Hiroshi Mabuchi, Department of Internal Medicine, Kanazawa University

Nobuhiro Yamada, Department of Internal Medicine, Tsukuba University

Hiroshige Itakura, Ibaraki Christian University

Yuichi Ishikawa, Faculty of Health Sciences, Kobe University

Tadayoshi Ouchi, Department of Geriatric Medicine, University of Tokyo

Hiroshi Horibe, Keisen Clinic

Tamio Teramoto, Department of Internal Medicine, Teikyo University

Hidenori Arai, Department of Geriatric Medicine, Kyoto University

Co-principal investigators (institutes): Kazuaki Shimamoto (Sappro Medical University), Takao Koike (Hokkaido University), Akizuki Morikawa (Asahikawa Red Cross Hospital), Makoto Tominaga (Yamagata University), Toshihiro Suda (Hirosaki University), Nobuyuki Sugawara (Mizusawa general hospital), Hideo Hamaguchi (Tsukuba University), Saburo Hori (St.Luka's International Hospital), Hideaki Bujo (Chiba University), Fumitaka Osuzu (National Defense Medical College), Koichi Kozaki (Tokyo University), Toshiro Murase (Toranomon Hospital), Katsuji Senda (Hamamatsu Social Insurance Hospital), Tomoo Okada (Nihon University), Akihiro Inazu and Toshinori Higashikata (Kanazawa University), Isamu Miyamori and Koji Oida (University of Fukui Faculty of Medical Sciences), Susumu Miyamoto (Himi Municipal Hospital), Akihisa Iguchi (Nagoya University), Nagahiko Sakuma (Nagoya City University), Taku Yamamura (National Cardiovascular Center), Shizuya Yamashita (Osaka University), Toshiko Kawakita and

Atsuhiko Sato (Kyoto Center for preventive medicine), Mitsuhiro Yokoyama (Kobe University), Genshi Egusa (Egusa clinic), Masunori Matsuzaki (Yamaguchi University), Masayoshi Kihata (Chugoku Central Hospital), Hitoshi Kukida (Udazima Social Insurance Hospital), Shoji Kohori (National Hospital organization Kumamoto Medical Center), Kyosuke Yamamoto (Saga University Faculty of Medicine), Sadatoshi Birou (Kagosima University), Takao Ota (University of Ryukyus), Masato Ageta (Miyazaki Prefectural Nichinan Hospital)

References

1. Anderson KM, Castelli WP, Levy D: Cholesterol and mortality. 30 years of follow-up from the Framingham study. JAMA 257:2176-2180, 1987

2. Stamler J, Wentworth D, Neaton JD: Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 256:2823-2828, 1986

3. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA 251:351-364, 1984

4. Holme I: Cholesterol reduction and its impact on coronary artery disease and total mortality. Am J Cardiol 76:10C-17C, 1995

5. Ericsson CG, Hamsten A, Nilsson J, Grip L, Svane B, de Faire U: Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. Lancet 347:849-853, 1996

6. Shepherd J. Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ: Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med 333:1301-1307, 1995

7. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, Braunwald E: The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 335:1001-1009, 1996

8. Levy RI, Moskowitz J: Cardiovascular research: decades of progress, a decade of promise. Science 217:121-129, 1982

9. Vartiainen E, Puska P, Pekkanen J, Tuomilehto J, Jousilahti P: Changes in risk factors explain changes in mortality from ischaemic heart disease in Finland. *Bmj* 309:23-27, 1994

10. Marmot MG, Syme SL, Kagan A, Kato H, Cohen JB, Belsky J: Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: prevalence of coronary and hypertensive heart disease and associated risk factors. Am J Epidemiol 102:514-525, 1975

11. Johnson CL, Rifkind BM, Sempos CT, Carroll MD, Bachorik PS, Briefel RR, Gordon DJ, Burt VL, Brown CD, Lippel K, et al.: Declining serum total cholesterol levels among US adults. The National Health and Nutrition Examination Surveys. JAMA 269:3002-3008, 1993

12. Konishi T: Total Serum Cholesterol Levels in Normal Subjects in Japan. Jpn Circ J 29:505-510, 1965

13. Sekimoto H, Goto Y, Naito C, Yasugi T, Okido M, Kuzuya F, Takeda R, Yamamoto A, Fukuzaki H, et al.: Changes of serum total cholesterol and triglyceride levels in normal subjects in Japan in the past twenty years. Research committee on familial hyperlipidemia in Japan. Jpn Circ J 47:1351-1358, 1983

14. Current state of and recent trends in serum lipid levels in the general Japanese population. Research Committee on Serum Lipid Level Survey 1990 in Japan. J Atheroscler Thromb 2:122-132, 1996

15. Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL, Buring JE: Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. *Circulation* 96:2520-2525, 1997

16. Yamamoto A, Yamamura T, Kawaguchi A, Kameda K, Matsuzawa Y: Triglyceride and glucose intolerance as a risk factor for coronary heart disease. *Cardiology* 78:185-193, 1991

17. Lauer MS, Fontanarosa PB: Updated guidelines for cholesterol management. JAMA 285:2508-2509, 2001

18. Grundy SM: United States Cholesterol Guidelines 2001: expanded scope of intensive low-density lipoprotein-lowering therapy. Am J Cardiol 88:23J-27J, 2001

19. Wood D, De Backer G, Faergeman O, Graham I, Mancia G, Pyorala K: Prevention of coronary heart disease in clinical practice: recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention. Atherosclerosis 140:199-270, 1998

Figure legends

Fig. 1

Trends of serum cholesterol levels in Japanese in 40 years from 1960 to 2000. Results of the surveys carried out by the members of Japan Atherosclerosis Society. The mean cholesterol level in men and women of 20-39 years, men of 40-59 years, and women of 50-69 years.

Fig. 1

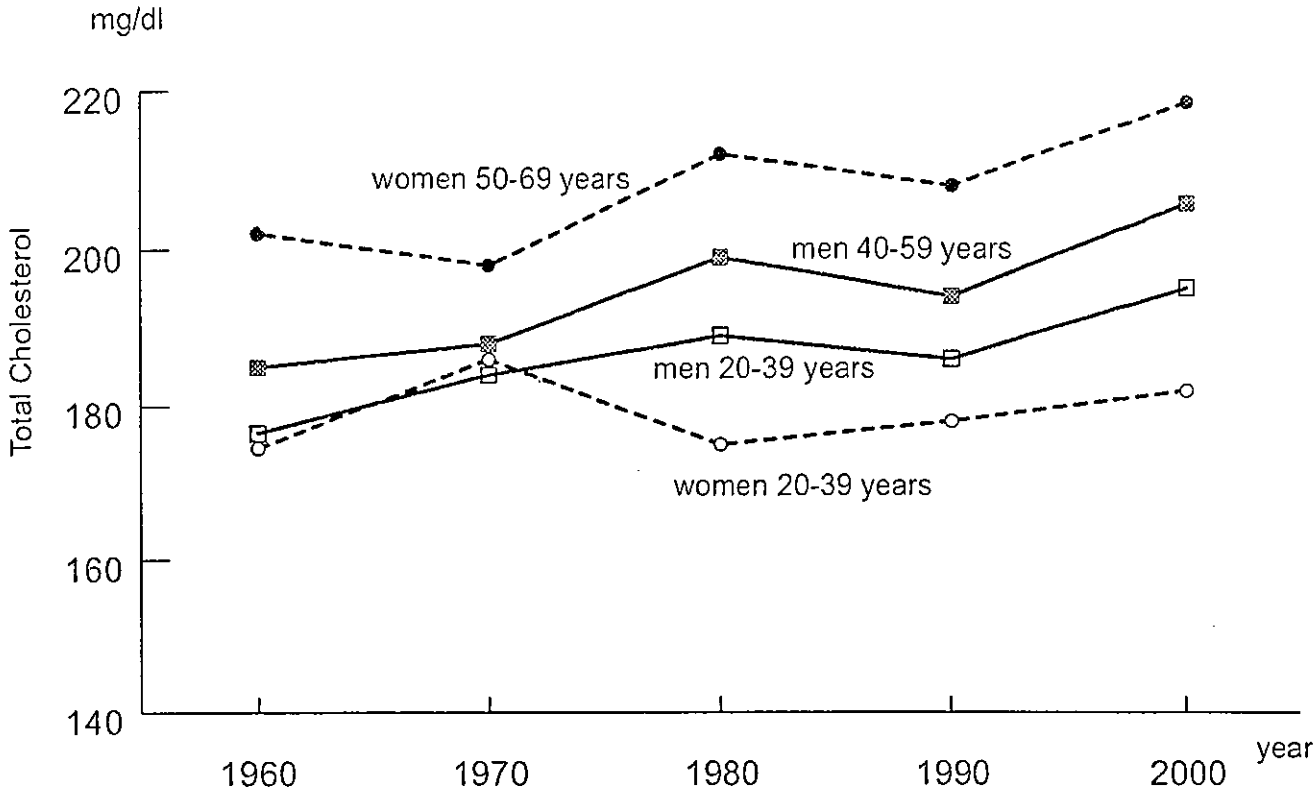
mg/dl
220
200
180
160
140
Total Cholesterol
women 50-69 years
men 40-59 years
men 20-39 years
women 20-39 years
1960
1970
1980
1990
2000
year

Table 1. Serum total cholesterol (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	216	186	27	102	185	26	114	186	27
10-19	465	181	28	196	178	28	269	183	27
20-29	1,256	180	31	394	181	32	861	180	31
30-39	1,642	195	34	1,101	200	34	541	185	31
40-49	3,564	201	33	2,399	204	32	1,165	195	32
50-59	3,467	211	34	2,328	207	33	1,139	218	34
60-69	1,625	209	34	844	200	34	780	218	32
70-79	551	206	33	271	198	32	280	214	32
80-89	53	197	33	23	181	29	30	208	32
Total	12,839	201	34	7,658	202	34	5,179	200	35

Table 2. Serum triglyceride (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	216	56	30	102	53	30	114	59	30
10-19	465	67	36	196	66	39	269	68	33
20-29	1,256	83	65	394	105	74	861	73	58
30-39	1,642	118	109	1,101	142	123	541	70	42
40-49	3,564	129	103	2,399	150	112	1,165	87	63
50-59	3,467	129	102	2,328	139	115	1,139	108	66
60-69	1,625	123	83	844	128	98	780	117	64
70-79	551	118	63	271	123	67	280	113	59
80-89	53	100	44	23	93	38	30	105	47
Total	12,839	118	96	7,658	136	109	5,179	92	62

Table 3. Serum HDL-cholesterol (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	216	69	15	102	70	15	114	68	16
10-19	465	65	14	196	63	14	269	66	13
20-29	1,255	64	14	393	56	13	861	68	14
30-39	1,637	58	15	1,096	54	14	541	67	14
40-49	3,545	58	15	2,380	55	14	1,165	65	15
50-59	3,434	59	16	2,295	56	15	1,139	65	16
60-69	1,614	57	14	833	55	14	780	60	14
70-79	551	57	15	271	55	15	280	60	15
80-89	53	58	16	23	54	12	30	61	18
Total	12,770	59	15	7,589	55	14	5,179	65	15

Table 4. Serum LDL-cholesterol (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	154	104	22	70	101	22	84	106	22
10-19	162	103	24	51	101	21	111	104	25
20-29	713	97	24	240	105	26	472	93	22
30-39	751	112	29	484	119	29	267	101	25
40-49	1,179	121	30	750	124	31	429	116	29
50-59	1,243	127	30	733	125	30	510	130	30
60-69	726	129	31	387	124	30	338	135	29
70-79	246	126	28	117	120	27	129	130	28
80-89	32	123	29	10	113	27	22	127	30
Total	5,206	118	31	2,842	121	30	2,362	115	31

Table 5. Serum RLP-cholesterol (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	154	1.9	0.6	70	2.0	0.6	84	1.9	0.7
10-19	161	2.5	1.2	51	2.5	1.1	110	2.5	1.3
20-29	712	3.5	3.1	240	4.5	4.2	471	2.9	2.2
30-39	762	5.0	6.0	493	6.2	6.9	269	2.7	2.6
40-49	1,211	5.2	7.7	774	6.2	8.7	437	3.2	4.9
50-59	1,322	4.8	6.2	791	5.2	7.4	531	4.3	3.7
60-69	662	4.6	7.3	363	5.1	9.4	298	4.1	3.5
70-79	206	4.1	3.7	98	4.3	4.4	108	4.0	2.9
80-89	28	3.7	2.5	8	2.4	1.6	20	4.2	2.7
Total	5,218	4.5	6.2	2,888	5.4	7.6	2,328	3.4	3.5

Table 6. Fasting glucose (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	158	88	7	74	88	7	84	87	6
10-19	170	85	6	57	87	7	113	85	6
20-29	996	88	16	340	89	20	655	87	13
30-39	1,281	92	15	886	93	14	395	90	18
40-49	2,865	95	18	2,018	97	19	847	90	12
50-59	2,909	99	20	2,002	101	20	907	94	19
60-69	1,489	98	21	752	102	25	737	95	15
70-79	531	98	16	257	99	16	274	97	15
80-89	52	103	27	22	104	36	30	102	20
Total	10,451	95	19	6,408	98	20	4,042	92	16

Table 7. HbA1c for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	155	4.7	0.2	72	4.7	0.2	83	4.7	0.2
10-19	171	4.7	0.3	58	4.7	0.3	113	4.6	0.3
20-29	1,147	4.6	0.4	374	4.6	0.6	772	4.6	0.3
30-39	1,261	4.7	0.5	871	4.7	0.5	390	4.7	0.4
40-49	2,536	4.9	0.6	1,844	4.9	0.7	692	4.8	0.5
50-59	2,676	5.1	0.7	1,879	5.1	0.7	797	5.1	0.7
60-69	1,141	5.2	0.8	614	5.3	0.9	527	5.2	0.6
70-79	443	5.3	0.7	209	5.3	0.7	234	5.4	0.8
80-89	52	5.4	0.8	22	5.4	1.0	30	5.3	0.6
Total	9,582	4.9	0.7	5,943	5.0	0.7	3,638	4.9	0.6

Table 8. Serum insulin (μU/ml) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	216	7	5	102	7	6	114	7	4
10-19	463	7	7	196	6	5	267	8	8
20-29	1,171	11	13	382	10	11	788	12	14
30-39	1,410	8	9	942	8	9	468	8	9
40-49	2,734	7	5	1,877	7	5	857	7	6
50-59	2,636	6	6	1,731	6	4	905	7	8
60-69	1,118	6	5	589	6	5	528	6	5
70-79	440	6	15	211	5	6	229	7	20
80-89	53	6	5	23	6	6	30	6	4
Total	10,241	7	8	6,053	7	6	4,186	8	10

Table 9. Serum uric acid (mg /dl) for each 10-year group in Japanese

Age	all			men			women		
	N	mean	S.D.	N	mean	S.D.	N	mean	S.D.
0-9	0	-	-	0	-	-	0	-	-
10-19	3	6.7	0.7	3	6.7	0.7	0	-	-
20-29	410	4.7	1.4	137	6.1	1.3	273	4.0	0.8
30-39	927	5.6	1.5	714	6.0	1.3	213	4.0	0.9
40-49	2,425	5.5	1.5	1,763	6.1	1.3	662	4.1	0.9
50-59	2,459	5.5	1.4	1,762	6.0	1.3	697	4.3	0.9
60-69	1,141	5.2	1.4	618	5.8	1.3	523	4.5	1.0
70-79	296	5.1	1.5	152	5.8	1.4	144	4.4	1.1
80-89	25	4.9	1.6	8	5.0	0.9	17	4.9	1.8
Total	7,686	5.4	1.4	5,157	6.0	1.3	2,529	4.3	1.0

Survey of gene polymorphisms on five genes related to triglyceride and HDL-cholesterol in the general Japanese population in 2000

Hidenori Arai, Akira Yamamoto, Yuji Matsuzawa, Yasushi Saito, Nobuhiro Yamada, Shinichi Oikawa, Hiroshi Mabuchi, Tamio Teramoto, Jun Sasaki, Noriaki Nakaya, Hiroshige Itakura, Yuichi Ishikawa, Yasuyoshi Ouchi, Hiroshi Horibe, Tohru Egashira, Hiroaki Hattori, and Toru Kita on behalf of the Research group on Serum Lipid Level Survey 2000 in Japan

Running title: Survey of gene polymorphism in Japanese

To whom correspondence should be addressed:

H. Arai, M.D., Ph.D., Department of Geriatric Medicine, Kyoto University School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

E-mail: harai@kuhp.kyoto-u.ac.jp

Tel&fax: 81-75-751-3463

Key words; Hyperlipidemia, polymorphism, cholesterol ester transfer protein, lipoprotein lipase, triglyceride lipase, apolipoprotein CIII

Abstract

We studied the association of six common gene polymorphisms of four genes related to lipid metabolism with serum lipid levels. We selected single-nucleotide polymorphisms (SNPs) in the cholesteryl ester transfer protein (*CETP*), lipoprotein lipase (*LPL*), hepatic triglyceride lipase (*LIPC*), and apolipoprotein CIII (*APOC3*), and studied 2267 individuals in the general Japanese populations. There was a significant interaction in *CETP* polymorphism (D442G, Int14 +1 G_A, and TaqIB), *LPL* polymorphism (S447X), and *LIPC* polymorphism (-514_CT) with HDL-cholesterol levels. We also found a significant interaction in *LPL* polymorphism (S447X) and *APOC3* polymorphism (SstI) with triglyceride levels. This is the largest database showing the association of common genetic variants in lipid metabolism with serum lipid levels in the general Japanese population. Further study is necessary to elucidate the role of these gene polymorphisms on cardiovascular events.

Introduction

Hyperlipidemia is a major risk factor for coronary artery disease (CAD) [1]. In contrast to the sharp decline in both serum cholesterol and the mortality from CAD in the United States and Western Europe, remarkable increases in serum cholesterol levels as well as CAD mortality have been anticipated in the Asian-Pacific area due to industrialization and modernization of the lifestyle [2]. The importance of lifestyle is also proved by the fact that Japanese who migrated to Hawaii and California, for example, showed higher levels of serum cholesterol and a higher incidence of CAD than people in Japan [3]. Thus, dietary habits and other environmental factors affect serum cholesterol levels and CAD mortality in the population. However, the genetic trait is also an important determinant of serum lipid levels.

Major mutations have been described coding for the low-density lipoprotein (LDL) receptor, apolipoprotein B, and so forth, affecting mainly the serum LDL-cholesterol levels [4, 5]. However, plasma triglyceride (TG) and high-density lipoprotein (HDL) cholesterol levels are also considered established risk factors for CAD [6]. Therefore, association of common gene variants at candidate genes with changes in TG and HDL-cholesterol levels would be important determinants for CAD risk. Considering the recent prevalence of the metabolic syndrome, it would be also intriguing to examine the effect of these gene polymorphisms on the development of the metabolic syndrome. So far in Japan, however, a large-scale analysis has not been performed on common gene variants related to lipid metabolism.

In 2000 we have conducted the lipid survey in the general Japanese population of 12,839 people all over the country (J Atheroscler Thromb, in press). In this survey we also tried to examine the frequency of common gene polymorphisms of four genes related to lipid metabolism and show the association with serum lipid levels. Among the proteins involved in lipid metabolism, we chose the following 4 genes because of the association with TG or HDL-cholesterol. Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester from HDL to apolipoprotein B-containing lipoproteins [7]. CETP is a key protein in reverse cholesterol transport and its deficiency is associated with hyperalphalipoproteinemia [8-10]. Among several polymorphisms of the *CETP* gene, G to A substitution at the 5' splice donor site of intron 14 (Int14 +1 G_A) and a missense mutation of exon 15 (D442G) in the *CETP* gene are common mutations of hyperalphalipoproteinemia in Japanese [11, 12]. The Int14 +1 G_A mutation results in a null allele: homozygotes with the mutation have no CETP in plasma and have marked elevation of HDL-cholesterol [9]. TaqIB polymorphism of the *CETP* gene is one of the most studied polymorphisms worldwide. The D442G mutant is near the carboxy terminal region of CETP shown to be essential for the function [13, 14]. The B2 allele of TaqIB polymorphism in intron 1 is associated with decreased CETP levels and high HDL-cholesterol levels [15] and with coronary heart disease risk in Framingham Study [16]. Therefore, we selected these three polymorphisms for our analysis.

Lipoprotein lipase (LPL) is one of the key enzymes in the metabolism of the TG-rich lipoproteins. Among the several polymorphisms of the *LPL* gene we chose S447X polymorphism,

which is common with the allele frequency being approximately 20% in healthy individuals [17-19]. Hepatic lipase (LIPC) is also a member of the lipase superfamily and plays an important role in the metabolism and modeling of both pro- and anti-atherogenic lipoproteins [20]. Among the several polymorphisms we selected -514C_T SNP. This polymorphism, located in the promoter region of the *LIPC* gene, has been demonstrated to influence LIPC activity levels [21]. Apolipoprotein CIII (apoCIII) can inhibit LPL and reduces the uptake of TG-rich remnant particles and the SstI polymorphism of the *APOC3* gene has been shown to associate with hypertriglyceridemia and CAD in various human populations [22-26]. Therefore, we also examined these polymorphisms in the general Japanese population.

The aim of this study was, therefore, to examine the incidence of these gene polymorphisms and their contribution to lipid concentrations in the general Japanese populations.

Methods

Designs and Data Collection

This work is part of the Serum Lipid Level Survey 2000 from various areas around Japan. The Ethics committee, graduate school and faculty of Medicine, Kyoto University approved the study protocol and all subjects provided written informed consent for participation of gene analysis. The handling of DNA samples was followed by the guideline from the Ministry of Health, Labor, and Welfare. In the Serum Lipid Survey 2000, a total of 12,839 subjects were recruited at 36 hospitals across the country. These subjects in the present study were participants in the survey at 9 hospitals