ゲノムワイド連鎖解析による原因遺伝子座の探索

家系内や集団中で複数の遺伝子座が同時に分離(遺伝)されていく現象を連鎖(linkage)とよび，それは各遺伝子座が同一染色体上の近傍に位置することを意味する．この“連鎖”の概念に基づき遺伝子マーカーと原因遺伝子との間の組換え率から，原因遺伝子の染色体上の位置を推定する方法が連鎖解析法である．ヒトゲノムの解読によりゲノム全体に高密度に散在するマイクロサテライトマーカーや単一塩基多型(SNP)などの遺伝子多型のカタログ化が進み，ゲノム全体を俯観した連鎖解析や相関解析が可能となりつつある．

1. 複数の形質を同時に対象とした解析

Framingham Heart Study の登録者を対象に，MetS に関連する 5 つの形質(収縮期血圧，トリグリセリド，HDL コレステロール，血糖値，BMI)およびこれら 5 つを複合した形質(MSS＝metabolic syndrome score と定義)に対してマイクロサテライトマーカーを用いた全ゲノム解析を行った結果が報告されている〔この場合の対象形質はすべて数量化可能なものであるため，quantitative trait locus(QTL)解析ともよばれる〕[14]．その結果，それぞれの形質や MSS に比較的弱い連鎖を示すピークがいくつかの染色体に散在してみられている．各形質および MSS の遺伝度はやはり 0.39～0.62 と高く，とくに 5 つの形質を合算した MSS の遺伝度が 0.61 ときわめて高いことは，MetS の成立に遺伝因子が大きく関与していることを示すものである．しかし一方，連鎖のピークに関しては，血糖値，HDL コレステロール，収縮期血圧のそれぞれの最大 LOD スコアが，それぞれ 2.37，2.27，1.93 と suggestive なレベルの連鎖が確認されたのに対して，MSS では 1.82 とむしろそれらを下まわる LOD スコアしか得られなかった[14]．言い換えるとこの結果は，MetS の診断基準に用いられる各形質に関して，それらの組合せや重みづけに関する考慮をせずに単純にその総和をもって MetS を定義するとすれば，遺伝因子の検出力はそれぞれの形質単独の場合に比べてかならずしも改善されないことを示している．

2. インスリン抵抗性と肥満あるいは高血圧を対象とした解析例

罹患同胞対を用いて複数の形質を対象としたゲノムワイド解析として，Kissebah と Rotter らによる研究が有名である．インスリン抵抗性と肥満に関連した表現形質に重点をおいた Kissebah らによる MetS の全ゲノムマッピングの結果では，第 3 染色体の長腕領域(3q27)に，BMI，ウエストおよびヒップ周囲径，体重，血漿インスリン値，血中インスリン/ブドウ糖比に対する連鎖のピークが重複して存在し，この領域に MetS の主要な疾患感受性遺伝子の存在が示唆された[15]．この第 3 染色体領域には候補遺伝子としてアディポネクチン遺伝子が存在する．

同様に，Rotter らによってインスリン抵抗性と高血圧に関連した表現形質に重点をおいた MetS の全ゲノムマッピングの結果，第 7 染色体の長腕領域(短腕端から 112～137cM の領域)に，空腹時インスリン値，血圧(収縮期血圧および平均動脈圧)，インスリン抵抗性指数 HOMA(homeostasis model assessment)，および血漿レプチン値に関する連鎖のピークがやはり重複して存在することが明らかとなった[16]．この領域の候補遺伝子としては，レプチン遺伝子やプロテインホスファターゼ 1 調節サブユニット 3 遺伝子(*PPP1R3*)などがあげられている．

3. 構成因子を対象とした解析例

MetS に注目したゲノムワイド連鎖解析は，当初は上記の 2 例のように MetS に関連した複数の形質を同時に対象としたものが主であったが，最近では因子分析によって抽出された各構成因子(component)を解析対象として全ゲノムワイド連鎖解析を行ったものも報告されつつある．HERITAGE Family Study では，456 名の白人および 217 名の黒人を対象としたそのような研究結果が報告されている[17]．この研究では因子分析の結果抽出された 2 つの主要な構成因子に関して全ゲノム解析が行われ，いくつかの連鎖を示唆する領域が得られている．しかし驚いたことに，白人と黒人で示された連鎖領域は大きく異なっており，重複する領域はまったく認められていない．このことは MetS の成因に人種差がきわめて大きく関係

表 3　メタボリックシンドロームのゲノムワイド連鎖解析の結果

疾患感受性遺伝子座の染色体上の局在位置	解析対象(人種など)	文献
各構成因子(component)を対象とした研究		
6q24-25, 7q21-31	Mexican Americans	*Diabetes*, **51**：841-847, 2002.
1p34, 1q41, 2p22, 7q31, 9q13-21, 10p11, 19q13	Whites and Blacks	文献[17]
2q36, 7q31, 12q21	Americans	*Diabetes*, **52**：2840-2847, 2003.
1p36, 3p12, 4p15, 6q13	Mexican Americans	*Hum. Biol.*, **76**：651-665, 2004.
3p26, 8p23, 11q24, 13p12, 15q15	Americans	*Obes. Res.*, **13**：1885-1890, 2005.
17q23, 18p11	Whites, Blacks, Hispanics, Asians	*Hypertension*, **45**：751-757, 2005.
複数の形質(multiple trait)を対象とした研究		
1q21, 7q22-31	Pima Indians	*Diabetes*, **48**(Suppl.)：A182, 1999.
3q27, 17p12	Caucasians	文献[15]
7q31-32	Hispanics	文献[16]
6q22-26	Mexican Americans	*Am. J. Hum. Genet.*, **68**：1149-1164, 2001.
2q14-21, 5p13, 6q22, 17pter	Framingham	*BMC Genet.*, **4**：S57, 2003.
12q13	Framingham	文献[14]
1q21-31	Hispanics	*Diabetes*, **53**：1170-1174, 2004.
1q21-25	Chinese	*Diabetes*, **53**：2676-2683, 2004.
7q11.23	Mexican Americans	*Hum. Biol.*, **77**：231-246, 2005.

することを意味し，わが国における MetS はやはりわが国の独自のデータに基づいて診断および解釈されるべきであることを示している．

4. ゲノムワイド連鎖解析のまとめ

現在までに多数の疾患感受性遺伝子座位が報告され，表 3 におもな報告の結果をまとめて示す．当然ながら重点をおく構成因子や形質によって解析結果が異なり，また対象となる人種の違いも結果に大きな影響を及ぼしうる．しかし，第 7 染色体の長腕(7q21-q31)や第 6 染色体の長腕(6q22-q26)，および第 1 染色体の長腕(1q21-q31)の各領域は複数の研究で報告されており，これらの領域に MetS の主要な疾患感受性遺伝子が存在する可能性が高い．Rotter らのヒスパニックを対象とした研究で同定された第 7 染色体の長腕領域には，HERITAGE Family Study のゲノムワイド解析においても黒人における MetS の主要な原因遺伝子が存在する可能性が示されており，とくに注目される．また，第 1 染色体の長腕領域(1q21)には，やはりインスリン抵抗性に関連した高脂血症と高率な冠動脈疾患の合併を特徴とする家族性複合型高脂血症(FCHL)の原因候補遺伝子として最近，USF1(upstream transcription factor 1)遺伝子が同定され[18]，FCHL と MetS の臨床的なオーバーラップを考えると USF1 遺伝子はおおいに注目される．USF1 は糖・脂質代謝に関連する多くの標的遺伝子の包括的な転写制御にかかわる転写因子であり，ヒト USF1 遺伝子のイントロン内に存在する 20 塩基の領域内の多型が主としてアポ蛋白 E 遺伝子の転写レベル制御を介して，FCHL や MetS にみられる脂質(リポ蛋白質)異常の原因となる可能性が示唆されている[19]．

おわりに

MetS に関する因子分析の結果，およびその疾患感受性を規定する因子といくつかの候補遺伝子，そしてゲノムワイド連鎖解析の結果を中心に述べた．MetS の遺伝素因は主要な生活習慣病の上流に位置づけられ，その解明は MetS の成因を理解するうえだけでなく，生活習慣病に起因する動脈硬化症の早期からのリスク診断と適切な介入による予防，および新規治療薬の開発などを可能にしうるという点で，臨床上非常に重要である．

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Ⅲ.基礎

遺伝素因

後藤田貴也

メタボリックシンドロームは生活習慣病の1つであるが，同じ生活環境下にあってもメタボリックシンドロームを発症しやすい人とそうでない人（あるいは家系）があることから，明らかに遺伝素因（複数の疾患感受性遺伝子の組合せ）が発症に関わるものと考えられている．メタボリックシンドロームの原因となる遺伝子に関しては現時点ではまだ不明な点が多いが，メタボリックシンドロームの成因的基盤をなす「インスリン抵抗性」や「内臓肥満」に関連する遺伝子は，有力な候補遺伝子となる．実際に，インスリンシグナル伝達にかかわる分子やアディポサイトカインなどの多数の候補遺伝子上の変異や多型とメタボリックシンドロームとの相関が報告されている．また，連鎖解析を用いた研究も盛んに行なわれ，いくつかの染色体領域にメタボリックシンドロームの疾患感受性を規定する遺伝子が存在する可能性が示唆されている．メタボリックシンドロームの原因（疾患感受性）遺伝子は人種や民族によって大きく異なるといわれ，わが国独自のデータに基づいた解析が重要と考えられる．

キーワード　因子分析　相関解析　連鎖解析　LODスコア

複合遺伝形質としてのメタボリックシンドローム

メタボリックシンドロームを構成する疾患はいずれも生活習慣病の代表であり，メタボリックシンドローム自体も複数の遺伝因子の存在を背景として，そこに過食や運動不足などの環境因子の負荷が加わり発症にいたるいわゆる複合遺伝形質（complex trait）あるいは多因子遺伝性疾患（multifactorial disease）と考えられている．双生児研究は，疾患や表現型の遺伝度（heritability）を調べる基本的な方法の1つであるが，実際にメタボリックシンドロームを対象とした双生児研究の結果によると，メタボリックシンドロームに特徴的な糖，脂質，血圧，および体重の異常は，いずれも52～80%と高い遺伝度をもつ遺伝形質であることが示されている[1]．

一方，複合遺伝形質としての観点からメタボリックシンドロームに関する大規模疫学調査の結果を，因子分析（factor analysis）（用語解説）によって解析した結果ではいずれも，メタボリックシンドロームは遺伝的には少なくとも3～4個以上の互いに独立した構成成分からなることが示されている（**表1**）．約2千名の日本人を対象として筆者らが行った因子分析の結果でも，1）

表1　因子分析を用いたメタボリックシンドロームの構成因子の抽出

Kaiser Permanente Women Twin Study	Framingham Offspring Study	Strong Heart Study of American Indians	Honolulu Heart Program of Japanese American	日本人健診受診者
体重	BMI	BMI	体重	インスリン
脂肪分布	W/H比	グルコース	胴囲	BMI
グルコース	インスリン	インスリン	インスリン	血圧
インスリン	中性脂肪	血圧	グルコース	グルコース
血圧	HDL-C	インスリン	インスリン	中性脂肪
中性脂肪	グルコース	中性脂肪	血圧	HDL-C
HDL-C	インスリン	HDL-C	中性脂肪	BMI
	BMI		HDL-C	
	血圧			

肥満関連因子，糖代謝関連因子，高血圧関連因子，脂質代謝関連因子

表2　メタボリックシンドロームの候補遺伝子

遺伝子名	染色体上の局在	多型／変異*	関連する表現型**
ADRB2（β2アドレナリン受容体）	5q32-q34	G16R	MetS（男性で）
ADRB3（β3アドレナリン受容体）	8p12-p11	W64R	Ob, IR
APM1（アディポネクチン）	3q27	I164T, SNP	IR, Ob & MetS
APOC3（アポ蛋白C3）	11q23	455T-C	TG, Ob & MetS
APOC3/A4/A5（アポ蛋白C3/A4/A5）	11q23	SNP	Ob, HT, IR, TG
AGT（アンジオテンシノーゲン）	1q42-q43	T174M	HT & MetS
CAPN10（カルパイン10）	2q37	SNP-43	BS, TG（肥満者で）
FABP2（脂肪酸結合蛋白2）	4q28-q31	A54T	TG & MetS
GCCR（グレリン受容体）	3q26	SNP	Ob & MetS
GHSR（グルココルチコイド受容体）	5q31	RFLP, N363S	Ob, HT, IR
GNB3（G蛋白質β3サブユニット）	12p13	C825T	HT & MetS
IL6（インターロイキン6）	7p21	SNP (promoter)	IR, TG, Ob & MetS
INPPL1 (SHIP2)	11q23	SNP	HT & MetS
INS（インスリン）	11p15	RFLP	TG & MetS
LEP（レプチン）	7q31	SNP, VNTR	Ob, HT
LMNA（ラミンA/C）	1q21	H566H	TG, HDL & MetS
LPL（リポ蛋白リパーゼ）	8p22	SNP	IR, TG & MetS
LTA（リンフォトキシンα）	6p21	T60N	BS & MetS
NOS3（内皮依存性NO合成酵素）	7q36	7164G-T, D298D, ほか.	HT & MetS
PPARG（PPAR-γ）	3p25	P12A	TG, HT, IR & MetS
PPARGC1A（PGC-1α）	5q32	G482S	HT, HDL-C & MetS
PPARD（PPAR-δ）	6p21	+294T/C	Ob, TG & MetS
PTPN1（蛋白チロシン脱リン酸化酵素1B）	20q13	SNP	TG, HDL, Ob, BS &HT
UBL5 (BEACON)	19p13	SNP	BS, TG, Ob
UCP1（脱共役蛋白1）	4q31	SNP	Ob, IR
USF1（上流刺激因子1）	1q22-q23	SNP	TG & MetS

* SNP=単一塩基多型，RFLP=制限酵素切断断片長多型 **TG=高トリグリセリド血症，HDL=低HDL-コレステロール血症，MetS=その他のメタボリックシンドロームの表現型，BS=糖代謝異常，HT=高血圧，R=インスリン抵抗性，Ob=肥満

インスリン抵抗性と肥満に関連した因子，2）血圧に関連した因子，3）糖代謝異常に関連した因子，4）脂質代謝異常と肥満に関連した因子，の計4つの因子によってメタボリックシンドロームにみられる表現型の変動の7割以上が説明可能であった．つまり，メタボリックシンドロームは遺伝的な観点から見て単一の病態ではなく，それぞれに高い遺伝度を有する少なくとも3～4個以上の独立した病態を内包するものと考えられる．一方，因子分析では，インスリンが糖や脂質，肥満といった複数の因子と同時に抽出される（表1）ことから，インスリン抵抗性が危険因子重複の背景に共通して存在することが示唆される．

メタボリックシンドロームの候補遺伝子

メタボリックシンドロームの成因的基盤をなすものとして，「インスリン抵抗性の亢進」が重要であり，また，インスリン感受性や糖・脂質代謝，血圧の調節に与る様々な生理活性物質（アディポサイトカイン）を産生する脂肪細胞自体のさまざまな異常（内臓脂肪蓄積に代表されるような質的・量的・機能的異常）も重要である．このようなメタボリックシンドローム成立の根幹にかかわる因子の遺伝子は，メタボリックシンドロームの疾患感受性を規定する有力な候補遺伝子となる．実際に，多数の候補遺伝子上の変異や多型とメタボリックシンドロームに関連する表現型との間で広く相関解析（association study）が行われ，複数の表現型との間に有意な相関がみられる遺伝子がいくつか報告されている（表2）．たとえば，アディポネクチン遺伝子のIle164Thr変異は，日本人において低アディポネクチン血症と糖尿病に関連し，メタ

ボリックシンドロームや冠動脈疾患とも関連することが報告されている[2].

ゲノムワイド連鎖解析

ヒトゲノムの解読によりゲノム全体に高密度に散在するマイクロサテライトマーカーや単一塩基多型（SNP）などの遺伝子多型のカタログ化が進み，ゲノム全体を俯瞰した連鎖解析（linkage study）（用語解説）や相関解析も行われている.

◆複数の形質を同時に対象とした解析

フラミンガム研究の登録者を対象に，メタボリックシンドロームに関連する5つの形質（収縮期血圧，トリグリセリド，HDL-コレステロール，血糖値，ボディマスインデックス［BMI］）およびこれら5つを複合した形質（メタボリックシンドロームスコア：MSS）に対して，マイクロサテライトマーカーを用いて行った全ゲノム解析の結果が報告されている[3]．それによると，血糖値，HDL-コレステロール，収縮期血圧ではそれぞれの最大LODスコアが，2.37，2.27，1.93とsuggestiveなレベルの連鎖が確認されたのに対して，MSSでは1.82とむしろそれらを下回るLODスコアしか得られていない．すなわち，メタボリックシンドロームを構成する主要な5つの形質に関して，それらの組み合わせや重みづけに関する考慮をせずに単純にその総和をもってメタボリックシンドロームを定義するとすれば，遺伝因子の検出力はそれぞれの形質単独の場合に比べて必ずしも改善されないことになる．これは，「メタボリックシンドロームは複数の独立した構成因子（病態）からなる」という因子分析の結果に合致し，メタボリックシンドロームの遺伝解析を行う上では，各構成因子に焦点を合わせた解析が重要になるものと思われる.

◆インスリン抵抗性と肥満あるいは高血圧に焦点を絞った解析例

罹患同胞対を用いて複数の形質を対象としたゲノムワイド解析として，KissebahとRotterらによる研究が有名である．インスリン抵抗性と肥満に関連した表現形質に重点をおいた白人における全ゲノムマッピングの結果では，3番染色体の長腕領域（3q27）に，BMI，ウエストおよびヒップ周囲径，体重，血漿インスリン値，血中インスリン/ブドウ糖比に対する連鎖のピークが重複して存在し，この領域にメタボリックシンドロームの主要な疾患感受性遺伝子の存在が示唆された[4]．この3番染色体領域には候補遺伝子としてアディポネクチン遺伝子などが存在する.

同様に，インスリン抵抗性と高血圧に関連した表現形質に重点をおいたヒスパニック系を対象とした全ゲノムマッピングの結果，7番染色体の長腕領域（短腕端から112-137cMの領域）に，空腹時インスリン値，血圧（収縮期血圧および平均動脈圧），インスリン抵抗性指数HOMA（homeostasis model assessment），および血漿レプチン値に関する連鎖のピークがやはり重複して存在することが明らかとなった[5]．この領域の候補遺伝子としては，レプチン遺伝子やプロテインフォスファターゼ1調節サブユニット3遺伝子（PPP1R3）などがあげられている.

◆構成因子を対象とした解析例

メタボリックシンドロームに注目したゲノムワイド連鎖解析は，従来は上記のように関連する複数の形質を同時に対象としたものが主であったが，最近では因子分析によって抽出された各構成因子（コンポーネント）を解析対象として全ゲノムワイド連鎖解析を行ったものも報告されつつある．HERITAGE Family Study対象者におけるこのような研究では，いくつかの連鎖を示

表3　メタボリックシンドロームのゲノムワイド連鎖解析の結果

疾患感受性遺伝子座の染色体上の局在位置	解析対象 (人種など)	文　献
各構成因子(component)を対象とした研究		
66q24-25, 7q21-31	Mexican Americans	Diabetes 51: 841-7, 2002.
1p34, 1q41, 2p22, 7q31, 9q13-21, 10p11, 19q13	Whites & Blacks	JCEM 88: 5935-5943, 2003.
2q36, 7q31, 12q21	Americans	Diabetes 52: 2840-7, 2003.
1p36, 3p12, 4p15, 6q13	Mexican Americans	Hum Biol 76: 651-65, 2004.
3p26, 8p23, 11q24, 13p12, 15q15	Americans	Obes Res 13: 1885-90, 2005.
17q23, 18p11	Whites, Blacks, Hispanics, Asians	Hypertension 45: 751-7, 2005
複数の形質 (multiple trait)を対象とした研究		
1q21, 7q22-31	Pima Indians	Diabetes 48 (Suppl A182), 1999.
3q27, 17p12	Caucasians	PNAS 97: 14478-83, 2000.
7q31-32	Hispanics	Circulation 104: 1255-60, 2001.
6q22-26	Mexican Americans	AJHG 68: 1149-64, 2001.
2q14-21, 5p13, 6q22, 17pter	Framingham	BMC Genetics 4 (S57), 2003.
12q13	Framingham	BMC Genetics 4 (S96), 2003.
1q21-31	Hispanics	Diabetes 53: 1170-4, 2004.
1q21-25	Chinese	Diabetes 53: 2676-83, 2004.
7q11.23	Mexican Americans	Hum Biol 77: 231-46, 2005.

唆する領域が得られたが，驚いたことに，示された連鎖領域は白人と黒人の対象者の間で大きく異なっており，重複する領域は全く認められていない．このことは，メタボリックシンドロームの成因に人種差が極めて大きく関与することを意味し，わが国におけるメタボリックシンドロームはやはりわが国の独自のデータに基づいて診断および解釈されるべきであることを示している．

◆ゲノムワイド連鎖解析のまとめ

現在までに多数の疾患感受性遺伝子座位が報告され，**表3**に主な報告の結果をまとめて示す．当然ながら，重点を置く構成因子や形質によって解析結果が異なり，また対象となる人種の違いも結果に大きな影響を及ぼしうる．しかしながら，7番染色体の長腕（7q21-31）や6番染色体の長腕（6q22-26），および1番染色体の長腕（1q21-31）の各領域は複数の研究で報告されており，これらの領域にメタボリックシンドロームの主要な疾患感受性遺伝子が存在する可能性が高い．以前より，この1番染色体の長腕領域（1q21）には，やはりインスリン抵抗性に関連した高脂血症と高率な冠動脈疾患の合併を特徴とする家族性複合型高脂血症（FCHL）の原因遺伝子が存在するとされてきた．近年，その有力な候補遺伝子としてUSF1（upstream transcription factor 1）遺伝子が同定され[6]，FCHLとメタボリックシンドロームの臨床的なオーバーラップを考えると，USF1はメタボリックシンドロームとの関連でも注目される．

用語解説

因子分析　体重や血圧，インスリン値といった互いに密接な関連をもつ量的形質に関して行なう多変量解析の一つであり，複雑に絡み合った因子を幾つかの互いに独立した因子のもとに抽出する方法である．

相関解析　いわゆるcase-control studyであり，患者群と正常対照群との間で候補遺伝子の変異や多型の頻度を比較して，疾患との相関（association）を調べる．検出力は高いが，偽陽性が多いことが問題となる．

連鎖解析　家系内や集団中で複数の遺伝子座が同時に分離（遺伝）されていく現象を連鎖（linkage）と呼び，それは各遺伝子座が同一染色体上の近傍に位置することを意味する．こ

の「連鎖」の概念に基づき遺伝子マーカーと原因遺伝子との間の組み換え率から原因遺伝子の染色体上の位置を推定する方法が連鎖解析法である.

LODスコア　連鎖の有無を判定する基準として用いられ，連鎖の有る無しを仮定した場合の各々の尤度（likelihood）の比の対数として表される．通常，LODスコアにおいて3.3以上で有意（significant）な，1.9以上で示唆的（suggestive）な連鎖が認められるとされる.

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ORIGINAL ARTICLE

Elevated C-Reactive Protein Is a Predictor of the Development of Diabetes in a General Japanese Population

The Hisayama Study

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OBJECTIVE — We examined the association between high-sensitivity C-reactive protein (CRP) levels and the development of diabetes in a general Japanese population.

RESEARCH DESIGN AND METHODS — A total of 1,759 Japanese subjects, aged 40–79 years and without diabetes (according to American Diabetes Association fasting criteria), were stratified into three groups according to CRP tertiles by sex and followed up prospectively for a mean of 9.0 years.

RESULTS — During the follow-up, 131 subjects (67 men and 64 women) developed diabetes. In both sexes, the age-adjusted cumulative incidence of diabetes increased significantly as the tertiles of CRP levels increased. In multivariate analyses, the risk of developing diabetes was significantly higher in the highest CRP tertile than in the lowest after adjustment for a number of confounding factors (odds ratio 2.63 [95% CI 1.23–5.65] for men and 2.25 [1.01–5.01] for women). In stratified analyses, this CRP-diabetes association was stronger in subjects without obesity or other risk factors related to insulin resistance and in nondrinking subjects.

CONCLUSIONS — Our findings suggest that elevated CRP concentration is a significant predictor of diabetes in the general Japanese population, independent of obesity and insulin resistance.

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In some cohort and nested case-control studies in Western countries, an elevated C-reactive protein (CRP) level has been an independent predictor of diabetes (1–10). Recent cross-sectional studies have also demonstrated clear associations of elevated serum CRP levels with obesity and insulin resistance (11–13). These findings suggest that the inflammatory state illustrated by elevated CRP concentrations is associated with hyperglycemia and diabetes through obesity or increased insulin resistance. However, epidemiological findings concerning this issue are still controversial; several studies have reported a significant positive association between elevation in CRP levels and the future risk of diabetes even after adjustment for BMI (1,2,4,7,9,10), whereas in other studies (3,6) this association disappeared after adjustment for BMI.

Japanese are characterized by low BMI levels and low CRP concentrations in blood compared with Westerners (14). Moreover, there have been no reports on the relationship between CRP levels and the development of diabetes among general populations in Japan. The aim of the present study is to examine the effects of serum CRP levels on the development of diabetes in a prospective study of a defined Japanese population, taking into account comprehensive risk factors.

RESEARCH DESIGN AND METHODS

Study population and follow-up survey

In 1988, a screening survey for the present study was performed in the Town of Hisayama in Japan. A total of 2,587 residents aged 40–79 years (80.2% of the total population of this age-group) participated in the baseline survey. The diabetes classification was based on the fasting criteria of the American Diabetes Association (15), i.e., subjects with fasting plasma glucose levels ≥7.0 mmol/l or those who were taking diabetes medications were considered diabetic.

After the exclusion of 80 subjects who had already eaten breakfast before the examination, 233 subjects with diabetes, and 67 subjects whose CRP concentrations could not be measured due to insufficient quantities of stored sera, the remaining 2,207 subjects (926 men and 1,281 women) were enrolled in the baseline examination. Among those, 1,759 subjects (694 men and 1,065 women) underwent follow-up examinations in 1993–1998 (follow-up rate 79.7%). We considered a subject to have developed diabetes when he/she met the above-mentioned baseline criteria. During this period, 131 subjects (67 men and 64 women) developed diabetes.

Laboratory measurements

Plasma glucose levels were determined by a glucose-oxidase method, and serum insulin was measured by radioimmunoassay. HbA_{1c} levels were measured by high-pressure liquid chromatography. Total cholesterol, HDL cholesterol, and triglycerides were all determined enzymatically. Serum specimens collected at the time of CRP measurement were stored at −20°C until used in 2002. High-sensitivity CRP

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Abbreviations: CRP, C-reactive protein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

concentrations were determined using a modification of the Behring latex-enhanced CRP assay. Sitting blood pressure was obtained three times and the average values used in the analyses. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or current treatment with antihypertensive agents. BMI (kilograms per meters squared) was used as an indicator of obesity.

Diabetes in first- or second-degree relatives indicated a family history of diabetes. Those subjects engaging in sports at least three times a week during their leisure time comprised a regular exercise group. Information on smoking habits and alcohol intake was used to classify subjects as having current habits or not.

Table 1—*Characteristics of subjects by sex*

	Men	Women
n	694	1,065
Age (years)	58 ± 10	57 ± 10
High-sensitivity CRP (mg/l)	0.49 (0.07–7.14)	0.36 (0.06–3.22)
Fasting plasma glucose (mmol/l)	5.6 ± 0.5	5.5 ± 0.5
HbA_{1c} (%)	5.5 ± 0.5	5.4 ± 0.5
Family history of diabetes (%)	9.3	7.3
Fasting insulin (pmol/l)	30.0 (18.0–72.0)	36.0 (18.0–72.0)
BMI (kg/m^2)	22.9 ± 2.9	23.0 ± 3.1
Total cholesterol (mmol/l)	5.10 ± 1.04	5.57 ± 1.05
HDL cholesterol (mmol/l)	1.26 ± 0.30	1.35 ± 0.29
Triglycerides (mmol/l)	1.25 (0.58–3.49)	1.02 (0.49–2.33)
Systolic blood pressure (mmHg)	131 ± 19	130 ± 20
Diastolic blood pressure (mmHg)	80 ± 11	75 ± 11
Hypertension (%)	41.5	32.7
Current drinking (%)	61.0	8.5
Current smoking (%)	47.6	5.4
Regular exercise (%)	16.1	4.9

Data are means ± SD or medians (95% CI) unless otherwise indicated.

Statistical analysis

Because the distributions of CRP, fasting insulin, and triglycerides were skewed, these variables were natural log transformed for statistical analyses. To analyze CRP levels as categorical variables, these levels were divided into tertiles by sex (0.05–0.28, 0.29–0.77, and 0.78–13.5 mg/l for men and 0.05–0.24, 0.25–0.57, and 0.58–5.78 mg/l for women). The age-adjusted cumulative incidence of diabetes was calculated by the direct method and compared by the Mantel-Haenszel χ^2 test using 10-year age-groupings. Age- and multivariate-adjusted odds ratios (ORs) and 95% CIs were calculated by logistic regression analysis. $P < 0.05$ was considered statistically significant in all analyses.

This study was conducted with the approval of the Ethics Committee of Kyushu University, and written informed consent was obtained from all participants.

RESULTS — The clinical characteristics of the subjects by sex are shown in Table 1. The mean age was 58 years for men and 57 years for women.

In both sexes, the age-adjusted cumulative incidence of diabetes increased significantly with elevating tertiles of baseline serum CRP concentrations. The incidences in the 3rd tertile for both sexes and in the 2nd tertile for men were significantly higher than in the 1st tertile (Fig. 1). As shown in Table 2, the risk of future diabetes in either sex was more than threefold higher in the 3rd tertile than in the 1st tertile after adjustment for age. These associations remained substantially unchanged even after adjustment for the other confounding factors, including age, family history of diabetes, fasting insulin, BMI, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, current drinking, current smoking, and physical activity (adjusted OR 2.63 [95% CI 1.23–5.65], $P = 0.014$, for men and 2.25 [1.01–5.01], $P = 0.049$, for women).

We next estimated the age- and sex-adjusted ORs and 95% CIs for the development of diabetes by an increment of 1 log CRP in men and women together according to the other risk factor levels (Table 3). Analyses were performed by dividing the subjects into three groups according to tertiles of BMI, triglycerides, and HDL cholesterol levels or into two

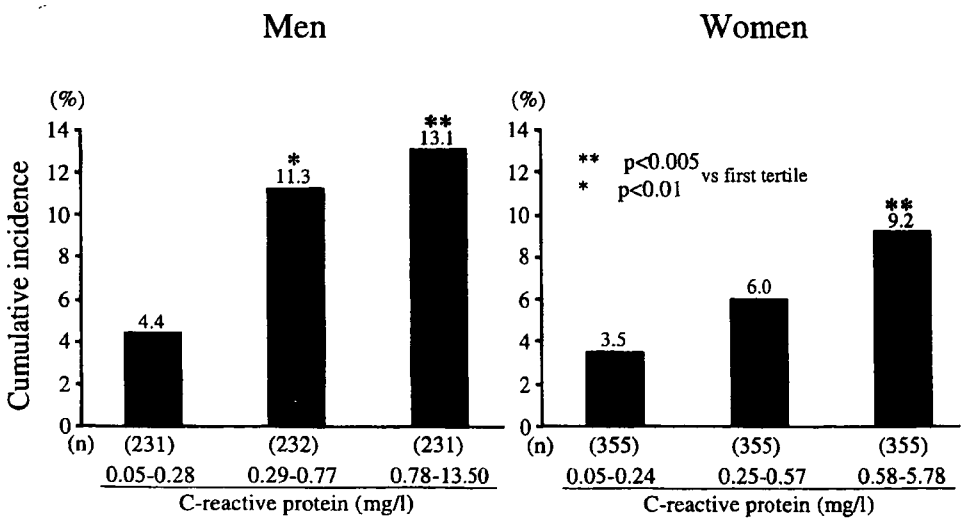

Figure 1—*Age-adjusted cumulative incidence of diabetes according to tertiles of serum high-sensitivity CRP levels by sex.*

Table 2—*Age- or multivariate-adjusted ORs and 95% CIs for occurrence of diabetes according to tertiles of serum high-sensitivity CRP levels by sex*

	High-sensitivity CRP level (mg/l)							
	Men				Women			
	0.05–0.28	0.29–0.77	0.78–13.50	*P* for trend	0.05–0.24	0.25–0.57	0.58–5.78	*P* for trend
Population at risk (*n*)	231	232	231		355	355	355	
Cases of diabetes (*n*)	11	26	30		10	21	33	
Age-adjusted OR (95% CI)	1 (referent)	2.67 (1.28–5.56)	3.23 (1.57–6.70)	0.002	1 (referent)	2.12 (0.98–4.58)	3.35 (1.60–7.03)	0.001
Multivariate-adjusted OR (95% CI)	1 (referent)	1.96 (0.92–4.19)	2.63 (1.23–5.65)	0.014	1 (referent)	1.76 (0.80–3.87)	2.25 (1.01–5.01)	0.049

Multivariate adjustment was made for age, family history of diabetes, fasting insulin, BMI, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, current drinking, current smoking, and physical activity.

groups by hypertension status, current drinking, and current smoking. Significant positive associations between CRP levels and incident diabetes were observed among subjects in the 1st tertile of BMI, among subjects in the 1st and 2nd tertiles of triglycerides, among subjects of the 2nd and 3rd tertiles of HDL cholesterol, and among subjects without hypertension or current drinking. Significant associations were also observed in both smokers and nonsmokers. However, clear CRP-diabetes associations were not seen in the other categories of any risk factors.

CONCLUSIONS— We demonstrated in a prospective study of a general Japanese population that elevated CRP level is an independent predictor of diabetes for both sexes even after adjustment for comprehensive risk factors. In stratified analyses, the CRP-diabetes association was stronger in subjects without risk factors related to insulin resistance, such as obesity, dyslipidemia, and hypertension, and among nondrinkers, whereas the presence of a current smoking habit did not affect this association.

To our knowledge, this is the first report to indicate that the low-grade inflammatory state illustrated by increased CRP is an independent risk factor for developing diabetes in a general Japanese population. Similar findings were observed in a Japanese-American population (13) as well as in some other Western populations (5–12,14). Since Japanese Americans have a Western lifestyle, their findings cannot be generalized to Japanese living in Japan. Our subjects were thinner than those in previous reports (1–10). Our findings suggest that the subclinical inflammatory process has an important role in the development of diabetes in relatively lean Asian populations, as it does in Western populations.

Recent cross-sectional epidemiological data have demonstrated that elevated serum CRP levels are associated with obesity, insulin resistance, and glucose intolerance (11–13). These findings suggest that the inflammatory state affects glucose levels in blood and increases the risk of diabetes via obesity or insulin resistance. However, our study showed that the association between CRP levels and the development of diabetes is independent of serum insulin levels as well as BMI. These findings are in accord with those of several other cohort studies (1,9). Additionally, our stratified analyses showed that the CRP-diabetes association was stronger particularly in individuals with low levels of risk factors related to insulin resistance. Therefore, a low-grade inflammatory state can be considered a risk factor for diabetes independent of obesity and insulin resistance, and unknown mediators are also thought to be involved in the development of diabetes.

In our subjects, the influence of CRP on the incidence of diabetes was stronger in nondrinkers than in drinkers. Some studies have shown that moderate alcohol

Table 3—*Age- and sex-adjusted ORs and 95% CIs for occurrence of diabetes by an increment of 1 log high-sensitivity CRP in all subjects according to risk-factor levels*

Risk factor	Population at risk (*n*)	Cases of diabetes (*n*)	Age- and sex-adjusted OR (95% CI)	*P*
BMI (kg/m^2)				
≤21.5	586	29	1.36 (1.05–1.75)	0.017
21.6–24.2	587	35	1.20 (0.92–1.57)	NS
≥24.3	586	67	1.25 (0.99–1.59)	NS
Triglycerides (mmol/l)				
≤0.88	587	30	1.30 (1.01–1.67)	0.042
0.89–1.34	582	29	1.50 (1.12–2.01)	0.007
≥1.35	590	72	1.16 (0.94–1.43)	NS
HDL cholesterol (mmol/l)				
≤1.14	572	49	1.04 (0.82–1.31)	NS
1.15–1.40	583	44	1.43 (1.13–1.81)	0.003
≥1.41	604	38	1.57 (1.20–2.07)	0.001
Hypertension				
Without	1,123	54	1.45 (1.18–1.77)	0.0003
With	636	77	1.16 (0.95–1.41)	NS
Current drinking				
Without	1,246	77	1.43 (1.20–1.71)	0.0001
With	513	54	1.14 (0.92–1.42)	NS
Current smoking				
Without	1,372	95	1.29 (1.09–1.53)	0.003
With	387	36	1.34 (1.04–1.72)	0.022

consumption is associated with lower CRP concentrations (16,17). Additionally, recent cohort studies have revealed that moderate alcohol consumption reduced the risk of future type 2 diabetes (18,19). Therefore, the intake of alcohol may attenuate the influence of CRP on the development of diabetes.

A recent cohort study has reported a significant association between inflammation and future diabetes among nonsmokers but not among smokers (8). In our subjects, however, an association between elevated CRP levels and incident diabetes was observed in both nonsmokers and smokers. This suggests that the CRP-diabetes association is independent of current smoking.

Several limitations of our study should be discussed. The primary limitation is that a diagnosis of diabetes was not based on a 75-g oral glucose tolerance test, but on a single reading of fasting glucose level, as was the case in other epidemiological studies (2,8,9). Subjects with diabetes having normal fasting glucose levels were misdiagnosed in our study. Additionally, some of the participants who were classified as having worsening fasting glucose status may not have been so categorized after repeated testing. These misclassifications should weaken the association found in this study. Therefore, the true association may be stronger than that shown in our findings. A secondary limitation is that CRP concentrations were measured in serum conserved for a long period at −20°C. However, the stability of CRP concentrations in serum preserved at this temperature for an average of 12 years was confirmed in the Reykjavik Study (20). The last limitation is that our study lacked information on drug use, which could affect serum CRP levels. It is known that several medications can alter CRP levels, including statins, ACE inhibitors, fibrates, niacin, thiazolidinedione, and estrogen/progestogen hormone (21). However, these medications were rarely used in our country in 1988. This suggests that such a bias does not invalidate the present findings.

In conclusion, we showed that subclinical elevation in CRP concentrations is an independent predictor of diabetes in a general Japanese population. CRP was an effective predictor of diabetes in individuals with the lowest BMI as well as in individuals without other risk factors related to insulin resistance. These findings add to the notion that low-grade inflammation is an important factor in the pathogenesis of type 2 diabetes. Further study is necessary to clarify the role of inflammation in the cascade to the development of diabetes.

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HIGH LIGHT

Decreasing incidence of lacunar vs other types of cerebral infarction in a Japanese population

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Abstract—*Background:* There is scant information on secular trends in the incidence and survival of ischemic stroke subtypes. *Methods:* The authors established three cohorts of Hisayama residents age ≥40 years in 1961 (1,618 subjects), 1974 (2,038 subjects), and 1988 (2,637 subjects). They followed up with each cohort for 12 years, comparing the incidence and survival rate of ischemic stroke subtypes. Morphologic examinations by autopsy or brain imaging was performed on most of the ischemic stroke cases in all cohorts. *Results:* The age-standardized incidence of lacunar infarction significantly declined by 59% for men and by 28% for women from the first to the second cohort. It continued to decline by 41% for men, but the decline decelerated for women between the second and third cohort. The age-standardized incidence of atherothrombotic infarction tended to decline from the first to the second cohort, whereas it was sustained between the second and third cohort for both sexes. The age-standardized incidence of cardioembolic infarction was unchanged throughout the cohorts. In these cohorts, mean blood pressure levels among hypertensive subjects and the prevalence of current smoker decreased with time, though the prevalence of hypertension remained stable. The 5-year survival rate after lacunar infarction significantly improved among the cohorts, but those of atherothrombotic and cardioembolic infarction did not. *Conclusions:* These data suggest that, in the Japanese population, the incidence of lacunar infarction steadily declined for the last 40 years. The improvement of hypertension control and decreasing prevalence of smoking might be responsible for this trend.

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Stroke is the major cause of mortality and the third leading cause of death in Japan and in Western countries.[1] Ischemic stroke is the most common type of stroke in developed countries, and it can be further divided into several subtypes based on the size and location of the affected cerebral arteries and their pathogenesis: that is, lacunar (LI), atherothrombotic (ATI), and cardioembolic (CEI) infarction.[2] A few cohort studies in Japan, including ours, have shown that the incidence of ischemic stroke significantly declined in the 1970s, but that in recent years, the rate of decline has decreased.[3,4] As risk factors, prognosis, and treatment among subtypes of ischemic stroke are different,[5-7] it would be informative to examine trends in the incidence and long-term survival of ischemic stroke by subtypes to improve our understanding of its pathogenesis and assist in establishing preventive measures. However, there has been very little information on this issue, as the definitive classification of ischemic stroke into subtypes requires detailed clinical data, including information on the disease course, neurologic symptoms, and morphologic features.

The Hisayama study is a population-based study that has established three cohorts at times corresponding to periods of remarkable lifestyle changes in Japan.[3,8-10] In this study, study-team physicians performed physical and neurologic examinations on the subjects who developed stroke and collected detailed clinical information. Furthermore, morphologic examinations by autopsy or brain imaging were performed in most of the stroke cases in each cohort.[3,6] These characteristics of the study design enabled us to examine secular trends in the incidence and survival rate of ischemic stroke subtypes.

Methods. *Study population.* Hisayama Town is a suburban community adjacent to Fukuoka City, a metropolitan area on Kyushu Island in southern Japan. The population of the town has been stable for many years (annual variation rate <5%)[3] and has been shown to be representative of Japan as a whole on the basis of data from the national census.[8,10] The study design and characteristics of the subject population have been described in detail elsewhere.[8-10] In brief, we established three study cohorts from Hisayama residents age ≥40 years in 1961, 1974, and 1988 after screening examinations. In 1961, a total of 1,658 subjects in that age group consented to participate in the screening examination (participation rate 90.1%). After the exclusion of 28 subjects with

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a history of stroke or myocardial infarction and 12 subjects who died or moved out of town during the examination, 1,618 subjects were enrolled as the first cohort. In the same manner, we established the second cohort consisting of 2,038 subjects from 2,135 participants (participation rate, 81.2%) in 1974 and the third cohort of 2,637 subjects from 2,742 participants (participation rate, 80.9%) in 1988.

Follow-up. We followed up with each cohort for 12 years by repeated health examinations. Health status was checked every year by mail or telephone for any subjects who did not undergo a regular examination or who moved out of town. We also established a daily monitoring system organized by the study team, local physicians, and members of the local health and welfare office. When the subjects died, autopsy examinations were performed at the Department of Pathology of Kyushu University. During the follow-up period of each cohort, autopsy examinations were performed on 372 subjects (81.6% of the deceased subjects) in the first cohort, 342 subjects (86.2%) in the second cohort, and 366 subjects (75.5%) in the third cohort. Only two subjects in the first cohort, two in the second cohort, and one in the third cohort were lost to follow-up.

Definition of ischemic stroke subtype. The diagnosis of stroke was determined on the basis of clinical information and autopsy findings. In principle, stroke was defined as a sudden onset of nonconvulsive and focal neurologic deficit persisting for >24 hours and was classified as ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage, or undetermined type.[3] Two stroke neurologists reviewed all gathered information about stroke cases and made the diagnoses of ischemic stroke subtypes separately on the basis of the Classification of Cerebrovascular Disease III proposed by the National Institute of Neurological Disorders and Stroke[2] as well as on the basis of the diagnostic criteria of the Trial of Org 10172 in Acute Stroke Treatment (TOAST) Study[11] and Cerebral Embolism Task Force.[12] Their diagnoses agreed in 94% of cases, and in the remaining cases, the diagnoses were determined by a detailed panel discussion. When sufficient clinical and morphologic information was obtained, a diagnosis of ischemic stroke subtype was defined as "definite." When the amount of either type of information was insufficient, the diagnostic level was defined as "probable."

Details of the diagnostic criteria of ischemic stroke subtypes have been described previously.[6] In brief, LI was diagnosed as the presence of a relevant brainstem or subcortical hemispheric lesion with a diameter of <1.5 cm demonstrated on brain imaging or autopsy and no evidence of cerebral cortical or cerebellar impairment. ATI was diagnosed when the subjects had significant stenosis (>50%) or occlusion of a major cerebral artery with infarct size ≥1.5 cm on brain imaging or autopsy. The diagnosis of CEI was made on the basis of primary and secondary clinical features suggestive of CEI as reported by the Cerebral Embolism Task Force.[12] The category of undetermined subtype (UND) included all ischemic stroke cases for which the subtype could not be determined because of insufficient clinical or morphologic information. We considered morphologic findings significant and used clinical features as reference information.

During the follow-up period of each cohort, first-ever ischemic stroke developed in 122 subjects (78 cases of LI, 26 of ATI, 13 of CEI, and 5 of UND) in the first cohort, 124 in the second cohort (67 of LI, 26 of ATI, 28 of CEI, and 3 of UND), and 137 in the third cohort (67 of LI, 37 of ATI, 33 of CEI, and 0 of UND). Among these, morphologic examinations by autopsy or brain imaging were performed on 110 patients (90.2%) in the first cohort, 118 (95.2%) in the second cohort, and 137 (100%) in the third cohort. In this study, we present the data regarding definite and probable ischemic stroke subtype cases together, as these combined data were almost identical to those for definite cases only.

Risk factors. Recumbent blood pressures were measured three times at every examination, and hypertension was defined as a mean systolic blood pressure of ≥140 mm Hg or a mean diastolic blood pressure of ≥90 mm Hg or a current use of antihypertensive agents. Glucose intolerance was defined by an oral glucose tolerance test in the subjects with glycosuria in 1961, by fasting and postprandial glucose concentrations in 1974, and by a 75-g oral glucose tolerance test in 1988, in addition to medical history of diabetes. Serum cholesterol levels were measured by the Zak-Henly method with a modification by Yoshikawa in 1961, by the Zurkowski method in 1974, and by the enzymatic method in 1988.[6] Hypercholesterolemia was defined as total cholesterol level of ≥6.2 mmol/L (240 mg/dL). Body height and weight were measured in light clothing without shoes, and obesity was defined as body mass index of ≥25.0 kg/m^2. Information on antihypertensive treatment, alcohol intake, and smoking habits was obtained with the use of a standard questionnaire and was categorized as current habitual use or not.

Statistical analysis. The incidence rates of ischemic stroke and its subtypes were calculated by the person-year method and adjusted for the age distribution of the World Standard Population by the direct method. The differences in the incidence among the three cohorts were tested by sex with the use of the Cox proportional hazards model after adjustment for age. Subjects who developed ischemic stroke were also followed up for the subsequent 5 years or to the end of the follow-up in every cohort, and survival rates were estimated with the Cox proportional hazards model. All statistical analyses were performed with the SAS program package. Values of $p < 0.05$ were considered significant in all analyses.

Results. *Trends in risk factors.* We compared the prevalence of cardiovascular risk factors at the baseline examination among the three cohorts by sex (table 1). In both sexes, the prevalence of hypertension was not different among the cohorts, but the proportion of individuals using antihypertensive agents consistently increased with time. As a result, among hypertensive subjects, mean blood pressures significantly decreased from the first to the third cohort in both sexes. The prevalence of glucose intolerance, hypercholesterolemia, and obesity increased progressively with time. The proportion of current smokers in both sexes and that of male drinkers declined linearly over the cohorts.

Trends in incidence of ischemic stroke subtype. The age-standardized incidence of ischemic stroke for men declined throughout the cohorts (table 2; $p < 0.05$). For women, the incidence also declined from the first to the second cohort ($p < 0.05$), but this declining trend was slowed between the second and third cohort. The age-standardized incidence of LI for men declined by 59% from the first to the second cohort ($p < 0.01$), and it continued to decline by 41% from the second to the third cohort ($p < 0.05$). The age-standardized incidence of LI for women also declined by 28% from the first to the second cohort, but the decline decelerated between the second and third cohort (15%). The age-standardized incidence of ATI declined by 41% from the first to the second cohort for both sexes, but the difference was not significant probably owing to the small number of events. The age-standardized incidence of ATI for women was slightly decreased in the third cohort (11%), but that for men was not. The age-standardized incidence of CEI did not change significantly among the cohorts for either sex.

The proportions of ischemic stroke subtypes among the cohorts by sex are shown in table 3. For men, the proportion of the subjects with LI steadily decreased from the first to the third cohort, whereas those of ATI and CEI increased. For women, the proportion of the subjects with CEI increased slightly from the first to the third cohort, but the proportions of the other subtypes were constant among the cohorts.

Trend in age-specific incidence of ischemic stroke subtype. The age-specific incidence rates of ischemic stroke subtypes for men and women combined among the three cohorts are shown in figure 1. The incidence of each subtype of ischemic stroke increased with advancing age in every cohort. The incidence of LI consistently decreased from the

Table 1 Prevalence of cardiovascular risk factors at baseline among three cohorts in 1961, 1974, and 1988 of the Hisayama study by sex

Variables	Men				Women			
	First cohort, n = 705	Second cohort, n = 855	Third cohort, n = 1,110	p for trend	First cohort, n = 913	Second cohort, n = 1,183	Third cohort, n = 1,527	p for trend
Age, y	55 ± 11	56 ± 11	57 ± 12	<0.001	57 ± 12	58 ± 12	59 ± 12	0.002
Hypertension, %	38.6	40.4	41.5	0.22	37.4	44.0	38.4	0.98
Antihypertensive agents, %	2.1	8.5	14.3	0.001	2.2	8.3	15.5	0.001
Systolic blood pressure,* mm Hg	161	158	152	<0.001	163	162	155	<0.001
Diastolic blood pressure,* mm Hg	91	87	84	<0.001	88	86	81	<0.001
Glucose intolerance, %	12.1	13.8	31.9	0.001	4.8	8.1	27.2	0.001
Hypercholesterolemia, %	1.7	5.3	14.9	0.001	3.2	9.6	25.9	0.001
Obesity, %	7.4	11.6	23.2	0.001	12.9	20.8	23.4	0.001
Current smoker, %	76.3	73.0	49.9	0.001	16.8	10.7	6.9	0.001
Current drinker, %	69.4	64.0	60.2	0.001	8.3	5.6	8.7	0.41

Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or a current use of antihypertensive agents. Hypercholesterolemia was defined as total cholesterol level ≥6.2 mmol/L (240 mg/dL). Obesity was defined as body mass index ≥25.0 kg/m^2.

* Mean systolic and diastolic blood pressures among hypertensive subjects in each cohort.

first to the third cohort mainly in the aged subjects. The incidence of ATI in the subjects age <80 decreased from the first to the second cohort but was unchanged in the third cohort. In contrast, the incidence of ATI remained high and showed no significant trend in the subjects age ≥80. The incidence of CEI showed no significant change in any age group.

Trends in survival of ischemic stroke subtype. Age- and sex-adjusted 5-year survival curves after ischemic stroke by its subtypes are shown in figure 2. The 5-year survival after LI was better than those after other subtypes and improved from the first (54%) to the third cohort (86%; $p < 0.05$). The 5-year survival after ATI tended to improve from the first (17%) to the second cohort (40%; $p = 0.08$) but showed no further improvement in the third cohort (40%). The 5-year survival after CEI was lowest among ischemic stroke subtypes and remained low throughout the study period (16% in the first, 24% in the second, and 26% in the third cohort).

Discussion. To our knowledge, this is the first report to examine secular trends in the incidence and survival rates of ischemic stroke by its subtype. Among three cohorts established at different times in a Japanese community, the incidence of LI declined significantly from the first to the third cohort, especially for men. The incidence of ATI tended to decline from the first to the second cohort, but it was sustained in the third cohort for both sexes. The incidence of CEI was unchanged throughout the study period. As a result, for men, the proportion of individuals with LI decreased from the first to the third cohort, and an opposite trend was observed for ATI and CEI. The 5-year survival rate after LI improved significantly among the cohorts, but those of ATI and CEI did not.

In our three cohorts, blood pressure levels were significantly decreased with time as a result of the

*Table 2 Age-standardized incidence rate (per 100,000 person-years) of ischemic stroke and its subtypes among three cohorts of the Hisayama study by sex, with a 12-year follow-up in each cohort**

	Men						*Women*					
	First cohort, 1961–1973		*Second cohort, 1974–1986*		*Third cohort, 1988–2000*		*First cohort, 1961–1973*		*Second cohort, 1974–1986*		*Third cohort, 1988–2000*	
	n	*Rate*	*n*	*Rate*	*n*	*Rate*	*n*	*Rate*	*n*	*Rate*	*n*	*Rate*
Ischemic stroke	63	801	59	506*	60	357*†	59	450	65	304*	77	260*
Lacunar	44	559	28	229*	24	134*†	34	259	39	186	43	158*
Atherothrombotic	12	165	12	98	19	116	14	105	14	62	18	55*
Cardioembolic	6	67	18	169	17	107	7	57	10	47	16	47
Undetermined	1	10	1	10	0	0	4	29	2	9	0	0

* $p < 0.05$ vs first cohort; † $p < 0.05$ vs second cohort.

Table 3 *Proportion of subjects with subtypes of ischemic stroke among three cohorts of the Hisayama study by sex*

	Men						Women					
	First cohort, 1961–1973		Second cohort, 1974–1986		Third cohort, 1988–2000		First cohort, 1961–1973		Second cohort, 1974–1986		Third cohort, 1988–2000	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Lacunar	44	(69.9)	28	(47.5)	24	(40.0)	34	(57.6)	39	(60.0)	43	(55.8)
Atherothrombotic	12	(19.0)	12	(20.3)	19	(31.7)	14	(23.7)	14	(21.5)	18	(23.4)
Cardioembolic	6	(9.5)	18	(30.5)	17	(28.3)	7	(11.9)	10	(15.4)	16	(20.8)
Undetermined	1	(1.6)	1	(1.7)	0	(0.0)	4	(6.8)	2	(3.1)	0	(0.0)

sevenfold increment in the use of antihypertensive medication, though the prevalence of hypertension remained stable. The prevalence of smoking habits for men was 4.5-fold higher than that for women in the first cohort, and it decreased significantly for both sexes in the third cohort. Contrary to these declining trends of the risk factors, the prevalences of glucose intolerance, hypercholesterolemia, and obesity were greatly increased over the study period for both sexes. These changes in risk factors might have affected trends in the incidence of ischemic stroke subtype.

In our Japanese population, LI was the most common subtype of ischemic stroke, contrary to the previous reports of Western populations.[13,14] An autopsy study comparing small intracerebral arteriosclerosis between Japanese and Japanese American men demonstrated that small intracerebral artery lesions

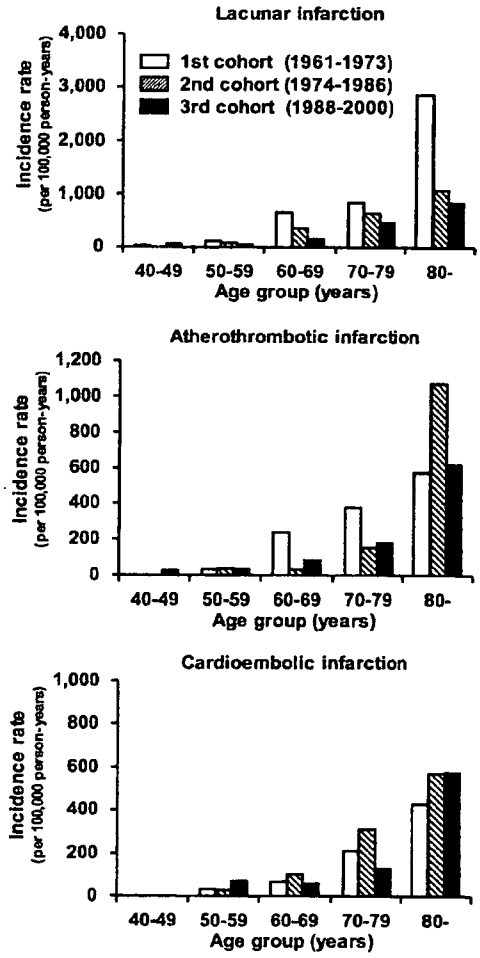

Figure 1. Age-specific incidence of ischemic stroke subtype of men and women combined among three cohorts of the Hisayama study, with a 12-year follow-up in each cohort.

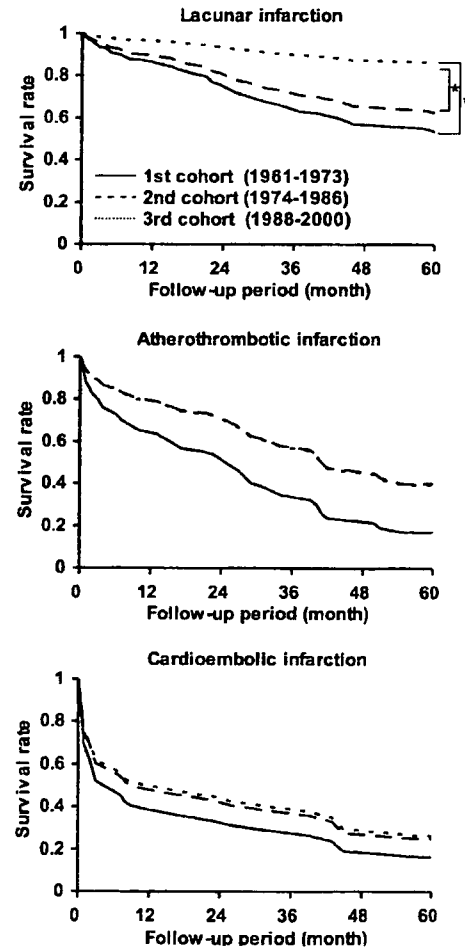

*Figure 2. Age- and sex-adjusted 5-year survival rates after ischemic stroke subtype among three cohorts of the Hisayama study. *p < 0.01.*

were more common in Japanese at every age.[15] Moreover, high blood pressure and a typical Asian diet were significantly associated with small intracerebral artery lesions.[15] The differences in race and lifestyle-related factors might contribute to the difference in the proportion of ischemic stroke subtypes between Japan and Western countries.

During the study period, the incidence of LI declined steeply, especially in men. The improvement of hypertension control and the decreasing prevalence of smoking may have been responsible for this finding. In contrast to the dynamic changes in the incidence of LI, the incidence of ATI has remained stable in recent years. One of the reasons for this finding may have been that the steep increase in metabolic disorders, such as glucose intolerance, dyslipidemia, and obesity, hindered the beneficial effects of the secular improvement of hypertension control and the cessation of smoking. Another possible reason is that hypertension control might be less effective for prevention of ATI. The Systolic Hypertension in the Elderly Program has also shown that the active treatment of hypertension significantly reduced the risk of LI, whereas such treatment appeared to have no effect on the occurrence of ATI.[16]

Despite the marked changes in cardiovascular risk factors among the cohorts, the incidence of CEI showed no significant change in this study. The effect of cardiovascular risk factors on the incidence of CEI was weaker than the effect on other subtypes.[6] In addition, the prevalence of atrial fibrillation, the most common risk factor for CEI, increased from 0.7% in the first cohort to 1.4% in the third cohort. These factors may have contributed to the sustained incidence of CEI. As a result of dynamic changes in risk factors, the proportion of ischemic stroke subtypes in our subjects has become closer to that of Western populations in recent years. However, it is important to note that this trend was caused not by the increase in the incidence of ATI and CEI, but by the steep decrease in the incidence of LI.

Consistent with previous studies,[17-19] we found that the 5-year survival rate was higher for LI, and lower for CEI in each cohort. Moreover, the survival rate improved significantly with time in the subjects with LI, but not in the subjects with ATI or CEI. Stroke is more severe in subjects with ATI and CEI than in those with LI. In addition, the incidence of coronary heart disease, a more common comorbidity in ATI and CEI,[19] is increasing among elderly individuals in Japan.[3] These factors may have contributed to the sustained low survival rate in ATI and CEI.

Our study had several possible limitations. First, the method for diagnosing stroke has been remarkably changed by the improvement of diagnostic techniques, and this may have affected the incidence rate.[20,21] It is possible that the decrease in the LI incidence could be artificial, that is, correspond to inclusion of the same patients into another category, for example, small deep infarction due to cardioembolism. In this study, however, methods for case ascertainment and the criteria for ischemic stroke subtypes were consistent among the cohorts, and the classification of ischemic stroke subtype was confirmed by detailed clinical and morphologic examination, the latter of which was performed in most of the ischemic stroke cases (90 to 100%). These facts make it unlikely that this bias invalidates the findings of the current study. Second, we established three cohorts independently in the same manner, but the subjects in later cohorts included many survivors of the former cohorts. This may have affected the development of stroke; however, we enrolled most of the unselected residents in every cohort, and the prevalence rate of cardiovascular risk factors in the third cohort was similar to that of the National Nutritional Survey of Japan.[3] Third, there were a small number of cases in each cohort, indicating a larger chance of bias in the results of this study. Nonetheless, we believe that the findings of our study represent precise secular trends, as we performed this study using a highly accurate method for determining all cardiovascular events.

Our findings indicate that correction of increasing metabolic disorders such as obesity, dyslipidemia, and glucose intolerance as well as strict management of hypertension have become more important to prevent ischemic stroke in contemporary Japanese individuals.

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NeuroImages

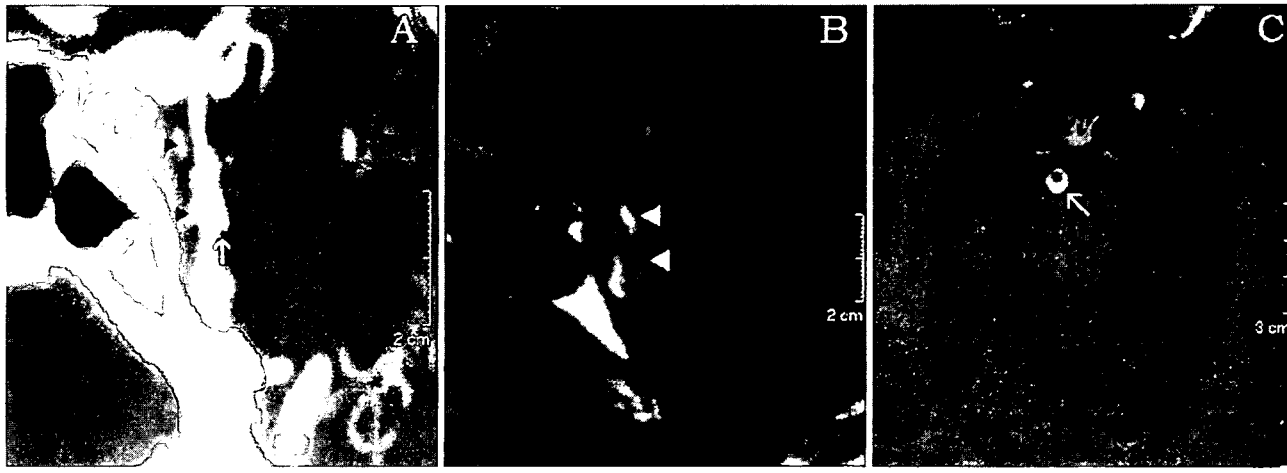

Figure. Sagittal computed tomographic angiography image depicts basilar artery (A) with atheroma (arrowhead) and proximal segment of dissection (arrow). T1-weighted images reveal clot in the atheromata (arrowheads, B) and T1 fat-suppressed image depicts circumferential clot in the vessel wall (arrow, C).

Intraplaque dissection of the basilar artery

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A 61-year-old right-handed man with hypertension, hyperlipidemia, and tobacco abuse presented with sudden dysarthria, left hemiparesis, and hemianesthesia. Examination also revealed left hemiataxia and hemianopsia. MRI revealed multiple acute infarctions in the right posterior cerebral artery territory. Magnetic resonance angiography revealed a narrowed and irregular basilar artery. Computed tomographic angiography demonstrated extensive calcific atherosclerotic changes with an intraluminal filling defect in the mid-basilar artery (figure). Fat-suppressed axial T1-weighted images confirmed intraplaque dissection (figure); T2 images showed low signal consistent with subacute intraplaque clot.

MRI can characterize complicated atheroma and distinguish intraplaque from juxtaluminal thrombosis in the anterior circulation.[1,2] In this case, CT and MRI were complementary for the characterization of the symptomatic lesion and helped guide choice of antithrombotic therapy.

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C-Reactive Protein and Risk of First-Ever Ischemic and Hemorrhagic Stroke in a General Japanese Population

The Hisayama Study

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Background and Purpose—The role of high-sensitivity C-reactive protein (hsCRP) in the development of stroke is not clearly understood. We investigated the relationship between serum hsCRP levels and stroke occurrence in a general Japanese population.

Methods—We followed 2692 subjects ≥40 years of age for 12 years. The relative risks and 95% CIs for ischemic and hemorrhagic stroke occurrence were calculated according to the hsCRP quintiles.

Results—During the follow-up, 129 first-ever ischemic and 59 hemorrhagic strokes occurred. In men, the age-adjusted incidence of ischemic stroke significantly increased with elevated serum hsCRP levels; the difference between the first and fifth quintiles was statistically significant (1.4 versus 6.6 per 1000 person-years; $P=0.02$). This association remained significant even after adjustment for other confounding factors, such as age, systolic blood pressure, ECG abnormalities, diabetes, body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking habits, alcohol intake, and regular exercise (adjusted relative risks, 3.11; 95% CI, 1.04 to 9.32; $P=0.04$). However, such associations were not observed for ischemic stroke in women or in hemorrhagic stroke in either sex. Among male subjects who were both in the fifth hsCRP level and had hypertension, diabetes, obesity, hypercholesterolemia, or a smoking habit, the risk of ischemic stroke was extremely increased, even after adjustment for other risk factors.

Conclusions—Our findings suggest that elevated serum hsCRP levels are an independent risk factor for future ischemic stroke in Japanese men and that the coexistence of a high hsCRP level with another risk factor extremely increases the risk of ischemic stroke. (***Stroke.*** **2006;37:27-32.)**

Key Words: C-reactive protein ■ hemorrhage, brain ■ ischemic stroke

C-reactive protein (CRP), an acute-phase reactant, increases significantly in inflammatory disorders[1] and enhances immune reactivity.[2] Recently, the role of endothelial cells and monocytes in the inflammatory process has become better understood,[3] and inflammation has emerged as an important factor in atherosclerosis. Consequently, high-sensitivity CRP (hsCRP) levels have attracted clinical attention as a predictive marker of atherosclerosis. Several epidemiological studies have reported that hsCRP levels were positively associated with the risk of cardiovascular disease.[4–9] Most of those studies examined coronary heart disease[4–6] or combined end points of coronary heart disease and ischemic stroke,[7–9] whereas only a few studies examined ischemic stroke.[10–12] The subjects of the latter studies were limited to the elderly[10,11] or men,[12] and we found no studies on hemorrhagic stroke.

The purpose of the present study was to examine the relationship between serum hsCRP levels and the development of ischemic and hemorrhagic stroke in a prospective study of a general population consisting of middle-aged and elderly Japanese men and women.

Methods

Study Population

Since 1961, we have been conducting a long-term prospective cohort study of cardiovascular disease in the town of Hisayama, a suburb of Fukuoka City in Southern Japan. In 1988, a screening survey for the present study was performed in the town.[13] A total of 2742 residents ≥40 years of age (80.9% of the total population of this age group) consented to participate in the examination. After excluding 96 subjects with a history of stroke or myocardial infarction and 54

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subjects whose frozen blood samples were insufficient for the measurement of serum hsCRP, the remaining 2592 individuals were enrolled in this study.

Follow-Up Survey

This population was followed up for 12 years, from December 1988 through November 2000, by repeated health examinations or by a daily monitoring system established by the study team and local physicians or members of the Health and Welfare Office for the town. A detailed description of the study methods was published previously.[14,15]

During the follow-up period, 188 subjects were moved out of town, and only 1 subject declined to be followed up. For subjects who did not undergo regular examinations or who moved out of town, their health status was checked by mail or telephone once a year. When new neurological symptoms were suspected, study-team physicians evaluated the subject's detailed diagnostic information. The clinical diagnosis of stroke was based on the detailed history, neurological examinations, and ancillary laboratory examinations.

Stroke Classification

Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for >24 hours and was classified as either ischemic or hemorrhagic (cerebral hemorrhage or subarachnoid hemorrhage). Rare causes of cerebrovascular disease, such as collagen disease, hematologic disorder, trauma, chronic subdural hematoma, or moyamoya disease, were not considered in stroke cases. The diagnosis and classification of stroke were based on clinical information, ancillary laboratory examinations (such as brain imaging including computed tomography and MRI, cerebral angiography, echocardiography, and carotid duplex imaging), and autopsy findings.

During the follow-up period, 188 subjects developed first-ever stroke. During the follow-up, 92 of the 188 first-stroke cases died, and, of these, 71 (77.2%) underwent autopsy examination. The first-stroke cases were classified as 129 ischemic strokes (56 men and 73 women) and 59 hemorrhagic strokes (25 men and 34 women).

Risk Factors

Plasma glucose levels were determined by the glucose-oxidase method, and diabetes mellitus was defined by a 75-g oral glucose tolerance test and by fasting (≥7.0 mmol/L) or postprandial blood glucose level (≥11.1 mmol/L) or by the use of hypoglycemic agents. Total cholesterol and high-density lipoprotein (HDL) cholesterol levels were determined enzymatically. Hypercholesterolemia was defined as a serum cholesterol level of ≥5.69 mmol/L. Serum specimens collected at the time of CRP measurement were stored at −20°C until they were used in 2002. Serum hsCRP levels were analyzed using a modification of the Behring latex-enhanced CRP assay on a Behring nephelometer BN-100 with a 2% interassay coefficient of variation.

Sitting blood pressure was measured 3 times at the right upper arm using a sphygmomanometer after ≥5 minutes of rest; the average of the 3 measurements was used in the analysis. Hypertension was defined as systolic blood pressure of ≥140 mm Hg and diastolic blood pressure of ≥90 mm Hg and current treatment with antihypertensive agents. Height and weight were measured in light clothes without shoes, and the body mass index (BMI, kg/m^2) was calculated. Obesity was defined as a BMI of ≥25 kg/m^2. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code,[16] 3-1) and ST depression (4-1,2,3) and atrial fibrillation (8-3).

Information on smoking habits, alcohol intake, and physical activity during leisure time was obtained with the use of a standard questionnaire. Smoking habits and alcohol intake were classified as either current or not. Those subjects engaging in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

Statistical Analysis

In both men and women combined, we found a significant interaction between sex and hsCRP levels on the risk of ischemic stroke, so the additional analyses were performed separately for men and women by using sex-specific quintiles of hsCRP: Q1, 0.05 to 0.20; Q2, 0.21 to 0.40; Q3, 0.41 to 0.71; Q4, 0.72 to 1.56; and Q5, 1.57 to 14.20 mg/L for men and 0.05 to 0.17, 0.18 to 0.30, 0.31 to 0.53, 0.54 to 1.09, and 1.10 to 13.00 mg/L, respectively, for women. The incidence rates were calculated by the person-year method and adjusted for age by the direct method using 10-year age groupings. The multivariate-adjusted relative risks (RRs) and 95% CIs were calculated according to the hsCRP quintile distribution, using the stepwise Cox proportional hazards model with $P<0.2$ required for entering or remaining in the model. The interaction between 2 risk factors on the risk of stroke was tested by the χ^2 test. A $P<0.05$ was considered to indicate statistical significance.

Results

The baseline characteristics of the subjects are shown in Table 1. The mean age was 58 years for men and 59 years for women. Compared with women, men had higher mean levels of serum hsCRP and systolic and diastolic blood pressures, as well as higher frequencies of hypertension, ECG abnormalities, diabetes mellitus, current smoking, current drinking, and regular exercise, whereas women had higher mean levels of BMI, total cholesterol, and HDL cholesterol.

Figure 1 shows the age-adjusted incidence rates of first-ever ischemic stroke according to quintiles of baseline serum hsCRP. The incidence rates of ischemic stroke were 1.4, 1.9, 5.8, 4.2, and 6.6 per 1000 person-years from the first to fifth quintiles of hsCRP for men and 2.0, 3.4, 5.4, 2.9, and 2.7 per 1000 person-years, respectively, for women. In men, the incidence of stroke rose significantly with rising serum hsCRP levels ($P<0.01$ for trend), and the incidence for subjects in the fifth quintile was ÷5-fold that of subjects in the first quintile ($P=0.02$). However, such an association was not seen in women ($P=0.71$ for trend). On the other hand, the age-adjusted incidence rates of first-ever hemorrhagic stroke were 2.4, 1.1, 2.2, 1.9, and 2.7 per 1000 person-years, respectively, for men, and 1.1, 2.6, 1.0, 1.3, and 1.6 per 1000 person-years, respectively, for women, and there were no significant trends in either sex (Figure 2).