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# UNIVERSITÀ DEGLI STUDI DI TORINO

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## Annals of Human Biology

Baseline frequency of Chromosomal Aberrations and Sister Chromatid Exchanges in peripheral blood lymphocytes of healthy individuals living in Turin (North-Western Italy): assessment of the effects of age, sex and GSTs gene polymorphisms on the levels of genomic damage.

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## Abstract

*Background*: The increased exposure to environmental pollutants has led to the awareness of the necessity for constant monitoring of human populations, especially those living in urban areas.

*Aim*: We evaluated the background levels of genomic damage in a sample of healthy subjects living in the urban area of Turin (Italy). The association between DNA damage with age, sex and *GSTs* polymorphisms was assessed.

*Subjects and methods*: 101 individuals were randomly sampled. Sister Chromatid Exchanges (SCEs) and Chromosomal Aberrations (CAs) assays, as well as genotyping of *GSTT1* and *GSTM1* genes were performed.

*Results*: Mean values of SCEs and CAs were 5.137±0.166 and 0.018±0.002, respectively. Results showed age and gender associated to higher frequencies of CAs but not to those of SCEs. Eldest subjects (51-65 years) showed significantly higher levels of CAs than younger individuals. GST polymorphisms did not appear to significantly influence the frequencies of either cytogenetic markers.

*Conclusion*: The CAs background frequency observed in this study is one of the highest reported among European populations. Turin is one of the most polluted cities in Europe in terms of air fine PM<sub>10</sub> and ozone, and the clastogenic potential of these pollutants may explain the high frequencies of chromosomal rearrangements here reported.

Key words: CA, SCE; glutathione S-transferase; environmental pollution; Italian population.

## Introduction

The assessment of genotoxic potential of environmental xenobiotics and their biological effects is essential for ensuring primary prevention of cancer diseases. In our daily activity, most of us are exposed to a wide variety of environmental pollutants, some of which are known for their genotoxic properties. Chemical air pollution is a mixture of many elements, produced by a variety of sources (mainly traffic, heating systems and industrial plants), several of which have been proven to have mutagenic and carcinogenic properties (Claxton et a., 2004; Traversi et al., 2008). The evaluation of control baseline data for cytogenetic markers in human populations is crucial, not only for the general health risk assessment, but also as a general guideline to estimate the potential risk in other populations exposed to similar pollutants (Carbonell et al., 1996). Cytogenetic rearrangements, such as Sister Chromatid Exchanges (SCEs) and Chromosomal Aberrations (CAs), have been considered as indicators of genomic instability and early biological effects of carcinogenic exposure. SCEs occur as a consequence of reciprocal changes between DNA replication products at apparently homologous chromosomal loci. These exchanges involve DNA breakage and reunion (Knudsen and Hansen, 2007), and are considered to be the consequence of DNA-replication errors on a damaged template (Garcia-Sagredo, 2008). On the other hand, the CAs assay allows a rapid overall analysis of cytogenetic damage and allows the detection of cells carrying unstable aberrations (chromosome and chromatid breaks, deletions, fragments, rings, dicentrics and chromatid exchanges) that will lead to cell death during proliferation (Garcia-Sagredo, 2008). Previous studies have provided strong evidence in support of the hypothesis that high CAs frequencies in peripheral blood lymphocytes is a powerful predictor of cancer risk and could be associated with early events of carcinogenesis (Hagmar et al., 1998).

The main objective of the present study was to evaluate the frequency of SCEs and CAs in human peripheral blood lymphocytes of non-occupationally exposed, non-smoking, healthy subjects, living in the city of Turin (North-Western Italy). Turin is located in the higher part of the Po river valley,

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an area where air exchanges are limited by the surrounding mountains, dominant winds are weak, and air pollutants can accumulate easily (Traversi et al., 2008). For these reasons Turin is one of the most polluted European cities in terms of particulate matters ( $PM_{2.5}$  and  $PM_{10}$ ) and ozone (ISTAT, 2011; WHO, 2013; Schilirò et al., 2010). For many years, the average annual PM2.5 pollution in Turin was higher than the limits set by the World Health Organization ( $35 \mu g/m^3$ , WHO, 2006), representing a potential environmental health problem (Traversi et al., 2008; Gilli et al., 2007). Indeed, a number of investigations have provided convincing evidence about the clastogenicity of high concentrations of particulate matters and ozone (Merz et al., 1975; Hüttner et al., 1999; Buschini et al., 2001; Díaz-Llera et al., 2002; Wei et al., 2006).

The role of age and gender, as potential confounding factors, on SCEs and CAs frequencies has been addressed in several studies. It was clearly proven that older individuals and females exhibit increased levels of DNA damage compared to younger subjects and males (Bonassi et al., 1995; Bolognesi et al., 1997; Wojda et al., 2006).

During eukaryotic evolution, organisms developed various detoxification systems able to protect the cells from DNA damage caused by reactive substances present in their environment. Glutathione S-transferases M1 and T1 (*GSTM1* and *GSTT1*) are phase II xenobiotic-metabolizing enzymes involved in the detoxification of reactive electrophiles metabolites providing protection against toxic substances present in the environment (Nebert et al., 1996). *GSTT1* and *GSTM1* genes are known to be polymorphic among humans for a deletion that prevents protein synthesis and consequently results in reduced detoxification capability in homozygous individuals. The deleted, non-functional alleles of the *GST* genes were found associated with the development of some types of cancer (Bajpai et al., 2007; Cha et al., 2007), as well as to an increased susceptibility to DNA damage (Palma et al., 2007). On the basis of these findings, the influence of age, gender and *GSTs* polymorphism on the spontaneous numbers of CAs and SCEs were also evaluated.

# Methods

## *Population sample*

The study population included 101 blood donors randomly sampled in Turin (Italy). Individuals selected for this study were subjects without any known exposure to specific xenobiotics, except for those of the routine household, traffic and clerical work. All subjects were living in the city, away from landfills, and their houses were equipped with modern heating systems. However, in the inner suburbs of the city there is an important automotive factory and numerous other smaller industrial installations that significantly contribute to the air pollution of the urban area.

In order to assess the possible influence of the age on the level of DNA damage, subjects were split into four age groups of 21-30, 31-40, 41-50, and 51-65 years old, respectively.

Subjects received detailed information about aims and experimental procedures of the study and gave their informed consent. Volunteers, in healthy conditions when sampling was conducted, were selected and anonymously identified by a numeric code.

It is well known that cigarette smoke contains a number of proven and suspected genotoxic agents, so smokers were excluded from the investigation. We also excluded individuals who reported alcohol consumption, treatment for acute infections and/or chronic non-infectious diseases, history of cancer, exposure to diagnostic X-rays, for at least two years prior the analysis. The study was approved by the University of Turin ethics committee and was performed in agreement with the ethical standards laid down in the 1964 Declaration of Helsinki.

# Blood Sample Collection and Cell Cultures

Blood samples were obtained by venipuncture (5-10 mL) and collected in heparinised tubes. All blood samples were coded, stored at 4°C, and processed within 2 hours after collection. Heparinized venous blood (0.3 mL) was cultured in 25 cm<sup>2</sup> flasks in 6 mL RPMI-1640 (Biological Industries) supplemented with 20% fetal calf serum, 2% mitogenic agent phytohemagglutinin-M (PHA, Difco, 0.2 mL), L-glutamine (2 mM), antibiotics (100 IU/mL penicillin, and 100 µg/mL streptomycin). Cell cultures were incubated for 72 and 48 hours (for SCEs and CAs assay, respectively) at 37 °C in an atmosphere of 5% CO<sub>2</sub> in the air. To arrest cells in mitosis, colchicine (Sigma, 0.25 µg/mL) was added at a concentration of 0.06 µg/mL during the last 2 hours of culture. Chromosome preparation was done following standard procedures. Cells were centrifuged, slowly re-suspended in 10 mL of pre-warmed hypotonic solution (0.075 M KCl, at 37 °C), and incubated for 15 minutes in a 37 °C water bath. Cells were then centrifuged again and fixed in cold methanol: acetic acid (3:1) for 20 minutes at room temperature. The treatment with the fixative was repeated three times. Finally, the supernatant was discarded and the pellet, dissolved in a minimal volume of fixative, was seeded onto the slides.

# Sister Chromatid Exchanges Assay

Bromodeoxyuridine (BrdUrd, 5 µg/mL) was added to the cultures after 24 hours to measure SCEs in second division metaphases. BrdUrd closely resembles thymidine and is efficiently incorporated into the elongating DNA strands during replication. After two cell cycles in BrdUrd medium, the two sister chromatids differ in the amount of BrdUrd present and the chromatid with more BrdUrd appears lighter ("bleaching" effect).

Sister chromatid differentiation was obtained by staining the fixed cells with fluorescence dye Hoechst 33258 (Sigma, 10 µg/mL, 20 minutes, at room temperature in the dark) followed by irradiation with an 8-W UV lamp (254 nm) at a distance of about 20 cm for 30 minutes. The slides were then incubated in 2x SSC (standard saline concentration) for 1 hour at 60°C and stained with 5% Giemsa (Sigma) in the Sörensen buffer for 10 minutes. Microscopic analysis was performed at 1000 X magnification on a light microscope (CX40, Olympus, Tokyo, Japan).

In order to determine the number of SCE/cell for each subject we scored 50 well-spread seconddivision metaphases containing 46 chromosomes. A total of 100 cells from each donor were scored for the determination of the replication index (RI), calculated according to the formula:  $RI = (M_I +$ 

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 $2M_2 + 3M_3$ /N, where  $M_1$ ,  $M_2$  and  $M_3$  represent the number of cells undergoing first second and third mitosis and N is the total number of scored metaphase (NSM).

## Chromosomal Aberrations Assay

For each subject, a total of 200 well-spread fixed metaphases were analysed for the following categories of CAs: chromatid breaks (B'), chromosome breaks (B"), dicentrics (Dic), acentric fragments (AF), and rings (R). Gaps (a-chromatid lesions) were not scored as CAs. Cells containing any type of chromosomal aberrations were scored as "Aberrant Cells" (Ab.C). Microscopic analyses were performed at 1000 X magnification on a light microscope (CX40, Olympus, Tokyo, 20, Japan).

# DNA Extraction and Genotyping

Genomic DNA was extracted using the Chelex solution protocol described by Walsh et al. (1991). GSTM1 and GSTT1 genotypes were determined by polymerase chain reaction (PCR), using the following primers: 5'-TTCCTTACTGGTCCTCACATCTC-3' and 5'-

TCACCGGATCATGGCCAGCA-3' for GSTT1 locus and 5'-CTGGATTGTAGCAGATCATGC-3' and 5'-CTGCCCTACTTGATTGATGGG-3' for GSTM1 locus. In addition, a fragment of the βglobin gene was co-amplified as internal control using primers 5'-CAACTCATCCACGTTCACC-3' and 5'-ACACAACT-GTGTTCACTAGC-3'. PCR reactions were carried out in a total volume of  $25 \,\mu$ l containing 10 ng of DNA (template), with a final concentration of 1X Reaction Buffer, 1.5 mM of MgCl2, 5% of DMSO, 250  $\mu$ M of dNTPs, 0.5  $\mu$ M of GSTs and  $\beta$ -globin primers, and 1U/sample of Tag DNA polymerase (Fischer, U.S.). The thermo-cycling procedure was set with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min before a final extension at 72°C for 10 min. GSTs PCR products were separated by electrophoresis on a 3% agarose gel and visualized by ethidium bromide (250 ng/ml) staining. The expected sizes of amplified GSTT1, GSTM1 and  $\beta$ - globin products were 480, 273 and 110 bp, respectively. Genotypes with homozygous deletion of the GST genes were identified as "GST-null" (GST-), whereas genotypes having at least one copy of the gene were scored as "GST-positive" (GST+).

#### Statistical analysis

Statistical analyses were conducted using the SPSS software statistical package programme (version 20.0, Inc., Chicago, Illinois, USA). Differences between sexes and among age groups were evaluated by Wilcoxon Mann-Whitney U test and/or one-way analysis of variance (ANOVA). The statistical significance of the effect of different factors was also evaluated by a multifactor analysis of variance (MANOVA). All *P*-values were two tailed and the level of statistical significance was PRset at *P*<0.05 for all tests.

#### Results

# *General characteristics of the population studied*

The demographic and genotypic features of the urban population investigated are reported in Table 1. Forty-eight subjects were male, with a mean age ( $\pm$ S.D.) of 39.479 ( $\pm$ 8.939), and 53 were female, with a mean age ( $\pm$ S.D.) of 36.302 ( $\pm$ 8.933). The age of the individuals ranged from 21 to 65 years, with a mean ( $\pm$ S.D.) of 37.812 ( $\pm$ 9.033).

The total sample was split into four groups according to the age of the subjects: 21-30 (n = 26, mean age  $26.846\pm2.723$ ; 31-40 (n = 45, mean age  $36.867\pm2.634$ ); 41-50 (n = 19, mean age  $45.211\pm2.299$ ); and 51-65 (n = 11, mean age  $54.818\pm4.309$ ).

Overall, seventy-three individuals resulted GSTT positive (72.28%, mean age 38.247±9.251) and 28 (27.72%, mean age 36.679±8.494) were GSTT null genotypes. Fifty-seven subjects resulted GSTM positive (56.44%, mean age 38.175±8.140), while the GSTM null genotypes were 44 (43.56%, mean age 37.341±10.152).

# SCEs and CAs assay

Detailed results of the SCEs and CAs analyses are summarized in Tables 2, 3 and 4. A total of 5,050 and 10,100 cells were available for the SCEs and RI analyses, respectively, whereas the total number of metaphases scored for the CAs assay was 20,200. The observed SCEs/cell value was  $5.137\pm0.166$  (range 2.26-9.76), while the mean RI value was  $1.898\pm0.021$  (range 1.46 - 2.28). Cells with 8 or more SCEs were defined as high frequency cells (HFCs) according to Carrano and Moore (1982). Subjects for whom more than 6 cells contained more than 8 SCEs were classified as high frequency individuals (HFIs). Twenty-four individuals were identified as HFIs and they showed a significantly higher value of SCEs/cell (*P*<0.001) compared to Non-HFIs. The total numbers of observed CAs and Ab.C, excluding gaps, were 360 (CAs/ cell =  $0.018\pm0.002$ ) and 349 (Ab.C/cell =  $0.017\pm0.002$ ), respectively.

# Age, gender, GSTs polymorphisms and cytogenetic markers

The MANOVA analysis revealed a significant role of age on CAs frequency (P = 0.012 and P = 0.015 for CAs including and excluding gaps, respectively), but not on SCEs levels (P = 0.186) (Table 7). These results were further confirmed by the ANOVA (P = 0.029 and P = 0.263 for CAs and SCEs, respectively). Moreover, when the total sample was split into four age groups, the eldest subjects (aged 51-65) showed significant higher CAs and Ab.C values (but not of SCEs) compared to the younger groups (Tables 2 and 4). Similarly, gender appeared to affect the amount of CAs observed (Tables 4 and 7) but not the frequency of SCEs (Tables 2 and 7). Finally, *GSTs* gene polymorphisms did not show significant overall associations with the frequencies of either cytogenetic markers, with the exception of *GSTT1* positive genotypes

associated to an increase of SCEs among women, as well as in the group of individuals aged 31-40

(Tables 5 and 6).

## Discussion

The exposure to increasing levels of environmental pollution in urban areas has progressively boosted public awareness of the need to constantly monitor human populations. Information provided by cytogenetic markers about factors potentially affecting the background level of chromosomal aberrations are of great importance in assessing both the general health risk and the frequency of expected genomic damage prior to occupational exposure. In order to evaluate the level of DNA damage in peripheral blood lymphocytes of nonoccupationally exposed, non-smoking, healthy subjects living in an urban area, we analysed the frequencies of SCEs and CAs observed in a sample of individuals from the city of Turin (Italy). The frequency Ab.C (0.017) observed in our dataset resulted in being one of the highest reported in literature among control samples used in several cytogenetic studies of European populations (Table 8). This result indicates that some local environmental factors may be involved in determining the high frequency of cytogenetic damage observed. Turin is one of the most polluted cities in Europe, mainly in terms of air fine PM (Schilirò et al., 2010; COTEC, 2005) whose clastogenic potential has been suggested in a number of studies (Hüttner et al., 1999; Buschini et al., 2001; Wei et al., 2006). In recent years, the monitoring of  $PM_{10}$  in Torino has revealed concentrations frequently higher than the daily and yearly quality targets set by Italian laws (Schilirò et al., 2010). Turin is a city with a high traffic density and consequent high levels of atmospheric genotoxic substances, such as benzene, toluene, and xylenes (Bono et al., 2003). Moreover a large automotive industrial complex is located in the inner suburbs of the city, as well as other smaller industrial installations that, with their discharge products, considerably contribute to air pollution. In this scenario, the city council opted to implement policies to limit the circulation of private vehicles in emergency (as a result of excess pollutants) and/or scheduled (as a preventive or progressive reduction of emissions) periods, also encouraging the use of the urban public transport. However, despite these air pollution prevention measures, in 2013 the concentrations of some pollutants exceeded the limits set by the Italian law (Table 9).

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Although air pollution seems to play a significant role in increasing DNA damage (Krytopoulos et al., 2001), it must be emphasized that spontaneous CAs can also be induced by a variety of exogenous and endogenous factors, such as genotoxicants in food (Santovito et al., 2012), age of individuals (Bolognesi et al., 1997), infection diseases (Freire-Maia et al., 1997), genetic susceptibility (Schlade-Bartusiak et al., 2000), as well as individual damage repair capacity (Durante et al., 2013; Cebulska-Wasilewska et al., 2005). For all these reasons it is not surprising that different levels of spontaneous CAs have been found in different human populations.

The observed frequency of SCEs in our sample was 5.137±0.166, with HFIs showing a significant higher frequency of SCEs than to Non-HFIs (Table 2). It was suggested that cells with high SCEs frequencies represent long-living subsets of lymphocytes that accumulated SCE-inducing lesions over time (Bozkurt et al., 2003). Therefore, the evaluation of HFCs and HFIs is considered an informative approach for assessing the effect of chronic exposure to genotoxic agents. The higher frequency of SCEs recorded among HFIs seem to indicate the presence of a subset of individuals more susceptible to genomic damage resulting from daily environmental exposure. Higher SCEs rates among HFIs could also reflect potential defects in DNA repair processes in these subjects (Garcia-Sagredo, 2008). These defects in cellular DNA repair have been linked to genome instability, heritable cancers, premature ageing syndromes and neurological diseases (Rass et al., 2007). Moreover, accumulation of DNA lesions in repair-defective individuals may cause cell death, either by progressively depriving the cell of vital transcripts or through apoptosis (Ljungman and Lane, 2004).

It is known that age and gender are important factors to be taken into consideration when designing human population studies. According to a number of studies on age-related genomic damage incidence in control populations (Bolognesi et al., 1997; Bender et al., 1988; Stephan and Pressl, 1999), the results obtained in this study seem to suggest an influence of the age on CAs and Ab.C rates. Indeed, we observed a significant increase of the frequency of these two cytogenetic markers

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in the older group compared to the younger ones (Table 4). This pattern could be an important signal because various studies have provided evidence that increased background levels of CAs in peripheral blood lymphocytes can be associated to a higher risk of cancer (Garcia-Sagredo, 2008; Hagmar et al., 1998). Moreover, the high SCEs frequency recorded among older subjects (Table 2), although statistically not significant, should also require attention as it could be interpreted as a signal of a potential increase of defects in DNA repair processes with age (Garcia-Sagredo, 2008), resulting in a progressive accumulation of genomic damage.

An association between CAs, SCEs and sex, with a tendency toward a higher number of CAs/cell and SCEs/cell in women than in men, has also been put forward by some authors (Bender et al., 1988; Bonassi et al., 1995; Wulf and Niebuhr, 1985). Additionally our sample showed females with significantly higher CAs and Ab.C frequencies than males (Table 4). Several hypotheses have been put forward to explain this sex bias (differences in the dimension of sexual chromosomes, hormonal levels, etc.), but so far a convincing explanation is still missing.

Finally, the individuals enrolled for our study were also genotyped for *GSTs* xenobiotic metabolizing enzymes involved in cellular detoxification mechanisms. The evidence about a relationship between GSTs polymorphisms and an increase of genomic damage are controversial. Kadioglu et al. (2012), analysing a sample of healthy Turkish subjects, observed higher CAs and micronuclei frequencies in individuals carrying a *GSTT1*-null allele. In contrast, Salnikova et al. (2012) found higher chromosomal aberration frequencies among *GSTM1/GSTT1* positive genotypes in a sample of workers from the Chernobyl nuclear power plant accident. In a previous analysis of a sample of subjects occupationally exposed to formaldehyde we did not find any association between *GSTs* polymorphisms and CAs (Santovito et al., 2011). Similarly to the results obtained by other authors (Rossi et al., 2009; Vodicka et al., 2009), the *GST* genotypes found in the Turin population did not appear to be associated to cytogenetic damage, as both SCEs and CAs

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frequencies were not statistically different between the *GSTs*-null and the *GST*-positives genotypes (Tables 2 and 3). However, it should be emphasized that our study group only included individuals not occupationally exposed to specific xenobiotics. Thus, the lack of a relationship between GSTs polymorphisms and chromosomal damage may be due to the relatively low concentrations of xenobiotics affecting the general urban population.

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## **Declaration of interest statement**

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Table 1 - General characteristics of the studied population.

Т

| Subjects   | N (%)      | Age (Mean $\pm$ S.D.) | Age range |
|------------|------------|-----------------------|-----------|
| Total      | 101        | 37.812±9.033          | 21-65     |
| Sex        |            |                       |           |
| Males      | 48 (47.52) | 39.479±8.939          | 27-65     |
| Females    | 53 (52.48) | 36.302±8.933          | 21-57     |
| Age groups |            |                       |           |
| 21-30      | 26 (25.74) | 26.846±2.723          | -         |
| 31-40      | 45 (44.56) | 36.867±2.634          | -         |
| 41-50      | 19 (18.81) | 45.211±2.299          | -         |
| 51-65      | 11 (10.89) | 54.818±4.309          | -         |
| Genotype   |            | Q                     |           |
| GSTT+      | 73 (72.28) | 38.247±9.251          | 21-65     |
| GSTT-      | 28 (27.72) | 36.679±8.494          | 23-53     |
| GSTM+      | 57 (56.44) | 38.175±8.140          | 21-54     |
| GSTM-      | 44 (43.56) | 37.341±10.152         | 23-65     |

N = number of studied subjects; S.D. = Standard Deviation;

LT = Long Time; ST = Short Time

|            |     | inan marriaduro    | ,     |       | <u></u>                  | P5.  |                  |       |                       |                   |
|------------|-----|--------------------|-------|-------|--------------------------|------|------------------|-------|-----------------------|-------------------|
| Groups     | Ν   | Age<br>(mean±S.D.) | Cells | SCEs  | SCEs/cell±S.E.           | HFCs | $\mathbf{M}_{1}$ | $M_2$ | <b>M</b> <sub>3</sub> | RI ±S.E.          |
| Total      | 101 | 37.812±9.033       | 5050  | 25942 | 5.137±0.166              | 450  | 3566             | 3967  | 2556                  | 1.898±0.021       |
| HFIs       | 24  | 40.208±10.455      | 1200  | 8598  | 7.165±0.144 <sup>a</sup> | 149  | 904              | 981   | 503                   | $1.823 \pm 0.031$ |
| Non-HFIs   | 77  | 37.013±8.693       | 3850  | 17344 | $4.505 \pm 0.154^{a}$    | 301  | 2662             | 2986  | 2053                  | $1.921 \pm 0.025$ |
| Sex        |     |                    |       |       |                          |      |                  |       |                       |                   |
| Males      | 48  | 39.479±8.939       | 2400  | 12573 | 5.239±0.190              | 218  | 1733             | 1892  | 1177                  | $1.885 \pm 0.024$ |
| Females    | 53  | 36.302±8.933       | 2650  | 13369 | 5.045±0.267              | 232  | 1833             | 2075  | 1379                  | $1.909 \pm 0.033$ |
| Age groups |     |                    |       |       |                          |      |                  |       |                       |                   |
| 21-30      | 26  | 26.846±2.723       | 1300  | 6467  | 4.978±0.460              | 112  | 952              | 969   | 679                   | $1.895 \pm 0.049$ |
| 31-40      | 45  | 36.867±2.634       | 2250  | 10592 | 4.708±0.244              | 184  | 1517             | 1788  | 1192                  | $1.926 \pm 0.028$ |
| 41-50      | 19  | 45.211±2.299       | 950   | 5378  | 5.661±0.300              | 93   | 685              | 793   | 409                   | $1.841 \pm 0.050$ |
| 51-65      | 11  | 54.818±4.309       | 550   | 3505  | 6.373±0.273              | 61   | 412              | 417   | 76                    | $1.885 \pm 0.053$ |

Table 2 – Frequency of SCEs and RI values in metaphases of lymphocytes from a sample of healthy Italian individuals, according to sex and age groups.

<sup>a</sup>P <0.001 (Wilcoxon Mann-Whitney *U* test)

N = Number of analysed subjects; S.D. = Standard Deviation; Cells = number of scored metaphases; SCEs = Sister chromatid exchanges; S.E. = Standard Error; HFCs = High Frequency Cells; HFIs = High Frequency Individuals; *RI (Replication Index)* =  $(M_1 + 2M_2 + 3M_3)/N$ , where  $M_1$ ,  $M_2$  and  $M_3$  represent the number of cells undergoing first second and third mitosis and N is the total number of scored metaphase.

| population $(N = 101)$            |                 |
|-----------------------------------|-----------------|
| Class                             | N (Mean% ± SD)  |
| Chromatid                         |                 |
| Gaps                              | 233 (1.15±0.85) |
| Breaks                            | 209 (1.03±1.20) |
| <i>Rearrangements<sup>a</sup></i> | 31 (0.153±0.31) |
| Chromosome                        |                 |
| Gaps                              | 39 (0.19±0.24)  |
| Breaks                            | 58 (0.29±0.63)  |
| <i>Rearrangements<sup>b</sup></i> | 62 (0.307±0.60) |
| % Chromosomal Aberrations         |                 |
| Excluding gaps                    | 360 (1.80±1.85) |
| Including gaps                    | 632 (3.13±2.45) |
| % Aberrant Metaphases             |                 |
| Excluding gaps                    | 349 (1.73±1.70) |
| Including gaps                    | 603 (2.99±2.13) |

| Table 3 - Analysis of CAs distribution in the studied |  |
|---|--|
| population $(N = 101)$                                |  |

<sup>a</sup> Including acentric fragments

<sup>b</sup> Including dicentrics, rings and acentric fragments

| Table 4 - Frequencies | y of CAs and Ab | etaphas            | ses of | lym  | mphocytes from a sample of healthy Italian individuals, according to sex and age |            |    |    |     |          |          |          |          |                           |                   |                         |                   |
|-----------------------|-----------------|--------------------|--------|------|--|------------|----|----|-----|----------|----------|----------|----------|---------------------------|-------------------|-------------------------|-------------------|
| ~                     |                 |                    | ~ "    | ~    |  |            |    |    |     | Total    | Total    | Total    | Total    | CAs/cell                  | CAs/cell          | Ab.C/cell               | Ab.C/cell         |
| Groups                | Ν               | (mean+S D)         | Cells  | Gaps | <b>B</b> '   | <b>B</b> " | AF | R  | Dic | CAs      | CAs      | Ab.C     | Ab.C     | (-Gaps)                   | (+Gaps)           | (-Gaps)                 | (+Gaps)           |
|                       |                 | (incan=5.D.)       |        |      |  |            | -  |    |     | (- Gaps) | (+ Gaps) | (- Gaps) | (+ Gaps) | mean±S.E.                 | mean±S.E          | mean±S.E.               | mean±S.E.         |
| Total                 | 101             | 37.812±9.033       | 20200  | 272  | 209  | 58         | 54 | 28 | 11  | 360      | 632      | 349      | 603      | 0.018±0.002               | 0.031±0.002       | 0.017±0.002             | 0.030±0.002       |
| Sex                   |                 |                    |        |      |  |            |    |    |     |          |          |          |          |                           |                   |                         |                   |
| Males                 | 48              | 39.479±8.939       | 9600   | 136  | 63   | 20         | 24 | 11 | 5   | 123      | 259      | 121      | 249      | $0.013 \pm 0.002^{a}$     | $0.027 \pm 0.002$ | $0.013 \pm 0.002^{b}$   | $0.026 \pm 0.002$ |
| Females               | 53              | $36.302 \pm 8.933$ | 10600  | 136  | 146  | 38         | 30 | 17 | 6   | 237      | 373      | 228      | 354      | $0.022 \pm 0.003^{a}$     | $0.035 \pm 0.004$ | $0.022 \pm 0.003^{b}$   | $0.033 \pm 0.004$ |
| Age Groups            |                 |                    |        |      |  |            |    |    |     |          |          |          |          |                           |                   |                         |                   |
| 21-30                 | 26              | 26.846±2.723       | 5200   | 49   | 56   | 14         | 10 | 2  | 2   | 84       | 133      | 83       | 130      | $0.016 \pm 0.002^{\circ}$ | $0.026 \pm 0.003$ | $0.016 \pm 0.002^{e}$   | $0.025 \pm 0.003$ |
| 31-40                 | 45              | $36.867 \pm 2.634$ | 9000   | 120  | 78   | 19         | 26 | 18 | 5   | 146      | 266      | 40       | 254      | $0.016 \pm 0.003^{d}$     | $0.030 \pm 0.004$ | $0.016 \pm 0.003^{f}$   | $0.028 \pm 0.003$ |
| 41-50                 | 19              | 45.211±2.299       | 3800   | 59   | 36   | 13         | 11 | 5  | 0   | 65       | 124      | 64       | 120      | $0.017 \pm 0.004$         | $0.033 \pm 0.006$ | $0.017 \pm 0.004$       | $0.032 \pm 0.005$ |
| 51-65                 | 11              | 54.818±4.309       | 2200   | 44   | 39   | 12         | 7  | 3  | 4   | 65       | 109      | 62       | 99       | $0.030 \pm 0.009^{c,d}$   | $0.050 \pm 0.012$ | $0.028 \pm 0.007^{e,f}$ | $0.045 \pm 0.009$ |

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 $^{a,b}P = 0.011$ ;  $^{c,d}P = 0.007$ ;  $^{e,f}P = 0.022$  (Wilcoxon Mann-Whitney U test)

N = Number of analysed subjects; Cells = number of scored metaphases; Gaps' = chromatidic gaps; Gaps'' = chromosomal gaps; B': chromatid breaks; B'': chromosome breaks; Dic = Dicentric chromosome; AF = Acentric Fragments; R = Rings; CAs = Chromosome Aberrations; Ab.C = Aberrant Cells; (-Gaps) = excluding gaps; (+Gaps) = including gaps; S.E. = Standard Error.

| Table 5 – Freq | uency   | of SC    | Es and  | RI values in meta   | phases | s of ly | mpho  | cytes f | from a sample | of |
|----------------|---------|----------|---------|---------------------|--------|---------|-------|---------|---------------|----|
| healt          | thy Ita | ilian in | dividua | lls, according to C | GSTs g | ene p   | olymc | orphisn | ns.           |    |
|                |         |          |         |                     |        |         |       |         |               |    |

|              | <u> </u>       |       |       |                          | 0                | · · I |                       | r                     |                                    |
|--------------|----------------|-------|-------|--------------------------|------------------|-------|-----------------------|-----------------------|------------------------------------|
| Groups       | Ν              | Cells | SCEs  | SCEs/cell±S.E.           | HFCs             | $M_1$ | <b>M</b> <sub>2</sub> | <b>M</b> <sub>3</sub> | RI ±S.E.                           |
| Total        |                |       |       |                          |                  |       |                       |                       |                                    |
| GSTT +       | 73             | 3650  | 19201 | 5.261±0.195              | 333              | 2666  | 2821                  | 1802                  | 1.879±0.025                        |
| GSTT –       | 28             | 1400  | 6741  | 4.815±0.314              | 117              | 900   | 1146                  | 754                   | 1.948±0.035                        |
| GSTM +       | 57             | 2850  | 14532 | 5.099±0.235              | 252              | 2104  | 2243                  | 1355                  | 1.869±0.029                        |
| GSTM –       | 44             | 2200  | 11410 | 5.186±0.233              | 198              | 1462  | 1724                  | 1201                  | 1.935±0.029                        |
| GSTT+/GSTM+  | 46             | 2300  | 11958 | 5.199±0.258              | 208              | 1755  | 1779                  | 1066                  | 1.850±0.032                        |
| GSTT-/GSTM-  | 17             | 850   | 4167  | 4.902±0.377              | 72               | 551   | 682                   | 465                   | 1.947±0.044                        |
| HFIs         |                |       |       |                          |                  |       |                       |                       |                                    |
| GSTT +       | 19             | 950   | 6867  | 7.228±0.173              | 119              | 725   | 772                   | 391                   | 1.812±0.038                        |
| GSTT –       | 5              | 250   | 1731  | 6.924±0.194              | 30               | 179   | 209                   | 112                   | $1.866 \pm 0.046$                  |
| GSTM +       | 13             | 650   | 4773  | 7.343±0.238              | 83               | 506   | 515                   | 280                   | $1.828 \pm 0.042$                  |
| GSTM -       | 11             | 550   | 3825  | $6.955 \pm 0.123$        | 66               | 398   | 466                   | 223                   | $1.817 \pm 0.050$                  |
| GSTT+/GSTM+  | 12             | 600   | 4406  | $7.343 \pm 0.258$        | 76               | 469   | 482                   | 250                   | $1.819 \pm 0.044$                  |
| GSTT-/GSTM-  | 4              | 200   | 1364  | 6.820±0.212              | 24               | 142   | 176                   | 82                    | $1.850 \pm 0.056$                  |
|              |                |       |       |                          |                  |       |                       |                       |                                    |
| Non-HFIs     |                |       |       |                          |                  |       |                       |                       |                                    |
| GSTT +       | 54             | 2700  | 12330 | 4.567±0.177              | 214              | 1942  | 2053                  | 1408                  | 1.902±0.031                        |
| GSTT –       | 23             | 1150  | 5014  | 4.360±0.306              | 87               | 720   | 933                   | 645                   | 1.966±0.040                        |
| GSTM +       | 44             | 2200  | 9759  | 4.436±0.209              | 169              | 1598  | 1728                  | 1075                  | 1.882±0.035                        |
| GSTM –       | 33             | 1650  | 7585  | 4.597±0.758              | 132              | 1064  | 1258                  | 978                   | 1.902±0.031                        |
| GSTT+/GSTM+  | 34             | 1700  | 7548  | 4.440±0.219              | 131              | 1287  | 1301                  | 813                   | 1.861±0.041                        |
| GSTT-/GSTM-  | 12             | 600   | 2671  | 4.452±0.346              | 46               | 377   | 467                   | 356                   | 1.983±0.058                        |
| Males        |                |       |       |                          |                  |       |                       |                       |                                    |
| GSTT +       | 32             | 1600  | 8465  | $5.291 \pm 0.240$        | 147              | 1184  | 1233                  | 785                   | $1.877 \pm 0.033$                  |
| GSTT –       | 16             | 800   | 4108  | 5.135±0.314              | 71               | 549   | 659                   | 392                   | $1.902 \pm 0.030$                  |
| GSTM +       | 21             | 1050  | 5708  | 5.436±0.295              | 99               | 839   | 828                   | 437                   | $1.812 \pm 0.039$                  |
| GSTM -       | 27             | 1350  | 6865  | $5.085 \pm 0.248$        | 119              | 894   | 1064                  | 740                   | $1.941 \pm 0.026$                  |
| GSTT+/GSTM+  | 17             | 850   | 4618  | $5.433 \pm 0.332$        | 80               | 697   | 665                   | 340                   | $1.792 \pm 0.047$                  |
| GSTT-/GSTM-  | 12             | 600   | 3018  | 5.030±0.354              | 52               | 407   | 496                   | 295                   | 1.903±0.039                        |
| Fomalos      |                |       |       |                          |                  |       |                       |                       |                                    |
| GSTT +       | 41             | 2050  | 10736 | 5 237+0 295 <sup>a</sup> | 186              | 1482  | 1588                  | 1017                  | 1 880+0 037                        |
| GSTT -       | 12             | 600   | 2633  | $4388\pm0508^{a}$        | 46               | 351   | 487                   | 362                   | $2009\pm0.057$                     |
| $GSTM \perp$ | 36             | 1800  | 2033  | 4.002+0.328              | 153              | 1265  | 1/15                  | 018                   | $1.003\pm0.008$                    |
| GSTM -       | 17             | 850   | 4545  | 5347+0465                | 70               | 568   | 660                   | /61                   | $1.905\pm0.050$<br>$1.924\pm0.064$ |
| CSTT+/CSTM+  | $\frac{1}{20}$ | 1450  | 7340  | 5 062±0 362 <sup>b</sup> | 127              | 1058  | 1111                  | 726                   | $1.924\pm0.004$<br>$1.884\pm0.042$ |
| GSTT-/GSTM-  | 5              | 250   | 1149  | $4596+1033^{b}$          | $\frac{127}{20}$ | 1058  | 186                   | 170                   | $2.052\pm0.042$                    |
|              | 5              | 230   | 1117  | 4.570±1.055              | 20               | 111   | 100                   | 170                   | 2.032±0.111                        |
| 21-30        |                |       |       |                          |                  |       |                       |                       |                                    |
| GSTT +       | 18             | 900   | 4480  | 4.975±0.373              | 78               | 676   | 657                   | 467                   | $1.884 \pm 0.059$                  |
| GSTT-        | 8              | 400   | 1987  | 4.968±0.679              | 34               | 276   | 312                   | 212                   | 1.920±0.093                        |
| GSTM +       | 14             | 700   | 3762  | 5.374±0.564              | 65               | 578   | 510                   | 312                   | 1.810±0.062                        |
| GSTM –       | 12             | 600   | 2705  | 4.508±0.458              | 47               | 374   | 459                   | 367                   | 1.994±0.070                        |
| GSTT+/GSTM+  | 12             | 600   | 3139  | 5.232±0.643              | 54               | 501   | 422                   | 277                   | 1.813±0.068                        |
| GSTT-/GSTM-  | 6              | 300   | 1364  | 4.547±0.814              | 24               | 199   | 224                   | 177                   | 1.963±0.111                        |
|              |                |       |       |                          |                  |       |                       |                       |                                    |

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| 31-40       |    |      |      |                          |     |      |      |      |                   |
|-------------|----|------|------|--------------------------|-----|------|------|------|-------------------|
| GSTT +      | 30 | 1500 | 7462 | 4.975±0.313 <sup>c</sup> | 130 | 1061 | 1170 | 7658 | 1.902±0.035       |
| GSTT –      | 15 | 750  | 3130 | 4.173±0.357 <sup>c</sup> | 54  | 456  | 618  | 424  | 1.976±0.042       |
| GSTM +      | 23 | 1150 | 5065 | 4.404±0.367              | 88  | 778  | 927  | 594  | 1.919±0.044       |
| GSTM –      | 22 | 1100 | 5527 | 5.025±0.316              | 96  | 739  | 861  | 598  | 1.934±0.034       |
| GSTT+/GSTM+ | 16 | 800  | 3775 | 4.719±0.451              | 66  | 575  | 633  | 391  | 1.884±0.053       |
| GSTT-/GSTM- | 8  | 400  | 1840 | 4.600±0.413              | 32  | 253  | 324  | 221  | 1.995±0.047       |
|             |    |      |      |                          |     |      |      |      |                   |
| 41-50       |    |      |      |                          |     |      |      |      |                   |
| GSTT +      | 18 | 900  | 5138 | 5.709±0.313              | 89  | 637  | 743  | 406  | $1.856 \pm 0.050$ |
| GSTT –      | 1  | 50   | 240  | 4.800                    | 4   | 48   | 50   | 3    | 1.570             |
| GSTM +      | 16 | 800  | 4507 | 5.634±0.344              | 78  | 567  | 664  | 368  | 1.874±0.053       |
| GSTM –      | 3  | 150  | 871  | 5.807±0.621              | 15  | 118  | 129  | 41   | 1.663±0.093       |
| GSTT+/GSTM+ | 16 | 800  | 4507 | 5.634±0.344              | 78  | 567  | 664  | 368  | 1.874±0.053       |
| GSTT-/GSTM- | 1  | 50   | 240  | 4.800                    | 4   | 48   | 50   | 3    | 1.570             |
|             |    |      |      |                          |     |      |      |      |                   |
| 51-65       |    |      |      |                          |     |      |      |      |                   |
| GSTT +      | 7  | 350  | 2228 | 6.366±0.402              | 39  | 278  | 258  | 169  | $1.858 \pm 0.083$ |
| GSTT –      | 4  | 200  | 1277 | 6.385±0.342              | 22  | 134  | 159  | 107  | 1.933±0.015       |
| GSTM +      | 4  | 200  | 1344 | 6.720±0.324              | 23  | 162  | 153  | 88   | 1.830±0.087       |
| GSTM –      | 7  | 3509 | 2161 | 6.174±0.383              | 38  | 250  | 264  | 188  | 1.917±0.068       |
| GSTT+/GSTM+ | 2  | 100  | 683  | 6.830±0.430              | 12  | 94   | 71   | 38   | $1.750 \pm 0.180$ |
| GSTT-/GSTM- | 2  | 100  | 616  | 6.160±0.420              | 11  | 66   | 77   | 57   | 1.955±0.005       |

 ${}^{a}P = 0.005; {}^{b}P = 0.043; {}^{c}P = 0.026$  (Wilcoxon Mann-Whitney U test)

N = Number of analysed subjects; Cells = number of scored metaphases; SCEs = Sister chromatid exchanges; S.E. = Standard Error; HFCs = High Frequency Cells; HFIs = High Frequency Individuals; *RI (Replication Index)* =  $(M_1 + 2M_2 + 3M_3)/N$ , where  $M_1$ ,  $M_2$  and  $M_3$  represent the number of cells undergoing first second and third mitosis and N is the total number of scored metaphase;

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| Groups         | Ν  | Cells | Gaps | B'  | В" | AF | R  | Dic | Total CAs<br>(- Gaps) | Total CAs<br>(+ Gaps) | Total Ab.C<br>(- Gaps) | Total Ab.C<br>(+ Gaps) | CAs/cell<br>(-Gaps)<br>mean±S.E.       | CAs/cell<br>(+Gaps)<br>mean±S.E | Ab.C/cell<br>(-Gaps)<br>mean±S.E. | Ab.C/cell<br>(+Gaps)<br>mean±S.E. |
|----------------|----|-------|------|-----|----|----|----|-----|-----------------------|-----------------------|------------------------|------------------------|--|---------------------------------|-----------------------------------|-----------------------------------|
| Fotal          |    |       |      |     |    |    |    |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| GSTT +         | 73 | 14600 | 183  | 152 | 40 | 39 | 19 | 9   | 259                   | 442                   | 250                    | 421                    | $0.018 \pm 0.002$                      | $0.030 \pm 0.003$               | $0.017 \pm 0.002$                 | 0.029±0.00                        |
| GSTT –         | 28 | 5600  | 89   | 57  | 18 | 15 | 9  | 2   | 101                   | 190                   | 99                     | 182                    | $0.018 \pm 0.003$                      | $0.034 \pm 0.004$               | $0.018 \pm 0.003$                 | 0.032±0.00                        |
| GSTM +         | 57 | 11400 | 138  | 117 | 23 | 29 | 21 | 7   | 197                   | 335                   | 191                    | 321                    | $0.017 \pm 0.002$                      | $0.029 \pm 0.002$               | $0.017 \pm 0.002$                 | 0.028±0.00                        |
| GSTM-          | 44 | 8800  | 134  | 92  | 35 | 25 | 7  | 4   | 163                   | 297                   | 158                    | 282                    | 0.019±0.003                            | $0.034 \pm 0.004$               | $0.018 \pm 0.003$                 | 0.032±0.00                        |
| GSTT+/GSTM+    | 46 | 9200  | 98   | 88  | 14 | 28 | 14 | 7   | 151                   | 249                   | 145                    | 238                    | $0.016 \pm 0.003$                      | $0.027 \pm 0.003$               | $0.016 \pm 0.002$                 | 0.026±0.00                        |
| GSTT-/GSTM-    | 17 | 3400  | 49   | 28  | 9  | 14 | 2  | 2   | 55                    | 104                   | 53                     | 99                     | 0.016±0.005                            | $0.031 \pm 0.005$               | $0.016 \pm 0.004$                 | 0.029±0.00                        |
| Males          |    |       |      |     |    |    |    |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| GSTT +         | 32 | 6400  | 86   | 44  | 13 | 20 | 8  | 3   | 88                    | 174                   | 86                     | 168                    | $0.014 \pm 0.002$                      | $0.027 \pm 0.002$               | $0.013 \pm 0.002$                 | 0.026±0.00                        |
| GSTT-          | 16 | 3200  | 50   | 19  | 7  | 4  | 3  | 2   | 35                    | 85                    | 35                     | 81                     | 0.011±0.003                            | $0.027 \pm 0.005$               | 0.011±0.003                       | 0.025±0.00                        |
| GSTM +         | 21 | 4200  | 53   | 29  | 7  | 17 | 8  | 2   | 63                    | 116                   | 61                     | 109                    | 0.015±0.003                            | $0.028 \pm 0.004$               | 0.015±0.003                       | 0.026±0.00                        |
| GSTM-          | 27 | 5400  | 83   | 34  | 13 | 7  | 3  | 3   | 60                    | 143                   | <b>6</b> 0             | 140                    | 0.011±0.002                            | $0.026 \pm 0.003$               | 0.011±0.002                       | 0.026±0.00                        |
| GSTT+/GSTM+    | 17 | 3400  | 38   | 22  | 5  | 17 | 7  | 2   | 53                    | 91                    | 51                     | 87                     | $0.016 \pm 0.004$                      | $0.027 \pm 0.004$               | $0.015 \pm 0.003$                 | 0.026±0.00                        |
| GSTT-/GSTM-    | 12 | 2400  | 35   | 12  | 5  | 4  | 2  | 2   | 25                    | 60                    | 25                     | 59                     | 0.010±0.003                            | $0.025 \pm 0.004$               | 0.010±0.003                       | 0.025±0.00                        |
|                |    |       |      |     | -  |    |    |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| Females        |    |       |      |     |    |    |    |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| GSTT +         | 41 | 8200  | 97   | 108 | 27 | 19 | 11 | 6   | 171                   | 268                   | 164                    | 253                    | 0.021±0.004                            | $0.033 \pm 0.005$               | 0.020±0.003                       | 0.031±0.00                        |
| GSTT-          | 12 | 2400  | 39   | 38  | 11 | 11 | 6  | 0   | 66                    | 105                   | 64                     | 101                    | $0.028 \pm 0.006$                      | $0.044 \pm 0.007$               | 0.027±0.005                       | 0.042±0.00                        |
| GSTM+          | 36 | 7200  | 85   | 88  | 16 | 12 | 13 | 5   | 134                   | 219                   | 130                    | 212                    | 0.019±0.003                            | $0.030 \pm 0.004$               | $0.018 \pm 0.003$                 | $0.029 \pm 0.00$                  |
| GSTM-          | 17 | 3400  | 51   | 58  | 22 | 18 | 4  | 1   | 103                   | 154                   | 98                     | 142                    | $0.030 \pm 0.007$                      | $0.045 \pm 0.009$               | $0.029 \pm 0.006$                 | 0.042±0.00                        |
| GSTT+/GSTM+    | 29 | 5800  | 60   | 66  | 9  | 11 | 7  | 5   | 98                    | 158                   | 94                     | 151                    | $0.017 \pm 0.003$                      | $0.027 \pm 0.005$               | $0.016 \pm 0.003$                 | 0.026±0.00                        |
| GSTT-/GSTM-    | 5  | 1000  | 14   | 16  | 4  | 10 | 0  | 0   | 30                    | 44                    | 28                     | 40                     | $0.030 \pm 3.868$                      | $0.044 \pm 0.014$               | $0.028 \pm 0.012$                 | 0.040±0.01                        |
| Age Groups     | -  |       |      |     | -  |    | -  |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| 21-30          |    |       |      |     |    |    |    |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| GSTT +         | 18 | 3600  | 34   | 38  | 9  | 9  | 2  | 2   | 60                    | 94                    | 59                     | 91                     | $0.017 \pm 0.003$                      | $0.026 \pm 0.004$               | $0.016 \pm 0.003$                 | 0.026±0.00                        |
| GSTT-          | 8  | 1600  | 15   | 18  | 5  | 1  | 0  | 0   | 24                    | 39                    | 24                     | 39                     | $0.015\pm0.004$                        | $0.024\pm0.005$                 | $0.015\pm0.004$                   | $0.024\pm0.00$                    |
| GSTM +         | 14 | 2800  | 20   | 34  | 9  | 6  | 2  | 2   | 53                    | 73                    | 52                     | 72                     | $0.019\pm0.003$                        | $0.02 \pm 0.001$                | $0.019\pm0.003$                   | $0.02 \pm 0.00$                   |
| GSTM -         | 12 | 2400  | 29   | 22  | 5  | 4  | 0  | 0   | 31                    | 60                    | 31                     | 58                     | $0.013\pm0.003$                        | $0.025\pm0.001$                 | $0.013\pm0.003$                   | 0.020=0.00                        |
| GSTT + /GSTM + | 12 | 2400  | 16   | 28  | 7  | 5  | 2  | 2   | 44                    | 60                    | 43                     | 59                     | $0.013 \pm 0.003$<br>$0.018 \pm 0.004$ | $0.025 \pm 0.001$               | $0.013 \pm 0.003$                 | 0.025+0.00                        |
| GSTT-/GSTM-    | 6  | 1200  | 11   | 12  | 3  | 0  | 0  | 0   | 15                    | 26                    | 15                     | 26                     | $0.010\pm0.001$                        | $0.023\pm0.003$<br>0.022+0.005  | $0.010\pm0.005$<br>0.013+0.005    | $0.023\pm0.00$                    |
| 0511-/05111-   | 0  | 1200  | 11   | 12  | 5  | U  | U  | U   | 15                    | 20                    | 15                     | 20                     | 0.015±0.005                            | 0.022-0.005                     | 0.015±0.005                       | 0.022-0.00                        |
| 31-40          |    |       |      |     |    |    |    |     |                       |                       |                        |                        |  |                                 |                                   |                                   |
| GSTT +         | 30 | 6000  | 67   | 51  | 8  | 15 | 10 | 3   | 87                    | 154                   | 83                     | 146                    | $0.015\pm0.003$                        | $0.026\pm0.004$                 | 0.014±0.003                       | $0.024\pm0.00$                    |
| GSTT-          | 15 | 3000  | 53   | 27  | 11 | 11 | 8  | 2   | 59                    | 112                   | 57                     | 108                    | $0.020\pm0.006$                        | $0.037\pm0.006$                 | $0.019\pm0.005$                   | $0.036\pm0.00$                    |
| GSTM+          | 23 | 4600  | 52   | 34  | 8  | 14 | 14 | 1   | 71                    | 123                   | 69                     | 120                    | $0.015\pm0.003$                        | $0.027\pm0.004$                 | $0.015\pm0.003$                   | $0.026\pm0.00$                    |
| GSTM_          | 22 | 4400  | 68   | 11  | 11 | 12 | 1  | 1   | 75                    | 1/3                   | 71                     | 134                    | $0.017 \pm 0.005$                      | $0.027 \pm 0.001$               | $0.016 \pm 0.003$                 | 0.020=0.00                        |

| Page | 28 | of | 31 |
|------|----|----|----|
|------|----|----|----|

| GSTT+/GSTM   | + 16   | 3200    | 28                   | 18    | 3           | 14  | 8   | 1     | 44        | 72        | 42          | 69          | 0.014±0.004           | 0.022±0.004                    | 0.013±0.003       | 0.022±0.004        |
|--------------|--------|---------|----------------------|-------|-------------|-----|-----|-------|-----------|-----------|-------------|-------------|-----------------------|--------------------------------|-------------------|--------------------|
| GSTT-/GSTM-  | - 8    | 1600    | 29                   | 11    | 6           | 11  | 2   | 2     | 32        | 61        | 30          | 57          | $0.020 \pm 0.009$     | $0.038 \pm 0.010$              | 0.019±0.009       | 0.036±0.008        |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
| 41-50        |        |         |                      |       |             | _   | _   | -     |           |           |             |             |                       |                                |                   |                    |
| GSTT +       | 18     | 3600    | 58                   | 36    | 13          | 9   | 5   | 0     | 63        | 121       | 62          | 117         | 0.018±0.005           | $0.034 \pm 0.006$              | $0.017 \pm 0.004$ | $0.0321 \pm 0.005$ |
| GSTT –       |        | 200     | 1                    | 0     | 0           | 2   | 0   | 0     | 2         | 3         | 2           | 3           | 0.010                 | 0.015                          | 0.010             | 0.015              |
| GSTM + CSTM  | 15     | 3000    | 42                   | 24    | 4           | 5   | 4   | 0     | 3/        | /9        | 37          | /8          | $0.012 \pm 0.002$     | $0.026\pm0.003$                | $0.012\pm0.002$   | $0.026\pm0.003$    |
| GSIM -       | 4      | 800     | 1/                   |       | 9           | 6   |     | 0     | 28        | 45        | 27          | 42          | $0.035\pm0.019$       | $0.056\pm0.021$                | $0.034\pm0.017$   | $0.052 \pm 0.019$  |
| GSTT+/GSTM   | + 13   | 200     | 42                   | 24    | 4           | 2   | 4   | 0     | 37        | 79        | 3/          | 18          | $0.012 \pm 0.002$     | $0.020\pm0.003$                | $0.012 \pm 0.002$ | $0.020\pm0.003$    |
| 0511-/051M   | - 1    | 200     | 1                    | 0     | U           | 2   | 0   | 0     |           | 3         | 2           | 5           | 0.010                 | 0.015                          | 0.010             | 0.015              |
| 51-65        |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
| GSTT +       | 7      | 1400    | 24                   | 27    | 10          | 6   | 2   | 4     | 49        | 73        | 46          | 67          | 0.035±0.013           | $0.052\pm0.018$                | 0 033±0 011       | $0.048\pm0.015$    |
| GSTT -       | 4      | 800     | 20                   | 12    | 2           | 1   | 1   | 0     | 16        | 36        | 16          | 32          | $0.020\pm0.015$       | $0.032\pm0.018$<br>0.045±0.008 | $0.020\pm0.011$   | $0.040\pm0.015$    |
| GSTM +       | 5      | 1000    | 24                   | 25    | $\tilde{2}$ | 4   | 1   | 4     | 36        | 60        | 33          | 51          | $0.020\pm0.005$       | $0.060\pm0.022$                | $0.033\pm0.013$   | $0.051\pm0.016$    |
| GSTM-        | 6      | 1200    | 20                   | 14    | 10          | 3   | 2   | 0     | 29        | 49        | 29          | 48          | $0.024\pm0.009$       | $0.041\pm0.012$                | $0.024\pm0.009$   | $0.040\pm0.012$    |
| GSTT+/GSTM   | + $3$  | 600     | 12                   | 18    | 0           | 4   | 0   | 4     | 26        | 38        | 23          | 32          | $0.043\pm0.028$       | $0.063 \pm 0.039$              | $0.038\pm0.024$   | $0.053\pm0.029$    |
| GSTT-/GSTM   | - 2    | 400     | 8                    | 5     | Õ           | 1   | 0   | 0     | 6         | 14        | 6           | 13          | 0.015±0.010           | 0.035±0.010                    | 0.015±0.010       | 0.032±0.008        |
| N = Number o | fanaly | sed sub | iects <sup>.</sup> ( | Cells | s = r       | ามm | ber | ofs   | cored met | aphases   | Gaps' = cht | omatidic ga | $aps^{.} Gaps^{} = c$ | hromosomal                     | gaps. B'. chroi   | matid breaks.      |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |
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|              |        |         |                      |       |             |     |     |       |           |           |             |             |                       |                                |                   |                    |

| Table 7 - Factors affecting Chromosome A | berration frequency and Sister Chromatid Exchanges |
|--|--|
| analyzed by MANOVA                       |  |

|                           | CAs exclu | ding gaps | CAs inclu | ding gaps | SCE   |       |
|---------------------------|-----------|-----------|-----------|-----------|-------|-------|
| Factors                   | F         | р         | F         | р         | F     | р     |
| Age                       | 1.920     | 0.012     | 1.873     | 0.015     | 1.290 | 0.186 |
| Sex                       | 8.039     | 0.006     | 4.271     | 0.042     | 0.184 | 0.670 |
| Interactions<br>Age x Sex | 1.933     | 0.040     | 2.466     | 0.011     | 1.014 | 0.452 |
|                           |           |           |           |           |       |       |
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| Table  | 8 – | Frequ | iencies | of ( | CAB | /Cell | in  | different | Euro | nean | local  | ities |
|--------|-----|-------|---------|------|-----|-------|-----|-----------|------|------|--------|-------|
| 1 auto | 0   | ricqu | icheres | UI V |     | Con   | 111 | uniterent | Luio | pean | iocai. | nues  |

| Country (City/District)              | Ν    | Cell    | % CAB/Cell | References               |
|--------------------------------------|------|---------|------------|--------------------------|
| Italy (Turin)                        | 101  | 20,200  | 1.70       | Present study            |
| Italy (Toscana region)               | 210  | 3,900   | 1.93       | Milillo et al., 1996     |
| Czech Republic (different districts) | 5430 | 543,000 | 1.38       | Rössner et al., 1998     |
| Great Britain (Carshalton, Surrey)   | 106  | 49,490  | 1.27       | Anderson et al. 1988     |
| Hungary (Budapest)                   | 175  | 17,500  | 0.88       | Gundy and Varga, 1983    |
| Germany (Sachsen-Anhalt)             | 51   | 10200   | 0.98       | Hüttner et al., 1999     |
| Greece (Athens)                      | 117  | 8,500   | 0.87       | Kyrtopoulos et al., 2001 |
| Poland (Silesia)                     | 54   | 5,400   | 1.38       | Michalska et al., 1999   |
| Poland (Bialystok)                   | 49   | 4,900   | 0.69       | Michalska et al., 1999   |

Cell = Number of Scored Metaphases; CAB = Cells with Aberrations

cen = Number of Scored Metaphases, CAB = Cens with Abertations

Table 9 - List of the main pollutants, with their concentrations, measured in the year 2013 in the city of Turin. Data available on: http://www.comune.torino.it/ambiente/aria/aria torino/index.shtml and http://rsaonline.arpa.piemonte.it/indicatori/aria.htm. Access date: 02-02-2015

| Pollutant                     | Concentration<br>(µg/m <sup>3</sup> ) | Limits set by<br>the Italian law<br>(µg/m <sup>3</sup> ) <sup>a</sup> |
|-------------------------------|---------------------------------------|---|
| SO <sub>2</sub>               | 9                                     | 125**   |
| C <sub>6</sub> H <sub>6</sub> | 2.45*                                 | 5   |
| СО                            | 1.7                                   | 10  |
| NO <sub>2</sub>               | 86.5*                                 | 40  |
| O <sub>3</sub>                | 87                                    | 40  |
| PM <sub>10</sub>              | 44*                                   | 20  |
| PM <sub>2.5</sub>             | 29                                    | 25  |

<sup>a</sup> maximum annual average concentrations imposed by Italian law

\* average values from 2 different detection stations

\* daily avenue times a yeu \*\* daily average not to be exceeded more than 3

times a year