Supplementary Methods

Blood collection and processing

Whole blood (15 mL) was collected from each subject into six PAXgene tubes via antecubital fossa venipuncture from each subject. PAXgene tubes were frozen at _801C after 2 h at room temperature. Total RNA was isolated according to the manufacturer's protocol (PAXgene blood RNA kit; Pre-AnalytiX) on an automated workstation Qiacube (Qiagen, Valencia, CA). The RNA is from polymorphonuclear cells (neutrophils, basophils, and eosinophils), mononuclear cells (lymphocytes and acrophages/monocytes), platelets, and red blood cell precursors.

Microarray hybridization

biotin-labeled cDNA was synthesized from 50 ng of total RNA using the Ovation™ Whole Blood Solution system according to the manufacturer's protocol(NuGEN Technologies Inc., San Carlos, CA,USA). Ovation® Whole Blood Solution system Consists of optimized protocols for global gene expression profiling of whole blood RNA without the need for globin reduction procedures. There is an amplification component based on Ribo-SPIA technology, and a fragmentation and labeling module to attach biotin to the amplified target. The Ribo-SPIA 3' Amplification Process is a three-step process that generates micrograms of cDNA from nanograms of total RNA. (1) First Strand Synthesis: Single stranded cDNA is prepared from total RNA using a chimeric DNA/RNA primers and reverse transcriptase. The primer has a DNA portion that hybridizes specifically to poly(A) sequences. The primer has a 3' DNA portion that hybridizes to the mRNA and a unique 5' RNA portion that does not hybridize to the

mRNA. The resulting cDNA/mRNA complex includes the unique RNA sequence at the 5' end of the cDNA; (2) Second Strand Synthesis: After partial fragmentation of mRNA in the cDNA/mRNA complex, DNA polymerase generates a second strand, including DNA complementary to the 5' RNA unique sequence incorporated into the first strand, resulting in a double-stranded cDNA with an RNA/DNA heteroduplex of unique sequence at one end; (3) SPIA Amplification: The isothermal linear amplification step uses an additional DNA/RNA chimeric primer, DNA polymerase and RNase H in an isothermal reaction. RNase H removes the unique RNA sequence in the double-stranded cDNA revealing a site for binding the DNA/RNA chimeric primer. DNA polymerase synthesizes cDNA starting at the 3' end of the primer and displacing the existing forward strand. RNA at the 5' end of the newly synthesized strand is again cleaved by RNase H, exposing the priming site for initiation of the next round of DNA synthesis. The entire process is repeated continuously with multiple DNA polymerase molecules participating in DNA synthesis along the same template molecule, leading to the rapid accumulation of micrograms of amplified single stranded (opposite sense) cDNA products. Subsequent biotin-labeled cDNA hybridization to Affymetrix Human U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA, USA) was performed according to standard Affymetrix Protocols (Affymetrix Expression Analysis Technical Manual;). Affymetrix Human U133 Plus 2.0 Arrays contain more than 54,000 probe sets, which analyze the expression level of over 47,000 transcripts and variants, including 38,500 well-characterized human genes.”

Supplementary Figure S1. Principal Components Analysis (PCA). The 50 genes that were differentially expressed in extensive WMH subjects versus minimal WMH controls ($p<0.005$ and fold change >1.5) were used for a PCA. The top three principal components were represented on the X, Y and Z axes. Each symbol represents one subject with red being subjects with extensive WMH (n=20) and green being controls with low WMH (n=18). The distance between samples in the 3-D space shows their differences based on the expression pattern. Each of the two ellipsoids represents a two-standard deviation space from the mean of each group of samples.