Aromatic DNA adducts and number of lung cancer risk alleles in Map-Ta-Phut Industrial Estate workers and nearby residents

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The Map-Ta-Phut Industrial Estate (MIE) in Rayong, Thailand, is the location of one of the largest industrial complexes in southeastern Asia. The MIE complex produces a mixture of air pollutants, including polycyclic aromatic hydrocarbons, compounds capable to induce the generation of DNA adducts. DNA adducts are considered to be a biomarker of carcinogen exposure; however, their production can be modulated by genetic susceptibility. Thus, we analysed the influence of EPHX1 His139Arg (A>G, rs2234922) and NQO1 Pro187Ser (C>T, rs1800566) in the metabolism of polycyclic aromatic hydrocarbons; MnSOD2 Val16Ala (C>T, rs1799725) a gene that acts against the free radical generation; APE1/Ref-1 Asp148Glu (T>G, rs3136820) a gene involved in the repair of DNA adducts and the activities of cytochrome P450, the manganese superoxide dismutase (MnSOD2), a gene that can inhibit the redox-cycling of PAHs, and MnSOD2 Val16Ala (C>T, rs1799725) a gene that acts against the free radical generation; APE1/Ref-1 Asp148Glu (T>G, rs3136820) a gene involved in the repair of DNA, and in the control of cell-cycle and apoptosis on leucocyte DNA adducts in 77 MIE workers, 69 Map-Ta-Phut residents, and 50 rural controls, Rayong, Thailand. We searched for associations with the ‘sum of at-risk alleles’ by combining the variant alleles of EPHX1, NQO1 and MnSOD2 together with the wild-type allele of APE1, since they appeared to influence lung cancer risk. Although our findings revealed significant associations between DNA adducts and the EPHX1 His139Arg and NQO1 Pro187Ser polymorphisms, the combination of at-risk alleles was found to affect DNA damage much stronger. DNA adducts were significantly increased in the individuals bearing 4 and ≥5 at-risk alleles [mean ratio (MR) = 1.55, 95% CI 1.10–2.18, P = 0.012, and MR = 2.11, 95% CI 1.27–3.51, P = 0.004, respectively]. After correction for residence/employment categorisation, a significant increment was present in the MIE workers with ≥5 alleles [MR = 2.88, 95% CI 1.46–5.71, P = 0.003). Our data indicate relationships between the generation of DNA adducts and the enzymatic activities of EPHX and NQO1. The combination of unfavourable genetic variants seems to determine the individuals’ susceptibility, rather than a single polymorphism.

Introduction

The Map-Ta-Phut Industrial Estate (MIE) in Rayong, Thailand, is the location of one of the largest steel, oil-refinery, petrochemical and power plant complexes in southeastern Asia. MIE complex can produce a mixture of air pollutants, including nitrogen oxides, benzene, heavy metals and polycyclic aromatic hydrocarbons (PAHs) (1–4). The PAHs can cause the generation of aromatic DNA adducts (5), which, if unrepaired, can induce mutations, including mutations in the TP53 tumor-suppressor gene (6). DNA adducts are considered to be a biomarker of carcinogen exposure (7), predictive of lung cancer risk (8–10).

Recently, we have analysed the peripheral blood levels of leucocyte DNA adducts in the MIE workers and the Map-Ta-Phut residents with respect to controls living in a rural district in the same province of Rayong (11). The amount of DNA adducts in the MIE workers were significantly increased compared with rural controls. The Map-Ta-Phut residents living in the surrounding areas also had enhanced DNA damage levels relative to controls (11).

Although the production of DNA adducts is induced by external exposures to carcinogens, their levels can be modulated by individual genetic susceptibility, such as the ability to activate-deactivate carcinogens, and to repair the DNA damage (12). Our previous studies showed significant associations of DNA adducts with single nucleotide polymorphisms (SNPs) in metabolic and DNA repair genes (10,12–19). For instance, we found significant relationships between the levels of DNA adducts and the activities of cytochrome P450, microsomal epoxide hydrolase (EPHX), myeloperoxidase and N-acetyl-transferase enzymes, and with the lack of glutathione S-transferase M1 detoxification (12,17–19). Our studies indicated a novel function of XRCC1 (X-ray repair cross complementing) and XRCC3, representing the base excision repair (BER) and the double-strand breaks repair pathways, in the repair of aromatic DNA adducts (10,14–16).

Herein, we addressed the issue of genetic susceptibility with special emphasis on DNA polymorphisms in genes involved in PAH metabolism, in the defence against free radicals, and in the control of cell-cycle and apoptosis in 77 MIE workers, 69 Map-Ta-Phut residents and 50 rural controls. We analysed the associations of DNA adducts with DNA polymorphisms in the microsomal EPHX1 (A>G, His139Arg, rs2234922), a gene involved in the production of diol-epoxide (20), the NAD(P)H:quinone oxidoreductase (NQO1, C>T, Pro187Ser, rs1800566), a gene that can inhibit the redox-cycling of PAHs into quinones (21), the manganese superoxide dismutase (MnSOD2, C>T, Val16Ala, rs1799725), a gene that acts against the generation of free radicals (22), highly reactive compounds capable to activate directly some PAHs, such as the cyclopenteno[c,d]pyrene (23) and the apurinic/apyrimidinic (AP) endonuclease 1 (APE1)/Ref-1 (T>G, Asp148Glu, rs3136820), a BER gene (24), also involved in the repair of aromatic DNA adducts (24), and also known as the redox sensitive regulator of cell-cycle and apoptotic response (25,26) (see Figure 1 for expected effects on DNA adducts).

These DNA polymorphisms were also selected because they appeared to influence the risk of lung cancer (27–31). The variant
affect DNA adduct levels. In the current study to study whether genetic susceptibility can be expected carcinogens. The study was approved by the Institutional Review Board of the National Cancer Institute, Bangkok, Thailand. A standard questionnaire, tested in small pilot study (unpublished results), was used to collect demographic and life-style data, i.e. age, gender, smoking habit (last three months), residence and occupation. The questionnaire was administered to the participants before blood sampling and after signed informed consent was obtained.

Materials and methods

Study population

The study population consisted of 77 MIE workers, 69 Map-Ta-Phut residents and 50 rural controls for which DNA adduct data and leucocyte DNA sequences were available (11). The MIE workers were identified and recruited through the industrial health service. The Map-Ta-Phut residents and the rural controls were contacted and recruited by local health personnel. Nearby residents were living in the surrounding residential areas near the MIE complex, whereas the rural controls lived in a district from the same province of Rayong, but with no proximity of relevant air pollution sources. The nearby residents and rural controls had no work history in industries entailing exposure to known or suspected carcinogens. The study was approved by the Institutional Review Board of the National Cancer Institute, Bangkok, Thailand. A standard questionnaire, tested in a small pilot study (unpublished results), was used to collect demographic and life-style data, i.e. age, gender, smoking habit (last three months), and subsequently genotyped by single base extension (SBE) (33).

DNA adduct levels

The combination of the variant alleles of EPHX1 His139Arg, NQO1 Pro187Ser and MnSOD Val16Ala have been associated to increased risk of lung cancer (27–29). Conversely, the variant allele of APE1/Ref-1 Asp148Glu SNP has been associated with a reduction of lung cancer risk (30,31). Thereby, we investigated the effects of the combination of the variant alleles of EPHX1 His139Arg, NQO1 Pro187Ser, MnSOD Val16Ala with the wild-type allele of APE1/Ref-1 Asp148Glu, by calculating the so-called ‘sum of at-risk alleles’, on the generation of DNA adducts to evaluate whether the presence of unfavourable at-risk allele combination can influence the level of DNA damage. The list of alleles that were considered to be the lung cancer risk alleles, included allele G for His139Arg of EPHX1, allele T for Pro187Ser of NQO1, allele T for Val16Ala of MnSOD-1, allele T and allele A for APE1/Ref-1 Asp148Glu.

Our previous cross-sectional study that compared the prevalence of aromatic DNA adducts in groups of subjects experiencing various degrees of air pollution in the Rayong Province, Thailand (11), provided the DNA adduct data that were used in the current study to study whether genetic susceptibility can affect DNA adduct levels.

DNA polymorphism analysis

The choice of the following DNA polymorphism, e.g. EPHX1 His139Arg, NQO1 Pro187Ser, MnSOD Val16Ala and APE1/Ref-1 Asp148Glu, included in this study was based on (a) the known effects on enzyme activity (20,22,24–26) (b) their association with lung cancer risk (27–31); and (c) their expected influence on DNA adduct levels (Figure 1). The SNPs were analysed using a multiplex polymerase chain reaction (PCR) method developed by Knaapen and coworkers (33). DNA sequences containing the SNPs of interest were amplified by multiplex PCR and subsequently genotyped by single base extension (SBE) (33).

Briefly, PCR was carried out in a 10-µl reaction volume containing water, 10x PCR buffer, 0.2 mM dNTPs, 0.5 mM MgCl₂, 1.25 U Platinum Taq polymerase, 40 ng template DNA and 0.2 mM/1 PCR primer. After a denaturation period of 3 min at 95°C, amplification was accomplished in 30 cycles of 94°C (30 s), 60°C (30 s) and 72°C (30 s). The final extension was done for 5 min at 72°C. Subsequent genotyping was done using the SNaPshot procedure, and SBE products were analysed on an ABI Prism 3100 genetic analyser using Genescan Analysis software (version 5.7). Both PCR and SBE primers were designed using primer 3 (http://www-genome.wi.mit.edu/cgi-bin/ primer3_www.cgi) and net primer (http://www.premierbiosoft.com/netprimer/netpr-launch/netprlaunch.html).

Fig. 1. Selection of genes involved in polycyclic aromatic hydrocarbon metabolism, in the defence against free radical production, and in the DNA repair and control of cell-cycle and apoptosis, e.g. microsomal epoxide hydrolase (EPHX1) His139Arg, NAD(P)H quinone oxidoreductase (NQO1) Pro187Ser, manganese superoxide dismutase (MnSOD) Val16Ala, apurinic/apyrimidinic endonuclease 1 (APE1) Asp148Glu genes and their expected effects on DNA adducts.
Table I. Characteristics of the study population

<table>
<thead>
<tr>
<th>Study population</th>
<th>Rural controls</th>
<th>Map-Ta-Phut residents</th>
<th>Map-Ta-Phut Industrial Estate workers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Gender</td>
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<td>17</td>
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<td>Smoking habit</td>
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<td>27</td>
</tr>
<tr>
<td>Former smokers</td>
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<td>5</td>
<td>3</td>
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<tr>
<td>Current smokers</td>
<td>120</td>
<td>24</td>
<td>39</td>
</tr>
</tbody>
</table>

Statistical analysis

The different genotypes were coded as wild-type (major allele homozygote), and variant genotypes (heterozygote and minor allele homozygote). The individuals were also grouped according to the number of alleles relevant for lung cancer risk (27–31).

The levels of DNA adducts across the different genotypes involved in PAH metabolism, in the defence against free radicals, and in the control of cell-cycle and apoptosis (21–23,25–27) were compared by multivariate statistical analyses using log-normal regression models. The regression parameters estimated from the model were interpreted as ratios (means ratio) between the means of DNA adducts of each level of the study variables with respect to the reference level, adjusted by age, gender, smoking status and residence and employment.

The relationship between DNA adducts and genotypes was also analysed by grouping individuals for residence and employment.

We used multivariate log-normal regression models, including age, gender, tobacco smoking and residence/employment as independent variables, to evaluate the effect of the combination of selected lung cancer risk alleles on the generation of aromatic DNA adducts. The analysis of the potential association of DNA adducts with the combination of selected at-risk alleles was performed among all the study population and stratifying by residence/employment. P < 0.05 (two-tailed) was considered significant. Data were analysed using SPSS 13.0 (SPSS, USA).

Results

Study population

The demographic characteristics of the study population and after categorisation for residence and employment are presented in Table I. The mean age of the study population was 34 ± 7 (SD) years; 81.2% of the volunteers, including 78% of the rural controls, 75.4% of the Map-Ta-Phut residents and 88.4% of MIE workers were male; 61.2% of the participants, including 48% of the rural controls, 56.5% of the nearby residents and 74% of the MIE workers, reported to be current smokers. Further demographic details were reported previously (12,34).

DNA polymorphisms and aromatic DNA adducts

The genotype distribution of the EPHX1 His139Arg, NQO1 Pro187Ser, MnSOD2 Val16Ala and APE1/Ref-1 Asp148Glu polymorphisms and the means of DNA adducts per 10^9 nucleotides ± standard deviation (SD) in overall the study population and by grouping the volunteers for residence and employment are reported in Table II. We used multivariate statistical analysis, including age, gender, smoking and residence/employment as independent variables, to examine the changes in the levels of aromatic DNA adducts across to the different genotypes and stratifying by residence and employment.

These multivariate models showed higher amount of DNA adducts in EPHX1-139Arg and NQO1-187Ser homozygotes with intermediate levels in the EPHX1-His/Arg and NQO1-Pro/Ser heterozygotes. The MIE workers carrying the MnSOD2-16Ala wild-type genotype presented increased levels of DNA adducts. Conversely, the APE1/Ref-1-148Glu homozygotes and the Asp/Glu heterozygotes showed decreased levels of DNA adducts (Table II).

In detail, the means of DNA adducts ± SD for the EPHX1 His139Arg polymorphism were 8.0 ± 7 DNA adducts per 10^9 nucleotides in wild-type homozygotes and 16 ± 2 DNA adducts per 10^9 nucleotides in Arg/Arg homozygotes in all subjects combined [mean ratio (MR) = 1.26, 95% CI 0.64–2.51, P = 0.499]. After correction for residence and employment categorisation, the increment of DNA adducts was found to be borderline significant in the MIE workers carrying the Arg/Arg in respect to the wild-type homozygotes [20 ± 2 vs. 9.2 ± 7, MR = 2.16, 95% CI 0.97–4.78, P = 0.058]. Also a significant trend of DNA adduct formation with increased levels in EPHX1-139Arg homozygotes and with intermediate levels in EPHX1-His/Arg heterozygotes in respect to His/His homozygotes was found in the MIE workers (P for trend = 0.019). No significant associations were detected in the Map-Ta-Phut residents and the rural controls.

The means of DNA adducts ± SD in adducts per 10^9 nucleotides for the NQO1 Pro187Ser polymorphism were 7.9 ± 9 in NQO1-187Ser homozygotes, 9.3 ± 7 in Ser/Ser heterozygotes and 9.1 ± 7 in Ser/Arg homozygotes in all study groups combined (MR = 1.50, 95% CI 1.13–1.99, P = 0.005, and MR = 1.75, 95% CI 1.14–2.68, P ≤ 0.011, respectively). A significant trend of DNA adduct generation with enhanced levels in NQO1-187Ser homozygotes and with intermediate amount in NQO1-Pro/Ser heterozygotes in respect to the wild-type was observed in the general population (P for trend = 0.002). After adjustment for residence and employment categorisation, significant changes were observed in the Map-Ta-Phut residents carrying the Ser/Ser and Pro/Ser genotypes with respect to the wild-type homozygotes (11.6 ± 6 and 9.7 ± 6 vs. 6.1 ± 6, MR = 2.45, 95% CI 1.12–5.35, P = 0.025 and MR = 2.03, 95% CI 1.26–3.28, P = 0.004, respectively). A significant trend was present in the Map-Ta-Phut residents (P for trend = 0.004). The MIE workers with the Ser/Ser showed a nonsignificant increment of DNA adducts with respect to the wild-type homozygotes (13 ± 8 vs. 11 ± 1, MR = 1.84, 95% CI 0.83–4.10, P = 0.132). A borderline significant trend was found in the MIE workers (P for trend = 0.093). No other associations were found.

When the MnSOD2 Val16Ala polymorphism was considered, the means of DNA adducts ± SD were 8.8 ± 7 DNA adducts per 10^9 nucleotides in the MnSOD2-Val16 homozygotes, 11 ± 8 in those carrying the Ala/Ala genotype in all groups combined (MR = 0.91, 95% CI 0.51–1.63, P = 0.759). A nonsignificant increment of DNA adducts was present in the MIE workers carrying the Ala/Ala genotype with respect to the wild-type homozygotes (14 ± 9 vs. 12 ± 8, MR = 1.19, 95% CI 0.51–2.78, P = 0.691). No other associations were detected.

The means of DNA adducts ± SD for the APE1/Ref-1 Asp148Glu polymorphism were 11 ± 9 adducts per 10^9 nucleotides in APE1/Ref-1-148Asp genotype, 6.8 ± 5 in Asp/Glu heterozygotes and 7.5 ± 6 in Glu/Glu homozygotes in all groups combined (MR = 0.71, 95% CI 0.54–0.94, P = 0.019, and MR = 0.80, 95% CI 0.51–1.28, P = 0.335, respectively). After correction for residence and employment categorisation, a nonsignificant decrement of DNA adducts was found in the MIE workers carrying the Glu/Glu and Asp/Glu genotypes with respect to the wild-type homozygotes (6.2 ± 5 and 8.3 ± 5 vs. 13 ± 11, MR = 0.49, 95% CI 0.21–1.19, P = 0.114, and MR = 0.73, 95% CI 0.46–1.15, P = 0.175, respectively).
Table II. Mean values and means ratio (MR) of aromatic DNA adducts per 10^9 normal nucleotides according to DNA polymorphisms in microsomal epoxide hydrolase (EPHX1) His139Arg, NAD(P)H quinone oxidoreductase (NQO1) Pro187Ser, manganese superoxide dismutase (MnSOD2) Val16Ala, apurinic/apyrimidinic endonuclease 1 (APE1) Asp148Glu genes and to the number of lung cancer risk alleles in all subjects combined, and in the rural controls, in the Map-Ta-Phut residents and in the Map-Ta-Phut Industrial Estate (MIE) workers.

<table>
<thead>
<tr>
<th>Gene-genotype (amino acid)</th>
<th>Study population</th>
<th>Rural controls</th>
<th>Map-Ta-Phut residents</th>
<th>MIE workers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
<td>Mean ratio</td>
<td>P</td>
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<tr>
<td>EPHX1 His139Arg</td>
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<td>A/A (His/His)</td>
<td>137</td>
<td>8.0 ± 7</td>
<td>Ref.</td>
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<tr>
<td>A/G (His/Arg)</td>
<td>51</td>
<td>9.6 ± 7</td>
<td>1.17 (0.86–1.60)</td>
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<tr>
<td>G/G (Arg/Arg)</td>
<td>8</td>
<td>16 ± 2</td>
<td>1.26 (0.64–2.51)</td>
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<tr>
<td>P value for trend</td>
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<tr>
<td>NQO1 Pro187Ser</td>
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<tr>
<td>C/C (Pro/Pro)</td>
<td>75</td>
<td>7.9 ± 9</td>
<td>Ref.</td>
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<td>C/T (Pro/Ser)</td>
<td>96</td>
<td>9.3 ± 7</td>
<td>1.50 (1.13–1.99)</td>
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<td>T/T (Ser/Ser)</td>
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<td>9.1 ± 7</td>
<td>1.75 (1.14–2.68)</td>
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<td>P value for trend</td>
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<td>MnSOD2 Val16Ala</td>
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<tr>
<td>C/C (Val/Val)</td>
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<td>8.8 ± 7</td>
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<td>C/T (Val/Ala)</td>
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<td>8.2 ± 8</td>
<td>0.79 (0.60–1.04)</td>
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<td>T/T (Ala/Ala)</td>
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<td>11 ± 8</td>
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<tr>
<td>APE1 Asp148Glu</td>
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<td>T/T (Asp/Asp)</td>
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<td>11 ± 9</td>
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<td>T/G (Asp/Glu)</td>
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<td>6.8 ± 5</td>
<td>0.71 (0.54–0.94)</td>
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<td>G/G (Glu/Glu)</td>
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<td>7.5 ± 6</td>
<td>0.80 (0.51–1.28)</td>
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<tr>
<td>P value for trend</td>
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</tbody>
</table>

| Lung cancer risk alleles  |                  |                |                       |             |            |                |                       |             |            |                |                       |             |
| ≤2 at risk alleles        | 58               | 6.5 ± 5        | Ref.                  | 0.480       | 16         | 5.5 ± 4       | Ref.                  | 0.617       | 22         | 6.8 ± 5        | Ref.                  | 0.976      |
| 3 at risk alleles         | 65               | 8.0 ± 7        | 1.13 (0.81–1.57)      | 0.400       | 22         | 5.0 ± 4       | 0.85 (0.44–1.63)      | 1.001       | 21         | 8.6 ± 8        | 1.01 (0.54–1.87)      | 0.976      |
| 4 at risk alleles         | 56               | 10.2 ± 7       | 1.55 (1.10–2.18)      | 0.012       | 16         | 5.9 ± 3       | 1.16 (0.52–2.56)      | 0.712       | 22         | 9.9 ± 6        | 1.37 (0.73–2.57)      | 0.318      |
| ≥5 at risk alleles        | 17               | 15 ± 14        | 2.11 (1.27–3.51)      | 0.004       | 4          | 10 ± 3        | 1.65 (0.56–4.87)      | 0.356       | 11         | 18 ± 16.28     | 1.46 (1.46–5.71)      | 0.003      |
| P value for trend         |                  |                |                       |             |            |                |                       |             |            |                |                       |             |

*The percentage of nondetectable DNA adducts was of 13% in all the study population, 26% in the rural controls, 12% in the Map-Ta-Phut residents, and 7.5% in the MIE workers.

*The effect of each variable (means ratio) is the ratio between the mean DNA adducts of each levels of study variables with respect to the reference level, adjusted by gender, age, smoking status and residence/employment.

*The lung cancer risk alleles considered in our study were the allele G for His139Arg of EPHX1, the allele T for Pro187Ser of NQO1, the allele T for Val16Ala of MnSOD2, and the allele T for Asp148Glu of APE1/Ref1.
A borderline significant trend was observed in the whole study population and in the MIE workers (P for trend = 0.057 and P for trend = 0.057, respectively). No associations were found in the Map-Ta-Phut residents and the rural controls.

**Lung cancer risk alleles and aromatic DNA adducts**

Then, we examined the effect of the combination of the variant alleles of EPHX1 (allele G), NQO1 (allele T) and MnSOD2 (allele T) together with the wild-type allele of APE1 (allele T) (27–31) on the levels of DNA adducts by multivariate statistical analysis, by calculating the so-called ‘sum of at-risk alleles.’ The combination of at risk alleles in the four genes could theoretically add up to eight risk alleles. However, in this study population a maximum sum of risk alleles of seven was observed. The combination analysis has been performed to identify individuals more susceptible to the effect of air pollution.

We used multivariate regression models, including age, gender, smoking and residence and employment as independent variables, to examine the levels of DNA adducts considering the number of lung cancer risk alleles (28–32) in the general population and grouping by environment.

The means of DNA adducts were 6.5 ± 5.5 adducts per 10⁹ nucleotides for all the subjects combined bearing ≤2 lung cancer risk alleles, 10.2 ± 7.0 for those with four at-risk alleles, and 15 ± 14 for those with ≥5 at-risk alleles (MR = 1.55, 95% CI 1.10–2.18, P = 0.012, and MR = 2.11, 95% CI 1.27–3.51, P = 0.004, respectively). A significant trend of DNA adduct formation with increased levels in individuals with ≥5 at-risk alleles and with intermediate levels in those with four at-risk alleles with respect to the reference category was found (P for trend = 0.002). After adjustment for residence and employment categorisation, the means of DNA adducts of the MIE workers were 6.9 ± 5.5 adducts per 10⁹ nucleotides for those bearing ≤2 at-risk alleles, 12.2 ± 8.4 for the MIE workers with four at-risk alleles and 18 ± 16 for those with ≥5 at-risk alleles (MR = 1.92, 95% CI 1.11–3.33, P = 0.020 and MR = 2.88, 95% CI 1.46–5.71, P = 0.003, respectively). A significant trend was present in the group of MIE workers (P for trend = 0.001). Nonsignificant associations were observed in the groups of the MaP-Ta-Phut residents and the rural controls.

**Discussion**

In the present study, we compared values for aromatic DNA adducts determined by ³²P-post-labelling in the general population and in subjects with different levels of exposure to air pollution and evaluated for genotype. We examined the associations between DNA adducts and the DNA polymorphisms in four genes relevant for PAH metabolism as well as for oxidative stress and the regulation of cell-cycle and apoptosis, e.g. the EPHX1 His139Arg, the NQO1 Pro187Ser, the MnSOD2 Val16Ala and the APE1 Asp148Glu DNA polymorphisms (20–22,25,26).

Although our findings revealed that EPHX1 His139Arg and the NQO1 Pro187Ser polymorphisms predispose for higher levels of DNA adducts, the combination of at-risk alleles, which were ‘a priori’ expected to influence the risk of lung cancer (27–31), was found to affect the production of DNA damage much stronger. We found for the study as a whole, and for the MIE workers, that DNA adduct levels were increased with increasing numbers of at-risk alleles. Our study supports the hypothesis that the combination of unfavourable genetic alleles determines the individuals’ susceptibility, rather than a single SNP.

The knowledge of the nature of DNA adducts can give relevant information regarding the mutational effects resulting from exposure to air pollution. Herein, DNA adducts were measured by the post-labelling methodology, which is known to measure all kinds of hydrophobic bulky DNA damage, including cross-links between bases, induced from some aromatic amines and oxidative agents (35). Identification of DNA adducts is not possible by post-labelling. However, the present findings of the relationships between the specific SNPs relevant for the metabolism of PAHs (at least some of them) give indirect evidence that the observed ³²P-post-labelled DNA adducts in the white blood cells of the MIE workers and the Map-Ta-Phut residents were derived at least in part from PAHs. This in combination with the known effects of the same SNPs on cancer risk (27–31), which may provide some support for a causative link of PAHs with cancer.

The genetic variant in EPHX influences the metabolic pathway of epoxide hydrolysis and has been found to play a major role in the formation of DNA adducts in current smokers and in the healthy participants from the Spanish cohort of the European Prospective Investigation into Cancer and nutrition study (18,19). In our results, we found positive associations of adducts with the EPHX1-139Arg variant allele that reached the significance in the MIE workers. The EPHX1 enzyme is involved in the ‘classical’ pathway of PAH activation via cytochrome P450’s in which EPHX1 transforms epoxide intermediates from PAHs into more reactive carcinogens, such as B(a)P-7,8-diol-9,10 epoxide (5). The 139Arg variant allele in EPHX1 enhances the activity of the microsomal enzyme (19), and, thereby, could predispose to increased concentrations of reactive diol-epoxides in the tissues of the susceptible individuals exposed to air pollution.

The reactive diol-epoxides formed by EPHX1 can be converted through a redox-cycling reaction into quinones (36), which are highly reactive metabolites capable to form DNA adducts (37). Redox-cycling of PAHs can be inhibited by NQO1, a cytosolic flavoenzyme that reduces the formation of quinones to hydroquinones utilising NAD(P)H as an electron donor (21). In our results, the NQO1-187Ser variant allele, leading to reduced enzymatic activity (38), significantly influenced the formation of adducts in all subjects combined. After adjustment for residence and employment categorisation, the relationship with the 187Ser allele was borderline significant in the MIE workers and reached significance in the Map-Ta-Phut residents. A decrement of the protection provided by NQO1 could result in increased amount of reactive carcinogens and DNA adducts in the exposed individuals carrying the variant allele with reduced enzymatic activity.

Superoxide anions are formed during quinone redox-cycling, which can be counteracted by MnSOD₂, an enzyme that catalyses the dismutation of superoxide into oxygen and hydrogen peroxide (22). This study found a nonsignificant increment of adducts in the MIE workers with the Ala variant allele, a variant that has been associated with a reduced transport of the enzyme into the mitochondria and a decreased defence against oxidative stress (22). The decreased defence against oxidative stress conferred from the Ala variant allele could contribute to enhance the levels of free radicals, which can inhibit DNA repair activity (38) and activate directly some PAHs, such as the cyclopenteno[c,d]pyrene (23), in the tissues of the exposed MIE workers with the variant allele.
The APE1 is an enzyme involved in the repair of AP sites produced after enzymatic removal of damaged bases (24), and, potentially, in the repair of aromatic DNA adducts (24). In our study, we observed a borderline significant decrement of adducts in the general population and, after correction for residential/occupational categorisation, in the MIE workers, carrying the APE1-148Glu variant allele. This is not in line with the normal rate of DNA repair reported for that variant (25); therefore, it is likely, that not the repair activity of APE1/Ref1 is involved in the observed effects, but rather its role in redox homeostasis. Indeed, although it was originally identified as a DNA-repair enzyme, accumulating evidence supports a main role of APE1/Ref1 in many biological processes, such as cell-cycle control and apoptosis (26). For example, APE1 has the ability to activate transcription factors, such as p53 and Egr-1, involved in controlling cell-cycle and apoptotic response (26). Furthermore, the 148Glu variant has been significantly associated with the arrest of cell-cycle and, possibly, with apoptosis (25). Accordingly, the decrement of the DNA adduct levels in the Asp/Glu heterozygotes and Glu/Glu homozygotes may be caused by an increased apoptotic response after a DNA-damaging challenge. The exact role of this particular genetic polymorphism on p53 and Egr-1 signalling needs additional study, before further conclusions can be drawn.

Our previous studies provided preliminary evidence that the levels of aromatic DNA adducts in the peripheral blood cells are predictive of lung cancer risk (8–10); therefore, we additionally analysed the relationships between adducts and the combination of four SNPs relevant for this disease (27–31) with the ‘sum of at-risk alleles’ by combining the variant alleles of EPHX1 (allele G), NQO1 (allele T) and MnSOD2 (allele T) together with the wild-type allele of APE1 (allele T).

Our findings show that the formation of DNA adducts was affected by the combination of the four functional polymorphisms. The amount of DNA damage was significantly higher in the general population bearing 4 and ≥5 at-risk alleles (MR = 1.55, 95% CI 1.10–2.18, and MR = 2.11, 95% CI 1.27–3.51, respectively). Furthermore, a significant gene-dose effect was observed. Splitting the population into the groups of the MIE workers, the Map-Ta-Phut residents and rural controls, a significant difference was observed in the MIE workers with 4 and ≥5 at-risk alleles (MR = 1.92, 95% CI 1.11–3.33, and MR = 2.68, 95% CI 1.46–5.71, respectively), the subjects that were increasingly exposed to air pollution.

This is consistent with our previous studies (10,17,18,39,40) and suggests that the combination of DNA polymorphisms is more important than the presence of single low penetrance SNP, even if the single SNP does not show a significant individual effect. Because the functional impact of a single protein is low, it is possible that the interaction of several variant proteins with slightly modified functional activity in more than one pathway is necessary to sum up to a significant increment of aromatic DNA adduct levels and, perhaps, lung cancer risk. Future studies should include multiple genotypes (preferably selected within certain pathways relevant for the type of exposure) and should account for the level of exposure to air pollution.

Taken together, our results indicate positive associations between aromatic DNA adducts and the functional DNA polymorphisms associated with variation of the EPHX and the NQO1 enzymatic activities. Furthermore, the presence of an unfavourable combination of at-risk alleles for genes involved in the metabolism of PAHs, the defence against free radicals, and the control of cell-cycle and apoptotic response seems to predispose to higher levels of aromatic DNA adducts. At the individual level, the combined analysis of at-risk alleles may be more important than the investigation of a single DNA polymorphism to define an individual’s vulnerability for a certain exposure, in this particular case exposure via polluted air. An understanding of the relationships between DNA polymorphisms and the corresponding functional impact may further contribute to the interpretation of the results obtained from cancer case–control association studies.

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