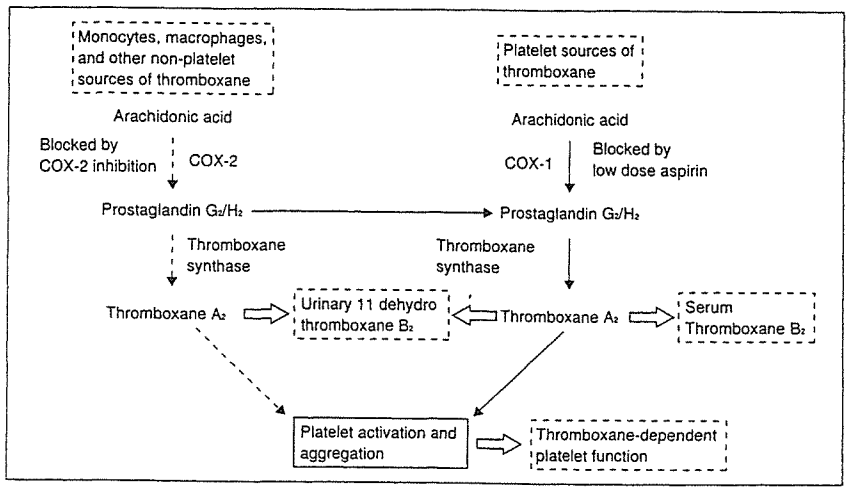

図　Thromboxan合成経路とアスピリンの抗血小板作用
文献[1]より許諾を得て転載.
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程度にとどまり，薬効は必ずしも満足できるものではない．血小板機能は個体差が大きく，例えば健常人の血小板凝集能を例にとっても，凝集物質に対し非常によく反応し凝集を起こす個体と，全く凝集を起こさない個体が存在する．この現象は経時的に観察しても同一個体ではよく保存され再現性が良好である．また血小板機能の個体差には遺伝的な要因が想定されている．

「アスピリンレジスタンス」はこれまでさまざまな状態に用いられてきた．すなわち，*in vitro*や*ex vivo*での血小板機能検査におけるアスピリン作用の減弱，*in vivo*でのトロンボキサンA_2(TXA_2) 合成抑制作用の減弱，そして臨床的効果の減弱すなわち血栓性疾患の予防効果の減弱などである．同様の現象はアスピリンに限らず，全く作用機序の異なる抗血小板薬，例えばクロピドグレルなどでも報告されている．Grotemeyerら[5]は検査上のアスピリンレジスタンスと，心血管イベントの関連を報告した181人のアスピリン服用脳卒中症例のうち，アスピリンレジスタンスと診断された60名は，2年間のフォローアップで40%の症例が重大な心血管イベントを経験したのに対し，アスピリン感受性と診断された残りの患者ではわずか4.4% が心血管イベントを経験した(相対危険率9.1)．2003年，Gumら[6]は325mg/dayのアスピリンで治療されている326人の冠状動脈疾患や脳血管障害患者を解析し，アスピリンレジスタンスと診断された17名中5名(29%)が2年間のフォローアップで重大な心血管イベントを起こした一方，非アスピリンレジスタンスと診断された309人での発症は30名(10%)にとどまったと報告している．

II アスピリンレジスタンスのメカニズムと評価法

本号のEikelboomの論文(38ページ掲載)には，考えうるアスピリンレジスタンスの機序が記載されている．アスピリンが抗血小板作用を発揮できない理由として，アスピリン投与量の不適切，アスピリンの吸収が悪い製剤(腸溶錠など)，服薬の不遵守などの問題のほか，併存する状態により血小板が活性化されていると思われる場合(喫煙，糖尿病)，イブプロフェンなど他の抗炎症薬のCOXへの作用が先行するとアスピリンによるアセチル化が阻害される可能性，ADPやコラーゲンに対する血小板の感受性亢進，赤血球や単球/マクロファージによる血小板活性化抑制の障害，マクロファージ，血管内皮細胞のCOX-1の再生，COX-2の誘導，そして血小板自体の遺伝的個体差(GPIIb/IIIaの遺伝的多型，COX-1遺伝子多型)などが提唱されている．他の抗炎症薬による干渉については，例えばアスピリンがCOX-1の^{120}Argに結合する際，イブプロフェンやナプロキセンなどがこれを競合的に阻害するためと考えられている．このような作用は他のNSAIDには観察されていないが，抗炎症薬がアスピリン療法，特に少量アスピリン治療における臨床的効果にどのような影響を与えるかは今後の研究を待たねばならない．

以下にこれまで用いられてきたアスピリンレジスタンスの評価法を紹介する．

1. 血小板凝集計

上述のGum らによる前向き研究では透過光変化を測定

する通常の血小板凝集計が用いられた．PRPでのADPやアラキドン酸凝集に対するアスピリンの効果を評価したもので，約5.2%の患者がアスピリンレジスタンスであったと報告している．この頻度は他の評価法を用い研究に比べかなり低い．しかし，臨床的アウトカムとの相関が非常に良好であったため，信頼性の高いデータと考えられている．

2. PFA-100

PFA-100 (Platelet Function Analyzer 100, Dade Behring, Deerfield, IL) は，高ずり応力下 (5,000～6,000/s) での血小板の血栓形成能を測定する装置である[7]．血管壁への血小板粘着および凝集，そして血栓形成を*in vitro*で再現する．ADPまたはエピネフリンとコラーゲンを含有する膜にクエン酸全血を通過させ，血栓形成による閉塞時間を測定する．閉塞時間はフォンビルブランド病などの出血性疾患で延長し，また一部の抗血小板薬でも延長する．アスピリンについてはエピネフリンとコラーゲンを含有するカートリッジで閉塞時間が延長する．この評価法では，アスピリンレジスタンスは9.5～40%とされており，喫煙者に多いと報告されている．またアスピリンレジスタンスにアスピリン用量依存性が報告されている．

3. 尿中11-dehydro thromboxane B_2排泄量

尿中11-dehydro thromboxane B_2排泄量が高い人では心筋梗塞や心血管死の頻度が高いとされるが，必ずしもアスピリンレジスタンスと関連するとは考えられていない．これは11-dehydro thromboxane B_2の一部が血小板以外の組織に由来することに起因するとされており，アスピリンの血小板への作用を確実に反映するものではない．

このようにアスピリンレジスタンスにはいくつかの評価法があるが，残念ながら現時点では理想的な検査法は存在しない．言い換えれば服薬中の患者の評価としてルチンに推奨される検査は存在しない．上述のごとくアスピリンレジスタンスの定義はコンプライアンス，体内動態にはじまり，評価法 (*in vivo*, *ex vivo*, *in vitro*) がまちまちである．血小板機能のみに着目したアスピリンレジスタンス評価を行ったとしても，その方法が研究によりさまざまであるため，標準化にはほど遠いのが現状である．

III アスピリンレジスタンスを検査すべきか？

心血管病の増加に伴い，動脈硬化を基盤にして発症するこれらの心血管イベントを効率的に予防するため，アスピリンレジスタンスの分子機序の早急な解明が望まれる．抗血小板薬に対する反応性の個体差が虚血性脳血管障害や冠状動脈疾患における血栓症予防効果に影響している可能性が高く，事実アスピリン不応症患者では実際に冠状動脈疾患での再発率が高いことは既述した．アテローム血栓症 (athrothrombosis) の成因を考えれば，たとえ抗血小板薬を服用していても血栓症の発症が高頻度にみられること，抗血小板薬の臨床的効果が20～30%のイベント減少にとどまることは予想できることである．1つの抗血小板薬が無効であったからといって直ちに別の抗血小板薬に変更する，という論理は抗血小板療法においては一般的ではない．アスピリンレジスタンスの頻度は定義によりまちまちであり，さらにアスピリンに対する血小板の反応性は，いくつかの血小板機能評価法の間で相関が低いことが問題となる．「不応」という用語に反対する向きもあり，「アスピリン反応性の個体差」などの表現がより適切かもしれない．現時点では十分な感度・特異度を有する血小板機能検査が存在せず，またアスピリンレジスタンスの定義，メカニズムも不明確であるため，アスピリンレジスタンスの診断を安易に行うべきではない．しかし，アスピリンレジスタンスの病態が十分に解明されれば，少なくとも一部の患者においては抗血小板薬の種類の変更に対する理論的裏付けが可能となる．動脈硬化の終末イベントである閉塞性血栓形成過程に介入して心血管病を予防する抗血小板薬効果の個人差の原因を見つけだすことの恩恵は大きい．

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Short Communication

G501C polymorphism of oxidized LDL receptor gene (OLR1) and ischemic stroke

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ABSTRACT

The human lectin-like oxidized low-density lipoprotein receptor 1 (OLR1/LOX-1) is the major endothelial scavenger receptor against oxidized low-density lipoprotein (Ox-LDL), which has been implicated in the pathogenesis of atherosclerosis. We investigated the G501C mutation in the OLR1 gene in 235 Japanese patients with ischemic cerebrovascular disease (CVD) and 274 age- and sex-matched healthy controls using single nucleotide primer extension analysis (SNuPe). There was no significant difference in the polymorphism between patients with ischemic CVD and controls (GC+CC versus GG, $p=0.48$). The C allele was not significantly different between the patients and controls (C versus G, $p=0.91$). Our results show that the OLR1 gene polymorphism has little effect on an increased risk for ischemic CVD in the Japanese population.

Human lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), encoded by the OLR1 gene, was identified as a cell surface endocytosis receptor for oxidized low-density lipoprotein (Ox-LDL) on vascular endothelial cells. In the physiological state, LOX-1 expression is seen *in vivo* in vascular rich organs, such as placenta, lungs, brain, and liver, and *in vitro* in normal aortic endothelial cells (Sawamura et al., 1997). On the other hand, Ox-LDL and inflammatory cytokines can upregulate the expression of LOX-1 and induce the endothelial expression of leukocyte adhesion molecules and smooth muscle growth factors, which are involved in atherosclerosis (Kume and Gimbrone, 1994). LOX-1 on the endothelium mediates the auto-activation of platelets, the platelet–endothelium interaction, and the release of endothelin-1 from endothelial cells that introduces endothelial dysfunction (Kakutani et al., 2000).

A single nucleotide polymorphism G501C of the OLR1 gene that results in an amino acid dimorphism (Lys/Asn) at residue 167 in LOX-1 protein was found in patients with ischemic heart disease from a single family, and G501C+C501C genotype increased the risk of myocardial infarction or the severity of coronary artery disease significantly (Tatsuguchi et al., 2003; Ohmori et al., 2004).

Interestingly, a recent study demonstrated that acetylsalicylic acid (aspirin), which could prevent ischemic stroke, inhibited Ox-LDL-mediated LOX-1 expression and metalloproteinase-1 in human coronary endothelial cells (Mehta et al., 2004). The expression of LOX-1 was greater than tenfold

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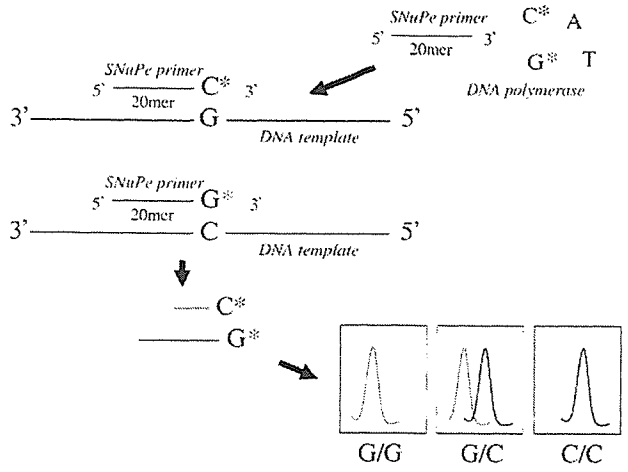

Fig. 1 – The principal of SNuPe genotyping. The constructed SNuPe primers anneal the amplified DNA templates in front of 501 sites. Subsequently, the different fluorescent dyes labeled ddGTP or ddCTP react with the target SNP site and stop the annealing on and after 501 sites. Using a scanner, we can genotype the G501C single nucleotide polymorphism.

at a transient ischemic core site compared with the non-ischemic side in the rat middle cerebral artery occlusion model (Schwarz et al., 2002). These data suggest that LOX-1 expression induces atherosclerosis in the brain and is the precipitating cause of ischemic stroke. Although several studies have shown the positive relationship between A501C polymorphism of OLR1 gene and atherosclerotic heart disease, the association between the polymorphism and ischemic stroke remains unclear. Using the SNuPe assay (Greenwood and Burke, 1996), we examined whether the OLR1 single nucleotide polymorphism is associated with ischemic cerebrovascular disease in the Japanese population.

We analyzed 235 unrelated Japanese patients with ischemic cerebrovascular disease (CVD) and 274 age- and sex-matched controls. All patients with CVD had attended the outpatient clinic of Keio University Hospital, Tokyo, for regular follow-up examinations. All controls worked for Keio University and had visited Keio University Hospital for their annual health examinations. We selected patients with CVD who were ≤70 years of age at the onset of CVD. On the basis of the Classification of Cerebrovascular Diseases III report from the committee established by the National Institute of Neurological Disorders and Stroke, patients with CVD who were given a diagnosis of atherothrombotic infarction (AT), lacunar infarction, or transient ischemic attack (TIA) were enrolled in this study. Those with cardioembolic cerebral infarction or cerebral hemorrhage were excluded. Those with a clinical history of cerebrovascular disease, myocardial infarction, or peripheral vascular disease were excluded. Written informed consent was obtained from all subjects after a full explanation of the study and a guarantee of total confidentiality. Brain computed tomography (CT) and/or magnetic resonance imaging (MRI) studies were performed on all patients with CVD. Hypertension, hypercholesterolemia, and smokers were defined as described previously (Ito et al., 2000).

Whole blood was collected into tubes containing sodium citrate. After genomic DNA was extracted, polymerase chain reaction (PCR) was carried out in a 25-μl volume containing 1.5 μl of 25 mM $MgCl_2$, 2 μl of 2 mM dNTP, 2.5 μl of 10× PCR buffer, 0.2 μl of each primer, 0.125 μl of Taq Gold, 17.475 μl of distilled water, and 1 μl of the extracted DNA. Amplification of a 441-bp fragment of the OLR1 gene was performed with the 5′ primer 5′-CTGGAGGGACAGATCTCAGC-3′ and 3′ primer 5′-TAAGTGGGGCATCAAAGGAG-3′. Amplification by PCR consisted of an initial 5 min of denaturation at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 61 °C, and 30 s at 72 °C, followed by 7 min at 72 °C in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) or a PLT-225 DNA Engine Tetrad (MJ Research, Waltham, MA, USA).

To analyze the G501C polymorphism of the OLR1 gene, a commercial MegaBACE SNuPe Genotyping Kit (Amersham Biosciences, Piscataway, NJ, USA) was used (Fig. 1). The 10-μl SNuPe reaction volume contained 1 μl purified PCR product, 4 μl SNuPe Premix, and 2 pmol SNuPe primer. The HPLC-purified SNuPe primer 5′-GGCTCATTTAACTGGGAAAA-3′ was constructed. The SNuPe reaction consisted of 25 cycles of 10 s at 94 °C, 5 s at 58 °C, and 10 s at 60 °C, and the sequence was completed by the MegaBACE 1000 DNA sequencing system (Amersham Biosciences, Piscataway, NJ, USA).

The differences in genotype frequencies and other risk factors were analyzed by the χ^2 test. The mean age and allele frequencies in the two groups were compared by Student's t-test. Multiple logistic regression methods were used to control for possible confounding factors. All statistical analyses were performed using Statview software (Ver. 5.0 for Windows, SAS Institute, CA, USA).

The 235 patients with CVD and 274 controls were well matched in terms of age and sex (Table 1). The risk factors hypertension, hypercholesterolemia, diabetes mellitus, and smoking were significantly more common in patients with CVD than in controls ($P<0.001$). The frequencies of the G501C polymorphism showed no deviation from Hardy–Weinberg equilibrium.

The frequencies of GC+CC genotype carrier were not statistically different between all patients with CVD and

Table 1 – Clinical characteristics of patients with CVD and controls

	Controls ($n=274$)	Patients with CVD ($n=235$)	P[a]
Male, %	71.9	77.9	NS[b]
Age, mean±SD, y	59.1±3.4	58.3±7.8	NS[b]
Hypertension, %	25.3	56.2	<0.001
Hypercholesterolemia, %	15.1	38.6	:0.001
Diabetes mellitus, %	5.53	25.8	<0.001
Smoking, %	25.6	53.3	<0.001
Body % mass index >27.3 kg/m^2, %	5.68	9.02	0.062
Family history, %	18.5	29.3	0.012

[a] χ^2 tests were used to compare values of patients with CVD and controls for all parameters except for age, which was compared by Student's t-test.
[b] Not significant.

Table 2 – Genotype and allele frequencies of the G501C polymorphism of the OLR1 gene in patients with CVD and controls

Genotype	Patients with CVD (n=235)	AT (n=69)	Lacunar (n=142)	TIA (n=24)	Controls (n=274)
GC+CC, %	39.1 (92)	44.9 (31)	37.3 (53)	33.3 (8)	36.1 (99)
GG, %	60.9 (143)	55.1 (38)	62.7 (89)	66.7 (16)	63.9 (175)
P[a]	0.484	0.182	0.811	0.783	–
C, %	21.1	24.6	20.1	16.7	21.4
G, %	78.9	75.4	79.9	83.3	78.6
P[a]	0.911	0.410	0.666	0.433	–

The number of subjects (X).
[a] χ^2 tests were used to compare genotype and allele frequencies between controls and all patients with CVD and between controls and individual groups of patients with CVD.

controls: 39.1% versus 36.1%, odds ratio (OR)=1.14 [95% confidence interval (CI)=0.98 to 2.00, p=0.484]. The C allele frequencies were not significantly different between all patients with CVD and controls: 21.1% versus 21.4%, OR=0.98 (95% CI=0.73 to 1.33, p=0.911) (Table 2). As seen in Table 2, a stepwise increase in the percentage of patients with CVD and GC+CC genotype was found depending on the severity of CVD: 33.3% in TIA, 37.3% in lacunar infarction, and 44.9% in AT. However, we failed to show a statistical difference between these subtypes of CVD.

In logistic regression analysis, sex, age, hypertension, hypercholesterolemia, diabetes mellitus, current smoking, body mass index, family history of stroke, and OLR1 genotypes (GC+CC vs. GG or C allele vs. G allele) were included as independent variables. This analysis revealed that the presence of the C allele was independent of these acquired risk factors.

The present study examined the relation between ischemic CVD and the G501C single nucleotide polymorphism (SNP) on exon 4 of the OLR1 gene and did not show the statistical influence of the SNP. As shown in Table 3, there are three other studies regarding the relation between acute myocardial infarction (AMI) and the polymorphism, but two of the three failed to show any relation. In detail, Ohmori et al. identified the significant stepwise decrease in the percentage of patients with GC+CC genotype depending on the severity of coronary artery disease (Ohmori et al., 2004), but the other group showed a significant association between GG genotype and the severity of coronary artery disease when the patients having three obstructed vessels were compared to those with one or two obstructed vessels (Trabetti et al., 2006). In addition, we found a stepwise increase in the percentage of patients with CVD and GC+CC genotype depending on the severity of CVD, but it was not significant. Based on these findings, we suppose that the association between atherosclerotic diseases and the genotype seems weak because we found the paradoxical results associated with disease severity in both studies.

Several reasons may explain why the present study failed to show an association between stroke and G501C genotype. First, the G501C genotype and other polymorphisms on intron 4, intron 5, and 3′UTR of the OLR1 gene were studied by two other groups of researchers. They showed that there was a significant association between the 3′UTR/T allele carrier (TC+TT genotypes) and acute myocardial infarction or coronary artery disease severity (Mango et al., 2003; Chen et al., 2003). Although the G501C genotype of the OLR1 gene, which resulted in the missense mutation of K167N in the extracellular ligand-binding domain of the LOX-1 protein, may affect the binding affinity between Ox-LDL and LOX-1, the effect of these SNPs in the untranslated portion remains unclear. However, Mango et al. suggested that SNPs in the untranslated portion may affect the alternative splicing of the OLR1 gene, which could express not only LOX-1 but also LOXIN, which lacked exon 5. They also reported that LOXIN blocked the apoptosis of endothelial cells, smooth muscle cells, and macrophages associated with LOX-1 activation (Mango et al., 2005). In a comparative expression analysis between the wild-type and mutant OLR-1 in intron 4, intron 5, and 3′UTR, LOXIN expression in the mutant was higher than that in the wild-type plasmid. Taken together, they suggested that alteration of LOXIN expression in untranslated SNPs is directly associated with susceptibility to AMI. If these SNPs are also important to the susceptibility to ischemic stroke, LOX-1 activation without Ox-LDL binding is more important than the Ox-LDL scavenger function of LOX-1. Thus, G501C may not have such a strong effect on the susceptibility to stroke.

Second, the mechanism of the relation between LOX-1 and ischemic stroke was proposed to be based on oxidative stress (Gorelick, 2001). Primarily, angiotensin II stimulates oxidation of LDL and expression of LOX-1 in pathogenesis of ischemic stroke, and the binding of Ox-LDL to LOX-1 increased production of superoxide, which inactivated nitric oxide in a chemical reaction (Cominacini et al., 2001). Subsequently, this reduced nitric oxide has been linked to impaired endothelium-dependent relaxation and the progression of atherosclerosis. If that is the main reason of atherosclerotic progression in patients with ischemic CVD, the GC+CC genotype has adverse effects against atherosclerosis, which means that

Table 3 – Association results between OLR1 gene polymorphism and atherosclerotic diseases in the previous studies and the present study

The first authors	Sample size[a]	Risk genotype	Odds ratio (95% CI)	P-value	Associated phenotype (results)
Tatsuguchi et al., 2003	102 vs. 102	G501C+C501C	2.89 (1.51–5.53)	<0.002	Acute myocardial infarction (associated)
Ohmori et al., 2004	171 vs. 248	G501C+C501C	34 vs. 37%[a]	Not significant	Acute myocardial infarction (failed)
Trabetti et al., 2006	190 vs. 160	G501C+C501C	0.97 (0.55–1.68)	0.88	Acute myocardial infarction (failed)
Present study	235 vs. 275	G501C+C501C	1.14 (0.98–2.00)	0.484	Ischemic cerebrovascular disease (failed)

[a] Patients vs. controls.

the genotype may decrease atherosclerotic vessels. That is another reason of our failure.

In conclusion, this study showed no association between the G501C polymorphism in the OLR-1 gene and ischemic cerebrovascular disease in the Japanese population. G501C may not influence the susceptibility to ischemic stroke. One reason is that LOX-1 activation without Ox-LDL binding may be more important than the scavenger function of LOX-1 for intracranial atherosclerosis. Another reason is that the interaction between Ox-LDL and LOX-1 may have an accelerative effect on endothelium dysfunction. Although recent studies indicate that LOX-1 is implicated in the mechanism of atherosclerosis and ischemic stroke, further studies are needed to evaluate whether the G501C genotype may change the binding affinity of LOX-1.

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REGULAR ARTICLE

Increased basal platelet activity, plasma adiponectin levels, and diabetes mellitus are associated with poor platelet responsiveness to in vitro effect of aspirin

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Abstract

Introduction: Aspirin is one of the most effective antiplatelet agents and is now commonly used to prevent vascular events. In some patients, however, recurrent vascular events have been demonstrated despite aspirin therapy. Our objective was to characterize individuals showing poor response to in vitro effect of aspirin, using PFA-100.

Methods: One hundred sixty-eight healthy male subjects were analyzed. We assessed platelet function tests, including PFA-100, whole blood aggregation, and optical platelet aggregation. Also measured were hemostatic and other parameters including von Willebrand factor (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo), soluble vascular adhesion molecule-1 (sVCAM-1), high sensitive C-reactive protein (hs-CRP), and adiponectin. Poor responders were defined as having a collagen/epinephrine-induced closure time (CEPI-CT) under 250 s with PFA-100

Abbreviations: CEPI-CT, collagen/epinephrine-induced closure time; CADP-CT, collagen/ADP-induced closure time; PATI, platelet aggregatory threshold index; TXB_2, thromboxane B_2; VWF:Ag, von Willebrand factor antigen; VWF:RCo, VWF ristocetin cofactor activity; sVCAM-1, soluble vascular adhesion molecule-1; hs-CRP, high sensitive C-reactive protein.

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when incubated with 10 μM aspirin, whereas good responders were defined as having a CEPI-CT of more than 250 s.
Results and conclusions: PFA-100 tests revealed that 40 subjects (24%) were poor responders (PR) and 128 (76%) were good responders (GR). Poor responsiveness was significantly associated with (1) higher basal platelet activities in PFA-100, as well as in whole blood aggregation and aggregometer;(2) increased level of adiponectin (8.8 ± 4.1 μg/mL [PR] vs 7.3 ± 2.9 μg/mL [GR], $p=0.010$);and (3) the presence of diabetes mellitus (17.5% [PR] vs 4.7% [GR], $p=0.009$). Importantly, whereas 24% of the subjects showed insufficient inhibition in PFA-100 when incubated with 10 μM aspirin, almost all subjects showed maximum inhibition with 30 μM aspirin. These observations suggest that higher doses of aspirin might overcome aspirin resistance.

Introduction

Acetylsalicylic acid (aspirin) has been shown to be one of the most effective antiplatelet agents and is now commonly used to prevent vascular events. Aspirin irreversibly inhibits cyclooxygenase-1 in platelets, resulting in inhibition of proaggregatory thromboxane A_2 (TXA_2) generation [1]. Results of a recent meta-analysis revealed that aspirin reduces the risk of secondary cardiovascular events by approximately 25% [2]. In some patients, however, recurrent vascular events have been demonstrated despite aspirin therapy [3–7]. This phenomenon is known as aspirin resistance. Indeed, previous ex vivo studies have revealed that aspirin resistance, judged by laboratory tests, increases the risk of recurrent vascular events [8,9]. In many laboratories, insufficient inhibition of platelet function regardless of aspirin intake was observed by platelet function tests or measurements of urinary level of 11-dehydro thromboxane B_2, a stable hydrolysis product of TXA_2 [8]. The prevalence of aspirin resistance varies from 5.5% to 60%, depending on the definitions of aspirin resistance and the population studied [10].

Although much effort has been focused on understanding aspirin resistance, its mechanism remains unclear. Aspirin resistance can be classified as either pharmacodynamic or pharmacokinetic [11]. Pharmacodynamic aspirin resistance means incomplete inhibition of platelet function despite of a sufficient blood concentration of aspirin, whereas pharmacokinetic type means incomplete inhibition of platelet function because of an insufficient blood concentration of aspirin. Individual pharmacokinetics after low-dose aspirin intake is highly variable [12,13]. Therefore, in the current study, in order to focus on platelet sensitivity to the same concentration of aspirin, we assessed platelet functions after in vitro incubation with aspirin (vehicle, 10 μM, or 30 μM) for 30 min in healthy subjects by three different methods, PFA-100, WBA-neo, and optical platelet aggregometer. In terms of an area under the blood concentration-time curve (AUC), in vitro incubation of 10 μM aspirin for 30 min is comparable to 80 mg aspirin intake (approximately 0.9 μg·h/mL) [13].

To characterize individuals showing poor response to aspirin, we assessed platelet function tests, including PFA-100, whole blood aggregation, and optical platelet aggregation. In addition, we measured plasma or serum levels of VWF:Ag, VWF:RCo, sVCAM-1 and hs-CRP as a marker of endothelial dysfunction or inflammation [14], and adiponectin, an adipocyte-derived plasma protein, that acts as both an anti-atherogenic [15,16] and anti-diabetic [16] adipokine.

Materials and methods

Subjects

All experiments were performed with the approval of the ethics committees of the participating research institutes (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan and Keio University Hospital, Tokyo, Japan). Informed written consent was obtained from all subjects before enrollment. Three hundred and twenty-five male subjects were initially enrolled at their regular check-ups. Those who had any apparent hematologic, cardiovascular, hepatic, or renal diseases, and those who took any NSAIDs or cold medicine within 10 d of testing were then excluded. Blood samples that showed basal CEPI-CT over 250 s and that resulted in incomplete studies in PFA-100 testing when incubated with vehicle or 10 μM aspirin were also excluded from the analysis. As a result, 168 subjects, whose mean age ± S.D. was 46.9 ± 5.1 y, were available for the analyses.

Blood sampling

Blood samples were drawn into evacuated tubes containing 3.8% buffered sodium citrate solution. Platelet rich plasma was obtained by 10-min centrifugation at 180 $\times g$. Platelet poor plasma was obtained by 10-min centrifugation at 2000 $\times g$.

In vitro aspirin incubation for platelet function tests

For evaluation of CEPI-CT using PFA-100 and of collagen-induced aggregation using WBA-neo, aspirin (vehicle, 10 μM, or 30 μM, final concentrations) was added to whole citrated blood (0.38% sodium citrate, final concentration). For evaluation of collagen-induced aggregation using optical platelet aggregometer, aspirin was added to platelet rich plasma that had been adjusted to 30×10^4 platelets/μL with autologous platelet poor plasma. The samples were incubated for 30 min at room temperature. Ethanol was used as the vehicle for aspirin.

PFA-100 testing

PFA-100 (Platelet Function Analyzer-100, Dade Behring, USA) is a relatively new point-of-care device for assessing platelet function in vitro under high shear flow condition (5000–6000/s) characterized by high sensitivity and easy-to-use operation [17]. A CEPI cartridge was employed to determine the responsiveness to aspirin because aspirin usually prolongs the CEPI-CT, whereas it has little effect on collagen/ADP-induced closure time (CADP-CT) [18]. In practice, any CEPI-CT over 250 s can be considered to be equivalent to nonclosure [19]. Thus, poor responders were defined as having CEPI-CT values of less than 250 s when treated with 10 μM aspirin, whereas good responders were defined as having CEPI-CT of 250 s or more. Concomitantly, CADP-CT was also measured. The maximum measurable value for either cartridge is 300 s. A previous report showed that the CV% of the duplicate measurements were 12% for CEPI-CT and 10% for CADP-CT [20].

Optical platelet aggregation and determination of TXB_2

Aggregation was induced by 1 or 2 μg/mL collagen (Collagenreagent Horm, Nycomed, Munchen, Germany) or by 2 or 5 μM ADP at 37 °C using an optical platelet aggregometer (ET-800, TOKYO KODEN, Tokyo, Japan). After aggregation induced by collagen, platelets were removed by centrifuging the samples at 11,100 $\times g$ for 3 min. The resulting plasma samples were stored at −80 °C until assay. TXB_2 level in the plasma samples was measured using a TXB_2 ELISA kit (Cayman Chemical, MI, USA).

WBA-Neo

The effects of aspirin on whole blood aggregation were measured using WBA-neo (ISK, Tokyo, Japan), a whole blood aggregation system based on the screen filtration pressure method [21]. Platelet aggregation was induced by 0.25, 0.5, 1, or 2 μg/mL collagen or by 1, 2, 4, or 8 μM ADP at 37 °C. The concentration of collagen or ADP needed to induce 50% aggregation was calculated as platelet aggregatory threshold index (PATI).

Laboratory measurements

After centrifugation of blood samples at 2000 $\times g$ for 10 min, plasma samples were stored at −80 °C until assay. VWF:Ag was measured using a microlatex particle-mediated immunoassay (LIA) (Roche Diagnostics, Mannheim, Germany). VWF:RCo levels were assessed by the ristocetin-induced aggregation of platelets (Dade Behring, Marburg, Germany). Serum levels of hs-CRP were measured using N High Sensitivity CRP (Dade Behring). Plasma adiponectin levels were determined using an adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The plasma level of sVCAM-1 was determined using a human sVCAM-1 ELISA kit (R&D Systems Inc., MN, USA).

Statistical analysis

Results are expressed as mean ± S.D. For continuous variables, differences between groups were detected using unpaired t-test. For the categorical variables, differences between groups were detected with χ^2 test. Differences were considered significant when p values were less than 0.05. These analyses were performed using StatView ver. 5.0. Linear regression plot analysis was performed using JMP ver. 4.1 (SAS Institute Inc., Cary, NC, USA).

Results

Individual differences in CEPI-CT values after in vitro treatment with 10 μM aspirin

When incubated with vehicle, the overall mean of CEPI-CT was 142 ± 38 s. When incubated in vitro

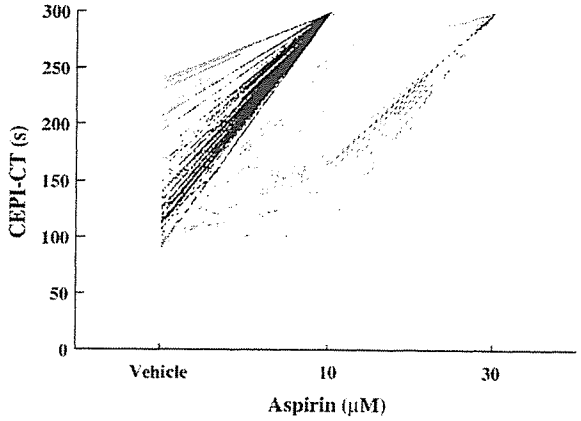

Figure 1 Individual changes in collagen/epinephrine-induced closure time (CEPI-CT) after in vitro incubation with aspirin. Dotted lines represent poor responders and solid lines represent good responders.

with 10 μM aspirin, substantial individual differences in CEPI-CTs were observed among subjects (Fig. 1). We defined poor responders as having a CEPI-CT less than 250 s despite 10 μM aspirin incubation and good responders as having a CEPI-CT of 250 s or more. According to these criteria, of the 168 male subjects enrolled, 40 (24%) were poor responders to aspirin and 128 (76%) were good responders. Notably, when incubated with 30 μM aspirin, all but 3 samples showed a maximum CEPI-CT of 300 s.

Demographics

Demographics and biochemical parameters of the subjects are listed in Table 1. Univariate analysis revealed that poor responders had less hemoglobin (14.6 ± 0.8 g/dL [PR] vs 14.9 ± 0.9 g/dL [GR], $p = 0.037$) and less total protein (7.12 ± 0.38 g/dL [PR] vs 7.26 ± 0.37 g/dL [GR], $p = 0.035$) compared to good responders. Interestingly, poor responders had a greater prevalence of diabetes mellitus (17.5% [PR] vs 4.7% [GR], $p = 0.009$), defined as fasting blood sugar ≥126 mg/dL or in treatment, than good responders. The level of HbA1c (5.4 ± 0.8% [PR] vs 5.1 ± 0.4% [GR], $p = 0.002$) is also larger in poor responders. There was no significant difference between poor and good responders with regard to age (48.2 ± 4.8 y [PR] vs 46.4 ± 5.1 y [GR], $p = 0.059$), BMI (23.2 ± 2.4 [PR] vs 24.2 ± 3.1 [GR], $p = 0.066$), cigarette smoking (35% [PR] vs 46% [GR], $p = 0.217$), and original platelet counts ($24.2 \pm 4.8 \times 10^4/\mu L$ [PR] vs $24.2 \pm 4.2 \times 10^4/\mu L$ [GR], $p = 0.999$).

Inflammatory parameters and adiponectin

To determine whether inflammatory status of blood vessels is associated with aspirin responsiveness, serum levels of VWF:RCo, VWF: Ag, sVCAM-1 [14], and hs-CRP were evaluated. As shown in Table 2, poor responders had larger levels of VWF:RCo

Table 1 Demographic and biochemical characteristics in poor and good responders

Characteristic	Poor responders	Good responders	p
Age (years)	48.2 ± 4.8	46.4 ± 5.1	0.059
BMI (kg/m^2)	23.2 ± 2.4	24.2 ± 3.1	0.066
Current smoker (%)	35 (14/40)	46 (59/128)	0.217
Hypertension[a] (%)	30 (12/40)	21 (26/126)	0.219
Hyperlipidemia[b] (%)	43 (17/40)	53 (68/128)	0.240
Diabetes mellitus[c] (%)	17.5 (7/40)	4.7 (6/127)	0.009**
HbA1c (%)	5.4 ± 0.8	5.1 ± 0.4	0.002**
WBC (/μL)	6100 ± 1800	6000 ± 1600	0.624
RBC (×10^4/μL)	468 ± 31	472 ± 33	0.491
Platelet (×10^4/μL)	24.2 ± 4.8	24.2 ± 4.2	0.999
Hematocrit (%)	44.8 ± 2.3	45.3 ± 2.5	0.216
Hemoglobin (g/dL)	14.6 ± 0.8	14.9 ± 0.9	0.037*
Fibrinogen (mg/dL)	246 ± 53	240 ± 60	0.574
Total protein (g/dL)	7.12 ± 0.38	7.26 ± 0.37	0.035*
Creatinine (mg/dL)	0.86 ± 0.11	0.89 ± 0.10	0.153
GOT (AST) (U/L)	24.2 ± 14.0	23.3 ± 10.2	0.633
GPT (ALT) (U/L)	27.3 ± 18.7	27.9 ± 15.1	0.836
γ-GTP (U/L)	54.7 ± 68.5	61.9 ± 58.5	0.511
HDL cholesterol (mg/dL)	54.5 ± 13.7	56.1 ± 13.1	0.500
LDL cholesterol (mg/dL)	132 ± 27	129 ± 32	0.645

Values are given as mean ± S.D. or % (number).

[a]Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or in treatment. [b]Total cholesterol ≥220 mg/dL or triglyceride ≥150 mg/dL or in treatment. [c]Fasting blood sugar ≥126 mg/dL or in treatment. *$p < 0.05$, **$p < 0.01$ by t-test or χ^2 test.

Table 2 Inflammatory parameters and adiponectin in poor and good responders

Parameter	Poor responders ($n=40$)	Good responders ($n=128$)	p
sVCAM-1 (ng/mL)	601 ± 261	493 ± 119	0.0004**
Adiponectin (μg/mL)	8.8 ± 4.1	7.3 ± 2.9	0.010*
VWF:RCo (%)	109.1 ± 37.2	90.4 ± 39.1	0.009**
VWF:Ag (%)	136.5 ± 55.8	105.0 ± 37.4	<0.0001**
hs-CRP (μg/mL)	1573 ± 6355	1201 ± 7011	0.765

Values are given as mean ± S.D.

sVCAM-1, soluble vascular adhesion molecule-1; VWF:RCo, VWF ristocetin cofactor activity; VWF:Ag, von Willebrand factor antigen; hs-CRP, high sensitive C-reactive protein. *$p<0.05$, **$p<0.01$ by t-test.

(109.1 ± 37.2% [PR] vs 90.4 ± 39.1% [GR], $p=0.009$), VWF: Ag (136.5 ± 55.8% [PR] vs 105.0 ± 37.4% [GR], $p<0.0001$), higher sVCAM-1 (601 ± 261 ng/mL [PR] vs 493 ± 119 ng/mL [GR], $p=0.0004$) than good responders. There were no significant differences in serum level of hs-CRP between poor and good responders (1573 ± 6355 μg/mL [PR] vs 1201 ± 7011 μg/mL [GR], $p=0.765$).

The serum level of adiponectin was also evaluated to determine whether this has association with aspirin responsiveness. Interestingly, poor responders had increased levels of adiponectin than good responders (8.8 ± 4.1 μg/mL [PR] vs 7.3 ± 2.9 μg/mL [GR], $p=0.010$). Consistent with previously reported results [22,23], adiponectin was positively correlated with HDL ($r=0.279$, $p=0.002$) and negatively correlated with BMI ($r=-0.339$, $p<0.0001$) and triglyceride ($r=-0.296$, $p<0.0001$) in the current study; additionally, no association between adiponectin and basal platelet function was observed.

Poor response was associated with basal hyperactivity in platelet function tests

The PFA-100 results indicate poor responders have a significantly shorter basal closure time than good responders. This result was observed for both CEPI cartridge (120 ± 33 s [PR] vs 149 ± 37 s [GR], $p<0.0001$) and CADP cartridges (89 ± 14 s [PR] vs 101 ± 24 s [GR], $p=0.002$) (Table 3). These results were also observed for the WBA-neo and optical aggregometry results (Table 3). In contrast, the TXB_2 production during platelet aggregation in aspirin-free condition was not significantly different between poor and good responders.

Discussion

Although much effort has focused on understanding aspirin resistance, its mechanism remains unclear. One possible reason would be the considerable individual variation in pharmacokinetics after aspirin intake [12,13]. This heterogeneity in aspirin blood concentration might make it difficult to determine the mechanism of aspirin resistance. In fact, even after repeated administration of low aspirin dose of 80 mg, there is room for further inhibition of platelet function [12]. In this study, to exclude the effect of individual pharmacokinetic variation and to focus on platelet responsibility to the same concentration of aspirin, platelet functions were evaluated after in vitro incubation of aspirin.

The results of the present study demonstrate that responsiveness to aspirin varies widely among subjects, even when 10 μM aspirin was added in

Table 3 Basal platelet functions in poor and good responders

Method	Platelet function test	Poor responders ($n=40$)	Good responders ($n=128$)	p
PFA-100	CEPI-CT (s)	120 ± 33	149 ± 37	<0.0001**
	CADP-CT (s)	89 ± 14	101 ± 24	0.002**
WBA-neo PATI	Collagen (μg/mL)	0.26 ± 0.06	0.32 ± 0.13	0.029*
	ADP (μM)	2.5 ± 1.6	4.0 ± 1.9	0.001**
Aggregometry	Collagen 1 μg/mL (%)	56 ± 21	44 ± 24	0.015*
	Collagen 2 μg/mL (%)	77 ± 12	72 ± 16	0.143
	ADP 2 μM (%)	44 ± 19	36 ± 15	0.008**
	ADP 5 μM (%)	73 ± 10	67 ± 13	0.004**
TXB_2 production	Collagen 1 μg/mL (ng/mL)	28.5 ± 10.6	25.5 ± 14.3	0.311
	Collagen 2 μg/mL (ng/mL)	54.6 ± 19.4	55.2 ± 26.0	0.924

Values are given as mean ± S.D.

CEPI-CT, collagen/epinephrine-induced closure time; CADP-CT, collagen/ADP-induced closure time; PATI, platelet aggregatory threshold index; TXB_2, thromboxane B_2. *$p<0.05$, **$p<0.01$ by t-test.

vitro (Fig. 1). Although most subjects showed CEPI-CT over 250 s when incubated with 10 μM aspirin, some required 30 μM. Univariate analysis revealed that poor aspirin responsiveness is significantly associated with several factors, including (1) higher basal platelet activities in PFA-100, as well as in aggregometer and WBA-neo, (2) increased levels of adiponectin, and (3) higher prevalence of diabetes mellitus or increased percentage of HbA1c. Association of sVCAM-1 levels and aspirin responsiveness might not be surprising because we observed that sVCAM-1 was associated with VWF:RCo ($r=0.2915$, $p=0.0002$), VWF:Ag ($r=0.5458$, $p<0.0001$) and VWF levels had been reported to be associated with aspirin responsiveness, judged by PFA-100 [18].

As shown in Table 3, poor responders had significantly shorter basal CEPI-CT and CADP-CT. This result held true for VWF-independent measurements (i.e., aggregometry and WBA-neo). Therefore, basal hyperactivity of poor responders was not likely due to solely to VWF levels. Notably, the same can be said when poor responsiveness was defined by optical platelet aggregation, instead of PFA-100. That is, when 1 μg/mL collagen-induced maximum aggregation in presence of 10 μM aspirin was analyzed by quartile, the uppermost quartile had higher basal aggregation than the others ($73.3\pm6.6\%$ vs $37.8\pm20.6\%$, $p<0.0001$). These results strongly indicate that those subjects who have higher basal platelet activity tend to respond poorly to aspirin treatment, which property is independent of apparatus used for measurements. In agreement with this, several studies demonstrated that incomplete inhibition by aspirin is associated with higher platelet activity. Kawasaki et al. concluded that aspirin resistance, defined by impaired prolongation of bleeding time, is associated to increased platelet optical aggregation by collagen [24]. Macchi et al. showed that aspirin-resistant patients, defined by CEPI-CT after aspirin intake, had shorter CADP-CT than aspirin-sensitive patients [25].

It is also notable that poor responders had significantly higher plasma level of adiponectin compared to good responders (Table 2). Adiponectin, a recently identified plasma protein specifically secreted by adipose tissue, is abundantly expressed in human plasma [26] and has a number of protective effects toward vascular endothelial cells [15,27]. Two receptors, adipoR1 and adipoR2, are known for adiponectin and mRNAs for both receptors are detected in human platelets by RT-PCR [28]. The recent study [28] revealed that adiponectin itself may act as an antiplatelet factor in human and mouse. According to our results, physiological level of plasma adiponectin did not correlate with platelet function determined by PFA-100, WBA-neo, and optical aggregometry (data not shown). On the other hand, there may be molecular mechanisms yet to be clarified underlying the association between increased plasma level of adiponectin and poor responsiveness to aspirin.

Notably, although a meta-analysis showed a clear benefit of aspirin intake for reduction in recurrence of vascular events, in the diabetic patients subgroup aspirin therapy failed to show a significant reduction in vascular events [2]. Indeed, several reports have described that patients with diabetes have reduced sensitivity to aspirin in ex vivo or in vitro platelet function tests [29,30]. Consistent with previous reports or evidence, our results indicate that poor response to aspirin is associated with diabetes mellitus or HbA1c. Several factors, such as enhanced platelet protein glycation that reduced platelet response to aspirin [31], hypersensitivity of platelets to agonists [32], or high rate of platelet turnover in diabetic patients [33] had been suggested as possible mechanisms. Its mechanism has not been fully elucidated, but our in vitro study might suggest that abnormal platelet turnover is not likely the cause of resistance. Although we did not find any correlation of diabetes with increased basal platelet function tests in the current study (data not shown), higher platelet activities in poor responders may be partially due to higher rate of diabetics in poor responder group, considering previous reports of platelet hyperfunction in diabetes [34,35].

Finally, platelet function was fully inhibited in almost all the subjects when incubated with an aspirin concentration of 30 μM. This finding indicates that higher doses of aspirin would be necessary for poor responders if the standard dose did not inhibit platelet functions. Indeed, several reports have suggested that higher doses of aspirin yield more effective results in platelet function tests, and that the dose of aspirin should be optimized for each patient, depending on the observed inhibitory effect. However, determining the optimal dose of aspirin might not be a straightforward process. A meta-analysis demonstrated that higher doses of aspirin (160–325 mg daily and 500–1500 mg daily) are no better than lower dose (75–150 mg daily) for the prevention of vascular events among patients at high risk of occlusive vascular events [2]. The implication of the meta-analysis is that aspirin would not reduce serious vascular events on the whole even if higher doses were given. The reason for the discrepancy remains unclear, but one possible explanation might be that platelet sensitivity to agonists is diverse, and the optimal dose of aspirin to

prevent vascular events will, of necessity, vary from patient to patient. According to previous reports [8,9], even limited inhibition of platelet function increases the risk of recurrence of vascular events. Thus, at least when incomplete inhibition was confirmed with low dose of aspirin, higher dose of aspirin would be effective for better clinical outcome.

In conclusion, responsiveness to aspirin varies widely among subjects, even if aspirin is added in vitro (i.e., individual pharmacokinetic variance was excluded). Higher basal platelet function, elevated adiponectin level and higher prevalence of diabetes were observed in the subjects showing poor response to aspirin. Importantly, our in vitro study demonstrated that almost all the subjects show maximum inhibition in PFA-100 when incubated with 30 μM aspirin, suggesting that aspirin resistance can be overcome with appropriate doses of aspirin and by monitoring the platelet inhibitory effects. Further understanding of aspirin resistance at the molecular level and a better designed clinical trial will be required to confirm these observations.

Limitations

There are some limitations to this study. First, we studied only male subjects, and the results might not be applicable for females. Second, participants taking NSAIDs or cold medicine were excluded from analysis based on answers to questionnaires only. Therefore, it is possible that some of the subjects had actually taken such drugs influencing platelet function tests. Third, those who had basal CEPI-CT over 250 s were excluded from analysis because the value is outside the baseline range [19]. This process might influence the prevalence of poor or good responders.

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血栓症――危険因子としての遺伝的背景

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要旨

現在，血栓の形成過程に関与する因子（脂質代謝，血管調節，凝固・線溶や血小板，炎症関連物質）の遺伝子変異あるいは遺伝子多型の中で，血栓症の危険因子となるものの報告が集積されている。また，血栓症の薬物療法の際の薬物代謝や薬物受容体に関与する遺伝子多型が，疾患のマネージメントに影響を及ぼす可能性は大きいと考えられている。これら遺伝的要因と血栓症の関係の研究は，疾患のリスク診断やテーラーメイド医療の可能性に繋がるとして近年注目を集めている。

はじめに

血栓形成に関与する因子の遺伝子変異や遺伝子多型が血栓症の危険因子として報告されている。それら遺伝的要因が疾患のマネージメント（特にリスク診断とテーラーメイド医療）向上の可能性として，近年注目を集めている。多因子性疾患である血栓症の発症・進展の過程に関与する因子は個人差があり，その差には遺伝的多様性（遺伝子変異や遺伝子多型）が影響を及ぼすことが報告されている[1]。

頻度は低いが遺伝性疾患の原因としてその意義が確立されている変異としては，血液凝固関連因子の遺伝子変異に加えて，動脈硬化を高頻度に発症する家族性（遺伝性）高脂血症の原因となる遺伝子変異が知られている。一方，より頻度の高い（集団の1%以上に存在する）遺伝子置換である遺伝子多型については，血栓症に関連する因子（脂質代謝，血管調節，凝固・線溶や血小板，炎症関連物質）の遺伝子多型がそれら遺伝子産物の濃度や機能の個体差に影響を及ぼすことによる関与や，血栓症に対する薬物療法の際の薬物代謝や薬物受容体に関与する遺伝子多型が疾患のマネージメントに影響を及ぼす可能性は大きいと考えられている。

2005年10月には国際共同研究チームによるヒト全染色体のハプロタイプ地図作成プロジェクト（アフリカ，アジア，ヨーロッパのそれぞれを起源とする複数のヒト集団から計270人のDNAサンプルを収集し，100万種類以上の遺伝子多型の頻度や相互関連性の程度を解明して，ヒト全染色体にわたるDNA多型性パターンの地図を作成し医学や医療の発展のために使用することを目的とするプロジェクト）の地図が完成した[2]。このハップマップの完成によって，疾患感受性に関与する遺伝子の研究がより効率的になり，急速に推進されていくと期待されている。

血栓には，静脈で形成され深部静脈血栓症に代表される「静脈血栓」と，動脈で形成され脳梗塞や心筋梗塞の原因となる「動脈血栓」に大別される。

静脈での血栓形成は，血液のうっ滞している箇所で，血液凝固因子が主体となる凝固反応の亢進が起こり，トロンビン生成を促進させることからはじまる。血流が遅いために高濃度のトロンビン

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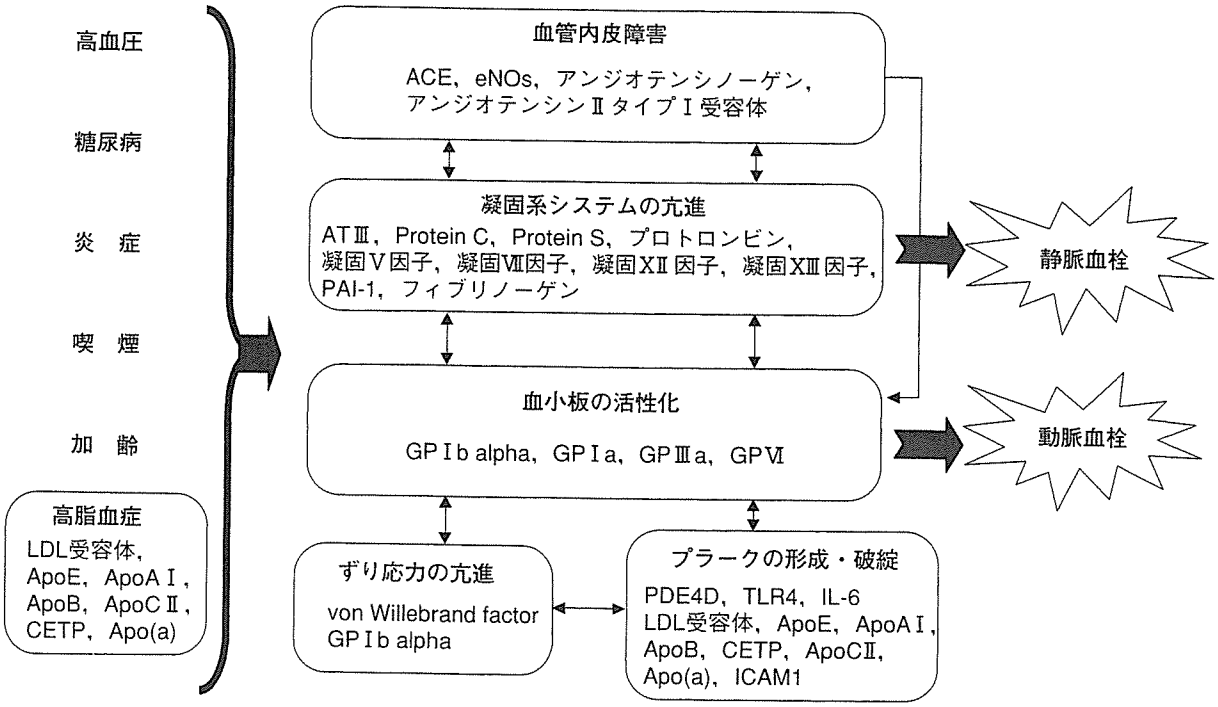

図1　血栓形成機序と血栓症に関与する遺伝的要因

血栓症に関与する遺伝子変異や遺伝子多型が報告されている主な因子を ▭ に示す。
LDL：低比重リポ蛋白，Apo：アポリポ蛋白，CETP：コレステロールエステル輸送蛋白，ACE：アンジオテンシン変換酵素，ATⅢ：アンチトロンビンⅢ，PAI-1：プラスミノーゲンアクチベーターインヒビター 1，GP：膜糖蛋白，PDE4D：ホスホジエステラーゼ 4D，TLR：toll-like receptor 4，ICAM1：intracellular adhesion molecule-1

が局所に存在してフィブリン塊を形成する。また，トロンビンは強力な血小板惹起物質であり血小板凝集塊形成も促す。

一方，動脈での血栓形成は，動脈硬化性のプラーク（粥腫）を基盤とし，プラークの進展・破綻とともに血管内皮が露呈，その血管損傷部位に対して速い血流のずり応力依存性に血小板の膜受容体が司る血小板活性化→血小板凝集反応により直接血栓形成に関与する。また，血小板はその活性化に伴って発現する炎症性物質や活性化血小板から放出される顆粒内物質によりプラーク破綻の機序にも関与，すなわち正のフィードバックに働きかけている。

このように，静脈と動脈のそれぞれ血管内における血流速度の違い（静脈内では遅く，動脈内では速い）により形成機序が異なるので，疾患に対する重要な因子も異なっている。

また，遺伝子型と環境因子，あるいは遺伝子型と遺伝子型といった組み合わせの効果が報告されているものがあり，それらの効果がある閾値を超えると発症に大きな影響を与えると考えられている。**図1**に血栓形成の機序と，血栓症の危険因子となる遺伝的要因が報告されている主なものを示す。

遺伝的要因を考慮した診療は，血栓症のマネージメントを個別に行うためにより有用と期待されているが，現在，先天性血栓傾向として臨床的に有用と考えられている遺伝子変異は存在するものの，臨床的有用性を議論されている遺伝子多型は凝固第Ⅴ因子の Leiden 変異のみである。この変異は欧米では認められているが，日本人には存在しない。したがって，日本人を対象として血栓症の日常診療に有用と考えられるものは存在せず，現在は遺伝子多型の臨床的意義を確立するために多くのデータの蓄積，解析が進行中である。

今回，血栓症に関係する遺伝子変異や遺伝子多型の主なものについて概説したい。

遺伝子変異

動脈硬化/動脈血栓を高頻度に発症する家族性(遺伝性)高脂血症をもたらす遺伝子変異が知られている。家族性（遺伝性）高脂血症の原因となり，動脈硬化・動脈血栓に対する惹起性が指摘されている遺伝子変異が報告されている因子は，低比重（LDL）受容体，アポリポ蛋白E，アポリポ蛋白AI，アポリポ蛋白CII，アポリポ蛋白CIII，アポリポ蛋白B，コレステロールエステル輸送蛋白，リポ蛋白リパーゼ，レシチンコレステロールアシル転換酵素である。この中でLDL受容体の遺伝子変異による家族性（遺伝性）高脂血症は，人口の約0.2%と頻度が高い。LDL受容体の遺伝子変異は700以上の報告があり，データベースにまとまっているのでそのアドレスを紹介したい(http://www.umd.necker.fr/LDLR/research.html)。

血液凝固系の制御因子であるアンチトロンビンIII（ATIII），プロテインC，プロテインSの遺伝子変異により，これら物質の量的・質的異常が血栓傾向をもたらすことが明らかとなっている。ATIIIはヘパリンを補因子として，血液凝固系の活性化の結果生じたトロンビン，活性化第X因子，あるいは活性化第IX因子の作用を阻害する。したがって，ATIIIの遺伝子変異による量的・質的異常は，過剰な血液凝固反応の進行を招く。ATIII異常を招く遺伝子変異の情報は，わかりやすいデータベースにまとまっているのでここにそのアドレスを紹介したい（http://www1.imperial.ac.uk/medicine/about/divisions/is/haemo/coag/antithrombin/default.html)。

プロテインCやプロテインSの遺伝子変異による，これら因子の欠乏・減少が認められている。一般人口における頻度は，それぞれ1,500人に1人，29,000人に1人である。プロテインCはトロンビンと血管内皮細胞上のトロンボモジュリンとの結合により活性化され，活性化プロテインC（APC）となる。APCはプロテインSを補酵素としながら第V因子や凝固第VIII因子を特異的に分解，失活化することで凝固系を抑制する。したがって，プロテインCやプロテインSの欠乏・減少は，生体内の抗凝固作用が減少し，血栓傾向を招来する。

遺伝子多型

血栓症に関連する因子として，脂質代謝，血管調節，凝固・線溶や血小板の因子，さらに最近では炎症関連物質の因子の遺伝子多型が，それら遺伝子産物の濃度や機能の個体差に影響を及ぼすことにより，血栓症の疾患感受性に関与するという報告がある。

心筋梗塞や脳梗塞の原因となる動脈血栓形成において重要な役割を有しており，近年，抗血小板療法のターゲットとして注目されている血小板膜受容体のglycoprotein（GP）Ibα，GP Ia，GP IIIa，GPVIの多型で血栓症と関係するものが報告されている。この関係の機序としてGP Ibα，GP IIIaはリガンドとの反応性，GP Iaは発現量とリガンドとの反応性を示唆する報告がある。GPIIIa多型には民族差が認められており，日本人での^{33}Pro型の頻度は，白人に比べて非常に低いため，少なくともわが国においてこの多型が血栓症に関与している可能性は低い。

GP Ibαの13個のアミノ酸が1〜4回の繰り返しVNTR多型において，3回，4回の繰り返し型は血栓症の危険因子と報告されている。VNTR多型もその頻度に民族差が認められている。この4回繰り返し型は，日本人での頻度は高い。われわれはこの4回の繰り返し型と1回の繰り返し型（それぞれに連鎖が報告されている^{145}Thr/Met）の配列を有する組み換え蛋白を作成し，GP Ibαのリガンドである von Willebrand factor (VWF) との反応性を流動状態下で検討した結果，この4回の繰り返し/^{145}Met型を有する配列がVWFと高い反応性を示すことを見いだしている[3]。

静脈血栓の形成過程で中心的役割を有する，血液凝固・線溶に関与する因子の血中レベルに影響する遺伝子多型が報告されており，そのレベル変化が血栓形成に影響を与えていると考えられている。また動脈血栓への関与も報告されている。脂

質代謝に関与する因子の遺伝子多型はその血中レベルに影響し，血栓症の疾患感受性に関与する。

LDL とは異なる代謝制御を受けるリポ蛋白(LP) (a) は，アポリポ蛋白 B100 に SS 結合したアポリポ蛋白(a) をその表面に有している。高 LP(a)血症は血栓性疾患の独立した危険因子である。注目すべきことは，血中脂質レベルの多くは環境因子の影響を受けているにもかかわらず，約 1,000 倍の個体差を示す LP(a) の血中レベルの調節は，遺伝的要因に依存しているという点である。LP(a) の血中レベルは，アポリポ蛋白(a) の遺伝子多型のクリングル反復数や 5'非翻訳領域の遺伝子多型と関係する。

また，血栓症の薬物療法の際の薬物代謝や薬物受容体に関与する遺伝子多型が，疾患のマネージメントに影響を及ぼす可能性は大きいと考えられている。最近注目されているのは VKORC1 の遺伝子多型である。2004 年にワーファリンの標的酵素のサブユニットである VKORC1 がクローニングされた[4,5]。その遺伝子多型がワーファリンに対する感受性に影響を及ぼしている(図 2)。さらに，薬剤代謝酵素である CYP2C9 との組み合わせでもワーファリンの効果に影響を及ぼすことが報告されている[6]。この VKORC1 の遺伝子型頻度には民族差が報告されており，今後日本人におけるこれらデータの集積が必要である。

他の多型では，GPIIIa 多型は抗 GPIIa/IIIa モノクローナル抗体であるアブキシマブやアスピリンに対する影響，GPIa 多型はクロピドグレル，アポリポ蛋白 E 多型は statins に対する影響の報告がある[7]。

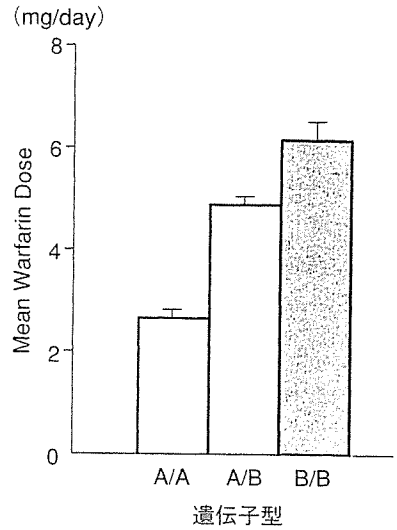

図 2　VKORC1 遺伝子多型とワーファリン投与量の関係（文献[6]より引用し改変）

おわりに

現在，疾患と遺伝的要因の研究は加速を増し，血栓症の予防，診断，治療において，より有用性の高いテーラーメイド医療の開発が期待されている。今後，診療において遺伝的素因の診断の導入を行う，すなわちテーラーメイド医療における標的因子の同定のためには，疾患と遺伝子型の疫学的検討とともに多型と疾患の関係の機序を理解する実験研究を行うことによる，個体の遺伝子型と表現型の統一的理解が必要であると考えられる。

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シンポジウム：臨床検査医学の進歩

3. 遺伝子多型検査は医療に貢献するか？

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遺伝子検査のなかでも生殖細胞系列(germline)の変異を同定するものは現在の保険診療では殆ど認められていない．多因子病で「病気になりやすさ」を調べる遺伝子検査は重要であるが，あくまで確率の予測であり，遺伝子検査の結果をもって診断が確定することは殆どない．遺伝子検査の結果が陽性でも人種差など遺伝的背景の違いや交絡因子などからその意義はcase-by-caseで考慮される必要がある．特に後天的な因子との寄与率の比較は常に頭に置くべきであろう．一方，単一遺伝病では遺伝学的検査の結果によって診断が確定し，治療方針が決定することが少なからずある．いずれにしても診療に直結する遺伝学的検査を，医師が研究費を用いて遺伝学的検査を行うことには多くの問題があり，早急な解決が望まれる．

2005年10月HapMap計画とよばれる国際協力プロジェクトの成果が報告された[1]．このプロジェクトはヒトの病気や薬に対する反応性に関わる遺伝子の基盤を整備するプロジェクトで，病気の診断，薬剤反応性の個体差の予測に大きく貢献すると考えられる．病気になりやすいか，なりにくいかは遺伝的要因と環境要因が複雑に絡み合って決定される．疾患によって遺伝的要因の関与は大きく異なり，外傷など関与が少ないと思われるものから，単一遺伝病のように殆どが遺伝的要因で決定するものまで様々である．多因子遺伝病，例えば生活習慣病は，複数の遺伝子座における正常遺伝子の組み合わせによって起こった生体機能の量的な偏りが環境の作用と呼応して発症する．代表的な例である高血圧，糖尿病，心筋梗塞，脳卒中などは同一家系内で多発し，しかもそれが単純なメンデルの遺伝形式では説明できない．正常人にみられる遺伝的多型(genetic polymorphism)はその遺伝子，牽いては蛋白の機能に影響し個体のphenotypeを決定する．

ヒトゲノムには約1000塩基に1個の割合で一塩基多型(single nucleotide polymorphism, SNP)が存在すると言われていた．2001年2月に報告されたヒトゲノムのdraft sequenceでは，ヒトゲノム3×10^9塩基対の中に，約1.42×10^6個のSNPが見い出されており，このデータからは約2000塩基に1 SNPということになる．遺伝子の中，特に翻訳領域には1遺伝子あたり平均2～3個のSNPsが存在するとされる．したがってヒトの全遺伝子数を3万個としても，全体で6～9万のSNPが翻訳領域に存在することになる．これらはいずれも直接，間接的に表現型に影響する可能性があり，一塩基多型の対立遺伝子数が仮に2個としても，その組み合わせは天文学的数字になる．多型は，転写活性の違いやmRNAの安定性の違いの結果生成される蛋白量に影響する場合と，出来た蛋白のアミノ酸変化により蛋白の細胞内輸送やproteaseに対する安定性，蛋白の構造変化(活性変化)を来たす場合が考えられる．いずれにしても，個体の表現形の違いを惹き起こし，疾患への易罹病性や薬物への反応性と関連することがある．一方で遺伝子多型は生体機能には何ら影響せず単に個体識別のマーカーとなるものもある．

多型と疾患の関連を解析する為には幾つかの方法があるが，多型の出現頻度を疾患群と正常群で比較する，患者-対照研究は最も一般的方法である．また責任遺伝子は未同定だが染色体上のおよ