1961 年に久山町の住民検診を受診した 40 歳以上の住民 1,621 名（当該年齢人口の約 90%）のうち，アンジオテンシン変換酵素遺伝子型を判定することのできた 937 名（男性 445 名；女性 492 名）をこの研究の対象とした．

アンジオテンシン変換酵素遺伝子型の判定には，血液標本あるいは剖検時に採取した新鮮凍結標本あるいはパラフィン標本を用いた．血液標本，新鮮凍結標本およびパラフィン標本からの DNA 抽出にはそれぞれ Lahiri らの方法，SDS-Percoll- Chloroform- GuSCN 法，および核酸自動分離装置（クラボー，NA-2000）を用いた．血液標本および新鮮凍結標本より抽出した DNA からの遺伝子型判定には Evans らのポリメラーゼ連鎖反応(PCR)法を用いた．パラフィン標本からの遺伝子型判定には二重 PCR 法を用いた．最終的に血液標本 77 検体および剖検標本 860 検体からアンジオテンシン変換酵素遺伝子型を判定することができた．

この集団を，1961 年 11 月から 1993 年 10 月までの 32 年間追跡した．われわれ研究チーム，地域開業医，久山町健康福祉課の間に設定した追跡システムを通じて，死亡者の情報を収集した．腫瘍死は，検診・臨床記録と剖検所見に基づいて診断した．

統計解析には Cox 比例ハザードモデルを用いた．

（倫理面の配慮）

本研究は「疫学研究に関する倫理指針」および「ヒトゲノム・遺伝子解析研究に関する倫理指針」に準拠し，九州大学医学部倫理委員会の承認の元で行われた．本研究は，健診受診者を対象とした疫学調査で，対象者が研究によって不利益を被ることはない．研究者は，対象者の個人情報の漏洩を防ぐうえで細心の注意を払い，その管理に責任を負っている．

C．研究結果

対象者の追跡開始前の平均年齢は 58 歳であった．アンジオテンシン変換酵素遺伝子型は，II 型 38%，ID 型 49%，DD 型 13%であり Hardy-Weinberg の法則に従っていた．

32 年間の追跡期間中に 176 名が悪性腫瘍のため死亡した．喫煙レベルと腫瘍死の関連を検討したところ，悪性腫瘍による死亡率は喫煙レベルの増加とともに上昇した（p trend<0.0001）．喫煙の他には年齢，性が腫瘍死の有意な危険因子であった．一方，アンジオテンシン変換酵素遺伝子多型と腫瘍死の間には有意な関連を認めなかった．

多変量解析を行ったところ，年齢，喫煙，body mass index に加えて，喫煙とアンジオテンシン変換酵素遺伝子多型の間の交互作用（ハザード比 3.31）が有意な危険因子となった．つまり，喫煙の悪性腫瘍に対する影響はアンジオテンシン変換酵素遺伝子型により異なることが示唆された．

喫煙の腫瘍死に対する影響をアンジオテンシン変換酵素遺伝子型別に検討したところ，遺伝子型に関わらず喫煙は腫瘍死の有意な危険因子であった．しかし，DD 型における喫煙の影響（ハザード比 4.51）は，II 型（ハザード比 2.00）や ID 型（ハザード比 2.24）における関連よりも約 2 倍大きかった．

D．考　察

福岡県久山町の地域一般住民を対象にした前向き追跡研究の成績から，喫煙の腫瘍死に対する影響は，アンジオテンシン変換酵素遺伝子多型により異なることが示唆された．

動物実験により，アンジオテンシン変換酵素は血管新生などを介して悪性腫瘍化のProgression の段階に関与する可能性が示唆されてきた．また，アンジオテンシン変換酵素遺伝子 DD 型では血液および組織のアンジオテンシン変換酵素活性が上昇していることが知られている．したがって，アンジオテンシン変換酵素遺伝子 DD 型では悪性腫瘍化の Progression の段階が促進される可能性がある．

悪性腫瘍化には，Initiation，Promotion，Progression の 3 段階が存在すると考えられている．喫煙はおもに初期の Initiation と Promotion の段階，アンジオテンシン変換酵素遺伝子 DD 型は後半の Progression の段階に関与すると推測されているので，両者の併存により腫瘍死の危険が高まったものと推測された．

アンジオテンシン変換酵素阻害薬と悪性腫瘍の関連を検討した報告がいくつかある．Lever らは，アンジオテンシン変換酵素阻害薬が悪性腫瘍の発生および腫瘍死を減少させたと報告した．一方，他の研究ではそのような関連を認めなかった．アンジオテンシン変換酵素遺伝子多型と腫瘍の関連を検討した患者対照研究においても一定の見解が得られていない．本研究は喫煙とアンジオテンシン変換酵素遺伝子多型との間に交互作用があることが示した．過去の研究における見解の違いには，対象者の喫煙頻度の差が関与している可能性がある．

本研究の最大の問題点は，対象者の数が少ないことである．今後この問題についてさらなる大規模研究が必要である．もう一つの問題点は選択バイアスである．本研究の対象者はアンジオテンシン変換酵素遺伝子型を判定できたものだけに限定されている．しかし，危険因子および悪性腫瘍による死亡率が遺伝子型を判定できた対象者とできなかった対象者の間でほぼ同等だったので，結論には影響しないと考えられる．

E．結　論

喫煙の腫瘍死に対する影響は，アンジオテンシン変換酵素遺伝子多型により異なっていた．ハイリスク者を早期発見し，禁煙をはたらきかけることで，腫瘍死を効率的に予防することが可能かもしれない．

F．健康危険情報

アンジオテンシン変換酵素遺伝子 DD 型は，喫煙の腫瘍死に対する影響を増強する可能性がある．喫煙者には禁煙を働きかけてゆく必要があるが，アンジオテンシン変換酵素遺伝子 DD 型を有する対象者には特に積極的に禁煙を働きかける必要がある．

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3．特許取得　　なし

4．実用新案登録　なし

5．その他

厚生労働科学研究費補助金（健康科学研究事業）
分担研究報告書

動脈硬化の発生・進展と脈管新生に関する病理学的研究

分担研究者　居石克夫　九州大学大学院医学研究院・教授

研究要旨　我々はこれまでに、ヒト冠状動脈硬化内膜における VEGF-A を基盤とした血管新生が動脈硬化の進展に寄与していることを示唆してきた。本研究では、剖検症例から得たヒト冠状動脈を用いて、動脈硬化型と内膜内脈管新生（血管新生とリンパ管新生）との関係、リンパ管新生因子として知られる VEGF-C、VEGF-D の発現を免疫組織化学的に検討し、硬化内膜にはリンパ管形成は極めて希であること、主にマクロファージにより発現された VEGF-C は内膜内血管新生に関与している可能性があることを明らかにした。以上より、VEGF-C は血管新生を軸にした炎症・修復過程を促進して動脈硬化の進展に寄与していることが示唆された。

A．研究目的

粥状硬化症は慢性炎症性疾患であるという仮説が提唱されている。血管新生は炎症巣で生じる現象であり、我々はこれまでに、ヒト冠状動脈硬化内膜における新生血管数は炎症細胞浸潤の程度と動脈硬化の進行度と相関し、血管内皮細胞増殖因子 VEGF-A(vascular endothelial growth factor-A)の発現細胞数は動脈硬化の進展と相関することを示した。また、動脈中膜へ VEGF-A を遺伝子導入することにより、血管を伴う内膜肥厚が促進することを示し、動脈硬化の進展に VEGF-A が関与していることを示唆してきた。本研究では、剖検症例から得たヒト冠状動脈を、米国心臓協会(AHA)分類による冠状動脈硬化型と内膜内脈管新生（血管新生とリンパ管新生）との関係、リンパ管新生因子として知られる VEGF-C、VEGF-D 発現との関係を免疫組織化学的に検討し、脈管新生とリンパ管新生因子の発現が動脈硬化の進展に寄与するかを検討した。

B．研究方法

九州大学病院の剖検症例で、23 人の日本人患者（男性 14 人、女性 9 人）における心臓を採取し、冠状動脈を 4%パラホルムアルデヒドにて潅流固定し、右冠状動脈と左冠状動脈前下行枝を 3mm 間隔に割断し、パラフィンに包埋した。採取した 169 ブロックを連続切片に薄切し、AHA 分類に従って動脈硬化型を判定した。免疫組織化学法は、ヒト VEGF-C、ヒト VEGF-D、ヒト CD68、ヒトαSMA、ヒト CD34、ヒトリンパ管内皮細胞ヒアルロン酸受容体-1 (LYVE-1)、ヒト podoplanin に対する特異抗体を使用した。

C．研究結果

VEGF-C 陽性細胞は、早期病変より進行病変により多く観察された($p<0.0001$、表 1A)。早期粥状硬化病変(I 型、II 型病変)において、VEGF-C 陽性細胞は主に内膜の泡沫化マクロファージと内膜と中膜のいくつかの紡錘形平滑

筋細胞に認められた(図 1a)。III 型から IV 型病変まで、VEGF-C の免疫反応は内膜の数多くの泡沫化マクロファージの集簇といくつかの紡錘形平滑筋細胞に認められた(図 2a)。VEGF-D は、内膜と中膜の多くの平滑筋細胞と泡沫化したマクロファージに豊富に発現を認め(図 1, 2b)、VEGF-D 陽性細胞数と動脈硬化の進行度との間に有意な相関を認めなかった($p=0.3507$、表 1B)。

血管新生に関しては、動脈硬化病変が進行するに従って新生内膜内に多くの CD34 陽性の新生血管が認められた($p<0.0001$、表 2A)。リンパ管新生に関しては、動脈硬化内膜において、LYVE-1 陽性リンパ管数は 13 しか認められなかった(表 2A、図 3)。同様の結果が、podoplanin 抗体を使っても認められた。定量的には、外膜と比べて、内膜におけるリンパ管数は非常に少ないことが分かった($p<0.0001$、表 2B)。

粥状硬化内膜において、VEGF-C 陽性細胞の出現程度は、内膜内の新生血管数と正の相関を認めた($p<0.0001$、図 4a)。VEGF-D の陽性細胞と新生血管数との間に相関は認めなかった($p=0.0789$、図 4b)。粥状硬化内膜において、VEGF-C 陽性細胞の出現程度は、内膜内の LYVE-1 陽性リンパ管数と正の相関を認めた($p=0.0322$、図 5a)。VEGF-D 陽性細胞数とリンパ管数との相関は認めなかった($p=0.1439$、図 5b)。

D. 考察

VEGF-C と VEGF-D は、VEGF-A と同様に、動脈硬化動物モデルの血管新生を促進することが知られている。VEGF-C の発現は、VEGF-A とは異なり、低酸素では制御されず、炎症性サイトカインに反応して増加する。この研究において、我々はヒト冠状動脈粥状硬化において、マクロファージが主な VEGF-C を発現細胞であることを実証した。このことは、最近の報告で、VEGF-C がヒトの腫瘍関連マクロファージに発現していること、単球は TNFαやリポポリサッカライドの刺激で VEGF-C を発現する報告からも支持される。これらのことから、VEGF-C は冠状動脈粥状硬化巣において炎症性サイトカインの刺激により単球/マクロファージからの発現が亢進し、血管新生に作用する可能性が考えられる。VEGF-D の発現が、動脈硬化病変や脈管新生と相関がない理由は不明である。冠状動脈硬化巣において、VEGF-D は、活性型になるための蛋白分解酵素が十分でない可能性が考えられる。また、これまでにヒトの冠状動脈粥状硬化巣におけるリンパ管新生に関する報告は認められない。過去の報告では、ヒトの潰瘍病変や褥創のような慢性創傷において、リンパ管が欠乏していることは創傷治癒を遅延する可能性が示唆されている。冠状動脈粥状硬化巣におけるリンパ管の相対的な減少は、炎症反応の治癒過程を遷延し、組織圧の増加により、動脈硬化の進展を促進する可能性が考えられる。

E. 結論

ヒト冠状動脈粥状硬化において、VEGF-C の発現の程度は動脈硬化の進展と相関し、また内膜内の新生脈管数と相関することを証明した。また、冠状動脈の硬化内膜はリンパ管新

生に不適切な環境である可能性があり、VEGF-Cはリンパ管新生より血管新生を内因的に促進し、冠状動脈硬化の進展に関与している可能性が示唆された。

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G. 知的財産権の出願・登録状況（予定を含む。） 特になし。

厚生労働科学研究費補助金・がん予防等健康科学研究事業
（総括・分担）研究報告書

ヒト悪性腫瘍の細胞学的特性および悪性度と遺伝子異常に関係する検討

（分担）研究者　恒吉　正澄　　九州大学大学院医学研究院形態機能病理学教授

研究要旨

消化管と骨軟部組織に発生するさまざまな腫瘍において種々の細胞増殖関連因子や遺伝子異常、薬剤耐性関連遺伝子について調べ、それらの因子と腫瘍の発生から発育進展様式や悪性度との関連を解析した。

さまざまな悪性腫瘍において遺伝子異常と腫瘍の発育進展との関連が見いだされ、細胞分化（発現形質）や遺伝子異常が腫瘍の生物学的態度の評価や組織発生の解明に有用であると考えられた。また、薬剤耐性関連遺伝子の解析結果は新しい化学療法への応用が期待される。

A. 研究目的

消化管と骨軟部組織に発生するさまざまな腫瘍において種々の細胞増殖関連因子や遺伝子異常、薬剤耐性関連遺伝子を調べ、それらの因子と腫瘍の発生から発育進展様式および臨床病理学的事項との関連について解析し、腫瘍の組織発生の解明や悪性度評価と治療への応用を目的とする。

B. 研究方法

消化管の腫瘍は、胃癌（若年者胃癌、Hepatoid 胃癌）と肝転移を来した大腸癌を用い、軟部腫瘍は平滑筋肉腫や滑膜肉腫、横紋筋肉腫、悪性末梢神経鞘

軟部腫瘍では、遺伝子異常（p53、p14、RB1、PTEN 、CHFR）や薬剤耐性関連遺伝子の腫瘍の DNA 抽出を行いメチレーションと遺伝子変異をオートシークエンサーにより検出した。CHFR は免疫染色に加え、染色体分析を行った。

C. 研究結果

1．若年者胃癌の臨床病理学的特徴と H. pylori 感染胃炎の関連。

腫瘍などを用いた。消化管腫瘍では、gastric mucin（胃腺窩上皮のマーカー）、MUC2（腸杯細胞のマーカー）、CD10（小腸刷子縁のマーカー）による免疫染色を行い、胃型・腸型の形質発現と腫瘍の発育進展様式や悪性度、細胞増殖との関連や、Helicobacter pylori（H. pylori）感染胃炎の発癌における意義を調べた。肝　p53、p14、RB1、PTEN の解析はそれぞれに対する抗体を用いた免疫染色に加え、腫瘍からの発育における意義や悪性度との関連を解析した。転移をきたした大腸癌では、上記の形質発現に加え、転移関連因子（血管新生因子や接着分子）の発現を調べた。

30 歳以下の若年者に発生した胃癌（粘膜内癌）は、ほとんどが胃型形質の低分化型腺癌であり、癌の背景粘膜には萎縮と腸上皮化生は軽度であるが、炎症の程度は強く、H. pylori 感染も高率であった。このことより若年者胃癌の発癌では H. pylori 感染が発癌において重要な意義があり、慢性萎縮性胃炎-腸上皮化生-癌化という経路を経ない発癌機序が想定された。

2．　Hepatoid 胃癌。

Hepatoid 胃癌では高率に分化型腺癌成分を伴い、形質的には分化型成分も Hepatoid 成分も腸型を示し、p53 蛋白発現のパターンも一致した。この結果から Hepatoid 胃癌は腸型形質の分化型腺癌として発生した後 Hepatoid 胃癌へ進展していくことが示唆された。

3．　大腸癌の転移予測因子の解析。

肝転移を来した大腸癌では、CD10 発現に加え、血管新生関連因子である VEGF や TGF-α、細胞接着因子 CD44v6 の発現が高率であった。多変量解析ではリンパ節転移陽性、CD10 発現、VEGF 発現が肝転移危険因子であり、それらの組み合わせが肝転移危険性予測に応用できることが示唆された。

4．　軟部腫瘍における細胞増殖関連因子と遺伝子異常の解析。

脂肪肉腫の亜型である粘液型/円形細胞型では、円形細胞成分の出現が予後不良因子でありその悪性化と進展には p14/p53 経路の異常が関与していることが示唆された。また、RB1 遺伝子の変異、LOH、メチル化などなんらかの異常が脂肪肉腫の脱分化に関与していることも示唆された。

悪性末梢神経鞘腫瘍では、CHFR の発現減弱は増殖活性亢進に関連があり、予後不良因子であった。また、染色体分析で正常な核型であったものは CHFR が強発現を示し、CHFR が腫瘍増殖抑制因子であることを示唆する結果であった。

種々の軟部肉腫において PTEN 遺伝子異常や蛋白消失のあるものは低頻度であったが、その異常のあるものは高い増殖活性を有していた。

5．　軟部腫瘍における薬剤耐性関連遺伝子の解析。

種々の軟部肉腫における薬剤耐性関連遺伝子である ABC transporter mRNA の発現を検索したところ、悪性末梢神経腫瘍において MDR1 および MRP3 発現レベルが他の腫瘍に比較して有為に高値であり、この腫瘍の化学療法抵抗性に関与している可能性が示唆された。

また、多剤耐性を示す骨肉腫および Ewing 肉腫培養細胞株に P 糖蛋白の阻害剤である verapamil や MRP1 蛋白阻害剤である MK571 を投与することにより HDAC inhibitor の抗腫瘍効果が増強された。

D．考察

胃癌の解析では、若年者胃癌の発生機序の特殊性、とくに H. pylori 感染が直接癌化に関与していることが分かり、H. pylori 除菌の重要性が発癌予防に重要であることが再認識された。また、肝癌様分化をしめす特殊な胃癌の亜型である Hepatoid 胃癌では、その組織発生が腸型形質の分化型癌と関連があることが判明したことで、その高悪性化の機序の解明の手がかりとなると思われる。

肝転移の危険因子による転移危険性予測に有用な因子は判明したが、CD10 の肝転移における作用機序は未だ不明である。

軟部腫瘍の解析では、腫瘍の発生から発育進展において種々の細胞増殖関連因子遺伝子異常が関与し、それらと悪性度との関連がわかってきた。そして、それらを規定するさまざまな因子の相互関係も少しずつ解明されてきた。今後は、各腫瘍に特異的な異常と共通する異常をもっと明らかにして行く必要がある。

また、軟部腫瘍における薬剤耐性関連遺伝子の解析は将来の新しい化学療法へ応用が期待される。

E．結論

さまざまな悪性腫瘍において遺伝子異常と腫瘍の発育進展との関連が見いだされ、細胞分化（発現形質）や遺伝子異常の解析が腫瘍の悪性度評価や組織発生の解明に有用であると考えられた。また、薬剤耐性関連遺伝子の解析結果は新しい化学療法への応

用が期待される。

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

The Hisayama Study

Yoshiyuki Wakugawa, MD; Yutaka Kiyohara, MD, PhD; Yumihiro Tanizaki, MD, PhD; Michiaki Kubo, MD, PhD; Toshiharu Ninomiya, MD, PhD; Jun Hata, MD; Yasufumi Doi, MD, PhD; Ken Okubo, MD; Yoshinori Oishi, MD; Kentaro Shikata, MD; Koji Yonemoto, PhD; Daisuke Maebuchi, MD; Setsuro Ibayashi, MD, PhD; Mitsuo Iida, MD, PhD

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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From the Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Correspondence to Yoshiyuki Wakugawa, MD, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka City 812-8582, Japan. E-mail wakuwaku@intmed2.med.kyushu-u.ac.jp

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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).

TABLE 1. Baseline Characteristics of Study Subjects, the Hisayama Study, 1988

Characteristic	Men (n=1092)	Women (n=1500)
Age, y	58.1±11.4	59.4±11.9
High-sensitivity C-reactive protein, mg/L		
Median	0.54	0.40
Mean	2.07±8.31	1.30±5.45
Systolic blood pressure, mm Hg	134.7±20.1	132.9±22.2
Diastolic blood pressure, mm Hg	80.5±11.4	75.8±10.8
Hypertension, %	45.2%	38.5%
Use of antihypertensive agents, %	14.2%	15.4%
ECG abnormalities, %	20.7%	14.7%
Diabetes mellitus, %	15.1%	9.6%
BMI, kg/m^2	22.8±2.9	22.9±3.3
Total cholesterol, mmol/L	5.09±1.07	5.54±1.07
HDL-cholesterol, mmol/L	1.25±0.31	1.33±0.30
Current smoking, %	49.8%	6.7%
Current drinking, %	60.6%	9.0%
Regular exercise, %	11.8%	9.1%

Data are mean±1 SD or percent, unless otherwise specified.

Table 2 shows the multivariate-adjusted RRs and their 95% CIs for the development of ischemic and hemorrhagic stroke according to hsCRP quintile categories. In men, the risk of ischemic stroke significantly increased with rising hsCRP levels even after adjustment for age, systolic blood pressure, ECG abnormalities, diabetes, BMI, total cholesterol, HDL cholesterol, smoking habits, alcohol intake, and physical activity (P=0.02 for trend), and the multivariate-adjusted RR of subjects in the fifth quintile was significantly higher than that of subjects in the first quintile (RR, 3.11; 95%CI, 1.04 to 9.32; P=0.04). However, such associations were not observed for ischemic stroke in women or for hemorrhagic stroke in either sex (Table 2). To examine the combined effects of elevated hsCRP levels and other cardiovascular risk factors on ischemic stroke occurrence, we estimated the age-adjusted RRs of ischemic stroke among 4 groups of male subjects according to the presence or absence of a high-hsCRP level (the fifth quintile, ≥1.57 mg/L) and each risk factor (Table 3). Compared with the reference group having neither high-hsCRP levels nor hypertension, the risk of ischemic stroke for the groups with either high-hsCRP levels or hypertension was not significant, but the risk for the group having both high-hsCRP levels and hypertension was significantly higher (RR, 2.77; 95% CI, 1.31 to 5.83; P<0.01). A similar pattern was observed for the coexistence of high-hsCRP levels and diabetes (RR, 4.30; 95% CI, 1.89 to 9.79; P<0.01), obesity (RR, 4.00; 95% CI, 1.53 to 10.46; P< 0.01), hypercholesterolemia (RR, 3.74; 95% CI, 1.71 to 8.19; P<0.01), or smoking habits (RR, 2.29; 95% CI, 1.78 to 4.87; P=0.03). There were significant interactions between high-hsCRP levels and diabetes (χ^2=5.370; P=0.02), as well as hypercholesterolemia (χ^2=6.052; P=0.01), and a marginally significant interaction (χ^2=3.39; P=0.06) between high-hsCRP levels and hypertension. However, interactions for obesity and smoking were not significant. These associations were substantially unchanged even after adjustment for other risk factors in the multivariate analysis.

Discussion

In a 12-year follow-up examination of a general Japanese population, we demonstrated that elevation of serum hsCRP levels was an independent risk factor for future ischemic stroke in men but not in women, whereas there was no association between serum hsCRP levels and the risk of future hemorrhagic stroke in either sex. Moreover, the coexistence of a high-hsCRP level and another risk factor, such as hypertension, obesity, diabetes, hypercholesterolemia, or smoking, extremely increased the risk of future ischemic stroke in our male subjects.

Recently, the Framingham Study[10] and Cardiovascular Health Study,[11] both which had elderly subjects (mean age,

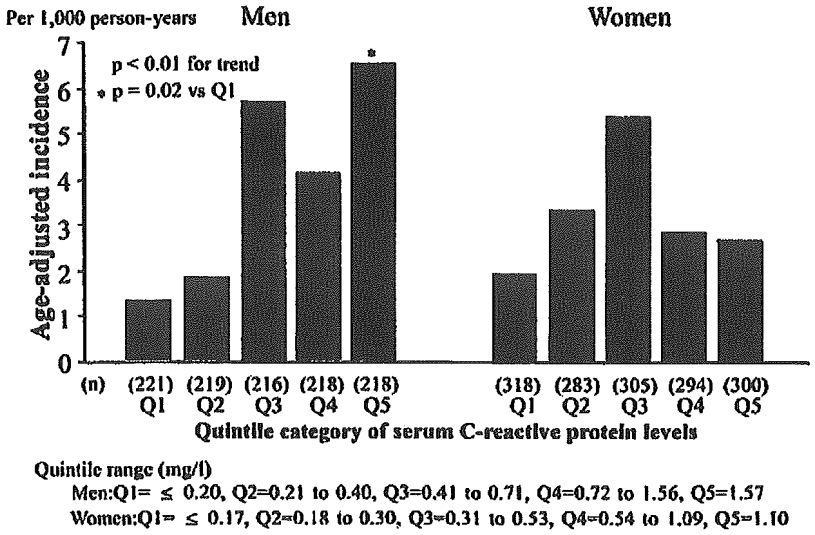

Figure 1. Age-adjusted incidence rates of first-ever ischemic stroke according to serum high-sensitivity C-reactive protein levels.

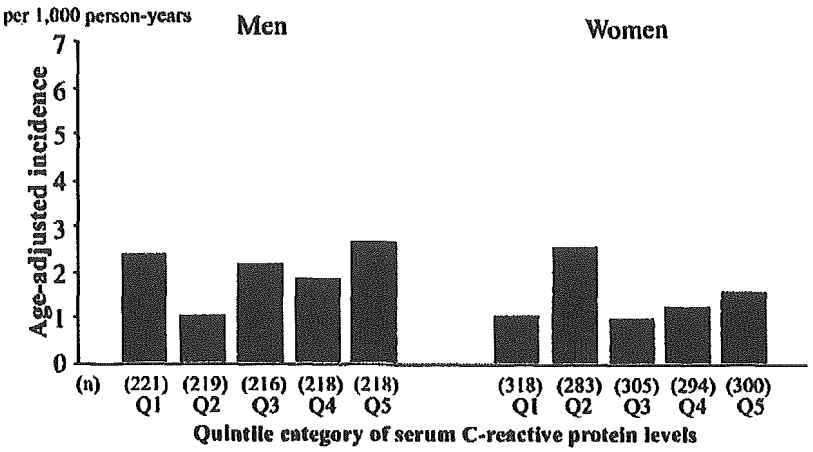

Figure 2. Age-adjusted incidence rates of first-ever hemorrhagic stroke according to serum high-sensitivity C-reactive protein levels.

Quintile range (mg/l)
Men:Q1= ≤ 0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=1.57
Women:Q1= ≤ 0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=1.10

69.8 and 72.6 years, respectively), and a nested case-control study of Japanese-American men[12] in Hawaii have investigated the association between hsCRP level and the risk of future ischemic stroke. In those studies, the elevation of serum hsCRP was clearly associated with ischemic stroke in men, which support our findings. For women, on the other hand, the effects of high levels of serum hsCRP on ischemic stroke were ambiguous. In the Framingham study women, hsCRP levels were significantly associated with the risk of ischemic stroke,[10] whereas no significant association was observed for the women in the Cardiovascular Health Study,[11] which was in accord with the findings of our study. Recent clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis[17,18] and that estrogen induces the elevation of hsCRP levels.[19] In women, such conflicting effects of sex hormone might weaken the association of hsCRP elevation with ischemic stroke. Another reason for the sex difference in the risk of ischemic stroke might stem from the difference in the atherosclerotic process between men and women. Generally, it is considered that atherosclerosis is more severe in men than in women. Thus, it may be easier to detect the association between hsCRP levels and ischemic stroke in men.

TABLE 2. Multivariate-Adjusted RRs of First-Ever Ischemic and Hemorrhagic Stroke according to Serum High-Sensitivity C-Reactive Protein Levels

Quintiles of Men/Women	RR	95% CI	*P* Value	RR	95% CI	*P* Value
Ischemic stroke						
Q1	1.00	1.00				
Q2	1.08	0.29 to 4.03	0.91	1.27	0.55 to 2.94	0.58
Q3	2.81	0.93 to 8.51	0.07	1.56	0.71 to 3.39	0.27
Q4	2.24	0.73 to 6.92	0.16	1.05	0.46 to 2.42	0.90
Q5	3.11	1.04 to 9.32	0.04	1.34	0.61 to 2.91	0.46
P for trend	0.02	0.65				
Hemorrhagic stroke						
Q1	1.00			1.00		
Q2	0.33	0.07 to 1.65	0.18	2.66	0.82 to 8.61	0.10
Q3	0.58	0.17 to 1.91	0.37	1.00	0.24 to 4.06	0.99
Q4	0.78	0.26 to 2.37	0.67	2.10	0.63 to 7.04	0.23
Q5	0.68	0.21 to 2.26	0.53	1.74	0.51 to 5.85	0.37
P for trend	0.92	0.64				

Men, mg/L: Q1=≤0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=≥1.57. Women, mg/L: Q1=≤0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=≥1.10. Multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, BMI, total cholesterol, HDL cholesterol, smoking habits, alcohol intake, and physical activity.

In our subjects, we did not find a clear association between hsCRP levels and hemorrhagic stroke occurrence. Because cerebral hemorrhage develops from the rupture of small vessels, such as cerebral perforating arteries, damaged by hypertension causing lipohyalinosis,[20] or by amyloid angiopathy,[21] it is suggested that elevated hsCRP levels have little or no association with small vessel disease. Although hypertension and smoking may accelerate the development and growth of intracranial aneurysm,[22] which is a main cause of subarachnoid hemorrhage, the association between atherosclerosis and intracranial aneurysm is considered weak.[23] Thus, our finding that there is no association between serum hsCRP levels and hemorrhagic stroke is reasonable.

Our stratified analysis showed an extremely increased risk of ischemic stroke in men who have both a high-hsCRP level and another risk factor. Although the mechanism underlying this phenomenon is not clearly understood, several possible explanations have been proposed. Because inflammation is strongly related to atherosclerosis, elevated hsCRP levels may reflect the existence of advanced atherosclerosis induced by other cardiovascular risk factors. Accordingly, it is conceivable that the coexistence of elevated hsCRP levels and other risk factors is a marker of a group at high risk of atherosclerosis, and, thus, the risk of ischemic stroke is considerably high in that group. Additionally, recent clinical

TABLE 3. Age-Adjusted RRs of First-Ever Ischemic Stroke according to High-Sensitivity C-Reactive Protein Levels and Risk Factors in Men

Risk Factor	CRP Levels	Events/Populations (n)	RR	95% CI	*P* Value
Hypertension					
No	Low	16/472	1.00		
Yes	Low	22/363	1.34	0.69 to 2.56	0.39
No	High	5/105	1.27	0.46 to 3.47	0.65
Yes	High	13/96	2.77	1.31 to 5.83	<0.01
Diabetes mellitus					
No	Low	30/719	1.00		
Yes	Low	8/116	1.65	0.75 to 3.59	0.21
No	High	11/167	1.42	0.71 to 2.84	0.32
Yes	High	7/34	4.30	1.89 to 9.79	<0.01
Obesity					
No	Low	47/635	1.00		
Yes	Low	11/200	1.91	0.93 to 3.93	0.08
No	High	13/162	1.69	0.87 to 3.29	0.12
Yes	High	5/39	4.00	1.53 to 10.46	<0.01
Hypercholesterolemia					
No	Low	31/617	1.00		
Yes	Low	7/218	0.77	0.34 to 1.75	0.54
No	High	10/145	1.15	0.56 to 2.35	0.71
Yes	High	5/56	3.74	1.71 to 8.19	<0.01
Current smoking					
No	Low	21/432	1.00		
Yes	Low	17/403	1.11	0.59 to 2.12	0.74
No	High	8/87	1.48	0.65 to 3.36	0.35
Yes	High	10/114	2.29	1.78 to 4.87	0.03

CRP levels: "high" indicates the fifth quintile; low, the first to fourth quintiles. Hypertension: systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive agents. Diabetes: fasting blood glucose ≥7.0 mmol/L, or postprandial blood glucose level ≥11.1 mmol/L, or current use of hypoglycemic agents. Obesity: BMI ≥25 kg/m^2. Hypercholesterolemia: total cholesterol level ≥5.69 mmol/L.

reviews, as well as experimental and clinical studies, have shown that inflammation is directly associated with the development of atherosclerosis[24] and instability of atheroma.[25,26] It is, therefore, speculated that chronic inflammation directly and extremely enhances the risk of ischemic stroke by such atherogenic effects of inflammation in people whose arterial walls have already been damaged by other risk factors.

Several limitations of our study should be discussed. The primary limitation is that our findings are based on a 1-time measurement of serum hsCRP, which may not accurately reflect the status of the study participants. However, this source of variability could not account for the relationship observed in the present study, because a random misclassification of such nature would tend to underestimate study findings and bias the results toward the null hypothesis. Thus, the true association may be stronger than that observed in our study. A second limitation is that the serum samples were measured after being stored at −20°C for a long period. However, the Reykjavik Study confirmed the stability of CRP concentrations in serum preserved at this temperature for an average of 12 years.[27] The last limitation is that our study lacked information on drug use, which could affect serum CRP levels. It is known that several medications, including statin, angiotensin-converting enzyme inhibitors, fibrates, niacin, thiazolidinedione, and estrogen/progestogen hormone can alter CRP levels.[28] However, these medications were rarely used in our country in 1988, when the serum samples for our study were collected. This suggests that such a bias did not invalidate the present findings.

In conclusion, our study found that, in a general Japanese population, the elevation of serum hsCRP levels was an independent risk factor for future ischemic stroke in men but not for hemorrhagic stroke in either sex. The addition of elevated serum hsCRP levels to the risk factor profile may significantly increase the predictability of ischemic stroke. Moreover, our study revealed that the risk of future ischemic stroke was considerably high in subjects who had both high-hsCRP levels and another risk factor. For such individuals, an elevated serum hsCRP level may provide additional

motivation for both the treating physician and the patient to control these risk factors strictly.

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Angiotensin I-converting enzyme gene polymorphism modifies the smoking–cancer association: the Hisayama Study

Hisatomi Arima[a], Yutaka Kiyohara[a], Yumihiro Tanizaki[a], Yusaku Nakabeppu[b], Michiaki Kubo[a], Isao Kato[a], Katsuo Sueishi[c], Masazumi Tsuneyoshi[d], Masatoshi Fujishima[a] and Mitsuo Iida[a]

We examined the long-term contribution of smoking and angiotensin I-converting enzyme (ACE) gene I/D polymorphism to total cancer deaths in a prospective study of a general Japanese population. A total of 937 subjects aged 40 years or older were selected from an original cohort of 1621 subjects and were followed up for 32 years. During the follow-up period, 176 subjects died of cancer. Cancer mortality increased significantly with increasing current smoking levels. Although no clear relationship was observed between ACE genotypes and fatal cancer, the interaction term between current smoking and ACE genotype DD was found to be significant. In stratified analysis by ACE genotype after controlling for age, sex, alcohol intake, body mass index, glucose intolerance, serum total cholesterol and systolic blood pressure, the risk of fatal cancer in currently smoking subjects with genotype DD was twofold greater than that in subjects with genotypes II and ID. Among current smokers, subjects with genotype DD also showed a significantly greater risk of death due to cancer compared with those with genotypes II and ID combined (hazard ratio (HR) 1.77; 95% confidence interval (CI) 1.04–3.00; $P=0.03$). In conclusion, our findings suggest that ACE genotype DD enhances the association between smoking and cancer death in the general population. *European Journal of Cancer Prevention* **15:000–000 © 2006 Lippincott Williams & Wilkins.**

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Keywords: angiotensin I-converting enzyme, cancer, cohort study, polymorphism, smoking

[a]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, [b]Division of Neurofunctional Genomics, Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, Kyushu University and CREST, JST, [c]Pathophysiological and Experimental Pathology, Department of Pathology, Graduate School of Medical Sciences, Kyushu University and [d]Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Correspondence to Hisatomi Arima, The George Institute for International Health, The University of Sydney, PO Box M201, Missenden Road, NSW 2050, Australia
Tel: +61-2-9993-4500; fax: +61-2-9993-4501;
e-mail: harima@thegeorgeinstitute.org

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Introduction

Cancer kills about 7 million people each year, making malignant neoplasm one of the leading causes of death worldwide (World Health Organization, 2002). Malignant respiratory neoplasm is the leading cause of cancer death, representing one-sixth of cancer mortalities, and approximately 80% of the respiratory malignant neoplasm burden is attributable to smoking (World Health Organization, 2002). Tobacco also causes malignant neoplasms in other sites, such as the oral cavity, pharynx, larynx, oesophagus, stomach, bile ducts, liver, pancreas, bladder, etc. (World Health Organization 2002; Doll *et al.*, 2004; Jee *et al.*, 2004).

The products of tobacco combustion cause DNA base modifications and adducts, leading to an accumulation of mutational changes in oncogenes and in tumour suppressor genes (Denissenko *et al.*, 1996; Pryor, 1997) and to the worsening of the biological properties of tumours (Suzuki *et al.*, 1992). Thus, it is likely that smoking is closely involved in the initiation and promotion steps of carcinogenesis. On the other hand, an enhanced renin–angiotensin system has been suggested to be involved in neoplastic cell proliferation (Muscella *et al.*, 2002), angiogenesis and metastasis (Fujita *et al.*, 2002). It is well known that angiotensin I-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism influences circulating ACE levels, and that the DD genotype induces the highest ACE levels in blood (Rigat *et al.*, 1990). Thus, the ACE genotype DD may modify and enhance the association between smoking habits and malignant neoplasm.

In order to clarify this issue, we examined the long-term prospective contribution of smoking and ACE gene I/D polymorphism to fatal cancer in a cohort study of a general Japanese population.

Subjects and methods

Study design and participants

The Hisayama Study is an ongoing population-based epidemiological study designed to investigate the mor-