selected from the JSNP database (http://snp.ims.u-tokyo.ac.jp/) using the criteria that minor allele frequencies were more than 10% in the Japanese population. Five SNPs in the *FOXO1A*, *IRS1* and *PIK3CB* gene loci (1, 1, 1, and 2 SNPs, respectively) were from Bonafe et al. (2003). Additionally 2 non-synonymous SNPs in the *PPARGC1A* gene locus were selected from Ek et al. (2001) (Table 2). The genomic DNA sequences of *FOXO1A*, *INSR*, *IRS1*, *PIK3CB*, *PIK3CG*, and *PPARGC1A* were obtained from the National Center for Biotechnology Information (NCBI, USA) (accession numbers NT_024524, NT_011255, NT_005403, NT_005612, NT_079596, and NT_006316, respectively). For each polymorphism not obtained from JSNP, we ensured that there was a sufficiently high frequency in our subjects by testing 24 control subjects. Polymorphisms were typed by DNA sequencing using the BigDye Terminator cycle sequencing kit and an ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA) or by real-time pyrophosphate DNA sequencing (Ronaghi et al., 1996, 1998) using a PSQ 96 system (Pyrosequencing AB, Uppsala, Sweden) according to the manufacturer's instructions.

2.3. Statistical analysis

The chi-square test was performed between SSCs and control subjects for each allelic and haplotypic frequency. Statistical significance was inferred when $P<0.05$. Pairwise linkage disequilibrium (LD) was estimated as $D=x_{11}-p_1q_1$, where x_{11} is the frequency of haplotype A_1B_1, and p_1 and q_1 are the frequencies of alleles A_1 and B_1 at locus A and B, respectively. A standardized LD coefficient, r, is given by $D/(p_1p_2q_1q_2)^{1/2}$ where p_2 and q_2 are the frequencies of the other alleles at locus A and B, respectively (Hill and Robertson, 1968). Lewontin's coefficient D' is given by D/D_{max}, where $D_{max}=\min[q_1p_2,p_1q_2]$ when $D>0$ (Lewontin, 1964). Haplotype frequencies for multiple loci were estimated by the expectation-maximization method.

Computations were performed using SNPAlyze software (Dynacom, Mobara, Japan).

3. Results

3.1. Pairwise LD in 5 genes

Among the SNPs not from the JSNP database, 3B2 in *PIK3CB* was not polymorphic in our 24 control samples (Table 2). Consequently this SNP was excluded from further experiments. The 92 healthy controls were genotyped for each of the 17 selected SNPs. The strength of LD for each SNP pair within each gene was measured using the $|D'|$ and the r^2 values (Fig. 1). This figure shows that FO1 and FO4 in *FOXO1A* locus are in very tight LD with each other ($r^2=0.789$). FO1 was selected as the representative SNP for this SNP pair and was examined in further analysis. FO4 was excluded from further analysis.

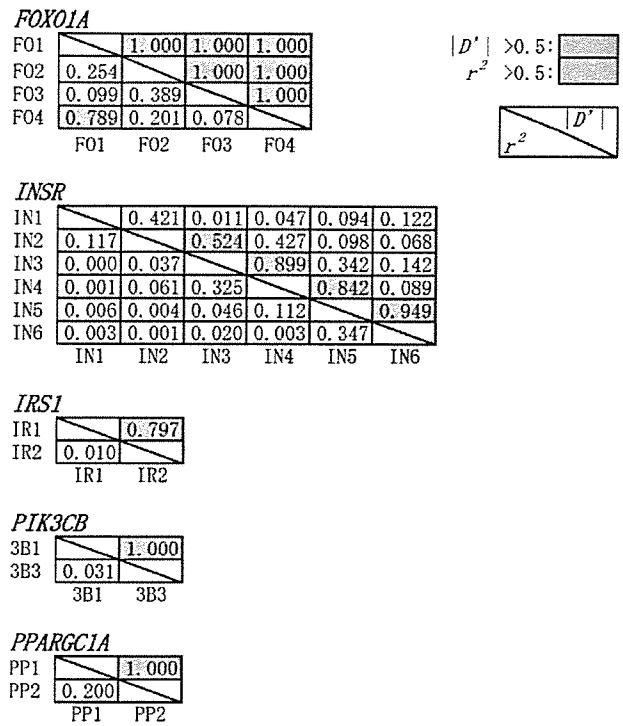

Fig. 1. Pairwise LD in *FOXO1A*, *INSR*, *IRS1*, *PIK3CB*, and *PPARGC1A* evaluated by $|D'|$ and r^2 estimations. The LD between all pairs of SNPs was evaluated by measuring $|D'|$ and r^2 values. Designated SNP IDs are shown in Table 2. Pairwise LD was determined in 92 younger controls. SNP pairs in high LD ($|D'|>0.5$, $r^2>0.5$) are shown as gray boxes. Upper right triangles show values of $|D'|$ and lower left triangles show values of r^2.

3.2. Allele and haplotype frequency distributions in young people and semisupercentenarians

An additional 122 SSCs and 30 healthy younger controls were genotyped for an association analysis using 16 SNPs in six genes (Table 2). Two SNPs (IN3 and IN4) in *INSR* showed a weak difference between SSCs and controls. These SNPs are in LD with each other ($|D'|=0.899$) and are within 2.4 kb of each other (Fig. 1).

Haplotypes were constructed on the basis of the genotype data from these SNPs in *INSR*. The expectation-maximization algorithm, with phase-unknown samples, was used to estimate haplotype frequencies. The MM haplotype (M: major allele) was more frequent in SSCs (57.0%) than in controls (47.3%) ($P=0.030$) (Table 3).

Table 3
Case control study of SSCs and controls using estimated haplotype frequencies in *INSR*

Haplotype ID	SNP ID		Frequency		χ^2	P
	IN3	IN4	SSC	Control		
1	M	M	0.570	0.473	4.729	0.030
2	m	M	0.197	0.224	0.603	0.437
3	M	m	0.000	0.011	3.019	0.082
4	m	m	0.234	0.292	2.076	0.150

M, major allele; m, minor allele.

4. Discussion

To date many genetic variations in the *INSR* locus have been reported to be associated with diseases including diabetes mellitus, leprechaunism, and Rabson–Mendenhall syndrome (Online Mendelian Inheritance in Man # 147670). To our knowledge this is the first report showing associations between genetic polymorphisms of *INSR* and human longevity. Through a study of Japanese centenarians, we found the prevalence of diabetes mellitus in centenarians to be significantly lower than that in the general population (manuscript in preparation). A common variant in the *PPARGC1A* gene has been reported to be associated with type II diabetes mellitus (Ek et al., 2001). The PPARGC1A protein interacts with FOXO1 in an insulin-regulated mechanism of gluconeogenesis (Puigserver et al., 2003). The risk variant (PP1 in Table 2) present frequently in both SSCs and controls (about 50%) and no association with the common variation and longevity was found in this study.

Although a significant association was observed between the IN3-M/IN4-M haplotype in *INSR* and longevity, both SNPs are located in introns and the functional implication of this haplotype association remains uncertain. Very recently a polymorphic variation of *IGF1R* was reported to affect human longevity in the Italian population (Bonafe et al., 2003) but the functional implication of the polymorphic variation also remains to be elucidated. It is noteworthy that both INSR and IGF1R are members of the insulin receptor tyrosine kinase family. Further comprehensive studies of the *INSR* locus, especially on the region including IN3 and IN4, together with the *IGF1R* locus are needed to identify the causal variations that enable or prevent human longevity and to clarify the molecular mechanisms of human longevity.

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Short communication

Blood type B might imply longevity

Kenichiro Shimizu[a,b,*], Nobuyoshi Hirose[b], Yoshinori Ebihara[b], Yasumichi Arai[b], Michiyo Hamamatsu[b], Susumu Nakazawa[b], Yukie Masui[c], Hiroki Inagaki[c], Yasuyuki Gondo[c], Junko Fujimori[d], Yoshiko Kanno[d], Kanoko Konishi[d], Koji Kitagawa[e]

[a]*Health Care Center, Shoko-Chukin Bank, 2-10-17 Yaesu, Chuo-ku, Tokyo 104-0028, Japan*
[b]*Division of Geriatric Medicine, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan*
[c]*Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*
[d]*Faculty of Nursing, Keio University, Kanagawa, Japan*
[e]*Gunma Paz Gakuen College, Gunma, Japan*

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Abstract

The aim of the present study was to investigate the association between blood groups and life expectancy. We compared frequencies of ABO blood group in 269 centenarians (persons over 100 years) living in Tokyo and those in regionally matched controls ($n=7153$). Frequencies of blood types A, O, B, and AB in centenarians were 34.2, 28.3, 29.4, and 8.2%, respectively, while those in controls were 38.6, 30.1, 21.9, and 9.4%, respectively. Blood type B was observed more frequently in centenarians than in controls ($\chi^2=8.41$, $P=0.04$). This tendency also was true in comparison between centenarians and 118 elderly old individuals of the 7153. Approximate one-third of the centenarians were free from serious diseases such as malignancy. However, blood types were not associated with such medical records. Our findings suggest that blood type B might be associated with exceptional longevity. Responsible mechanisms need to be investigated.

Keywords: Centenarian; Blood group; Longevity

1. Introduction

A variety of medical literature has been concerned with blood groups. However, only a small number of issues have been proven to be of clinical importance: the ABO blood type in transfusion, the Rh antigen in incompatible pregnancy, and the Duffy antigen in malarial infection. Recently, blood type O individuals have been reported to have lower plasma concentrations of von Willebrand factor (VWF), a marker of blood coagulability, than persons with other blood types (O'Donnell and Laffan, 2001). Since elevated VWF carries increased risk for ischemic heart disease, cardiovascular events might be less frequent in individuals with blood type O. In other words, associations are possible between blood groups and life expectancy. We therefore investigated frequencies of ABO blood groups in the very old, specifically centenarians.

2. Methods

Of 1206 centenarians living in Tokyo at the time of our study, 269 individuals, 202 women and 67 men, in ages from 100 to 109 years (Mean 101.2 [Std Dev 1.8]) gave informed consent and agreed to a visit for our medical examinations. We identified the ABO blood group using their blood samples and examined their medical records with respect to hypertension, cardiovascular disease, apoplexy, diabetes, femoral fracture, malignancy, and chronic lung disorder. As a regionally matched control group, we selected 7153 individuals (1673 women and 5480 men) aged 17–93 years (mean 54.8 [Std Dev 11.0]) who came to the Keio Health Consulting Center for annual medical check-ups in 2003. Of the 7153, the following

* Corresponding author. Tel.: +81 3 3272 6111x430; fax: +81 3 3271 5296.
E-mail address: shimizu_kenichiro@1986.jukuin.keio.ac.jp (K. Shimizu).

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Table 1
Comparison of blood group frequencies

	Blood type			
	A	O	B	AB
Observation				
Centenarians (n=269)	92 (34.2)	76 (28.3)	79 (29.4)	22 (8.2)
Controls (n=7153)	2759 (38.6)	2153 (30.1)	1570 (21.9)	671 (9.4)
Old controls (n=740)	288 (38.9)	219 (29.6)	159 (21.5)	74 (10.0)
Elderly old controls (n=118)	48 (40.7)	34 (28.8)	27 (22.9)	9 (7.6)
Expectation				
General population[a]	109 (38.7)	83 (29.3)	63 (22.2)	28 (10.0)
Tokyo area[b]	108 (38.3)	83 (29.1)	63 (22.4)	29 (10.2)

Data are numbers followed by percentages in parentheses. Differences between centenarians and controls and between observed and expected frequencies were investigated by χ^2-tests. Observation in centenarians was significantly different from that in controls (χ^2 [d.f.=3]=8.41, P=0.04) and from expectations (χ^2 [d.f.=3]=12.68, P=0.005 for Japan; χ^2 [d.f.=3]=11.91, P=0.007 for metropolitan Tokyo). Notably, blood type B was observed more frequently in centenarians. This predominance of blood type B, although not being statistically significant, was observed in comparison between centenarians and old controls (χ^2 [d.f.=3]=7.17, P=0.06) and between centenarians and elderly old controls (χ^2 [d.f.=3]=2.25, P=0.52).

[a] Calculated from data for 4464349 individuals in a 1978 survey throughout Japan.

[b] Calculated from data for 293688 Tokyo-area individuals among the above 4464349.

two subgroups were constituted: Old control group consisting of 740 individuals over 70 years (mean 74.8 [Std Dev 4.4]) and elderly old control group of 118 over 80 years (mean 82.8 [Std Dev 2.8]). Differences in frequencies were investigated by χ^2-tests. A $P<0.05$ was considered to be statistically significant.

3. Results

Frequencies of blood types A, O, B, and AB in the centenarian group were 34.2, 28.3, 29.4, and 8.2%, respectively; those in the control group were 38.6, 30.1, 21.9, and 9.4%, respectively (Table 1). Observed frequencies differed significantly between these two groups (χ^2 [d.f.=3]=8.41, P=0.04). Notably, blood type B was observed more frequently in centenarians than in controls. This predominance of blood type B, although not being statistically significant, was observed in comparison between centenarian group and old control subgroup (χ^2 [d.f.=3]=7.17, P=0.06) and between centenarian group and elderly old control subgroup (χ^2 [d.f.=3]=2.25, P=0.52). We next compared the frequencies of ABO blood groups in the centenarians with those in a general Japanese population as calculated from a 1978 survey conducted in 4464349 individuals throughout Japan (Fujita et al., 1978). A similar result showing an increased frequency of blood type B in centenarians was obtained (χ^2 [d.f.=3]=12.68, P=0.005). This also was true when the centenarians were compared with 293688 Tokyo-area individuals among the 4464349 (χ^2 [d.f.=3]=12.02, P=0.007). The frequency distribution of blood types in the 1978 survey was almost the same as that in a 1933 survey of 121200 individuals (Furuhata, 1933) and that for 5819007 blood donors profiled in an annual report of the Japanese Red Cross (year 2000) (The Japanese Red Cross Society, 2002). Our findings suggest that to some degree blood type B might be associated with exceptional longevity.

The following important diagnoses were recorded in centenarians: hypertension (n=78), cardiovascular disease (n=51), apoplexy (n=37), diabetes (n=9), femoral fracture (n=66), malignancy (n=24), and chronic lung disorder (n=29). Approximate one-third of the centenarians were free from these important diseases. However, blood types were not associated with such medical records (Table 2) (χ^2 [d.f.=3]=4.16, P=0.25). This finding implies that blood type B might be related to surviving serious diseases rather than escaping them.

4. Discussion

One would expect an abundance of centenarians with blood type O, since plasma concentrations of VWF, a cardiac risk factor, are lower in blood type O individuals. However, the frequency of blood type O in centenarians tended to be lower than expected. Instead, we found

Table 2
Relationship between blood groups and medical history

Blood groups	Medical history of important diseases	
	Absence	Presence
A (n=92)	32 (34.8)	60 (65.2)
O (n=76)	19 (25.0)	57 (75.2)
B (n=79)	18 (22.8)	61 (77.2)
AB (n=22)	8 (36.4)	14 (63.6)
Total (n=269)	77 (28.6)	192 (71.4)

Data are numbers followed by percentages in parentheses. Relationship between blood groups and medical history was investigated by χ^2-tests (χ^2 [d.f.=3]=4.16, P=0.25).

a possible association of blood type B with exceptional longevity. Differences in ABO blood groups are determined by antigens in the glycocalyx on the surface of the erythrocyte. These antigens are present in most tissues as well as on erythrocytes. Therefore, differences in the glycocalyx expressed by cells might elicit differing responses in biomedical phenomena apart from hemagglutination. Henry et al. summarized patterns in which blood types may be associated with various diseases, stating that bacterial infections tend to attack individuals with blood type A, while viral infections tend to be associated with blood type O. Also, cancers and clotting disorders tend to be associated with blood type A, while autoimmune diseases and bleeding disorders are associated with blood type O (Henry and Samuelsson, 2000). According to these tendencies, blood type B individuals might be more likely to escape serious illnesses, and therefore show longevity. On the other hand, our findings imply that blood type B might contribute to longevity via biomedical mechanisms favorable for surviving serious diseases rather than escaping them. In future, blood groups will need to be investigated from an aspect of glyconomics, or the study of sugar-modifications to proteins that affect structure and function.

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Reviews

Aging and HDL Metabolism in Elderly People More Than 100 Years Old

Yasumichi Arai and Nobuyoshi Hirose

Department of Geriatric Medicine, Keio University School of Medicine, Tokyo Japan.

Epidemiological studies have enhanced the importance of high-density lipoprotein (HDL) as a risk factor for CAD, as well as disability and frailty in the oldest elderly. Therefore, HDL and molecules involved in HDL metabolism seem to be attractive candidates for longevity-promoting factors. A series of observational studies has demonstrated that the predominance of the larger, more lipid-rich HDL2 subclass is a reproducible phenotype among centenarians. This finding was recently evolved by nuclear magnetic resonance technology in quantification of lipoprotein particle size. However, results of investigations into the mechanisms underlying the lipoprotein profiles in the oldest elderly have been conflicting. Genetic variation in cholesteryl ester transfer protein (CETP), which is a carrier protein in reverse cholesterol transport, was demonstrated to have no association with longevity in one study, but to have positive impacts on large HDL particles and longevity in another. Regarding environmental factors, acute phase reactant and nutritional status are frequently associated with HDL-C levels in the oldest elderly, however, the causality of the association remains to be elucidated. Determination of the association between cognitive function and HDL in the oldest elderly is also a future task. To obtain further insight into the mechanistic roles of low HDL in the pathophysiology of geriatric syndrome, a much greater effort should be invested in this research field. *J Atheroscler Thromb, 2004; 11: 246–252.*

Key words: High-density lipoprotein, Reverse cholesterol transport, Cholesteryl ester transfer protein, Nutrition, Interleukin 6

Introduction

Numerous epidemiological studies have confirmed that a low level of high-density lipoprotein cholesterol (HDL-C) is a strong risk factor for coronary artery disease (CAD) (1). Moreover, clinical trials have also established that increasing HDL-C levels by drugs could recuce CAD risk (2, 3). Recently, the importance of HDL as a risk factor for CAD or all-cause mortality has been emphasized, especially in the oldest population. Low HDL-C level, but not high LDL-C level, has been demonstrated to be associated with increased risk of CAD and stroke in the elderly over 85 years old (4). Low HDL-C combined with low levels of serum albumin indicate higher mortality among elderly population (5). Based on this evidence, one can hypothesize that intervention by elevating HDL levels or mechanism(s) for maintaining high HDL levels could reduce CAD risk, thus bringing about longer life expectancy. This hypothesis is compatible with observations that familial hyperalphalipoproteinemia often coexists with longevity (6), and that higher HDL levels are found among healthy elderly aged 85–89 years as compared to those in middle-aged subjects (7). Accordingly, HDL and molecules involved in HDL metabolism seem to be attractive candidates for longevity-promoting factors, and informative findings in this research field are accumulating. This article reviews the current status of our knowledge of HDL metabolism and its regulatory mechanism(s) in the oldest elderly, including centenarians.

Address for correspondence: Nobuyoshi Hirose, Department of Geriatric Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan.
E-mail: hirosen@sc.itc.keio.ac.jp
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HDL Cholesterol and Its Subpopulations in Centenarians

In the general population, HDL-C levels as well as total cholesterol decrease in older ages (8). Menopause, change in body mass index, and age itself may be important determinants of decline in HDL levels among the elderly. However, results from centenarian studies have been conflicting. Some investigators have reported that centenarians showed a lipid profile similar to that of the middle-aged population (9), but others have demonstrated lower total and HDL cholesterol in centenarians (10, 11). This discrepancy may be attributable to differences in sampling, and the nutritional and functional status of centenarians (this issue is also described in later paragraphs).

HDL particles are heterogeneous in apolipoprotein composition, density, particle size, and electrophoretic mobility. To fractionate HDL into subpopulations, various procedures including ultracentrifugation, polyanion precipitation, and gradient gel electrophoresis have been applied. HDL comprises two subclasses: the larger, more lipid-rich HDL2, and the smaller, denser HDL3. Lipoprotein profiles and HDL subclasses among elderly people have been extensively studied. Ettinger *et al.* measured HDL subfractions by a precipitation method in 1,127 female and 825 male subjects over 65 year old, and demonstrated that HDL2-C, but not HDL3-C, slightly increased with age in both men and women (12). Although the analytic procedure was different, predominance of the HDL2 subfraction was observed among octogenarians (13) and centenarians. Barbagallo *et al.* examined HDL subfractions using polyacrylamide gradient gels in 16 female centenarians and 32 healthy normolipidemic postmenopausal women. They did not find any significant difference in lipid or apolipoprotein levels, however, the HDL2b level was increased, and the HDL3a level decreased in centenarians, compared to those in the controls (9). We have also demonstrated relatively higher levels of HDL2-C among 42 centenarians by ultracentrifugation (11). Recently, nuclear magnetic resonance (NMR) spectroscopy was efficiently applied for the quantification of lipoprotein particle size (14, 15). Using these new methods, Barzilai *et al.* analyzed lipoprotein subclasses among Ashkenazi Jews over 95 years old and their offspring (16). Although the differences in cholesterol levels were modest, they found that the particle sizes of both HDL and LDL were remarkably higher in the long-lived subjects compared with those in elderly controls from the Ashkenazi population. In addition, the offspring of long-lived subjects had intermediate HDL and LDL particle sizes between those of their parents and those of the controls, indicating that these lipoprotein phenotypes are heritable, and may be a causal biological candidate for longevity-promoting factors. Although these findings were obtained from cross-sectional studies and should be confirmed by longitudinal observation, the predominance of larger particles in HDL subclasses is the most reproducible phenotype among subjects who have reached extreme old age.

Factors Which Regulate HDL Metabolism in Centenarians

Gene regulation of HDL cholesterol

The levels of plasma HDL-C and its subpopulations are regulated by a number of environmental, physiological, and genetic factors. Family and twin studies have estimated that the heritability of HDL-C levels varies from 35 to 66% (17, 18). One report has suggested that the genetic influence of lipoproteins is not dependent on age (18), however, another demonstrated that the genetic components in lipoprotein variations increased after menopause (19). It will never be practicable to estimate the gene effects on lipoprotein levels in centenarians by a similar method, however, several genetic variations affecting HDL metabolism have been nominated as human longevity genes (Table 1).

Cholesteryl ester transfer protein gene

The anti-atherogenic property of HDL particles is largely explained via its role in reverse cholesterol transport (RCT). Cholesteryl ester transfer protein (CETP) is a carrier protein in RCT, that mediates the transfer of cholesteryl esters from HDL to apoB-containing lipoproteins, thus enhancing RCT. Despite much accumulation of clinical and experimental data, the role of CETP in atherosclerosis remains to be elucidated. Several mutations at the CETP gene locus, which cause depletion of CETP activity and consequently high HDL-C in plasma, have been described, especially in Japanese subjects (20–22). CETP deficiency has been demonstrated to regulate lipoprotein profiles in an antiatherogenic manner, inducing high levels of HDL subclass 2, and accordingly to be associated with longevity (20). However, these mutations exhibited proatherogeneity when combined with a serum HDL-C level < 60 mg/dl (23), or with low activity of hepatic lipase (24). Furthermore, in Omagari city, in which CETP deficiency caused by a G-to-A mutation at intron 14 is extremely frequent, the prevalence of the mutation was higher in patients with CAD, and lower in elderly subjects over 80 years old, as compared to that in control subjects, suggesting a negative impact of the mutation on longevity (25). The roles of CETP gene variations in longevity are also in debate. Recently, we investigated 256 centenarians and 190 controls (mean age, 40 years old; range, 22–65) for the implication of CETP deficiency and Taq1B polymorphisms of the CETP gene as a longevity factor (26). In this study, we explored the finding that, although heterozygous CETP deficiency as well as

Table 1. Associations between genetic variations that modulate HDL metabolism and longevity.

Genes	Polymorphisms	Population	Results	Reference No.
APOA1	*Msp* I	Italian cohort	P allele was dominant in the oldest elderly	41
CETP	Intron 14 G to A	Japanese cohort	Intron 14 splicing defect was less frequent in subjects > 80 years old	25
CETP	Intron 14 G to A	Japanese centenarians	No association with longevity	26
CETP	G445A	Japanese centenarians	No association with longevity	26
CETP	*Taq* IB	Japanese centenarians	No association with longevity	26
CETP	I405V	Ashkenazi	VV genotype was dominant in probands with longevity	16
LPL	*Pvu* II	Japanese centenarians	No association with longevity	26
LPL	*Hind* III	Japanese centenarians	No association with longevity	26
HL	– 514 C/T	Japanese centenarians	No association with longevity	26
ABCA1	219 R/K	Japanese centenarians	No association with longevity	26
PON1	Codon 192	Italian centenarians	B allele was more frequent in centenarians	42

APOA1: apolipoprotein A1, CETP: cholesteryl ester transfer protein, LPL: lipoprotein lipase, HL: hepatic lipase, ABCA1: ATP-binding cassette transport 1, PON1: paraoxonase 1
G: glycine, A: alanine, I: isoleucine, V: valine, C: cytosine, T: thymine, R: arginine, K: lysine

the B2 allele of Taq1B polymorphisms were consistently associated with lower CETP mass and higher HDL-C concentrations, neither of these allelic variations were significantly associated with longevity. In contrast, at least one variant in the CETP gene was recently shown to have a strikingly higher impact on longevity. Barzilai *et al.* demonstrated in the above-mentioned study (16) that long-lived Ashkenazi individuals and their offspring had a dramatically higher frequency of homozygosity for the 405 valine allele of CETP (VV genotype) as compared to that in ethnicity-matched controls. They also found that the VV genotype was associated with lower CETP concentration and increased HDL and LDL particle size, suggesting that this variant could be a genetic component for this unique lipoprotein phenotype among long-lived subjects, and therefore a promising candidate for a longevity gene. Although these findings await testing in other populations, Barzilai's study has raised the encouraging possibility that longitudinal observations of the offspring of long-lived subjects will determine whether large lipoprotein particles and the I405V polymorphism of the CETP gene are causal candidates for longevity.

Hepatic and lipoprotein lipase

In addition to CETP, hepatic lipase (HL), lipoprotein lipase (LPL), and ATP-binding cassette transporter 1 (ABCA1) are important components of RCT. Because these lipases and ABCA1 may regulate lipoprotein metabolism in concert with CETP, we simultaneously investigated associations of genetic variations involved in RCT and longevity (Table 1).

LPL is a key enzyme that regulates the lipolysis of triglyceride-rich lipoproteins (TRL). Several polymorphisms of the LPL gene have been shown to be associated with atherogenic lipid profiles (27–29), CAD (28, 29), and diabetes (28), yet the results have been conflicting. In a study of 256 centenarians (26), we demonstrated a significant association between *Pvu* II (-/-) variants of the LPL gene and higher HDL-C concentration in centenarians, however, we found no association between this genetic polymorphism and longevity. Neither ABCA1 219R/K genotype nor the HL – 514C/T promoter polymorphism had significant associations with longevity or lipid profiles in the centenarians or the controls. The negative results from our study do not necessarily contradict the putative roles of RCT in longevity. Exposure to Japanese traditional food with very low fat (10% of total calories) during the most susceptible period for CAD may exempt the obligation of RCT in longevity. Further study with a sample size large enough to calculate gene-gene interactions and to evaluate the overall efficacy of RCT is required to investigate what combination of candidate gene polymorphisms could be optimum for human longevity.

Effects of nutritional status and inflammation

Other than genetic components, numerous environmental factors, including weight change, diet and nutrition, use of medications, smoking habit, acute phase response, undiagnosed disease, physical activities, and aging itself, may substantially affect HDL-C levels. Recently, as evidence suggesting clinical implications of C-reactive protein (CRP) in predicting cardiovascular risk has been increasing, associations between acute phase reactants such as CRP and interleukin 6 (IL-6), and HDL have been frequently investigated. In an acute phase model of rabbits, remodeling of HDL composition includ-

ing displacement of apo A1 by serum amyloid A (SAA), an inflammation marker, was reported (30). HDL has potentially antioxidant, anti-inflammatory (31), and anticoagulant properties, however, it was demonstrated to have proinflammatory activities during acute phase response (32). In epidemiological studies, an inverse correlation of CRP with HDL-C levels was observed among general populations of elderly (33) and hospitalized elderly patients (34). Regarding the oldest elderly, however, we should consider the effects of age-related immune alteration or activation, which is ubiquitously observed in this exceptional population (35). A modest increase in CRP (11, 26), IL-6 (11), and tumor nocrosis factor-α (TNF-α) (36) was demonstrated in centenarians even when they were in good health. Moreover, low-grade inflammatory activation is often associated with undernutrition indicated by hypoalbuminemia, poor health status, and chronic conditions such as atherosclerosis and dementia in the oldest elderly. Therefore, we conducted a comprehensive analysis of diet and nutritional status, inflammatory markers, and physical and cognitive function as well as apo E polymorphisms, as predictors for lipid profiles in centenarians (Table 2). In the study, the levels of albumin, prealbumin, and transferrin showed a strong and positive correlation with HDL-C and apo A1. Log-transformed CRP and IL-6 were inversely correlated with HDL and apo A1. Multiple regression analysis was performed to evaluate independent contributions of these factors to the variance of lipid profiles, and indicated that the level of albumin was the strongest predictor of HDL-C in centenarians (11). However, in the oldest elderly, malnutrition often coexists with inflammatory activation, and the albumin level does not necessary reflect nutritional status. So, it seems to be difficult to distinguish the independent effects of nutritional status and inflammation markers on lipoprotein profiles, even by longitudinal observation.

Cognitive function and HDL

Several lines of evidence have suggested that low HDL-C is associated with cognitive decline in the oldest elderly. Although the pathophysiology of dementia in this population has not been fully uncovered, vascular components have been generally implied. This may explain in part the association between HDL and dementia in those of extremely old age, however, van Exel *et al.* (37) demonstrated that cognitive impairment in the oldest elderly was at least partially independent of atherosclerotic disease. They examined 561 subjects at least 85 years old for associations between serum lipids, cognitive impairment, and CAD, and demonstrated that a low MMSE score was associated with a low HDL-C level, even when subjects with CAD and stroke were excluded. They proposed that the antiaggregation and anti-inflammatory properties of HDL might explain the associations between HDL and cognitive impairment. In centenarians, we demonstrated that the level of HDL-C, but not TC or LDL-C, was significantly lower in the subset with cognitive impairment (11). In addition to cross-sectional design, the accumulation of lower albumin, higher CRP and IL-6, lower ADL scores, and the higher rate of apo E4 in this subset made it difficult to determine the causality of as-

Table 2. Pearson's correlation coefficients of nutritional indices, inflammation markers, Apo ε alleles, dietary intake, and ADL score with lipid parameters in centenarians.

	TC ($n = 75$)	LDL-C ($n = 42$)	HDL-C ($n = 75$)	HDL2-C ($n = 42$)	HDL3-C ($n = 42$)	apo A1 ($n = 75$)	apo B ($n = 75$)
BMI	0.047	0.069	0.002	0.023	0.082	0.079	0.116
Albumin	0.226	0.176	0.411†	0.401†	0.420†	0.539†	0.113
Prealbumin	0.446†	0.087	0.295*	0.095	0.330*	0.442†	0.051
Transferrin	0.258*	0.039	0.303	0.148	0.236	0.419†	0.046
CETP mass	0.136	0.009	0.068	0.076	0.106	0.146	0.047
log (CRP)	−0.125	0.239	−0.215	−0.253	0.040	−0.298*	0.168
log (IL-6)	−0.143	0.133	−0.213	−0.222	−0.216	−0.309†	0.024
Number of ε 2 alleles	−0.239*	−0.449†	0.009	0.032	0.055	0.043	−0.316*
Number of ε 4 alleles	0.158	0.002	−0.288*	0.078	−0.268	−0.325†	0.016
Energy intake	0.005	0.257	0.060	0.017	0.103	0.050	0.060
Fat intake	0.049	0.330*	0.101	0.133	0.012	0.045	0.095
ADL score	0.121	0.128	0.326†	0.133	0.469†	0.328†	0.013

* $p < 0.05$, † $p < 0.01$, BMI: body mass index, CETP: cholesteryl ester transfer protein, CRP: C-reactive protein, IL-6: Interleukin-6, ADL: activity of daily living

Adapted from Arai Y *et al.*: J Am Geriatr Soc, 49: 1434–1441, 2001 with permission of ©Blackwell Publishing Ltd.

sociation between dementia and low HDL. Recently, statin treatment for more than 5,000 elderly over 70 years old lowered LDL-C by 34%, elevated HDL by 5% and reduced cardiovascular death, but had no impact on cognitive function and disability (38). Based on these findings, cognitive impairment in the oldest elderly is multifactorial, and low HDL-C may be a reproducible marker, rather than a determinant.

Conclusion

There is no doubt that low HDL-C is a potent risk factor for CAD in the elderly. Furthermore, low HDL-C can be a reliable marker for frailty and poor prognosis among the oldest elderly, when combined with hypoalubuminemia and/or proinflammatory cytokinemia. A series of epidemiological studies has described a significant association of large particle size in HDL, and presumably in LDL, with human longevity. Very recently, Geesaman et al. first identified a genetic variant of microsomal transfer protein (MTP) as a human longevity gene by a genome-wide linkage study using 137 sibships of long-lived subjects, followed by haplotype-based mapping (39, 40). These findings suggest a strong implication of lipoprotein metabolism in the process of living an extremely long life. Compared with the fundamental evidence from the clinical research field of cardiology, longitudinal and interventional studies from the view point of gerontology have been lacking. It is of special interest whether elevation of HDL-C by pharmacological intervention could improve cogniftive function in the oldest elderly. To obtain further insight into the mechanistic roles of low HDL in the pathophysiology of geriatric syndrome, a much greater effort should be invested in this research field.

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