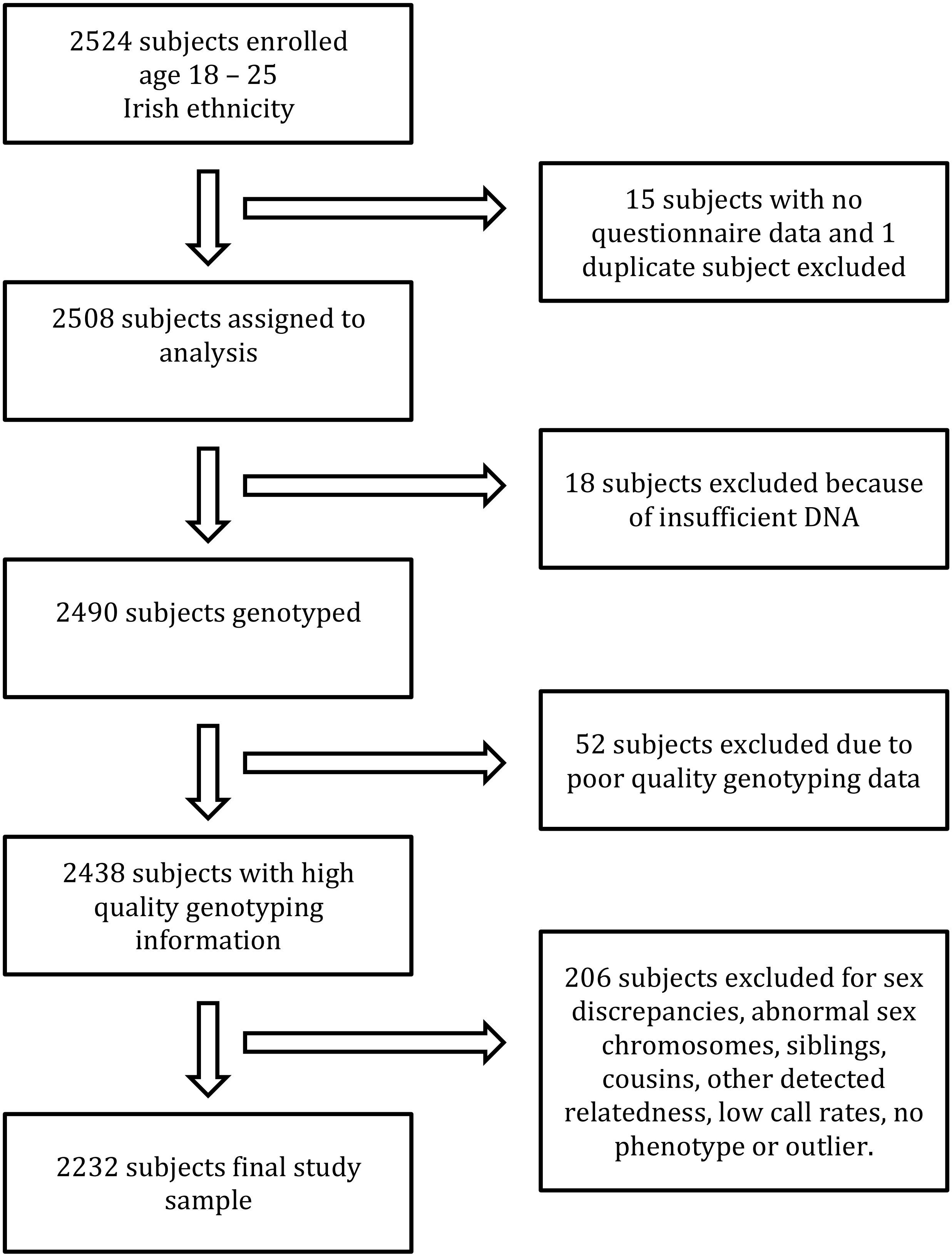
Supplemental Figure 1. Flow Chart for Study Population



Supplemental Table 1. 76 SNPs used in Candidate Gene Analyses

Gene	SNP	chromosome	position
MTHFR	rs4846048	1	11768839
MTHFR	rs2184226	1	11770023
MTHFR	rs3737966	1	11770346
MTHFR	rs1537516	1	11770448
MTHFR	rs1476413	1	11774887
MTHFR	rs1801131	1	11777063
MTHFR	rs1801133	1	11778965
MTHFR	rs1572151	1	11780298
MTHFR	rs4846052	1	11780538
MTHFR	rs2066471	1	11783045
MTHFR	rs13306567	1	11783052
MTHFR	rs17037397	1	11784750
MTHFR	rs9651118	1	11784801
MTHFR	rs3753584	1	11787173
MTHFR	rs3737964	1	11789631
MTHFR	rs4846054	1	11791817
DHFR	rs1650723	5	79957786
DHFR	rs11951910	5	79975227
DHFR	rs863215	5	79983761
DHFR	rs1650697	5	79986537
DHFR	rs6151599	5	79986788
DHFR	rs380691	5	79987790

MFTC/SLC25A32	rs3098259	8	104479596
MFTC/SLC25A32	rs1061196	8	104480104
MFTC/SLC25A32	rs3133810	8	104484294
MFTC/SLC25A32	rs17803441	8	104486221
MFTC/SLC25A32	rs3134295	8	104496535
MFTC/SLC25A32	rs750606	8	104497706
FPGS	rs1544105	9	129602546
FPGS	rs7856096	9	129606360
FPGS	rs10760503	9	129618225
FOLH1	rs6485964	11	49122813
FOLH1	rs16906158	11	49127350
FOLH1	rs34033751	11	49132803
FOLH1	rs7113251	11	49143860
FOLH1	rs3974729	11	49153523
FOLH1	rs588458	11	49170624
FOLH1	rs202700	11	49173275
FOLR1	rs2071010	11	71578612
MTHFS	rs685487	15	77923184
MTHFS	rs8923	15	77924615
MTHFS	rs2733103	15	77925626
MTHFS	rs17284990	15	77931252
MTHFS	rs16971450	15	77941626
MTHFS	rs6495446	15	77942037

MTHFS	rs7177659	15	77945097
MTHFS	rs6495449	15	77945214
MTHFS	rs17285431	15	77949320
MTHFS	rs6495451	15	77951108
MTHFS	rs2562744	15	77961443
MTHFS	rs2733106	15	77964742
MTHFS	rs12438477	15	77965338
MTHFS	rs2586182	15	77970821
MTHFS	rs2733088	15	77971712
MTHFS	rs12440798	15	77973395
MTHFS	rs2115540	15	77977363
MTHFS	rs2163005	15	77981535
PCFT/SLC46A1	rs4795434	17	23741044
PCFT/SLC46A1	rs11656835	17	23741176
PCFT/SLC46A1	rs2239910	17	23747793
PCFT/SLC46A1	rs739439	17	23747949
PCFT/SLC46A1	rs2239907	17	23749871
PCFT/SLC46A1	rs17719944	17	23753580
RFC/SLC19A1	rs2838952	21	45754591
RFC/SLC19A1	rs12483377	21	45755537
RFC/SLC19A1	rs2236484	21	45756112
RFC/SLC19A1	rs7499	21	45756756
RFC/SLC19A1	rs17004785	21	45757046
RFC/SLC19A1	rs7867	21	45757080
RFC/SLC19A1	rs2838956	21	45769452

RFC/SLC19A1	rs2838958	21	45772995
RFC/SLC19A1	rs4818789	21	45773255
RFC/SLC19A1	rs12659	21	45775984
RFC/SLC19A1	rs1051269	21	45776434
RFC/SLC19A1	rs3788205	21	45788806
RFC/SLC19A1	rs1023159	21	45790608

Supplemental Table 2. SNPs that reached genome-wide significance in association with log-transformed red cell folate in 2232 Irish participants ($p < 5 \times 10^{-08}$). Data adjusted for age and gender.

gene	SNP	CHR	BP	N	BETA	SE	R^2	$^1H^2a$	P value	^{2}P value 677CC
MTHFR	RS1801133	1	11778965	2229	-0.0463	0.005379	0.03221	0.0680	1.37×10^{-17}	-
MTHFR	RS13306561	1	11788391	2228	0.04649	0.006782	0.02067	0.0202	9.22×10^{-12}	8.93×10^{-4}
MTHFR	RS3753584	1	11787173	2230	0.04631	0.006781	0.02051	0.0200	1.09×10^{-11}	1.01×10^{-3}
MTHFR	RS17367504	1	11785365	2229	0.04631	0.006784	0.02049	0.0200	1.12×10^{-11}	7.36×10^{-4}
MTHFR	RS17037390	1	11783430	2227	0.04542	0.006754	0.01992	0.0193	2.22×10^{-11}	8.74×10^{-4}
CLCN6	RS2050265	1	11802286	2230	0.04538	0.006789	0.01966	0.0191	2.93×10^{-11}	1.86×10^{-3}
CLCN6	RS198358	1	11826663	2229	0.0392	0.005873	0.01961	0.0196	3.12×10^{-11}	.014
CLCN6	RS7537765	1	11809890	2230	0.04522	0.006788	0.01953	0.0190	3.39×10^{-11}	2.06×10^{-3}
CLCN6	RS17037452	1	11818262	2228	0.0452	0.006792	0.0195	0.0190	3.57×10^{-11}	2.02×10^{-3}
CLCN6	RS12567136	1	11806318	2228	0.04501	0.006799	0.01931	0.0189	4.48×10^{-11}	2.48×10^{-3}
CLCN6	RS503040	1	11813282	2229	0.03372	0.005154	0.01886	0.0184	7.49×10^{-11}	> 0.05
CLCN6	RS198401	1	11810971	2227	0.03364	0.005149	0.01882	0.0183	7.94×10^{-11}	> 0.05
CLCN6	RS535107	1	11812055	2230	0.03369	0.005157	0.0188	0.0183	7.95×10^{-11}	> 0.05
CLCN6	RS198406	1	11820179	2230	0.03337	0.005133	0.01862	0.0179	9.77×10^{-11}	> 0.05
CLCN6	RS198408	1	11820717	2224	0.03319	0.005138	0.01843	0.0177	1.28×10^{-10}	> 0.05
CLCN6	RS198391	1	11799004	2228	0.03312	0.005165	0.01814	0.0177	1.74×10^{-10}	> 0.05
CLCN6	RS198393	1	11802272	2230	0.03293	0.005136	0.01811	0.0175	1.76×10^{-10}	> 0.05
CLCN6	RS12406667	1	11796438	2230	0.03297	0.005157	0.01802	0.0176	1.97×10^{-10}	> 0.05
CLCN6	RS198392	1	11801749	2229	0.03291	0.005148	0.01802	0.0174	1.99×10^{-10}	> 0.05

MTHFR	RS6541003	1	11778454	2230	0.03278	0.005164	0.01777	0.0172	2.63×10^{-10}	> 0.05
MTHFR	RS3737966	1	11770346	2229	0.03296	0.005221	0.01759	0.0175	3.28×10^{-10}	> 0.05
NPPB	RS198389	1	11841858	2229	0.0327	0.005204	0.01742	0.0173	3.96×10^{-10}	> 0.05
CLCN6	RS17037425	1	11792970	2230	0.044	0.007033	0.01726	0.0168	4.73×10^{-10}	2.77×10^{-3}
MTHFR	RS4846052	1	11780538	2229	0.03212	0.00515	0.01717	0.0166	5.31×10^{-10}	> 0.05
CLCN6	RS4846054	1	11791817	2229	0.03166	0.005171	0.01656	0.0160	1.08×10^{-9}	> 0.05
NPPA	RS632793	1	11833264	2229	0.03198	0.005249	0.01639	0.0162	1.31×10^{-9}	> 0.05
NPPB	RS198388	1	11839927	2229	0.03166	0.005224	0.01622	0.0162	1.60×10^{-9}	> 0.05
NPPB	RS198375	1	11836344	2230	0.03161	0.005269	0.01589	0.0157	2.32×10^{-9}	> 0.05
NPPB	RS1318408	1	11848368	2229	0.04467	0.007682	0.01495	0.0146	6.95×10^{-9}	8.14×10^{-4}
NPPB	RS12562952	1	11849643	2230	0.0464	0.008024	0.01479	0.0147	8.39×10^{-9}	2.35×10^{-3}
KIAA2013	RS2639453	1	11905131	2229	-0.03421	0.006251	0.01327	0.0276	4.93×10^{-8}	> 0.05
MTHFR	RS4846049	1	11772952	2229	0.02969	0.005436	0.01322	0.0125	5.22×10^{-8}	> 0.05

SNPs were tested using a simple linear regression model executed in PLINK v.1.7 under the assumption of an additive genetic model.

[1] H^2a is locus specific heritability (variance of additive model / variance of phenotype)

[2] P-value for SNP association in MTHFR 677CC homozygous subjects (n = 989).

Supplemental Table 3. SNPs that reached genome-wide significance in association with log-transformed serum folate in 2232 Irish participants ($p < 5 \text{ X } 10^{-08}$). Data adjusted for age and gender.

gene	SNP	CHR	BP	N	BETA	SE	R^2	P value	[1]P value 677CC
MTHFR	RS1801133	1	11778965	2230	-0.04932	0.007373	0.01969	2.82×10^{-11}	-
LOC390997	RS7545014	1	11857240	2229	-0.04013	0.006977	0.01464	1.01×10^{-8}	1.35×10^{-4}
LOC390997	RS7554327	1	11859991	2230	-0.03998	0.006987	0.01448	1.20×10^{-8}	1.07×10^{-4}
RNU5E	RS1999594	1	11881803	2230	0.03981	0.006996	0.01433	1.43×10^{-8}	7.74×10^{-3}
RNU5E	RS12085006	1	11881310	2229	0.03964	0.007004	0.01418	1.71×10^{-8}	7.52×10^{-3}

SNPs were tested using a simple linear regression model executed in PLINK v.1.7 under the assumption of an additive genetic model.

[1] P-value for SNP association in MTHFR 677CC homozygous subjects (n = 989).

Supplemental Table 4. SNPs that reached genome-wide significance in association with log-transformed plasma total homocysteine in 2232 Irish participants ($p < 5 \text{ X } 10^{-08}$). Data adjusted for age and gender.

gene	SNP	CHR	BP	N	BETA	SE	R^2	[1]H2a	P value	[2]P value 677CC
MTHFR	RS1801133	1	11778965	2229	0.03087	0.003374	0.03623	0.0353	1.26×10^{-19}	-
RNU5E	RS1999594	1	11881803	2229	-0.02195	0.003217	0.02048	0.0173	1.14×10^{-11}	> 0.05
RNU5E	RS12085006	1	11881310	2228	-0.02186	0.003221	0.02028	0.0172	1.45×10^{-11}	> 0.05
RNU5E	RS12724129	1	11880226	2229	-0.0197	0.003292	0.01583	0.0114	2.51×10^{-9}	> 0.05
MFN2	RS4240897	1	11965342	2229	-0.01945	0.003287	0.01548	0.0124	3.76×10^{-9}	> 0.05
TWISTNB	RS17354370	7	19566392	2230	-0.02096	0.003546	0.01544	0.0134	3.93×10^{-9}	.0047
LOC390997	RS2050267	1	11869160	2227	-0.01981	0.003356	0.01541	0.0077	4.14×10^{-9}	> 0.05
LOC390997	RS4846069	1	11871618	2220	-0.01973	0.00336	0.01531	0.0082	4.95×10^{-9}	> 0.05
LOC390997	RS2050270	1	11869043	2230	-0.01968	0.003354	0.01522	0.0076	5.08×10^{-9}	> 0.05
TWISTNB	RS2024353	7	19545401	2230	-0.02142	0.003651	0.01522	0.0102	5.09×10^{-9}	.011
LOC390997	RS2050268	1	11869131	2230	-0.01968	0.003355	0.01521	0.0076	5.14×10^{-9}	> 0.05
MFN2	RS730123	1	11992879	2230	0.01915	0.003298	0.0149	0.0148	7.32×10^{-9}	> 0.05
LOC390997	RS2336377	1	11872557	2222	0.0187	0.003251	0.01469	0.0142	1.00×10^{-8}	> 0.05
LOC390997	RS1321072	1	11873905	2229	-0.0193	0.003357	0.01463	0.0083	1.02×10^{-8}	> 0.05
TWISTNB	RS4721780	7	19515433	2229	-0.02012	0.00351	0.01453	0.0101	1.13×10^{-8}	.029
LOC390997	RS12749498	1	11875214	2230	-0.01913	0.003354	0.01438	0.0080	1.34×10^{-8}	> 0.05
LOC390997	RS1321073	1	11874213	2230	-0.01913	0.003354	0.01438	0.0080	1.34×10^{-8}	> 0.05
LOC390997	RS17037520	1	11874993	2230	-0.01913	0.003354	0.01438	0.0080	1.34×10^{-8}	> 0.05
LOC390997	RS2065611	1	11876113	2230	-0.01913	0.003354	0.01438	0.0080	1.34×10^{-8}	> 0.05

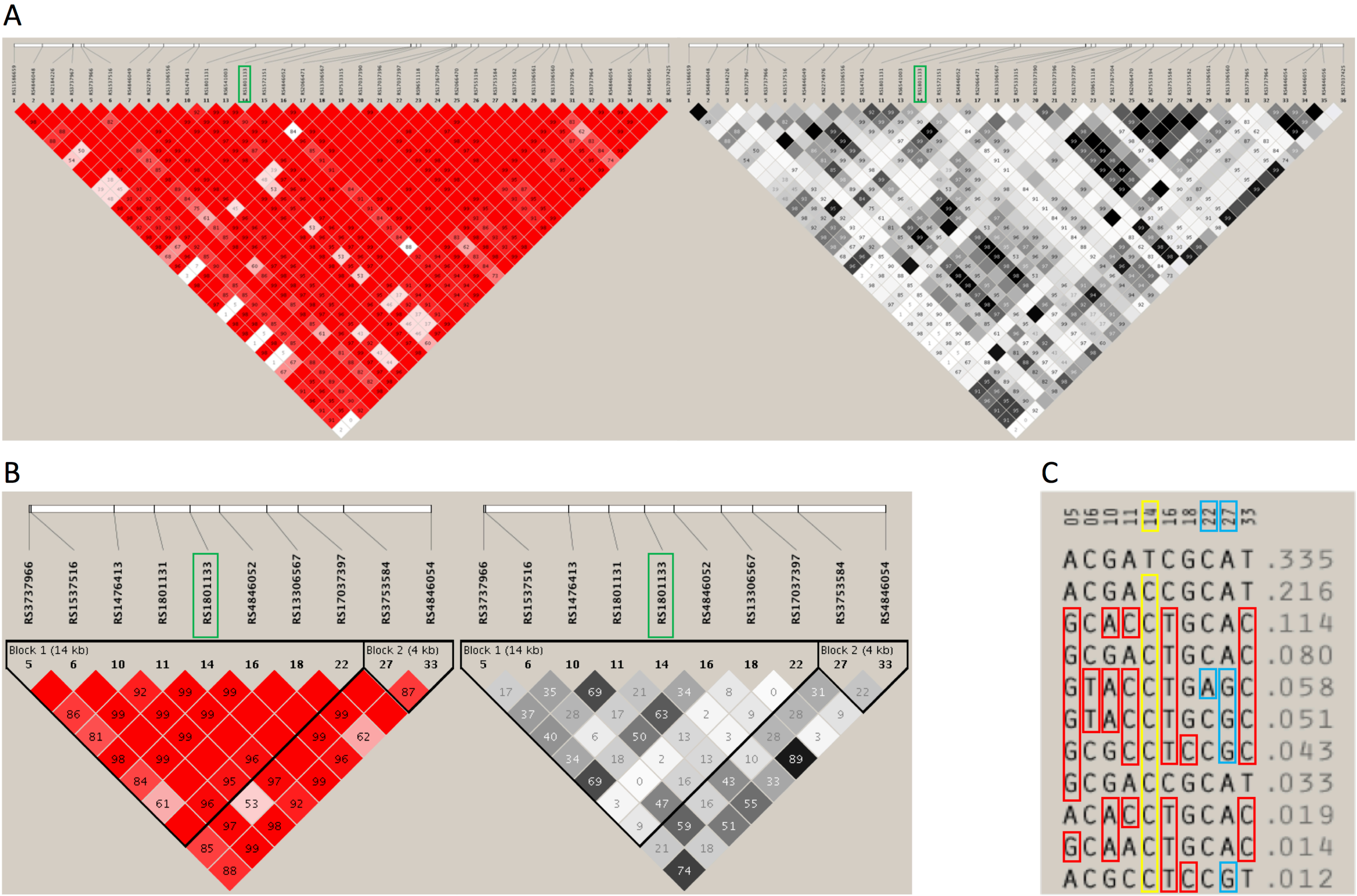
LOC390997	RS4845886	1	11871247	2230	-0.01913	0.003354	0.01438	0.0080	1.34×10^{-8}	> 0.05
LOC390997	RS12036266	1	11875901	2229	-0.0191	0.003355	0.01434	0.0080	1.41×10^{-8}	> 0.05
RNU5E	RS12756891	1	11879071	2226	-0.01911	0.003358	0.01435	0.0080	1.44×10^{-8}	> 0.05
RNU5E	RS4846076	1	11879352	2219	-0.01916	0.003368	0.01439	0.0080	1.45×10^{-8}	> 0.05
MFN2	RS3753579	1	11961178	2229	-0.01895	0.003386	0.01387	0.0073	2.45×10^{-8}	> 0.05
TWISTNB	RS10245919	7	19516175	2224	-0.01919	0.003448	0.01375	0.0102	2.93×10^{-8}	.030
LOC390997	RS4314892	1	11872424	2186	-0.01866	0.00338	0.01376	0.0075	3.81×10^{-8}	> 0.05
CHMP1A	RS164746	16	88236520	2228	0.01776	0.003219	0.01349	0.0131	3.85×10^{-8}	5.76×10^{-5}
CHMP1A/DPEP1	RS154657	16	88235597	2230	0.01774	0.003217	0.01346	0.0131	3.91×10^{-8}	5.49×10^{-5}
RNU5E	RS10779767	1	11886826	2202	-0.01948	0.003546	0.01354	0.0059	4.38×10^{-8}	> 0.05

SNPs were tested using a simple linear regression model executed in PLINK v.1.7 under the assumption of an additive genetic model.

[1] H^2a is locus specific heritability (variance of additive model / variance of phenotype)

[2] P-value for SNP association in MTHFR 677CC homozygous subjects (n = 989).

Online Supporting Material



Supplemental Figure 2. Linkage disequilibrium (LD) in the *MTHFR* gene region. **A**) D' (left) and r^2 (right) plots of pairwise LD measures are shown for 35 SNPs in *MTHFR* and its 5kb flanks. SNPs are numbered sequentially from left to right, with the SNP IDs shown above. *MTHFR* 677C>T (rs1801133) is boxed in green in each plot. A subset of 16 tagSNPs were selected for association analyses. **B**) D' (left) and r^2 (right) plots of pairwise LD measures are shown for 10 SNPs in *MTHFR* that are associated with red cell folate (RCF) after correction for multiple tests (Table 2). SNPs numbering is preserved from (A), and the SNP IDs are shown above. The *MTHFR* 677C>T rs1801133 label is boxed in green in each plot. Adjacent to it is MTHFR 1298 A>C (rs1801131, SNP 11). **C**) Haplotypes and their frequencies (right column) are shown for the 10 SNPs in (B) (numbering and SNP IDs are preserved). Alleles that are associated with higher levels of RCF are boxed in color: yellow, *MTHFR* 677C>T (rs1801133); red, SNP alleles that are not associated with changes in RCF in *MTHFR* 677CC homozygotes; blue, SNP alleles that are associated with increased RCF in *MTHFR* 677CC homozygotes.