Ⅲ.研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表　（平成１９年度）

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
尾本　暁子 池田　智明	産婦人科専門医にきく最新の臨床　I 周産期　E　妊娠後期と分娩		新しい胎児心拍数図ー新しい胎児心拍数図についてその要点を教えてください。 ー 回答 ー	中外医学社	東京	2007	68-71
川村　淳 野々木宏	血行動態異常の評価法と左心不全/治療		新・目でみる循環器病シリーズ10心筋梗塞症	株式会社メディカルビュー社	東京	2007	
野々木宏	心肺停止	井村裕夫	わかりやすい内科学	文光堂	東京	2008	129-133

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kitakaze M, sakura M,Kim J, Shintani Y, Asanuma H, Hamasaki T, Seguchi O, Myoishi M, Minamino T, Ohara T, Nagai Y, Nanto S, Watanabe K, Fukuzawa S, Hirayama A Nakamura N, Kimura K, Fujii K, Ishihara M, Saito Y, Tomoike H, Kitamura S	Human atrial natriureteic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction(J-WIND)	Lancet	370 (9597)	1483-93	2007

Sugiyama S, Hirota H, Kimura R, Kokubo Y, Kawasaki T, Suehisa E, Okayama A, Tomoike H, Hayashi T, Nishigami K, Kawase I, Miyata T	Haplotype of thrombomodulin gene associated with plasma thrombomodulin level and deep vein thrombosis in the Japanese population.	Thromb Res.	119	35-43	2007
Banno M, Hanada H, Kamide K,Kokubo Y, Kada A, Yang J, TanakaC, Takiuchi S, Horio T, MatayoshiT,Yasuda H, Nagura J, TomoikeH,Kawano Y, Miyata T	Association of genetic polymorphisms of endothelin-converting enzyme-1 gene with hypertension in a Japanese population and rare missense mutation in preproedothelin-1 in Japanese hypertensives.	Hypertens Res	30	513-20	2007
Sakata T, Okamoto A, MoritaT,Kokubo Y, Sato K, OkayamaA,Tomoike H, Miyata T	Age-and gender-related differenced of plasma prothrombin activity levels.	Thromb Haemost	97(6)	1052-3	2007
Shimizu W, Matsuo K, Kokubo Y, Satomi K, Kurita T, Noda T, Nagaya N, Suyama K, Aihara N, Kamakura S, Inamoto N, Akahoshi M, Tomoike H	Sex hormone and gender difference- role of testosterone on male predominance in Brugada syndrome.	J Cardiovasc Electrophysiol.	18(4)	415-21	2007
Miyake Y, Kimura R, Kokubo Y,Okayama A, Tomoike H, YamamuraT, Miyata T	Genetic variations in PCSK9 in the Japanese population : rare genetic variants in PCSK9 might collectively contribute to plasma LDL choresterol levels in the general population.	Atherosclerosis	196	29-36	2007
Takashima N, Niwa, Y, Mannami T, Tomoike H, Iwai N	Characterization of subclinical thyroid dysfunction from cardiovascular and metabolic viewpoints.	The Suita Study, Circ J.	71(2)	191-5	2008

Kamide K, Kokubo Y, Yang J, Matayoshi T, Inamoto N, Takiuchi S, HorioT, Miwa Y, Yoshii M, Tomoike H, Tanaka C, Banno M, Okuda T, Kawano Y, Miyata T	Association of genetic polymorphisms of ACARDAB and COMT with human hypertension.	J. of Hypertension	25(1)	103-10	2007
Kimura R, Miyashita K, Kokubo Y, Akaiwa Y, Otsubo R, Nagatsuka K, Otuski T, Okayama A, Minematsu K, Naritomi H, Honda S, Tomoike H, Miyata T	Genotypes of vitamine K epoxide reductase, gamma-glutamyl carboxylase, and cytochrome P450 2C9 as determinants of daily warfarin dose in Japanese patients.	Thromb Res.	120 (2)	181-6	2007
Noor JI, Ueda Y, Ikeda T, Ikenoue T	Edaravone inhibits lipid peroxidation in neonatalbhypoxic-ischemic rats: an in vivo microdialysis study.	Neurosci Lett.	414	5-9	2007
Parer JT, Ikeda T	A framework for standardized management of intrapartum fetal heart rate patterns.	Am J Obstet Gynecol	197	26.el-6	2007
Ang X, Hagberg H, Nie c, Zhu C,Ikeda T, Mallad C	Dual role of intrauterine immune challenge on neonatal and adult brain vulnerability to hypoxia-ischemia.	J europathol Exp Neurol	66	552-561	2007
Orito K, Gotanda N, Murakami M, Ikeda T, Egashira N, Mishima K, Fujiwara M	Prenatal exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB126) promotes anxiogenic behavior in rats.	Tohoku J exp med	212(2)	151-15	2007
Nakano S, Sugimoto T, Kawasoe T, Koreeda A, Kondo K, Ikeda T, Kai K, Wakisaka S	Staged operations for posthemorrhagic hydrocephalus in extremely low-birth-weight infants with preceding stoma creation after bowel perforation ; surgical strategy.	Childs Nerv Syst	23	459-463	2007

Ito A, Suganami T, Miyamoto Y, Yoshimasa Y, Takeya M, Kamei Y, Ogawa Y	Role of MAPK phosphatase-1 in the induction of monocyte chemoattractant protein-1 during the course of adipocyte hypertrophy.	J Biol Chem	35	25445-25452	2007
Kawamura M, Itoh H, Yura S, Mogami H, Suga S, Makino H, Miyamoto Y, Yoshimasa Y, Sagawa N, Fujii S	Undernutrition in utero augments systolic blood pressure and cardiac remodeling in adult mouse offspring: possible involvement of local cardiac angiotensin system in developmental origins of cardiovascular disease.	Endocrinology	148 (3)	1218-1225	2007
Suzuki M, Takamisawa I, Yoshimasa Y, Harano Y	Association between insulin resistance and endothelial dysfunction in type 2 diabetes and the effects of pioglitazone.	Diabetes Res Clin Pract	76	12-17	2007
Makino H, Doi K, Hiuge A, Nagumo A, Okada S, *Miyamoto Y*, Suzuki M, Yoshimasa Y	Impaired flow-mediated vasodilatation and insulin resistance in type 2 diabetic patients with albuminuria.	Diabetes Res Clin Pract	79(1)	177-182	2007
Okada S, Makino H, Nagumo A, Sugisawa T, Fujimoto M, Kishimoto I, Miyamoto Y, Kikuchi-Taura A, Soma T, Taguchi A, Yoshimasa Y	Circulating CD34-positive cell number is associated with brain natriuretic peptide level in type 2 diabetes patients.	Diabetes Care	31	157-158	2008
Tomiyama M, Horio T, Kamide K, Nakamura S, Yoshihara F, Nakata H, Nakahama H, Kawano Y	Reverse white-coat effect as an independent risk for left ventricular concentric hypertrophy in patients with treated essential hypertension.	J Hum Hypertens	21	212-219	2008
Kato T, Horio T, Tomiyama M, Kamide K, Nakamura S, Yoshihara F, Nakata H, Nakahama H, Kawano Y	Reverse white-coat effect as an independent risk for microalbuminuria in treated hypertensive patients.	Nephrol Dial Transplant	22	911-916	2007

Kawano Y, Ando K, Matsuura H, Tsuchihashi T, Fujita T, Ueshima H	Report of the Working Group of the Japanese Society of Hypertension: (1) Rationale for dietary salt reduction and salt-restriction target level for the management of hypertension.	Hypertens Res	30	879-886	2007
Kawano Y, Tsuchihashi T, Matsuura H, Ando K, Fujita T, Ueshima H	Report of the Working Group of the Japanese Society of Hypertension: (2) Assessment of saltintake in the management of hypertension.	Hypertens Res	30	887-893	2007
Iwashima Y, Horio T, Kamide K, Rakugi H, Ogihara T, Kawano Y	C-reactive protein, left ventricular mass index, and risk of cardiovascular disease in essential hypertension.	Hypertens Res	36	1177-1185	2007
Yasuda S, Miyazaki S, Kinoshita H, Nagaya N, Kanda M, Goto Y, Nonogi H	Enhanced cardiac production of matrix metalloproteinase-2 and -9 and its attenuation associated with pravastatin treatment in patients with acute myocardial infarction.	Clinical Science (in Britain)	112	43-49	2007
Yui Y, Hirayama A, Nonogi H, Kimura K, Kodama K, Hosoda S, Kawai C	Unstable Angina and Non-ST Elevation Acute Coronary Syndrome –Epidemiology and Current Management in Japan(Japan Multicenter Investigation for Cardiovascular Disease-D(JMIC-D)Committee)-	Circulation journal	71	1335-1347	2007
Yoshiki Yui, Eiji Shinoda, Kazuhisa Kodama, Atsushi Hirayama, Hiroshi Nonogi, Kazuo Haze, Tetsuya Sumiyoshi, Saichi Hosoda and Chuichi Kawai	Multicenter Investigation for Cardiovascular Diseases B(JMIC-B)Study Group: Nifedipine retard prevents hospitalization for angina pectoris better than angiotensin-converting enzyme inhibitors in hypertensive Japanese Patients with previous myocardial infarction(JMIC-B substudy).	Journal of Hypertension	25	2019-2026	2007
Takashima N, Shioji K, Kokubo Y, Okayama A, Goto Y, Nonogi H, Iwai N	Validation of the Association Between the Gene Encoding Proteasome Subunit α Type 6 and Myocardial Infarction in a Japanese Population.	Circulation Journal	71	495-498	2007

Koichi Akutsu, Hiroko Morisaki, Satoshi Takeshita, Shingo Sakamoto, Yuiichi Tamori, Tsuyoshi Yoshimuta, Naoyuki Yokoyama, Hiroshi Nonogi, Hitoshi Ogino, Takayuki Morisaki	Phenotypic Heterogeneneity of Marfan-Like Connective Tissue Disorders Associated With Mutations in the Transforming Growgh Factor-β Receptor Genes.	Circulation Journal	Vol.71 No.8	1305-1309	2007
Shimizu W, Kamakura S, et al	Sex hormone and gender difference--role of testosterone on male predominance in Brugada syndrome.	J Cardiovasc Electrophysiol	18	415-21	2007
Moss AJ, Kamakura S, et al	Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene.	Circulation	115	2481-9	
Ohgo T, Kamakura S, et al	Acute and chronic management in patients with Brugada syndrome associated with electrical storm of ventricular fibrillation.	Heart Rhythm	4	695-700	2007
Otomo K, Kamakura S, et al	Participation of a concealed atriohisian tract in the reentrant circuit of the slow-fast type of atrioventricular nodal reentrant tachycardia.	Heart Rhythm	4	703-10	2007
Otomo K, Kamakura S, et al	Implications of 2:1 atrioventricular block during typical atrioventricular nodal reentrant tachycardia.	J Interv Card Electrophysiol	19	109-19	2007
Yokokawa M, Kamakura S, et al	Comparison of long-term follow-up of electrocardiographic features in Brugada syndrome between the SCN5A-positive probands and the SCN5A-negative probands.	Am J Cardiol	100	649-55	2007
Aiba T, Kamakura S, et al	Electrophysiologic study-guided amiodarone for sustained ventricular tachyarrhythmias associated with structural heart diseases.	Circ J	72	88-93	2007
Nakajima H, Kobayashi J, Funatsu T, Shimahara Y, Kawamura M, Kawamura A, Yagihara T, Kitamura S	Predictive factors for the intermediate-term patency of arterial grafts in aorta no-touch off-pump coronary revascularization	Eur J Cardiothorac Surg	32 (5)	711-7	2007

Nakajima H, Kobayashi J, Tagusari O, Niwaya K, Funatsu T, Brik A, Yagihara T, Kitamura S	Graft design strategies with optimum antegrade bypass flow in total arterial off-pump coronary artery bypass				2007
Kawamura M, Itoh H, Yura S, Mogami H, Suga S, Makino H, Miyamoto Y, Yoshimasa Y, Sagawa N, Fujii S.	Undernutrition in utero augments systolic blood pressure and cardiac remodeling in adult mouse offspring: possible involvement of local cardiac angiotensin system in developmental origins of cardiovascular disease	Endocrinology	148 (3)	1218-25	2007
Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S.	Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene	Circulation	115 (19)	2481-9	2007
Makino H, Doi K, Hiuge A, Nagumo A, Okada S, Miyamoto Y, Suzuki M, Yoshimasa Y	Impaired flow-mediated vasodilatation and insulin resistance in type 2 diabetic patients with albuminuria.	Diabetes Res Clin Pract	79(1)	177-82	2007

Nishijima T, Nakayama M, Yoshimura M, Abe K, Yamamuro M, Suzuki S, Shono M, Sugiyama S, Saito Y, Miyamoto Y, Nakao K, Yasue H, Ogawa H	The endothelial nitric oxide synthase gene -786T/C polymorphism is a predictive factor for reattacks of coronary spasm.	Pharmacogenet Genomics	17(8)	581-587	2007
Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T, Seguchi O, Myoishi M, Minamino T, Ohara T, Nagai Y, Nanto S, Watanabe K, Fukuzawa S, Hirayama A, Nakamura N, Kimura K, Fujii K, Ishihara M, Saito Y, Tomoike H, Kitamura S.	Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND)	Two randomised trials. Lancet	370	1483-93	2007
Asakura M, Asanuma H, Kim J, Liao Y, Nakamaru K, Fujita M, Komamura K, Isomura T, Furukawa H, Tomoike H, and Kitakaze M.	Impact of Adenosine Receptor Signaling and Metabolism on Pathophysiology in Patients with Chronic Heart Failure	Hypertens Res	30	781-787	2007
Ikeda T	Stem cells and neonatal brain injury.	Cell Tissue Res			2007
野々木 宏	わが国からのデータ発信：国際ガイドライン改訂インパクト	MMj,3	No.9	711	2007

野々木　宏	心肺蘇生法の事始め：日本循環器学会が動いた	Osaka　Heart Club	31	1-3	2007
野々木　宏	J-PULSE　院外心停止者の救命率向上に対する自動体外式序除細動器を用いた心肺蘇生法の普及とエビデンス確立のためのウツタイン様式を用いた大規模臨床研究	不整脈 News＆Views	24	13-14	2007
野々木　宏	Evidence, Pay for Performannce and Appropriateness : New Changes in the Practice of Cardiology	AHA		144-150	2007
野々木宏, 白井伸一、田原良雄、安田聡	虚血性心疾患にともなう致死性不整脈の緊急治療	座談会		1-8	2007

Ⅳ.研究成果の刊行物・別刷

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Genetic variants in *PCSK9* in the Japanese population: Rare genetic variants in *PCSK9* might collectively contribute to plasma LDL cholesterol levels in the general population

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Abstract

The aim of this study was to investigate whether plasma low-density lipoprotein cholesterol (LDL-C) levels in the general population are influenced by rare sequence variations in the *PCSK9* gene. We sequenced the promoter and coding regions of the *PCSK9* gene in individuals from the general population ($n = 3655$) with the lowest ($n = 78$) and highest ($n = 96$) LDL-C levels and in individuals taking antihypercholesterolemia medication ($n = 96$). We identified 33 sequence variants in the *PCSK9* gene among which 24 were specific for Japanese. Statistical analysis showed that one missense mutation, R93C, was associated with low LDL-C levels. The other variants had no association with LDL-C levels or the numbers of individuals with the variants were too small for statistical analysis. A comparison of the numbers of individuals with nonsynonymous mutations between the low LDL-C and high LDL-C/treatment groups found that four missense mutations and one nonsense mutation were identified only in the low LDL-C group and six missense mutations were identified only in the high LDL-C/treatment group. As we have analyzed groups at opposite ends of the LDL-C spectrum, it is likely that some of these nonsynonymous mutations may be associated with either low or high LDL-C in the Japanese population. Based on the extremely high frequencies of the nonsynonymous mutations in *PCSK9* compared with those of *LDLR* or *apoB-100*, *PCSK9* mutations could be important factors that cumulatively influence plasma LDL-C levels in the general population.
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Keywords: *PCSK9*; Plasma LDL cholesterol; Rare genetic variants; General population; Missense mutation

1. Introduction

Elevated plasma concentration of low-density lipoprotein cholesterol (LDL-C) is a major risk factor for the development and progression of atherosclerosis. Plasma concentrations of LDL-C are determined primarily by the activity of the LDL receptor (LDLR) in the liver. Recently, the *proprotein convertase subtilisin/kexin type 9* (*PCSK9*) gene has been found to be involved in the post-transcriptional regulation of the LDLR. *PCSK9* encodes a protein of 692 amino acids which is a member of the subtilisin-like protein convertase family [1,2] and is expressed most abundantly in the liver, kidneys and small intestine [2]. PCSK9 consists of several domains: a signal peptide, a prosegment, a subtilase-like catalytic domain and a C-terminal domain [3]. It is synthesized as a soluble zymogen which undergoes autocatalytic intramolecular cleavage in the endoplasmic reticulum (ER) between the prosegment and the catalytic domain [1,2].

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After cleavage, the mature PCSK9 exits the ER and is efficiently secreted [2]. The only known substrate of PCSK9 is itself; no other substrate(s) for PCSK9 have yet been identified. Although even the physiological substrate remains unknown, PCSK9 has been shown to play a role in cholesterol metabolism by regulating the number of cell-surface LDLRs [3–5].

Overexpression of the wild-type *Pcsk9* gene in mice results in hypercholesterolemia because of a reduced number of LDLRs [3–5]. The reduced number of LDLRs due to PCSK9 is not accompanied by changes in *LDLR* mRNA levels; therefore, it is likely that PCSK9 is involved in the post-transcriptional regulation of the LDLR [4,5]. Degradation of the LDLR by PCSK9 is dependent on the catalytic activity of PCSK9 [5,6]. In contrast, mice expressing no PCSK9 have markedly increased hepatic LDLR levels, resulting in accelerated LDL clearance [7]. These findings indicate that PCSK9 normally acts to limit the number of LDLRs at the cell surface. Thus, *PCSK9* mutations which disrupt normal function, i.e., loss-of-function mutations, are presumed to increase the number of LDLRs, resulting in hypocholesterolemia. In fact, the nonsense mutations identified by Cohen et al. are associated with a 40% reduction in mean plasma levels of LDL-C [8]. On the other hand, some mutations in the *PCSK9* gene cause hypercholesterolemia [9–11], which are probably due to gain-of-function mechanisms. These mutations in *PCSK9* might promote the degradation of LDLRs in hepatocytes [3–5]. Recently, Cameron et al. demonstrated that loss-of-function mutations in *PCSK9* increase the number of cell-surface LDLRs, while gain-of-function mutations decrease the number of LDLRs, based on studies on HepG2 cells transfected with mutant *PCSK9* constructs [12].

Since mutations in *PCSK9* can cause severe hypercholesterolemia [9–11] as well as hypocholesterolemia [8,13], sequence variants of *PCSK9* might contribute to variations in the plasma levels of LDL-C. Shioji et al. [14] have identified the two common single nucleotide polymorphisms (SNPs), and Chen et al. [15] identified a haplotype associated with differences in plasma LDL-C levels. Kotowski et al. performed a systematic examination of the relationship between sequence variations in *PCSK9* and plasma levels of LDL-C in the general population [16]. They analyzed sequence variations in *PCSK9* in individuals of the examined population who had lower and higher LDL-C levels and found that three missense mutations and two noncoding sequence variants were significantly associated with lower levels of LDL-C, while a single noncoding variant was associated with a modest increase in LDL-C levels. They concluded that sequence variants in *PCSK9* contribute significantly to interindividual variations in plasma LDL-C levels, and report that the spectrum of *PCSK9* alleles associated with LDL-C levels spanned a wide range of allele frequencies and magnitude of phenotypic effects.

In order to verify whether sequence variants in *PCSK9* could be a determinant of LDL-C plasma levels in the Japanese general population, we performed sequence analyses in the proximal promoter and all exons of *PCSK9* in individuals from the general population with the lowest and highest LDL-C levels and also in individuals taking antihypercholesterolemia medication since these individuals are presumed to have originally high levels of plasma LDL-C. Finally, we performed statistical analyses and compared the numbers of individuals with certain genetic variants between groups.

2. Methods

2.1. General population and the three investigated groups of individuals

DNA analysis was performed in individuals selected from the participants of the Suita cohort study, whose total sample included 3655 subjects. The study design of the Suita study has been described previously [17–19]. Briefly, the individuals were randomly selected from the municipal population registry, taking into consideration group stratification by gender and 10-year age divisions. The subjects visited the National Cardiovascular Center every 2 years for general health checkups. In addition to performing routine blood examinations, including lipid profiles, glucose levels, blood pressure and anthropometric measurements, a physician or nurse administered questionnaires regarding the individual's personal history of cardiovascular disease, including angina pectoris, myocardial infarction and stroke. Leukocyte DNA was collected from individuals who visited the National Cardiovascular Center between April 2002 and February 2004, and written informed consent was obtained from each individual before proceeding with genetic analysis. All clinical data, sequencing results and genotyping results were anonymous, and the study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

From the 3655 participants of the Suita study, we selected the 96 individuals who showed the lowest levels of LDL-C to form the low LDL-C group. After analysis, it became evident that 18 of these individuals were under antihypercholesterolemia treatment; these subjects were excluded, leaving a total of 78 individuals in this group. Additionally, there were 498 individuals who were under antihypercholesterolemia medication in the total population. From this treated population we selected the 96 individuals with the highest LDL-C levels to form the treatment group. Sixteen individuals in the treatment group had ischemic heart diseases. From the untreated 3139 individuals, we selected the 96 showing the highest LDL-C levels to form the high LDL-C group.

2.2. Lipid measurements

Total serum cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) levels were measured with an autoanalyzer (Toshiba TBA-80; Toshiba, Tokyo, Japan) using a fasting blood sample. LDL-C levels were calculated

using the Friedewald formula [20]. Individuals with triglyceride levels higher than 400 mg/dl were omitted.

2.3. DNA analysis

Genomic DNA was isolated from blood leukocytes using an NA-3000 nucleic acid isolation system (Kurabo, Osaka, Japan). The proximal promoter (−380 bp upstream) and all coding regions (including 10 bases of each exon–intron boundary sequence) were directly sequenced. Descriptions of the primers used for amplifying and sequencing fragments are given in the electronic appendix. Polymerase chain reaction (PCR) was performed with 10 ng of genomic DNA as the template in a 10-μl reaction mixture using a HotStar Taq Master Mix Kit (Qiagen, Hilden, Germany) as follows: *Taq* polymerase was activated at 95 °C for 15 min, followed by 40 cycles of denaturing at 95 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 30 s. The PCR products were then treated with shrimp alkaline phosphatase and exonuclease I (PCR product pre-sequencing kit; USB Corporation, Cleveland, OH, USA), and used as templates for direct single-pass sequencing with a BigDye Terminator v3.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA). The reaction products were purified with Sephadex G-50 (Amersham Biosciences, Uppsala, Sweden) and analyzed on an ABI PRISM 3700 DNA analyzer (Applied Biosystems). The acquired sequences were examined for the presence of variants using the Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) followed by visual inspection. Each detected variant was confirmed by repeat sequencing from the opposite direction. The A of the initiating ATG codon is denoted as nucleotide +1, and the nucleotide sequence (GenBank accession no: NT032977) was used as the reference sequence.

2.4. Statistical analysis

Lipid levels and ages were compared by Student's *t*-test. Deviations in the distributions of the genetic variants in the low LDL-C and high LDL-C/treatment groups were assessed by Fisher's two-sided exact test using the version 4 SNPAlyze statistical software (Dynacom Co. Ltd., Mobara, Japan). Pairwise linkage disequilibrium (LD) between two polymorphisms was evaluated by r^2 using SNPAlyze version 4.

3. Results

3.1. Participant characteristics

The characteristics of the individuals in the low LDL-C, high LDL-C and treatment groups, and those of the treated and untreated individuals in the total population, are shown in Table 1. The LDL-C levels of the individuals in the low LDL-C group ranged from 29.2 to 88.0 mg/dl (mean ± S.D., 70.3 ± 13.2 mg/dl), and those in the high LDL-C group ranged from 169.8 to 300.8 mg/dl (mean ± S.D., 196.7 ± 19.2 mg/dl). The LDL-C levels of

Table 1
Characteristics of individuals in the low LDL-C, high LDL-C and treatment groups, and of the total population of the Suita study

	Low LDL-C group	High LDL-C group	Treatment* group	Total population	
				Treated*	Untreated
No. (men/women)	78 (35/43)	96 (36/60)	96 (23/73)	516 (171/345)	3139 (1538/1601)
Age					
Range	39–88	40–85	44–85	44–90	35–93
Mean ± S.D.	64.2 ± 12.1	64.3 ± 10.2	69.2 ± 7.9$^{+,++}$	69.9 ± 8.5	63.9 ± 11.5
LDL-C (mg/dl)					
Range	29.2–88.0	169.8–300.8	148.0–204.4	52.4–204.4	29.2–300.8
Mean ± S.D.	70.3 ± 13.2	196.7 ± 19.2**	165.1 ± 13.4$^{+,++}$	126.9 ± 27.0	126.8 ± 29.8
Total cholesterol (mg/dl)					
Range	88–223	229–380	208–318	124–350	87–380
Mean ± S.D.	156.6 ± 22.9	277.4 ± 23.5**	246.3 ± 20.1$^{+,++}$	210.4 ± 30.1	207.6 ± 32.9
Triglycerides (mg/dl)					
Range	21–396	40–230	49–289	33–435	18–1868
Mean ± S.D.	100.7 ± 71.4	113.3 ± 43.6	118.4 ± 48.1	119.5 ± 61.9	106.2 ± 76.2
HDL-C (mg/dl)					
Range	27–125	32–106	31–91	26–108	22–140
Mean ± S.D.	66.1 ± 20.6	58.0 ± 12.4**	57.5 ± 12.6^{+}	59.4 ± 14.6	60.3 ± 15.8

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

* Treatment refers to antihypercholesterolemia medication. The lipid values of the individuals in the treatment group were taken under treatment. *P* values were obtained by Student's *t*-test.

** $P < 0.005$, low LDL-C group vs. high LDL-C group.

\+ $P < 0.005$, low LDL-C group vs. treatment group.

++ $P < 0.005$, high LDL-C group vs. treatment group.

the subjects in the treatment group ranged from 148.0 to 204.4 mg/dl (mean ± S.D., 165.1 ± 13.4 mg/dl), which were lower than those of the high LDL-C group but significantly higher than those of the untreated population (mean ± S.D., 126.8 ± 29.8 mg/dl, P value by t-test <0.005).

Triglyceride levels did not differ significantly between the three groups and these values were also similar to those of treated and untreated individuals in the total population. HDL-C levels in the low LDL-C group were significantly higher than those of all other groups. Lower LDL-C levels in general tended to be accompanied by higher HDL-C levels, however, the reason for this remains unclear. There was no difference in age between the low and high LDL-C groups, however, ages in the treatment group were higher than those of the other groups. In the total population, the treated individuals also had higher ages than the untreated individuals. This may be because exercise or diet therapy is preferred for the treatment of hypercholesterolemia in younger patients, while lipid-lowering drug therapy is often adopted in older patients.

3.2. *PCSK9 polymorphisms found*

We sequenced 156 alleles from 78 individuals with low LDL-C levels, 192 alleles from 96 individuals with high LDL-C levels, and 192 alleles from the individuals taking antihypercholesterolemia medication, identifying a total of 33 genetic variants. A list of the genetic variants and their genotype distribution for each group are shown in 2Table 2. 'Allele 1' refers to the allele shown in the GenBank reference (accession no. NT032977), and 'Allele 2' is the variant allele. An LD defined by an r^2 value greater than 0.5 is indicated in the LD column; there are four LD groups (a–d). The minor allele frequencies of the variants in groups a (−64C > T, L21-22ins, and A53V) and b (c.658 − 7C > T and c.799 + 3A > G) were greater than 0.1, indicating that these variants are relatively common. With respect to group d, the two missense mutations, A514T and V624M, were found in the same individual. The r^2 values in groups a and c were both 1.00, indicating tight LD, and the value in group b was 0.83.

Fourteen of the 33 detected variants have previously been reported (see the columns for dbSNP ID and the references in Table 2), leaving 19 novel variants. Among 14 of the variants that have been already reported, five were found to date only in the Japanese population [14], thus, a total of 24 variants are specific to the Japanese population. The genetic variants that are specific to the Japanese population are shown in boldface type in Tables 2 and 3 and Fig. 1. Twenty of the 33 variants were nonsynonymous and resulted in an amino acid change, including one nonsense mutation (W428X) in exon 8 and one trinucleotide insertion resulting in the addition of an extra leucine in a leucine repeat (L21-22ins) in exon 1. The others were either synonymous variants in coding regions or variants in untranslated regions. The sites of the nonsynonymous mutations found in the present study are shown in Fig. 1; they were distributed along the entire structure of the *PCSK9* gene.

3.3. *Contribution of the PCSK9 gene variants to plasma LDL-C levels*

Statistical analysis was carried out in order to determine whether sequence variants in the *PCSK9* gene affect plasma LDL-C levels. Deviations in the distribution of each genetic variant in either the low LDL-C group or the high LDL-C/treatment group were assessed by Fisher's two-sided exact test. Of the tested 33 genetic variants, only a single missense mutation, R93C, was found to be significantly associated with the low LDL-C group (P = 0.003).

Next, we compared the numbers of individuals with nonsynonymous mutations in the low LDL-C and high LDL-C/treatment groups (Table 3), since nonsynonymous mutations are presumed to have stronger and more direct effects than synonymous or noncoding sequence variants. The allele frequencies of R93C in the low LDL-C group and in the high LDL-C/treatment group were 0.051 and 0.008, respectively (Table 3). Although the other nonsynonymous mutations did not show significant statistical values in distribution, several nonsynonymous mutations were found only in the low LDL-C group (Q219E, A239D, W428X, G452D and S668R) or only in the high LDL-C/treatment group (V4I, E32K, E54A, R104C, A514T and V624M). The sequence analysis on the *LDLR* gene was performed in the individuals who had the *PCSK9* mutations found only in the high/treatment group, confirming that there was no *LDLR* mutation in these individuals. L21-22ins, A53V, A68T, G263S, I424V, V474I, V644I and G670E were found in both the low LDL-C and high LDL-C/treatment groups.

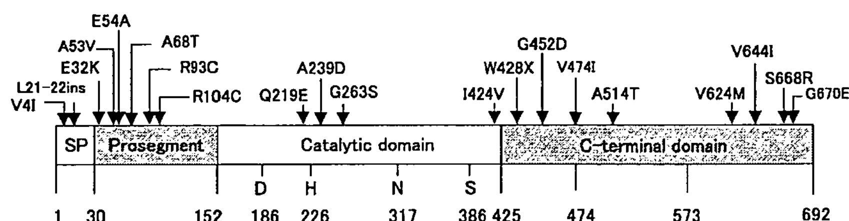

Fig. 1. Sites of the nonsynonymous mutations found in the present study. SP indicates signal peptide. The positions of the canonical aspartic acid (D), histidine (H), serine (S) catalytic triad, and oxyanion-hole asparagine (N) [3], are shown. The amino acid numbers are indicated at the bottom, and the sites of mutations found in the present study are indicated by arrows. Mutations shown in boldface type are those found only in the Japanese population.

Table 2
Genetic variants found and their genotypic distribution in each group

SNPs (allele 1 > allele 2)	LD (r^2 > 0.5)	Region	Amino acid change	No. of subjects											Flanking sequences	dbSNP ID	Reference
				Low LDL-C group			High LDL-C group			Treatment group			Allele frequency				
				Allele 1 Homo	Hetero	Allele 2 Homo	Allele 1 Homo	Hetero	Allele 2 Homo	Allele 1 Homo	Hetero	Allele 2 Homo	Allele 1	Allele 2			
−253G > A		Exon1		77	1	0	95	1	0	96	0	0	0.996	0.004	GCGGGCGCC[G/A]CCGTTCAGT		
−64C > T	a	Exon 1		63	15	0	76	18	2	72	22	2	0.883	0.117	AGGCGCCGC[C/T]GGCGTGGAC		[9,11,14,15]
c.10G > A		Exon 1	V4I	78	0	0	95	1	0	90	6	0	0.987	0.013	ATGGGCACC[G/A]TCAGCTCCA		[14]
c.63–64insCTG	a	Exon 1	L21-22ins	63	15	0	76	18	2	72	22	2	0.883	0.117	CTGCTGCTG[-/CTG]CTCCTGGGT		[9,11,14,15]
c.94G > A		Exon1	E32K	78	0	0	91	5	0	93	3	0	0.985	0.015	CGTGCGCAG[G/A]AGGACGAGG		
c.158C > T	a	Exon 1	A53V	63	15	0	76	18	2	72	22	2	0.883	0.117	ACGGCCTGG[C/T]CGAAGCACC	rs11583680	[9,11,14]
c.161A > C		Exon 1	E54A	78	0	0	96	0	0	95	1	0	0.998	0.002	GCCTGGCCG[A/C]AGCACCCGA		
c.202G > A		Exon 1	A68T	77	1	0	95	1	0	96	0	0	0.996	0.004	CACCGCTGC[G/A]CCAAGGTGC		
c.277C > T		Exon 2	R93C	70	8	0	95	1	0	94	2	0	0.980	0.020	CAGTCAGAG[C/T]GCACTGCCC		
c.310C > T		Exon 2	R104C	78	0	0	95	1	0	96	0	0	0.998	0.002	CAGGCTGCC[C/T]GCCGGGGAT		
c.336G > A		Exon 2	L112L	74	4	0	85	10	1	90	5	1	0.957	0.043	CAAGATCCT[G/A]CATGTCTTC		[14]
c.420C > T		Exon 3	V140V	78	0	0	96	0	0	95	1	0	0.998	0.002	GCCCCATGT[C/T]GACTACATC		
c.655C > G		Exon 4	Q219E	77	1	0	96	0	0	96	0	0	0.998	0.002	TTCCACAGA[C/G]AGGTAAGCA		
c.657 + 9G > A		Intron 4		73	5	0	91	5	0	93	3	0	0.976	0.024	GGTAAGCAC[G/A]GCCGTCTGA	rs11800243	[9,11,15]
c.658 − 7C > T	b	Intron 4		47	28	3	54	34	8	48	44	4	0.748	0.252	TGTGTTCGT[C/T]GAGCAGGCC	rs2483205	[9,11,15]
c.716C > A		Exon 5	A239D	77	1	0	96	0	0	96	0	0	0.998	0.002	GCCGGGATG[C/A]CGGCGTGGC		
c.787G > A		Exon 5	G263S	77	1	0	94	2	0	94	2	0	0.991	0.009	ACGGTTAGC[G/A]GCACCCTCA		
c.799 + 3A > G	b	Intron 5		42	32	4	48	39	9	46	44	6	0.715	0.285	CTCATAGGT[A/G]AGTGATGGC	rs2495477	[9,11,15]
c.993C > T		Exon 6	P331P	76	1	1	95	0	1	94	2	0	0.987	0.013	CTCAGCTCC[C/T]GAGGTAGGT		[14]
c.1227C > T		Exon 8	A409A	78	0	0	96	0	0	95	1	0	0.998	0.002	CACCCTGGC[C/T]GAGTTGAGG		
c.1270A > G		Exon 8	I424V	75	3	0	95	1	0	96	0	0	0.993	0.007	AAAGATGTC[A/G]TCAATGAGG		[14]
c.1284G > A		Exon 8	W428X	77	1	0	96	0	0	96	0	0	0.998	0.002	TGAGGCCTG[G/A]TTCCCTGAG		
c.1355G > A		Exon 9	G452D	77	1	0	96	0	0	96	0	0	0.998	0.002	TTTTTGCAG[G/A]TTGGCAGCT		
c.1380A > G	c	Exon 9	V460V	0	6	72	0	5	91	0	6	90	0.031	0.969	CAGGACTGT[A/G]TGGTCAGCA	rs540796	[9,11,14,15]
c.1420G > A	c	Exon 9	V474I	0	6	72	0	5	91	0	6	90	0.031	0.969	GCCACAGCC[G/A]TCGCCCGCT	rs562556	[9,11,14,15]
c.1540G > A	d	Exon 10	A514T	78	0	0	96	0	0	95	1	0	0.998	0.002	GCCCACAAC[G/A]CTTTTGGGG		
c.1863 + 6G > A		Intron 11		78	0	0	96	0	0	95	1	0	0.998	0.002	GCAGGTGAA[G/A]AGGCCCGTG		
c.1870G > A	d	Exon 12	V624M	78	0	0	96	0	0	95	1	0	0.998	0.002	CAGGTGACC[G/A]TGGCCTGCG		
c.1878C > T		Exon 12	C626C	78	0	0	95	1	0	96	0	0	0.998	0.002	CGTGGCCTG[C/T]GAGGAGGGC		
c.1930G > A		Exon 12	V644I	77	1	0	94	2	0	96	0	0	0.994	0.006	ACCTCCCAC[G/A]TCCTGGGGG		[14]
c.1947C > T		Exon 12	A649A	78	0	0	96	0	0	95	1	0	0.998	0.002	GGCCTACGC[C/T]GTAGACAAC		
c.2004C > A		Exon 12	S668R	77	1	0	96	0	0	96	0	0	0.998	0.002	CAGCACCAG[C/A]GAAGGGGCC		
c.2009G > A		Exon 12	G670E	0	6	72	0	13	83	0	6	90	0.046	0.954	CCAGCGAAG[G/A]GGCCGTGAC	rs505151	[9,11,15]

The mutations found only in Japanese are shown in boldface type. SNP, single nucleotide polymorphism; LD, linkage disequilibrium; homo, homozygote; hetero, heterozygote.

Table 3
No. of individuals with nonsynonymous mutations in the low LDL-C and high LDL-C/treatment groups

Amino acid change	Domain	No. of subjects			Allele frequency		LDL-C (mean ± S.D.) (mg/dl)	Identity with rodents
		Low LDL-C group ($n=78$)	High LDL-C group ($n=96$)	Treatment group ($n=96$)	Low LDL-C group ($n=78$)	High LDL-C/treatment group ($n=192$)		
Mutation significantly associated with the low LDL-C group								
R93C	**Prosegment**	**8**	**1**	**2**	**0.051***	**0.008***	**96.2 ± 43.0**	**N**
Mutations found only in the low LDL-C group								
Q219E	**Catalytic**	**1**	**0**	**0**	**0.006**	**0.000**	**83.6**	**Y**
A239D	**Catalytic**	**1**	**0**	**0**	**0.006**	**0.000**	**49.8**	**Y**
W428X	**C-terminal**	**1**	**0**	**0**	**0.006**	**0.000**	**70.4**	**Y**
G452D	**C-terminal**	**1**	**0**	**0**	**0.006**	**0.000**	**73.6**	**Y**
S668R	**C-terminal**	**1**	**0**	**0**	**0.006**	**0.000**	**87.6**	**Y**
Mutations found only in the high LDL-C/treatment group								
V4I	**Signal peptide**	**0**	**1**	**6**	**0.000**	**0.018**	**168.5 ± 14.4**	**N**
E32K	**Prosegment**	**0**	**5**	**3**	**0.000**	**0.021**	**167.5 ± 10.4**	**N**
E54A	**Prosegment**	**0**	**0**	**1**	**0.000**	**0.003**	**153.0**	**N**
R104C	**Prosegment**	**0**	**1**	**0**	**0.000**	**0.003**	**192.2**	**Y**
A514T	**C-terminal**	**0**	**0**	**1**	**0.000**	**0.003**	**192.2**	**Y**
V624M	**C-terminal**	**0**	**0**	**1**	**0.000**	**0.003**	**192.2**	**Y**
Mutations found in both groups								
L21-22ins	Signal peptide	15	18,2**	22,2+	0.096	0.125	146.3 ± 50.3++	N
A53V	Prosegment	15	18,2**	22,2+	0.096	0.125	146.3 ± 50.3++	N
A68T	**Prosegment**	**1**	**1**	**0**	**0.006**	**0.003**	**129.3 ± 89.2**	**N**
G263S	**Catalytic**	**1**	**2**	**2**	**0.006**	**0.010**	**172.4 ± 52.8**	**Y**
I424V	**Catalytic**	**3**	**1**	**0**	**0.019**	**0.003**	**104.0 ± 52.6**	**Y**
V474I	C-terminal	6	5	6	0.038	0.029	141.2 ± 53.6	N
V644I	**C-terminal**	**1**	**2**	**0**	**0.006**	**0.005**	**145.9 ± 50.4**	**N**
G670E	C-terminal	6	13	6	0.038	0.049	158.8 ± 55.2	Y

Catalytic, catalytic domain; C-terminal, C-terminal domain; * P-value by Fisher's exact test was 0.003; N, amino acid residue in human wild-type has no identity with those of mouse and rat; Y, amino acid in human wild-type has identity with those of mouse and rat; ** 18 heterozygotes and 2 homozygotes; + 22 heterozygotes and 2 homozygotes; ++ mean ± S.D. was calculated from the values of the heterozygotes. The mutations specific to Japanese are shown in boldface type.

4. Discussion

In order to identify the genetic variants in *PCSK9* affecting plasma LDL-C levels in the Japanese population, we screened the proximal promoter and the entire coding region sequences in 78 individuals with low LDL-C levels, 96 individuals with high LDL-C levels, and 96 individuals currently taking antihypercholesterolemia medication. All subjects were selected from a large sample of the general population ($n = 3655$).

Among the 33 detected sequence variants, only one missense mutation, R93C, was found to be significantly associated with the low LDL-C group ($P = 0.003$). This mutation has not been detected to date in the other populations; thus, it may be a specific genetic factor causing low LDL-C levels in the Japanese population. The other variants showed no statistical significance in their association to either low or high LDL-C levels. However, when the numbers of individuals with nonsynonymous mutations were compared between the low LDL-C group and the high LDL-C/treatment group (Table 3), four missense mutations, Q219E, A239D, G452D and S668R, and one nonsense mutation, W428X, were found only in the low LDL-C group. All of these have not been detected to date in the other populations. Five (or six) missense mutations, V4I, E32K, E54A, R104C, A514T (and/or V624M) were found only in the high LDL-C/treatment group. Since A514T and V624M were found in the same individual, the contribution of each mutation to the individual's high LDL-C level is less clear. All of these mutations are also specific to Japanese. The numbers of individuals bearing each mutation were very small and nine of these mutations were identified in single individuals and therefore they might have arisen by chance, however, because we have analyzed groups at opposite ends of the LDL-C spectrum, it is likely that some of these rare variants in the *PCSK9* gene are associated with either low or high LDL-C levels in the Japanese population. Family studies or similar investigations in other Japanese populations are necessary to fully understand the effect of each mutation.

Although at present it is difficult to determine the effect of each mutation, the frequencies of nonsynonymous mutations in the *PCSK9* gene were extremely high compared to those of *LDLR* and *apo B-100*. Heterozygotes with the *LDLR* mutations are found in approximately 0.2% of most general populations, and those with *apoB-100* are found in about 0.1% [21]. *ApoB-100* mutations have not yet been detected in the Japanese population [22]. Among the 20 nonsynonymous *PCSK9* mutations detected in the present study, L21-22ins, A53V, G670E and V474I were found to be distributed almost equally between the two examined groups (Table 3), and their allele frequencies were 0.117, 0.117, 0.046 and 0.031, respectively (Table 2). As these mutations have been found with high frequencies in several populations, they are known as common polymorphisms [11]. The allele frequencies of the remaining 16 nonsynonymous mutations ranged from 0.002 to 0.02. Collectively, these rare nonsynonymous mutations appeared in 47 of the 270 examined individuals (17%) in the present study, indicating extraordinary high frequencies of the mutations in this gene. Because of these high frequencies, the *PCSK9* gene might be an important determinant of plasma LDL-C levels in the general population.

When we examined the sites of the nonsynonymous mutations, the mutations detected only in the low LDL-C group were found to be concentrated in the catalytic and C-terminal domains, while the mutations found only in the high LDL-C/treatment group were distributed in the signal peptide, the prosegment and the C-terminal domain (Table 3). The amino acid residues of the mutations found only in the low LDL-C group are all conserved between rodents and humans (Table 3). Although the numbers of each mutation were too small to reach any definitive conclusions, we speculate that the mutations occur in the catalytic or C-terminal domain, which are expected to be important for the function of the protein, are prone to be hypocholesterolemic. These mutations, together with nonsense mutations, are prone to be hypocholesterolemic due to a loss-of-function mechanism. This speculation is consistent with the finding that the two nonsense mutations found in African Americans, Y142X and C679X, are hypocholesterolemic [8]. The present W428X mutation was also found in low the LDL-C group and the individual heterozygous for this mutation had 70.4 mg/dl of plasma LDL-C (Table 3), corresponding to a 44% decrease from the average LDL-C level in the untreated population (126.8 mg/dl, Table 1). This observation is consistent with the finding that the two African-American nonsense mutations were associated with a 40% reduction in plasma LDL-C levels [8].

Although selected mutations in the *PCSK9* gene have been found to cause severe phenotypes of hypercholesterolemia [9–11], more frequent genetic variants in *PCSK9* are associated with modest differences in plasma LDL-C [8,16]. Even if the effect of each mutation is small, *PCSK9* mutations might cumulatively contribute to differences in LDL-C levels in the general population.

We previously performed large-scale sequence analyses of five hypertension candidate genes (*WNK4*, *SCNN1B*, *SCNN1G*, *NR3C2*, and *RGS2*) to evaluate whether rare genetic mutations contribute collectively to quantitative trait variations, such as those found in blood pressure, finding that a low but significant subset of hypertensive subjects had missense/frameshift mutations [23–26]. Therefore, rare mutations collectively and partially contribute to quantitative trait variations, such as plasma levels of HDL-C [27,28] and LDL-C, and hypertension.

Based on the present analyses, nonsynonymous mutations in the *PCSK9* gene might contribute to either high or low levels of LDL-C in the Japanese general population.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2006.12.035.

References

[1] Naureckiene S, Ma L, Sreekumar K, et al. Functional characterization of Narc 1, a novel proteinase related to proteinase K. Arch Biochem Biophys 2003;420:55–67.
[2] Seidah NG, Benjannet S, Wickham L, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proc Natl Acad Sci USA 2003;100:928–33.
[3] Benjannet S, Rhainds D, Essalmani R, et al. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. J Biol Chem 2004;279:48865–75.
[4] Maxwell KN, Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. Proc Natl Acad Sci USA 2004;101:7100–5.
[5] Park SW, Moon YA, Horton JD. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. J Biol Chem 2004;279:50630–8.
[6] Maxwell KN, Fisher EA, Breslow JL. Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. Proc Natl Acad Sci USA 2005;102:2069–74.
[7] Rashid S, Curtis DE, Garuti R, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. Proc Natl Acad Sci USA 2005;102:5374–9.
[8] Cohen J, Pertsemlidis A, Kotowski IK, et al. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in *PCSK9*. Nat Genet 2005;37:161–5.
[9] Abifadel M, Varret M, Rabes JP, et al. Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. Nat Genet 2003;34:154–6.
[10] Timms KM, Wagner S, Samuels ME, et al. A mutation in *PCSK9* causing autosomal-dominant hypercholesterolemia in a Utah pedigree. Hum Genet 2004;114:349–53.
[11] Leren TP. Mutations in the *PCSK9* gene in Norwegian subjects with autosomal dominant hypercholesterolemia. Clin Genet 2004;65:419–22.
[12] Cameron J, Holla OL, Ranheim T, et al. Effect of mutations in the PCSK9 gene on the cell surface LDL receptors. Hum Mol Genet 2006;15:1551–8.
[13] Berg KE, Ose L, Leren TP. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. Arterioscler Thromb Vasc Biol 2006;26:1094–100.
[14] Shioji K, Mannami T, Kokubo Y, et al. Genetic variants in *PCSK9* affect the cholesterol level in Japanese. J Hum Genet 2004;49:109–14.
[15] Chen SN, Ballantyne CM, Gotto Jr AM, et al. A common *PCSK9* haplotype, encompassing the E670G coding single nucleotide polymorphism, is a novel genetic marker for plasma low-density lipoprotein cholesterol levels and severity of coronary atherosclerosis. J Am Coll Cardiol 2005;45:1611–9.
[16] Kotowski IK, Pertsemlidis A, Luke A, et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. Am J Hum Genet 2006;78:410–22.
[17] Mannami T, Konishi M, Baba S, Nishi N, Terao A. Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. Stroke 1997;28:518–25.
[18] Mannami T, Baba S, Ogata J. Potential of carotid enlargement as a useful indicator affected by high blood pressure in a large general population of a Japanese city: the Suita study. Stroke 2000;31:2958–65.
[19] Mannami T, Baba S, Ogata J. Strong and significant relationships between aggregation of major coronary risk factors and the acceleration of carotid atherosclerosis in the general population of a Japanese city: the Suita Study. Arch Intern Med 2000;160:2297–303.
[20] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[21] Goldstein JL, Brown MS. Molecular medicine. The cholesterol quartet. Science 2001;292:1310–2.
[22] Nohara A, Yagi K, Inazu A, et al. Absence of familial defective apolipoprotein B-100 in Japanese patients with familial hypercholesterolaemia. Lancet 1995;345:1438.
[23] Kamide K, Takiuchi S, Tanaka C, et al. Three novel missense mutations of *WNK4*, a kinase mutated in inherited hypertension, in Japanese hypertensives: implication of clinical phenotypes. Am J Hypertens 2004;17:446–9.
[24] Kamide K, Tanaka C, Takiuchi S, et al. Six missense mutations of the epithelial sodium channel β-and γ-subunits in Japanese hypertensives. Hypertens Res 2004;27:333–8.
[25] Kamide K, Yang J, Kokubo Y, et al. A novel missense mutation, F826Y, in the mineralocorticoid receptor gene in Japanese hypertensives: its implications for clinical phenotypes. Hypertens Res 2005;28:703–9.
[26] Yang J, Kamide K, Kokubo Y, et al. Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. J Hypertens 2005;23:1497–505.
[27] Cohen JC, Kiss RS, Pertsemlidis A, et al. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 2004;305:869–72.
[28] Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in *ABC transporter A1* contributes to HDL cholesterol in the general population. J Clin Invest 2004;114:1343–53.

Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials

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Summary

Background Patients who have acute myocardial infarction remain at major risk of cardiovascular events. We aimed to assess the effects of either human atrial natriuretic peptide or nicorandil on infarct size and cardiovascular outcome.

Methods We enrolled 1216 patients who had acute myocardial infarction and were undergoing reperfusion treatment in two prospective, single-blind trials at 65 hospitals in Japan. We randomly assigned 277 patients to receive intravenous atrial natriuretic peptide (0·025 μg/kg per min for 3 days) and 292 the same dose of placebo. 276 patients were assigned to receive intravenous nicorandil (0·067 mg/kg as a bolus, followed by 1·67 μg/kg per min as a 24-h continuous infusion), and 269 the same dose of placebo. Median follow-up was 2·7 (IQR 1·5–3·6) years for patients in the atrial natriuretic peptide trial and 2·5 (1·5–3·7) years for those in the nicorandil trial. Primary endpoints were infarct size (estimated from creatine kinase) and left ventricular ejection fraction (gauged by angiography of the left ventricle).

Findings 43 patients withdrew consent after randomisation, and 59 did not have acute myocardial infarction. We did not assess infarct size in 50 patients for whom we had fewer than six samples of blood. We did not have angiographs of left ventricles in 383 patients. Total creatine kinase was 66459·9 IU/mL per h in patients given atrial natriuretic peptide, compared with 77878·9 IU/mL per h in controls, with a ratio of 0·85 between these groups (95% CI 0·75–0·97, p=0·016), which indicated a reduction of 14·7% in infarct size (95% CI 3·0–24·9%). The left ventricular ejection fraction at 6–12 months increased in the atrial natriuretic peptide group (ratio 1·05, 95% CI 1·01–1·10, p=0·024). Total activity of creatine kinase did not differ between patients given nicorandil (70520·5 IU/mL per h) and controls (70852·7 IU/mL per h) (ratio 0·995, 95% CI 0·878–1·138, p=0·94). Intravenous nicorandil did not affect the size of the left ventricular ejection fraction, although oral administration of nicorandil during follow-up increased the left ventricular ejection fraction between the chronic and acute phases. 29 patients in the atrial natriuretic peptide group had severe hypotension, compared with one in the corresponding placebo group.

Interpretation Patients with acute myocardial infarction who were given atrial natriuretic peptide had lower infarct size, fewer reperfusion injuries, and better outcomes than controls. We believe that atrial natriuretic peptide could be a safe and effective adjunctive treatment in patients with acute myocardial infarction who receive percutaneous coronary intervention.

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Introduction

Despite availability of effective medical treatments, chronic heart failure remains a major cause of morbidity and mortality worldwide.[1-3] Ischaemic heart disease, in turn, is one of the main causes of chronic heart failure.[4] The most important treatment objectives are prevention of acute myocardial infarction, and, in individuals who have an acute myocardial infarction, reduction in infarct size and ischaemia or reperfusion injury.[5] Only a few medications have been shown to decrease ischaemia or reperfusion injury.[6-8]

Reperfusion of ischaemic myocardium reduces infarct size and improves left ventricular function, both of which contribute to better clinical outcomes in patients with acute myocardial infarction.[9-11] However, reperfusion can also cause tissue damage.[12] Several drugs have been trialled for the prevention or amelioration of such injuries, but results have not been consistently satisfactory.[13-15] Recently, human atrial natriuretic peptide and nicorandil have both been shown to be effective for reduction of myocardial damage after acute myocardial infarction in basic and clinical studies.[16-25] Atrial natriuretic peptide is a candidate for adjunctive treatment after acute myocardial infarction, because it has been shown to suppress the renin–angiotensin–aldosterone system and endothelin-1, both of which which modulate infarct size and cardiac remodelling.[17] Nicorandil is a combined adenosine triphosphate (ATP)-sensitive potassium channel opener and nitrate preparation that has also shown promise as an adjunctive treatment for acute myocardial infarction. In the clinical setting, however,