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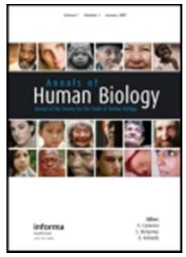

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Baseline frequency of Chromosomal Aberrations and Sister Chromatid Exchanges in peripheral blood lymphocytes of healthy individuals living in Turin (North-Western Italy): assessment of the effects of age, sex and *GSTs* gene polymorphisms on the levels 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_{10} 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, 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 μg/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^2 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_2 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 second-division 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: $RI = (M_1 +$

$2M_2 + 3M_3)/N$, 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 (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, 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 β-globin 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 μl containing 10 ng of DNA (template), with a final concentration of 1X Reaction Buffer, 1.5 mM of MgCl2, 5% of DMSO, 250 μM of dNTPs, 0.5 μM of *GSTs* and β-globin primers, and 1U/sample of Taq 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 β-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 set 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 (±S.D.) of 39.479 (±8.939), and 53 were female, with a mean age (±S.D.) of 36.302 (±8.933). The age of the individuals ranged from 21 to 65 years, with a mean (±S.D.) of 37.812 (±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±2.723); 31-40 (n = 45, mean age 36.867±2.634); 41-50 (n = 19, mean age 45.211±2.299); and 51-65 (n = 11, mean age 54.818±4.309).

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±0.166 (range 2.26-9.76), while the mean RI value was 1.898±0.021 (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±0.002) and 349 (Ab.C/cell = 0.017±0.002), 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 non-occupationally 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).

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 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 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.

Subjects	N (%)	Age (Mean ± 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			
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

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.

Groups	N	Age (mean±S.D.)	Cells	SCEs	SCEs/cell±S.E.	HFCs	M_1	M_2	M_3	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[a]	149	904	981	503	1.823±0.031
Non-HFIs	77	37.013±8.693	3850	17344	4.505±0.154[a]	301	2662	2986	2053	1.921±0.025
Sex										
Males	48	39.479±8.939	2400	12573	5.239±0.190	218	1733	1892	1177	1.885±0.024
Females	53	36.302±8.933	2650	13369	5.045±0.267	232	1833	2075	1379	1.909±0.033
Age groups										
21-30	26	26.846±2.723	1300	6467	4.978±0.460	112	952	969	679	1.895±0.049
31-40	45	36.867±2.634	2250	10592	4.708±0.244	184	1517	1788	1192	1.926±0.028
41-50	19	45.211±2.299	950	5378	5.661±0.300	93	685	793	409	1.841±0.050
51-65	11	54.818±4.309	550	3505	6.373±0.273	61	412	417	76	1.885±0.053

[a]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.

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

Class	N (Mean% ± SD)
Chromatid	
Gaps	233 (1.15±0.85)
Breaks	209 (1.03±1.20)
Rearrangements[a]	31 (0.153±0.31)
Chromosome	
Gaps	39 (0.19±0.24)
Breaks	58 (0.29±0.63)
Rearrangements[b]	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)

[a] Including acentric fragments
[b] Including dicentrics, rings and acentric fragments

Table 4 - Frequency of CAs and Ab.C in metaphases of lymphocytes from a sample of healthy Italian individuals, according to sex and age

Groups	N	Age (mean±S.D.)	Cells	Gaps	B'	B"	AF	R	Dic	Total CAs (- Gaps)	Total CAs (+ Gaps)	Total Ab.C (- Gaps)	Total Ab.C (+ Gaps)	CAs/cell (-Gaps) mean±S.E.	CAs/cell (+Gaps) mean±S.E	Ab.C/cell (-Gaps) mean±S.E.	Ab.C/cell (+Gaps) 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±0.002[a]	0.027±0.002	0.013±0.002[b]	0.026±0.002
Females	53	36.302±8.933	10600	136	146	38	30	17	6	237	373	228	354	0.022±0.003[a]	0.035±0.004	0.022±0.003[b]	0.033±0.004
Age Groups																	
21-30	26	26.846±2.723	5200	49	56	14	10	2	2	84	133	83	130	0.016±0.002[c]	0.026±0.003	0.016±0.002[e]	0.025±0.003
31-40	45	36.867±2.634	9000	120	78	19	26	18	5	146	266	40	254	0.016±0.003[d]	0.030±0.004	0.016±0.003[f]	0.028±0.003
41-50	19	45.211±2.299	3800	59	36	13	11	5	0	65	124	64	120	0.017±0.004	0.033±0.006	0.017±0.004	0.032±0.005
51-65	11	54.818±4.309	2200	44	39	12	7	3	4	65	109	62	99	0.030±0.009[c,d]	0.050±0.012	0.028±0.007[e,f]	0.045±0.009

[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 – Frequency of SCEs and RI values in metaphases of lymphocytes from a sample of healthy Italian individuals, according to *GSTs* gene polymorphisms.

Groups	N	Cells	SCEs	SCEs/cell±S.E.	HFCs	M_1	M_2	M_3	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±0.046
GSTM +	13	650	4773	7.343±0.238	83	506	515	280	1.828±0.042
GSTM -	11	550	3825	6.955±0.123	66	398	466	223	1.817±0.050
GSTT+/GSTM+	12	600	4406	7.343±0.258	76	469	482	250	1.819±0.044
GSTT-/GSTM-	4	200	1364	6.820±0.212	24	142	176	82	1.850±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±0.240	147	1184	1233	785	1.877±0.033
GSTT –	16	800	4108	5.135±0.314	71	549	659	392	1.902±0.030
GSTM +	21	1050	5708	5.436±0.295	99	839	828	437	1.812±0.039
GSTM -	27	1350	6865	5.085±0.248	119	894	1064	740	1.941±0.026
GSTT+/GSTM+	17	850	4618	5.433±0.332	80	697	665	340	1.792±0.047
GSTT-/GSTM-	12	600	3018	5.030±0.354	52	407	496	295	1.903±0.039
Females									
GSTT +	41	2050	10736	**5.237±0.295**[a]	186	1482	1588	1017	1.880±0.037
GSTT –	12	600	2633	**4.388±0.598**[a]	46	351	487	362	2.009±0.068
GSTM +	36	1800	8824	4.902±0.328	153	1265	1415	918	1.903±0.038
GSTM –	17	850	4545	5.347±0.465	79	568	660	461	1.924±0.064
GSTT+/GSTM+	29	1450	7340	**5.062±0.362**[b]	127	1058	1114	726	1.884±0.042
GSTT-/GSTM-	5	250	1149	**4.596±1.033**[b]	20	144	186	170	2.052±0.111
Age groups									
21-30									
GSTT +	18	900	4480	4.975±0.373	78	676	657	467	1.884±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

31-40									
GSTT +	30	1500	7462	**4.975±0.313**[c]	130	1061	1170	7658	1.902±0.035
GSTT –	15	750	3130	**4.173±0.357**[c]	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±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±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±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;

Table 5 - Frequency of CAs and Ab.C in metaphases of lymphocytes from a sample of healthy Italian individuals, according to *GSTs* gene polymorphisms.

Groups	N	Cells	Gaps	B’	B”	AF	R	Dic	Total CAs (- Gaps)	Total CAs (+ Gaps)	Total Ab.C (- Gaps)	Total Ab.C (+ Gaps)	CAs/cell (-Gaps) mean±S.E.	CAs/cell (+Gaps) mean±S.E	Ab.C/cell (-Gaps) mean±S.E.	Ab.C/cell (+Gaps) mean±S.E.
Total																
GSTT +	73	14600	183	152	40	39	19	9	259	442	250	421	0.018±0.002	0.030±0.003	0.017±0.002	0.029±0.003
GSTT –	28	5600	89	57	18	15	9	2	101	190	99	182	0.018±0.003	0.034±0.004	0.018±0.003	0.032±0.004
GSTM +	57	11400	138	117	23	29	21	7	197	335	191	321	0.017±0.002	0.029±0.002	0.017±0.002	0.028±0.002
GSTM –	44	8800	134	92	35	25	7	4	163	297	158	282	0.019±0.003	0.034±0.004	0.018±0.003	0.032±0.004
GSTT+/GSTM+	46	9200	98	88	14	28	14	7	151	249	145	238	0.016±0.003	0.027±0.003	0.016±0.002	0.026±0.003
GSTT-/GSTM-	17	3400	49	28	9	14	2	2	55	104	53	99	0.016±0.005	0.031±0.005	0.016±0.004	0.029±0.005
Males																
GSTT +	32	6400	86	44	13	20	8	3	88	174	86	168	0.014±0.002	0.027±0.002	0.013±0.002	0.026±0.002
GSTT –	16	3200	50	19	7	4	3	2	35	85	35	81	0.011±0.003	0.027±0.005	0.011±0.003	0.025±0.004
GSTM +	21	4200	53	29	7	17	8	2	63	116	61	109	0.015±0.003	0.028±0.004	0.015±0.003	0.026±0.003
GSTM –	27	5400	83	34	13	7	3	3	60	143	60	140	0.011±0.002	0.026±0.003	0.011±0.002	0.026±0.003
GSTT+/GSTM+	17	3400	38	22	5	17	7	2	53	91	51	87	0.016±0.004	0.027±0.004	0.015±0.003	0.026±0.003
GSTT-/GSTM-	12	2400	35	12	5	4	2	2	25	60	25	59	0.010±0.003	0.025±0.004	0.010±0.003	0.025±0.004
Females																
GSTT +	41	8200	97	108	27	19	11	6	171	268	164	253	0.021±0.004	0.033±0.005	0.020±0.003	0.031±0.004
GSTT –	12	2400	39	38	11	11	6	0	66	105	64	101	0.028±0.006	0.044±0.007	0.027±0.005	0.042±0.006
GSTM +	36	7200	85	88	16	12	13	5	134	219	130	212	0.019±0.003	0.030±0.004	0.018±0.003	0.029±0.004
GSTM –	17	3400	51	58	22	18	4	1	103	154	98	142	0.030±0.007	0.045±0.009	0.029±0.006	0.042±0.008
GSTT+/GSTM+	29	5800	60	66	9	11	7	5	98	158	94	151	0.017±0.003	0.027±0.005	0.016±0.003	0.026±0.004
GSTT-/GSTM-	5	1000	14	16	4	10	0	0	30	44	28	40	0.030±3.868	0.044±0.014	0.028±0.012	0.040±0.012
Age Groups																
21-30																
GSTT +	18	3600	34	38	9	9	2	2	60	94	59	91	0.017±0.003	0.026±0.004	0.016±0.003	0.026±0.003
GSTT –	8	1600	15	18	5	1	0	0	24	39	24	39	0.015±0.004	0.024±0.005	0.015±0.004	0.024±0.005
GSTM +	14	2800	20	34	9	6	2	2	53	73	52	72	0.019±0.003	0.026±0.004	0.019±0.003	0.026±0.004
GSTM –	12	2400	29	22	5	4	0	0	31	60	31	58	0.013±0.003	0.025±0.004	0.013±0.003	0.024±0.004
GSTT+/GSTM+	12	2400	16	28	7	5	2	2	44	60	43	59	0.018±0.004	0.025±0.005	0.018±0.003	0.025±0.005
GSTT-/GSTM-	6	1200	11	12	3	0	0	0	15	26	15	26	0.013±0.005	0.022±0.005	0.013±0.005	0.022±0.005
31-40																
GSTT +	30	6000	67	51	8	15	10	3	87	154	83	146	0.015±0.003	0.026±0.004	0.014±0.003	0.024±0.003
GSTT –	15	3000	53	27	11	11	8	2	59	112	57	108	0.020±0.006	0.037±0.006	0.019±0.005	0.036±0.006
GSTM +	23	4600	52	34	8	14	14	1	71	123	69	120	0.015±0.003	0.027±0.004	0.015±0.003	0.026±0.004
GSTM –	22	4400	68	44	11	12	4	4	75	143	71	134	0.017±0.005	0.032±0.006	0.016±0.004	0.030±0.005

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±0.009	0.038±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±0.006	0.017±0.004	0.0321±0.005
GSTT –	1	200	1	0	0	2	0	0	2	3	2	3	0.010	0.015	0.010	0.015
GSTM +	15	3000	42	24	4	5	4	0	37	79	37	78	0.012±0.002	0.026±0.003	0.012±0.002	0.026±0.003
GSTM –	4	800	17	12	9	6	1	0	28	45	27	42	0.035±0.019	0.056±0.021	0.034±0.017	0.052±0.019
GSTT+/GSTM+	15	3000	42	24	4	5	4	0	37	79	37	78	0.012±0.002	0.026±0.003	0.012±0.002	0.026±0.003
GSTT-/GSTM-	1	200	1	0	0	2	0	0	2	3	2	3	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±0.018	0.033±0.011	0.048±0.015
GSTT –	4	800	20	12	2	1	1	0	16	36	16	32	0.020±0.005	0.045±0.008	0.020±0.005	0.040±0.006
GSTM +	5	1000	24	25	2	4	1	4	36	60	33	51	0.036±0.016	0.060±0.022	0.033±0.013	0.051±0.016
GSTM –	6	1200	20	14	10	3	2	0	29	49	29	48	0.024±0.009	0.041±0.012	0.024±0.009	0.040±0.012
GSTT+/GSTM+	3	600	12	18	0	4	0	4	26	38	23	32	0.043±0.028	0.063±0.039	0.038±0.024	0.053±0.029
GSTT-/GSTM-	2	400	8	5	0	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 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; S.E. = Standard Error.

Table 7 - Factors affecting Chromosome Aberration frequency and Sister Chromatid Exchanges analyzed by MANOVA

	CAs excluding gaps		CAs including gaps		SCE	
Factors	F	p	F	p	F	p
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						
Age x Sex	1.933	**0.040**	2.466	**0.011**	1.014	0.452

Table 8 – Frequencies of CAB/Cell in different European localities

Country (City/District)	N	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

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 ($\mu g/m^3$)**	**Limits set by the Italian law ($\mu g/m^3$) [a]**
SO_2	9	125**
C_6H_6	2.45*	5
CO	1.7	10
NO_2	86.5*	40
O_3	87	40
PM_{10}	44*	20
$PM_{2.5}$	29	25

[a] maximum annual average concentrations imposed by Italian law
* average values from 2 different detection stations
** daily average not to be exceeded more than 3 times a year