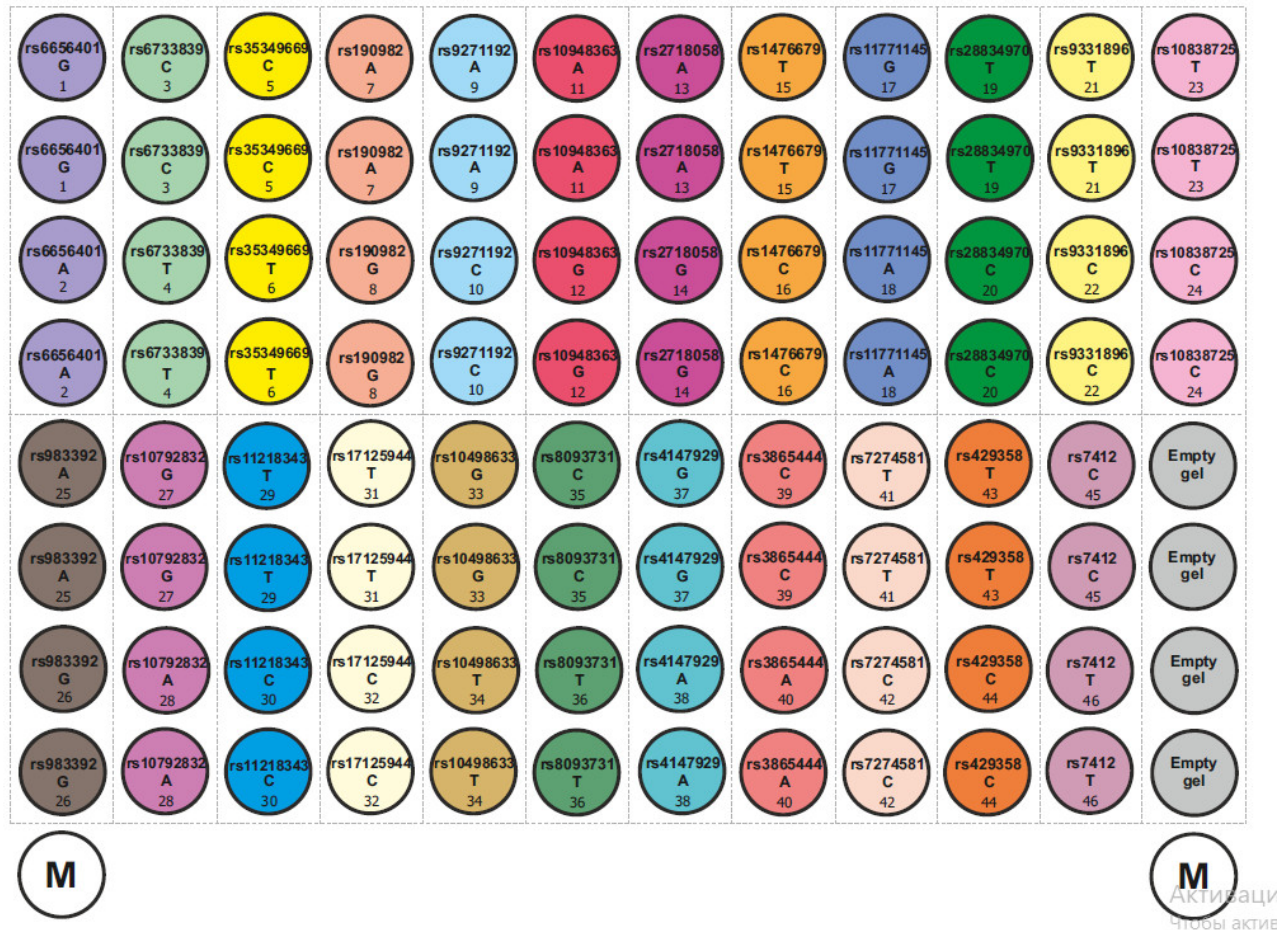


Supplementary file 1. Identification of genetic markers associated with the risk of Alzheimer's disease using DNA microarray

Microarray layout:



List of oligonucleotides for immobilization on the microarray

	Gene	SNP	Reference Allele	Effect Allele	Sequence, 5'-3'
1	CR1	rs6656401	G	A	ATCTTCTCGTCGCCTTC
2					ATCTTCTCATCGCCTTCT
3	BIN1	rs6733839	C	T	TTCTTAAAAACACCCTTTTCC
4					TTCTTAAAAATACCCTTTTCC
5	INPP5D	rs35349669	C	T	TGATAGCTTCCCACTGAG
6					TGATAGCTTTCCCACTGAGT
7	MEF2C	rs190982	A	G	CTCAAAGATAAACCAGGACA
8					CTCAAAGATGAACCAGGAC
9	HLA-DRB5/1	rs9271192	A	C	CCCCTCTCATAAAAAGTCATA
10					CCCTCTCATACAAAGTCATA
11	CD2AP	rs10948363	A	G	GGGCAAAACAAATTTTGTTA
12					GGGCAAAACGAATTTTGTT
13	NME8	rs2718058	A	G	TTTTTCCTGTTAATTTCTATCT

14					TTTTTCCTGTTGATTTCTATC
15	ZCWPW1	rs1476679	T	C	GATCCTGTTATGGACTCCT
16					ATCCTGTTACGGACTCCT
17	EPHA1	rs11771145	G	A	TAAACTTTATGAAACGGAATC
18					TAAACTTTATAAAACGGAATCA
19	PTK2B	rs28834970	T	C	GTGGAATTGTACAACACTG
20					TGGAATTGCACAACACTG
21	CLU	rs9331896	T	C	ACAGCTTTGTGGAGGA
22					ACAGCTTCGTGGAGGA
23	CELF1	rs10838725	T	C	GCCAACATTGCACCAC
24					AGCCAACACTGCACCA
25	MS4A6A	rs983392	A	G	CTAAAATTCCACATGCAATTTT
26					CTAAAATTCCACGTGCAATTTT
27	PICALM	rs10792832	G	A	AAAAATGTAGGAGCAAAACA
28					GAAAAATGTAGAAGCAAAACA
29	SORL1	rs11218343	T	C	AGCCGGTAATAGAGTATTTT
30					AGCCGGTAACAGAGTATTT
31	FERMT2	rs17125944	T	C	GTTTCAGATCTCCTTGATAG
32					TTTCAGATCCCCCTTGATAG
33	RIN-SLC24A	rs10498633	G	T	CTCTCAGTCAGTAAACAGTT
34					CTCTCAGTCATTAAACAGTTA
35	DSG2	rs8093731	C	T	TCTAATGCCACTCAAGCT
36					TTCTAATGCTACTCAAGCT
37	ABCA7	rs4147929	G	A	GCCCATTGTGCCCCT
38					TGCCCATTATGCCCCT
39	CD33	rs3865444	C	A	TAAACACCCCATGGATCT
40					TAAACACCCAATGGATCTA
41	CASS4	rs7274581	T	C	TCTGGCCTGGCGCG
42					TCTGGCCCGGCGC
43	APOE	rs429358	T	C	AGGACGTGTGCGGC
44					GGACGTGCGCGGC
45		rs7412	C	T	TGCAGAAGCGCCTGG
46					GCAGAAGTGCCTGGC

Multiplex PCR

Amplification was carried out in two rounds in the same reaction volume. At the first round, PCR products were generated using locus-specific primers (See Table below) and human genomic DNA as a template. The reaction mixture (25 µl in total) contained 5×PCR Buffer (Asfogen, Ltd, Moscow, Russia), dNTPs (Evrogen, LLC, Moscow, Russia), 5 units of HotTaq Multi DNA polymerase (Asfogen, Ltd, Moscow, Russia), 1 µl of human genomic DNA with typical concentrations of 20-60 ng/µl, and mixture of corresponding forward and reverse primers (see Table below). For the second round of amplification, PCR fragments obtained at

the 1st round were used as a template. A universal Cy5-fluorescently labeled Uni-Cy5 primer was used (see Table below) to yield predominantly single-stranded fluorescently labeled PCR products. PCR was carried out in an S1000 thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: denaturation for 3 min at 95°C, 50 cycles at 95°C - 30 s, 65°C - 30 s, 67°C - 30 s, 69°C - 30 s, 72°C - 20 s, then 50 cycles at 95° C - 20 s, 56° C - 10 s, 72° C - 30 s. The PCR product mixture after the 2nd round of amplification containing predominantly single-stranded fluorescently labeled fragments was used for hybridization on microarray.

List of primers used in multiplex PCR

Gene	SNP	Direction	Sequence, 5'-3'	Concentration in the reaction mixture (mM)
CR1	rs6656401	F	ATCATTTCTTCTCTGTCTCCATCTTCTC	0,04
		R	tcattggatctcattaGAAGAGCAAAGGACACACACAGA G	0,04
BIN1	rs6733839	F	AAAAGGAAAGAAATCTCTGTTCTGCTTC	0,04
		R	tcattggatctcattaCTAGTCTTCTCTGGTTCCTTCTTCTG	0,04
INPP5D	rs35349669	F	GGTGCAGGCCACAGTGATA	0,04
		R	tcattggatctcattaCAGCCACCCCTGATGCTTT	0,04
MEF2C	rs190982	F	CTTTTAAGTGTAGTTTTCTATGTGCTCTTCAC	0,04
		R	tcattggatctcattaTTAAAAAGACTTTAATAATTCCTGT CCTGG	0,04
HLA- DRB5/1	rs9271192	F	GGTATCTAAAGTAAAATTCCAATACCCCTCTCA	0,04
		R	tcattggatctcattaGAACCCCAAGGAGCTCTGATAAAG TA	0,04
CD2AP	rs10948363	F	GGATTTGAGTATAAGTTCTAACTTCTTAGGGC	0,04
		R	tcattggatctcattaGGTCATTTTTCTAAACACAACACTT TAGTTCCA	0,04
NME8	rs2718058	F	CTAGGTCTTTTCAGAGAACGAGCATTGG	0,04
		R	tcattggatctcattaAAATTACAACATAAATCAACACAG TTGAAGATAGG	0,04
ZCWP W1	rs1476679	F	CAAAAAACGTCACATGGTACTTAGACTG	0,04
		R	tcattggatctcattaCCATTCTCCCGATCTGTTCTGGC	0,04
EPHA1	rs11771145	F	CCTGACTTAAAACACCACGGAGTG	0,04
		R	tcattggatctcattaGACACCAAAGAATGCATATCAGAT GATTCCG	0,04
PTK2B	rs28834970	F	TTATGGATCTGCCTTTTCTGGTCATTCC	0,04
		R	tcattggatctcattaGCTAAGTGAAAGCAGCCGTCGC	0,04
CLU	rs9331896	F	CTCCGGTGGTCCAGACACAG	0,04
		R	tcattggatctcattaCATTCAGCTCTTCCCTCCAGG	0,04
CELF1	rs10838725	F	CTTCTGGAGACTGAGGCACGAGAATTA	0,04
		R	tcattggatctcattaCTTGTCGCCACGATGGAGTA	0,04
MS4A6 A	rs983392	F	AGGAACAACAATTGAACAACAGGTGTTTC	0,04
		R	tcattggatctcattaGTTTTAGACAATAAGCTTGTGGTA AGTTTCTAC	0,04
PICAL M	rs10792832	F	GAGGCCACTTAAAGGAAGTGGGA	0,04
		R	tcattggatctcattaGGCCATCCTTTCTCACACTTACAGC	0,04
SORL1	rs11218343	F	CACTGCACCCAGCCGGTAA	0,04

		R	tcattggatctcattaACTCAAACAAGGATGTGGGAACAT GTG	0,04
FERMT 2	rs17125944	F	GCTTTTGTCTGGCCTAGTTTGTGG	0,04
		R	tcattggatctcattaCTGGTACTGGTGCATGATTTTGCC	0,04
RIN- SLC24A	rs10498633	F	CTTCAGGCACTTAGCAGACAAGATGAT	0,04
		R	tcattggatctcattaCCATGGCTGTCCTGTTCTACCTG	0,04
DSG2	rs8093731	F	GACACTCTAAGGCGGGACTCAGTAATC	0,04
		R	tcattggatctcattaTTCTAATTGGGATGTTAACAGTGGT TTTCAAGC	0,04
ABCA7	rs4147929	F	TGTCCCATTCTCACATTTGCCC	0,04
		R	tcattggatctcattaGCCAGCATTGAGTGTGGAGCA	0,04
CD33	rs3865444	F	CCCAGACACTCACACGGACC	0,04
		R	tcattggatctcattaACACCAGGGCTGATCACTGC	0,04
CASS4	rs7274581	F	GTCAGACCGGGTAAGCAGCTTG	0,04
		R	tcattggatctcattaGCCTCAGCCTCCCAAAGTGGAAG	0,04
APOE	rs429358	F	TGGGCGCGGACATGGAG	0,04
		R	tcattggatctcattaCAGCTCCTCGGTGCTCTGG	0,04
	rs7412	F	GCGATGCCGATGACCTGC	0,04
		R	tcattggatctcattaAGGCGCTCGCGGATGG	0,04
Universal adapter primer Uni-Cy5			taatgagatccaatga	4

F – forward primer\$ R – reverse primer

Microarray hybridization

Multiplex PCR product mixture (20 µL) was added to 10 µL of 20×SSPE (Thermo Fisher Scientific, MA, USA) and 10 µl of formamide (Panreac, Spain). Microarray hybridization chambers were filled with 36 µL of resulting mixtures, and microarrays were incubated at 37°C for 6-12 hours. The microarray was washed after removal of the hybridization mixture with 1×SSPE for five minutes twice, then washed with distilled water and air dried.