Head and neck paragangliomas : genetics, heredity and clinical characteristics

Hensen, E.F.

Chapter 7

Parent-of-origin-dependent inheritance in SDHD-linked paragangliomas

Erik F. Hensen
Ekaterina S. Jordanova
Ivonne J.H.M. van Minderhout
Pancras C.W. Hogendoorn
Peter E. Taschner
Andel G.L. van der Mey
Peter Devilee
Cees J. Cornelisse

Published as:
Somatic loss of maternal chromosome 11 causes parent-of-origin-dependent inheritance in SDHD-linked paraganglioma and phaeochromocytoma families.
Oncogene. 23 (2004) 4076-4083.

Abstract

Germline mutations in succinate dehydrogenase subunits B, C and D (*SDHB*, *SDHC* and *SDHD*), genes encoding subunits of mitochondrial complex II, cause hereditary paragangliomas and phaeochromocytomas. In *SDHB* (1p36)- and *SDHC* (1q21)-linked families, disease inheritance is autosomal dominant. In *SDHD* (11q23)-linked families, the disease phenotype is expressed only upon paternal transmission of the mutation, consistent with maternal imprinting. However, *SDHD* shows biallelic expression in brain, kidney and lymphoid tissues. Moreover, consistent loss of the wild-type (wt) maternal allele in SDHD-linked tumors suggests expression of the maternal *SDHD* allele in normal paraganglia. Here we demonstrate exclusive loss of the entire maternal chromosome 11 in *SDHD*-linked paragangliomas and phaeochromocytomas, suggesting that combined loss of the wt *SDHD* allele and maternal 11p region is essential for tumorigenesis. We hypothesize that this is driven by selective loss of one or more imprinted genes in the 11p15 region. In paternally, but not in maternally derived *SDHD* mutation carriers, this can be achieved by a single event, that is, non-disjunctional loss of the maternal chromosome 11. Thus, the exclusive paternal transmission of the disease can be explained by a somatic genetic mechanism targeting both the *SDHD* gene on 11q23 and a paternally imprinted gene on 11p15.5, rather than imprinting of *SDHD*.

Introduction

Paragangliomas (PGL) of the head and neck are neuroendocrine tumors arising in branchiomeric and intravagal paraganglia. They are rare, highly vascular, mostly benign tumors usually characterized by an indolent growth pattern. Paragangliomas, like normal paraganglia, consist of two cell types: the type I or chief cells, which represent the neoplastic population in paragangliomas, and the type II or sustentacular cells[1]. The most common site is the carotid body, a chemoreceptive organ in the bifurcation of the carotid artery that senses oxygen levels in peripheral blood in a way that is not yet fully understood. Most paragangliomas appear to be sporadic, but a significant minority of the cases (10-50%) has been shown to be familial. Recently, several genes have been implicated in these familial forms of the disease. Analysis of families carrying the PGL1 gene revealed germline mutations in the succinate dehydrogenase complex-subunit D (SDHD) gene on 11q23[2]. This gene encodes a mitochondrial protein, an anchoring subunit of the mitochondrial respiratory chain complex II.

Subsequently, mutations in other subunits of the same mitochondrial complex II were also found to be associated with hereditary paraganglioma. The SDHB gene (1p36.1-p35) encodes a catalytic subunit of mitochondrial complex II and has been implicated in familial paraganglioma of the head and neck as well as in familial paraganglioma of the adrenal medulla, better known as pheochromocytoma[3]. Both *SDHD* and *SDHB* appear to act as tumor suppressor genes in hereditary paraganglioma. The *SDHC* gene (1q21) encodes the second anchoring subunit of the mitochondrial complex II and mutations in this gene have recently been shown to cause hereditary paraganglioma as well[4]. Furthermore, a hereditary paraganglioma family with linkage to a region on 11q13.1, the PGL2 locus, has been described[5]. However, no mitochondrial complex II genes are known to be located in this region.

Interestingly, strikingly different inheritance patterns have been found for paragangliomas of different genetic background. Whereas *SDHB*- and *SDHC*-linked pedigrees show autosomal dominant inheritance, *SDHD*- and PGL2-linked pedigrees exhibit a clear parent-of-origin effect: inheritance of paraganglioma occurs in an autosomal dominant way only when paternally transmitted, while no phenotype develops after maternal transmission. This pattern is consistent in all *SDHD*-linked pedigrees, and suggests sex-specific epigenetic modification of the maternal *SDHD* allele, consistent with genomic imprinting[6]. However, no evidence of a physical imprint, for example, methylation of the 11q22.1-23 region, has been found. Furthermore, the *SDHD* gene is biallelicly expressed in human brain, kidney and lymphoid tissue[2]. It has been suggested that the imprinting of *SDHD* is restricted

to the paraganglia cells, but loss of the maternal *SDHD* allele is frequently observed in paraganglioma from *SDHD*-mutation carriers, an event that is unlikely to promote tumor growth when the maternal allele is already silenced by an imprint[2,7,8]. We hypothesized that somatic, selective loss of the whole maternal chromosome 11 could explain the exclusive paternal inheritance of the disease, mimicking maternal imprinting of the *SDHD* gene. We performed fluorescent in situ hybridization (FISH) studies on 23 *SDHD*-linked tumors using different probe sets in order to test for loss of chromosome 11, and loss of heterozygosity (LOH) analysis using several microsatellite markers to determine the parental origin of the lost chromosome. Complete loss of a chromosome 11 copy was found in all tumors, and LOH analysis on a subset of seven tumors from patients for whom parental DNA samples were available revealed the exclusive maternal origin of the lost chromosome. We propose that the selective loss of the maternal chromosome 11 copy is driven by the allelic phasing of the *SDHD* germline mutation and a paternally imprinted tumor suppressor gene on 11p15.

Materials and methods

Patients and families

Diagnosis of paraganglioma was based on medical history, physical and otolaryngological examination, radiological imaging and histopathology of the excised tumor. After obtaining informed consent, peripheral blood was obtained from patients and their parents for genomic DNA isolation. Routinely processed archival paraffin-embedded carotid body paraganglioma or phaeochromocytoma tissue from patients with the D92Y Dutch founder mutation in the *SDHD* gene were obtained from the archives of the Department of Pathology of the Leiden University Medical Center[2,29].

Mutation detection

The D92Y mutation in the *SDHD* gene was detected by direct sequencing of PCR products obtained from peripheral blood lymphocyte (PBL) DNA as described previously[2].

Interphase FISH on paraffin-embedded tissue sections

We performed interphase FISH on paraffin-embedded sections as previously described[30]. The pLC11A probe and the PUC1.77 probe for the centromeric alphoid repeat DNA of chromosomes 11 and 1, respectively, were kindly provided by Dr. J. Wiegant (Department of Molecular Cell Biology, LUMC, Leiden, The Netherlands)[31,32]. We have chosen the PUC1.77 probe as a reference because of our extensive experience with the interpretation of the signals given by this probe and a previous LOH study did not indicate involvement

of chromosome 1 in PGL1/SDHD-linked paragangliomas[33]. The probes were labeled by standard nick translation with biotin-16-aUTP or digoxigenin-11-dUTP (Roche, Basel, Switzerland). A total of 200 nuclei were analyzed for each sample by two independent investigators (EFH and ESJ).

Triple color interphase FISH on nuclei isolated from paraffin-embedded tissue

Isolation of intact nuclei, hybridization and immunodetection were performed as previously described, with slight modifications[34]. The hybridization mix contained 50% formamide, 3 ng/µl of each of the three probes (either PUC1.77, pLC11A and 3F7 or PUC1.77, 371C18 and 469N6) and a 50-fold excess of human Cot-1 DNA (Invitrogen Life tech., Paisley, UK). A volume of 5 µl of the mix was applied directly onto the slides and covered with an 18 x 18mm^2 coverslip. After a denaturation step of 8 min at 80°C, the slides were incubated overnight at 37°C in a moisture chamber. The BAC probes 371C18 (telomere 11p), 469N6 (telomere 11q) and 3F7 (11q23, containing the *SDHD* gene) were obtained from the Children's Hospital Oakland Research Institute (Peter de Jong BAC library RP11). All probes were labeled by standard nick translation with biotin-16-aUTP, digoxigenin-11-dUTP or fluorescein-12-dUTP (Roche). A total of 200 nuclei were analyzed for each sample and probe combination by two independent investigators (EFH and ESJ).

Flow cytometry analysis and flow sorting

Cell preparation and staining procedures were performed as described elsewhere[35]. Pepsin digestion was used to isolate whole nuclei from 45 mm thick paraffin sections. Nuclei were subsequently stained with propidium iodide. DNA content was determined with a FACscan flow cytometer (Becton & Dickson, Immunocytometry Systems, San Jose, CA, USA). On average, 100.000 nuclei were measured in each sample. If the DNA histogram showed a single $G_{0,1}$ and G_2 peak both populations were subsequently sorted on a FACsorter (FACSVantage SE, Becton & Dickson, Immunocytometry Systems, San Jose, CA, USA). Owing to the G_2 arrest often detected in paraganglioma cells, the $G_{2,M}$ population was considered enriched for tumor cells[12]. If the DNA histogram showed $G_{0,1}$ peaks, the left peak was considered to represent the diploid and the right peak the aneuploid population. Cells were sorted directly into 1.5 ml microfuge tubes and DNA was subsequently isolated as previously described[36].

LOH analysis

LOH analysis was performed as previously described[1]. Genotypes of patients and their parents were established for the markers D11S1984 and D11S2362 (11p15), D11S4183 (11p11), D11S1335, D11S1765 and D11S4075 (11q13) and D11S1647, D11S3178 and pDJ159Ogt1R (11q23). Markers were informative if they were heterozygous in the patient,

and the parental origin of the alleles could be unambiguously derived. Subsequently, in informative cases both diploid and aneuploid or diploid and the $G_{2,M}$ fractions were tested.

Results

We started with FISH experiments on tissue sections from five paragangliomas from D92Y carriers. The rationale for initially choosing sections rather than cell suspensions was the expectation that this would facilitate the visual selection of nuclei of the type I (chief) cells. The sections were hybridized with centromere probes for chromosomes 11 and 1, the latter chromosome serving as a ploidy reference. Loss of centromere 11 relative to centromere 1 was found in all tumors, in 45-65% of nuclei (Figure 1). Of the nuclei with three signals for chromosome 1, 13-54% had two signals for chromosome 11, 5-54% had one signal for chromosome 11 and 5-32% had no signals for chromosome 11. Of the nuclei with two signals for chromosome 1, 44-66% had one signal for chromosome 11 and 8–31% had no signals for chromosome 11, whereas of the nuclei with only one signal for chromosome 1, 0-7% had no signals for chromosome 11.

To exclude the possibility that loss of signals due to tissue sectioning could have interfered with the results, we next hybridized isolated whole nuclei of 10 paragangliomas, three of which were also studied in the first study. Whereas the use of suspensions precluded the selection of type I cells, evaluation of an unselected sample of 200 nuclei still demonstrated the relative loss of centromere 11 in all samples in 35-63% of nuclei (Figure 2).

To discriminate between loss of the entire chromosome and subchromosomal loss due to complex rearrangements, we next analyzed isolated whole nuclei of nine paragangliomas and two pheochromocytomas from D92Y mutation carriers that were not used in the previous studies, using a triple color FISH technique. This allows simultaneous detection of two probes on chromosome 11 and one probe on centromere 1 (Figure 3). First, we studied the centromere 1 and 11 probes in combination with a BAC probe that covers the *SDHD* gene on 11q23 (Figure 3a and c). Concomitant loss of both probes located on chromosome 11 relative to centromere 1 was observed in all samples, in 24-65% of paraganglioma and 31-62% of pheochromocytoma nuclei (Figure 4a).

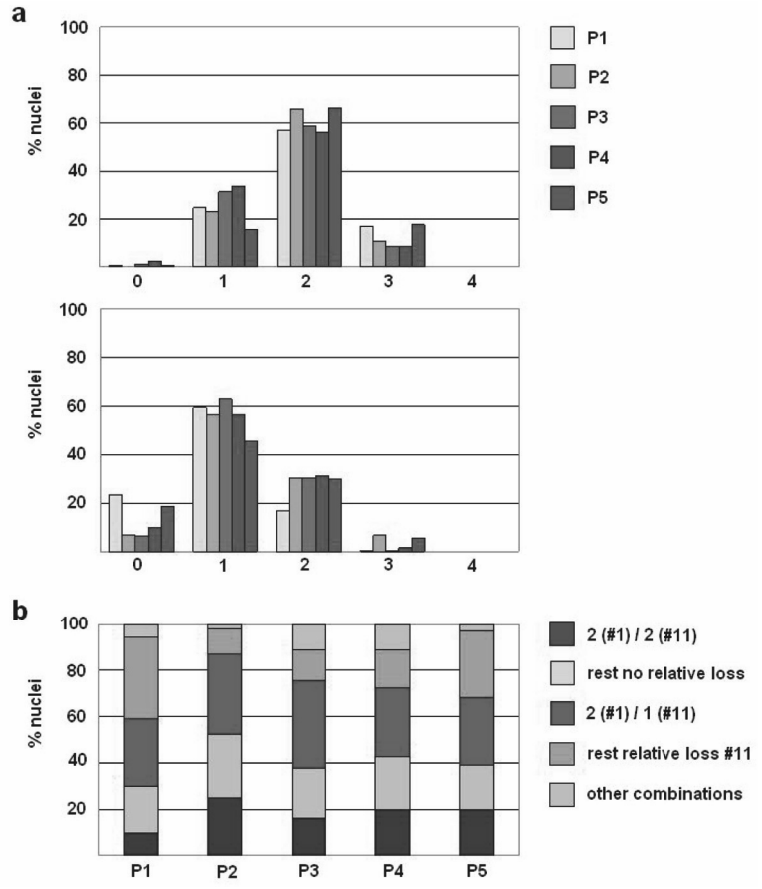

Figure 1. Results obtained from interphase FISH analysis of paraffin-embedded sections of five SDHD-linked paragangliomas (P1- P5). (**a**) Frequency distribution of signals obtained with the centromere 1 (PUC1.77) probe (upper panel) and the centromere 11 (pLC11A) (lower panel). Compared to chromosome 1, there is a clear loss of chromosome 11 centromere signals. More than two chromosome 1 signals are observed in 9-17% of the nuclei, indicating aneuploidy or tetraploidy. (**b**) Loss of centromere 11 relative to centromere 1 signals (red and orange) is observed in 46-65% of the nuclei. Loss of centromere 1 signals relative to centromere 11 ('other combinations') is 2-11%.

Next, we used BAC probes for the subtelomeric regions of 11p and 11q, with the centromere 1 probe as a reference (Figure 3b and d). Concomitant loss of both probes located on chromosome 11 relative to centromere 1 was found in 26-70% of paraganglioma and 23-54% of pheochromocytoma nuclei (Figure 4b). In both triple-color experiments, loss of one of the two probes located on chromosome 11 relative to the other was observed in only a small minority of nuclei (1-7% and 2-4%, respectively), demonstrating that the

observed relative loss of chromosome 11 involves the entire copy. Thus, relative loss of chromosome 11 signals was observed in all 23 tumors, ranging from 23 to 70% (mean = 40%).

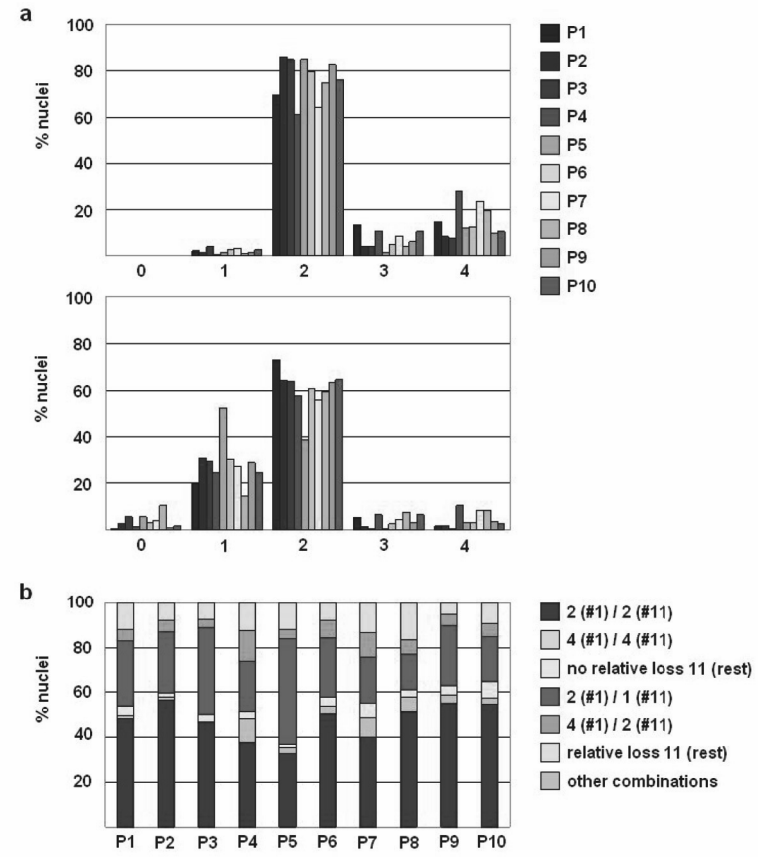

Figure 2. Interphase FISH results from isolated whole nuclei of 10 SDHD-linked paragangliomas (P1- P10). (**a**) Frequency distribution of signals obtained with the centromere 1 (PUC1.77) probe (upper panel) and the centromere 11 (pLC11A) probe (lower panel). Compared to chromosome 1, there is a clear loss of chromosome 11 centromere signals. More than two chromosome 1 signals are observed in 12-40% of the nuclei, indicating aneuploidy or tetraploidy. (**b**) Loss of centromere 11 relative to centromere 1 signals (red and orange) is observed in 35-63% of the nuclei. Loss of centromere 1 signals relative to centromere 11 ('other combinations') is negligible (0-1%).

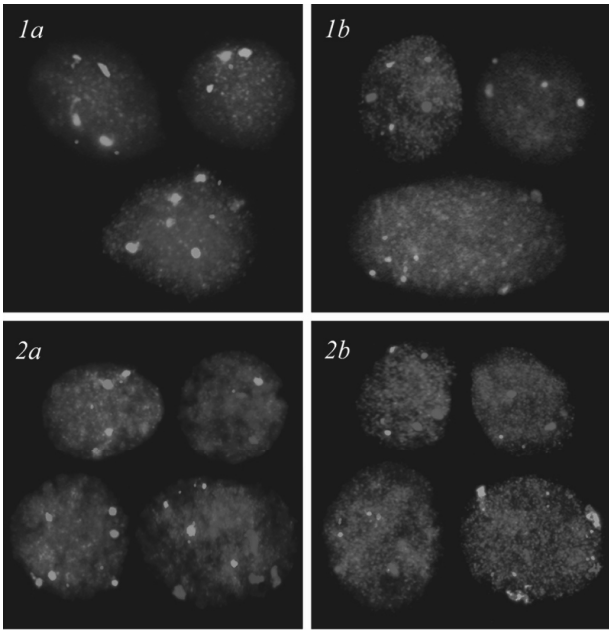

Figure 3. Triple colour FISH on whole nuclei isolated from paraffin-embedded tissue. Probe/colour combinations are centromere 11 (pLC11A, green), centromere 1 (PUC1.77, blue) and 11q23 (RP11-3F7, red) **(1a, 2a)**, and subtelomere 11p (RP11-645I8, green), subtelomere 11q (RP11-469N6, red) and centromere 1 (blue) **(1b, 2b)**. Each panel is a composite of individually captured nuclei. **(1a)** Paraganglioma cell nuclei. Top left : diploid nucleus with two signals for each probe, top right: monosomy for chromosome 11, bottom: tetraploidy for centromere 1 and diploidy for each chromosome 11 probe. **(1b)** Paraganglioma cell nuclei. Top left : diploid nucleus, top right: chromosome 11 monosomy and relative chromosome 11 loss in a tetraploid nucleus (bottom). **(2a)** Phaeochromocytoma cell nuclei. Top left : diploid nucleus, top right: chromosome 11 monosomy, bottom left : relative chromosome 11 loss in a tetraploid nucleus, bottom right: tetraploid nucleus without relative chromosome 11 loss. **(2b)** Phaeochromocytoma cell nuclei. Top left : diploid nucleus, top right: chromosome 11 monosomy, bottom left : relative chromosome 11 loss in a tetraploid nucleus, bottom right: a tetraploid nucleus without relative chromosome 11 loss.

To determine the parental origin of the lost chromosome 11, we performed LOH analysis on seven paragangliomas and two pheochromocytomas that were also analyzed by triple-color FISH. For these cases, patient- as well as parental PBL-derived DNA samples were available. LOH analysis was performed after tumor cell populations were enriched by fluorescence activated cell sorting (FACS) of the aneuploid $G_{0,1}$ fraction, or the often increased $G_{2,M}$ fraction of diploid tumors, with the diploid $G_{0,1}$ fraction as a reference[1,9]. We used three markers on 11p and five on 11q. In five paragangliomas and two pheochromocytomas, LOH analysis was informative for at least one marker on both chromosome arms. For two paragangliomas, the analysis was informative for only

one marker, either on 11p or 11q. In aneuploid- or $G_{2,M}$-cell populations, all evaluable LOH experiments showed loss of maternal alleles. As expected, retention of heterozygosity was not observed (Figure 5). In the diploid cell populations and patient PBL DNA samples, no LOH was found (data not shown).

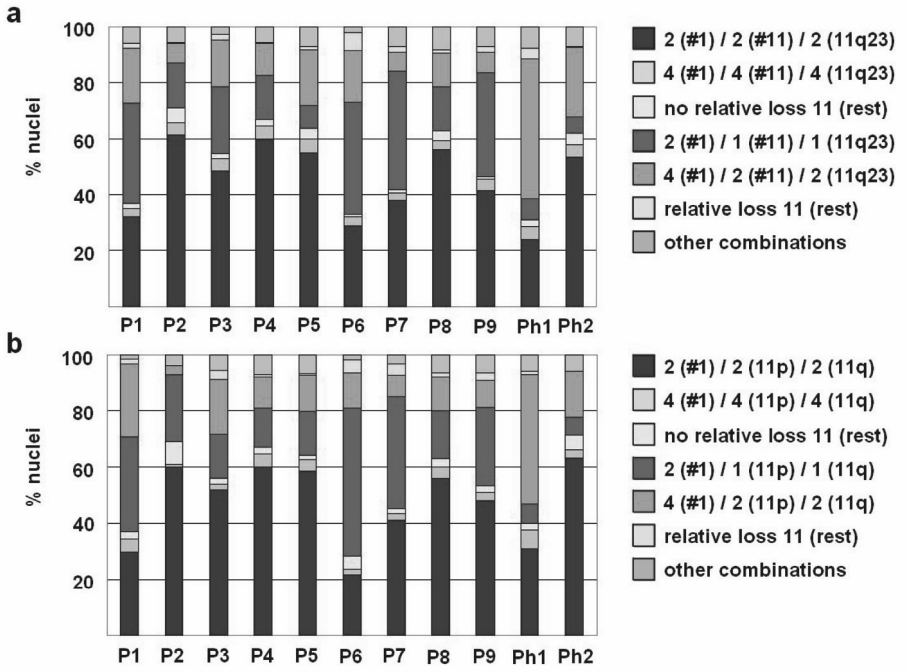

Figure 4. Counts of whole nuclei isolated from paraffin-embedded material of paragangliomas (P6-P14) and phaeochromocytomas (Ph1- Ph2), analysed by triple colour interphase FISH. (**a**) Results for centromere 11 (pLC11A), centromere 1 (PUC1.77) and 11q23 (RP11-3F7) probes. Simultaneous loss of both chromosome 11 probes relative to centromere 1 (red and orange) was observed in 24-65% of paragangliomas and 31-62% of phaeochromocytomas. (**b**) Results for centromere 1, subtelomeric 11p (RP11-645I8) and 11q (RP11-469N6) probes. Simultaneous loss of both chromosome 11 probes relative to centromere 1 (red and orange) was observed in 26-70% of paragangliomas and 23-54% of phaeochromocytomas. For each tumor, distributions are very similar in (a) and (b) indicating high reproducibility of the technique. Note that in both (a) and (b) nonsimultaneous loss of chromosome 11 probes or loss of centromere 1 signals relative to chromosome 11 signals (white) is infrequent (3-8% and 2-7%, respectively).

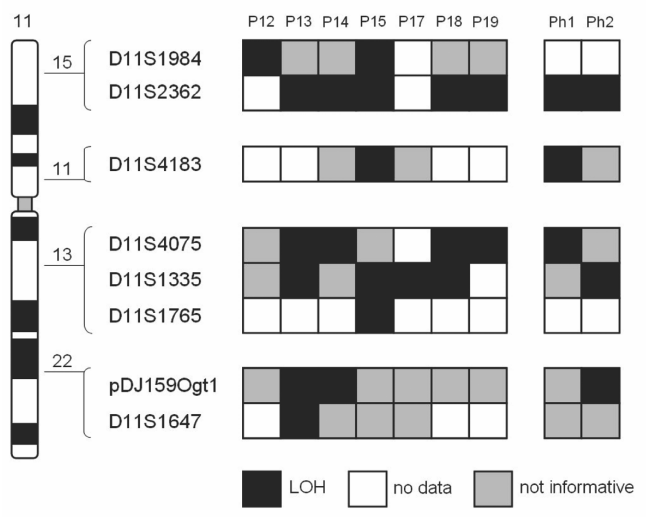

Figure 5. LOH analysis of sorted aneuploid $G_{0,1}$ or diploid $G_{2,M}$ fractions of isolated nuclei of paraffin-embedded paragangliomas (P12- P19) and phaeochromocytomas (Ph1- Ph2). LOH involved the maternal allele in all cases in which the parental origin of the lost allele could be assessed (black). Retention of heterozygosity was not found for any of the informative markers.

Discussion

The results obtained in this study demonstrate the loss of an entire copy of chromosome 11 in all investigated *SDHD*-linked paragangliomas. By LOH analysis, we were able to unequivocally demonstrate the maternal origin of the lost chromosome copy in a subset of seven paraganglioma and two phaeochromocytoma cases from which parental blood DNA samples were available. However, even without this direct proof, Knudson's two-hit model predicts that in case of paternal transmission of the germline mutation, loss of the wildtype maternal allele should have occurred in the tumor.

Although loss of a centromere 11 already indicates loss of the entire chromosome 11, we obtained additional evidence by the triple color FISH experiments with telomeric probes and the 3F7 probe containing the *SDHD* gene. Since it was not possible to accurately discriminate type I cells in the FISH experiments on isolated nuclei, the evaluation of an unselected sample of 200 nuclei unavoidably included non-neoplastic cells as well. This explains most of the variation in loss of chromosome 11 between the different cases and the concordance of the results obtained with different probe sets for the individual tumors (Figure 4). FISH on tissue sections, while permitting selection of type I cell nuclei, did not

yield significantly higher percentages of nuclei with relative chromosome 11 loss because of loss of signals from sliced nuclei. The latter problem would have seriously complicated, if not precluded, the interpretation of triple color FISH experiments on tissue sections and thus nuclear suspensions were used in all further experiments.

The selective loss of the entire maternal chromosome 11 explains why *SDHD*-linked tumors appear to arise only upon paternal transmission of the mutation, even though the *SDHD* gene itself is not imprinted. The latter is supported by the observed biallelic expression of *SDHD* in several human tissues[2]. Although it is not uncommon for the somatic 'second hit' in the Knudson model of tumorigenesis to involve a gross chromosomal mechanism such as non-disjunctional chromosome loss, it is intriguing that in *SDHD*-linked paragangliomas this appears to be the preferred mechanism for the second hit. We hypothesize that a second target gene on chromosome 11, which is subject to genomic imprinting, is involved in tumor formation. A growth advantage is gained when the wild-type maternal *SDHD* allele on 11q23 and the active maternal copy of this second, paternally imprinted gene are lost simultaneously. As the only region known to harbor an imprinted gene cluster on chromosome 11 is 11p15, we further hypothesize that this second gene is located here. Within that model, the most parsimonious mechanism would be a single event, viz. the loss of the entire maternal chromosome 11 copy in case of a maternal wt *SDHD* allele and paternal inheritance of the *SDHD* mutation (Figure 6a). Loss of the maternal wt *SDHD* allele only, for example, by loss of a part of 11q, would not target the second tumor-suppressor gene on 11p15 and therefore not lead to tumor formation (Figure 6b). In case of maternal inheritance of the *SDHD* mutation, loss of paternal alleles would not lead to tumor formation for the same reason (Figure 6c and d). At least two events caused by different chromosomal mechanisms will be required to inactivate both *SDHD* and the imprinted gene on 11p15 when *SDHD* is maternally transmitted. These are successive loss of the paternal wt *SDHD* allele by, for example, mitotic recombination, followed by loss of the recombined paternal chromosome containing the paternal 11q23 region and the maternal 11p15 region (Figure 6e). Given the evidence for complex LOH mechanisms in solid tumors, it is somewhat surprising that the probability of this occurring in paraganglioma formation appears to be very low or zero, since no cases of maternal transmission have been reported to date[10,11]. One explanation might be that the number of cell divisions in normal paraganglia is simply too low since in most head and neck paragangliomas the growth fraction is lower than 1%[12]. Selective loss of the whole maternal chromosome 11 would explain the exclusive paternal transmission of disease in paraganglioma linked to the PGL2 locus as well, because it is also located on 11q[5]. It would also explain the absence of generation skipping of tumor susceptibility in *SDHB* (1p36-p35)- and *SDHC* (1q21)-linked families. In the latter two, loss of the maternal 11p15 region is probably

also essential for tumor development, since *SDHB*, *SDHC* and *SDHD* encode subunits from the same mitochondrial complex. Dannenberg et al. detected loss of 11p in two out of nine sporadic paragangliomas by comparative genomic hybridization, but the mutation status of *SDHB*, *SDHC* or *SDHD* was not investigated[8]. Furthermore, loss of 11p has been reported in 45% of 11 sporadic abdominal paragangliomas[13]. Since data on the parental origin of the 11p losses are lacking, a major role for loss of maternal 11p in sporadic paragangliomas, although likely, still remains to be proven.

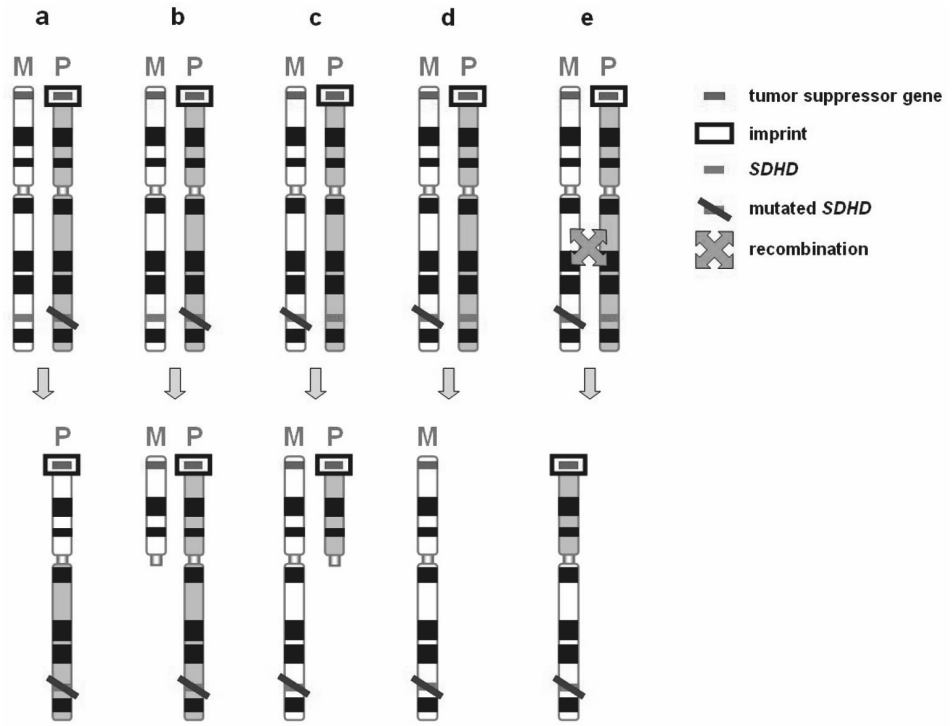

Figure 6. Model for the imprinted transmission of SDHD-linked paraganglioma. Maternal (white) and paternal (grey) chromosomes are depicted. (**a**) Both the maternal 11q region, containing the wt SDHD allele, and the maternal 11p region, containing the active tumor suppressor allele, are targeted. In case of an event targeting only the wt maternal SDHD allele on 11q (**b**), the active maternal tumor suppressor allele on 11p15 is not affected and tumor development is inhibited. In case of maternal inheritance of the SDHD mutation, a second hit targeting the wt paternal allele by, for example, a deletion of the paternal 11q region (**c**) or even the whole paternal chromosome 11 (**d**) will leave the maternal 11p15 region intact and tumor formation is not initiated. When the SDHD mutation is maternally transmitted, at least two events caused by different chromosomal mechanisms will be required to inactivate both the wt SDHD allele and the active maternal allele of the imprinted tumor-suppressor gene on 11p15, namely loss of the paternal wild-type SDHD allele by, for example, mitotic recombination, followed by loss of the recombined paternal chromosome containing the paternal 11q23 region and the maternal11p15 region (**e**). Apparently, this sequence of events is very unlikely in vivo.

Interestingly, a high percentage (86%) of loss of chromosome 11 was also found in 31/36 (86%) of Von Hippel-Lindau (VHL) related pheochromocytomas, of which 25/31 had loss of both 11p and 11q whereas six had only 11p loss[14]. The investigators suggested that this observation might indicate the involvement of a different but essential and complementary genetic pathway in VHL-linked pheochromocytoma tumorigenesis. The results of our study emphasize a role for loss of 11p, and in particular the maternal copy, in *SDHD*-linked pheochromocytoma formation as well. LOH of maternal 11p15, often with duplication of paternal 11p15, occurs frequently in human pediatric tumors including Wilm's tumors, embryonal rhabdomyosarcomas, hepatoblastoma and adrenocortical carcinomas[15,16].

There is convincing evidence that LOH of 11p15 leads to disruption of the regulation of expression of oppositely imprinted genes, in particular *H19* and *IGF2*, in a variety of tumors[15,16]. The *IGF2* gene product is a survival factor and strong mitogen that is overexpressed in a variety of human tumors including hereditary paragangliomas and pheochromocytomas[17]. *H19* codes for an untranslated RNA that acts a negative trans regulator of *IGF2* expression[18,19].

Disruption of imprinted expression of 11p15 has also been implicated in the Beckwith–Wiedemann syndrome and focal hyperplasia of Langerhans islets causing congenital hyperinsulinism (FoCHI)[20-22]. There is an interesting parallel between our findings of maternal chromosome 11 loss in hereditary paraganglioma and loss of maternal 11p15 in FoCHI[22]. This disease is caused by a paternally inherited, recessive mutation of the *ABCC8*- or *KCNJ11*-gene, which is located on 11p15.4, that is, outside the imprinted region. The lesions show a strongly decreased expression of *H19* and increased expression of *IGF2*. Thus, like in paraganglioma, a single somatic event targets the wild-type allele of a non-imprinted susceptibility gene on the maternal chromosome 11 as well as the maternally imprinted 11p15 region, and in both types of diseases this results in exclusive paternal transmission. Although the development of solid tumors in general is a multi-step genetic evolution process, it is unclear why tumor development in *SDHD* mutation carriers specifically requires loss of a putative maternally expressed tumor-suppressor gene, in addition to loss of wt *SDHD*. It has been speculated that the tumorigenic effects of *SDHD* inactivation might be explained by either mitogenic effects of elevated levels of reactive oxygen species or blocking of apoptosis due to mitochondrial dysfunction[23,24]. On the other hand, oxidative stress may trigger pro-apoptotic signaling and create a selection pressure for mutational activation of anti-apoptotic pathways. Since the IGF pathway has found to be involved in anti-apoptotic signaling, loss of the maternally expressed

H19 gene, a known suppressor of *IGF2*, might be an essential step in paraganglioma development[25,26].

SDHD-linked paraganglioma is a striking, and to our knowledge, first example of the effect of allelic phasing on the penetrance of a hereditary tumor syndrome in man. Recently, allelic phasing of mouse chromosome 11 deficiency was found to influence p53 tumorigenicity[27]. The deletion on chromosome 11 elevated the tumor susceptibility and modified the tumor spectrum when in trans with the p53 mutation. Many genes display differential expression of parental alleles, due to genomic imprinting or genetic regulation[28]. Conceivably, a certain dosage ratio of cancer-related alleles, which are coincidentally located on the same chromosome in cis-configuration, may provide a selective growth advantage. The tumorigenic potential of acquired chromosome aneuploidy, a hallmark of many solid tumors, would then be dependent on the allelic phasing or imprinting status of these genes. Our study provides a clear-cut example of this mechanism, which might also apply to an individual's overall susceptibility to more common forms of cancer.

Acknowledgements

We thank C.J. Haven for help with the FISH experiments and N.J. Kuipers-Dijkshoorn for technical assistance. We acknowledge T. van Wezel and M. Lombaerts for helpful comments on the manuscript. This research was supported by a grant from the JANIVO foundation.

References

1. Van Schothorst EM, Beekman M, Torremans P et al., Paragangliomas of the head and neck region show complete loss of heterozygosity at 11q22-q23 in chief cells and the flow-sorted DNA aneuploid fraction. Hum Pathol. 29 (1998) 1045-1049.
2. Baysal BE, Ferrell RE, Willett-Brozick JE et al., Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287 (2000) 848-851.
3. Astuti D, Latif F, Dallol A et al., Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet. 69 (2001) 49-54.
4. Niemann S and Muller U, Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet. 26 (2000) 268-270.
5. Mariman EC, Van Beersum SE, Cremers CWRJ et al., Analysis of a second family with hereditary non-chromaffin paragangliomas locates the underlying gene at the proximal region of chromosome 11q. Hum Genet. 91 (1993) 357-361.
6. Van der Mey AGL, Maaswinkel-Mooy PD, Cornelisse CJ et al., Genomic imprinting in hereditary glomus tumors: evidence for new genetic theory. Lancet 2 (1989) 1291-1294.
7. Baysal BE, Farr JE, Rubinstein WS et al., Fine mapping of an imprinted gene for familial nonchromaffin paragangliomas, on chromosome 11q23. Am J Hum Genet. 60 (1997) 121-132.
8. Dannenberg H, de Krijger RR, Zhao J et al., Differential loss of chromosome 11q in familial and sporadic parasympathetic paragangliomas detected by comparative genomic hybridization. Am J Pathol. 158 (2001) 1937-1942.
9. Van der Mey AGL, Cornelisse CJ, Hermans J et al., DNA flow cytometry of hereditary and sporadic paragangliomas (glomus tumors). Br J Cancer 63 (1991) 98-302.
10. Devilee P, Cleton-Jansen AM and Cornelisse CJ, Ever since Knudson. Trends Genet. 17 (2001) 569-573.
11. Balmain A, Gray J and Ponder B, The genetics and genomics of cancer. Nat Genet. 33(Suppl.) (2003) 238-244.
12. Douwes Dekker PB, Kuipers-Dijkshoorn N, Hogendoorn PCW et al., G2M arrest, blocked apoptosis, and low growth fraction may explain indolent behavior of head and neck paragangliomas. Hum Pathol. 34 (2003) 690-698.
13. Edstrom E, Mahlamaki E, Nord B et al., Comparative genomic hybridization reveals frequent losses of chromosomes 1p and 3q in pheochromocytomas and abdominal paragangliomas, suggesting a common genetic etiology. Am J Pathol. 156 (2000) 651-659.
14. Lui WO, Chen J, Glasker S et al., Selective loss of chromosome 11 in pheochromocytomas associated with the VHL syndrome. Oncogene 21 (2002) 1117-1122.
15. Gicquel C, Raffin-Sanson ML, Gaston V et al., Structural and functional abnormalities at 11p15 are associated with the malignant phenotype in sporadic adrenocortical tumors: study on a series of 82 tumors. J Clin Endocrinol Metab. 82 (1997) 2559-2565.
16. Tycko B, Epigenetic gene silencing in cancer. J Clin Invest. 105 (2000) 401-407.
17. Stewart CE and Rotwein P, Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. Physiol Rev. 76 (1996) 1005-1026.
18. Li YM, Franklin G, Cui HM et al., The H19 transcript is associated with polysomes and may regulate IGF2 expression in trans. J Biol Chem. 273 (1998) 28247-28252.
19. Runge S, Nielsen FC, Nielsen J et al., H19 RNA binds four molecules of insulin-like growth factor II mRNA-binding protein. J Biol Chem. 275 (2000) 29562-29569.
20. Waziri M, Patil SR, Hanson JW and Bartley JA, Abnormality of chromosome 11 in patients with features of Beckwith-Wiedemann syndrome. J Pediatr. 102 (1983) 873-876.
21. Weksberg R and Squire JA, Molecular biology of Beckwith-Wiedemann syndrome. Med Pediatr Oncol. 27 (1996) 462-469.

22. Fournet JC, Mayaud C, De Lonlay P et al., Unbalanced expression of 11p15 imprinted genes in focal forms of congenital hyperinsulinism: association with a reduction to homozygosity of a mutation in ABCC8 or KCNJ11. Am J Pathol. 158 (2001) 2177-2184.
23. Astrom K, Cohen JE, Willett-Brozick JE et al., Altitude is a phenotypic modifier in hereditary paraganglioma type 1: evidence for an oxygen-sensing defect. Hum Genet. 113 (2003) 228-237.
24. Eng C, Kiuru M, Fernandez MJ and Aaltonen LA, A role for mitochondrial enzymes in inherited neoplasia and beyond. Nat Rev Cancer 3 (2003) 193-202.
25. Datta SR, Dudek H, Tao X et al., Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91 (1997) 231-241.
26. Burns JL and Hassan AB, Cell survival and proliferation are modified by insulin-like growth factor 2 between days 9 and 10 of mouse gestation. Development 128 (2001) 3819-3830.
27. Biggs PJ, Vogel H, Sage M et al., Allelic phasing of a mouse chromosome 11 deficiency influences p53 tumorigenicity. Oncogene 22 (2003) 3288-3296.
28. Schadt EE, Monks SA, Drake TA et al., Genetics of gene expression surveyed in maize, mouse and man. Nature 422 (2003) 297-302.
29. Van Schothorst EM, Jansen JC, Grooters E et al., Founder effect at PGL1 in hereditary head and neck paraganglioma families from the Netherlands. Am J Hum Genet. 63 (1998) 468-473.
30. Haralambieva E, Kleiverda K, Mason DY et al., Detection of three common translocation breakpoints in non-Hodgkin's lymphomas by fluorescence in situ hybridization on routine paraffin-embedded tissue sections. J Pathol. 198 (2002) 163-170.
31. Cooke HJ and Hindley J, Cloning of human satellite III DNA: different components are on different chromosomes. Nucleic Acids Res. 6 (1979) 3177-3197.
32. Waye JS, Creeper LA and Willard HF, Organization and evolution of alpha satellite DNA from human chromosome 11. Chromosoma 95 (1987) 182-188.
33. Devilee P, Van Schothorst EM, Bardoel AF et al., Allelotype of head and neck paragangliomas: allelic imbalance is confined to the long arm of chromosome 11, the site of the predisposing locus PGL. Genes Chromosomes Cancer 11 (1994) 71-78.
34. Jordanova ES, Riemersma SA, Philippo K et al., Hemizygous deletions in the HLA region account for loss of heterozygosity in the majority of diffuse large B-cell lymphomas of the testis and the central nervous system. Genes Chromosomes Cancer 35 (2002) 38-48.
35. Beerman H, Kluin PM, Hermans J et al., Prognostic significance of DNA-ploidy in a series of 690 primary breast cancer patients. Int J Cancer 45 (1990) 34-39.
36. Abeln EC, Corver WE, Kuipers-Dijkshoorn N et al., Molecular genetic analysis of flow-sorted ovarian tumor cells: improved detection of loss of heterozygosity. Br J Cancer 70 (1994) 255-262.