Multiple hypothesis testing

Following the Kruskal-Wallis test, multiple hypothesis testing was performed using the Dunn's multiple comparisons test to identify the significant pairwise differences among groups.

When AATD patients were compared among themselves, a gradation was observed, so that high-risk (ZZ) patients showed a significantly higher GSSG/GSH ratio (p=0.01, p<0.0001; respectively), MDA (p=0.02, p=0.01), 8-OHdG (p=0.003, p<0.0001) and PC (p=0.04, p<0.001) and lower TG levels (p=0.02, p<0.0001) than intermediate (MZ; SZ) and low-risk patients (MS; SS). Regarding GSH levels, high-risk patients showed significantly higher levels than low-risk patients (p=0.004) and also higher levels than intermediate-risk patients, although these differences were not significant (p=0.56). Similarly, intermediate-risk patients showed a significantly higher GSSG/GSH ratio (p=0.01), MDA (p=0.02), 8-OHdG (p=0.02) and PC (p=0.04) and lower TG (p=0.02) and GSH (p=0.04) levels than low-risk patients.

Regarding antioxidant enzymatic activities, high-risk patients showed significantly lower CAT activity than intermediate- and low-risk patients (p=0.02, p=0.005; respectively), whereas no significant differences were observed between low and intermediate-risk patients (p=0.09). A significant increase in GPx activity was observed in high-risk compared to intermediate- (p=0.02) and low-risk patients (p=0.002) and no significant differences were observed between low- and intermediate-risk patients (p=0.35). No significant differences were observed in SOD and GRd activities.

High-risk patients showed significantly higher H_2O_2 levels than either intermediate- (p=0.002) or low-risk patients (p=0.0004). In addition, intermediate-risk patients showed significantly higher H_2O_2 levels than low-risk patients (p=0.03).

Compared to controls and low-risk patients, diminished catalase activity and higher H_2O_2 levels were observed in high- and intermediate-risk patients. GPx activity (an enzyme that catalyses the reduction of H_2O_2 to H_2O using GSH as the reducing factor) is increased in these patients, probably to compensate for the accumulation of H_2O_2. However the significant low levels of GSH would prevent the removal of the H_2O_2, which accumulates in these patients. Moreover, no differences in the activity of GRd (an enzyme that catalyses the reduction of GSSG to GSH) were observed, as would be expected to compensate for low levels of GSH observed in these patients to maintain the reducing environment of the cell. Since H_2O_2 is itself a powerful oxidant and central precursor to both ˙OH and HOCl (two extremely potent oxidants), its accumulation

would explain the significantly increased levels of oxidative stress biomarkers (MDA, 8-OHdG and PC) observed in high- and intermediate-risk patients (see Figure E5 online data supplement).

Overall, these results show a gradation in oxidative stress parameters suggesting that the expression of the Z allele produces higher oxidative stress status in homozygous (ZZ) than in heterozygous (MZ; SZ) patients.