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Evaluation of baseline frequency of sister chromatid exchanges in an Italian population according to age, sex, smoking habits, and gene polymorphisms

Alfredo Santovito | **Claudio Gendusa** | **Piero Cervella**

University of Turin, Department of Life Sciences and Systems Biology, Via Accademia Albertina n. 13, Torino 10123, Italy

Correspondence
Alfredo Santovito, Department of Life Sciences and Systems Biology, Via Accademia Albertina n. 13, 10123 Torino, Italy.
Email: alfredo.santovito@unito.it

Abstract

Objectives: Increased SCEs frequencies in human lymphocytes are an indicator of spontaneous chromosome instability and could be influenced by different exogenous and endogenous factors. In this study, we evaluated the influence of age, sex, smoking habits, and genetic polymorphisms on the background levels of SCEs in peripheral blood lymphocytes.

Methods: Two hundred-thirty healthy Italian subjects were recruited. Data about age, sex and smoking habits were recorded. Subjects were also genotyped for *GSTT1*, *GSTM1*, *GSTP1 A/G*, *CYP1A1 Ile/Val*, *CYP2C19 G/A*, *ERCC2/XPD Lys751Gln*, *XRCC1 Arg194ATrp, XRCC1 Arg399Gln, and XRCC1Arg208His* gene polymorphisms.

Results: The frequency of SCEs/cell was 5.15 ± 1.87, with females showing a significantly higher SCEs value with respect to males (5.36 ± 2.10 and 4.82 ± 1.39, respectively). Smokers showed significantly increased levels of SCEs with respect to nonsmokers (5.93 ± 1.75 and 4.70 ± 1.79, respectively) whereas no differences were observed between heavy and light smokers. Age correlated with the RI value ($P = .01$) but not with the SCEs frequency ($P = 07$), although the 31-40 age group showed a significantly lower SCEs frequency with respect to the other age groups. A significant association was also found between *GSTP2C19-AA*, *GSTT1*-null, *GSTM1-null*, *ERCC2/XPD Gln751Gln*, and *XRCC1 His208His* genotypes, and higher frequencies of SCEs.

Conclusion: We describe the association between some phase I, phase II, and DNA-repair gene polymorphisms with increased SCEs frequencies, reinforcing the importance of genetic analysis in biomonitoring studies. Sex and age were found to be important endogenous factors that affect the level of genomic damage and the replicative capacity of cells, respectively.

1 | INTRODUCTION

The frequency of sister chromatid exchanges (SCEs) in peripheral blood lymphocytes is extensively used as a biomarker of chromosomal damage and genome stability in human populations. SCEs occur as a consequence of interchanges between DNA replication products at homologous chromosomal loci, and these exchanges involve DNA

Abbreviations: BrdUrd, bromodeoxyuridine; Cas, chromosomal aberrations; CYP, cytochrome P; GST, glutathione-*S*-transferase; HFC, high frequency cells; HFI, high frequency individuals; LT, long time; NER, nucleotide excision repair; RI, replication index; SCEs, sister chromatid exchanges; ST, short time; XPD, Xeroderma pigmentosum complementation group D.

breakage and reunion (Knudsen & Hansen, 2007). Nevertheless, some compounds are able to form covalent adducts with the DNA or to interfere with DNA metabolism and repair, with consequent induction of genomic damage.

The SCEs test is also used in the surveillance of work environments with low-dose exposures to mutagens or carcinogens. Indeed, increased frequencies of SCEs are found to be associated with a higher risk of various types of cancers (Baltaci, Kayikçioğlu, Alpas, Zeyneloğlu, & Haberal, 2002; Medves et al., 2016). They also serve as indicators of spontaneous chromosome instability among human populations (Salah et al., 2011). In this context, one of the objectives of the present study was to evaluate, by means of the SCEs assay, the level of genomic damage in the peripheral blood lymphocytes of non-occupationally exposed, healthy subjects. Indeed, it is known that the level of genomic damage can be partly influenced by a variety of external factors such as chemical and physical agents, life styles (smoking and drinking habits, nutrition), or residential and/or working areas, as well as by endogenous factors, including those of biological origins such as sex and age (Santovito, Cervella, & Delpero, 2015, 2016).

Individual genetic susceptibility was found to play an increasingly important role in determining the levels of genomic damage. From a genetic point of view, this susceptibility is due to a battery of gene polymorphisms, principally those of metabolic genes (such as cytochrome *P* (*CYP*) 450 and glutathione-*S*-transferase (*GST*) family genes) (Autrup, 2000; Wang et al., 2013). In particular, phase I cytochrome P450 (*CYP1*) gene products are involved in the oxidative metabolism of xenobiotics, producing compounds subsequently processed by phase II enzymes, such as GSTT1. It was observed that variants of *CYP1* and *GSTs* metabolic genes were associated with increased levels of chromosomal aberrations and SCEs (Hemminki et al., 2015; Kumar et al., 2011; Laczmanska et al., 2006).

In order to prevent the potentially mutagenic consequences of DNA modifications, cells have evolved different mechanisms of DNA repair, depending on the specific type of DNA damage. These mechanisms include Base Excision Repair (BER) and Nucleotide Excision Repair (NER) that correct nonbulky damage and lesions that distort the DNA double helical structure, respectively (Cleaver, Lam, & Revet, 2009; Collins & Azqueta, 2012). Most of the genes encoding DNA-repair enzymes are polymorphic, and some of these polymorphisms are directly related to increased levels of chromosomal aberrations and SCEs (Laczmanska et al., 2007; Toolaram, Kümmerer, & Schneider, 2014; Vodicka et al., 2004).

We decided to evaluate the relationships between some phase I (*CYP1A1 Ile/Val* and *CYP2C19 G/A*), phase II (*GSTT1 positive/null, GSTM1 positive/null* and *GSTP1 A/G*), and DNA-repair (*ERCC2/XPD Lys751Gln, XRCC1 Arg194Tr, XRCC1 Arg399Gln* and *XRCC1 Arg208His*) gene polymorphisms, as they are known to be associated with an increased susceptibility to DNA damage (Laczmanska et al., 2006; Santovito et al., 2015; Toolaram et al., 2014), and the levels of genomic damage measured by SCEs assay.

2 | MATERIALS AND METHODS

2.1 | Study population

The present work is part of a national study designed to analyze the health of the urban Italian population. Our group is interested in analyzing the relationships between life style, endogenous factors and levels of genomic damage. The demographic characteristics of the studied group are reported in Table 1. The study population comprised 230 blood donors sampled in Turin (Piedmont, North-Western Italy). We recruited subjects without any known exposure except those of the routine household, traffic and/or clerical work. Subjects were randomly recruited among different university departments, hospital workers of the administrative staff, and healthy volunteers enrolled in these structures. The equal representation of both sexes and of all age-groups was the only adopted selection criteria.

TABLE 1 General characteristics of the studied subjects

Subjects	*N*	Age (mean ± SD)	Age range
Total	230	38.45 ± 9.51	21–70
Sex			
Males	90	42.01 ± 9.34	22–70
Females	140	36.16 ± 8.94	21–58
Smoking habits			
Smokers	83	39.60 ± 10.65	22–70
Nonsmokers	147	37.80 ± 8.79	21–66
Heavy smokers	66	40.20 ± 10.61	22–70
Light smokers	17	37.29 ± 10.81	22–58
LT smokers	47	46.92 ± 7.44	33–70
ST smokers	36	30.06 ± 5.25	22–42
Age groups			
A	54	26.41 ± 2.84	21–30
B	91	36.42 ± 2.60	31–40
C	59	44.88 ± 2.81	41–50
D	26	55.96 ± 4.83	51–70

N = number of studied subjects; SD = standard deviation.
Heavy smokers = >10 cig/die; light smokers = ≤10 cig/day.
LT smokers = long time smokers = >10 years of smoking habit.
ST smokers = short time smokers = ≤10 years of smoking habit.

Subjects were divided into four groups, from A to D, according to age: 21–30 (group A), 31–40 (group B), 41–50 (group C), and 51–70 (group D). The last group included a wider age range because only three individuals were found to belong to the age group 61–70. Therefore, we decided to join the classes of age 51–60 and 61–70 into a single age-group for this analysis.

In order to analyze the influence of smoking on the level of genomic damage, the total population sample was subdivided in two groups, smokers and nonsmokers. Smokers were then subdivided into four subgroups based on the number of cigarettes smoked/day (cig/day) [heavy smokers, >10 cig/day; light smokers, ≤10 cig/day] and on the number of years of smoking [LT-smokers = long time smokers, >10 years; ST smokers = short time smokers, ≤10 years].

The present study was performed in accordance with the ethical standards of the University of Turin bioethics committee and with the 1964 Declaration of Helsinki. Blood donors were informed about the aim and the experimental details of the study, gave their informed consent, and volunteered to donate blood for sampling. They were healthy at the moment of blood sampling and interviews. In our sample, we exclusively considered individuals who did not consume drugs or alcohol and were not exposed to X-ray for a period of at least two years prior to the analysis.

2.2 | Blood sampling and SCEs assay

Blood samples were obtained by venipuncture (5–10 ml) and collected in heparinized tubes. After collection, all blood samples were coded, cooled (4°C), and processed within 2 h after collection. Approximately 0.4 mL of each sample was cultured using RPMI 1640 medium supplemented with 20% fetal calf serum, 2 mM L-glutamine, 0.2 mL of the mitogenic agent phytohemagglutinin (PHA), and antibiotics (100 IU/mL penicillin, and 100 μg/mL streptomycin). To arrest cells in mitosis, colchicine (0.25 μg/mL) was added at a concentration of 0.06 μg/mL during the last 2 h of culture. The cultures were incubated for 72 h at 37°C in an atmosphere of 5% CO_2 in the air. To measure SCEs in second division metaphases, bromodeoxyuridine (BrdUrd, 5 μg/mL) was added at 24 h. 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 and the chromatid with more BrdUrd is lighter in appearance (a "bleaching" effect). Chromosome preparation was done following standard procedures, as described in Santovito, Cervella, and Delpero (2014). For each subject, we scored 50 well-spread second-division metaphases containing 46 chromosomes. A total of 100 cells from each donor was scored for the determination of the replication index (RI), calculated according to the following 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 metaphase scored.

Together with the individual mean value of SCEs per cell, in order to increase the sensitivity of the assay, we calculated the high frequency cells (HFC) and the number of high frequency individuals (HFI). These measures took into account cells with a high frequency of SCEs that represented a subpopulation of more sensitive cells or of long living lymphocytes which accumulated DNA lesions *in vivo* (Carrano & Moore, 1982). The evaluation of HFC was performed to assess individual variability in susceptibility to genotoxic agents. The occurrence of HFC in population studies is generally evaluated using aggregate measures such as the HFI, ie, subjects who show a high proportion of HFC (Bonassi, Fontana, Ceppi, Barale, & Biggeri, 1999).

2.3 | DNA extraction and genotyping

DNA extraction was conducted using a Chelex solution, according to the following protocol: 10 μL of peripheral blood was diluted in 1 mL of sterile distilled water for 15 min at room temperature. After centrifugation at 14,000 rpm for 1 min, the pellet was re-suspended in 200 μL of 5% Chelex solution in Tris-EDTA at pH 8, heated to 56°C for 15 min and, after vortex for 10 sec, at 100°C in boiler water for 8 min. For PCR reactions we used 19 μL of this solution containing extracted DNA. Primers and methodologies are described in Bonello et al. (2010), Chen, Xue, Xu, Ma, and Wu (2001), García-Gonzalés et al. (2012), Li et al. (2009), Matullo et al. (2001), Pemble et al. (1994), Wang et al. (2010), and Zhong, Wyllie, Barnes, Wolf, and Spurr (1993). PCR reactions were performed in a 25 μL volume containing about 10 ng DNA (template), with a final concentration of 1× reaction buffer, 1.5 mM of $MgCl_2$, 5% of DMSO, 250 μM of dNTPs, 0.5 μM of each primer, and 1 U/sample of *Taq* DNA polymerase (Fischer, US). Cycles were set as follows: 35 cycles, 1 min at 95°C, 1 min at 60°C, 1 min at 72°C, and a final extension step 10 min at 72°C. Amplification products were detected by ethidium bromide staining after 3% agarose gel electrophoresis. In order to improve our results, all null genotypes and a random 20% (n = 75) of the subjects were regenotyped for all analyzed polymorphisms. Only when the results of the two genotyping efforts were similar, did we include the subject in our sample.

2.4 | Statistical analysis

Statistical analysis was assessed using the SPSS software statistical package program (version 23.0 SPSS, Chicago, IL).

TABLE 2 Frequency of SCEs in the studied population according to sex and smoking habits

Groups	*N*	Cells	SCEs	SCEs/cell ± SD	M_1	M_2	M_3	RI ± SD
Total	230	11,500	59,172	5.15 ± 1.87	8027	8992	5940	1.90 ± 0.24
HFI	62	3100	23,379	7.54 ± 0.99[a]	2338	2662	1165	1.80 ± 0.26[d]
Non-HFI	168	8400	35,793	4.26 ± 1.23[a]	5690	6328	4776	1.95 ± 0.22[d]
Sex								
Males	90	4500	21,685	4.82 ± 1.39[b]	3102	3505	2389	1.92 ± 0.19
Females	140	7000	37,487	5.36 ± 2.10[b]	4926	5485	3552	1.90 ± 0.27
Smoking habits								
Nonsmokers	147	7350	34,573	4.70 ± 1.79[c]	5022	5763	3902	1.92 ± 0.22
Smokers	83	4150	24,599	5.93 ± 1.75[c]	3006	3227	2039	1.88 ± 0.28
Heavy smokers	66	3300	20,026	6.07 ± 1.54	2404	2545	1623	1.87 ± 0.25
Light smokers	17	850	4573	5.38 ± 2.39	602	682	416	1.89 ± 0.36
LT smokers	47	2350	13,722	5.84 ± 1.63	1775	1891	997	1.81 ± 0.22[e]
ST smokers	36	1800	10,877	6.04 ± 1.92	1231	1136	1042	1.96 ± 0.33[e]

HFI = high frequency individuals; LT = long time smokers (>10 years od smoking habit); ST = short time smokers (≤10 years of smoking habit); *N* = number of analyzed subjects; SCEs = sister chromatid exchanges; metaphases; 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 metaphase scored; SD = standard deviation.
[a,c]$P < .001$; [b]$P = .01$; [d]$P = .03$; [e]$P = .04$ (Wilcoxon Mann-Whitney *U* test).

The Wilcoxon Mann-Whitney *U* test and the ANOVA with Tukey's correction test were used to analyze the differences in the frequency of SCEs between males and females, smokers and non-smokers, and age groups, as well as to test the influence of the analyzed gene polymorphisms on the level of genomic damage. Multiple regression analysis was also used to evaluate the influence of age, sex, and smoke on SCEs frequency. All *P* values were two tailed and the level of statistical significance was set at $P < .05$ for all tests.

3 | RESULTS

3.1 | Study population

The general characteristics of the study subjects are reported in Table 1. Ninety subjects were male (mean age ± SD 42.01 ± 9.34, range 22–70) and 140 were female (mean age ± SD 36.16 ± 8.94, range 21–58).

Eighty-three subjects were regular smokers (48 females and 35 males) while 147 were not (92 females and 55 males). Among smokers, there were 66 heavy smokers and 17 light smokers, with an average number of cigarettes smoked per day of 17.83 ± 6.42 (ranging from 8 to 40). There were 47 long time (LT) and 36 short time (ST) smokers, with an average number of years of smoking of 14.26 ± 9.04 (ranging from 2 to 38).

Fifty-four subjects fell into age group A (mean age 26.41 ± 2.84), 91 in age group B (mean age 36.42 ± 2.60), 59 in age group C (mean age 44.88 ± 2.81) and 26 in age group D (mean age 55.96 ± 4.83).

3.2 | SCEs analysis

The results of the SCEs analysis are summarized in Table 2. The mean frequency of SCEs and the value of RI in the total sample were 5.15 ± 1.87 and 1.90 ± 0.24, respectively. According to the Carrano and Moore (1982) methodology, 62 individuals were classified as HFI with a mean value of

TABLE 3 Factors affecting sister chromatid exchanges analyzed by ANOVA

Factors	*F* value	*P* value
Demographic characteristics		
Age groups	6.87	0.02
Sex	4.58	0.03
Smoking habits		
Smoke vs. nonsmokers	25.13	<0.001
Heavy vs. light	2.12	0.15
Long time vs. short time	0.28	0.60
Gene polymorphisms		
CYP1A1 Ile/Val	2.08	0.28
CYP2C19 G/A	14.62	<0.001
GSTT1±	18.88	<0.001
GSTM1±	6.93	0.01
GSTP1 A/G	2.22	0.20
ERCC2/XPD Lys751GLn	9.68	0.03
XRCC Arg194trp	0.97	0.49
XRCC Arg399Glc	1.39	0.22
XRCC Arg208His	12.62	0.00

TABLE 4 Multiple regression analysis of confounding factors on SCEs and RI values in lymphocytes of the study groups

	SCEs frequency			RI value		
CF	β-Co	*P* value	95% CI (lower) – (upper)	β-Co	*P* value	95% CI (lower)-(upper)
Age	0.10	0.07	(–0.89–24.85)	−0.16	0.01	(–0.01)-(–0.00)
Cig/day	0.23	0.03	(0.35)-(6.20)	0.04	0.79	(–0.62)-(0.88)
Years of smoking	−0.05	0.67	–(2.60)-(1.68)	0.04	0.69	(–0.43)-(0.64)

CF = confounding factor; β-co = β-coefficient; SCE = sister chromatid exchanges; RI = replication index.

SCEs/cell of 7.54 ± 0.99, whereas 168 subjects were classified as non-HFI, with a mean value of SCEs/cell of 4.26 ± 1.23. We observed significant differences between HFI and Non-HFI in terms of SCEs frequency ($P < .001$) and RI value ($P = .03$).

Similarly, sex seemed to play an important role in the determining the amount of genomic damage, as females showed a significantly ($P = .01$) higher level of SCEs with respect to males (5.36 ± 2.10 and 4.82 ± 1.39, respectively), as also confirmed by the ANOVA analysis ($P = .03$, Table 3).

With regard to smoking habits, smokers showed a significantly ($P < .001$) higher frequency of SCEs compared to non-smokers (5.93 ± 1.75 and 4.70 ± 1.79, respectively), whereas no statistical differences were found in terms of SCEs between heavy- and light-smokers (6.07 ± 1.54 and 5.38 ± 2.39, respectively) as well as between LT- and ST smokers (5.84 ± 1.63 and 6.04 ± 1.92, respectively). However, LT smokers showed a significantly lower RI value with respect to ST smokers ($P = .04$), indicating a possible cytotoxic effect of smoking over time. Finally, among smokers, the regression analysis indicated a significant correlation ($P = .03$) between the number of cigarettes/day and the level of SCEs (Table 4).

Age was found to correlate with the RI value ($P = .01$) but not with the SCEs frequency ($P = .07$) (Table 4), although the ANOVA indicated significant differences in the level of SCEs among age groups ($P = .02$; Table 3). Indeed, the 31–40 age group showed a significantly lower SCEs frequency with respect to all other age groups (Table 5).

3.3 | Gene polymorphisms

Finally, the influence of some gene polymorphisms on SCEs frequency was also evaluated (Tables 3 and 6). Our results showed that *GSTP2C19-AA*, *GSTT1*-null, *GSTM1-null*, *ERCC2/XPD Lys751Gln* and *XRCC1 Arg208His* gene polymorphisms were associated with significantly higher levels of SCEs, whereas this association was not found for the other analyzed genetic polymorphisms.

4 | DISCUSSION

In the present study, we evaluated, by means of the SCEs assay, the influence of some exogenous (smoking habits) and endogenous (age, sex, metabolic, and DNA-repair genes polymorphisms) parameters on the level of the cytogenetic damage in the peripheral blood lymphocytes of nonoccupationally exposed, healthy subjects living in the city of Turin (North Italy).

Although Turin is one of the most polluted cities in Europe (Santovito et al., 2016), mainly in terms of air fine particular matter whose mutagenic potential has been suggested in a number of studies (Buschini et al., 2001; Wei & Meng, 2006), we observed a frequency of SCEs generally

TABLE 5 SCEs frequency according to age groups

Age groups	*N*	Cells	SCEs	SCEs/cell ± SD	M_1	M_2	M_3	RI ± SD
A (21–30)	54	2700	14,530	5.38 ± 2.02[a]	1930	1959	1520	1.93 ± 0.30[d]
B (31–40)	91	4550	20,453	4.50 ± 1.81[a,b,c]	2971	3543	2582	1.95 ± 0.21[e,f]
C (41–50)	59	2950	16,757	5.68 ± 1.77[b]	2154	2492	1205	1.83 ± 0.24[e,f,g]
D (51–70)	26	1300	7432	5.72 ± 1.26[c]	972	998	633	1.87 ± 0.20[d,g]

N = number of analyzed subjects; SCEs = sister chromatid exchanges; metaphases; 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 metaphase scored; SD = standard deviation.
[a]$P = .01$; [b]$P = <.001$; [c]$P = .001$; [d]$P = .04$; [e]$P = .01$; [f]$P = .04$; [g]$P = .03$ (Wilcoxon Mann-Whitney *U* test).

TABLE 6 SCEs frequency according to genetic polymorphisms

Gene polymorphisms	*N*	Cells	SCEs	SCEs/Cell ± SD	M_1	M_2	M_3	RI ± SD
PHASE I								
CYP1A1 Ile/Ile	177	8850	44,395	5.02 ± 1.87	6082	6837	4738	1.92 ± 0.25
CYP1A1 Ile/Val	44	2200	12,093	5.50 ± 1.86	1615	1802	983	1.86 ± 0.18
CYP1A1 Val/Val	9	450	2684	5.96 ± 1.69	331	351	220	1.88 ± 0.28
CYP2C19 GG	202	10,100	51,179	5.07 ± 1.81[a,b]	7025	7838	5310	1.91 ± 0.24
CYP2C19 G/A	23	1150	6554	5.70 ± 1.97[a]	845	929	512	1.84 ± 0.24
CYPC192 A/A	5	250	1440	5.76 ± 3.34[b]	157	225	118	1.92 ± ± 0.29
PHASE II								
GSTT1+	38	9600	47,191	4.92 ± 1.69[c]	6679	509	4979	1.91 ± 0.24
GSTT1-	168	1900	11,981	6.31 ± 2.28[c]	1349	1481	962	1.89 ± 0.25
GSTM1+	62	8400	41,584	4.95 ± 1.84[d]	5727	6635	4397	1.92 ± 0.24
GSTM1-	125	3100	17,588	5.67 ± 1.86[d]	2301	2355	1544	1.88 ± 0.23
GSTT1+/GSTM+	34	6250	31,066	4.97 ± 1.71	4379	4971	3117	1.89 ± 0.25
`GSTT1-/GSTM1-	50	1700	9231	5.43 ± 2.06	1151	1359	889	1.93 ± 0.22
GSTT1+/GSTM1-	21	2500	12,574	5.03 ± 1.80	1779	1855	1367	1.92 ± 0.25
GSTT1-/GSTM1+	186	1050	6301	6.00 ± 2.40	718	807	567	1.92 ± 0.23
GSTP1 AA	36	9300	46,711	5.02 ± 1.80	6561	7238	4771	1.90 ± 0.25
GSTP1 AG	8	1800	10,088	5.60 ± 2.06	1190	1444	955	1.93 ± 0.23
GSTP1 GG	185	400	2373	5.93 ± 2.24	276	310	214	1.92 ± 0.24
DNA repair								
ERCC2/XPD Lys751Lys	29	9250	45,375	4.91 ± 1.72[e,f]	6420	7214	4834	1.91 ± 0.25
ERCC2/XPD Lys751Gln	16	1450	8423	5.81 ± ± 2.01[e]	1063	1161	677	1.87 ± 0.23
ERCC2/XPD Gln751Gln	192	800	5374	6.72 ± 2.31[f]	544	617	429	1.92 ± 0.26
XRCC1 Arg194Arg	31	9600	48,673	5.07 ± 1.89	6727	7497	4931	1.90 ± 0.25
XRCC1 Arg194Trp	7	1550	8504	5.49 ± 1.65	1070	1198	836	1.92 ± 0.25
XRCC1 Trp194Trp	178	350	1994	5.70 ± 2.25	230	297	1343	1.92 ± 0.18
XRCC1 Arg399Arg	45	8900	45,469	5.11 ± 1.91	6188	6938	4641	1.91 ± 0.25
XRCC1 Arg399Gln	7	2250	11,870	5.28 ± 1.73	1592	1759	1141	1.90 ± 0.24
XRCC1 Gln399Gln	200	350	1832	5.24 ± 1.91	247	295	158	1.87 ± 0.19
XRCC1 Arg208Arg	26	10,000	50,695	5.07 ± 1.88[g]	6956	7883	5133	1.90 ± 0.24
XRCC1 Arg208His	4	1300	7062	5.43 ± 1.69[h]	957	923	707	1.89 ± 0.27
XRCC1 His208His		200	1415	7.08 ± 1.22[g,h]	114	186	100	1.97 ± 0.16

N = number of analysed subjects; SCEs = sister chromatid exchanges; metaphases; 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 metaphase scored; SD = standard deviation.
[a,b,c,f,h]$P = <.001$; [d]$P = .01$; [e]$P = .01$; [g]$P = .001$ (Wilcoxon's Mann-Whitney *U* test).

similar to that found by Carere et al. (2002) for Rome and lower with respect to that observed by Barale et al. (1998) for Pisa (Tuscany Region, Central Italy). On the contrary, in our previous published work (Santovito et al., 2016), we reported a high baseline frequency of chromosomal aberrations (such as breaks, dicentrics, and rearrangements) in a control sample of subjects living in Turin, one of the highest value reported in literature for European control populations.

It is known that spontaneous genomic damage can also be induced by a variety of other endogenous and exogenous factors. Among them, smoking has been found to increase the level of SCEs and other cytogenetic biomarkers in peripheral blood lymphocytes of many human populations (Bonassi et al., 2011; Salah et al., 2011).

According to data reported by other authors (Salah et al., 2011; Sebastià et al., 2014), a significant increase in SCEs frequency was observed among smokers (Tables 2 and 3). Moreover, the chromosomal damage seemed to correlate with the number of cigarettes/day (Table 4), indicating a possible cumulative genotoxic effect of the cigarette smoke on human lymphocytes. This increased level of genomic damage observed among smokers can be explained by the fact that cigarette smoke contains several genotoxic compounds,

most of them also having carcinogenic properties, such as polycyclic aromatic hydrocarbons, aromatic amines, and metals (IARC, 1986).

Contrary to what was observed in other reports (Salah et al., 2011; for a review see DeMarini, 2004 and Husgafvel-Pursiainen, 2004), in our work and other published studies, no significant differences were found between light and heavy smokers and between LT- and ST smokers in terms of SCEs frequency. Finally, among smokers, no correlation was found between the number of years of smoking and SCEs frequency (Table 4). A possible interpretation of this pattern has been postulated by Costa et al. (2008), who hypothesized that various physiological systems (induction of metabolizing and detoxifying enzymes, induction of DNA-repair processes) had adapted over time. In this sense a few cigarettes per day may stimulate a cell-adaptive response, thereby causing an apparent lowering in SCEs frequency. Moreover, as suggested by Donmez-Altuntas and Bitgen (2012) for other cytogenetic markers, it could be that cells damaged by cigarette smoke may not survive in culture or may not divide because they are more likely to die of necrosis or apoptosis, making it impossible to carry out the SCEs assay with them.

As for the role of sex, although in some studies (Sebastià et al., 2014; Santovito et al., 2015) no sex effect was observed, in our work females showed significantly higher SCE values than males, while sex had no effect on the replicative capacity of the cells, as indicated by the RI values (Tables 2 and 3).

The influence of age on the frequencies of SCEs have been evaluated in many studies (for a review see Bolognesi et al., 1997). In the present work, although the regression analysis indicated that the level of SCEs did not correlate with age (Table 4), we observed a significantly lower frequency of SCEs for the 31–40 age group with respect to the 41–50 and 51–70 age groups (Tables 3 and 5). The increase in the amount of SCEs among subjects belonging to the last two age groups could be explained by a decreased efficiency in the repair of DNA damage, with consequent accumulation of "aberrant cells," in peripheral lymphocytes of older individuals. Indeed, it has been well documented that cells from older individuals exhibit increased levels of damaged DNA and chromosomal instability (Bukvic et al., 2001; Donmez-Altuntas & Bitgen, 2012; Milosevic-Djordjevic et al., 2002).

The individuals enrolled in our study were also genotyped for five phase I and phase II metabolic gene polymorphisms, as well as for four DNA-repair gene polymorphisms (Table 6). In agreement with data obtained by Kumar et al. (2011), our results showed that *GSTT1-null* and *GSTM1-null* gene polymorphisms were associated with increased cytogenetic damage. This result was not surprising because the reduced detoxification ability of the null genotypes has been related to an increased susceptibility to DNA damage (Palma et al., 2007), as well as to an increased cancer risk (Bajpai et al., 2007; Cha et al., 2007).

CYP2C19 A/A subjects also showed a frequency of SCEs significantly higher with respect to the *CYP2C19* G/G homozygote genotypes. Although other polymorphisms in genes belonging to *CYP2* family, such as those in the *CYP2E1* c1/c2 gene, were found to affect the frequency of SCEs (Laczmanska et al., 2006), this is the first study showing a possible association of the *CYP2C19 A/A* genotype with increased SCEs levels in a control population.

Finally, analyzing the effect of DNA-repair gene polymorphisms on the amount of genomic damage, we found, for the first time in a control population, an association between *XPD* Gln751Gln and *XRCC1 His208His* genotypes and increased levels of SCEs. It is known that high frequencies of SCEs are related to defects in the DNA-repair machinery (Garcia-Sagredo, 2008). At the same time, the *XPD* codon 751 Gln allele was found to be associated with lower DNA repair capacity and lower cell viability in *in vitro* systems (Xiao et al., 2016; Zhang et al., 2017), and with higher levels of DNA adducts (Matullo et al., 2003; Palli et al., 2001) and chromatid aberrations (Ma, Qui, Tie, & Guo, 2013). Similarly, the *XRCC1 His* allele results in defective DNA repair capacity, due to the inefficient localization of protein to the DNA damage site (Ji et al., 2010). In this scenario, we can postulate that the reduced DNA repair capacity could potentially contribute to the higher levels of SCEs observed among homozygous *XPD Gln751Gln* and *XRCC1 His208His* genotypes.

However, it should be emphasized that data related to the association of *ERCC2/XPD* Lys751Gln and the cytogenetic damage are contradictory. In previous published studies, the *XPD751Gln* variant allele was found to be associated with increased micronuclei frequencies (Pérez-Cadahía, 2008) but with decreased chromatid aberration frequencies (Lunn et al., 2000; Vodicka et al., 2015). *Vice versa*, in other studies the *Gln* allele failed to influence the levels of SCEs and DNA adducts (Duell et al., 2000). A possible explanation for these conflicting results could be that the accumulation of chromosomal aberrations requires a complex interplay between different DNA repair pathways. Gene-gene interactions in DNA repair genes could also influence enhanced or decreased chromosomal aberration frequencies. For example, Spitz et al. (2001) observed the best repair activity in cells from wild-type individuals who were homozygous at both Lys751Gln and Asp312Asn loci and the lowest repair capacity in those carrying at least two variant alleles.

5 | CONCLUSIONS

Beyond the classical endogenous and exogenous factors, such as sex, age and smoking habits, which are already known to

have in some cases a stronger effect on the level of genomic damage, we describe a positive association between CYP2C19 A/A, *GSTT-null, GSTM1-null, XPD 751 CC*, and *XRCC1 His208His* genotypes with increased frequencies of SCEs. This finding reinforces, in bio-monitoring studies of human populations, the importance of genetic analysis designed to evaluate more classic endogenous and exogenous factors that could influence the level of the genomic damage. Moreover, our data assume a more important connotation if we consider the fact that, in the present study, we analyzed a control population consisting of subjects not exposed for professional reasons to xenobiotics, but living in a city, like Turin, with many problems related to urban pollution (Bono et al., 2016; Raaschou-Nielsen et al., 2013; Santovito et al., 2016; Traversi et al., 2009). In this sense, we hope that the results of this study can be used as a stimulus for future biomonitoring programs in other Italian and globally distributed cities.

Finally, it should be emphasized that the results of the present work cannot be generalized for all people of European descent because this group is heterogeneous, with differences in the distribution of genetic polymorphisms and in life styles among individuals.

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AUTHOR CONTRIBUTIONS

Alfredo Santovito designed the study, performed the laboratory analysis, analyzed the data and drafted the manuscript. Claudio Gendusa, collected the data, contributed to draft the manuscript and to perform the laboratory analysis. Piero Cervella provided necessary logistical and economical support, analyzed the data and edited the manuscript for intellectual content providing critical comments on the manuscript.

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