ONLINE DATA SUPPLEMENT

Title: Decreased glutathione and low catalase activity contribute to oxidative stress in children with alpha-1 antitrypsin deficiency

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Table S1. Demographic and clinical characteristics in AATD and control children.

Figure E1. **Glutathione levels in AATD patients and control individuals.** High (ZZ) and intermediate-risk (MZ; SZ) patients showed significantly lower TG levels than control individuals. No significant differences were observed between low-risk and control individuals (A). GSH levels were significantly decreased in serum of AATD patients compared to control individuals (B) whereas no significant differences were observed in the GSSG levels (C). Cellular oxidative status as determined by the GSSG/GSH ratio showed a significant imbalance towards increased oxidative status in high and intermediate-risk patients (D). Abbreviations are found in the text. Asterisks indicate levels of statistical significance with respect to the control group (**p<0.001; ***p<0.0001).

Figure E2. **Oxidative stress biomarkers are increased in serum of AATD patients.** High (ZZ) and intermediate-risk (MZ; SZ) patients showed significantly higher levels of malonyldialdehyde (A), 8-hydroxydeoxyguanosine (B) and protein carbonylation (C) than control individuals (MM). No significant differences were observed between the low-risk (MS; SS) and the control group. Abbreviations are

found in the text. Asterisks indicate levels of statistical significance with respect to the control group (*p<0.01; **p<0.001; ***p<0.0001).

Figure E3. **Antioxidant enzyme capacity in AATD patients and control individuals.** High (ZZ) and intermediate-risk (MZ; SZ) patients showed significantly higher CAT (B) and GPx (C) activities than the control group (MM) whereas no differences in these enzymatic activities were observed between low-risk (MS; SS) and control groups. No significant differences were observed in SOD (A) and GRd activities (D) in any of the groups. Abbreviations are found in the text. Asterisks indicate levels of statistical significance with respect to the control group (**p<0.001; ***p<0.0001).

Figure E4. Hydrogen peroxide accumulates in leukocytes of AATD patients. High (ZZ) and intermediate-risk (MZ; SZ) patients showed significantly higher basal concentration of hydrogen peroxide determined by 2',7' dichlorofluorescein diacetate (DCFH) fluorescence than control individuals (MM). No significant differences were observed between the low-risk (MS; SS) and the control group. Asterisks indicate levels of statistical significance with respect to the control group (*p<0.01; **p<0.001).

Figure E5. Figure 1. Schematic overview of glutathione metabolism and enzymatic antioxidant defence mechanisms in patients with AATD as compared to control individuals. Left: Eukaryotic cells possess antioxidant enzymes that are responsible for neutralising reactive oxygen species, which may oxidize nucleic acids, lipids and proteins leading to cell malfunction if they

accumulate. Superoxide dismutase detoxifies superoxide anion (O_2^-), which is converted to hydrogen peroxide (H_2O_2). Reaction of O_2^- and H_2O_2 in the presence of ferrous iron (Fe^{++}) produces hydroxyl radicals (·OH). Superoxide anion is able to react with nitric oxygen (NO) to form the much more powerful oxidant peroxynitrite ($ONOO^-$). In the presence of neutrophil myeloperoxidase (MPO), H_2O_2 and chloride (Cl^-) form hypochorus acid (HOCl). Both ·OH and HOCl are potent oxidants. H_2O_2 accumulation is prevented by catalase (CAT) and glutathione peroxidase (GPx), the latter uses reduced glutathione (GSH) as the reducing factor. Oxidised glutathione (GSSG) is either exported from the cell or reduced to GSH by the action of glutathione reductase (GRd) using NADPH as the electron donor. **Right**: High- and intermediate-risk AATD patients show diminished CAT activity, which leads to H_2O_2 accumulation. GPx activity is increased in these patients to compensate for the accumulation of H_2O_2, however, low levels of GSH would prevent its removal. Since H_2O_2 is a potent oxidant and a precursor of ·OH and HOCl, its accumulation would explain the increased levels of oxidative stress biomarkers observed in these patients.