Am. J. Hum. Genet. 72:213–216, 2003

Early-Onset Brain Tumor and Lymphoma in *MSH2*-Deficient Children

To the Editor:

Homozygous germline mutations of *MLH1* have been reported so far in three families with hereditary nonpolyposis colorectal cancer (HNPCC [MIM 114500]) and have been shown to be associated with leukemia or lymphoma, CNS tumors, and the neurofibromatosis type I phenotype (Ricciardone et al. 1999; Wang et al. 1999; Vilkki et al. 2001). More recently, the first case of a homozygous germline mutation of *MSH2* was described in a child with leukemia and multiple café-au-lait spots (Whiteside et al. 2002). We report here the incidental discovery of a new case of *MSH2* deficiency, which is remarkable because of the presentation of the family and because of the association with an early-onset brain tumor.

The proband (III.2) and her husband (III.1), of French origin, were seen for genetic counseling in the dramatic context of the death of their two children (fig. 1). Individual IV.1 died at age 15 mo from a T mediastinal lymphoma; her brother (IV.2) died at age 4 years from a temporal glioblastoma. Their mother and father, age 29 years and 32 years, respectively, had no personal history of cancer. Although several second-degree relatives of the parents had developed cancers (fig. 1), the presentation of the family did not fulfill the criteria for a Mendelian genetic predisposition to cancer. The development of a CNS tumor and lymphoma in two sibs led us to consider initially the hypothesis of Li-Fraumeni syndrome (LFS) in this family. Since no DNA was available from the affected children, we analyzed the *TP53* gene in both unaffected parents. Sequencing analysis of *TP53* revealed no mutation. Stimulated by our recent finding of a family with LFS with complete heterozygous germline deletion of *TP53* (unpublished data), we completed the analysis of *TP53* by searching for a similar defect using quantitative multiplex PCR of short fluorescent fragments (QMPSF) (Charbonnier et al. 2000,

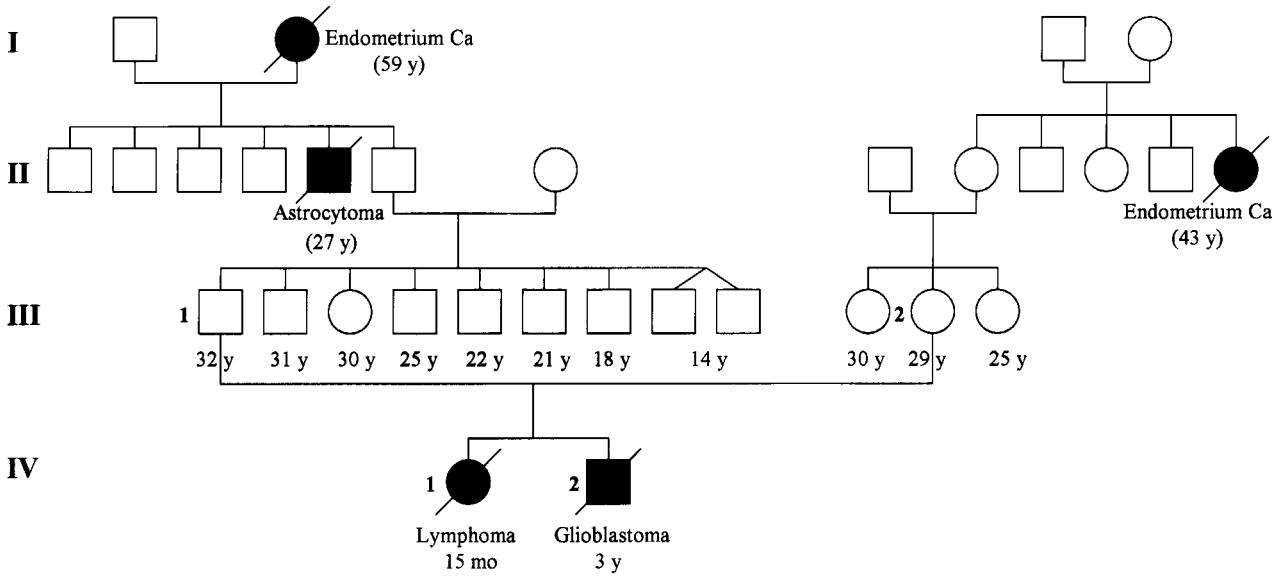

Figure 1 Partial pedigree of the family. Filled symbols denote affected subjects; open symbols denote asymptomatic subjects; oblique line denote deceased. Numbers beside symbols are subject identifiers. The ages of unaffected individuals are indicated. For affected subjects, the tumor type and the age at diagnosis or of death (in parentheses) are indicated. Ca = cancer; y = years; mo = months.

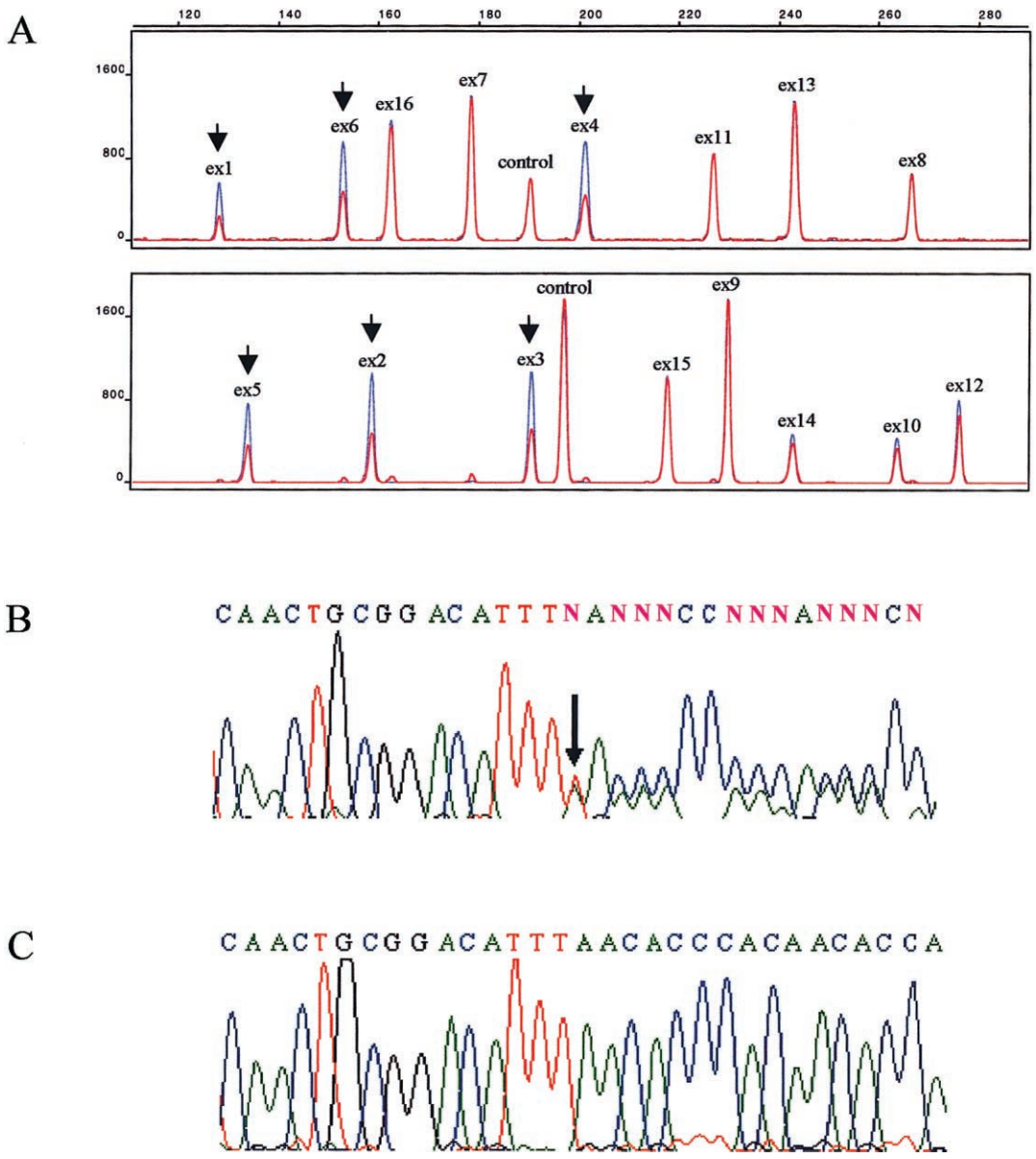

Figure 2 Detection of the *MSH2* alterations. *A*, Heterozygous deletion of *MSH2* exons 1–6 in the father (individual III.1) detected by QMPSF. The electropherogram of the father (*red*) was superposed on that of a control individual (*blue*). The *Y*-axis displays fluorescence in arbitrary units, and the *X*-axis indicates the size in bp. This result was obtained on two independent samples. *B*, Heterozygous 1-bp deletion within exon 3 detected in the mother (individual III.2). *C*, Hemizygous 1-bp deletion within exon 3, detected in the brain tumor developed in individual IV.2. In panels *B* and *C*, sequences correspond to the noncoding strand.

2002). QMPSF analysis of *TP53* performed in the father (III.1) demonstrated that the *TP53* gene was not affected, but, to our surprise, revealed a heterozygous deletion of *MSH2* exon 3 corresponding to the control amplicon. Therefore, we analyzed, by QMPSF, the 16 exons of *MSH2*, and this analysis showed the presence, in the unaffected father, of a heterozygous genomic deletion of *MSH2* removing exons 1–6 (fig. 2*A*). We then sequenced the *MSH2* gene in the unaffected mother (III.2) and identified a 1-bp heterozygous deletion at codon 153 within exon 3 (fig. 2*B*). In the absence of constitutional DNA from the affected children, we sequenced *MSH2* exon 3 from the glioblastoma DNA of individual IV.2. As shown in figure 2*C*, we detected only the mutant maternal allele, which strongly suggested that individual IV.2 had received from his father the mutant allele harboring the exons 1–6 deletion. Haplotype analysis at the *MSH2* locus confirmed the presence of two parental *MSH2* alleles within the tumor, ruling out a somatic loss of heterozygosity (data not shown).

Screening for microsatellite instability (MSI), as recommended by Boland et al. (1998), revealed no replication error (RER) phenotype within the glioblastoma.

This case report shows that *MSH2* deficiency in humans can result in early-onset CNS tumors. Homozygous *MLH1* mutations have been detected in two children who had developed a medulloblastoma (Wang et al. 1999) and a glioma (Vilkki et al. 2001). Compound heterozygous mutations of the *PMS2* gene, which is rarely involved in HNPCC, have been identified in two sisters with early-onset brain and colorectal tumors (De Rosa et al. 2000). These studies, together with the present report, indicate that germline *MMR* deficiency predisposes to primary early-onset neuroepithelial tumors. Turcot syndrome (MIM 276300) was originally defined by the association of CNS malignant tumors with familial polyposis of the colon, but molecular studies have subsequently distinguished two entities, resulting from *APC* and *MMR* gene mutations, respectively (Hamilton et al. 1995; Paraf et al. 1997). It is tempting to speculate, as suggested elsewhere (De Rosa et al. 2000), that, in some families with Turcot syndrome, the association of colorectal neoplasms with childhood brain tumors may be due to a complete MMR deficiency.

We were surprised that we could not detect an RER phenotype in the *MSH2*-deficient brain tumor. It is interesting that MSI was not also detected in the nontumoral DNA of the *MSH2*-deficient patient reported by Whiteside et al. (2002), in contrast to the case of the two *MLH1*-deficient subjects analyzed by Wang et al. (1999) and Vilkki et al. (2001). This result could suggest that, at least in certain tissues, *MSH2* deficiency could lead to tumorigenesis through a mechanism distinct from a defect in the repair of postreplicative mismatches affecting repetitive sequences. Indeed, MSH2, like its bacterial homolog, MutS, has been shown to play additional roles in genetic recombination, since these proteins prevent exchange between divergent DNA sequences (Modrich and Lahue 1996). Furthermore, MSH2 was recently shown to be associated with TP53 within recombinative repair complexes during S phase (Zink et al. 2002).

As in the previous report of a *MSH2* deficiency (Whiteside et al. 2002), the familial history presented in this study was not strongly suggestive of HNPCC, although the young age of the parents and their sibs could explain the absence of cancer within this generation. Therefore, as shown in this report, the presence of homozygous mutations of the different *MMR* genes must be considered in families with early-onset CNS tumors and hematological malignancies, even in the absence of a familial history of HNPCC.

Acknowledgments

We are grateful to Mario Tosi for critical review of the manuscript and to Lucy B. Rorke from The Children's Hospital of Philadelphia for the expert examination of the glioblastoma. This work was supported by l'Association pour la Recherche sur le Cancer, La Fondation pour la Recherche Médicale, and La Ligue Nationale Contre le Cancer. G.B. was supported by a grant from Le Ministère de la Recherche.

Gaëlle Bougeard,[1] Françoise Charbonnier,[2] Alexandre Moerman,[3] Cosette Martin,[2] Marie M. Ruchoux,[4] Nathalie Drouot,[2] and Thierry Frébourg[1,2]

[1]INSERM EMI 9906–IFRMP, Faculty of Medicine, and [2]Department of Genetics, Centre Hopitalo-Universitaire of Rouen, Rouen; and Departments of [3]Genetics and [4]Neuropathology, Hôpital Roger Salengro, Centre Hopitalo-Universitaire of Lille, Lille, France

Electronic-Database Information

Accession numbers and URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for hereditary nonpolyposis colorectal cancer [MIM 114500] and Turcot syndrome [MIM 276300])

References

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S (1998) A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58:5248–5257

Charbonnier F, Olschwang S, Wang Q, Boisson C, Martin C, Buisine MP, Puisieux A, Frébourg T (2002) MSH2 in contrast to MLH1 and MSH6 is frequently inactivated by exonic and promoter rearrangements in hereditary nonpolyposis colorectal cancer. Cancer Res 62:848–853

Charbonnier F, Raux G, Wang Q, Drouot N, Cordier F, Limacher JM, Saurin JC, Puisieux A, Olschwang S, Frébourg T (2000) Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments. Cancer Res 60: 2760–2763

De Rosa M, Fasano C, Panariello L, Scarano MI, Belli G, Iannelli A, Ciciliano F, Izzo P (2000) Evidence for a recessive inheritance of Turcot's syndrome caused by compound heterozygous mutations within the PMS2 gene. Oncogene 19: 1719–1723

Hamilton SR, Liu B, Parsons RE, Papadopoulos N, Jen J, Powell SM, Krush AJ, Berk T, Cohen Z, Tetu B, Burger PC, Wood PA, Taqi F, Booker SV, Petersen GM, Offerhaus GJA, Tersmette AC, Giardiello FM, Vogelstein B, Kinzler KW (1995) The molecular basis of Turcot's syndrome. N Engl J Med 332:839–847

Modrich P, Lahue R (1996) Mismatch repair in replication

fidelity, genetic recombination and cancer biology. Annu Rev Biochem 65:101–133
Paraf F, Jothy S, Van Meir EG (1997) Brain tumor-polyposis syndrome: two genetic diseases? J Clin Oncol 15:2744–2758
Ricciardone MD, Özçelik T, Cevher B, Özdag H, Tuncer M, Gürgey A, Uzunalimoglu Ö, Çetinkaya H, Tanyeli A, Erken E, Öztürk M (1999) Human MLH1 deficiency predisposes to hematological malignancy and neurofibromatosis type 1. Cancer Res 59:290–293
Vilkki S, Tsao JL, Loukola A, Pöyhönen M, Vierimaa O, Herva R, Aaltonen LA, Shibata D (2001) Extensive somatic microsatellite mutations in normal human tissue. Cancer Res 61: 4541–4544
Wang Q, Lasset C, Desseigne F, Frappaz D, Bergeron C, Navarro C, Ruano E, Puisieux A (1999) Neurofibromatosis and early onset of cancers in hMLH1-deficient children. Cancer Res 59:294–297
Whiteside D, McLeod R, Graham G, Steckley JL, Booth K, Somerville MJ, Andrew SE (2002) A homozygous germ-line mutation in the human MSH2 gene predisposes to hematological malignancy and multiple café-au-lait spots. Cancer Res 62:359–362
Zink D, Mayr C, Janz C, Wiesmüller L (2002) Association of p53 and MSH2 with recombinative repair complexes during S phase. Oncogene 21:4788–4800

Address for correspondence and reprints: Dr. Thierry Frébourg, INSERM EMI 9906, Faculty of Medicine, 22 Boulevard Gambetta, 76183 Rouen, France. E-mail: Frebourg@chu-rouen.fr

0002-9297/2003/7201-0024$15.00

Am. J. Hum. Genet. 72:216–218, 2003

Hereditary p16-Leiden Mutation in a Patient with Multiple Head and Neck Tumors

To the Editor:

Recent studies have shown that most Dutch families with atypical multiple-mole melanoma (FAMMM) have a 19-bp germline deletion (p16-Leiden) in exon 2 of the p16 gene (p16 [MIM 600160]) (Gruis et al. 1995; van der Velden et al. 1999, 2001). Incomplete penetrance and variable clinical expression in p16-Leiden carriers point to the fact that other genetic mechanisms can compensate for the p16 loss of function (Gruis et al. 1995). Indeed, van der Velden et al. (2001) reported in the *Journal* that variants in the melanocortin-1 receptor modify the risk of melanoma in p16-Leiden carriers. It is interesting that, apart from reports on simultaneous pancreatic tumors, other cancer types have never been found in such families with p16-Leiden (Gruis et al. 1995).

Recently, we found a hereditary heterozygous p16-Leiden mutation in a man who neither smoked more than five cigarettes daily nor abused alcohol, initially diagnosed at age 54 years, who simultaneously developed three carcinomas of the pharynx and oral cavity. The patient showed a familial accumulation of tumor diseases. Both of his parents and his only sister died of cancer very early (the mother of gynecologic cancer, the father of liver carcinoma, and the sister of leukemia). Dutch relatives are not known.

DNA from tumors and blood was extracted according to a standard protocol (Sambrook et al. 1989). Mutation analysis of exon 1 and exon 2 of the p16 gene was done by PCR-SSCP sequencing as described by Schneider-Stock et al. (1998).

The p16-Leiden mutation was found in the heterozygous constitution in all three tumors and in the blood of the patient. We investigated all DNA samples for p16 promotor methylation to check the status of the retained wild-type allele and, thus, to assess the real functional significance of this finding. The methylation status of the p16 promotor was determined by methylation-specific PCR (Herman et al. 1996). The primer sequences for the unmethylated reactions were (sense) 5′-TTA TTA GAG GGT GGG GTG GAT TGT-3′ and (antisense) 5′-CAA CCC CAA ACC ACA ACC ATA A-3′, which amplify a 151-bp product. The primer sequences for the methylated reaction were (sense) 5′-TTA TTA GAG GGT GCG GAT CGC-3′ and (antisense) 5′-GAC CCC GAA CCG CGA CCG TAA-3′, which amplify a 150-bp product. The two forward primers were labeled with FAM dye. PCR was done using 1/10 of bisulfite reaction and a MasterAmp Optimization Kit (Biozym) in an automated thermocycler (PTC 200) according to the manufacturer's instructions. PCR products were analyzed using an ABI310 sequencer (injection time between 20–30 s, Pop 4, dRhodamine Matrix standard ABI Prism [Perkin Elmer]). The p16 promotor was methylated in all three tumor samples but not in the blood of the patient (fig. 1).

This result led us to suggest a loss of p16 protein expression and, thus, a complete loss of p16 tumor suppressor function. For p16 immunohistochemistry, a monoclonal mouse antibody to p16 (1:100 dilution, Quartett) was used according to the manufacturer's instructions. Indeed, all tumor sections were immunohistochemically p16-negative. The third tumor showed an additional gross rearrangement in the p53 gene (75-bp insertion in exon 4).

This is the first report on p16-Leiden mutation in orolaryngeal carcinomas, although p16 alterations are very common in this tumor type (50% p16 promotor methylation [Bittencourt Rosas et al. 2001], 30% p16 mutations [Esteller et al. 2001*a*; Poi et al. 2001]). Because of the heterozygous p16-Leiden constitution, our proband was a suitable model for studying the type of p16 inactivation in the three metachronous carcinomas. To date, the methylation status of p16-Leiden carriers has

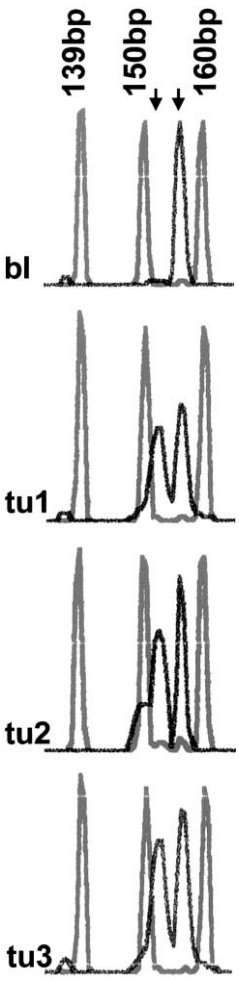

Figure 1 Analysis of $p16^{INK4}$ promotor methylation on ABI310 Prism. Methylated (*left arrow*) and unmethylated (*right arrow*) signals can be detected between the 150-bp and the 160-bp ROX matrix standards. There is a near-equal amplification of unmethylated and methylated DNA in the three head and neck tumors but no methylation in the blood DNA of the patient; bl = blood DNA; tu1–tu3 = tumors 1–3, respectively.

never been checked. To the best of our knowledge, this is one of the only a few clear examples that aberrant promotor methylation might function as the "second genetic hit" in a familial cancer syndrome. Whereas no methylation could be recognized in the blood of the patient's DNA, all three tumors showed inactivation of the retained wild-type allele, with the somatic event being aberrant promotor methylation. There are only a few reports demonstrating the role of promotor methylation for biallelic gene inactivation (Grady et al. 2000; Esteller et al. 2001*b*). Furthermore, it has to be mentioned that the p16-Leiden mutation should also affect the $p14^{ARF}$ transcript, because alternative splicing of the first exon and common downstream exons permit this locus to encode two different products that regulate the cell cycle via two distinct pathways.

It is noteworthy that contact with the patient began five months before the actual date of examination when the patient attended a talk. His presence was not because of symptoms of a tumor but because of genetic findings. This early meeting led to discovery of the tumor of the tongue while the tumor was still in a less-advanced stage. Therefore, the tumor could be easily resected, leaving the tongue almost unaffected. Consequently, the patient is now in good physical condition (Karnofsky 70%–80%). The patient was informed about the hereditary mutation and was included in the risk program for upper-abdominal screening because p16-Leiden mutation has been reported to be a risk factor for developing pancreatic carcinoma (Lal et al. 2000; Vasen et al. 2000; Lynch et al. 2002).

We think that regarding head and neck cancer, our data is novel and may have consequences for further studies of families with FAMMM.

Acknowledgments

We thank the patient who consented to the study and gave his blood sample. A.G. is a Ph.D. student supported by the Graduierten-Kollege of the Medical Faculty of the Otto-von-Guericke University, Magdeburg.

Regine Schneider-Stock,[1] Anja Giers,[2] Christiane Motsch,[2] Carsten Boltze,[1] Matthias Evert,[1] Bernd Freigang,[2] and Albert Roessner[1]

Departments of [1]Pathology and [2]Otohinolaryngology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany

Electronic-Database Information

The accession number and URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for p16 and p14 [MIM 600160])

References

Bittencourt Rosas SL, Koch W, da Costa Carvalho MG, Wu L, Califano J, Westra W, Jen J, Sidransky D (2001) Promotor hypermethylation pattern of p16, O^6-methylguanine-DNA-methyltransferase, and death-associated protein kinase in tumors and saliva of head and neck cancer patients. Cancer Res 61:939–942

Esteller M, Corn PG, Maylin SB, Herman JG (2001*a*) A gene hypermethylation profile of human cancer. Cancer Res 61: 3225–3229

Esteller M, Fraga M, Guo M, Gacia-Foncillas J, Hedenfalk I, Godwin AK, Trojan J, Vaurs-Barriere C, Bignon YJ, Ramus S, Benitez J, Caldes T, Akiyama Y, Yuasa Y, Launonen V, Canal MJ, Rodriguez R, Capella G, Peinado MA, Borg A,

Aaltonen LA, Ponder BA, Baylin SB, Herman JG (2001*b*) DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet 10:3001–3007

Grady WM, Willis J, Guilford PJ, Dunbier AK, Toro TT, Lynch H, Wiesner G, Ferguson K, Eng C, Park JG, Kim SJ, Markowitz S (2000) Methylation of the CDH1 promotor as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet 26:16–17

Gruis NA, van der Velden P, Sandkuijl LA, Prins DE, Weaver-Feldhaus J, Kamb A, Bergman W, Frants RR (1995) Homozygotes for CDKN2 (p16) germline mutation in Dutch familial melanoma kindreds. Nat Genet 10:351–353

Herman JG, Graff JR, Myohänen S, Nelkin BD, Maylin SB (1996) Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA 93:9821–9826

Lal G, Liu L, Hogg D, Lassam NJ, Redston MS, Gallinger S (2000) Patients with both pancreatic adenocarcinoma and melanoma may harbor germline CDKN2A mutations. Genes Chrom Cancer 27:358–361

Lynch HT, Brand RE, Hogg D, Deters CA, Fusaro RM, Lynch JF, Li L, Knezetic J, Lassam NJ, Goggins M, Kern S (2002) Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma-pancreatic carcinoma-prone families: the familial atypical mole melanoma-pancreatic carcinoma syndrome. Cancer 94:84–96

Poi MJ, Yen T, Li J, Song H, Lang JC, Schuller DE, Pearl DK, Casto B, Tsai MD, Weghorst CM (2001) Somatic INK4a-ARF locus mutations: a significant mechanism of gene inactivation in squamous cell carcinomas of the head and neck. Mol Carcinog 30:26–36

Sambrook J, Fritsch EF, Maniatis T (2nd ed) (1989) Molecular cloning—a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Schneider-Stock R, Walter H, Haeckel C, Radig K, Rys J, Roessner A (1998) Gene alterations at the CDKN2A (p16/MTS1) locus in soft tissue tumors. Int J Oncol 13:325–329

van der Velden PA, Sandkuijl LA, Bergman W, Hille ETM, Frants RR, Gruis NA (1999) A locus linked to p16 modifies melanoma risk in Dutch familial atypical multiple mole melanoma (FAMMM) syndrome families. Genome Res 9:575–580

van der Velden PA, Sandkuijl LA, Bergman W, Pavel S, van Mourik L, Frants RR, Gruis NA (2001) Melanocortin-1 receptor variant R151C modifies melanoma risk in Dutch families with melanoma. Am J Hum Genet 69:774–779

Vasen HFA, Gruis NA, Frants RR, van der Velden PA, Hille ETM, Bergman W (2000) Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-Leiden). Int J Cancer 87:809–811

Address for correspondence and reprints: Dr. Regine Schneider-Stock, Molecular Tumor Genetics Lab, Department of Pathology, Otto-von-Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany. E-mail: Regine.Schneider-Stock@medizin.uni-magdeburg.de

0002-9297/2003/7201-0025$15.00

Am. J. Hum. Genet. 72:218–219, 2003

Another Case of Imprinting Defect in a Girl with Angelman Syndrome Who Was Conceived by Intracytoplasmic Sperm Injection

To the Editor:

Intracytoplasmic sperm injection (ICSI) has been established as an efficient treatment for male infertility and also as a supplement to in vitro fertilization (IVF) without obvious male infertility. ICSI is now regarded as a procedure that is safe overall, and no increase in developmental delay was found in a follow-up of 221 ICSI-conceived children in the 2nd year of life (Sutcliffe et al. 2001). However, the possibility of an increased risk of imprinting defects has been raised (Manning et al. 2000). Two children conceived by ICSI who had Angelman syndrome (AS [MIM 105830]) due to a presumably sporadic imprinting defect have recently been reported (Cox et al. 2002).

We here report a 3.5-year-old girl with AS due to a sporadic imprinting defect, born of a pregnancy that was also the result of ICSI. The girl was the first child of a 35-year-old mother and a 36-year-old father. The father has a healthy daughter by another partner, and sperm analysis was normal on three different occasions. The mother had one spontaneous abortion and two extrauterine pregnancies before treatment with IVF. Traditional IVF did not result in fertilized eggs, and ICSI was therefore performed in spite of the normal sperm analysis of the father. The first ICSI pregnancy resulted in another spontaneous abortion, whereas the second ICSI procedure resulted in a normal pregnancy. Birth was at term, birth weight was 3,760 g, length was 54 cm, and head circumference was 36 cm (75th percentile). Development was considered normal for the first 3–4 mo, after which she started to have infections. She walked at age 2 years. She had no epilepsy but had an abnormal electroencephalogram with large-amplitude slow-spike waves. There was no language development. Chromosomes, including subtelomeres, were normal. At age 3 years, her height and weight were at the 50th percentile, whereas her head circumference was 1 cm below the 2.5th percentile. She was mentally retarded and atactic. She was dysmorphic, with a square face, deep-set eyes, and a protruding tongue.

FISH analysis using the *SNRPN* probe (MIM 182279), as well as microsatellite studies, revealed normal chromosomes 15 of biparental origin. A common large deletion of 15q11-q13 and uniparental paternal disomy could therefore be excluded. Methylation-specific Southern blot analysis and methylation-specific PCR (Zeschnigk et al. 1997) for the *SNRPN* locus showed the presence of a normal unmethylated paternal band and the complete ab-

sence of a methylated maternal band, indicating that the patient had an imprinting defect. Quantitative Southern blot analysis of the critical AS imprinting center (IC) region (Buiting et al. 1999) showed a normal dosage; therefore, an IC deletion was unlikely. This result was confirmed by sequence analysis of the 880-bp AS-IC element, where the patient was heterozygous for three different SNPs. Both parents had normal chromosomes and a normal methylation pattern. These findings suggest that the patient belongs to the group of patients with a sporadic imprinting defect (Buiting et al. 1998).

Both patients reported by Cox et al. (2002) had fathers with sperm abnormalities, and the possibility that the imprinting defect could be related to male infertility was discussed. The father of the child reported here had normal sperm, and a relationship to male infertility is therefore unlikely. However, similar to the mother, the maternal grandmother also had a history of reproductive difficulties. In addition to three healthy children, she had four spontaneous abortions and one daughter who was stillborn at term. Since both the maternal grandmother and the mother had reproductive difficulties, a maternal oogenesis defect cannot be excluded.

A sporadic imprinting defect is a very rare cause of AS, and Cox et al. (2002) therefore considered a relationship to the ICSI procedure to be likely. The report of a third patient with this rare disorder further supports the assumption that ICSI can lead to an increased risk for imprinting defects.

K. H. Ørstavik,[1,3] K. Eiklid,[5] C. B. van der Hagen,[4,5] S. Spetalen,[7] K. Kierulf,[6] O. Skjeldal,[2] and K. Buiting[8]

Departments of [1]Medical Genetics and [2]Pediatrics, Rikshospitalet, [3]Institute Group of Clinical Medicine and [4]Institute of Medical Genetics, University of Oslo, Departments of [5]Medical Genetics and [6]Gynecology, Ulleval University Hospital, and [7]Huseby Resource Center, Oslo; and [8]Institut für Humangenetik, Universität Essen, Essen

Electronic-Database Information

Accession numbers and the URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for the *AS* gene [MIM 105830] and the *SNRPN* probe [MIM 182279])

References

Buiting K, Dittrich B, Groß S, Lich C, Färber C, Buchholz T, Smith E, et al (1998) Sporadic imprinting defects in Prader-Willi syndrome and Angelman syndrome: implications for imprint-switch models, genetic counseling, and prenatal diagnosis. Am J Hum Genet 63:170–180

Buiting K, Lich C, Cottrell S, Barnicoat A, Horsthemke B (1999) A 5-kb imprinting center deletion in a family with Angelman syndrome reduces the shortest region of deletion overlap to 880 bp. Hum Genet 105:665–666

Cox GF, Bürger J, Lip V, Ulrike A, Mau UA, Sperling K, Wu BL, Horsthemke B (2002) Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet 71:162–164

Manning M, Lissens W, Bonduelle M, Camus M, De Rijcke M, Liebaers I, Van Steirteghem A (2000) Study of DNA-methylation patterns at chromosome 15q11-q13 in children born after ICSI reveals no imprinting defects. Mol Hum Reprod 6: 1049–1053

Sutcliffe AG, Taylor B, Saunders K, Thornton S, Liebermann BA, Grudzinskas JG (2001) Outcome in the second year of life after in-vitro fertilisation by intracytosplamic sperm injection: a UK case-control study. Lancet 357:2080–2084

Zeschnigk M, Lich C, Buiting K, Horsthemke B, Dörfler W (1997) A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the *SNRPN* locus. Eur J Hum Genet 5:94–98

Address for correspondence and reprints: Dr. Karen Helene Ørstavik, Department of Medical Genetics, Rikshospitalet, 0027 Oslo, Norway. E-mail: k.h.orstavik@klinmed.uio.no

0002-9297/2003/7201-0026$15.00