ONLINE MUTATION REPORT

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

T Hienonen, H Sammalkorpi, P Isohanni, R Versteeg, R Karikoski, L A Aaltonen

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Neuroblastoma is the most frequently occurring extracranial 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.^{1, 2}

Several acquired genetic changes have been described in neuroblastoma, the most frequent being *MYCN* oncogene amplification, 1p deletion, and 17q amplification.³ 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.⁴ 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.^{5, 6} However, the chromosome 16p region was tested for linkage with negative results in Italian and British families.^{2, 7} In addition, the known candidate neuroblastoma loci that are frequently altered sporadically have been excluded in some neuroblastoma families.^{5, 7} 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.⁸⁻¹⁰ 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.*¹¹

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,^{12,13} and of *TrkA*, the expression of which is associated with neuroblastomas with good prognosis.¹⁴ 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.^{15,16}

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 *Tom1l2*, 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	40S ribosomal s27a
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	TOM1L2	Target of myb1-like 2	Target of myb 1 src activating and signalling molecule
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	PRPSAP2	Phosphoribosyl pyrophosphate synthetase associated protein 2	Phosphoribosyl pyrophosphate synthetase associated
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	MFAP4	Microfibril associated glycoprotein 4 precursor	Precursor
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			Galectin
ENSG00000175417.2			Keratin type I
ENSG00000175415.1	NM_032249		Ambiguous
ENSG00000182906.2			Ubiquitin carboxyterminal hydrolase
ENSG00000124422.1	USP22	Ubiquitin carboxyterminal hydrolase 22	Ubiquitin carboxyterminal hydrolase 22

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 16,⁵⁻⁶ but other studies have produced negative results in chromosome 16 and some of the regions commonly altered in neuroblastomas.⁵⁻⁷ 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.¹⁷⁻¹⁹ 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.²⁰ 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.²¹ Recently, three such patients were identified with truncating mutations in *Rai1*,²¹ which was later cloned from a neuroblastoma cell line.²² *Rai1* has been associated with neuronal differentiation in mouse studies.²³ 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 hemizygoty of other genes in the 17p11.2 region.²¹

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	GTAGCAAAA	23	12	15	12	17	13
		GTAGCATAAA	32	17	43	57	55	48
		GCAATCGCAG	0	0	12	1	2	0
		Total	55	29	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	9	0	0	0	4	0
		CTTGTCCTC	0	0	5	0	0	0
<i>Prpsap2</i>	18 962 058	AGGGAGCAGA	2	15	12	5	0	21
<i>Mfap4</i>	19 448 786	GCCTTTCCT	0	9	6	1	4	4
<i>Usp22</i>	21 068 373	TTTATAAAA*	2	3	5	0	7	0
		Total	2	12	11	1	11	4

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.²⁴ 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.²⁵ The deletion detected in our neuroblastoma patient is larger than those typically seen in SMS patients.²⁶ 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 *Tom1l2*, 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.²⁷ 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.^{28–29} 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*.³⁰ 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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Authors' affiliations

T Hienonen, H Sammalkorpi, L A Aaltonen, Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland
P Isohanni, Department of Medical Genetics, The Family Federation of Finland, Helsinki, Finland
P Isohanni, Department of Pediatric Neurology, Hospital for Children and Adolescents, Helsinki University Central Hospital, Helsinki, Finland
R Versteeg, Departments of Human Genetics and Pediatric Oncology, Academic Medical Center, Amsterdam, The Netherlands
R Karikoski, Central Pathology Laboratory, Helsinki University Hospital Laboratory Diagnostics, Helsinki, Finland

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The first two authors contributed equally to this study.

Correspondence to: L A Aaltonen, Department of Medical Genetics, Biomedicum Helsinki, P.O. Box 63, FIN-00014 University of Helsinki, Finland; lauri.aaltonen@helsinki.fi

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