Cancer genetics

ORIGINAL ARTICLE

Indication for CDKN2A-mutation analysis in familial pancreatic cancer families without melanomas

Femme Harinck,1 Irma Kluft,2 Nienke van der Stoep,3 Rogier A Oldenburg,4 Anja Wagner,4 Coralie Aalfs,5 Rolf H Sijmons,6 Jan-Werner Poley,1 Ernst J Kuipers,1,7 Paul Fockens,8 Theo A M van Os,5 Marco J Bruno1

ABSTRACT

Background CDKN2A-mutation carriers run a high risk of developing melanomas and have an increased risk of developing pancreatic cancer (PC). Familial PC (FPC) patients with a personal history or family history of melanomas are therefore offered CDKN2A-mutation analysis. In contrast, CDKN2A testing in FPC families without a history of melanomas is not generally recommended. The aim of this study was to evaluate the frequency of CDKN2A-mutations in FPC families without melanomas.

Methods Data were gathered from PC family registers. FPC families were defined as families with clustering of PC without meeting diagnostic criteria of familial cutaneous malignant melanoma (familial CMM) or other inherited cancer syndromes. Blood samples were obtained for DNA isolation from PC patients or first degree relatives and analysed for CDKN2A-mutations.

Results Among 40 FPC families, DNA analyses were carried out in 28 families (70%), leading to identification of CDKN2A-mutations in six families (21%). None of the CDKN2A-mutation-positive families fulfilled the diagnostic criteria for familial CMM and in three CDKN2A families no melanomas were observed. Two CDKN2A-mutations were found; the Dutch founder mutation p16-Ser8fs-mutation. After disclosure of the CDKN2A-mutation in one of the families, a curable melanoma was discovered at dermatological surveillance in a 17-year-old family member.

Conclusion CDKN2A-mutation can be found in a considerable proportion of families with FPC. CDKN2A-mutation analysis should therefore be included in genetic testing in FPC families, even in the absence of reported melanomas. This strategy will enhance the recognition of individuals at risk for PC and facilitate the early detection of melanomas.

INTRODUCTION

Approximately 10% of all pancreatic cancer (PC) cases occur in a background of familial clustering. In about 20% of these cases the underlying gene mutation is recognised. One such inherited cancer syndrome with a known increased risk for PC is familial cutaneous malignant melanoma (familial CMM), referred to in the past as the familial atypical multiple mole melanoma syndrome (QMMIM 155600).2-5

This syndrome is characterised by the familial occurrence of melanomas and inherits as an autosomal dominant trait. Germ-line mutations in CDKN2A have been found in at least a quarter of all melanoma prone families.6 7

In addition to an increased risk of developing melanomas, CDKN2A-mutation carriers are also at risk of other types of cancer, particularly PC.2-4

Previous studies have shown that the risk of developing PC among CDKN2A carriers may be 50 times greater than in the general population.5

Therefore, families with any combination of PC and melanomas should be offered CDKN2A analysis.5-14 However, CDKN2A analysis is not recommended in families with a clustering of PC but without melanomas.10

The aim of this study was to evaluate the frequency of CDKN2A-mutations in familial PC (FPC) families without melanomas.

MATERIALS AND METHODS

Patients and families

We conducted a retrospective cohort study. Data were gathered from PC family registries from four Dutch clinical genetic centres (Academic Medical Center Amsterdam, Erasmus MC-University Medical Center Rotterdam, University Medical Center Groningen and the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital). The total number of PC families was 70. Based on their phenotype, PC families were divided into FPC families (n=40) and syndromic PC families (n=30).

FPC families were defined as families with clustering of PC (≥2 first degree relatives (FDR), ≥5 relatives (FDR or second degree relatives (SDR)) or two SDR relatives, one <50 years at diagnosis) and not meeting diagnostic criteria of known inherited cancer syndromes (listed below).10 Syndromic PC families were defined as families with a known inherited cancer syndrome predisposing them to PC (including familial CMM, Peutz-Jeghers, Lynch, Li-Fraumeni and hereditary breast and ovarian cancer syndromes). The diagnosis of familial CMM was made based on the Dutch clinical criteria of familial atypical multiple mole melanomas in either ≥2 affected FDR or ≥5 affected relatives (FDR and/or SDR).15 The total number of familial CMM families within the cohort of syndromic PC families was 16 (53%).

In this study, we included only the FPC families and analysed whether DNA-mutation analysis for CDKN2A/CDK4 was performed, and if so what the outcome of this mutation analysis was. DNA, either from blood samples or available paraffin embedded tumour samples, originated from individuals affected with PC or, in families without...
available DNA from affected individuals, from healthy FDRs. For each family, a complete three-generation pedigree was made. Clinical diagnoses reported by patients and family members were verified by a review of medical and pathological records, and by revision of histological slides whenever available. At the time of genetic counselling, patients or their family members had given written informed consent to use the DNA results for future research projects.

All individuals from CDKN2A-mutation-positive families were advised to undergo dermatological examination to detect dysplastic nevi and/or melanoma.

**CDKN2A/CDK4-mutation analysis**

Direct sequencing of all CDKN2A exons and CDK4 exon 2 was performed on samples from the index cases, and subsequently on relatives of mutation-positive cases. In brief, all exons with flanking intronic regions were amplified by PCR using the following primers: CDKN2A-EX1BF 5'-CTGCGTGGA-3', CDKN2A-EX1AR 5'-GAGAATCGAAGCGCTA-3', CDKN2A-EX2F 5'-TTCGCTAAGTGCTC-3', CDKN2A-EX2R 5'-GAGAATCGAAGCGCTA-3', CDKN2A-EX3R 5'-TTTACGGTAGTGGGG-A-3', CDKN2A-EX3F 5'-TTCGCTAAGTGCTC-3', CDKN2A-EX4F 5'-TTCGCTAAGTGCTC-3', CDKN2A-EX4R 5'-TTCGCTAAGTGCTC-3', CDKN2A-EX5F 5'-AATAATGAACTGTAAGTGGCAGTGGACTA-3', CDKN2A-EX5R 5'-TTCGCTAAGTGCTC-3'. All primers were flanked with respectively m13forward or reverse tags to allow direct sequencing. PCR reactions were carried out using GoTaq® DNA Polymerase (Promega, Benelux b.v.) based on the standard protocol at annealing temperature (Ta) as indicated at the primers. Subsequently, the sequence PCR products were analysed on an ABI37300 sequencer using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) and genotypes were assigned using SeqScape software (Applied Biosystems). The reference sequences for CDKN2A and for CDK4 respectively with GenBank accession numbers NT_008413 v17 and NC_000012 v10 were used to analyse the sequence results and all detected variants were described according to the HGVS nomenclature recommendations.

CNV analysis of CDKN2A was performed for all samples by Multiplex Ligation-dependent Probe Amplification using the MRC-Holland probe-mix ME024-A1 (MRC-Holland, Amsterdam, Netherlands) as indicated by the manufacturer. CNV data for the CDKN2A region specific probes were analysed using GeneMarker software package (SoftGenetics, Pennsylvania, USA).

**Data analyses**

Continuous variables are presented as mean (SD) or median (IQR), where appropriate. Continuous variables were compared using the t test or the Mann– Whitney test. Categorical variables were compared using the χ² or Fisher’s exact test. All analyses were conducted using the Statistical Package for the Social Sciences (V.17.0; SPSS Institute). A two-sided p value <0.05 was considered to be statistically significant.

**RESULTS**

In our series of 40 FPC families, DNA analyses were carried out in 28 families (70%). Of the remaining 12 families, DNA was not available. Twenty-seven of the 28 analysed families (96%) were of Caucasian descent and one family (3.6%) was of Indonesian descent (Maluku Islands). These 28 FPC families had a total of 74 affected patients with PC. In 14 families (50%) two family members were diagnosed with PC, in 12 families (45%) three family members were diagnosed with PC, in one family (5.6%) four family members were diagnosed with PC and in one family (5.6%) seven family members were diagnosed with PC. Of the 74 PC cases, 41 (55%) were male subjects. The mean age of diagnosis of PC was 59.0 years (range 30–84 years, SD 12.3). Nine patients (12%) were younger than 45 at the time of diagnosis. In addition to the PC cases, 24 families (36%) were affected by other types of cancer. Four families (14%) were affected by melanomas, and nine families (52%) were affected by breast cancer.

In 21 FPC families (75%), DNA was available from affected PC cases, isolated from blood samples in 19 families and from PC tumour tissue in two families. In the remaining families, DNA analyses were carried out in DNA of healthy FDR (six families: mean number of FDR tested 1.7, range 1–3) or suspected carriers (one family) because of their position in the pedigree. DNA analyses of mutations in CDKN2A/CDK4 led to the identification of a causal genetic factor in six (21.4%) FPC families. In three (9%) of these CDKN2A-mutation-positive FPC families, no melanomas and/or dysplastic nevi had been reported at the time of DNA analyses. In the other three CDKN2A-mutation-positive FPC families, two had one family member diagnosed with melanoma and in the third family two second degree members were diagnosed with melanoma.

Two different CDKN2A-mutations were found, the Dutch founder mutation p16-Leiden (c.225_245del, p.Ala76fs) and the c.19_23dup;p.Ser8fs-mutation. The p16-Leiden-mutation was found in all three melanoma-positive (100%) families and in two of the melanoma-negative (67%) families.

Table 1A shows the characteristics of the CDKN2A-mutation-positive FPC families without melanomas; supplementary figures A to C display the pedigrees. These three families included a total of eight PC cases of whom five were male subjects (65%) and the mean age at the time of diagnosis was 51.5 years (SD 9.2). Two patients (25%) were younger than 45 at the time of diagnosis. The Dutch founder mutation was found in two Caucasian families. The c.19_23dup;p.Ser8fs-mutation was found in the family of Indonesian descent.

The CDKN2A-mutation-positive FPC families without melanoma (table 1A) and with melanomas (table 1B) included a total of 19 PC cases of whom 10 were male subjects (53%) and the mean age at the time of diagnosis was 55.7 years (SD 10.2). These characteristics were not statistically significantly different from the PC cases of the other FPC families: mean age at the time of diagnosis was 60.1 (SD 12.8), 56% were male persons.

**DISCUSSION**

In six out of 28 FPC families tested, we identified a CDKN2A-mutation (21%), in three of whom (50%) no melanomas and/or dysplastic nevi had been reported at the time of DNA analysis. If the current recommendation would have been followed to test only for CDKN2A-mutations in FPC families with at least one melanoma case, these three families would have gone unnoticed.10 This recommendation is based on three previous studies on the role of CDKN2A-mutations in FPC families, which failed to identify CDKN2A-mutations in non-melanoma FPC families9 12 13 (table 2).

Recently, the prevalence of CDKN2A-mutations in a large series of unselected PC cases was studied and this turned out to be low (0.6%).16 A subanalysis of this series in which only FPC cases were included showed CDKN2A-mutations in 3.3% of cases. The discrepancy between these and our series is not readily explained, although the method of patient selection between our series (gathered from PC family registries) and the latter large series (unselected PC cases) may account for part of the discrepancy. It
does not seem likely that the differences in results can be explained by the type of CDKN2A-mutations that we found. The Dutch founder mutation p16-Leiden, which two of our CDKN2A-mutation-positive non-melanoma FPC families carried, is a well-researched mutation; the phenotype of its carriers is not restricted to clustering of PC only.3 Although, a part of the differences might be explained by the founder role of this mutation. The third CDKN2A-mutation-positive non-melanoma FPC family carried a c.19_23dup.p.Ser8fs-mutation. To our knowledge, this is a new mutation that explained by the founder role of this mutation. The third CDKN2A-mutation-positive non-melanoma FPC family carried a c.19_23dup. p.Ser8fs-mutation. To our knowledge, this is a new mutation that has not been previously reported. The frame shift that is caused by this mutation causes a stop-codon. Based on the results of this one family, it is too early to claim that this specific mutation causes a phenotype without melanomas. The family in whom the c.19_23dup.p.Ser8fs-mutation was detected is of Indonesian descent and their darker-skinned complexion might have offered them added protection from developing melanomas. It is well known that the risk of developing melanoma is higher in fair-skinned people, especially those with blond or red hair and who sunburn and freckle easily than in people with darker complexions.17 The two families with the Dutch founder mutation were Caucasian.

Differences in lifestyle may also explain some of the difference between the results of earlier studies and our series. For example, it is well known that cigarette smoking is associated with an increased risk of PC.18 Unfortunately, we lack detailed information about smoking status. We therefore cannot exclude the possibility that in our series the PC affected family members of non-melanoma CDKN2A-mutation-positive families smoked more than affected individuals in the previously published studies which also did not report on smoking status.

Another limitation of this current series is that in some families affected relatives were unavailable for DNA testing and, instead, unaffected FDRs were tested. A negative genetic test result in such cases does not exclude the presence of a pathogenic mutation unless a specific genetic mutation is found in another relative. This may have caused an underestimation of the prevalence of CDKN2A/CDK4 in this current study.

We were able to collect detailed information on the family history of all FPC families in whom DNA analyses were carried out. In addition, patients not known with any skin lesions before they were genetically tested were seen by a dermatologist, minimising the chance that we missed a diagnosis of melanoma or dysplastic nevi negligible. Since in both family B and family C some family members died of cancer of unknown origin, we cannot state with 100% certainty that none of these individuals died of melanoma.

The three non-melanoma FPC families in which a CDKN2A-mutation was found were of moderate to large size. It is therefore less likely that melanoma or dysplastic nevi were not observed because of a low a priori change based on numbers and RR. Interestingly, during dermatological follow-up of one of our CDKN2A-positive FPC families we detected an early stage melanoma in a 17-year-old female family member. A year after resection of a 5-mm superficial spreading melanoma (Breslow 0.8 mm), this patient is disease-free.

Table 2 Overview of literature on the role of CDKN2A-mutations in FPC

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population</th>
<th>CDKN2A-mutation found</th>
<th>Type(s) of mutation</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slater et al12</td>
<td>56 FPC families</td>
<td>No</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bartsch et al8</td>
<td>18 FPC families</td>
<td>No</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moskaluk et al12</td>
<td>21 FPC families</td>
<td>Yes</td>
<td>c.457 G&gt;T; c.19_23dup.p.Ser8fs</td>
<td>Mel-PC family</td>
</tr>
<tr>
<td>Current study</td>
<td>28 FPC families</td>
<td>Yes</td>
<td>c.19_23dup.p.Ser8fs; c.225_243del, p.Ala76fs</td>
<td>FPC family</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.225_243del, p.Ala76fs</td>
<td>FPC family</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.225_243del, p.Ala76fs</td>
<td>FPC family</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.225_243del, p.Ala76fs</td>
<td>Mel-PC family</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.225_243del, p.Ala76fs</td>
<td>Mel-PC family</td>
</tr>
</tbody>
</table>

*Family affected by PC and melanoma but that does not fulfil the diagnostic criteria of familial cutaneous malignant melanoma.
†After disclosure of the CDKN2A mutation, a melanoma was diagnosed in this family.
FPC, familial PC; PC, pancreatic cancer.
A number of previous reports have shown an indication for BRCA2-mutation analysis in FPC families that did not meet the criteria of familial breast and ovarian cancer.21 In a similar way, our findings emphasise the need to include CDKN2A-mutation analysis in genetic testing for FPC families, even in the absence of reported melanomas. It will help to better identify those at risk of developing PC and/or melanoma.

Surveillance of individuals at a high risk of malignant melanomas has proved to lead to early detection of melanomas and will consequently have a favourable effect in prognosis.22 Surveying of individuals at high risk of PC is emerging and may lead to an improvement of prognosis and a decline in PC incidence.23–25

In conclusion, the results of this series show that CDKN2A-mutation can be found in a considerable proportion of families with FPC. CDKN2A-mutation analysis should therefore be included in genetic testing in FPC families, even in the absence of reported melanomas. This strategy will enhance the recognition of individuals at risk for PC and facilitate the early detection of melanomas.


Competing interests None.

Ethics approval Approval provided by the Ethics Committee Erasmus MC, Ethics Committee Erasmus MC.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES


Indication for \textit{CDKN2A}-mutation analysis in familial pancreatic cancer families without melanomas

Femme Harinck, Irma Kluijt, Nienke van der Stoep, et al.

doi: 10.1136/jmedgenet-2011-100563