CHAPTER IV

RESULTS

SNP identification in *PSEN1* on HapMap website

Coverage of *PSEN1* located on chromosome 14 included the entire 90,901 kb of the gene with a MAF > 5% had been genotyped by HapMap using an r^2 cut-off value of 0.6. The 6 tag SNPs were chosen. MAFs, region, base change ancestral alleles and ancestral alleles in the HapMap dataset for the 6 tag SNPs were shown in Table 4.

Table 4 Identified tagSNPs in *PSEN1*

Identified rsSNPs in *PSEN1*	Minor Allele Frequency (MAF) (%)	Region (intron)	Base change Ancestral Alleles	Ancestral Alleles
rs362340	13.3	4	A/G	A
rs3025780	20.0	3	G/T	Not available
rs165932	28.9	8	T/G	T
rs10146743	10.0	9	T/G	G
rs214273	13.6	3	A/G	G
rs165933	38.9	8	G/A	G

Note: rsSNPs = referenceSNPs

Sample size determination, Alzheimer's disease patient and control samples collection

In this study, the selected genotype is estimated to be 38.9% (rs165933 in *PSEN1*). The relative risk for Null/Null (G/A) carriers, compared to normal, is 2.0 (Rg). Desired power is 80%, at a significance level of 0.05 with a 2-sided alternate hypothesis. The calculated result showed that the study should have 135 AD patients and 135 controls in order to perform SNPs association studies in *PSEN1* in Thai AD patients. However, this study used only 15 AD patients and 15 controls that given in Table 5 as the initial study.

Table 5 Demographic data

Groups	Sex (Male : Female)	Average of age (years)
Controls	3 : 12	58.33
Alzheimer's disease patients	7 : 8	71.73
Controls and Alzheimer's disease patients	10: 20	65.03

Primer design

The forward and reverse primers for 6 tag SNPs in *PSEN1* were shown in Table 6 and estimated PCR product of 6 tagSNPs in *PSEN1* was shown in Table 7.

Table 6 Forward and reverse primers used for 6 tagSNPs in *PSEN1* PCR amplification

Identified 5 tagSNPs in *PSEN1*	**Forward and reverse primer names**	**Sequence 5'- 3'**	**Tm * (°C)**
rs362340	F_rs362340	ATATAGGTTAGGGTTAGGTTTG	60
	R_rs362340	CCTGTGGCTGTAGCTTGC	62
rs3025780	F_rs3025780	GTCTCTTCCTCTAATCTTCAG	60
	R_rs3025780	GTTGCAGTTCCAGGTTTAAG	58
rs165932	F_rs165932	CTTCGTTAATTCCTCCCTAC	60
	R_rs165932	GGGAATGCTGGTTCCCCAA	58
rs10146743	F_rs10146743	GAGCTTGTTACTGGCATGTT	58
	R_rs10146743	CCATCGTCCATCCACAGAAC	60
rs214273	F_rs214273	CTGCAACTCTACTAGCTAATC	60
	R_rs214273	CAACTGCGGAATTCACGGAC	58
rs165933	F_rs165933	TTGTTGAGCAGAAGGGTAATG	58
	R_rs165933	ACCACTGAATATCTACGTAAG	62

Note: Tm = melting temperature

Table 7 PCR product sequences of 6 tagSNPs in *PSEN1*

6 tagSNPs in *PSEN1*	PCR products (5'-3')
rs362340 (65 bp)	ATATAGGTTAGGGTTAGGTTTGGCA**R**CGTATTATAGAACAAACATTGGCAAGCTACAGCCACAGG
rs3025780 (314 bp)	GTCTCTTCCTCTAATCTTCAGTCTACACTGTGACATGATAGGTATGCTCAAGTCTCTAACAGCTCTAAAATAACGTTTTCTGTTTTTTTTTTTGTCT**K**CATTTCTGGTGGGGTTGGATAAGAAAAGTTTTTCAAGTTAAATCACTCTCTATTGCAAGCCTCTTCCACCTTTTTTTTTTTTTTTTTTGCTTCGAAGAATGATTGCCTTTACCTTCTCAGTTCTCAGTTATTTTCCAGCACATTATGATTTTATGACCTTGATACAGAAGGGCTGGGCTCCTGGCTAAATTCCACCCTTAAACCTGGAACTGCAAC
rs165932 (427 bp)	CTTCGTTAATTCCTCCCTACCACCCATTTACAAGTTTAGCCCATACATTTTATTAGATGTCTTTTATGTTTTTCTTTTTCTAGATTTAGTGGCTGTTTTGTGTCCGAAAGGTCCACTTCGTATGCTGGTTGAAACAGCTCAGGAGAGAAATGAAACGCTTTTTCCAGCTCTCATTTACTCCTGTAAGTATTTGAGAA**K**GATATTGAATTAGTAATCAGTGTAGAATTTATCGGAACTGAAGCACATGTAACTATGGTCATTTTCATGGTACTTGTTCTCATCTTAAATGCACAGCATTCCTGGAACTCCTGCAGATCTCTTTGTTTCCTTGCAAGCAATTGTCTTCTACCTGATGTTGATTCAAGAGAGTTTTCAATATGAATAGAAAGAAAGAAAATGTTTAGATATTGGGGAACCAGCATTCCC
rs10146743 (678 bp)	GAGCTTGTTACTGGCATGTTGGCTGTGGGAAGTATATCTTGAAATTCACTAAAAGACCAACTATTGCTGGGCGCAGTGGCTCAAGCACCTGTAATCCCAGCACTTTGGAAGGCCAAGGCAGGCGGACCACCTGAGATCAGGAGTTCGAGACCAGCCCAACCAACATGGAGAAACCCCACCTCTACTAAAAATACAAAATTAGCCCATTGTGGTGGCACATGCCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCAGGAGAATCACTTGAACCTGGGAGGTGGAGGTTGCAGTGAGCTGAGATCATACCATTGCACTCCAGCCTGGGCAACAAGAGCGAAACTCCATCTCAAAAAAAAAAAAAAAAAAGACCAACTACCTTCACAATAAATAAAAATGTCTTACATTTCTAATTAAAATTTTTGTTCAGTTTTATTATTCAGCTTTAAAAAGGAAGGGAATTCTGACATGTTACAACATGAATAAATCTTGAG**K**ACTTTGTGCTAAGTGAAATAAGCTAGTTACAAAAAGAAAAATACTGTATGATTCCACTTACATCAGAGTAGTCAGAAAGTAGACTGGTGGTTGCCAGGGGTTAAGGGGAGGGGAAATGAAGGGTCGTTTAATGGGTGTAGAGTTTCAGTTTTACAAGATGAAAAGAGTTCTGTGGATGGACGATGG
rs214273 (858 bp)	CTGCAACTCTACTAGCTAATCATCATAAAGTGAGAGCAGAGGCCAGGCACGGTGGCTCATGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCGGGCAGATCACCTGAGGTTAGGAGTTCAATACCAGCTTGGCCAACATGGAGAAACCCCATCTCTACTAACAATACAAAAATTAGCCGGGCTTGGTGGCAGGTGCCTGTAATCCCAGCTACTCAGGAGGCTGAGCCAGGAGAATCGCTGGAACCCAGGAGGTGGAGGTTACAGTGAGCTGACGTCGCGCCATTGCACTCCAGCCTGGGCGACGAGCGAAACTCCATCTCAAAAATAAATAAATAAACAATAAATAAATAGAGAAAAAGAAAGAAAAGAAAAGAGAGAGAGCATGGATGTCCTGTGAGACAGTTGGAGTATCTATAGGATTTTTAGAGGTGGACCCAGAATAGAGGAAAACACAAGGCCAACTATAAGCTATCCAGTTGCACTAGGTGTGGGGTCAGATTTTCCTCTGTTAGGACCAGAGAGCAGAGCTAGGAACAGTGGTGGAAGTTACCAGGCAATAAGGTCTGGATCAATAAAAAGAACTTTCTCATAGCCTGGCATGGTGGTGCATGCTTGTAGTCCCAGCTACTCAGGAACTCAGGAGGCTGAGGTACGAGGATTGCTTGTCTAAGAGTTTGAGTCCAGCATGGGCAACATAGAGAGGTTCTGTGTTTTTTTTTTTTTAATTTTCCCACAGTTATGGTACATCTGGGCTGCCTTGTAGGCTTGTGTGTACATTTCACTACAAACAGTCACAAAAGGCTG**R**AAGGCCACTCCAGCAAGATCTGTAGAGGATTAGTCCGTGAATTCCGCAGTTG

Table 7 (Cont.)

6 tagSNPs in *PSEN1*	PCR products (5'-3')
rs165933 (1,023 bp)	TTGTTGAGCAGAAGGGTAATGTGAGCAGAGCCGTGCCTTTGTAAGCTGGCAGCACTGTGTGAGATGAATTGGTGGGTTGGAT ACTGAGATCATGAGAGGCATACTAAGCATAATTAAGATGATATTGCCATGATCTAGGTGGAAAGTAATGGGGGTTTGAATTA TG**R**TAGTGGCAGTAGCAATCAAGGGAAAGAGTTGATCAGAGGATTCAGAGGTAGAATCAATAGTTCTAGCAACTGAGGAGA GAAGTTGTAAGCTTGAAGGAAAGGTGATGAAGAAAAAATGCTTTCCTGTGTTTTCTTGTTGTTGTTGTTGAGATAGGGTCTCA CTCCCATCCAGGATGGAGTACAGTAGTGTGATCATGGCTCACTGCAGCCTCGACCTCCCAGGCTCAGGTGATCCACCCACCTC AGCCTCCCGAGTAGCTGTGACTACAGGCACGCACTACCAGGCCTGGCTAATTTTTTTGTGTTGTGTGTAGAGACTGGGTTTTGC CATGTGCCCAGGCTGGTCTTGAACTCCTCGGCTTAAGCGATCCTCCTGCCTTGACTTCACAAAGTGCTTGAGTTACAGGTGTGA GCTACCACGCCTGGCCATGTTTTCTTGTGTGAAGGATCTGTTTAGTTTTATATCTTTCTGTGGCTCATATCTAATTTAGTTGACA GTACCTGTGGGTCACTAGGTAGACATTGCTAGCAGACGTTTAGAAATGAAATACTAGAGCTTGGGAAAAAGTTGATATTTGA GATAGAGACTTGAAGAACATTAGCAGAGAGTTGGTAGTTAAGGTCTGTGAGCTGGTGAGCAATTCAAATAAAAGCAGAAGAG AAGAGGAAGACAAGGGTCAAACTTTGTCAACTACTGTGTTTAGAGAATGAGACAAGAGAGGATACTACAGGAAGTAGAGGA AAATAGTGGAAAATTGGGCAAGCCAGTATTTTTCACTTAAGAATATCATTACTGTTTTTTGATGTCAGCACATGAAATGGCTG CATAGTGTTCTCTTACGTAGATATTCAGTGGT

Note: DNA sequences from HapMap, underlines are forward and reverse primer positions. R and K alphabets are ambiguity code.

PCR amplification and agarosegel electrophoresis

The 5 tag SNPs PCR products of *PSEN1* in AD patients and controls were shown in Figures 26-28.

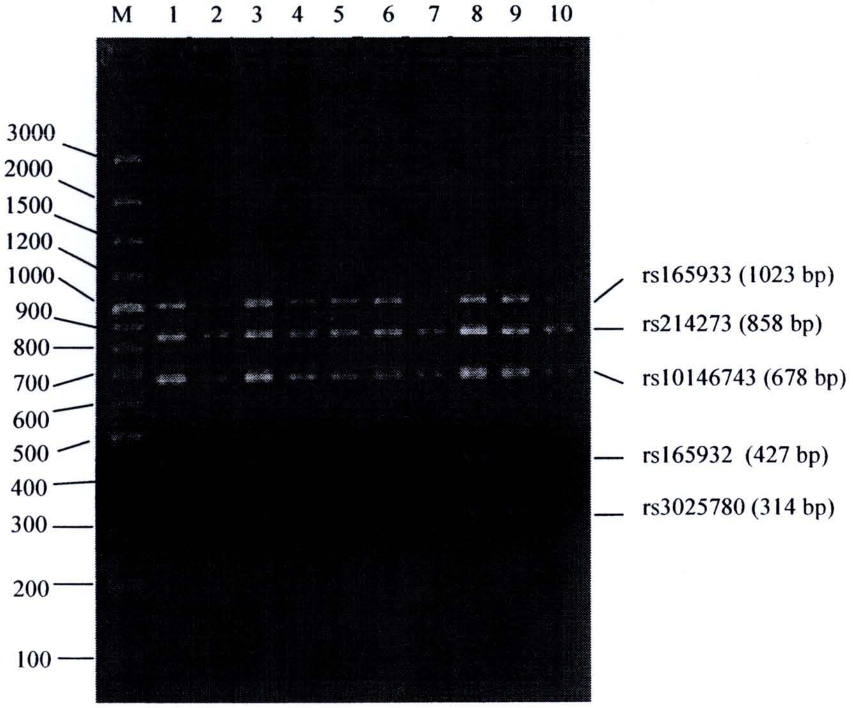

Figure 26 A 2 % agarose gel ethidium bromide stained showing PCR product sizes of 5 tagSNPs (rs3025780, rs165932, rs10146743, rs214273, rs165933) in *PSEN1* of Alzheimer's disease patients (AD). Lane M: 100 bp plus DNA Ladder, Lane 1: AD002, Lane 2:AD003, Lane 3:AD005, Lane 4:AD007, Lane 5:AD008, Lane 6:AD010, Lane 7:AD012, Lane 8:AD014, Lane 9:AD015, Lane 10:AD019.

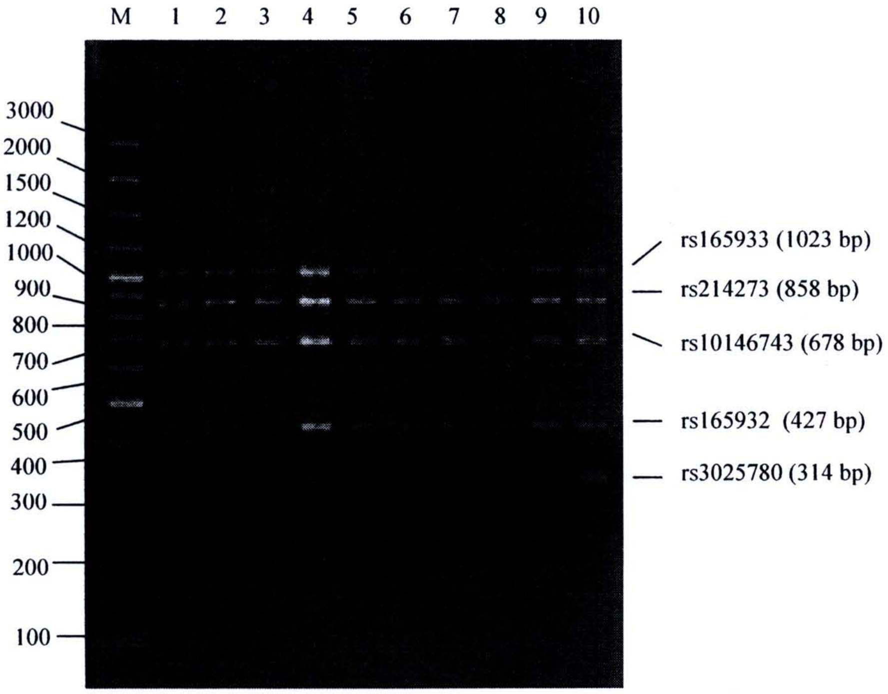

Figure 27 A 2 % agarose gel ethidium bromide stained showing PCR product sizes of 5 tagSNPs (rs3025780, rs165932, rs10146743, rs214273, rs165933) in *PSEN1* of Alzheimer's disease patients (AD) and Controls (CT). Lane M:100 bp plus DNA Ladder, Lane 1:AD020, Lane 2: AD021, Lane 3:AD022, Lane 4:AD025, Lane 5:AD027, Lane 6:CT001, Lane 7:CT002, Lane 8: CT004, Lane 9:CT005, Lane 10:CT006.

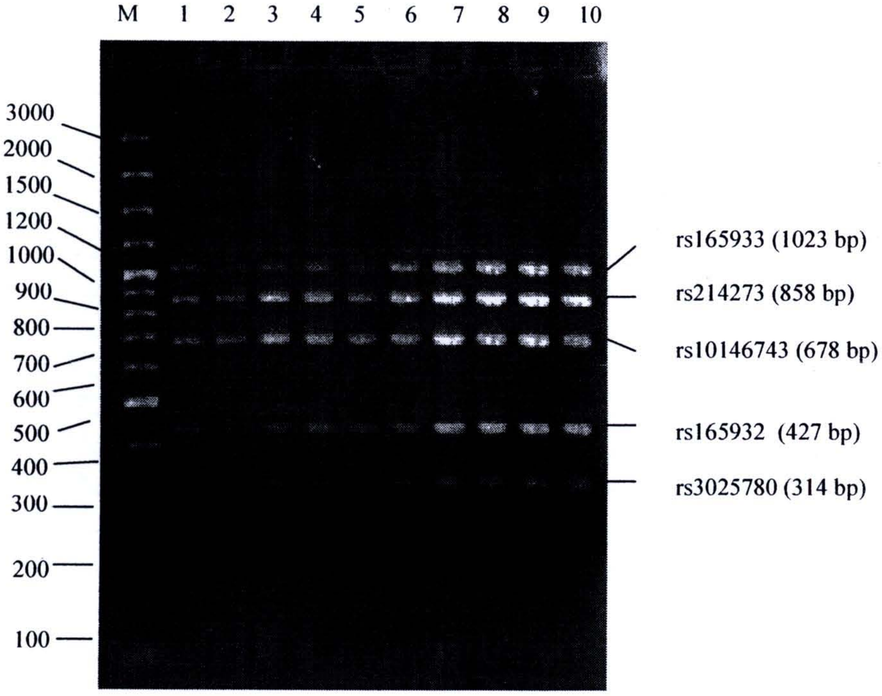

Figure 28 A 2 % agarose gel ethidium bromide stained showing PCR product sizes of 5 tagSNPs (rs3025780, rs165932, rs10146743, rs214273, rs165933) in *PSEN1* of Controls (CT). Lane M:100 bp plus DNA Ladder, Lane 1:CT007, Lane 2:CT008, Lane 3:CT009, Lane 4: CT012, Lane 5:CT014, Lane 6:CT015, Lane 7:CT016, Lane 8:CT017, Lane 9: CT018, Lane 10:CT019.

Genotyping of 5 tagSNPs by DNA sequencing technique and alignment

In this study, 5 tag SNPs (rs3025780, rs165932, rs10146743, rs214273 and rs165933 except rs362340) were genotyped by automated DNA sequencing of 15 controls (CT001, CT002, CT004, CT005, CT006, CT007, CT008, CT009, CT012, CT014, CT015, CT016, CT017, CT018 and CT019) and 15 AD patients (AD002, AD003, AD005, AD007, AD008, AD0010, AD012, AD014, AD015, AD019, AD020, AD021, AD022, AD025 and AD027). The 5 tagSNPs of *PSEN1* in controls ($N = 15$) and AD patients ($N = 15$) were shown in Table 8. The frequencies of the haplotypes at 5 tag SNPs in the *PSEN1* in all controls and AD patients are given in Table 8 .

Table 8 5 tagSNPs Genotyping in *PSEN1*

Groups	Subjects	Haplotype				
		rs3025780	**rs165932**	**rs10146743**	**rs214273**	**rs165933**
		G/T	**T/G**	**T/G**	**A/G**	**G/A**
Controls	CT001	TT	GG	TT	AA	GG
	CT002	TT	GG	TT	AA	GG
	CT004	TT	TT	TT	AA	AA
	CT005	TT	TT	TT	GG	GG
	CT006	GG	TT	TT	GG	GG
	CT007	TT	TT	TT	GG	AA
	CT008	TT	TT	TT	GG	AG
	CT009	GG	TT	TT	GG	AA
	CT012	TT	TT	TT	GG	AA
	CT014	TT	TT	TT	GG	AA
	CT015	TT	GG	TT	AA	GG
	CT016	TT	TT	TT	GG	GG
	CT017	TT	TT	TT	GG	AA
	CT018	TT	TT	TT	GG	GG
	CT019	TT	TT	GG	GG	GG
AD patients	AD002	TT	TT	GG	GG	AG
	AD003	TT	TT	GG	GG	AA
	AD005	TT	TT	TT	GG	AG
	AD007	TT	GG	GG	AA	GG
	AD008	TT	TT	TT	GG	AA
	AD010	GG	TT	TT	GG	GG
	AD012	TT	TT	TT	GG	AA
	AD014	TT	TT	TT	GG	GG
	AD015	TT	TT	GG	GG	GG
	AD019	TT	TT	TT	AA	AA
	AD020	TT	TT	GG	GG	GG
	AD021	TT	TT	TT	GG	AG
	AD022	TT	TT	TT	GG	AA
	AD025	TT	TT	GG	AA	GG
	AD027	GG	TT	GG	GG	GG

Note: CT = control, AD = Alzheimer's disease patient, AG is heterozygous allele was found only in rs165932. The AD patient is 20 % and 6.66% in controls. Black boxes indicate AG heterozygous alleles.

Statistical analysis

Genotype frequencies of 5 tagSNPs in *PSEN1* are shown in Table 9.

Table 9 Genotype frequencies of 5 tag SNPs in *PSEN1*

5 tagSNPs	Genotypes	AD Patients (%)	Controls (%)
rs3025780 (G/T)	GG	2 (13.33)	2 (13.33)
	GT	0 (0.00)	0 (0.00)
	*TT	13 (86.66)	13 (86.66)
rs165932 (T/G)	*TT	14 (93.33)	12 (80.00)
	TG	0 (0.00)	0 (0.00)
	GG	1 (6.66)	3 (20.00)
rs10146743 (T/G)	*TT	8 (53.33)	14 (93.33)
	TG	0 (0.00)	0 (0.00)
	GG	7 (46.66)	1 (6.66)
rs214273 (A/G)	AA	2 (13.33)	4 (26.66)
	AG	0 (0.00)	0 (0.00)
	*GG	13 (86.66)	11 (73.33)
rs165933 (G/A)	*GG	7 (46.66)	7 (46.66)
	GA	3 (20.00)	1 (6.66)
	AA	5 (33.33)	7 (46.66)

Note: * indicates the highest each tagSNPs in *PSEN1*.

Haplotype Frequencies

From the haplotype analyses of 5 tagSNP in *PSEN1* in controls and AD patients, the highest haplotype frequencies were found in TTTGA (black box in Table 10); 33.33% CT and 26.66%AD. Minor allele frequencies were shown in Table 11.

Table 10 Haplotypes frequencies of 5 tag SNPs in the *PSEN1* in all controls (*N* = 15) and AD patients (*N* = 15)

Haplotype						
rs3025780	**rs165932**	**rs10146743**	**rs214273**	**rs165933**	***CT (%)**	***AD %**
G/T	**T/G**	**T/G**	**A/G**	**G/A**		
T	G	T	A	G	3(20.00)	0(0.00)
T	T	T	A	A	1(6.66)	1(6.66)
T	T	T	G	G	3(20.00)	2(13.33)
G	T	T	G	G	1(6.66)	1(6.66)
T	T	T	G	A	5(33.33)	4(26.66)
G	T	T	G	A	1(6.66)	0(0.00)
T	T	G	G	G	1(6.66)	2(13.33)
T	T	G	G	A	0(0.00)	2(13.33)
T	G	G	A	G	0(0.00)	1(6.66)
T	T	G	A	G	0(0.00)	1(6.66)
G	T	G	G	G	0(0.00)	1(6.66)

Note: *CT = control, AD Alzheimer's disease, underline indicates minor allele. Black box shows highest haplotypes frequency.

Table 11 MAFs in controls and AD patients for case-control association for the *PSEN1*

5 tagSNPs	MAF in Control	Control	AD
rs3025780	G	2 (6.66)	2 (6.66)
rs165932	G	3(10.00)	1 (3.33)
rs10146743	G	1 (3.00)	7 (23.33)
rs214273	A	3 (10.00)	3 (10.00)
rs165933	A	7 (23.33)	7 (23.33)

Note: Black box shows the highest MAF in control.

Odd ratio (OR)

Genotypes of rs3025780, rs165932, rs10146743 and rs214273 were failed in the odd ratio calculation (as shown in Table 12). The odd ratio was calculated with frequencies of homologous allele and heterologous alleles of 5 tagSNPs in *PSEN1* compared between controls and AD patients (such as GG in rs3025780 was calculated with GT control frequencies, GT was calculated with TT and TT was calculated with GT). The calculated odd ratio of genotype in rs165933 was calculated that shown in Table 12.

Table 12 Genotype frequencies and associated odd ratios for risk of developing Alzheimer's disease

5 tagSNPs	Genotypes	AD	Controls	OR (95% CI)
rs3025780	GG	2 (13.33)	2 (13.33%)	0.00
	GT	0 (0.00)	0 (0.00)	0.00
	TT	13 (86.66)	13 (86.66)	0.00
rs165932	TT	14 (93.33)	12 (80.00)	0.00
	TG	0 (0.00)	0 (0.00)	0.00
	GG	1 (6.66)	3 (20.00)	0.00
rs10146743	TT	8 (53.33)	14 (93.33)	0.00
	TG	0 (0.00)	0 (0.00)	0.00
	GG	7 (46.66)	1 (6.66)	0.00
rs214273	AA	2 (13.33)	4 (26.66)	0.00
	AG	0 (0.00)	0 (0.00)	0.00
	GG	13 (86.66)	11 (73.33)	0.00
rs165933	GG	7 (46.66)	7 (46.66)	0.3330 (0.0275 - 4.0359)
	GA	3 (20.00)	1 (6.66)	4.2000 (0.3320 - 53.1253)
	AA	5 (33.33)	7 (46.66)	0.2381 (0.0188 - 3.0117)