

## OCCUPATIONAL EXPOSURE TO DYES, METALS, POLYCYCLIC AROMATIC HYDROCARBONS AND OTHER AGENTS AND K-*ras* ACTIVATION IN HUMAN EXOCRINE PANCREATIC CANCER

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***ras* genes are known critical DNA targets for chemical carcinogens. Exocrine pancreatic cancer (EPC) is the human tumor with the highest prevalence of K-*ras* mutations at diagnosis. We analyzed the relationship between past occupational exposure to dyes, metals, polycyclic aromatic hydrocarbons (PAHs) and other agents and mutations in codon 12 of the K-*ras* gene in 107 incident cases of EPC. Information on occupational and life-style factors was obtained from personal interviews conducted during hospital stay. Occupational exposures were examined using industrial hygienists (IH) assessment and the Finnish job-exposure matrix (Finjem). Specific occupational exposures among K-*ras* mutated EPC cases ( $n = 83$ ) were compared to those of K-*ras* wild-type EPC cases ( $n = 24$ ) (case-case analysis). Multivariate-adjusted odds ratios (OR) and their corresponding 95% confidence limits were estimated by unconditional logistic regression. Cases with K-*ras* mutations were significantly more likely than wild-type cases to have been exposed to dyes and organic pigments (OR 4.8;  $p < 0.05$ ). There was some indication of weaker associations between K-*ras* mutations and occupational exposure to lead, PAHs, benzo[a]pyrene, gasoline, nickel, inhalatory exposure to chromium and sedentary work. The association with chromium compounds was stronger for G to T transversions, a finding compatible with experimental studies on mutation spectra for chromium. Results lend moderate support to the hypothesis of indirect relationships between occupational exposure to dyes and organic pigments and the activation of the K-*ras* gene in the etiopathogenesis of human exocrine pancreatic cancer.**  
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The relationship between some occupational exposures and the risk of pancreatic cancer remains controversial.<sup>1–3</sup> Epidemiologic studies have often been negative, while positive findings have frequently been nonspecific and inconsistent.<sup>3</sup> However, results from a recent meta-analysis suggest that the risk of pancreatic cancer may be increased by occupational exposure to chlorinated hydrocarbon solvents, organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), nickel and chromium.<sup>4</sup> Some of these agents are thought to be directly genotoxic, while others might enhance the mutagenicity and carcinogenicity of directly acting genotoxic agents.<sup>5</sup>

Activation of *ras* genes by a point mutation is the most frequent oncogenic alteration in human cancer.<sup>6</sup> Oncogenic *ras* mutations are one of the fundamental initiating events in several types of cancer, including pancreatic cancer.<sup>7</sup> *Ras* functions as a molecular switch in a network of intracellular signaling pathways, mainly controlling cell differentiation or proliferation. *ras* activating mutations result in constitutive signaling, thereby stimulating cell proliferation and inhibiting apoptosis.<sup>8</sup> Some agents found in the occupational environment induce neoplasms with *ras* alterations in animal models.<sup>9–12</sup> Also, several epidemiological studies have

reported associations between *ras* mutations and specific occupational exposures in human populations: asbestos in lung cancer,<sup>13,14</sup> vinyl chloride in angiosarcoma of liver<sup>15</sup> and solvents in acute myeloid leukemia,<sup>16</sup> hence supporting a role for chemical activation of *ras* genes in human cancer, and a rationale for conducting studies that assess the influence of environmental exposures in the pathogenesis of *ras*-mutated neoplasms.

At diagnosis, pancreatic tumors exhibit the highest prevalence of K-*ras* mutations (ranging from 75 to 85%)<sup>7</sup> of all human cancers. In pancreatic neoplasms, all *ras* mutations are in the K-*ras* gene, and practically all occur in codon 12. Previous studies on pancreatic cancer have reported associations between K-*ras* activation and coffee consumption, occupational exposure to hydrocarbon solvents and serum levels of several organochlorine compounds.<sup>1,17–20</sup> The relation between tobacco smoking and alcohol consumption and K-*ras* activation is unclear in EPC.<sup>19–23</sup>

The objective of our study was to analyze the relationship between selected occupational exposures and mutations in the K-*ras* gene in human exocrine pancreatic cancer.

### SUBJECTS AND METHODS

Methods and subjects have been described in detail elsewhere.<sup>1,17,18,24–26</sup> Briefly, subject recruitment took place in 1992–1995 at 5 general hospitals in eastern Spain, where 185 incident cases of EPC were prospectively identified. All their discharge diagnoses were reviewed by a panel of experts blinded to molecular results.<sup>27</sup> All cases were also independently reviewed by the study reference pathologists, who were unaware of the original diagnoses. The study protocol was approved by the Ethics Com-

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mittee of the participating hospitals, and patients gave informed consent to be included in the study.

The present report is based on 107 EPC patients with known *K-ras* status and occupational data. There were no significant differences between them and the remaining EPC cases with respect to sex, education, study site, tumor stage, duration of the interview and consumption of coffee, tobacco and alcohol, except that the included subjects were slightly younger.<sup>1,17</sup> Of the 107 cases, 83 (77.6%) harbored a *K-ras* mutation and 24 did not. There were no differences in the frequency of mutations according to age, sex, education and tumor stage at diagnosis.<sup>17</sup> *K-ras* nonmutated cases had smoked slightly more and drunk significantly less coffee than mutated cases.<sup>17</sup>

#### Patient interviews

Trained monitors conducted interviews with patients during hospital stay, soon around the time of diagnosis. Questions focused on past clinical history, occupation and life-style (including tobacco, coffee and alcohol consumption). The respondent was the patient himself in 96% of the cases and a relative alone in 4%.<sup>1,17,24,25,27</sup> Detailed occupational information was requested for 10 activities that were *a priori* defined to be potentially related with pancreas and biliary cancers,<sup>2-4</sup> including pesticide use, handling of petroleum derivatives, chemical industry, metal industry, rubber industry, graphic arts, jewelry, manufacture or repair of automobiles, leather tanning and textile industry. Patients who reported having worked in any of these activities were asked for the duration of employment, specific activity and products to which they had been exposed. The same information was also requested for 2 additional activities performed for at least 6 years. Using all the information about occupational exposures collected in the questionnaire, 2 industrial hygienists evaluated exposures to 22 suspected carcinogens. They coded exposure as exposed, unexposed or unknown. Exposed required a substantiated source of exposure. If exposure were unsubstantiated but possible, the category unknown was used. The intensity of the exposure was coded as high, low, none or unknown.<sup>25</sup> The industrial hygienists developed algorithms for the exposure assessment. Two occupational epidemiologists evaluated and accepted the algorithms. In addition, we used the Finnish job-exposure matrix (Finjem)<sup>28</sup> to explore occupational exposure to 21 chemical agents and 2 physical exposures. Two investigators (TK and JA) performed the conversion from Spanish to Finnish occupational codes.<sup>25</sup> The exposure categories used were exposed and unexposed. The cut point between exposed and unexposed was set as close as possible to the median of the distribution of the product of the probability of exposure (range 0.06–1) and the intensity of exposure (most in mg/m<sup>3</sup> or in ppm). For the present study we also created a more specific criterion to define exposure, by which exposure was required to have taken place both on the basis of the IH assessment and on the basis of Finjem. Chromium exposure assessed with Finjem included inhalatory exposure to chromium dust or fume from welding, smelting, grinding or other processing of stainless steel and other materials containing chromium, including metallic chromium, Cr(III), Cr(VI) and other chromium compounds, while the IH evaluated inhalatory and dermal exposure to chromium (III) and chromium (VI).

#### Detection of *K-ras* mutations

Paraffin-embedded tumor samples were used for the molecular analyses. Methods to detect mutations have been described in detail.<sup>1,17,21</sup> Briefly, DNA was extracted and amplified in 2 steps by nested PCR; in the second amplification reaction, an artificial BstNI restriction endonuclease site was introduced to discriminate between wild-type and mutated *K-ras* codon 12 sequences. Products were analyzed by acrylamide gel electrophoresis and ethidium bromide staining. To characterize the nucleotide substitution in codon 12, all mutated samples were further analyzed using a similar RFLP-based approach, as described elsewhere.<sup>21</sup> DNA from oral mucosal scrapings was used as normal control and DNA

from pancreas cancer cell lines or tumors were used as controls for the Val, Asp, Arg, Cys and Ser mutations. Interpretation of digestion products' electrophoresis was performed independently by 2 investigators (NM and FXR). When discordant results were obtained, the analysis was repeated and results evaluated again. This strategy has been shown to yield an agreement of >95% for all enzyme digestions.<sup>21</sup>

#### Statistical analyses

In our case-case study,<sup>17</sup> occupational exposures of EPC cases with and without *K-ras* mutated tumors were compared. In contingency tables, comparison of 2 qualitative or categorical variables was performed with Pearson's chi-square test for homogeneity or independence; alternatively, when  $\geq 20\%$  of cells had expected counts less than 5, Fisher's exact test was applied. For ordered categorical variables the Mantel-Haenszel chi-square test for linear trend was used.<sup>29</sup> If the observed number of cases in 1 cell of the contingency table was 0, the Woolf-Haldane correction was applied.<sup>30</sup> Multivariate-adjusted odds ratios (OR), their corresponding 95% confidence limits (CL) and 2-sided *p* values were estimated by unconditional logistic regression. The following potential confounders were included in the models: sex, age, cumulative number of years smoked and coffee consumption during the year prior to the first symptom. Allowance for other potential confounding variables (*e.g.*, schooling, alcohol consumption and diabetes) did not substantially modify any of the estimates.

## RESULTS

Among agents evaluated by both exposure assessment approaches, usually a higher proportion of cases than controls was classified as exposed by Finjem than by IH (Table I). Indications of an association between *K-ras* mutation and lead exposure were found with both methods of exposure assessment, with a slightly higher risk estimate found by the IH. Mutated cases were over 4 times more likely to have been occupationally exposed to lead than wild-type cases (OR = 4.8), on the basis of IH. With the Finjem assessment, the risk increased 2.4-fold. Increased ORs were also seen for exposure to textile/cotton dust following both approaches (Finjem and IH). On the basis of Finjem, cases harboring a *K-ras* mutation were more than 3 times as likely to have inhaled chromium compounds than wild-type cases (few participants were deemed exposed by IH). This was similarly true for nickel but with a lower OR. Although no increases were seen using the IH evaluation, with an OR of 4.8 Finjem suggests that in pancreatic cancer an association may exist between *K-ras* mutations and exposure to PAHs. No association between exposure to cutting oils and *K-ras* mutations was found by IH nor by Finjem. Few cases were exposed to ionizing radiation.

Results for exposures assessed only by IH are presented in Table II. Only *K-ras* mutated patients had been exposed to aluminum, anilines, dyes and organic pigments and other inorganic pigments. However, the difference reached statistical significance only for exposure to dyes and organic pigments (OR 4.8; *p* < 0.05). All 4 cases exposed to anilines were also classified as exposed to dyes and organic pigments. Occupations entailing exposure to dyes and organic pigments included workers who reported dying or handling dyes as textile workers (*n* = 2), in the manufacture of dyes (2), as leather tanners (1), as shoemakers (1) and as hairdressers (1). Risk estimates for pesticides varied on the basis of the type of pesticides. While arsenical pesticides showed an increased risk (OR = 1.9), exposure to organochlorine pesticides was equally reported among patients with *K-ras* mutated and wild type pancreatic tumors. Exposure to the other types of pesticides was slightly more frequent among patients with *K-ras* wild-type tumors (Table II). The latter was true also for dermal and inhalatory exposure to chromium compounds (OR = 0.15). All 5 *K-ras* nonmutated cases considered by IH to be exposed to chromium compounds were deemed so at a low intensity of exposure.

**TABLE I** – OCCUPATIONAL EXPOSURES IN EXOCRINE PANCREATIC CANCER CASES WITH AND WITHOUT MUTATIONS IN THE K-RAS GENE<sup>1</sup>

	K- <i>ras</i> mutated ( <i>n</i> = 83)		K- <i>ras</i> wild-type ( <i>n</i> = 24)		Adjusted OR <sup>2</sup> (95% CI)
	Exposed		Exposed		
	<i>n</i>	(%)	<i>n</i>	(%)	
Lead <sup>3</sup>					
Industrial hygienists	7	(8.4)	0	(0.0)	4.80 <sup>4</sup> (0.67–UH <sup>5</sup> )
Finjem	10	(12.0)	1	(4.2)	2.15 (0.33–42.5)
Textile/cotton dust <sup>6</sup>					
Industrial hygienists	5	(6.0)	0	(0.0)	3.43 <sup>4</sup> (0.54–UH)
Finjem	6	(7.2)	0	(0.0)	4.11 <sup>4</sup> (0.65–UH)
Both <sup>7</sup>	2	(2.4)	0	(0.0)	1.50 <sup>4</sup> (0.07–UH)
Chromium compounds (inhalatory) <sup>8</sup>					
Industrial hygienists	2	(2.4)	0	(0.0)	1.50 <sup>4</sup> (0.25–UH)
Finjem	8	(9.6)	1	(4.2)	3.47 (0.50–71.6)
Both <sup>7</sup>	2	(2.4)	0	(0.0)	1.50 <sup>4</sup> (0.25–UH)
Nickel <sup>3</sup>					
Industrial hygienists	1	(1.2)	0	(0.0)	— — —
Finjem	6	(7.2)	1	(4.2)	2.33 (0.30–50.0)
Polycyclic aromatic hydrocarbons					
Industrial hygienists	9	(10.8)	2	(8.3)	0.91 (0.17–7.01)
Finjem	7	(8.4)	0	(0.0)	4.80 <sup>4</sup> (0.61–UH)
Both <sup>7</sup>	3	(3.6)	0	(0.0)	2.13 <sup>4</sup> (0.24–UH)
Cutting oils					
Industrial hygienists	2	(2.4)	1	(4.2)	1.01 (0.07–26.7)
Finjem	5	(6.0)	1	(4.2)	2.41 (0.29–53.6)
Both <sup>7</sup>	2	(2.4)	1	(4.2)	1.01 (0.07–26.7)
Pesticides					
Industrial hygienists	9	(10.8)	3	(12.5)	0.66 (0.16–3.39)
Finjem	3	(3.6)	2	(8.3)	0.43 (0.06–3.99)
Both <sup>7</sup>	2	(2.4)	1	(4.2)	0.33 (0.03–7.65)
Ionizing radiation <sup>9</sup>					
Industrial hygienists	0	(0.0)	1	(4.2)	0.00 (0.00–1.36)

<sup>1</sup>Estimates based on industrial hygienists and Finjem assessment of the exposure.—<sup>2</sup>Odds ratio adjusted by age, sex and tobacco and coffee consumption.—<sup>3</sup>No cases were deemed exposed by both the industrial hygienists and Finjem.—<sup>4</sup>Odds ratio based on the Woolf-Haldane correction.—<sup>5</sup>Unquantifiably high.—<sup>6</sup>Finjem assessed exposure to textile dust, while industrial hygienists assessed exposure to cotton dust.—<sup>7</sup>Exposed according to both industrial hygienists and Finjem.—<sup>8</sup>Dermal exposure to chromium was excluded to make comparable the exposure definition of the industrial hygienists and Finjem.—<sup>9</sup>No cases were deemed exposed by Finjem.

**TABLE II** – OCCUPATIONAL EXPOSURES IN EXOCRINE PANCREATIC CANCER CASES WITH AND WITHOUT MUTATIONS IN THE K-RAS GENE<sup>1</sup>

	K- <i>ras</i> mutated ( <i>n</i> = 83)		K- <i>ras</i> wild-type ( <i>n</i> = 24)		Adjusted OR <sup>2</sup> (95% CI)
	Exposed		Exposed		
	<i>n</i>	(%)	<i>n</i>	(%)	
Aluminium	2	(2.4)	0	(0.0)	1.50 <sup>4</sup> (0.23–UH <sup>5</sup> )
Anilines	4	(4.8)	0	(0.0)	2.77 <sup>4</sup> (0.47–UH)
Chromium compounds <sup>3</sup>	3	(3.6)	5	(20.8)	0.15 (0.03–0.76)
Dyes and organic pigments	7	(8.4)	0	(0.0)	4.80 <sup>4</sup> (1.02–UH)
Other inorganic pigments	2	(2.4)	0	(0.0)	1.50 <sup>4</sup> (0.06–UH)
Pesticides, arsenical	6	(7.2)	1	(4.2)	1.87 (0.26–38.5)
Pesticides, organochlorine	7	(8.4)	2	(8.3)	0.93 (0.19–6.98)
Pesticides, organophosphate	7	(8.4)	3	(12.5)	0.55 (0.13–2.91)
Pesticides, other	7	(8.4)	3	(12.5)	0.50 (0.11–2.71)

<sup>1</sup>Estimates based on industrial hygienists assessment of the exposure.—<sup>2</sup>Odds ratio adjusted by age, sex, and tobacco and coffee consumption.—<sup>3</sup>Includes dermal and inhalatory exposure to chromium compounds.—<sup>4</sup>Odds ratio based on the Woolf-Haldane correction.—<sup>5</sup>Unquantifiably high.

Several agents evaluated only with Finjem showed increased ORs, although none of the estimates was statistically significant (Table III). Occupational exposures as gasoline engine exhaust and sedentary work were over 4 times more likely among cases with K-*ras* mutated tumors than among K-*ras* wild-type cases. The OR was slightly over 2 for gasoline and low frequency magnetic fields.

All cases exposed to gasoline, gasoline engine exhaust, benzo-(A)pyrene, aluminum and lead were men. Upon stratification by age ( $\pm$  60), higher ORs were apparent for lead, nickel and inhalatory exposure to chromium in the group under 60 years.

When analyses were restricted to subjects whose exposures started at least 10 years before diagnosis, ORs decreased slightly (less than 25%) for most agents and increased for nickel (OR = 4.7; CL 0.8–90). Restricting the analyses to adenocarcinoma of the pancreas did not modify the ORs reported in Tables I–III.

Table IV shows the associations between selected occupational exposures and mutational spectra. As compared to wild-type cases, a higher proportion of cases with a mutation from glycine to valine (GGT→GTT) were deemed exposed to lead and to chromium (inhalatory exposure). Whilst some of the other putative associa-

**TABLE III** – OCCUPATIONAL EXPOSURES IN EXOCRINE PANCREATIC CANCER CASES WITH AND WITHOUT MUTATIONS IN THE K-RAS GENE<sup>1</sup>

	K-ras mutated (n = 83)		K-ras wild-type (n = 24)		Adjusted OR <sup>2</sup> (95% CI)
	Exposed		Exposed		
	n	(%)	n	(%)	
Gasoline engine exhaust	7	(8.4)	0	(0.0)	4.80 <sup>3</sup> (0.52–UH <sup>4</sup> )
Gasoline	3	(3.6)	0	(0.0)	2.13 <sup>3</sup> (0.24–UH <sup>4</sup> )
Sedentary work	7	(8.4)	0	(0.0)	4.80 <sup>3</sup> (0.50–UH <sup>4</sup> )
Low frequency magnetic fields	25	(30.1)	3	(12.5)	2.71 (0.75–13.2)
Benzo(A)pyrene	6	(7.2)	1	(4.2)	1.82 (0.22–40.4)
Volatile sulfur compounds	15	(18.1)	3	(12.5)	1.74 (0.44–8.95)
Diesel engine exhaust	5	(6.0)	1	(4.2)	1.02 (0.13–21.5)
Bitumen fumes	2	(2.4)	0	(0.0)	2.13 <sup>3</sup> (0.18–UH <sup>4</sup> )
Cadmium	6	(7.2)	2	(8.3)	0.94 (0.18–7.39)
Leather dust	1	(1.2)	0	(0.0)	— —
Wood dust	1	(1.2)	2	(8.3)	0.29 (0.01–3.96)
Synthetic polymer dust	7	(8.4)	3	(12.5)	0.50 (0.10–2.88)

<sup>1</sup>Estimates based on assessment of the exposure with Finjem.—<sup>2</sup>Odds ratio adjusted by age, sex and tobacco and coffee consumption.—<sup>3</sup>Odds ratio based on the Woolf-Haldane correction.—<sup>4</sup>Unquantifiably high.

**TABLE IV** – ASSOCIATIONS BETWEEN SELECTED OCCUPATIONAL EXPOSURES AND MUTATION SPECTRA<sup>1</sup>

	Valine vs. K-ras wild-type		Aspartic acid vs. K-ras wild-type	
	OR <sup>2</sup>	(95% CI)	OR <sup>2</sup>	(95% CI)
Chromium compounds (Finjem)	9.88	(0.48–424)	3.33	(0.10–121)
Lead (Finjem)	1.49	(0.08–43.0)	3.55	(0.37–79.6)
Lead (IH)	9.27 <sup>4</sup>	(1.25–UH <sup>5</sup> )	7.00 <sup>4</sup>	(0.18–UH <sup>5</sup> )
Benzo(A)pyrene	— <sup>3</sup>	—	3.24	(0.21–98.6)
PAHs (Finjem)	—	—	14.20 <sup>4</sup>	(0.99–UH <sup>5</sup> )
PAHs (IH)	2.02	(0.27–19.1)	0.58	(0.05–6.48)
Volatile sulfur compounds	1.03	(0.12–10.5)	0.55	(0.04–5.62)
Low frequency magnetic fields	2.78	(0.48–20.8)	2.15	(0.33–15.6)
Gasoline engine exhaust	—	—	10.40 <sup>4</sup>	(0.42–UH <sup>5</sup> )
Gasoline	—	—	7.00 <sup>4</sup>	(0.35–UH <sup>5</sup> )
Sedentary work	6.28 <sup>4</sup>	(0.39–UH)	—	—

<sup>1</sup>Number of cases mutated from glycine (GGT) to valine (GTT) = 21 number of cases mutated to aspartic acid (GAT) = 19.—<sup>2</sup>Odds ratio adjusted by age, sex and tobacco and coffee consumption.—<sup>3</sup>— None or only one case exposed.—<sup>4</sup>Odds ratio based on the Woolf-Haldane correction.—<sup>5</sup>Unquantifiably high.

tions shown in Table IV may deserve to be tested in new, independent studies, several hundred cases are likely to be required for the estimates to achieve sufficient precision.

#### DISCUSSION

We found that in exocrine pancreatic cancer occupational exposure to dyes and pigments might be associated to the activation of the K-ras gene. We also observed weaker associations between K-ras mutations and occupational exposure to lead, PAHs, gasoline engine exhaust, nickel, inhalatory exposure to chromium compounds and sedentary work. However, the number of subjects was small, most results were not statistically significant and, hence, caution should be used in the interpretation of all estimates.

Although dyes are a chemically heterogeneous group, most of them are aromatic amines, which are pancreatic carcinogens in animal models, and may play a role in human pancreatic carcinogenesis.<sup>20,25,31–35</sup> A significant correlation has been reported between aromatic DNA adducts in the pancreas and K-ras mutations.<sup>36</sup> We did not find a specific association between exposure to dyes and mutational spectra; this suggests that rather than causing direct K-ras damage, dyes might confer a proliferation advantage to K-ras mutated cell clones, or act through other indirect mechanisms.<sup>20</sup> Excesses of pancreatic cancer have been reported in association with exposure to dyes and aniline derivatives,<sup>37–40</sup> as well as among leather tanners.<sup>41–46</sup> However, such associations were not apparent in other cohorts of exposed workers.<sup>47,48</sup> In our study population exposure to dyes was a risk factor for pancreatic

cancer.<sup>25</sup> In this respect, a similarity may exist between pancreas cancer and bladder cancer, another site in which *ras* mutations are important.

Lead has been reported to induce mutations in eukaryotic cells.<sup>49,50</sup> Studies in cultured human cells suggest that the type of mutations induced by lead are dose-dependent, with point mutations being induced at low doses and deletions at higher concentrations.<sup>49</sup> Direct DNA damage by lead has only been reported at high concentrations.<sup>50</sup> Lead compounds have shown enhancing effects in combination with UV light and alkylating agents, putatively through interference with DNA repair *via* DNA polymerases inhibition.<sup>51</sup> Another mechanistic scenario compatible with our results is the interaction with enzymes involved in the metabolism of K-ras mutagens;<sup>1,17,20</sup> for instance, ionic lead may inhibit CYP1A2 and induce GSTP in rat liver.<sup>52</sup>

Results on the basis of Finjem support an association between inhalatory exposure to chromium compounds and K-ras mutations. Exposure of cells to chromium (VI) results in binding of chromium (III) to DNA, yielding binary Cr(III)-DNA adducts.<sup>53,54</sup> Studies assessing the mutational spectrum induced by chromium (III) and chromium (VI) in human cells concluded that single base substitutions at the G:C base pairs were the predominant type of mutations for chromium (III)<sup>54,55</sup> and for chromium (VI).<sup>56–58</sup> The most frequently base substitutions found were G to T transversions,<sup>54,56</sup> G to C transversions<sup>55</sup> and G to A transitions.<sup>57,58</sup> It has also been reported that the chromium (III)-induced base-substitution hot spots are different from those occurring spontaneously.<sup>55</sup> The odds

ratio between chromium compounds and *ras* mutations was stronger for G to T transversions, a finding compatible with results from experimental studies on mutation spectra for chromium. While Finjem considered only inhalatory exposure to chromium, the IH considered also dermal exposure to chromium compounds. The source of dermal exposure to chromium compounds was putative dermal contact with cement in all cases. Given the low dose of chromium in cement (1 to 173 mg/kg in different countries)<sup>59,60</sup> and the low dermal absorption for chromium compounds, dermal exposure to cement is quantitatively minor compared to inhalatory exposure. The inverse association with K-*ras* mutations and dermal exposure to chromium might be due to other factors present among construction workers.

Results for nickel exposure on the basis of Finjem support an association with K-*ras* mutations. A study biomonitoring carcinogenic metals in a general population found a positive association between nickel and oxidative DNA lesions, in line with a possible genotoxic effect of nickel.<sup>61</sup> Although nickel is not considered a direct mutagenic agent,<sup>62</sup> nickel compounds can induce chromosomal aberrations<sup>63</sup> and may increase DNA methylation.<sup>62</sup> Exposure to metals such as arsenic, chromium and nickel may enhance generation of reactive oxygen species (ROS), which may promote carcinogenesis.<sup>64</sup>

Holding a sedentary job was a risk factor for pancreatic cancer in our case-control study<sup>24</sup> and in others;<sup>33,65</sup> this may reflect a biological pathway involving obesity, lack of physical activity or both.<sup>2,66–68</sup>

Analyses of mutational spectra provided some support for the association with inhalatory exposure to chromium. However, special caution is required when using mutation spectra as a basis to assess the role of occupational exposure to mutagens or carcinogens in human cancer.<sup>69</sup> Mutations may result from various selection processes.<sup>20,69,70</sup> Variations in the capacity of target cells to repair mutagenic DNA lesions must also be taken into account,<sup>70,71</sup> and spectra may critically depend upon conditions of exposure.<sup>72</sup>

The possibility that some of the cases could belong to several exposure categories was assessed. Based on the evaluation of the industrial hygienists, only 1 out of the 7 cases exposed to dyes and organic pigments was exposed to solvents, whereas 3 out of the 7 cases were deemed as exposed to solvents on the basis of Finjem. Alcohol did not influence risk estimates for any of the associations reported. Potential confounding by cigarette and coffee consumption was accounted for in the logistic regression models.

One of the strengths of our study is the high percent of subjects (90%) with occupational information personally obtained soon around the time of diagnosis.<sup>24,25</sup> For pancreatic cancer, the proportion of cases who had both molecular and environmental data was also high.<sup>73</sup> All diagnoses were revised by a panel of experts

in pancreatic pathology.<sup>27</sup> Also remarkable is the lack of differences between patients with and without available tissue for genetic analyses, arguing against an important selection bias.<sup>17,73–75</sup> The observed prevalence of K-*ras* mutations (78%) agrees with that found by the larger studies.<sup>7,73</sup> Finally, since K-*ras* status and occupational exposures were assessed independently, misclassification of exposure would be nondifferential and associations would tend to be diluted.

Although several estimates were imprecise and some associations might have emerged by chance, all exposures tested were chosen *a priori* based on evidence for pancreatic cancer.<sup>4</sup> Most agents that we found associated with K-*ras* mutations also exhibited an increased risk in our case-control analyses.<sup>25</sup> Since controls used in such analyses included patients with disorders where *ras* alterations can exist (*e.g.*, chronic pancreatitis), we chose not to use the controls in the present study.

Each of the 2 methods we used to measure occupational exposures has strengths and weaknesses. The IH were familiar with the country working conditions and took advantage of information about gender, periods of employment and products to which patients referred having been exposed.<sup>25</sup> On the other hand, when a job-exposure matrix involves elements such as intensity, probability and time period of exposure (all 3 available in Finjem), estimates may be more consistent.<sup>76,77</sup>

*ras* activation has been proposed as a biomarker of occupational cancer risk for early detection and prevention.<sup>78</sup> However, before implementing systematic screening for *ras* alterations among workers, large cohort studies would need to evaluate the prognostic significance of *ras* alterations in asymptomatic subjects.<sup>79</sup>

In conclusion, results lend moderate support to the hypothesis of indirect relationships between occupational exposure to dyes and organic pigments and the activation of the K-*ras* gene in the etiopathogenesis of human EPC. However, since this is only the first study on these occupational exposures and K-*ras* mutations in EPC, special caution is required in the interpretation of results.

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