Mb distance (Ensembl, 22nd July 2004 update). Genes spanning the deleted region are presented in table 1. According to microsatellite analysis, there were no deletions in the DNA of the patient's parents, and the deleted allele was maternal.

The normal tissue DNA of the patient was analysed by array CGH. The results correlated with the microsatellite marker data in that the deleted region contained genes with nearly 50% intensity reduction. According to the array CGH, there were no other clear areas with copy number changes in the patient's normal tissue DNA, a finding compatible with the karyotyping result. Most of the successfully hybridised genes within the deletion have unknown functions.

The affected 17p11.2 region harbours at least 83 genes (table 1). To identify those genes that could have a role in neuroblastoma differentiation, we analysed *MYCN*, *Meis1*, and *TrkA* regulated gene expression in three neuroblastoma cell lines by SAGE. This approach identified a few hundred genes in the downstream pathways of *MYCN*, *Meis1*, and *TrkA*. In the 17p11.2 region, five genes displayed changes in their expression patterns. *MYCN* decreased the expression of *Ubb* and *Tom112*, whereas *Prpsap2*, *Mfap4*, and *Usp22* were down-regulated by *Meis1*. *Mfap4* expression was also upregulated by *TrkA* (table 2).

We also studied 11 sporadic normal/tumour tissue neuroblastoma samples for LOH by microsatellite marker analysis. We performed analysis on four markers in the deleted region but none of the samples showed allelic imbalance at these markers according to two independent viewers. At least two, typically three, markers were informative in each normal/tumour sample pair.

**Table 1** Genes spanning the 17p11.2 deletion

| Ensembl gene ID   | External gene ID | Gene description  | Protein family description   |
|-------------------|------------------|---|--|
| ENSG00000170315.1 | UBB              | Ubiquitin   | <b>40S ribosomal s27a</b>  |
| ENSG00000154050.1 |                  |   | Unknown  |
| ENSG00000154051.1 | NM_030970        |   | Unknown  |
| ENSG00000175061.1 | NM_152350        |   | Ambiguous  |
| ENSG00000181350.1 | Q8NAA5           |   | Unknown  |
| ENSG00000141040.3 | ZNF287           | Zinc finger protein 287   | Zinc finger  |
| ENSG00000181186.3 |                  |   | Arginine/serine rich pre-splicing factor   |
| ENSG00000170187.2 |                  |   | Unknown  |
| ENSG00000170160.1 | O60311           |   | Ankyrin repeat domain  |
| ENSG00000183855.1 |                  |   | UPF3   |
| ENSG00000128422.4 |                  |   | Keratin type I   |
| ENSG00000182235.1 |                  |   | Coactosin  |
| ENSG00000128438.1 | Q9H7M0           |   | Ubiquitin carboxyl terminal hydrolase  |
| ENSG00000108516.2 | TNFRSF13B        | Tumour necrosis factor receptor superfamily member 13b              | Tumour necrosis factor receptor superfamily member 13B transmembrane activator and CAML interactor |
| ENSG00000133030.3 | NM_015134        | Rho interacting protein 3   | TRIO and F actin binding protein   |
| ENSG00000179598.1 | NM_178836        | Similar to CG12314 gene product                                     | Ambiguous  |
| ENSG00000154803.2 | NM_144997        | Folliculin isoform 1  | Ambiguous  |
| ENSG00000183815.1 | NM_032686        |   | Unknown  |
| ENSG00000141030.1 | COPS3            | COP9 constitutive photomorphogenic homologue subunit 3              | COP9 homologue subunit 3   |
| ENSG0000010302.1  | NT5M             | 5',3'-nucleotidase, mitochondrial precursor                         | 5' 3' deoxyribonucleotidase cytosolic type   |
| ENSG00000141026.1 | NM_018019        |   | Ambiguous  |
| ENSG00000108551.1 | RASD1            | RAS, dexamethasone-induced 1  | RAS  |
| ENSG00000133027.4 | PEMT             | Phosphatidylethanolamine n-methyltransferase                        | Phosphatidylethanolamine n methyltransferase   |
| ENSG00000108557.4 | RAI1             | Retinoic acid induced 1 isoform 1                                   | Acid induced 1   |
| ENSG00000183564.1 | SMCR5            | Smith-Magenis syndrome chromosome region, candidate 5               | Smith-Magenis syndrome chromosome region candidate 5   |
| ENSG00000072310.2 | SREBF1           | Sterol regulatory element binding protein-1                         | Sterol regulatory element binding 1  |
| ENSG00000175715.3 |                  |   | Unknown  |
| ENSG00000182822.1 | Q9UF26           |   | Unknown  |
| ENSG00000175662.3 | <b>TOM1L2</b>    | <b>Target of myb 1-like 2</b>                                       | <b>Target of myb 1 src activating and signalling molecule</b>                                      |
| ENSG00000171962.3 | NM_031294        |   | E07 gene   |
| ENSG00000171953.2 | ATPAF2           | ATP synthase mitochondrial F1 complex assembly factor 2             | Ambiguous  |
| ENSG00000141034.1 | NM_024052        |   | Ambiguous  |
| ENSG00000108591.1 | DRG2             | Developmentally regulated GTP binding protein 2                     | Developmentally regulated GTP binding  |
| ENSG00000091536.3 | MYO15A           | Myosin XV   | Ambiguous  |
| ENSG00000091542.1 | NM_017758        |   | Alanine rich region/type I antifreeze protein  |
| ENSG00000131899.2 | LLGL1            | Lethal giant larvae homologue 1                                     | Lethal 2 giant larvae  |
| ENSG00000177731.1 | FLII             | Flightless-I protein homologue                                      | Actin  |
| ENSG00000177427.3 | SMCR7            | Smith-Magenis syndrome chromosome region, candidate 7               | Ambiguous  |
| ENSG00000177302.1 | TOP3A            | DNA topoisomerase III alpha   | DNA topoisomerase III  |
| ENSG00000176994.1 | SMCR8            | Smith-Magenis syndrome chromosome region, candidate 8               | Ambiguous  |
| ENSG00000176974.2 | SHMT1            | Serine hydroxymethyltransferase, cytosolic                          | Serine hydroxymethyltransferase  |
| ENSG00000170292.1 |                  |   | Unknown  |
| ENSG00000183027.2 |                  |   | Ubiquitin carboxyl terminal hydrolase  |
| ENSG00000131885.3 |                  |   | Keratin type I   |
| ENSG00000171916.3 |                  |   | Galectin   |
| ENSG00000167494.1 | NOS2B            | Nitric oxide synthase IIb   | Nitric oxide synthase  |
| ENSG00000178870.1 | NM_024974        |   | Ambiguous  |
| ENSG00000170299.2 |                  |   | Ankyrin repeat domain  |
| ENSG00000160516.1 | RPS28            | 40S ribosomal protein S28   | Unknown  |
| ENSG00000154874.1 | NM_182568        |   | Unknown  |
| ENSG00000174977.2 |                  |   | Polyadenylate binding interacting 1  |
| ENSG00000174969.2 | ZNF26            | Zinc finger protein 26  | Zinc finger  |
| ENSG00000174973.2 |                  |   | Unknown  |
| ENSG00000186060.1 |                  |   | Tripartite motif 16 oestrogen responsive b box   |
| ENSG00000171931.1 | C17orf1A         | Charcot-Marie-Tooth duplicated region transcript 1                  | Ambiguous  |
| ENSG00000171928.1 | FAM18B           |   | FAM18S   |
| ENSG00000183105.1 |                  |   | Unknown  |
| ENSG00000141127.1 | <b>PRPSAP2</b>   | <b>Phosphoribosyl pyrophosphate synthetase associated protein 2</b> | <b>Phosphoribosyl pyrophosphate synthetase associated</b>  |
| ENSG00000179727.2 | Q8N2W8           |   | Ambiguous  |
| ENSG00000154025.2 | NM_152351        |   | Sodium/glucose cotransporter   |
| ENSG00000154016.2 | GRAP             | GRB2-related adaptor protein  | Growth factor receptor bound 2 adapter/SH2/SH3 adapter   |
| ENSG00000072134.3 | EPN2             | Epsin 2 isoform B   | Ambiguous  |
| ENSG00000108641.1 | NM_015681        | B9 protein  | Unknown  |
| ENSG00000166484.4 | MAPK7            | Mitogen-activated protein kinase 7                                  | Mitogen activated kinase   |
| ENSG00000166482.3 | <b>MFAP4</b>     | <b>Microfibril associated glycoprotein 4 precursor</b>              | <b>Precursor</b>   |
| ENSG00000128482.2 | ZNF179           | Zinc finger protein 179   | Zinc finger 179 brain finger   |
| ENSG00000184001.1 | Q9BSW5           |   | 40S ribosomal S2   |
| ENSG00000142494.1 | NM_018242        |   | Ambiguous  |

**Table 1** Continued

| Ensembl gene ID          | External gene ID | Gene description                                    | Protein family description                     |
|--------------------------|------------------|---|--|
| ENSG00000072210.3        | ALDH3A2          | Fatty aldehyde dehydrogenase                        | Aldehyde dehydrogenase dimeric NADP preferring |
| ENSG00000180638.3        | NM_152908        |   | Ambiguous                                      |
| ENSG00000108602.4        | ALDH3A1          | Aldehyde dehydrogenase, dimeric nadp-preferring     | Aldehyde dehydrogenase dimeric NDAP preferring |
| ENSG00000083290.4        | ULK2             | UNC-51-like kinase 2                                | Serine/threonine kinase ULK1                   |
| ENSG00000108599.3        | AKAP10           | A kinase anchor protein 10, mitochondrial precursor | A kinase anchor 10 mitochondrial               |
| ENSG00000128487.4        | NM_152904        | Sperm antigen HCMOGT-1                              | Unknown  |
| ENSG00000154888.1        | Q9H3A7           |   | Ambiguous                                      |
| ENSG00000154895.4        | Q8IYA2           |   | Unknown  |
| ENSG00000154898.2        |                  |   | Ankyrin repeat domain                          |
| ENSG00000179302.2        |                  |   | UPF3   |
| ENSG00000170298.3        |                  |   | Galactin                                       |
| ENSG00000175417.2        |                  |   | Keratin type I                                 |
| ENSG00000175415.1        | NM_032249        |   | Ambiguous                                      |
| ENSG00000182906.2        |                  |   | Ubiquitin carboxyterminal hydrolase            |
| <b>ENSG00000124422.1</b> | <b>USP22</b>     | <b>Ubiquitin carboxyterminal hydrolase 22</b>       | <b>Ubiquitin carboxyterminal hydrolase 22</b>  |

The list was expanded to contain genes beyond the deletion borders since these genes might also be affected by the deletion. The genes are listed in their chromosomal order with *Ubb* as the most telomeric gene. Genes identified by SAGE are depicted in **bold**.

## DISCUSSION

Neuroblastoma is a complex disease with diverse clinical behaviour and largely unknown aetiology. Linkage studies in neuroblastoma families have suggested the existence of predisposing gene(s) in chromosomes 4 and 16,<sup>5 6</sup> but other studies have produced negative results in chromosome 16 and some of the regions commonly altered in neuroblastomas.<sup>5 7</sup> This suggests that multiple genes could be involved in neuroblastoma development and hence linkage found in some populations might not be detected in other populations. The observation of several deleted regions in neuroblastomas without gene identification raises the possibility that haploinsufficiency might also be involved. Thus, the genetic mechanisms involved in neuroblastoma development need to be clarified further, and additional analyses on neuroblastomas are of great importance as they can lead to better understanding, diagnosis, and management of the disease.

In the present work, we performed genetic analyses on a young patient with advanced stage neuroblastoma and metastasis of the disease. The patient was also diagnosed with SMS. According to prometaphase chromosome analysis, there was a 17p11.2 chromosome deletion in the normal tissue DNA. The deleted region was defined with microsatellite

marker analysis and was seen to lie between markers D17S1857 and D17S842.

In neuroblastoma, 17p deletions are rare.<sup>17-19</sup> However, chromosome 17p11.2 deletions have been frequently observed, for example in childhood primitive neuroectodermal tumours, where the commonly deleted region overlaps with the critical region deleted also in SMS.<sup>20</sup> Although 17p11.2 deletion is a characteristic aberration in SMS patients, there are rare cases where 17p11.2 deletions have not been detected. These cases are phenotypically consistent with SMS, but do not display some of the SMS associated features, such as heart defects or short stature.<sup>21</sup> Recently, three such patients were identified with truncating mutations in *Rai1*,<sup>21</sup> which was later cloned from a neuroblastoma cell line.<sup>22</sup> *Rai1* has been associated with neuronal differentiation in mouse studies.<sup>23</sup> Thus *Rai1* could be responsible for the behavioural, neurological, otolaryngological, and craniofacial anomalies of SMS, and the other features of this syndrome could result from hemizyosity of other genes in the 17p11.2 region.<sup>21</sup>

To our knowledge, there have been no reports of tumour predisposition associated with SMS, although the syndrome is characterised by variable phenotypic features. This argues

**Table 2** *MYCN*, *Meis1*, or *TrkA* regulated gene expression in SH-EP, SJ-NB8, and SH-SY5Y neuroblastoma cell lines

| Gene           | Start position, bp | SAGE tag(s) | SH-EP       |           | SJ-NB8       |          | SH-SY5Y     |           |
|----------------|--------------------|-------------|-------------|-----------|--------------|----------|-------------|-----------|
|                |                    |             | <i>MYCN</i> |           | <i>Meis1</i> |          | <i>TrkA</i> |           |
|                |                    |             | Off         | On        | Off          | On       | Off         | On        |
| <i>Ubb</i>     | 16 484 927         | GTAGCAAAAA  | 23          | 12        | 15           | 12       | 17          | 13        |
|                |                    | GTAGCATAAA  | 32          | 17        | 43           | 57       | 55          | 48        |
|                |                    | GCAATTCGCAG | 0           | 0         | 12           | 1        | 2           | 0         |
|                |                    | Total       | <b>55</b>   | <b>29</b> | 70           | 70       | 74          | 61        |
| <i>Tom1l2</i>  | 17 950 706         | TAAATACTC   | 9           | 0         | 0            | 0        | 2           | 0         |
|                |                    | TGGCAGCTG   | 0           | 0         | 0            | 0        | 2           | 0         |
|                |                    | Total       | <b>9</b>    | <b>0</b>  | 0            | 0        | <b>4</b>    | <b>0</b>  |
|                |                    | CTTGTCCTC   | 0           | 0         | <b>5</b>     | <b>0</b> | 0           | 0         |
| <i>Prpsap2</i> | 18 962 058         | AGGGAGCAGA  | <b>2</b>    | <b>15</b> | <b>12</b>    | <b>5</b> | <b>0</b>    | <b>21</b> |
|                |                    | GCCITTCCT   | 0           | 9         | 6            | 1        | 4           | 4         |
| <i>Mfap4</i>   | 19 448 786         | TTTTATAAAA* | 2           | 3         | 5            | 0        | 7           | 0         |
|                |                    | Total       | <b>2</b>    | <b>12</b> | <b>11</b>    | <b>1</b> | <b>11</b>   | <b>4</b>  |
| <i>Usp22</i>   | 21 068 373         | TTTTATAAAA* | 2           | 3         | 5            | 0        | 7           | 0         |
|                |                    | Total       | <b>2</b>    | <b>12</b> | <b>11</b>    | <b>1</b> | <b>11</b>   | <b>4</b>  |

The SAGE tag frequencies are normalised per 100 000 transcripts in the cell. Potentially relevant gene expression differences are depicted in **bold**. \*Tag can also belong to two other, unrelated genes.

against the existence of important tumour suppressors in the SMS commonly deleted region. The phenotypic variability of SMS can perhaps be partly explained by the differences in the 17p11.2 deletion size. The deletion size can vary because of the occurrence of alternative low copy repeats (LCRs) in the 17p11.2 region.<sup>24</sup> LCRs can act as substrates for non-allelic homologous recombination that results in deletion, duplication, or inversion of the genomic segments flanked by the LCRs.<sup>25</sup> The deletion detected in our neuroblastoma patient is larger than those typically seen in SMS patients.<sup>26</sup> Thus the genes locating closer to the deletion breakpoints, rather than the SMS commonly deleted region, might be of particular interest for neuroblastoma predisposition.

We analysed the deleted 17p11.2 region further with array CGH and SAGE. The array CGH data correlated well with the results obtained from the microsatellite analysis and no other clear copy number changes were detected in the patient's normal tissue DNA. However, most genes within the deleted region have unknown functions. SAGE data was used to identify any 17p11.2 genes that could be involved in neuroblastoma development. The downstream targets of *MYCN*, *Meis1*, and *TrkA* pathways were examined in three neuroblastoma cell lines. *MYCN* and *Meis1* are oncogenes, and their downregulated targets might be associated with differentiation. Haploinsufficiency of these target genes due to the deletion could potentially block a differentiation step and thereby promote pathogenesis of neuroblastoma. *TrkA* expression is associated with good prognosis of neuroblastoma, and may therefore upregulate genes with differentiation potential. Similarly, a deletion of the upregulated genes could block differentiation.

The SAGE analyses of the neuroblastoma pathways identified five genes in the deleted region that could have a role in neuroblastoma development. *MYCN* was shown to regulate the expression of *Ubb* and *Tom112*, which are involved in protein degradation and trafficking, respectively. An aberrant form of *Ubb* has also been detected in the cerebral cortex of Alzheimer's and Down's syndrome patients.<sup>27</sup> Other candidate genes downregulated by *Meis1* or upregulated by *TrkA* were *Mjap4*, *Usp22*, and *Prpsap2*, which have functions related to cell adhesion, protein degradation, and synthesis of nucleotides and histidine, respectively.<sup>28–29</sup> Our SAGE data suggest that these five genes could have a role in the previously identified neuroblastoma pathways, and further analyses of their roles in neuroblastoma may therefore be of interest.

In addition to the genes identified by SAGE, one of the possible candidates within the 17p11.2 region is *LLGL1*, a human homologue of the *Drosophila* lethal giant larvae gene, which is associated with neuroblast development in *Drosophila*.<sup>30</sup> However, *LLGL1* was not represented in the array CGH chip or SAGE.

In this paper, we report a germline 17p11.2 deletion in a neuroblastoma patient who had advanced stage and metastatic disease but was cured with extensive treatment. No LOH of the 17p11.2 region was found in a limited set of sporadic neuroblastomas. As the deletion might be a random event with no tumorigenic implications, additional samples of different clinical types need to be analysed to obtain more detailed information on the possible importance of the affected region in neuroblastoma development. Although some attractive candidate genes reside in the deleted region according to our array CGH analysis and SAGE data, genes with unknown or less attractive functions also have to be considered. Genotyping a more extensive neuroblastoma sample set might aid in confirming the putative association between 17p11.2 and neuroblastoma, and further fine map the relevant region to form solid basis for candidate gene analyses by sequencing and other methods.

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