Supplemental information SI1. Detailed information on biospecimens and genotyping methods.

Materials and Methods

Biospecimens

Germline DNA was extracted from 3-5 ml of whole blood obtained by venipuncture and anticoagulated with EDTA (ethylenediaminetetraacetic acid) using the CTAB (N-Cetyl-N,N,N-trimethyl-ammonium bromide; Merck, Darmstadt, Germany) protocol.

DNA concentration and quality were measured by absorbance at 260 nm and by the ratio A260 nm/A280 nm, respectively, in a GeneQuant™-Pro RNA/DNA calculator (Amersham-Biosicences). Some samples were also analyzed by electrophoresis on 1% agarose (Genbiotech SRL, Buenos Aires, Argentina) gels dyed with ethidium bromide (Promega, Wisconsin, USA).

All samples were kept at -80ºC for long-term storage; and a 50 ng DNA/µl aliquot (working solution) was stored at -20ºC.

Genotyping

We genotyped three polymorphisms in three GST genes: *GSTP1* c.313 A>G (NM_000852.3:c.313A>G; p.105 Ile>Val; rs1695), *GSTT1* null, and *GSTM1* null. The genotyping of *GSTP1* c.313 A>G was performed by PCR-RFLP assay. The PCR reaction included 1.5-2.5 ng/µl DNA, 1x PCR reaction buffer (Fermentas, Ontario, Canada), 200 µM

dNTP (Genbiotech SRL, Buenos Aires, Argentina), 2 mM $MgCl_2$ (Fermentas, Ontario, Canada), 0.05 U/µl Taq polymerase (Fermentas, Ontario, Canada), and 0.3 µM of each primer (Forward: 5'-TGTGTGGCAGTCTCTCATCC; Reverse: 5'-GAAGCCCCTTTCTTTGTTCA) (IDT, Iowa, USA). Cycling included a step at 94ºC for 5 minutes followed by 40 cycles of 94ºC for 30 seconds, 57ºC for 30 seconds, 72ºC for 45 seconds, with a final step at 72ºC for 5 minutes. Enzymatic digestion of PCR fragments was performed at 37ºC overnight and included 0.1-0.5 µg of PCR product, 10 U of Alw26I restriction enzyme (Fermentas, Ontario, Canada), and 1x buffer Tango (Fermentas, Ontario, Canada) in a final volume of 20 µl. Genotyping call rate for this SNP was 98%.

GSTT1 null and *GSTM1* null genotypes were assessed by multiplex-PCR reaction. The PCR reaction included 1.5-2.5 ng/µl DNA, 1x PCR reaction buffer (Fermentas, Ontario, Canada), 300 µM dNTP (Genbiotech SRL, Buenos Aires, Argentina), 2.5 mM $MgCl_2$ (Fermentas, Ontario, Canada), 5% DMSO, 0.08 U/µl Taq polymerase (Fermentas, Ontario, Canada), and 0.3 µM of each primer (*GSTT1* Forward: 5'-TTCCTTACTGGTCCTCACATCTC; *GSTT1* Reverse: 5'-TCACCGGATCATGGCCAGCA; *GSTM1* Forward: 5'-CTGCCCTACTTGATTGATGGG; *GSTM1* Reverse: 5'-CTGGATTGTAGCAGATCATGC) (IDT, Iowa, USA). Cycling included a step at 94ºC for 5 minutes followed by 40 cycles of 94ºC for 30 seconds, 58ºC for 30 seconds, 68ºC for 80 seconds, with a final step at 68ºC for 5 minutes. This method allowed us to discriminate the null genotype (homozygote deletion), determined by the absence of band in the electrophoresis, from the heterozygote and homozygote present genotypes. Because we amplified both genes in a single PCR reaction, we called the null genotype when there was an absence of the band for either *GSTT1* or *GSTM1* and when the other gene amplified (internal PCR control). Samples that did not amplify for both genes were repeated twice or three times to discard a PCR failure. These samples were called null for both *GSTT1* and *GSTM1* only when the following criteria were met: i) all replications were concordant, ii) other samples within the same PCR reaction

using the same PCR mix amplified (reaction control), and iii) PCR reactions for double-null samples showed the specific amplicon for other genes (DNA quality control). Genotyping call rates were 99% for *GSTT1* and 98% for *GSTM1*.

All PCR reactions were performed in a DNA Engine™ Thermocycler (Bio-rad, California, USA). PCR reactions and digested products were analyzed by 2% agarose (Genbiotech SRL, Buenos Aires, Argentina) gel electrophoresis in 1x TAE buffer (0.8 M Tris; 0.4 M sodium acetate; 0.04 M EDTA; pH 8.3) and dyed with ethidium bromide (Promega, Wisconsin, USA). Gels were photographed and analyzed with the G-Box system (Syngene, USA) and the Genesnap software (Syngene, USA).

Samples that failed were repeated once or twice as needed. Genotyping outputs were read by 2 independent laboratory members, and 10-12% of blindly random selected samples were re-analyzed as quality control of the experiments. The results were considered for the final analyses when there was 100% agreement between the two independent readers, and when there was a 100% concordance between samples and blinded repeats.