S2: Methods for *SOD2* Genotyping and Validation

Approximately 5 ml of whole blood at baseline was received from each site within 24 hours of collection and processed immediately for genomic DNA.  All samples were lysed with 5X Buffer EL Erythrocyte lysis buffer solution (Qiagen, Germantown, MD, USA). Samples were further processed according to Qiagen’s recommended protocol.  DNA purity and concentrations were determined by nanodrop spectrophotometer at 260 nm and 280 nm. Each DNA sample was stored in Qiagen’s EB (50 µl) buffer at -80oC until analysis.

DNA samples were processed by PCR-based allelic discrimination for germline genotyping single nucleotide polymorphism (SNP) using Taqman SNP genotyping assay from Applied Biosystems (Waltham, MA, USA).  The primer bioassay contained the VIC and FAM reporter dye with the sequence context, CTGCCTGGAGCCCAGATACCCCAAA[A/G], CCGGAGCCAGCTGCCTGCTGGTGCT.  Three ng/µl of extracted DNA from each study subject was used for the PCR reaction.  Five µl of PCR reaction mix was added to each DNA sample in a 384-well plate, and PCR was performed by 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). Reaction mix was made using 2.25 µl of ionized water and ABI master mix (with amperase, 2.5 µl, 20x primer = 0.25 µl).  The PCR was performed with an amperase-activating step of 50oC for 2 min, and 95oC for 10 min, followed by 40 cycles of 95oC for 15 sec and 60oC for 1 min. Allelic Discrimination Plate Documents were read using the SDS Allelic Discrimination Software, Version 2.3. Automatically assessed allelic fragment patterns were converted to genotypes. For quality control purposes, all blood samples were regenotyped using PCR-restriction fragment length polymorphism (PCR-RFLP) with no apparent discrepancies in genotyping found.

PCR-RFLP was used for genotyping *SOD2* 47T>C variant (rs4880) located on chromosome 6q25.3. Briefly, the *SOD2* PCR fragment containing the SNP was amplified using *SOD2* forward 5’-GCTGTGCTTTCTCGTCTTCAG-3’ and *SOD2* reverse 5’-TGGTACTTCTCCTCGGTGACG-3’. With the selected primers, DNA was amplified in a 30μl reaction volume containing 1x PCR buffer, 1.5 mM MgCl2, 0.2 μM primer (DNA Technologies Coralville, IA, USA), 200 μM each deoxynucleotide triphosphate (dNTPs, Fermentas, St. Leon-Rot, Germany), 30 ng genomic DNA, 0.5 U Taq DNA polymerase (ThermoFisher, Waltham, MA, USA). The PCR program was applied at 94°C for 3 min for initial denaturation; 35 cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 30 sec; and 72°C for 5 min for final extension. Genotyping was performed using restriction digestion under the following conditions: 10 ml PCR product, 2 ml buffer 2, 7.5 ml water, and 0.5 ml enzyme (5 U) in a total volume of 20 ml. The BsaWI digests were incubated at 37oC for 2 hr with expected fragment sizes of 167 and 40 bp for V/V homozygotes, 207, 167, and 40 bp for A/V heterozygotes, and 207 bp for A/A homozygotes. Digested PCR products were visualized on a 2% agarose gel stained with ethidium bromide under ultraviolet light. Laboratory personnel were unaware of treatment assignments of the samples.