Appendix 1. Details of Genotyping Assays

Neither genotyping lab had methodology developed to analyze all of the genetic variants, so each lab analyzed a subset of the polymorphisms on all DNA specimens available. The Core Genotyping Facility of the National Cancer Institute (NCI) determined the DNA concentration in each of the cell lysates with use of picogreen (Invitrogen Corporation, Carlsbad, California) and a semiquantitative real-time TaqMan assay (Applied Biosystems, Foster City, California) as previously described (1). Each DNA specimen was also characterized by a microsatellite profile of 15 short tandem repeat markers (AmpFLSTR Identifiler PCR Amplification kit, Applied Biosystems) for identification purposes that would be used as a quality control measure to check against duplication and contamination.

For each 5 µl TaqMan (5' nuclease assay) reaction, 5 ng of DNA was used. Reactions were set up by using 2.5 µl of 2X Universal Master Mix [Applied Biosystems (ABI)] and assay-specific concentrations of primers and dye-labeled probes. Reaction plates were thermocycled using conditions specific to the assay, and the ABI 7900HT sequence-detection system was used to read endpoints.

For MGB Eclipse Probe Systems assays (3' hybridization triggered fluorescence reaction) [Nanogen (formerly Epoch Biosciences), Bothell, Washington] that were performed at NCI, 5 ng of DNA were used for each 5 µl assay. Reactions were set up using 2.5 µl of the 2X Jumpstart Master Mix (Sigma-Aldrich, St. Louis, Missouri), 0.16 µl of 2.5 U/µl JumpStart Taq polymerase (Sigma-Aldrich), and assay-specific concentrations of primers and dye-labeled probes. Reaction plates were thermocycled under assay-specific conditions; and endpoint dissociation (melting) curves were generated on the ABI 7900HT sequence-detection system, by monitoring fluorescence while the reactions were heated from 30°C to 80°C at a 10% ramp rate. Dissociation curves of the first derivative of fluorescence and raw fluorescent values were then exported from the Sequence Detection Systems software (Applied Biosystems) in text format for further analysis with use of DynaDASH melt curve analysis software (Dyna Metrix, Stockholm, Sweden) for genotype scoring.

Details such as primer and probe concentrations and thermocycling conditions for all TaqMan assays and for each MGB Eclipse assay performed at NCI can be found at http://snp500cancer.nci.nih.gov/assay_list.cfm.

For assays performed at the National Center for Environmental Health (NCEH), which are noted in table A1 below, cell lysates were purified by using the ChargeSwitch Direct 96 gDNA kit (Invitrogen). Ten µl of cell lysate were added to ChargeSwitch wells, and purified DNA was eluted in 100 µl of elution buffer, according to the manufacturer's instructions. For MGB Eclipse assays, 2 µl of DNA were used for each 10 µl reaction. Reactions were set up by using 5.0 µl of 2X Jumpstart Master Mix (Sigma-Aldrich), 0.32 µl of 2.5 U/µl JumpStart Taq polymerase (Sigma-Aldrich), and 0.5 µl of each assay-specific 20X MGB primer and probe mix (Nanogen). Reaction plates were thermocycled under standard conditions per the manufacturer's recommendation, and endpoint dissociation (melting) curves were generated as described above. The MGB Eclipse melt curve macro (Nanogen) was used for genotype scoring. For the *CBS* 68-bp insertion polymorphism, the TET-labeled probe was specific to the insertion region and detected both heterozygous and homozygous insertion genotypes. Variation in peak heights of melting curves relative to melting curves produced by the wild-type control region FAM-labeled probe distinguished between one or two copies of the *CBS* insertion allele. The genotypes for these individuals were confirmed by polymerase chain reaction (PCR) as previously described (2).

Determination of the *ADRB2* (rs1042714) and *F2* (rs1799963) genotypes was carried out using pyrosequencing technology (Biotage AB, Uppsala, Sweden). Two µl of DNA were used for each 20 µl reaction mix containing 0.5 µM of a 5' biotinylated forward primer and 0.5 µM reverse primer (see table A1) and Applied Biosystems GeneAmp Fast PCR Master Mix. Amplification was performed by using the 9800 Fast PCR System according to the manufacturer's suggested protocol (Applied Biosystems). Single-stranded biotinylated templates for pyrosequencing analysis were prepared by using 5 µl of the PCR products in a Vacuum Prep Workstation (Biotage AB). The sequencing reactions were performed with 5 µM of sequencing primer and the PSQ 96A SNP Reagent Kit on a PSQ 96HS System, as described by the manufacturer (Biotage AB). Nucleotide dispensation orders and genotype calls were chosen automatically by the system software.

The *ACE* insertion-deletion polymorphism (rs4646994) was determined by capillary fragment analysis (3) with use of the ABI Prism 3730 Genetic Analyzer. Two µl of DNA were used for each 20 µl reaction mix containing 0.375 µM of a 5' FAM-labeled forward primer, 0.375 µM reverse primer (table A1) and ABI GeneAmp Fast PCR Master Mix. Amplification was performed with use of the ABI 9800 Fast PCR System according to the manufacturer's suggested protocol. PCR product (1 µl) was resuspended in 9 µl of Hi-Di Formamide (Applied Biosystems) along with 1 µl of ROX-labeled GeneFlow 625 DNA ladder (Chimerx, Madison, Wisconsin), denatured for 1 minute at 96°C, and chilled to 4°C. The denatured sample was injected into a 36 cm 3730 capillary array along with Performance Optimized Polymer 7 using the GeneMapper36-POP7-1-Run Module. Data collection and fragment sizing was achieved by using DataCollection 2.0 and GeneMapper 4.0 3730 system software programs (Applied Biosystems). A 190-bp fragment is generated in the absence of the insertion (deletion genotype), and a 490-bp fragment is generated in the presence of the insertion (insertion genotype).

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Appendix 2. Deviations From Hardy-Weinberg Proportions, Third National Health and Nutrition Examination Survey (NHANES III), Phase 2 (1991–1994)

Gene symbol	Variant (dbSNP ID)*	*P* value		
		Non-Hispanic white	Non-Hispanic black	Mexican-American
ABCB1	rs1045642	0.02	0.07	0.93
ACE	rs4646994	0.11	0.10	0.27
ADH1B	rs1229984	0.89	0.59	0.14
	rs17033	0.82	0.02	0.10
	rs2066702	0.86	0.52	0.67
ADH1C	rs1693482	0.91	0.37	0.35
	rs698	0.93	0.26	<0.01
ADRB1	rs1801252	0.11	0.78	0.83
ADRB2	rs1042713	0.96	0.43	0.14
	rs1042714	0.74	0.16	0.66
ADRB3	rs4994	0.27	0.78	0.82
ALAD	rs1800435	0.63	0.48	0.69
CAPN10	rs3792267	0.65	0.64	0.42
B9D2	rs1800468	0.90	0.67	0.99
	rs1800469	0.93	0.54	0.18
CAT	rs769214	0.68	0.82	0.32
CBS	844ins68bp	0.44	0.99	0.28
CCL5	rs2280788	0.04	0.79	0.63
CCR2	rs1799864	0.34	0.06	0.74
CXCL12	rs169097	<0.01	0.46	0.61
CYP1A1	rs2472299	0.01	0.61	0.65
	rs2606345	0.71	0.21	0.72
CYP1A2	rs11854147	<0.01	0.11	0.11
	rs2069514	0.09	0.29	0.29
	rs4886406	0.13	0.18	0.87
CYP1B1	rs1056836	0.42	0.14	0.02
	rs1056837	0.21	0.52	0.01
	rs162557	0.81	0.64	0.99
CYP2A6	rs1801272	0.79	0.79	0.93
CYP2C19	rs4986893	0.94	0.95	0.96
	rs4986894	0.75	0.30	0.55
CYP2C9	rs1057910	0.28	0.08	0.58
CYP2E1	rs2031920	0.16	0.73	0.61
CYP3A4	rs2740574	0.22	0.03	0.84
F2	rs1799963	0.51	0.88	0.63
F5	rs6025	0.49	0.80	0.03
FAM82A	rs163086	0.73	0.18	0.18
FCGR2A	rs1801274	0.06	0.32	0.50
FGB	rs1800790	0.81	0.01	0.82
IL10	rs1800871	0.06	0.66	0.02
	rs1800872	0.06	0.98	0.03
	rs1800896	0.89	0.35	0.36
IL1B	rs1143623	0.76	0.58	0.44
IL4	rs2243248	0.68	0.46	0.03
	rs2243250	0.53	0.03	0.07
	rs2243270	0.71	0.01	0.08

Gene symbol	Variant (dbSNP ID)*	*P* value		
		Non-Hispanic white	Non-Hispanic black	Mexican-American
IL4R	rs1801275	0.73	<0.01	0.58
	rs1805015	0.97	0.36	0.51
ITGA2	rs1126643	0.64	0.14	0.99
ITGB3	rs5918	0.81	0.59	0.22
MBL2	rs11003125	0.76	0.71	0.44
	rs1800450	0.01	0.02	0.66
	rs1800451	0.16	0.12	0.23
	rs5030737	0.27	0.63	<0.01
	rs7096206	0.41	0.97	<0.01
MTHFR	rs1801131	0.07	0.42	0.77
	rs1801133	0.10	0.49	0.93
	rs2066470	0.32	0.25	0.84
MTRR	rs1801394	0.74	0.82	0.04
NAT2	rs1041983	0.77	0.03	0.66
	rs1208	0.67	0.06	0.68
	rs1799930	0.83	0.04	0.70
	rs1801279	0.97	0.05	<0.01
	rs1801280	0.85	0.65	0.6
NOS2A	rs1800482	0.96	0.81	<0.01
	rs9282799	0.96	0.65	0.89
NOS3	rs1799983	0.35	0.10	0.11
	rs2070744	0.31	0.07	0.01
NQO1	rs10517	0.65	0.65	0.20
	rs1800566	0.51	0.93	0.93
	rs34755915	0.81	0.88	0.82
	rs689452	0.23	0.63	0.25
	rs689453	0.23	0.25	0.21
OGG1	rs1052133	0.11	0.14	0.12
PON1	rs662	0.37	0.98	0.90
	rs854560	0.40	0.30	0.55
PPARG	rs1801282	0.93	0.95	0.79
SERPINE1	rs1799762†	0.92	0.17	0.46
TGFB1	rs1982073	0.05	0.34	0.11
TLR4	rs4986790	0.50	0.73	0.39
TNF	rs1800629	0.78	0.87	0.88
	rs1800750	<0.01	0.41	0.22
	rs361525	0.06	0.53	0.20
VDR	rs2239185	<0.01	0.98	0.85
	rs731236	0.12	0.26	0.22
XRCC1	rs1001581	0.69	0.20	0.92
	rs1799782	0.97	0.13	0.12
	rs25486	0.70	0.26	0.88
	rs25487	0.67	0.16	0.76
	rs25489	0.99	0.25	0.44

* Unique identifier in the Entrez SNP (single-nucleotide polymorphism) database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/projects/SNP/).

† Variant is also known as rs1799768 and rs179988.

Appendix 3. Genes Genotyped in the Third National Health and Nutrition Examination Survey (NHANES III) DNA Bank Samples, Phase 2 (1991–1994), Listed by Pathway

Pathway*	Gene	Associated disease/outcome†
Apoptosis, Cell cycle, Cellular growth and differentiation	*CAPN10* *IL10* *IL1B* *IL4* *IL4R* *ITGB3* *PPARG* *TGFB1* *TNF* *VDR*	Cancer Infectious disease Neurodegenerative disease Obesity Osteoporosis Type 2 diabetes
Blood pressure regulation, Cardiac function	*ACE* *ADRB1* *ADRB2* *NOS2A* *NOS3*	Cardiovascular disease Renal disease Type 2 diabetes
Cellular adhesion, Cell migration/motility	*CCL5* *CCR2* *CXCL12* *F2* *FGB* *ITGA2* *ITGB3* *SERPINE1*	Cancer Cardiovascular disease Infectious disease
DNA repair	*OGG1* *XRCC1*	Cancer
Hemostasis	*F2* *F5* *FGB* *ITGA2* *ITGB3* *NOS3* *SERPINE1*	Cardiovascular disease
Immunity and inflammation	*CCL5* *CCR2* *CXCL12* *FCGR2A* *IL10* *IL1B* *IL4* *IL4R* *MBL2* *NOS2A* *PPARG* *TGFB1* *TLR4* *TNF* *VDR*	Asthma Cancer Cardiovascular disease Infectious disease Renal disease Rheumatoid arthritis Type 2 diabetes

Pathway*	Gene	Associated disease/outcome†
Metabolism of free radicals/Oxidative stress	*CAT* *NOS2A* *NOS3* *PON1*	Cancer Cardiovascular disease Infectious disease Neurodegenerative disease Renal disease Type 2 diabetes
Nutrient metabolism	*ACE* *ADH1B* *ADH1C* *ADRB1* *ADRB2* *ADRB3* *ALAD* *CAPN10* *CAT* *CBS* *CYP1A1* *CYP1A2* *CYP1B1* *CYP2A6* *CYP2C19* *CYP2C9* *CYP2E1* *CYP3A4* *MTHFR* *MTRR* *NOS2A* *NOS3* *NQO1* *PPARG* *SERPINE1* *TNF* *VDR*	Asthma Birth defects Cancer Cardiovascular disease Neurodegenerative disease Obesity Osteoporosis Renal disease Type 2 diabetes
Xenobiotic metabolism	*ABCB1* *ADH1B* *ADH1C* *ALAD* *CYP1A1* *CYP1A2* *CYP1B1* *CYP2A6* *CYP2C19* *CYP2C9* *CYP2E1* *CYP3A4* *NAT2* *NQO1* *PON1*	Asthma Cancer Cardiovascular disease Drug and alcohol dependency Drug/pharmacological adverse reactions Neurodegenerative disease Rheumatoid arthritis

* Not mutually exclusive. Inclusion of genes in pathways based on information gathered from the GeneCards database (http://www.genecards.org), the KEGG GENES database (http://www.genome.jp/kegg/genes.html), and selected publications for *ACE* (1, 2), *CAPN10* (3), and *SERPINE1* (4-6).

† Includes diseases and clinical outcomes of major public health importance with a proposed or established association with at least one of the genes listed, but the genetic associations are not limited to these diseases.

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