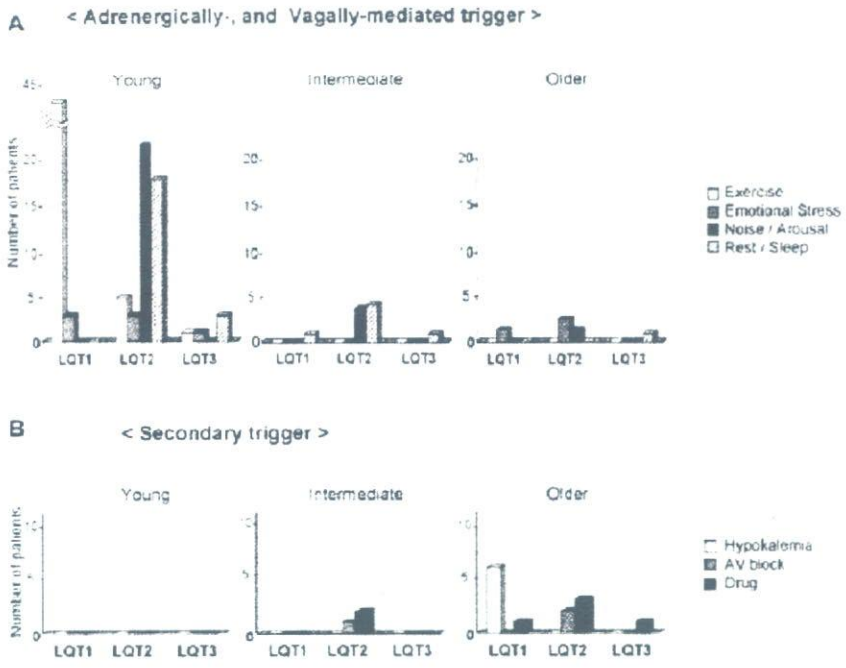

Figure 2. *Genotype-dependent difference of triggers for cardiac events in the young, intermediate, and older groups. A: autonomic triggers. B: secondary triggers: bar graphs indicate the number of patients and their patterns of a specific trigger as summarized in insets. Other triggers for cardiac events that were undefined were excluded.*

Results

Clinical Characteristics

Table 1 summarizes the clinical characteristics of the study subjects. The percentages of females, probands, and patients with positive family history were significantly different among the three groups. In the older group, the percentage of females and probands increased, but that of positive family history decreased. The intermediate group patients showed similar levels of QT prolongation, family history, and Schwartz scores as those of the young group patients. There were no significant differences in basal HR, QTc, and Schwartz scores among the three groups.

Genetic Characteristics

There were 58 LQT1, 75 LQT2, and 12 LQT3 patients (Table 1). In these genotyped patients, we identified 31 *KCNQ1*, 60 *KCNH2*, and 8 *SCN5A* mutations (total 99 different mutations). Among 58 LQT1 patients, most (48/58, 83%) of the first cardiac events occurred at young age. In contrast, first cardiac events occurred less at young age in LQT2 (51/75, 68%) and LQT3 (7/12, 58%) patients compared to the LQT1 patients ($P = 0.019$). The prevalence of transmembrane mutations in LQT1 and LQT3 patients and that of pore site mutations in LQT2 patients was evaluated, but no significant differences were observed among the three groups.

Triggers for Cardiac Events

Figure 1 illustrates the incidence of three categorical triggers in the three age groups. In Figure 1, left-sided bars indicate the number of patients in whom the event was induced by either adrenergically (open bar) or vagally (gray bar) mediated triggers. Right-sided black bars indicate those with secondary triggers. The vertical axis indicates the number of patients. In the young group, all 101 cardiac events were

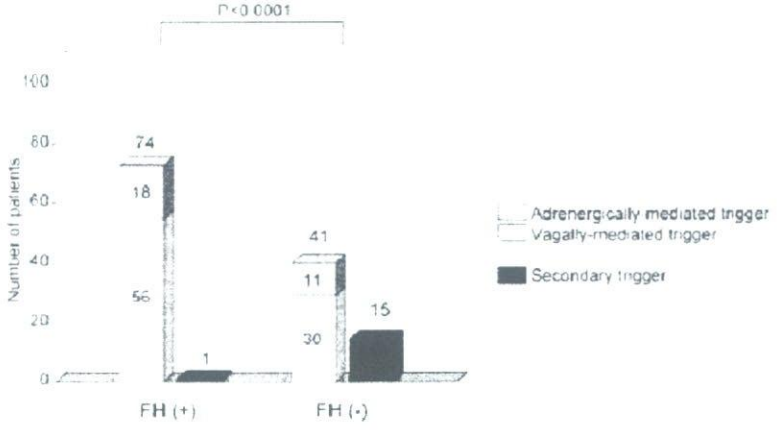

Figure 3. *Triggers for cardiac events in the patients with and without family history. Bar graphs show the number of symptomatic patients and their triggers to induce the cardiac events: open bars, adrenergically mediated; gray bars, vagally mediated; and black bars, secondary triggers. Other triggers for cardiac events that were undefined were excluded.*

TABLE 1

Clinical and Genetic Characteristics in the Three Groups

	Young (n = 106)	Intermediate (n = 20)	Older (n = 19)	P Value
Age at first cardiac event (years)	11.0 ± 0.4	28.0 ± 1.1	59.0 ± 3.5	
Female (%)	63 (59%)	16 (80%)	18 (95%)	0.004
Proband (%)	80 (75%)	18 (90%)	19 (100%)	0.035
Family history	68 (64%)	11 (55%)	3 (16%)	<0.001
HR (bpm)	65 ± 1.2	65 ± 2.8	65 ± 2.8	0.984
QTc (ms)	515 ± 5.7	523 ± 12	485 ± 10	0.084
Schwartz score	6.1 ± 0.2	6.2 ± 0.4	5.3 ± 0.5	0.335
Subtype				
LQT1 (n = 58)	48/58 (83%)	1/58 (2%)	9/58 (15%)	
LQT2 (n = 75)	51/75 (66%)	16/75 (21%)	8/75 (11%)	
LQT3 (n = 12)	7/12 (58%)	3/12 (25%)	2/12 (17%)	
Transmembrane mutation (LQT1)	39/48 (81%)	1/1 (100%)	7/9 (78%)	
Pore site mutation (LQT2)	25/51 (49%)	9/16 (56%)	1/8 (13%)	
Transmembrane mutation (LQT3)	6/7 (86%)	3/3 (100%)	1/2 (50%)	

Data are presented as the mean value ± SD or number (%) of subjects.
HR = heart rate; LQT1 = long QT syndrome caused by the *KCNQ1* potassium channel gene mutations; LQT2 = long QT syndrome caused by the *KCNH2* potassium channel gene mutations; LQT3 = long QT syndrome caused by the *SCN5A* sodium channel gene mutations; QTc = QT interval corrected by Bazett's formula.

TABLE 2

Clinical and Genetic Characteristics in Patients With or Without Family History

	FH (+) (n = 82)	FH (−) (n = 63)	P Value
Age at first cardiac event (years)	14.0 ± 1.1	26.0 ± 2.8	<0.001
Female (%)	55 (67%)	42 (67%)	0.960
Proband (%)	54 (66%)	63 (100%)	<0.001
HR (bpm)	65 ± 1.2	64 ± 1.4	0.728
QTc (ms)	512 ± 6.9	513 ± 7.0	0.890
Schwartz score	6.4 ± 0.2	5.6 ± 0.2	0.005
Subtype			
LQT1 (n = 58)	40/58 (69%)	18/58 (31%)	
LQT2 (n = 75)	36/75 (48%)	39/75 (52%)	
LQT3 (n = 12)	6/12 (50%)	6/12 (50%)	
Transmembrane mutation (LQT1)	35/40 (88%)	13/18 (72%)	
Pore site mutation (LQT2)	19/36 (53%)	16/39 (41%)	
Transmembrane mutation (LQT3)	6/6 (100%)	4/6 (67%)	

Data are presented as the mean value ± SD or number (%) of subjects.
FH = family history; HR = heart rate; LQT1 = long QT syndrome caused by the *KCNQ1* potassium channel gene mutations; LQT2 = long QT syndrome caused by the *KCNH2* potassium channel gene mutations; LQT3 = long QT syndrome caused by the *SCN5A* sodium channel gene mutations; QTc = QT interval corrected by Bazett's formula.

associated with autonomic triggers, among which 79 (78%) events were adrenergically mediated and 22 (22%) were vagally mediated. On the other hand, only 5 of 18 cardiac events (28%) were associated with autonomic triggers in the older group, and secondary triggers induced cardiac events in the majority of the older group patients (13/18, 72%). The percentage of secondary triggers was significantly larger in the older group than in the other two groups (0% in young [0/101] vs 23% in intermediate [3/13] vs 72% in older [13/18]: $P < 0.0001$). Among the cardiac events triggered by autonomic factors, the percentage of the adrenergically mediated triggers was significantly lower in the intermediate group patients (79/101 [78%] in young vs 3/10 [30%] in intermediate vs 4/5 [80%] in older group: $P < 0.001$). Thus, triggering factors were significantly different among the three groups.

Figure 2A shows autonomic triggers in the three genotypes in each age group. There were also genotype-dependent differences in triggers for cardiac events in the young group, as previously reported:[5] in young LQT1 patients, 92% (44/48) of the cardiac events occurred during exercise (open bar), but none with noise/arousal (black bar) or rest/sleep (hatched bar). This is in sharp contrast with the pattern in young LQT2 patients: 37% (19/51) of the events occurred during rest/sleep and 43% (22/51) with noise/arousal. Irrespective of onset age, cardiac events triggered by noise/arousal were very specific and observed in only LQT2 patients (35%, 26 of 75 LQT2 patients). In contrast, 44% (3/7) of LQT3 patients experienced cardiac events during rest/sleep. In opposition to the young LQT1 patients, only ~20% of total LQT2 and LQT3 patients experienced cardiac events triggered by exercise or emotional stress.

Figure 2B depicts secondary triggers in the three genotypes in each age group. Hypokalemia (open bar), complete AV block (gray), and drugs (black) were associated with cardiac events in total of 6, 3, and 7 patients, respectively, in the intermediate and older groups. Interestingly, hypokalemia was associated with cardiac episodes in only older LQT1 patients. On the other hand, drugs and AV block triggered cardiac events mainly in LQT2 patients of >20 years. Responsible drugs were amphetamine, aprindine, cisapride (plus pimenol), disopyramide, erythromycin, hydroxyzine, and procainamide.

Family History

Comparison of clinical and genetic characteristics between patients with and without family history is shown in Table 2. The age at first cardiac event was significantly younger and Schwartz score was significantly higher in the patients with family history than in those without it. LQT1 patients appeared to have more family history compared to those of LQT2 and LQT3 genotypes. Figure 3 illustrates the incidence of three categorical triggers in patients with and without family history. Triggers for cardiac events were also significantly different between the two groups, and secondary trigger was seen in only 1 patient with family history and in 27% (15 of 56) of patients without family history.

Discussion

In the genotyped/symptomatic LQTS patients, the present study demonstrated that factors triggering cardiac events were different depending on the age of their first onset. In general, syncope and sudden death in LQTS are believed to be due to TdP-type of ventricular tachycardia and occur usually in the young.[15,16] However, pathophysiological properties of LQTS-related events were found to be even different among the three groups that were divided by age of less than 20, 20-39, and greater than 40 years. In the young group (<20 years), triggers were closely related to the autonomic nervous tone. In contrast, secondary triggers induced cardiac events in 72% of the older patients (>40 years), suggesting

that "double hit" by secondary trigger(s) appeared to aggravate the clinical phenotype, in addition to genetic variants in ion channel genes, in the older group. The intermediate group patients were at in-between risk in clinical characteristics and the triggers of cardiac events. Interestingly, regarding the triggers of cardiac events, the percentage of the adrenergically mediated trigger was lower in the intermediate group. This may reflect a relatively small number of LQT1 patients in the intermediate group.

Although there was no statistically significant difference in the QTc interval among the three age groups ($P = 0.084$), the older group showed shorter QTc interval compared to that in the other two groups. The QTc in the young group was even shorter than that in the intermediate group. This was probably due to the fact that the QTc in the LQT1 patients was significantly shorter than that in the LQT2 and LQT3 patients (LQT1: 490 ± 6.6, LQT2: 534 ± 8.4, LQT3: 555 ± 26; $P < 0.0001$) and the percentage of LQT1 patients was higher in the young group.

Our results in the young group are consistent with previous reports:[5,7] LQT1 patients experienced the majority of their cardiac events during exercise or emotional stress and only a few occurred during rest/sleep, in opposition to the pattern in LQT2 and LQT3 patients. Cardiac events in LQT2 patients in the young group were mainly associated with noise and sudden arousal and other adrenergic triggers. Cardiac events occurred during rest/sleep in half of the young LQT3 patients.

Among the secondary triggers, hypokalemia was associated with cardiac episodes in only LQT1 patients. Lower extracellular K^+ concentrations are known to reduce outward conductance of both rapid component of delayed rectifier potassium (I_{Kr}) and background inward rectifier potassium (I_{K1}) currents.[17-19] In LQT1, the slow component of delayed rectifier potassium current (I_{Ks}) is impaired, and the function of I_{Kr} and I_{K1} channels remains normal or even upregulated to compensate the total net outward K^+ conductance. Therefore, hypokalemia may unveil the potential repolarization disorder by reducing both "healthy" I_{Kr} and I_{K1}.

On the other hand, AV block and drug intake associated with cardiac events as secondary triggers were seen to be present in most of the intermediate and older LQT2 patients. Tan and colleagues[20] reported that pause-dependence of TdP onset was predominant in LQT2 but absent or rare in LQT1, suggesting that this disparity may reflect different mechanisms. Experimental studies have shown that I_{Ks} blockade (LQT1) causes delayed afterdepolarizations (DAD) but not early afterdepolarizations (EAD);[21] on the contrary, I_{Kr} blockade (LQT2) causes EADs, predominantly at slower HRs.[22] Extreme bradycardia due to AV block may lead to EAD as well as TdP through the postpausal prolongation of action potential plateau. Both a smaller I_{Kr} due to complete deactivation and an enhanced inward Na^+/Ca^{2+} exchanger at low HR may contribute to EAD formation by providing time for recovery and reactivation of L-type Ca^+ channel. In the presence of pathological bradycardia, therefore, I_{Kr} plays a more important role in abbreviating the repolarization and, thereby, keeping the appropriate QT interval because little accumulation of outward I_{Ks} occurs at lower HR.[23]

In this connection, drug-induced TdP has been shown to depend on intervals of preceding pauses.[24] The above-mentioned mechanism on the bradycardia-induced TdP may give an explanation of our result that most of the drug-induced events were observed in LQT2. Because responsible drugs are known to block cardiac I_{Kr} (except hydroxyzine), preexisting repolarization abnormality due to gene mutations may predispose the patients to fatal arrhythmias by further reducing the outward K^+ conductance.[25,26] In preliminary experiments of biophysical assay with heterologous expression systems, we found that these *KCNH2* mutations identified in drug-induced TdP patients produced mild loss-of-function of I_{Kr}.

We evaluated only "already-symptomatic" genotyped patients in this study. The percentage of "still-asymptomatic" patients was 58% of all genotyped patients (198 of 343). The average ages of asymptomatic patients were 19.0 ± 1.9 years (5–67 years) for probands and 34.0 ± 1.8 years (2–68 years) for family members. Asymptomatic probands were still young; therefore, some of them would be symptomatic in the future, being exposed to higher risk of lethal events. The results of our study again emphasize the importance of a careful approach to asymptomatic (preclinical) LQTS patients to decrease their arrhythmic risk, particularly in older patients (≥20 years). Because of lack of apparent phenotypes, most of them were not diagnosed prior to the onset of symptoms. However, one of the most important missions of our genetic testing would be to achieve a preclinical diagnosis of LQTS, particularly in patients with forme-fruste phenotype. Because of low penetrance, inheriting a gene mutation per se does not always mean that the individual mutation carrier will present clinical manifestation,[27] but apparently "healthy" carriers have inherited the risk for developing the clinical phenotype. Once genetic information becomes available, we can introduce the timely beta-blocker therapy and conduct careful follow-up, including ECG recordings, lifestyle modifications (i.e., avoidance of QT-prolonging drugs), avoidance of hyopokalemia, bradycardia, other alarming symptoms, and family education (home automatic electrical defibrillator, etc.).

Study Limitations

Intermediate and older group patients may have a higher possibility to use more drugs. We, therefore, could not exclude such an age-dependent risk accumulation affecting the results of trigger distribution. As for another issue, carriers of milder mutations may induce cardiac events more likely in association with secondary triggers. Our study included only subjects with three major genotypes, although they account for the majority of LQTS patients. Patients with compound mutations of LQT1-3 and 5–7 genotypes were all excluded from analysis. However, we failed to exclude compound mutation carriers with other (LQT4, 8–10) or unknown genotypes, which may result in a minor selection bias.

We evaluated only the Japanese population and there remains a concern about ethnic differences. However, the prevalent mutations found in more than 4 patients were A341V-KCNQ1, A344A/sp-KCNQ1, and A614V-HERG and were all popular in other ethnic cohorts. The genotype-specific triggers were also similar to those observed in previous studies from other countries.

Although syncope may result from diseases other than LQTS-related ventricular arrhythmia, we considered sudden onset/offset nature of loss of consciousness in a genotyped LQTS patient as syncope due to ventricular arrhythmias, if there was no evidence of another explanation, and included as a study subject. In this connection, very short duration of

TdP that did not cause syncope was underestimated if Holter ECG failed to detect it.

Conclusion

Triggers of cardiac events were closely related to the autonomic nervous tone with a higher incidence of family history in younger patients. In contrast, arrhythmic events in older patients were associated with secondary triggers, such as drugs, hypokalemia, and AV block, with genotype specificity. Thus, arrhythmic triggers in LQTS differ depending on the age of the patients, stressing the importance of age- and genotype-related therapy for genotyped LQTS.

Acknowledgments: We gratefully acknowledge the technical assistance of Arisa Ikeda, Tamami Hirata (Department of Cardiovascular Medicine, Shiga University of Medical Science, Otsu, Japan), Toshiko Shibata, Naotaka Ohta, and Toshiro Ura (Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan).

References

1. Shimizu W: The long QT syndrome: Therapeutic implications of a genetic diagnosis. Cardiovasc Res 2005;67:347-356.
2. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foell JD, Li Z, Kamp TJ, Towbin JA: Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation 2006;114:2104-2112.
3. Medeiros-Domingo A, Kaku T, Tester DJ, Iturralde-Torres P, Itty A, Ye B, Valdivia C, Ueda K, Canizales-Quinteros S, Tusié-Luna MT, Makielski JC, Ackerman MJ: SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. Circulation 2007;116:134-142.
4. Schwartz PJ, Priori SG, Locati EH, Napolitano C, Cantù F, Towbin JA, Keating MT, Hammoude H, Brown AM, Chen LS: Long QT syndrome patients with mutations of the *SCN5A* and *HERG* genes have differential responses to Na^+ channel blockade and to increases in heart rate. Implications for gene-specific therapy. Circulation 1995;92:3381-3386.
5. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R: Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. Circulation 2001;103:89-95.
6. Schwartz PJ, Zara A, Locati E, Moss AJ: Stress and sudden death. The case of long QT syndrome. Circulation 1991;83(Suppl II):II70-II80.
7. Wilde AA, Jongbloed RJ, Doevendans PA, Düren DR, Hauer RN, van Langen IM, van Tintelen JP, Smeets HJ, Meyer H, Geelen JL: Auditory stimuli as a trigger for arrhythmic events differentiate *HERG*-related (LQTS2) patients from *KVLQT1*-related patients (LQTS1). J Am Coll Cardiol 1999;33:327-332.
8. Bazett HC: An analysis of the time-relations of electrocardiograms. Heart 1920;7:353-370.
9. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS: Diagnostic criteria for the long QT syndrome: An update. Circulation 1993;88:782-784.
10. Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, Washizuka T, Aizawa Y, Nakamura K, Ohe T, Aiba T, Miyamoto Y, Yoshinasa Y, Towbin JA, Priori SG, Kamakura S: Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: Multicenter study in Japan. J Am Coll Cardiol 2004;44:117-125.
11. 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 2007;115:2481-2489.
12. Moss AJ, Zareba W, Kaufman ES, Gartman E, Peterson DR, Benhorin J, Towbin JA, Keating MT, Priori SG, Schwartz PJ, Vincent GM, Robinson JL, Andrews ML, Feng C, Hall WJ, Medina A, Zhang L, Wang Z: Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. Circulation 2002;105:794-799.
13. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT: Genomic structure of three long QT syndrome genes: *KVLQT1*, *HERG*, and *KCNE1*. Genomics 1998;51:86-97.
14. Wang Q, Li Z, Shen J, Keating MT: Genomic organization of the human *SCN5A* gene encoding the cardiac sodium channel. Genomics 1996;34:9-16.
15. Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Robinson JL, Priori SG, Benhorin J, Locati EH, Towbin JA, Keating MT, Lehmann MH, Hall WJ: Influence of the genotype on the clinical course of the long-QT syndrome. N Engl J Med 1998;339:960-965.
16. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D: Risk stratification in the long-QT syndrome. N Engl J Med 2003;348:1866-1874.
17. Lopez-Barneo J, Hoshi T, Heinemann SH, Aldrich RW: Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. Receptors Channels 1993;1:61-71.
18. Choy AM, Lang CC, Chomsky DM, Rayos GH, Wilson JR, Roden DM: Normalization of acquired QT prolongation in humans by intravenous potassium. Circulation 1997;96:2149-2154.
19. Rasmusson RL, Morales MJ, Wang S, Liu S, Campbell DL, Brahmajothi MV, Strauss HC: Inactivation of voltage-gated cardiac K^+ channels. Circ Res 1998;82:739-750.
20. Tan HL, Bardai A, Shimizu W, Moss AJ, Schulze-Bahr E, Noda T, Wilde AAM: Genotype-specific onset of arrhythmias in congenital long-QT syndrome: Possible therapy implications. Circulation 2006;114:2096-2103.
21. Burashnikov A, Antzelevitch C: Block of $I_{(Ks)}$ does not induce early afterdepolarization activity but promotes beta-adrenergic agonist-induced delayed afterdepolarization activity. J Cardiovasc Electrophysiol 2000;11:458-465.
22. Liu J, Laurita KR: The mechanism of pause-induced torsade de pointes in long QT syndrome. J Cardiovasc Electrophysiol 2005;16:981-987.
23. Stengl M, Volders PGA, Thomsen M, Spatjens RL, Sipido KR, Vos MA: Accumulation of slowly activating delayed rectifier potassium current (I_{Ks}) in canine ventricular myocytes. J Physiol 2003;551:777-786.
24. Tan HL, Hou CJ, Lauer MR, Sung RJ: Electrophysiologic mechanisms of the long QT interval syndromes and torsade de pointes. Ann Intern Med 1995;122:701.
25. Sanguinetti MC, Jiang C, Curran ME, Keating MT: A mechanistic link between an inherited and an acquired cardiac arrhythmia: *HERG* encodes the I_{Kr} potassium channel. Cell 1995;81:299-307.
26. Mitcheson JS, Chen J, Lin M, Culberson C, Sanguinetti MC: A structural basis for drug-induced long QT syndrome. Proc Natl Acad Sci U S A 2000;97:12329-12333.
27. Priori SG, Napolitano C, Schwartz PJ: Low penetrance in the long-QT syndrome clinical impact. Circulation 1999;99:529-533.

tor gene in lean and obese Pima Indians. *Hum Mol Genet* 1997; **6**: 675–679.
7. Lakka TA, Rankinen T, Weisnagel SJ, *et al*: Leptin and leptin receptor gene polymorphisms and changes in glucose homeostasis in response to regular exercise in nondiabetic individuals. The HERITAGE family study. *Diabetes* 2004; **53**: 1603–1608.
8. Park KS, Shin HD, Park BL, *et al*: Polymorphisms in the leptin receptor (LEPR)—putative association with obesity and T2DM. *J Hum Genet* 2006; **51**: 85–91.
9. Matsuoka N, Ogawa Y, Hosoda K, *et al*: Human leptin receptor gene in obese Japanease subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. *Diabetologia* 1997; **40**: 1204–1210.
10. Loos RJ, Rankinen T, Chagnon Y, Tremblay A, Perusse L, Bouchard C: Polymorphisms in the leptin and leptin receptor genes in relation to resting metabolic rate and respiratory quotient in the Quebec Family Study. *Int J Obes* 2006; **30**: 183–190.
11. Kawai K, Sugimoto K, Nakashima K, Miura H, Ninomiya Y: Leptin as a modulator of sweet taste sensitivities in mice. *Proc Natl Acad Sci U S A* 2000; **97**: 11044–11049.
12. de Luca C, Kowalski TJ, Zhang Y, *et al*: Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. *J Clin Invest* 2005; **115**: 3484–3493.
13. Shigemura N, Ohta R, Kusakabe Y, *et al*: Leptin modulates behavioral responses to sweet substances by influencing peripheral taste structures. *Endocrinology* 2004; **145**: 839–847.
14. de Krom M, van der Schouw YT, Hendriks J, *et al*: Common genetic variations in CCK, leptin, and leptin receptor genes are associated with specific human eating patterns. *Diabetes* 2007; **56**: 276–280.
15. Bartoshuk LM, Duffy VB, Hayes JE, Moskowitz HR, Snyder DJ: Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. *Philos Trans R Soc Lond B Biol Sci* 2006; **361**: 1137–1148.
16. Karlin S, Cameron EC, Chakraborty R: Path analysis in genetic epidemiology: a critique. *Am J Hum Genet* 1983; **35**: 695–732.
17. Wang TN, Huang MC, Chang WT, *et al*: G–2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines. *Obesity* 2006; **14**: 183–187.
18. Mammes O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F: Association of the G–2548A polymorphism in the 5′ region of the LEP gene with overweight. *Ann Hum Genet* 2000; **64**: 391–394.
19. Le Stunff C, Le Bihan C, Schork NJ, Bougneres P: A common promoter variant of the leptin gene is associated with changes in the relationship between serum leptin and fat mass in obese girls. *Diabetes* 2000; **49**: 2196–2200.
20. Bernhardt SJ, Naim M, Zehavi U, Lindemann B: Changes in IP3 and cytosolic Ca^{2+} in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. *J Physiol* 1996; **490**: 325–336.
21. Lindemann B: Taste reception. *Physiol Rev* 1996; **76**: 719–766.
22. Harvey J, Ashford ML: Insulin occludes leptin activation of ATP-sensitive K^+ channels in rat CRI-G1 insulin secreting cells. *J Physiol* 1998; **510**: 47–61.
23. Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford ML: Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* 1997; **390**: 521–525.
24. Freidman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 1998; **395**: 763–770.
25. Tartaglia LA, Dembski M, Weng X, *et al*: Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; **83**: 1263–1271.
26. Fong TM, Huang RR, Tota MR, *et al*: Localization of leptin binding domain in the leptin receptor. *Mol Pharmacol* 1998; **53**: 234–240.
27. Shigematsu Y, Norimatsu S, Ohtsuka T, Okayama H, Higaki J: Sex-related differences in the relations of insulin resistance and obesity to left ventricular hypertrophy in Japanese hypertensive patients. *Hypertens Res* 2006; **29**: 499–504.
28. Sakurai M, Miura K, Takamura T, *et al*: Gender differences in the association between anthropometric indices of obesity and blood pressure in Japanese. *Hypertens Res* 2006; **29**: 75–80.
29. Fujiwara T, Saitoh S, Takagi S, *et al*: Development and progression of atherosclerotic disease in relation to insulin resistance and hyperinsulinemia. *Hypertens Res* 2005; **28**: 665–670.
30. Takeuchi H, Saitoh S, Takagi S, *et al*: Metabolic syndrome and cardiac disease in Japanese men: applicability of the concept of metabolic syndrome defined by the National Cholesterol Education Program–Adult Treatment Panel III to Japanese men—the Tanno and Sobetsu Study. *Hypertens Res* 2005; **28**: 203–208.

ATH-10105; No. of Pages 8

ELSEVIER

Atherosclerosis xxx (2007) xxx–xxx

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome

Masafumi Nakayama[a,*], Takashi Kudoh[a], Koichi Kaikita[a], Michihiro Yoshimura[e], Shuichi Oshima[b], Yoshihiro Miyamoto[c], Motohiro Takeya[d], Hisao Ogawa[a]

[a] *Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto City 860-8556, Japan*

[b] *Division of Cardiology, Kumamoto Central Hospital, 1-5-1 Tainoshima, Kumamoto City 862-0965, Japan*

[c] *Division of Atherosclerosis and Diabetes, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan*

[d] *Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto City 860-8556, Japan*

[e] *Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8, Nishi-Shimbashi, Minato-ku, Tokyo, 105-8461, Japan*

Received 3 April 2007; received in revised form 28 August 2007; accepted 6 September 2007

Abstract

Objective: Morbidity and mortality rates are still high among patients with acute coronary syndrome (ACS); moreover, it is clinically difficult to determine precisely which patients will progress satisfactorily. Unstable plaque is characterized by an increased number of activated inflammatory cells, including macrophages and lymphocytes, and an increased release of numerous inflammatory mediators and proteolytic enzymes. Mononuclear cells consist of monocytes/macrophages and lymphocytes and are able to be experimentally isolated. We searched for a specific risk factor for ACS in the peripheral blood mononuclear cells (PBMCs).

Methods and results: We examined the expression of 12,625 genes in PBMCs utilizing a gene chip microarray system in ACS patients in acute and chronic stable phases. The gene expression profiles revealed that class A macrophage scavenger receptors (SR-A), among the immune response factors and the receptor activity markers, were the most strongly increased in the acute phase. We examined SR-A gene expression levels of PBMCs using real time RT-PCR in 122 consecutive patients: 32 ACS patients; 41 stable angina patients; and, 49 control subjects. The SR-A gene expression levels of the PBMCs were highest in the ACS patients ($p < 0.0001$). The occurrence of a reattack of a cardiovascular event was significantly lower in the low SR-A group than in the high SR-A group ($p < 0.001$).

Conclusion: SR-A gene expression level in the PBMCs specifically increases in patients with ACS, and provides a predictive marker for a reattack of a cardiovascular event.

Keywords: Acute coronary syndrome; Class A macrophage scavenger receptor; Directional coronary atherectomy; Peripheral blood mononuclear cells; Microarray; Risk factor; Gene expression

1. Introduction

Morbidity and mortality rates are still high among patients with acute coronary syndrome (ACS). ACS, defined as acute myocardial infarction (AMI) with ST elevation, AMI without ST elevation, or unstable angina, is most often induced by coronary thrombosis as an acute complication of atherosclerosis: a rupture of the fibrous cap of an atherosclerotic plaque is one example [1–4]. The processes of thrombosis and inflammation are closely related and increasing evidence supports the involvement of inflammation in both the atherogenesis and pathogenesis of ACS [5–7]. It has always been

* Corresponding author. Tel.: +81 96 373 5175; fax: +81 96 362 3256.
E-mail address: nakama@kumamoto-u.ac.jp (M. Nakayama).

0021-9150/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2007.09.006

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

difficult to determine precisely which patients will progress satisfactorily.

Unstable plaque is characterized by an increased number of activated inflammatory cells, including macrophages and lymphocytes, and an increased release of numerous inflammatory mediators and proteolytic enzymes [5–7]. The inflammatory responses lead to the disruption of plaque and subsequent events in ACS. According to the previous studies, activated macrophages are believed to facilitate ongoing inflammation present within the plaque, and thus may be important in the initiation of ACS [8]. Macrophages within the plaque are scavenging cells that accumulate lipid to become foam cells. Macrophage and extracellular lipid accumulation expand the lipid core of lesions, destroying intimal tissue and fibrous caps, [9] and also result in progressive tissue destruction of the media and elastic lamina below the core [10,11]. Lymphocytes also infiltrate into unstable plaque, thereby resembling a delayed hypersensitivity reaction. Lymphocytes, including T-cells, secrete cytokines that regulate the activity of macrophages and may differentiate into effecter cells with tissue-damaging potential [12]. Although characteristics of inflammatory cells and pathogenesis in the unstable plaque have been well studied, characteristics of inflammatory cells and pathogenesis in the peripheral blood in ACS patients have rarely been studied. Mononuclear cells consist of monocytes/macrophages and lymphocytes and are able to be experimentally isolated.

In this study, we searched for risk factors and/or pathogenetic markers for ACS in the peripheral blood mononuclear cells (PBMCs).

2. Methods

2.1. Study population

Two female patients with unstable angina were included in the microarray protocol study: class IIIB-Tpos angina as defined by Braunwald for classification of unstable angina [13]. Neither patient had any known coronary risk factors, including hypertension, diabetes mellitus, smoking, or dyslipidemia. They immediately underwent coronary angiographies and percutaneous coronary interventions. We obtained blood samples at the time of admission (acute phase) and 3 weeks after the admission (chronic stable phase). In the acute phase, the blood samples were obtained before the emergency percutaneous interventions.

In larger population study, the study population consecutively admitted from January 2002 to December 2005 and consisted of three groups: 32 patients with ACS; 41 patients with stable angina; and, 49 control subjects. The patients with ACS consisted of 21 patients with acute myocardial infarction and 11 patients with unstable angina (class IIIB-Tpos angina). All the patients with ACS immediately upon admission received coronary angiographies and percutaneous coronary interventions. The blood samples were obtained at the time of admission (acute phase) and 3 weeks after the admission (chronic stable phase) in the patients with ACS. In the acute phase, the blood samples were obtained before the emergency percutaneous interventions. All stable angina patients had one or more stenotic coronary arteries with more than 75% stenosis after nitroglycerin administration. The control subjects all underwent diagnostic cardiac catheterization, including coronary angiography, for evaluation of their chest pain, and all had angiographically normal or nearly normal coronary arteries. We obtained blood samples from the patients with stable angina and the control subjects before the diagnostic cardiac catheterization.

Seventy-three patients with ACS or stable angina were followed up. We measured the SR-A gene expression levels in these 73 patients with stable state of ischemic heart disease and followed them up after obtaining informed consent. We assigned the 73 patients into two groups, according to their SR-A gene expression levels in the stable state of ischemic heart disease, as follows: the low SR-A group (the SR-A gene expression level <5.0) and the high SR-A group (the SR-A gene expression level $\geq$5.0) (median SR-A gene expression level: 5.0 [0.3–19.1]). We examined the following four events during the follow-up period: fatal myocardial infarction; nonfatal myocardial infarction; unstable angina; and, heart failure with NYHA class IV symptoms.

Written informed consent was obtained from all patients in this study. The study was in agreement with the guidelines approved by the ethics committee of Kumamoto University Graduate School of Medical Sciences.

Patients with evidence of congestive heart failure, atherosclerosis obliterans, cerebral infarction, severe angina unresponsive to medical therapy, serious ventricular arrhythmia, bronchial asthma, pneumonia, or other coexisting severe illnesses were excluded from this study. Additionally, some patients who withdrew their informed consent were excluded.

2.2. Separation of peripheral blood mononuclear cells and extraction of total RNA

We layered a maximum of 10 mL of whole blood with 0.38% sodium citric acid over 4 mL of Ficoll-Paque (Pharmacia Biotech, Sweden) and then we centrifuged this for 15 min at 3000 rpm at room temperature; next, we aspirated the mononuclear cells located at the plasma-Ficoll-Paque interface. We centrifuged this aspirated solution for 15 min at 15,000 rpm at room temperature; we then extracted total RNA of peripheral blood mononuclear cells (PBMCs) utilizing an RNeasy Mini Kit (QIAGEN, GmbH Hirden, Germany).

2.3. GeneChip microarray protocol

Ten micrograms (5 μg in each sample) of total RNA were used in the first-strand cDNA synthesis with T7-d(T)24 primer (GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG-[dT]24) and Superscript II (Gibco-BRL, Gaithersburg, Md). The cDNA which combined two

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

samples was prepared according to protocols provided with the Affymetrix U95 GeneChip System (Affymetrix, Santa Clara, CA). The second-strand cDNA synthesis was performed at 16 °C for 2 h by adding *Escherichia coli* DNA ligase, DNA polymerase I, and RNase H to the reaction, followed by T4 DNA polymerase to blunt the ends of newly synthesized cDNA. The cDNA was purified with phenol/chloroform and ethanol precipitation. Using a BioArray HighYield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY, USA), the purified cDNA was incubated for 5 h at 37 °C in an in vitro transcription reaction to produce cRNA-labeled with biotin.

cRNA was fragmented by incubating it in a buffered solution containing 200 mmol/L Tris-acetate, 500 mmol/L KOAc, and 150 mmol/L MgOAc for 35 min at 94 °C. Fifteen micrograms of fragmented cRNA were mixed with eukaryotic hybridization controls (containing control cRNA and oligonucleotide B2) and hybridized with a pre-equilibrated human U95 Affymetrix Chip for 16 h at 45 °C. The chips were then washed in a fluidic station with non-stringency wash buffered solution (6Xstandard saline phosphate with EDTA, 0.01% Tween 20, and 0.005% antifoam) for 10 cycles and in a high stringency buffered solution (100 mmol/L *N*-morpholino-ethanesulfonic acid, 0.1 mol/L NaCl, and 0.01% Tween 20) for 4 cycles and then stained with streptavidin phycoerythrin. This process was followed by incubation with normal goat immunoglobulin G and biotinylated mouse anti-streptavidin antibodies, and then re-stained with streptavidin phycoerythrin.

The resulting chips were scanned in an HP G2500A ChipScanner (Affymetrix) to detect hybridization signals. Image output files were visually examined for major chip defects and hybridization artifacts, and the raw data was then processed with GeneChip Microarray Analysis Suite 5.0 software (Affymetrix). The image from each chip was scaled such that the absolute signal intensity was adjusted to target just intensity and this resulting value was reported as a non-negative integer. We compared the gene expression of the PBMCs in patients with unstable angina at the time of admission and at 3 weeks after admission.

Functional ontological classification was based on Richardson et al.'s terms: cellular component; biological process; and, molecular function [14]. We compared frequencies of terms within the specified list with frequencies of terms within all genes in the Entrez Gene database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene). We calculated a *p*-value using Fisher's exact test to determine whether any gene ontology terms correlated to any specific list of genes. Statistical inference of enrichment of specific terms, in the list, was made with Fisher's exact test.

2.4. Quantitative real time reverse transcription-polymerase chain reaction analysis

Samples (500 ng) of total RNA were reverse transcribed utilizing the High Capacity DNA Archive Kit (Applied Biosystems, Foster City, CA, USA). Human class A macrophage scavenger receptor (SR-A) mRNA levels and human Glyseraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were quantified using a two step real time reverse transcription-polymerase chain reaction; the reaction mixture (10 μL) contained 250 ng of cDNA template, a set of primers and a related probe, and a TaqMan Universal Master Mix (Applied Biosystems). The primers and probe sets for SR-A (Hs00234007) and GAPDH (Hs99999905) were purchased from TaqMan® Gene Expression Assays (Applied Biosystems). The context sequences of the SR-A gene and GAPDH gene are 5′-GGA ATA GTG GCA GCT CAA CTC CTG A and 5′-GCG CCT GGT CAC CAG GGC TGC TTT T, respectively. Real-time polymerase chain reaction was performed according to the manufacturer's prescribed protocol using an ABI PRISM 7900 Sequence Detector (Applied Biosystems, Foster City, CA USA). Standard curves for human SR-A mRNA levels were generated using dilutions of samples of cDNA prepared from PBMCs. Gene expressions of SR-A and GAPDH were quantified using the critical threshold cycle. The critical threshold cycle is inversely proportional to the logarithm of the initial number of template molecules. We determined the SR-A gene expression level as follows: SR-A mRNA level/GAPDH mRNA level.

2.5. Statistical analysis

When the clinical characteristics of the study patients were considered, hypertension was operationally defined as blood pressures >140/90 mm Hg, diabetes mellitus was defined as fasting blood glucose levels 126 mg/dL or 200 mg/dL during an oral glucose tolerance test, and hypercholesterolemia was defined as total cholesterol level ≧220 mg/dL.

Multiple logistic regression analysis with forward stepwise selection (Wald) was performed with SPSS Advanced Statistics 14.0J for Windows (SPSS Japan Inc.). Independent variables were coded as the following dummy variables: SR-A gene expression level, 0 for <5.0 and 1 for ≧5.0; sex, 0 for female and 1 for male; age, 0 for <65 years and 1 for ≧65 years; body mass index, 0 for <25 kg/m^2 and 1 for ≧25 kg/m^2; total cholesterol level, 0 for <180 mg/dL and 1 for ≧180 mg/dL; HDL-cholesterol level, 0 for ≧40 mg/dL and 1 for <40 mg/dl; cigarette smoking, 0 for nonsmokers and 1 for smokers; hypertension, 0 for normotension and 1 for hypertension; diabetes mellitus, 0 for absence and 1 for presence; family history of cardiovascular disease, 0 for absence and 1 for presence; lipoprotein(a) level, 0 for <30 mg/dL and 1 for ≧30 mg/dL; and, high-sensitivity C-reactive protein (hs-CRP) level, 0 for <1.0 mg/L and 1 for ≧1.0 mg/L.

A Kaplan–Meier survival curve was used for determining the rate of reattack of cardiovascular disease in both low SR-A gene expression level (<5.0) groups and high SR-A gene expression level (≧5.0). Utilizing the Log-Rank test, we compared the reattack rates of cardiovascular disease

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

between the low and the high SR-A gene expression level groups.

Continuous variables are expressed as mean ± S.D. Continuous variables were compared using two-tailed unpaired or paired *t*-tests. Categorical variables were compared by Chi-square analysis; Fisher's exact probability was used. A probability value <0.05 was considered to indicate statistical significance.

3. Results

3.1. Gene expression profile

We compared the expression of 12,625 genes between the acute and chronic stable phases in ACS patients. Among the significant increased genes at the time of onset of ACS, in comparison with those in the chronic stable phase, we listed the genes whose expression level increased over twofold (Supplemental Table 1). According to our genetic ontological examination, class A macrophage scavenger receptors (SR-A), among the immune response factors and the receptor activity markers, were the most strongly increased in the acute phase (Supplemental Table 2). We remarked then that SR-A was a candidate for being a risk factor for ACS. Among the significant decreased genes at the time of onset of ACS, in comparison with those in chronic stable phase, we listed the genes whose expression significantly decreased in Supplemental Table 3 ($p < 0.05$).

3.2. Clinical characteristics of the patients who underwent peripheral blood sampling in the lager population

Peripheral blood mononuclear cells (PBMCs) were obtained from 49 control subjects: 41 patients with stable angina; and, 32 patients with ACS. We compared the clinical characteristics including age, gender, body mass index, hypertension, smoking, diabetes mellitus, hypercholesterolemia, total cholesterol level, low-density lipoprotein (LDL) cholesterol level, high-density lipoprotein (HDL) cholesterol level, triglycerides, lipoprotein (a) level, high-sensitivity C-reactive protein (hsCRP) level, family history of cardiovascular disease, and pharmacological medications among the 3 groups (Table 1). The incidence of hypertension was significantly lower in the control group than in the stable angina group ($p < 0.05$). The incidence of diabetes mellitus was significantly lower in the control group than in both the stable angina and the ACS groups ($p < 0.05$). Total cholesterol level, as well as HDL-cholesterol level, was significantly higher in the control group than in both the stable angina and the ACS groups ($p < 0.05$). The hsCRP levels in the control group were significantly lower than in both the stable angina and ACS groups ($p < 0.05$). Concerning the cardiovascular medications, the frequencies of use of beta-blockers, nitrates, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and diuretics were significantly lower in the control group than in either the stable angina or the ACS group. The frequency of use of nitrates was

Table 1
Clinical characteristics: peripheral blood samples analyses

	Control group ($n = 49$)	Stable angina group ($n = 41$)	Acute coronary syndrome group ($n = 32$)
Age (years)	68 ± 11	68 ± 11	71 ± 10
Male: female	29:20	32:9	23:9
Body mass index (kg/m^2)	24 ± 3	24 ± 4	23 ± 3
Hypertension	21/49(43%)	27/41 (66%)*	18/32 (56%)
Smoking	19/49 (39%)	24/41 (59%)	18/32 (56%)
Diabetes mellitus	10/49 (22%)	19/41 (46%)*	12/32 (38%)*
Hypercholesterolemia	15/49 (31%)	17/41 (41%)	9/32 (28%)
Total cholesterol (mg/dL)	197 ± 24	170 ± 37*	173 ± 30*
LDL-cholesterol (mg/dL)	116 ± 31	102 ± 35	110 ± 22
HDL-cholesterol (mg/dL)	57 ± 18	45 ± 14*	48 ± 14*
Triglycerides (mg/dL)	126 ± 51	124 ± 50	116 ± 46
Lipoprotein (a) (mg/dL)	23.7 ± 17.6	22.1 ± 21.0	30.6 ± 22.0
hsCRP (mg/L)	0.15 ± 0.28	1.02 ± 2.77*	1.19 ± 2.41*
Family history of CVD	11/49 (22%)	8/41 (20%)	7/32 (22%)
Medications			
Calcium channel blocker	20/49 (41%)	20/41 (49%)	17/32 (53%)
Beta blocker	2/49 (4%)	17/41 (41%)*	17/32 (53%)*
Niterate	0/49 (0%)	7/41 (17%)*	13/32 (41%)*#
ACE inhibitor	5/49 (10%)	20/41 (49%)*	22/32 (69%)*
Angiotensin receptor blocker	5/49 (10%)	14/41 (34%)*	7/32 (22%)
Diuretic	3/49 (6%)	18/41 (44%)*	14/32 (44%)*
Statin	5/49 (10%)	12/41 (29%)	4/32 (13%)

LDL: low-density lipoproteins; HDL: high-density lipoproteins; hsCRP: high-sensitive C-reactive protein; CVD: cardiovascular disease; ACE: angiotensin converting enzyme. Continuous values are mean ± S.D. Categorical values are expressed as number of patients (percentage). *$p < 0.05$ vs. control group. #$p < 0.05$ vs. stable angina group.

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

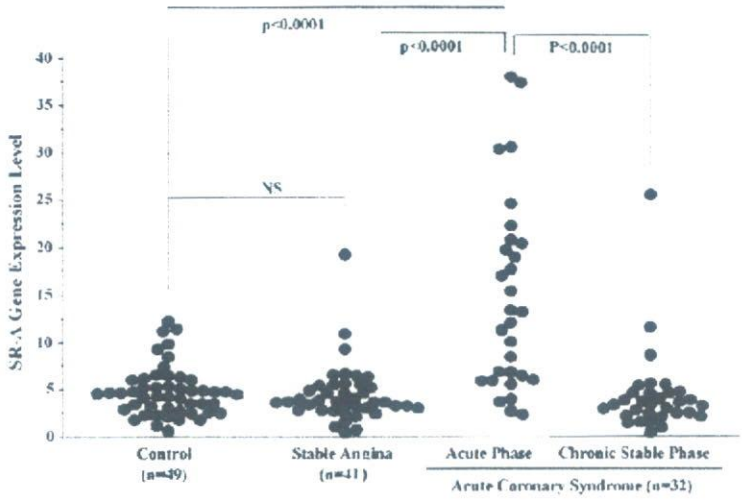

Fig. 1. The SR-A gene expression levels in the peripheral blood mononuclear cells (PBMCs) in the control, the stable angina, and the acute coronary syndrome groups. Values are expressed as the means ± S.E.M.

significantly higher in the ACS group than in the stable angina group.

3.3. The SR-A gene expression levels of the PBMCs

The SR-A gene expression levels of the PBMCs in the control, the stable angina, and the ACS groups were 4.7 ± 0.4, 4.4 ± 0.5, and 13.9 ± 1.8, respectively (Fig. 1). The SR-A gene expression levels of the PBMCs were significantly lower in both the control and stable angina groups than in the ACS group ($p < 0.0001$). There were no significant differences between the control and the stable angina groups.

The SR-A gene expression levels at 3 weeks after admission (chronic stable phase) was 5.1 ± 0.4. The SR-A gene expression levels were significantly suppressed in the chronic stable phase ($p < 0.0001$).

The patients with ACS included 21 patients with acute myocardial infarction and 11 patients with unstable angina (class IIIb-T_{pos}). The SR-A gene expression levels of the PBMCs were 12.1 ± 1.9 and 17.3 ± 3.6 in the patients with acute myocardial infarction and in the patients with unstable angina, respectively. There were no significant differences between the patients with acute myocardial infarction and the patients with unstable angina. In both groups, the SR-A gene expression levels were significantly higher than in either the control group or the stable angina group ($p < 0.0001$).

3.4. Multiple logistic regression analysis

We performed multiple logistic regression analysis with forward stepwise selection using all the clinical risk factors and the SR-A gene expression level. This analysis revealed that the most predictive independent risk factor for ACS was a high SR-A gene expression level ($p < 0.001$, relative risk: 14.005), followed by low HDL-cholesterol level ($p = 0.023$, relative risk: 5.649) and diabetes mellitus ($p = 0.037$, relative risk: 5.003) as is shown in Table 2. Also, the most strongly independent risk factor for stable angina was diabetes mellitus ($p = 0.011$, relative risk: 3.629), followed by low HDL cholesterol ($p = 0.023$, relative risk: 3.556), and hypertension ($p = 0.037$, relative risk: 2.693) (Table 3).

3.5. Follow-up study

The prognosis of patients in this study was followed up until 1 January 2007. The mean follow-up period was 745 days (range 320–1809 days) for the patients without reattack of cardiovascular event, with mean follow-up periods of 704

Table 2
Multiple logistic regression analysis for acute coronary syndrome

	β	S.E.	Wald	Relative risk (95% CI)	Significance
High SR-A gene expression	2.639	0.656	16.209	14.005 (3.875–50.618)	<0.001
Low HDL-C level diabetes	1.732	0.721	5.763	5.649 (1.374–23.224)	0.023
Mellitus	1.610	0.707	5.191	5.003 (1.252–19.987)	0.037
Constant	−2.551	0.603	17.872	0.078	<0.001

S.E.: standard error.

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

Table 3
Multiple logistic regression analysis for stable angina

Stable angina	β	S.E.	Wald	Relative risk (95%CI)	Significance
Diabetes mellitus	1.289	0.506	6.476	3.629(1.345–9.791)	0.011
Low HDL-C level	1.269	0.557	5.185	3.556 (1.193–10.609)	0.023
Hypertension	0.991	0.474	4.362	2.693 (1.063–6.823) 0.037	0.037
Constant	−1.444	0.430	11.279	0.236	0.001

S.E.: standard error.

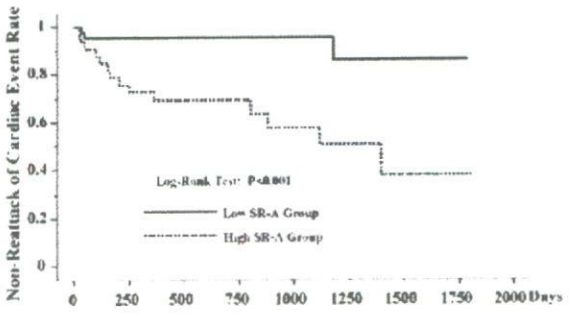

Fig. 2. Kaplan–Meier survival curves of cumulative reattack of cardiovascular event rates in patients with chronic coronary heart disease divided into two groups according to their SR-A gene expression levels in the stable state of ischemic heart disease, as follows: the low SR-A group (the SR-A gene expression level <5.0) and the high SR-A group (the SR-A gene expression level ≥5.0).

days (range 320–1792 days) for the low SR-A group and 832 days (range 340–1809 days) for the high SR-A group.

The clinical characteristics of the study patients are shown in Supplemental Table 4. We compared the clinical characteristics including age, gender, body mass index, hypertension, smoking, diabetes mellitus, hypercholesterolemia, total cholesterol level, LDL-cholesterol level, HDL-cholesterol level, triglycerides, lipoprotein (a) level, hsCRP level, family history of cardiovascular disease and pharmacological medications between the two groups. There were no significant differences between the low and high SR-A groups as regards these atherosclerotic risk factors and pharmacological medications.

Reattack of cardiovascular event occurred in 2 patients in the low SR-A group (5%): 1 patient with unstable angina; and, 1 patient with heart failure with NYHA class IV symptoms. On the other hand, reattack of cardiovascular event occurred in 14 patients in the high SR-A group (39%): 1 patient with fatal myocardial infarction; 2 patients with non-fatal myocardial infarction; 8 patients with unstable angina; and, 3 patients with heart failure with NYHA class IV symptoms. Kaplan–Meier analysis revealed that the occurrence of a reattack of a cardiovascular event was significantly lower in the low SR-A group than in the high SR-A group ($p < 0.001$) as is shown in Fig. 2.

Subsequently, we performed multiple logistic regression analysis with forward stepwise selection for a reattack of a cardiovascular event, using all the clinical risk factors and the SR-A gene expression level (Table 4). The analysis revealed that the most predictive independent risk factor for a reattack of a cardiovascular event was a high SR-A gene expression level ($p = 0.009$, relative risk = 18.333). Other classical coronary risk factors were not significant predictive factors for reattacks.

4. Discussion

It is well known that macrophages play a multifaceted role in inducing plaque rupture, blood coagulation, and fibrinolysis via the production of various enzymes, activators, inhibitors, and bioactive mediators [15–17]. During the development of atherosclerosis, macrophages interact with vascular endothelial cells, medial smooth muscle cells, and infiltrated inflammatory cells [18]. SR-A was identified, in the search for receptor molecules which are implicated in atherogenesis, through its receptor-mediated uptake of modified, including oxidized, LDL [19,20]. Immunoelectron microscopic studies indicate that SR-A localizes itself on the cell surface membranes of macrophages; however, SR-A is not expressed in either monocytes or in their precursor cells [21–23]. In human atherosclerotic lesions, SR-A is expressed on the cell surfaces of macrophages and macrophage-derived foam cells. The intensity of the immunoreactivity of these receptors is more marked in fatty streaks than in diffuse intimal thickening or in the atheroscle-

Table 4
Multiple logistic regression analysis for reattack of cardiovascular disease

	β	S.E.	Wald	Relative risk (95%CI)	Significance
High SR-A gene expression	2.909	1.109	6.885	18.333 (2.088–160.992)	0.009
Constant	−3.091	1.022	9.139	0.045	0.003

S.E.: standard error.

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

rotic plaque; moreover, the intensity is decreased in advanced complicated lesions [22,23]. These results indicate that SR-A is expressed, and its expression level is increased, during the progression of atherosclerosis, with its expression reaching a peak at the stage of the development of fatty streak lesions.

During the development and progression of atherosclerosis, lipoproteins from the circulating blood infiltrate the intimal and accumulate in the arterial walls, thus producing diffuse intimal thickening. Subsequently, monocytes migrate into the thickened intimal area and differentiate into macrophages, which absorb oxidized LDL via scavenger receptors, including the SR-A, and then these change into foam cells—leading to fatty streak lesions and atherosclerotic plaques [22]. In coronary atherosclerotic plaque, disruption occurs in the shoulder regions where macrophages have accumulated, leading to ulcerations or arterial occlusions [15–17].

The SR-A gene expression level in the PBMCs was highest in the ACS group. Contrarily, the SR-A gene expression levels were low in the control and the stable angina groups. There were no significant differences in the SR-A gene expression levels between the control and the stable angina groups. This result indicates that SR-A gene expression level in the PBMCs increases in patients with ACS; a parallel SR-A gene expression level increase is seen in atherosclerotic lesions. The SR-A gene expression possibly originates in macrophages/monocytes which originate in the unstable plaque. It appears likely that the unstable plaque discharges macrophages/monocytes with SR-A due to any of following: plaque rupture; atherosclerotic lesion macrophages seeding into the peripheral circulation; circulating monocytes inducing SR-A gene expression via unstable plaque; or, some other, as yet, unknown reason. Further study is needed to elucidate the mechanism for the increasing SR-A gene expression level in the PBMCs in patients with unstable plaque.

We performed multiple logistic regression analysis with forward stepwise selection using all the clinical risk factors and the SR-A gene expression levels. This analysis revealed that the most predictive independent risk factor for ACS was the high SR-A gene expression level, followed by low HDL-cholesterol level and diabetes mellitus. On the other hand, the independent risk factors for stable angina were diabetes mellitus, low HDL cholesterol, and hypertension. Among these clinical risk factors and the SR-A gene expression level, the high SR-A gene expression level was a specific independent risk factor for ACS. Further, the high SR-A gene expression level in the stable state of ischemic heart disease was the only independent predictive marker for a reattack of a cardiovascular event. Further study on larger and longer-term follow-up populations will be beneficial to further validate this point.

In conclusion, SR-A gene expression level specifically increases in the peripheral circulating blood in patients with ACS. The SR-A gene expression level in the peripheral circulating blood also provides a predictive marker for a reattack of a cardiovascular event.

Acknowledgments

We thank M. Tsukamoto for secretarial work. This study was supported in part by the following grants: Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Tokyo, Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2007.09.006.

References

[1] Burke AP, Farb A, Malcom GT, et al. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 1997;336:1276–82.
[2] Lee RT, Libby P. The unstable atheroma. Arterioscler Thromb Vasc Biol 1997;17:1859–67.
[3] Herrick J. Certain clinical features of sudden obstruction of the coronary arteries. Trans Am Assoc Physicians 1912;27:100–16.
[4] Fuster V, Lewis A. Conner memorial lecture. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. Circulation 1994;90:2126–46.
[5] Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999;340:115–26.
[6] Fuster V, Fayad ZA, Badimon JJ. Acute coronary syndromes: biology. Lancet 1999;353(Suppl II):SII 5–9.
[7] Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. JAMA 2001;286:2107–13.
[8] Anwaruddin S, Askari AT, Topol EJ. Redefining risk in acute coronary syndromes using molecular medicine. J Am Coll Cardiol 2007;49:279–89.
[9] Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first 40 years of life. Eur Heart J 1990;11(Suppl E):3–19.
[10] Tracy RE, Devaney K, Kissling G. Characteristics of the plaque under a coronary thrombus. Virchows Arch A Pathol Anat Histopathol 1985;405:411–27.
[11] Isner J, Donaldson R, Fortin A, et al. Attenuation of media of coronary arteries in advanced atherosclerosis. Am J Cardiol 1986;58:397.
[12] Weyand CM, Goronzy JJ, Liuzzo G, et al. T-cell immunity in acute coronary syndromes. Mayo Clin Proc 2001;76:1011–20.
[13] Hamm WC, Braunwald E. A classification of unstable angina revisited. Circulation 2000;102:118–22.
[14] Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: tool for the unification of biology. Nat Genet 2000;25:25–9.
[15] Ross R. Atherosclerosis: defense mechanisms gone awry. Am J Pathol 1993;143:987–1002.
[16] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–9.
[17] Kaikita K, Ogawa H, Yasue H, et al. Tissue factor expression on macrophages in coronary plaques in patients with unstable angina. Arterioscler Thromb Vasc Biol 1997;17:2232–7.
[18] Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low-density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci USA 1979;76:333–7.

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

[19] Kodama T, Freeman M, Rohrer L, et al. Type I macrophage scavenger receptor contains α-helical coil and collagen-like coiled coils. Nature 1990;343:531–5.

[20] Naito M, Suzuki K, Mori T, et al. Coexpression of type I and type II human macrophage scavenger receptors in macrophages of various organs and foam cells in atherosclerotic lesions. Am J Pathol 1992;141:591–9.

[21] Naito M, Kodama T, Matsumoto A, Doi T, Takahashi K. Tissue distribution, intracellular localization, and in vitro expression of bovine macrophage scavenger receptors. Am J Pathol 1991;139:1411–23.

[22] Takahashi K, Takeya M, Sakashita N. Multifunctional roles of macrophages in the development and progression of atherosclerosis in humans and experimental animals. Rev Med Electron Microsc 2002;35:179–203.

[23] Tomokiyo R, Jinnouchi K, Honda M, et al. Production, characterization, and interspecies reactivities of monoclonal antibodies against human class A macrophage scavenger receptors. Atherosclerosis 2002;161:123–32.

Please cite this article in press as: Nakayama M, et al., Class A macrophage scavenger receptor gene expression levels in peripheral blood mononuclear cells specifically increase in patients with acute coronary syndrome, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2007.09.006

Original Article

Impact of Metabolic Syndrome Components on the Incidence of Cardiovascular Disease in a General Urban Japanese Population: The Suita Study

Yoshihiro KOKUBO[1], Tomonori OKAMURA[1], Yasunao YOSHIMASA[2], Yoshihiro MIYAMOTO[2], Katsuyuki KAWANISHI[3], Yasushi KOTANI[3], Akira OKAYAMA[1),4)], and Hitonobu TOMOIKE[1]

Abdominal obesity is a prerequisite for some definitions of metabolic syndrome (MetS). We investigated the impact of MetS defined by two different criteria, which either did or did not require abdominal obesity as a prerequisite, on cardiovascular disease (CVD) incidence in an urban Japanese cohort study. We studied 5,332 Japanese (aged 30–79 years, without CVD at baseline), who completed a baseline survey (September 1989 to March 1994) and were followed up through December 2005. MetS was defined by the NCEP-ATPIII (modified by Asian obesity criteria) and the Japanese criteria. After 61,846 person-years of follow-up, we documented 317 CVD incidences. The MetS frequencies of the Japanese and of the modified NCEP-ATPIII criteria were 17.7% and 25.1% for men and 5.0% and 14.3% for women, respectively. The multivariate hazard ratios (HRs; 95% confidence intervals [CI]) of CVD incidence for MetS by the modified NCEP-ATPIII criteria were 1.75 (1.27–2.41) in men and 1.90 (1.31–2.77) in women, and those for MetS by the Japanese criteria were 1.34 (0.96–1.87) in men and 2.20 (1.31–3.68) in women. The multivariate HRs of CVD incidence for MetS for the Japanese and for the modified NCEP-ATPIII criteria were 2.92 (1.54–5.55) and 1.94 (0.98–3.82) in men under 60 years old, respectively. The CVD incidence risks increased according to the number of MetS components. The risks were similar among participants with the same number of MetS components, regardless of abdominal obesity. In conclusion, the number of MetS components (modified NCEP-ATPIII criteria) may be more strongly associated with CVD incidence than the abdominal obesity essential criteria (the Japanese criteria) in a general urban Japanese population. (*Hypertens Res* 2008; 31: 2027–2035)

Key Words: **metabolic syndrome, cardiovascular risk factor, cohort study, general population**

Introduction

Metabolic syndrome (MetS) is a clustering of impaired glucose metabolism, abdominal fat accumulation, dyslipidemia, and elevated blood pressure (*1*). Previous papers have shown an association between MetS and cardiovascular disease (CVD) (*2*), but most studies conducted thus far have been based on Western populations. There have been several well-designed prospective studies of Asian populations, and those

From the [1]Department of Preventive Cardiology and [2]Department of Atherosclerosis and Diabetes, National Cardiovascular Center, Suita, Japan; [3]The Suita Medical Foundation, Suita, Japan; and [4]Japan Anti-Tuberculosis Association, Tokyo, Japan.

This study was supported by grants-in-aid from the Japanese Ministry of Health, Labour and Welfare (H14-Kouka-027, H19-SeiShu-017, and H18-SeiShu-012) and by a Research Grant for Cardiovascular Disease from the Ministry of Health, Labour and Welfare (18S-2, 19K-8), Japan.

Address for Reprints: Yoshihiro Kokubo, M.D., Ph.D., Department of Preventive Cardiology, National Cardiovascular Center, 5–7–1, Fujishiro-dai, Suita 565–8565, Japan. E-mail: ykokubo@hsp.ncvc.go.jp

Received May 30, 2008; Accepted in revised form September 7, 2008.

studies had various limitations, including the use of body mass index (BMI) (*3*, *4*), non-fasting triglyceride and glucose levels (*3*, *4*), mortality (*4*, *5*), or small sample size (*4–7*). In order to properly define MetS, it is essential to use data on waist circumference and on the levels of both fasting glucose and fasting triglycerides.

MetS has been defined in several ways by several groups, including the World Health Organization (*8*), the European Group for the Study of Insulin Resistance (*9*), the American Association of Clinical Endocrinologists, and the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) (*10*). However, these definitions are aimed mainly at Western countries. The International Diabetes Foundation (IDF) (*11*) and the American Heart Association (*12*) have recently introduced alternative definitions that can be applied worldwide (*10*). Stroke incidence is relatively higher in Japan than in Western countries (*13*). It is uncertain whether these criteria can be applied well to Japanese populations. A MetS definition needs to be tailored to the epidemiological background of the area in question.

The Japanese Committee on the Criteria for MetS has recently proposed a definition of Japanese MetS (*14*, *15*). Under both the IDF and the Japanese definitions, the presence of abdominal obesity is necessary for a diagnosis of MetS. However, no prospective study has examined the association between MetS based on the Japanese criteria and CVD, particularly in urban areas, where most Japanese live. Therefore, we undertook this study to examine the impact of MetS under the Japanese and modified NCEP-ATPIII criteria on CVD incidence in a general urban Japanese population.

Methods

Study Population

The Suita study (*16*, *17*), an epidemiological survey of cerebrovascular disease and CVD, was based on a random sampling of 12,200 residents of Suita, a city of approximately 350,000 people in northern Osaka, Japan. As a baseline, in 1989, participants between the ages of 30 and 79 were arbitrarily selected from the municipality population registry and stratified into groups by sex and age in 10-year increments. Of these, 6,406 men and women participated in regular health checkups between September 1989 and March 1994. Since then, these participants have participated in regular health checkups at the National Cardiovascular Center every 2 years and answered health questionnaires every year.

Some cohort members in the study population were excluded from these analyses because they met one or more of the following criteria: past or present CVD illness at baseline ($n=208$), failure to fast for at least 10 h before venipuncture or missing data ($n=170$), or failure to follow up after their baseline examination ($n=696$). After these exclusions, 5,332 individuals remained for analysis.

Baseline Survey

We performed routine blood tests that measured fasting serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels. Physicians or nurses administered questionnaires covering the subjects' personal habits and any present illnesses. The subjects were classified as current smokers if they smoked at least one cigarette per day, as non-smokers if they had never smoked, and as past smokers if they had stopped smoking. Blood pressure was measured three times in a sitting position after at least 5 min of rest. Systolic and diastolic blood pressures (SBP and DBP) were taken to be the average of the second and third measurements that were recorded at least 1 min apart by well-trained doctors. Waist circumference was measured in a standing position at the umbilical level to the nearest 1 cm by well-trained technicians. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the National Cardiovascular Center.

Definitions of Metabolic Syndrome

MetS was defined using two criteria. First, in accordance with NCEP-ATPIII (*18*) criteria, it was defined as the presence of three or more of the following five components: 1) abdominal obesity modified by the International Obesity Task Force central obesity criteria for Asia (waist circumference ≥90 cm in men and ≥80 cm in women) (*19*), 2) elevated blood pressure (SBP/DBP ≥130/85 mmHg and/or current use of antihypertensive medication), 3) hypertriglyceridemia (serum triglyceride levels ≥1.7 mmol/L [150 mg/dL] and/or current use of cholesterol-lowering medication), 4) low HDL cholesterol (serum HDL levels of ≤1.0 mmol/L [40 mg/dL] in men and of ≤1.3 mmol/L [50 mg/dL] in women), and 5) elevated blood glucose levels (fasting blood glucose ≥6.1 mmol/L [110 mg/dL] and/or current use of insulin or oral medication for diabetes).

Second, we used the definition of MetS recommended by the Japanese Committee on the Criteria for MetS (*14*, *15*). MetS was defined by abdominal obesity (waist circumference ≥85 cm in men and ≥90 cm in women) (*20*) and least two of the following three components: 1) elevated blood pressure (SBP/DBP ≥130/85 mmHg), 2) hyperlipidemia (serum triglyceride levels ≥1.7 mmol/L [150 mg/dL] and/or HDL levels <1.0 mmol/L [40 mg/dL]), and 3) elevated blood glucose levels ≥6.1 mmol/L (110 mg/dL). Subjects taking medication for hypertension, hyperlipidemia, or diabetes were included as having that component.

Endpoint Determination

The endpoint of the follow-up period for each participant was whichever one of the following occurred first: 1) the date of the first myocardial infarction (MI) or stroke event, 2) the date of death, 3) the date the participant moved out of Suita,

Table 1. Baseline Distributions of Cardiovascular Disease Risk Factors According to Metabolic Syndrome under the NCEP-ATPIII Modified by Asian Obesity Definitions

	Men (n=2,492)			Women (n=2,840)		
	MetS(−) (n=2,043)	MetS(+) (n=449)	p*	MetS(−) (n=2,253)	MetS(+) (n=587)	p*
Age at baseline, years	55.4±13.3	58.1±11.5	<0.001	52.2±12.6	61.3±9.8	<0.001
Systolic blood pressure, mmHg	126±20	140±19	<0.001	120±20	141±20	<0.001
Diastolic blood pressure, mmHg	78±12	85±11	<0.001	73±11	83±12	<0.001
Total cholesterol, mg/dL	200±34	210±35	<0.001	210±38	227±38	<0.001
HDL cholesterol, mg/dL	51±13	40±10	<0.001	60±12	45±10	<0.001
Triglyceride, mg/dL#	121±73	241±156	<0.001	90±44	178±113	<0.001
Waist circumference, cm	81.0±7.3	89.7±7.0	<0.001	74.7±8.9	87.4±8.5	<0.001
Elevated blood pressure, %	41.8	85.8	<0.001	30.4	82.1	<0.001
Hypertriglyceridemia, %	21.6	82.9	<0.001	7.2	63.7	<0.001
Lower-HDL cholesterol, %	15.5	64.8	<0.001	18.7	80.1	<0.001
Hyperglycemia, %	8.9	43.9	<0.001	3.6	29.6	<0.001
Current smoker, %	50.5	47.6	0.278	11.9	11.8	0.958
Current drinker, %	75.5	72.6	0.207	34.6	25.4	<0.001

Elevated blood pressure: antihypertensive drug use or >130/85 mmHg; hypertriglyceridemia: antilipidemic drug use or triglyceride >150 mg/dL; lower-HDL cholesterol: HDL cholesterol <40 mg/dL. MetS, metabolic syndrome; HDL, high-density lipoprotein. *ANOVA or χ^2 tests were performed. #Log-transformed triglyceride was performed to statistical analysis.

Table 2. Age-Adjusted Hazard Ratios (Confidence Intervals) for Incidence of Cardiovascular Disease According to Abdominal Obesity at Baseline Examination

	Men				Women			
	Case, n	Person-year	HR (95% CI)	p	Case, n	Person-year	HR (95% CI)	p
Japanese criteria								
<85 cm (men)/<90 cm (women)	111	17,112	1		96	29,960	1	
≥85 cm (men)/≥90 cm (women)	77	11,247	0.97 (0.72–1.30)	0.844	33	3,890	1.64 (1.09–2.46)	0.019
Asian criteria								
<90 cm (men)/<80 cm (women)	145	23,136	1		53	21,139	1	
≥90 cm (men)/≥80 cm (women)	43	5,223	1.18 (0.84 1.67)	0.327	76	12,711	1.44 (1.00–2.07)	0.048
NCEP-ATPIII criteria								
<102 cm (men)/<88 cm (women)	182	27,976	1		91	28,730	1	
≥102 cm (men)/≥88 cm (women)	6	384	2.00 (0.88 4.54)	0.095	38	5,121	1.47 (1.00–2.17)	0.048

HR, hazard ratio; CI, confidence interval.

or 4) December 31, 2005 (censored). As a first-step survey to detect MI and stroke incidence, each participant's health status was checked during a clinical visit at the National Cardiovascular Center every 2 years. Furthermore, every year a health questionnaire was given to each participant *via* mail or telephone.

Confirmation of Strokes and Myocardial Infarctions

In total, five hospitals in this area were capable of performing computed tomographic scans and/or magnetic resonance imaging, and all were major hospitals that admitted acute stroke and MI patients. Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information. Strokes and MI events were registered if they occurred after the date on which the baseline health examination was held and before January 1, 2006. Strokes were defined according to the National Survey of Stroke criteria (*21*). These criteria require the rapid onset of a constellation of neurological deficits lasting at least 24 h or until death. For each stroke subtype (cerebral infarction [thrombotic or embolic infarction], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images, or autopsy. Defi-

Table 3. Age-Adjusted Hazard Ratios (95% Confidence Intervals) for Incidence of Cardiovascular Disease, Myocardial Infarction, and All Strokes According to Metabolic Syndrome under the Japanese and NCEP-ATPIII Definitions

	Men			Women		
	MetS(−)	MetS(+)	*p* value	MetS(−)	MetS(+)	*p* value
Cardiovascular disease						
MetS Japanese definition						
Cases, *n*	140	48		110	19	
Person-year	23,542	4,817		32,325	1,526	
Age-adjusted	1	1.31 (0.94–1.82)	0.109	1	2.16 (1.31–3.54)	0.002
Multivariate-adjusted	1	1.34 (0.96–1.87)	0.080	1	2.20 (1.31–3.68)	0.003
<60 years old						
Cases, *n*	27	15		25	4	
Person-year	14,752	2,366		22,085	529	
Age-adjusted	1	2.76 (1.46–5.23)	0.002	1	5.39 (1.82–15.98)	0.002
Multivariate-adjusted	1	2.92 (1.54–5.55)	0.001	1	6.25 (2.08–18.79)	0.001
≥60 years old						
Cases, *n*	113	33		85	15	
Person-year	8,790	2,451		10,240	997	
Age-adjusted	1	1.04 (0.70–1.53)	0.841	1	1.83 (1.05–3.18)	0.033
Multivariate-adjusted	1	1.06 (0.71–1.57)	0.764	1	1.80 (1.01–3.20)	0.046
MetS NCEP-ATPIII (Asian) definition						
Cases, *n*	133	55		73	56	
Person-year	23,373	4,986		27,405	6,446	
Age-adjusted	1	1.70 (1.23–2.34)	0.001	1	1.93 (1.35–2.77)	<0.001
Multivariate-adjusted	1	1.75 (1.27–2.41)	<0.001	1	1.90 (1.31–2.77)	<0.001
<60 years old						
Cases, *n*	30	12		19	10	
Person-year	14,509	2,606		19,872	2,742	
Age-adjusted	1	1.79 (0.91–3.52)	0.089	1	2.72 (1.23–5.99)	0.013
Multivariate-adjusted	1	1.94 (0.98–3.82)	0.055	1	2.96 (1.34–6.57)	0.007
≥60 years old						
Cases, *n*	103	43		54	46	
Person-year	8,864	2,381		7,533	3,704	
Age-adjusted	1	1.67 (1.16–2.40)	0.005	1	1.78 (1.19–2.66)	0.005
Multivariate-adjusted	1	1.73 (1.20–2.48)	0.003	1	1.70 (1.12–2.59)	0.012
Myocardial infarction						
MetS Japanese definition						
Cases, *n*	56	22		32	7	
Person-year	22,962	4,663		31,697	1,457	
Age-adjusted	1	1.48 (0.90–2.44)	0.117	1	2.36 (1.02–5.46)	0.043
Multivariate-adjusted	1	1.51 (0.91–2.48)	0.105	1	2.70 (1.15–6.35)	0.023
MetS NCEP-ATPIII (Asian) definition						
Cases, *n*	52	26		18	21	
Person-year	22,833	4,795		26,944	6,211	
Age-adjusted	1	2.09 (1.30–3.37)	0.002	1	2.68 (1.41–5.10)	0.003
Multivariate-adjusted	1	2.12 (1.31–3.43)	0.002	1	2.77 (1.44–5.32)	0.002
All strokes						
MetS Japanese definition						
Cases, *n*	84	26		78	12	
Person-year	23,177	4,659		32,078	1,487	
Age-adjusted	1	1.21 (0.78–1.89)	0.381	1	2.09 (1.12–3.88)	0.019
Multivariate-adjusted	1	1.27 (0.81–1.97)	0.292	1	2.05 (1.07–3.92)	0.031
MetS NCEP-ATPIII (Asian) definition						
Cases, *n*	81	29		55	35	
Person-year	23,010	4,826		27,266	6,299	
Age-adjusted	1	1.52 (0.99–2.34)	0.053	1	1.70 (1.09–2.64)	0.018
Multivariate-adjusted	1	1.58 (1.02–2.43)	0.037	1	1.62 (1.02–2.58)	0.041

Multivariate adjusted for age, smoking and drinking status. MetS, metabolic syndrome.

nite and probable MI was defined according to the criteria set out by the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project (*22*), which requires evidence from ECGs, cardiac enzymes, and/or autopsy. Sudden deaths of unknown origin were deaths that occurred within 24 h from onset and were included in MI. However, there was little difference in hazard ratios between the groups with and without sudden death from CVD, because sudden death constituted a small sample size ($n=6$).

To complete surveillance for fatal stroke and MI, we also systematically searched for death certificates, the purpose of which were permitted to use by the Ministry of Health, Labour and Welfare. We checked for possible stroke and MI using data from 1) the health examination and questionnaire for the stroke and MI registry, without informed consent for the medical records survey and 2) death certificates without registration of CVD incidence, which were defined as probable stroke or MI. CVD was defined as stroke and MI in this study. Informed consent to review in-hospital medical records was obtained from 86.2% of participants who were suspected of having any signs or information suggesting the incidence of stroke or MI. For 13.8% of subjects from whom informed consent was not obtained, final diagnoses of CVD were confirmed by physicians or epidemiologists who had been involved in the diagnostic process throughout the study, in order to avoid the misclassification of diagnoses.

Statistical Analysis

Analyses of variance and χ^2 tests were used to compare mean values and frequencies by sex, respectively, according to MetS based on the modified NCEP-ATPIII criteria. For each subject, the person-years of follow-up were calculated from September 1, 1989, to whichever came first: the first endpoint, MI or stroke event, death, emigration, or December 31, 2005. A Cox proportional hazards regression model was used to detect associations between abdominal obesity for Japanese (≥85 cm in men or ≥90 cm in women), Asian (≥90 cm in men or ≥80 cm in women), and American criteria (≥102 cm in men or ≥88 cm in women) and CVD during the follow-up period. The Cox proportional hazard regressions were fitted to the grouping (positive or negative MetS) after adjusting for age and the other potential confounding factors: baseline age, smoking status (never, ex-smoker, or current smoker), and drinking status (never, ex-drinker, or current drinker). Trend tests were conducted by assigning the number of MetS components to test the significance of these variables. All statistical analyses were conducted using the SAS statistical package (release version 8.2; SAS Institute Inc., Cary, USA).

Results

During the follow-up period (averaging 12.5 years), 200 strokes were documented (160 definite strokes and 40 probable strokes). These strokes comprised 130 cerebral infarctions, 31 intracerebral hemorrhages, 22 subarachnoid hemorrhages, and 17 unclassified strokes. In addition, 117 MIs were documented (61 definite MIs and 56 probable MIs or sudden cardiac deaths).

Table 1 shows the distribution of CVD risk factors at the baseline according to MetS as defined by the modified NCEP-ATPIII criteria. Compared with the non-MetS groups, men and women with MetS were more likely to be older and to have higher frequencies of each MetS component.

Table 2 presents the age-adjusted HRs (95% confidence intervals [CI]) for the incidence of CVD according to waist circumference by the NCEP-ATPIII, Japanese, and Asian obesity criteria. Regardless of the criteria set, abdominal obesity was associated with CVD only in women.

Table 3 shows the association of MetS by the Japanese and the modified NCEP-ATPIII criteria with CVD incidence according to age category and sex. Using the Japanese criteria, MetS was associated only in women with the incidence of CVD, MI, and all strokes (HR [95% CI]: 2.20 [1.31–3.68], 2.70 [1.15–6.35], and 2.05 [1.07–3.92], respectively), whereas in men overall MetS was not associated with the incidence of CVD or its subtypes. However, among men under 60 years old, MetS based on the Japanese criteria was associated with CVD incidence (HR=2.92, 95% CI: 1.54–5.55). Using the modified NCEP-ATPIII definition, MetS was associated with each CVD subtype in both men and women. Multivariate adjusted HRs of CVD incidence for MetS based on the NCEP-ATPIII criteria were 1.94 (0.98–3.82) and 1.73 (1.20–2.48) in men less than or equal to and over 60 years old, respectively.

Figure 1 shows that the frequency of MetS increased with age for men and women based on the NCEP-ATPIII (A) and Japanese (B) criteria, respectively. The frequency based on the NCEP-ATPIII modified by the Asian obesity criteria (25.1% for men and 14.3% for women) was higher than that based on the Japanese criteria (17.7% for men and 5.0% for women), especially in women.

The risk of CVD incidence increased according to the number of components combined in men and women with and without abdominal obesity (Fig. 2). In addition, compared with the non-abdominal obesity and non-component groups, the risks of CVD incidence were similar among participants who had the same numbers of components, regardless of the presence or absence of abdominal obesity in men and women combined.

Figure 3 shows the multivariate HRs for MetS based on the Japanese and NCEP-ATPIII definitions modified by the obesity criteria for waist circumference. When the Japanese definition was adopted and the risk of MetS was monitored through sequential waist circumference changes, the cut-off points for waist circumference, which conferred a risk of CVD in men and women, were 84 cm and 92 cm, respectively. When the definition of MetS indicative waist circumference was higher than those values, the risk was not statistically significant. When the NCEP-ATPIII definition

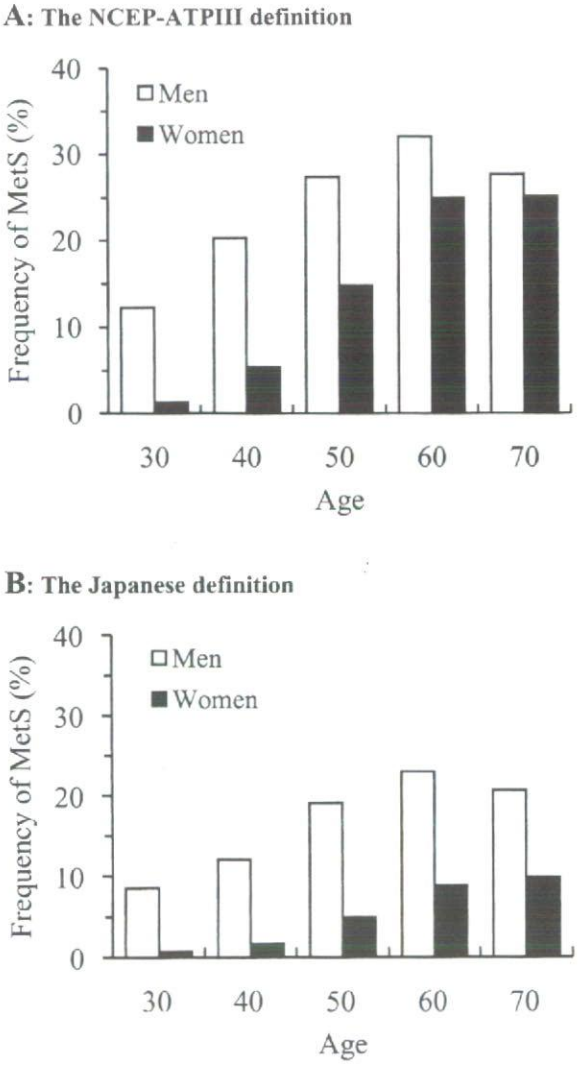

Fig. 1. *Frequencies of MetS components (A: the NCEP-ATPIII definition; and B: the Japanese definition, modified by the Asian waist circumference criteria) by sex. White and solid bars indicate men and women, respectively.*

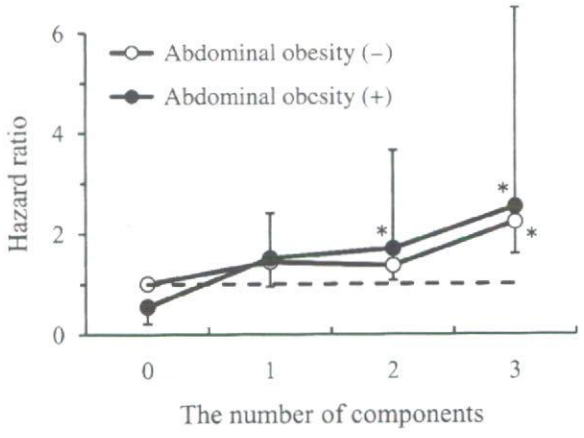

Fig. 2. *Multivariate HRs for the risks of CVD incidence according to the number of components based on the NCEP-ATPIII definition with and without abdominal obesity. White and solid circles indicate non-abdominal and abdominal obesity according to the Asian obesity criteria. *$p<0.05$ compared to the reference of non-abdominal obesity and no-components. Bars show 95% CI for the HRs.*

was used, the value of waist circumference did not modify the risk of CVD, implying that the clustering of risk factors may be more important than waist circumference itself for determining CVD risk.

Discussion

In the current cohort study of a general urban Japanese population, the association between MetS and CVD was significant when the NCEP-ATPIII (modified by the Asian criteria) definition was applied. MetS based on the Japanese criteria was associated with CVD incidence in women, whereas in men the association was found only in those under 60 years old. In addition, the risk of CVD incidence was similar among participants who had the same numbers of components regardless of whether they were abdominally obese. To the best of our knowledge, this is the first study of an urban Japanese cohort.

Compared to the previous studies, this study has several methodological strengths. First, previous Japanese cohort studies associating MetS with CVD were based predominantly on BMI (*3*, *4*), non-fasting blood collection (*3*, *4*), and mortality as the endpoint (*4*, *5*). Our baseline subjects were observed in the fasting state, and we used waist circumference and a wide age range. Second, we evaluated a large prospective cohort of people randomly selected from a general Japanese population. A prospective study has little recall bias as well as results from a general population cohort that is more representative than occupational, hospital-based, or volunteer cohorts. Third, our sample size was relatively large for a cohort study and we could therefore perform sub-analysis by age and CVD subtypes. Fourth, our cohort population was selected at random from an urban population, in contrast to most of the other MetS cohort populations, which were selected from rural populations. Our study is the first of its kind in an urban area. Finally, our study examined the risk of CVD incidence, which is a more direct measure of CVD risk than the rate of CVD mortality, because the time to death from CVD is influenced by treatment.

Abdominal obesity induces inflammation in adiposities (*23*), endothelial dysfunction (*24*, *25*), and oxidative stress (*26*), thereby contributing to CVD development (*27*, *28*).

Fig. 3. *Multivariate HRs for MetS based on the NCEP-ATPIII (A) and Japanese (B) definitions through sequential changes in waist circumference by sex. Solid and dotted lines indicate HRs and 95% CI, respectively.*

Accumulating evidence suggests that MetS increases the risk of CVD (*29*). However, there has been a lack of convincing evidence (*29*) that MetS is associated with CVD in Japan. Iso *et al.* reported that MetS was associated with a risk for ischemic CVD in Japan (*3*), although they used BMI as well as non-fasting blood glucose and triglyceride levels to define MetS. Ninomiya *et al.* reported that MetS was a significant risk factor for CVD in a rural Japanese population (*6*). However, that study examined a rural population half the size of that in our study. Takeuchi *et al.* reported that MetS was a risk factor for cardiac disease in a rural cohort (*7*), but their data were based on a small sample that comprised only men. Kadota *et al.* reported that MetS, defined by BMI and non-fasting blood samples, was associated with CVD mortality (*4*).

We have shown that the components of MetS synergistically increase CVD risk. Abdominal obesity did not affect the association between the number of MetS components and the risk of CVD incidence. The risk of CVD was also not related to waist circumference when the NCEP-ATPIII definition was applied (data not shown), suggesting that the combination of risk factors *per se* is more important than abdominal obesity for conferring risk.

The definition of MetS may be reconsidered on the basis of age and sex. According to our results, lifestyle modifications may not be needed for older men who are free of cardiovascular risk factors even if they have abdominal obesity. Therefore, to prevent CVD, it is not adequate for only subjects with MetS to change their lifestyles; subjects with one or two MetS components, even without abdominal obesity, should modify their lifestyles.

When the waist-circumference threshholds were sequentially changed in the Japanese criteria for MetS, our data showed that the clustering of metabolic risk factors was statistically significant for CVD at waist circumferences less than 85 cm for men and 93 cm for women. When the definition of MetS-indicative waist circumferences was higher than those values, the risk clustering was not statistically significant for