Polymorphism of glutathione S-transferases M1 and T1: susceptibility to solar keratoses in an Italian population

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Summary

Background. Polymorphisms of glutathione S-transferases (GSTs) are linked to skin cancer, but data on their association with solar keratosis (SK) are few and conflicting.
Aim. To verify the possible association between the development of SK and the 'null' GSTM1 and/or T1 genotype.
Methods. Analysis of the GSTM1 and T1 genotype of 33 subjects with ≥3 solar keratoses and of 150 controls, before and after stratification based on smoking habits, sun exposure and immunosuppression.
Results. The GST T1 null allele is significantly (P < 0.03) associated with increased prevalence of SK in our population.
Conclusions. Our study, the first on a Mediterranean population, shows the existence of a correlation between SK and the GST T1 null genotype. This result points out significant differences between subjects of different ethnic and geographical origin and warrants further investigation on a larger population, and ethnically different populations.

Glutathione S-transferases (GSTs) are a family of enzymes that catalyse detoxification of exogenous and endogenous electrophilic compounds through glutathione conjugation.1,2 Formerly thought to be predominantly hepatic enzymes, GSTs have since been found in several other organs including skin, and are now considered an important component of the defensive mechanism against oxidative stress.

Genetic polymorphisms are common in GSTs. Based on isoelectric points, four main groups of human GST polymorphisms (GSTA, GSTM, GSTP and GSTT1) have been identified. Particular interest has been recently focused on GSTM1 and GSTT1. A relevant percentage of the population (which varies depending on ethnicity) carries a 'null' genotype (i.e. homozygosity for a genetic deletion, resulting in no enzyme production) of these genes.4,5 Several studies have shown that the ‘null’ GSTM1 and/or GSTT1 genotype (and the consequently decreased antioxidant activity) is correlated with a higher risk for oxidative stress-related diseases, such as neoplastic and inflammatory diseases. In dermatology, this correlation has been found for psoriasis, vitiligo, allergic dermatoses, melanoma and other cancers.6–10

Surprisingly, very few data are available on the possible correlation between the ‘null’ GSTM1/GSTT1 genotype and solar keratosis (SK), a well-known precancerous condition. To our knowledge, only two studies, both carried out on white Australians, have been performed, and these gave conflicting results.11,12

In this study we aimed to define the role of the null GSTM1 and/or GSTT1 genotypes as a risk factor for the development of SK in subjects from Sicily and Calabria, two regions of southern Italy.

Methods

The study was approved by the local ethics committee, and all participants gave written informed consent.
Study population

In total, 33 consecutive patients (16 men, 17 women, mean ± SD age 68.13 ± 10.25 years; range 55–99) with ≥ 3 solar keratoses were enrolled into the study. Exclusion criteria were severe systemic diseases, tumours, diseases that cause immune depression or photosensitivity and history of sunburns. All selected patients were white, born and living in Sicily or Calabria. They completed a questionnaire about lifestyle (including sun exposure and smoking habits), past and present diseases, and pharmacological therapies used.

For the control group, the same questionnaire was given to subjects without SK, who were selected using the exclusion criteria mentioned above. Based on their clinical history and answers to the questionnaire, we chose 150 people who matched the study group for ethnic and geographical origin, age (67.81 ± 11.01 years, range 57–95), skin phototype, job, use of immunosuppressive drugs and exposure to the aforementioned risk factors. Univariate statistical analysis was performed to confirm that there were no significant differences in the above parameters between patients and controls.

DNA analysis

Buccal swabs were used to obtain a sample of cells for DNA analysis from all 183 participants. DNA was extracted using a standard method (Chelex® 100 method; Bio-Rad Laboratories, Hercules, CA, USA). The relevant genes were amplified by PCR using a thermal cycler (PCR Sprint; Thermo Hybaid, Franklin, NJ, USA) and creating a multiplex system for the simultaneous amplification of GSTM1, GSTT1 and β-globin (as control) genetic loci. Primers used were those reported previously. Each PCR reaction was performed with 2.5 μL extract (5–250 ng DNA), 0.5 μmol/L of each primer, 2.5 μL Taq buffer (10× PCR Buffer II; Applied Biosystems, Foster City, CA, USA), 2 μL MgCl2 25 mmol/L (Applied Biosystems), 0.5 μL dNTP mix (10 mmol/L PCR Nucleotide Mix; Promega, Madison, WI, USA), 1 U Taq polymerase (DyNAzyme II DNA Polymerase; Finnzymes, Espoo, Finland) in a total volume of 25 μL. Thirty amplification cycles were performed as follows: denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min. The PCR products were separated by vertical electrophoresis at 2000 V (maximum mA and W) for 150 min on ultrathin (0.4 mm) layer polyacrylamide denaturing gels (6% with 7 mol/L urea) in Tris–borate–EDTA buffer 1x and then visualised by silver staining. This resulted in very good resolution (Fig. 1) of the migration bands of interest (480 bp for GSTT1 and 215 bp for GSTM1), identified by comparison with the molecular weights of a specific standard marker (DNA pGEM® marker; Promega).

Statistical analysis

Differences between the two groups were evaluated using χ² analysis with Yates’ correction when needed or Fisher exact test when at least one value in the contingency table was ≤ 5. P < 0.05 was considered significant. Results were evaluated before and after stratification for smoking habit, prolonged sun exposure (> 3 h/day), immunosuppressive therapies, either alone or in combination.

Results

Contingency tables and χ² test results are shown in Table 1. Possession of the GSTT1 null allele was significantly (P < 0.03) associated with an increased prevalence of SK in our study population; this was not true for the M1 null allele (P > 0.05). Subjects with both M1 null and T1 null alleles were more likely than those with an ‘active’ phenotype (both M1 and T1 non-null) to have SK, with a difference close to statistical significance (P = 0.09) (Table 1).
Stratification based on environmental risk factors (tobacco smoke, prolonged sun exposure, immunosuppressive treatment, or a combination of these) did not show significant differences between patients and controls for GST genotype (Table 1).

**Discussion**

The relationship between GST genotype and SK has been investigated previously in only two studies, both carried out by the same research group. The first study, performed on 89 patients and 92 controls, found no apparent correlation between SK and the GSTM1 null allele. However, the authors concluded that this genetic marker should have been retested taking into account lifestyle and employment factors, because of the well-known role of ultraviolet (UV) irradiation in the pathogenesis of malignant and premalignant cutaneous lesions.

The second study was conducted on a larger sample (135 patients, 135 controls) with more detailed analysis of several genetic factors (GSTM1, T1, P1 and Z1 genes, skin type, ability to tan) and environmental/behavioural factors (outdoor exposure, smoking, β-carotene supplementation, sunscreen use). This study found a significant increase in the risk of SK development in subjects with the GSTM1 null allele (OR = 2.1, increasing to 3.4 when high outdoor exposure was concurrently present).

Although confirming that GST genotypes influence the individual susceptibility to develop SK, our results, using a typical Mediterranean population (ethnically different from white Australians), show some intriguing peculiarities.

First, the frequencies of the null alleles of GSTM1 and T1 in our control population (54.67% and 24.67%, respectively) are much lower than those in the Australian control population (36% and 8%, respectively). Our high frequencies agree with the data reported by Ada et al., which is, to date, the only epidemiological study on this topic concerning a white population (Turkish) in the Mediterranean area.

A second difference is that the GSTT1 null genotype, but not the GSTM1 null genotype, was significantly associated with increased risk of SK in our population. This was not significant (P = 0.09) for subjects with both M1 and T1 null alleles; this was probably due to the limited size of our sample, because the raw data showed a clear statistical trend towards association of this genotype with SK.

Third, in our population, the risk due to exogenous factors (alone or in combination) was not significantly modified by the possession of the GSTM1 and/or T1 null alleles.

Natural selection for various historical, geographical and genetic reasons is the most likely explanation for the discrepancies between our data and those published by Griffiths and co-workers. It is well documented that white Australians are more prone than other populations to sun-induced premalignant and malignant skin lesions. Epidemiological studies reported a prevalence

### Table 1 GSTM1 and GSTT1 genotype frequencies in subjects affected by solar keratosis and control population.

| Genotype frequencies | GSTM1 | | | GSTT1 | | | GSTM1 + GSTT1 | | |
|----------------------|------|-----|-----|------|-----|-----|------|-----|
|                      | Null | Non-null | P  | χ² | Null | Non-null | P  | χ² | Null | Non-null | P  | χ² |
| Total                |      |         |    |    |      |         |    |    |      |         |    |    |
| SK                   | 17   | 7       | > 0.05 | 0.29 | 16   | 7       | < 0.03 | 5.10 | 7    | 7       | > 0.05 | 2.91 |
| Control              | 82   | 49      |       |     | 37   | 49      |       |     | 18   | 49      |       |     |
| Smoker               |      |         |    |    |      |         |    |    |      |         |    |    |
| SK                   | 7    | 5       | > 0.05 | 0.08 | 7    | 5       | > 0.05 | 0.80 | 4    | 5       | > 0.05 | 1.03 |
| Control              | 37   | 22      |       |     | 17   | 22      |       |     | 8    | 22      |       |     |
| Immunosuppression    |      |         |    |    |      |         |    |    |      |         |    |    |
| Control              | 4    | 1       | > 0.05 |*   | 3    | 1       | > 0.05 |*   | 1    | 1       | > 0.05 |*   |
| Smoker               | 18   | 10      |       |     | 8    | 10      |       |     | 4    | 10      |       |     |
| Sun exposure (> 3 h/day) |      |         |    |    |      |         |    |    |      |         |    |    |
| Control              | 6    | 2       | > 0.05 |*   | 5    | 2       | > 0.05 |*   | 2    | 2       | > 0.05 |*   |
| Smoker               | 27   | 16      |       |     | 13   | 16      |       |     | 6    | 16      |       |     |
| Multiple environmental risk factors† |      |         |    |    |      |         |    |    |      |         |    |    |
| Control              | 4    | 2       | > 0.05 |*   | 6    | 2       | > 0.05 |*   | 3    | 2       | > 0.05 |*   |
| Smoker               | 23   | 13      |       |     | 10   | 13      |       |     | 5    | 13      |       |     |

SK, solar keratosis. *Fisher exact test; †smoking, high sun exposure, immunosuppression. Significant P values are shown in bold.
rate for SK of approximately 50% in people aged > 40 years\textsuperscript{17} and 80% in the seventh decade of life,\textsuperscript{18} while the figures for adults in the northern hemisphere are between 11% and 25%.\textsuperscript{19} This is due not only to the high solar irradiation found in Australia, but also to the skin phototype of Australian people, who often have a fair or medium complexion. The high frequency of such complexions, in spite of climatic conditions, is mainly due to the geographical position of Australia, which limits the possibility to expand the genetic pool of the residents, thus the current ‘average’ skin phototype in Australia is similar to that of the first colonizers of the continent, who came from northern Europe.

Conversely, Sicily and Calabria, situated in the middle of the Mediterranean sea, were and still are the crossroads of several populations, which have created a much larger gene pool. Climatic conditions here have favoured the selection of a darker ‘average’ complexion: in our population, which is a good representation of the phototypes in the general population, about 55% of subjects had Fitzpatrick skin phototype III, and the remainder had phototype IV.

It can be hypothesized that the different level of protection given by cutaneous pigmentation is a possible reason for the different prevalence of GST\textsubscript{M1}/T1 null genotypes between Mediterranean and Australian populations. A fair, less ‘protective’ skin is more prone to oxidative events and this leads, through time, to the selection of subjects able to neutralize electrophiles and other harmful compounds efficiently. Conversely, because darker skin reduces the likelihood of UV-induced molecular damage, the role of GSTs (and possibly other antioxidant systems) is probably less crucial and, consequently, selection of the ‘active’ genotypes is less stringent. As a possible indirect confirmation of this mechanism, it can be seen that possession of the GST\textsubscript{M1} null genotype in subjects with high sun exposure notably increased the risk given by prolonged sun exposure alone (OR 3.4 vs. 1.6, respectively) in the population described by Griffiths,\textsuperscript{12} whereas this was not the case for GST\textsubscript{M1} and/or T1 null alleles in our sample. Moreover, both studies agree on the insignificant effect of the GST genotype on the risk due to tobacco smoke. Taken together, all these data suggest that antioxidant mechanisms different from GST\textsubscript{M1} and T1 can neutralize a certain amount of oxidative stress. When this limit is overcome, the lack of contribution of the above enzymes to the antioxidant system in subjects bearing the GST\textsubscript{M1} and/or T1 null allele(s) becomes evident, and the risk of premalignant (and possibly malignant) lesions significantly increases. This is probably what happens in fair-skinned Australian subjects exposed to high levels of sun exposure, but not in our Mediterranean population, even when additional risk factors are present.

Immunosuppression, which was also considered in our study, does not increase oxidative stress, but favours the onset of SK by limiting the ability to eliminate damaged/mutated cell clones.

Overall, our data agree with those by Griffiths and co-workers\textsuperscript{12} with regard to the importance of the GST system in the protection against SK; however, whereas in Australians the major role in this field is played by the GST\textsubscript{M1} gene, in our population only GST\textsubscript{T1} had a correlation with SK (although GST\textsubscript{M1} data were borderline significant). The reasons are not known: however, looking at the differences between the two populations, the data concerning GST\textsubscript{M1} and T1 genotypes should be considered, in order to increase our understanding of the global genetic asset of the antiphoto-damage mechanisms.

**Conclusion**

Our study sheds some light on the correlation, never investigated previously in a Mediterranean population to our knowledge, between SK and the GST\textsubscript{T1} null genotype. Although our data need to be confirmed on a larger number of patients and in other Mediterranean countries, our data reveal significant differences between subjects of different ethnic origins and geographical locations. These differences, due to variable interaction between multiple genes and multiple environmental factors, should prompt further investigation in different geographical areas and in different populations. This will help to better elucidate the distribution of the various GST genotypes and the role of GSTs in the complex pathogenesis of premalignant skin lesions and, possibly, their evolution to cutaneous tumours.

**References**

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