Serum insulin-like growth factor-I and amyloid beta protein in Alzheimer's disease: Relationship with cognitive function

Ayako Kimoto[1], Koji Kasanuki[1,2], Ryo Kumagai[1,2], Nobuto Shibata[1], Yosuke Ichimiya[1,2] and Heii Arai[1]

1. Department of Psychiatry and Behavioral Science, Graduate School of Medicine, Juntendo University, Tokyo, Japan
2. Juntendo Tokyo Koto Geriatric Medical Center, Juntendo University School of Medicine, Tokyo, Japan

Correspondence to: Ayako Kimoto, MA
Department of Psychiatry and Behavioral Science, Graduate School of Medicine, Juntendo University
2-1-1, Hongo, Bunkyo-ku, Tokyo, Japan
Zip code: 113-8421
Email: akimoto@juntendo.ac.jp
TEL: +81(0)3-5802-1071
FAX: +81(0)3-5802-1071

ABSTRUCT

Previous studies have suggested that Insulin-like growth factor-I (IGF-I) deficiency may lead to cognitive deficits in neurodegenerative diseases such as Alzheimer's disease (AD). The present study aimed to investigate the possible relationship between cognitive function and concentration of IGF-I or Amyloid β protein (Aβ) in serum in AD patients. A total of 81 elderly Japanese patients were enrolled in this study. Concentrations of IGF-I, $A\beta_{42}$, and $A\beta_{40}$ in serum were measured. Neuropsychological tests; Mini-Mental State Examination (MMSE) and Hasegawa Dementia Scale-Revised (HDS-R), were also performed. Linear correlations among the age, serum IGF-I, serum $A\beta_{42}$ or $A\beta_{40}$, $A\beta_{42}/A\beta_{40}$ ratio, MMSE or HDS-R total score, and the scores for 6 HDS-R subscales were analyzed by regression analysis. IGF-I showed a significant negative correlation with age (β=-0.357, P=.002), and a positive correlation with $A\beta_{42}/A\beta_{40}$ ratio (β=0.318, P=.007). Serum IGF-I and both the MMSE and the HDS-R total score also correlated ((β=0.505, β=0.524, P<.01). Among the HDS-R subscales, "Recall" (ρ=.379, P<.01), "Verbal Fluency" (ρ=.360, P<.01), and "Attention and Calculation" (ρ=.389, P<.01) showed significant positive correlations with serum IGF-I. Those results, that lower serum IGF-I was associated with cognitive impairment, suggest that metabolism of IGF-I may be involved in the

pathogenesis of cognitive deficits in AD.

Key words: Alzheimer's disease, IGF-I, $A\beta_{42}$, $A\beta_{40,}$ Cognitive function, dementia

Short running title: IGF- Ⅰ, Amyloid β and Cognitive function

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia. AD is characterized by progressive degeneration of synapses and dendrites, leading to impairment of cognitive function. Dense accumulation of senile plaques, which are composed of amyloid beta protein (Aβ), and neurofibrillary tangles composed of hyperphosphorylated tau protein are the main pathological hallmarks of AD.[1]

Insulin-like growth factor-I (IGF-I) acts as a trophic factor for almost all neurons as well as glial cells, and has many beneficial effects on brain function and the nervous system. IGF-I has a neuroprotective effect, inhibits apoptosis, regulates neurogenesis, and increases neural excitability. [2,3,4,5] IGF-I deficiency may result in neurodegeneration and the development of neurofibrillary tangles. [6] Previous studies have shown that serum levels of IGF-I are altered in many neurodegenerative conditions.[7,8]

However, studies on the correlation between serum IGF-I and cognitive function have obtained conflicting results.[9-15] Healthy centenarians with a higher serum IGF-I level and IGF-I/IGF binding protein-3 (IGFBD3) ratio were found to have less cognitive impairment.[13] In addition, older subjects with high concentration of total IGF-I in serum were reported to have better cognitive performance,[9,10] while patients

with AD had significantly lower serum IGF-I.[14] On the other hand, it was reported that serum IGF-I is increased in AD patients.[12,15]

The present study was designed to investigate the possible relationship between cognitive function and the serum level of IGF-I or Aβ in AD patients. Since there have been few investigations of the detailed relations between cognitive function and serum IGF-I as well as serum Aβ, we examined the serum levels of IGF-I, $A\beta_{40}$, and $A\beta_{42}$, as well as $A\beta_{40}/A\beta_{42}$ ratio, in patients with AD, and then analyzed the correlations with cognitive functions.

MATERIALS AND METHODS

Participants

Eighty-one elderly Japanese patients, including 24 men and 57 women with a median age of 78 years (range: 46-99 years), who visited the outpatient clinic of Juntendo Tokyo Koto Geriatric Medical Center were enrolled in this study. Among them, 70 patients (21 men and 49 women with a median age of 80 years) were diagnosed as having AD according to the Diagnostic and Statistical Manual-Ⅳ-TR (DSM-Ⅳ-TR) criteria,[16] and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association

(NINCDS-ARDRA) criteria.[17] The other 11 subjects had MCI due to AD (3 men and 8 women with a median age of 72.5 years)[18]. None of the subjects were taking drugs that are known to influence cognitive function or the serum IGF-I level.

Neuropsychological examination – cognitive evaluation

Trained examiners performed two standardized cognitive tests (Mini-Mental State Examination (MMSE) and the Hasegawa Dementia Scale-Revised (HDS-R)[18,19] to assess the cognitive function of the subjects. These screening tests mainly assess cortical functions that are important for daily life. The score ranges from 0 to 30 for both tests. HDS-R includes questions on the age of the subject, orientation, immediate recall, serial subtraction of 7s, reciting digits backwards, recalling three words, recalling five objects, and language fluency (generating names of vegetables). We calculated both the total score and the score for each subscale [18,19]. Cognitive impairment was defined as being present when the score was less than 24/30 for the MMSE and 20/30 for the HDS-R.

Serum parameters

Blood samples were obtained from the subjects, and concentrations of IGF-I, amyloid β_{40}, and amyloid β_{42} in serum were determined by a Japanese laboratory company.

(SRL Inc., Tokyo, Japan)

1) Serum IGF-I

IGF-I concentration in serum was determined by immunoradiometric assay (IGF-I IRMA kit, Daiichi Radioisotope laboratories, Chiba, Japan).[20] The reference values of serum IGF-I in persons >60 years are 42-250 ng/ml for men, and 37-207 ng/ml for women. The intra- and inter-assay coefficients of variation were 2.4% and 1.8%, at analyte levels of 50 and 1000 ng/ml.

2) Serum Aβ40 and Aβ42

The concentration of Aβ40 and Aβ42 in serum were determined by sandwich ELISA method.[28,29] Sandwich Aβ enzyme-linked immunosorbent assay kits were used (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The assay sensitivity was 0.019 pmol/L (range: 1.0–100 pmol/L) for $A\beta_{40}$ and 0.06 pmol/L (range: 0.1–20 pmol/L) for $A\beta_{42}$.

3) Apo E

Apo E genotype was determined by PCR amplification and subsequent digestion with the restriction enzyme. Genotyping was done by isoelectric focusing and immunoblot.[21]

Statistical Analysis

SPSS 19.0J for Windows (SPSS Japan Inc., Tokyo, Japan) was used for all statistical procedures. Examination of normality by the Shapiro-Wilk test showed that the distribution of the variables was not normal, except for the HDS-R total score. Accordingly, non-parametric methods were adopted for further statistical analysis. Demographic characteristics were presented by calculating the median values and quartiles.

Linear correlations among the variables were analyzed by regression analysis. Spearman's method was used to assess correlations between age, serum IGF-I, serum Aβ42, serum Aβ40, the Aβ42/Aβ40 ratio, the HDS-R total score and its 6 subscales (Orientation, Rehearsal, Registration, Attention and Calculation, Recall, and Verbal fluency), and the MMSE total score. Relationships between the variables were also evaluated by stepwise multiple linear regression analysis using the MMSE or HDS-R as the dependent variable. In all analyses, $P<0.01$ was considered statistically significant.

RESULTS

The demographic profile of the subjects is shown in Table 1. The 25th percentile (1st quartile: Q1) is the score below which 25 percent of the observations may be found, and 75th percentile is the score below which 75 percent of the observations may be found. Results of linear regression analysis comparing serum IGF-

I with the other variables are shown in Table 2. Serum IGF-I and age showed a significant negative correlation (β=-0.357, P=.002). Neither serum $A\beta_{40}$ nor $A\beta_{42}$ displayed a significant correlation with serum IGF-I, but the $A\beta_{42}/A\beta_{40}$ ratio showed a significant positive correlation (β=0.318, P=.007). Significant positive correlations were detected between the serum IGF-I and both the MMSE (β=0.505, P<.001) and the HDS-R total score (β=0.505, P<.001). Scatter diagram which shows the relationship of serum IGF-1 and Neuropsychological exams; MMSE and HDS-R, are shown in Figure 1 and 2. However, neither age nor the serum $A\beta_{42}/A\beta_{40}$ ratio was correlated with the MMSE or HDSR, as well as with any of the subscales.

Among the HDS-R subscales, “Recall" (ρ=.379, P=.001), “Verbal Fluency” (ρ=.360, P=.002), and “Attention and Calculation” (ρ=.389, P=.001) showed significant positive correlations with serum IGF-I.

When multiple forward stepwise regression analysis was performed using MMSE or HDS-R as the dependent variables, serum IGF-I was a significant factor (P<.001, R=0.504, B=0.067). Other variables, including $ApoE_{4,}$ age, and the $A\beta_{42}/A\beta_{40}$ ratio, did not have a significant influence on MMSE or HDS-R in this model.

DISCUSSION

The results of the present study supported the hypothesis that the concentration of IGF-I in serum is related to cognitive decline.[9-11,13,14] Previous studies have shown that the serum and brain levels of IGF-I decrease with age,[22] and that many neurodegenerative conditions are associated with altered levels of IGF-I.[7,8] However, previous investigations of concentration of IGF-I in serum in AD patients have obtained conflicting results, with both reduced and increased circulating IGF- I concentrations being reported.[12,14] In our study, although our samples were obtained only from AD and MCI-AD patients, the concentration of IGF-I in serum showed a significant positive correlation with HDS-R or MMSE scores, indicating that serum IGF-I decreases as cognitive function becomes more severely impaired in AD.

In multiple linear regression analysis, IGF-I was shown to be an explanatory variable for the MMSE and HDS-R scores, whereas $ApoE_4$, age, and the $A\beta_{42}/A\beta_{40}$ ratio were not. When interpreting this result, it should be noted that no patient had $ApoE_{4/4}$ in this cohort and only 12 out of 81 had $ApoE_{4/3}$ or $ApoE_{4/2}$, so the genotype distribution might have partly influenced our findings. There have been a few studies about the association of IGF-I with detailed parameters of cognitive function. In healthy older male subjects, the association between the serum IGF-I level and cognitive function was assessed by using the WAIS and other neuropsychological tests.[9]

Significant correlations were found between serum IGF-I and Digit symbol substitution (cognitive and perceptual-motor processing speed) and the Concept shifting task (cognitive processing speed), which represent mental processing speed and executive processing. However, neither memory nor language functions were found to be correlated with the serum IGF-I level. In contrast, our study showed that memory and verbal fluency were associated with the concentration of IGF-I in serum, because "Recall" (short term memory), "Attention and Calculation" (working memory), and "Verbal fluency" (language ability) all displayed a significant positive correlation with serum IGF-I. This difference could be explained by the study subjects of the other study being healthy and not having AD. Short-term memory is known to be regulated by the hippocampus.[23] IGF-I protects and rescues hippocampal neurons from the effects of Aβ, and it has a neurotrophic effect in the hippocampus, which is involved in learning and memory.[24] In accordance with these reports, the present study showed an association between serum IGF-I and cognitive function, for which the hippocampal region is essential.

Aβ is excreted from the brain into the celebrospinal fluid (CSF), and then is transferred into the blood (Aβ clearance).[25] A lower serum $A\beta_{42}/A\beta_{40}$ is considered to indicate that less $A\beta_{42}$ than $A\beta_{40}$ is being excreted from the brain into the CSF or

blood,[26] so this ratio is considered to be an indicator of the accumulation of $A\beta_{42}$ in the brain. Some researchers have suggested that elevation of the plasma level of $A\beta_{42}$ increases the risk of AD.[27] However, a significant decrease of plasma $A\beta_{42}$ was reported after the onset of AD.[28,29] In healthy elderly persons, physiologically produced Aβ is processed and excreted from the brain in order to maintain homeostasis, while the balance between production and removal is lost in AD patients[27]. In AD, increased production and/or decreased processing/clearance of Aβ may lead to the formation of amyloid plaques,[28] and this appears to be the initial step in the pathogenesis of AD. Although it seems that the serum $A\beta_{42}/A\beta_{40}$ ratio does not have enough specificity and sensitivity for diagnosis, it has been suggested to be a useful biomarker for early detection/prediction of AD.[30] In our study, serum IGF-I and the serum $A\beta_{42}/A\beta_{40}$ ratio were significantly correlated. Some studies have suggested that IGF-I enhances Aβ clearance.[31,32] A negative correlation between the plasma IGF-I level and Aβ levels in brain tissue was observed in rats.[33] Young genetically engineered mice with low circulating IGF-I levels show an increase of Aβ in the brain, while the Aβ burden can be reduced in aging rats by increasing serum IGF-I.[32] Thus, researchers have focused on this Aβ-reducing effect of IGF-I, and have proposed that administration of IGF-I may be a new therapeutic option for treatment and prevention of AD.

As a limitation of the present study, our data were obtained only from AD and MCI-AD patients. For further elucidation, data from healthy controls would be also necessary to be obtained. Also, more detailed and reliable cognitive testing is necessary. Although used the MMSE, HDS-R and its subscales as indexes of cognitive function, detailed neuropsychological tests could detect smaller differences in cognitive function. Moreover, another limitation of this study may be a lack of data concerning nutritional status. In this study, none of the subjects were taking drugs that affected the IGF-I level, but nutrition is another factor that regulates IGF-I.[34,35] Excess intake of protein was reported to increase IGF-I in elderly subjects.[36] Therefore, adjustment for dietary protein intake is necessary when further research is undertaken. In this study, 10 of the participants had diabetes. Researchers have been investigating on phosphatidylinositol 3-kinase (PI3K) pathway, which is activated by both insulin and IGF-I, as potential targets for treatment of type Ⅱ diabetes.[37] Although there are few reports showing a direct relationship between IGF-I and diabetes, it is indicated that the inactivation of PI3K pathway relates to insulin resistance.[37] Thus, the effect of diabetes on the level of IGF-I should be taken into account. We analyzed the data, comparing 10 participants with diabetes and the other 71 non-diabetes participants by t-test. However, there was no significant difference on the level of IGF-I. (data not shown)

In the future, prospective studies are needed in order to assess the changes over time in individual subjects.

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Conflicts of Interests

None of the authors has any conflict of interest.

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Figure legends

Table1. Clinical data of participants.

Table2. Linear regression analysis of factors influencing IGF- I.

Table3. Multiple linear regression analysis; factors influencing MMSE scores.

Table4. The correlations between serum parameters and HDS-R subscales (Spearman's ρ).

Figure1. Correlation between serum IGF- I and the HDS-R score.

Figure2. Correlation between serum IGF- I and the MMSE score.

Figure3.Correlation between serum IGF- I and the age.

Figure4.Correlation between serum IGF- I and the $A\beta_{42}/A\beta_{40.}$

Supplementary table. Supplemental data

Figure legends

Table1. Clinical data of participants.

Table2. Linear regression analysis of factors influencing IGF- I.

Table3. Multiple linear regression analysis; factors influencing MMSE scores.

Table4. The correlations between serum parameters and HDS-R subscales (Spearman's ρ).

Figure1. Correlation between serum IGF- I and the HDS-R score (R^2=0.245).

Figure2. Correlation between serum IGF- I and the MMSE score (R^2=0.228).

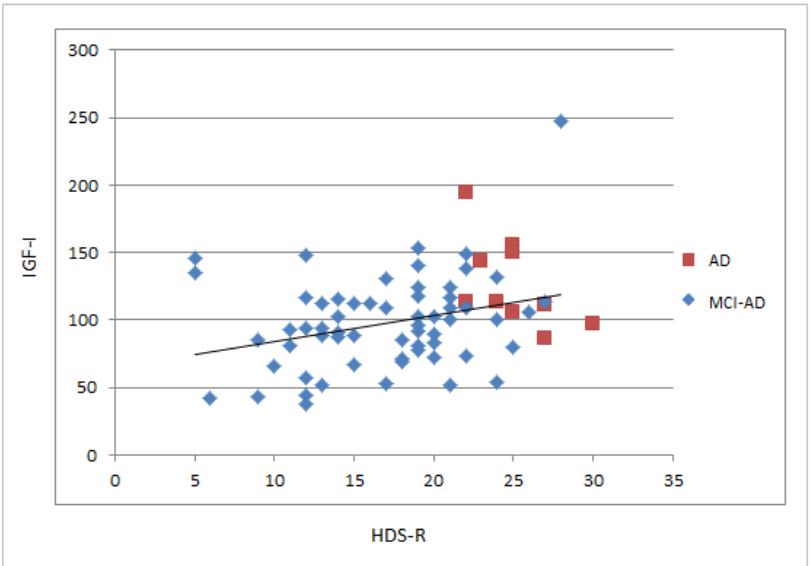

Figure1. Correlation between serum IGF- I and the HDS-R score.

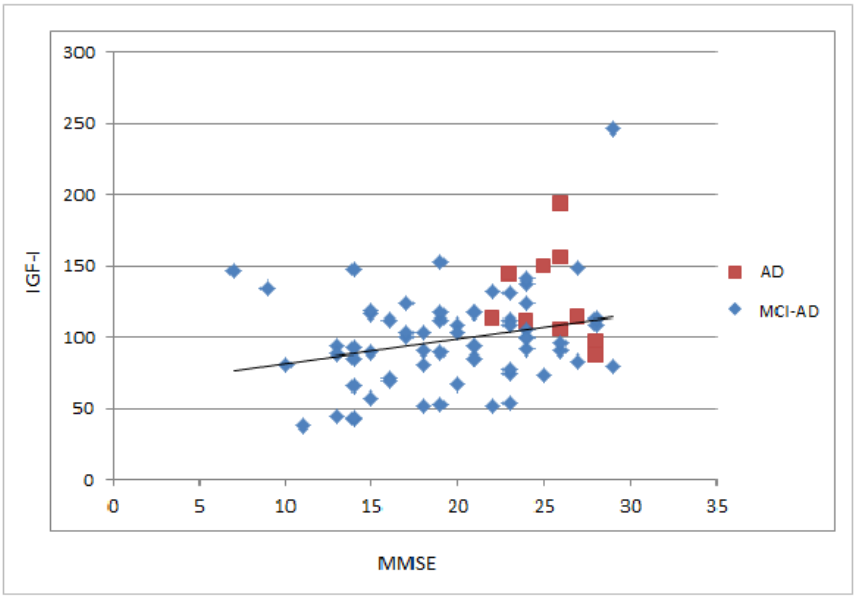

Figure2. Correlation between serum IGF- I and the MMSE score.

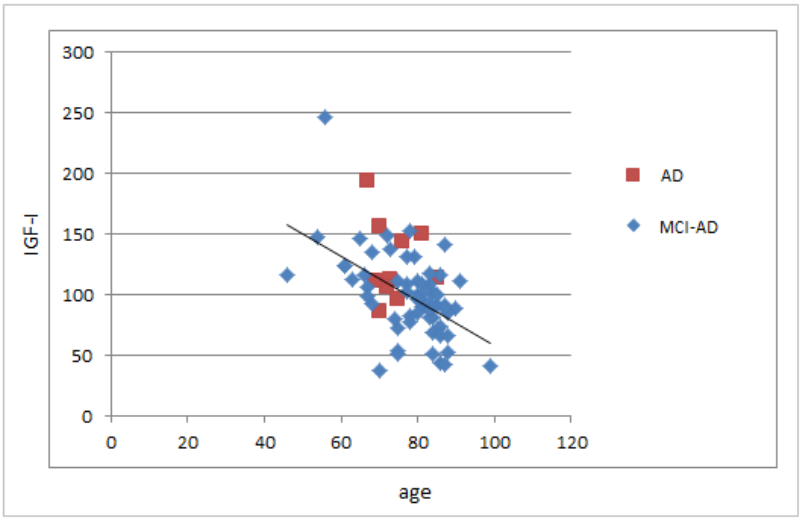

Figure3.Correlation between serum IGF- I and the age.

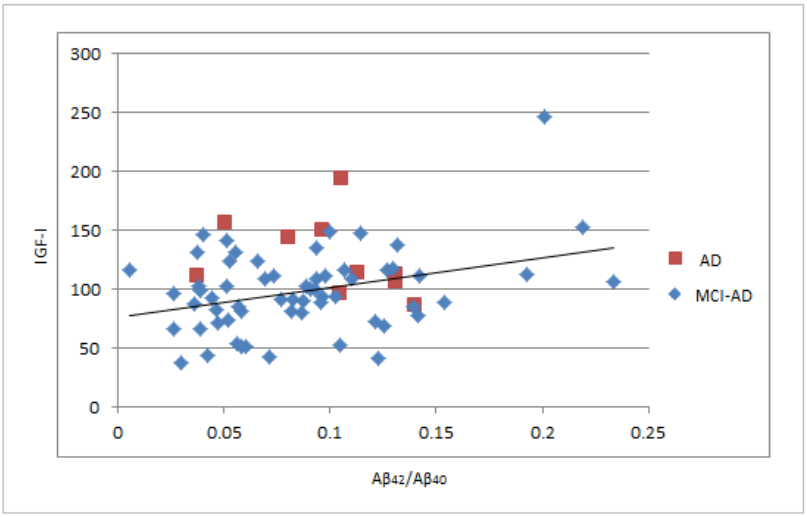

Figure4.Correlation between serum IGF- I and the $A\beta_{42}/A\beta_{40}$.

sex	age	ApoE	IGF1	Aβ 40	Aβ 42	ratio Aβ 42/40	HDS-R	MMSE	Orientation	Rehearsal	Recall	Registration	Verbal Fluency	Attention and Calculation
0	84	2	81	36.5	3	0.08	11	10	3	3	2	2	0	1
0	84	3	103	29.2	2.6	0.09	20	18	3	3	3	4	2	4
0	75	4	112	40.7	3	0.07	16	23	2	3	0	3	4	4
0	75	2	73	16.5	2	0.12	20	25	5	3	3	4	2	3
0	83	2	109	18.1	2	0.11	21	20	5	3	0	4	5	4
0	81	3	90	64.1	5.6	0.09	20	26	7	3	0	5	3	2
0	79	1	132	54.2	3	0.06	24	22	5	3	5	3	4	4
1	72	1	149	27	2.7	0.10	22	27	6	3	4	5	0	4
0	85	3	91	28.6	2.2	0.08	14	18	4	3	1	4	0	2
0	83	3	81	53.1	3.1	0.06	19	18	3	1	2	4	5	2
0	61	3	124	53.1	3.5	0.07	21	24	4	3	0	5	5	4
0	80	2	112	22.5	3.2	0.14	15	19	5	3	0	4	1	2
0	81	3	94	39.4	3.8	0.10	12	13	3	3	0	3	1	2
0	85	2	100	97.2	8.8	0.09	21	17	3	3	2	4	5	3
0	86	2	116	19.6	2.1	0.11	14	15	4	3	2	2	0	3
0	81	3	109	24.5	2.3	0.09	22	28	7	3	5	4	0	3
0	73	4	138	22.8	3	0.13	22	24	5	3	2	4	4	4
0	90	2	89	35.5	3.4	0.10	13	15	3	3	1	4	1	1
0	88	3	85	53	3	0.06	18	21	5	3	2	4	1	3
0	85	2	71	72.4	3.4	0.05	18	16	4	3	1	3	5	2
0	80	3	85	18.7	2.6	0.14	9	14	1	3	0	3	0	2
0	91	2	112	34.8	3.4	0.10	13	16	4	3	0	3	0	3
0	67	4	106	60	14	0.23	26	24	7	3	4	5	5	2
0	80	2	100	41.9	3.9	0.09	24	24	7	3	5	5	0	4
1	66	3	117	59.2	7.5	0.13	12	19	4	3	0	2	0	3
1	78	2	83	77.8	3.6	0.05	20	27	5	3	3	5	0	4
0	86	2	74	53.8	2.8	0.05	22	23	6	3	1	4	5	3
1	83	2	94	36.9	3.8	0.10	13	21	4	3	0	3	1	2
0	78	3	153	63.9	14	0.22	19	19	5	3	2	3	3	3
0	87	2	92	36.3	3	0.08	19	24	7	3	0	4	2	3
1	63	2	113	23.9	4.6	0.19	27	28	7	3	3	5	5	4
1	68	2	135	32	3	0.09	5	9	1	3	0	1	0	0
1	87	2	89	27.3	4.2	0.15	15	19	6	2	0	3	3	1
0	78	1	78	34.6	4.9	0.14	19	23	7	3	0	3	4	2
0	83	1	118	49.4	6.4	0.13	19	15	1	6	3	0	0	3
0	84	2	69	84.4	10.6	0.13	18	16	2	3	2	4	4	3
1	54	3	148	20.1	2.3	0.11	12	14	3	3	0	4	1	1
0	77	3	109	83.6	5.8	0.07	17	23	4	3	5	2	0	3
0	99	3	42	68.4	8.4	0.12	6	14	3	2	0	0	0	1
0	82	3	103	54.6	2.1	0.04	19	20	5	3	2	2	5	2
1	86	4	67	75.2	2	0.03	15	20	5	3	1	2	0	4
1	83	4	88	58	2.1	0.04	14	13	3	3	2	3	0	3
1	46	4	117	347.4	2	0.01	21	21	6	3	1	3	5	3
1	77	3	131	53.4	2	0.04	17	23	4	3	0	3	4	3
1	68	3	93	45.2	2	0.04	11	14	3	3	0	3	0	2
0	87	3	141	71.8	3.7	0.05	19	24	4	3	0	3	5	4
0	88	3	66	66.4	2.6	0.04	10	14	2	3	0	4	0	1
1	61	3	124	58.8	3.1	0.05	19	17	4	3	3	4	1	4
1	80	3	96	75.6	2	0.03	19	26	6	3	2	2	2	4
0	75	2	247	23.9	4.8	0.20	28	29	7	3	6	4	5	3
1	63	2	103	70.1	3.6	0.05	14	17	4	2	2	3	0	3
0	77	4	199	51.6	2	0.04		24						
1	65	3	46	50	2	0.04	5	7	2	3	0	0	0	0
0	70	4	38	73	2.2	0.03	12	11						
1	87	3	43	44.8	3.2	0.07	14	9	3	3	0	2	0	1
1	77	4		46.6	2.3	0.05	20	22	4	3	0	5	5	3
0	77			21.9	2.6	0.12	6	12	2	3	2	2	0	1
0	67			55.3	2.7	0.05	5	7	3	3	0	1	0	0
0	68			28.4	3	0.11	1	1	1	1	0	0	0	0
0	77			32.5	2	0.06	11	17	3	3	0	2	0	3
0	83			55.4	4.2	0.08	13	20	4	3	0	3	0	3
1	91	3					8	12	1	3	0	3	0	1
0	56	2					13	15	0	3	5	4	5	4
0	86	3	44	66	2.8	0.04	12	13	4	3	0	3	0	2
0	84	2	52	62.9	3.8	0.06	13	18	4	3	0	4	0	2
0	75	3	54	48.1	2.7	0.06	24	23	6	3	5	5	2	3
0	80	2	57				12	15	4	3	0	3	0	2
0	88	2	53	44	4.6	0.10	17	19	4	3	2	5	0	3
1	75	2	52	46.3	2.7	0.06	21	22	7	3	4	5	0	2
0	74	3	80	27.6	2.4	0.09	25	29	7	3	4	5	4	2
0	73	3	113	24.4	3.2	0.13	24	22	7	3	5	5	3	1
0	70	2	187	32.9	4.6	0.14	27	28	7	3	6	4	4	3
0	76	3	144	24.8	2	0.08	23	23	7	3	6	4	2	1
0	72	3	106	15.3	2	0.13	25	26	6	3	5	4	4	3
0	70	4	156	45.2	2.3	0.05	25	26	4	3	5	5	4	4
1	81	2	150	88	8.5	0.10	25	25	6	3	3	4	5	4
0	75	3	197	30.6	3.2	0.10	30	28	7	3	6	5	5	4
1	67	3	194	78.6	8.3	0.11	22	26	7	3	5	5	0	2
1	85	4	114	63.8	7.2	0.11	22	27	6	3	2	4	4	3
0	69	4	111	53.2	2	0.04	27	24	5	3	6	5	5	3
0	74	2	148	138	15.2	0.11	30	27	7	3	6	5	5	4

ApoE(1:2/2, 2:2/3, 3:3/3, 4:4/3)