B

		0	1	2
KCNQ1	rs757092	GG	GA	AA
KCNH2	rs1805123(K879T)	CC	CA	AA
KCNH2	rs3815459	GG	GS	AA

QT-Prolongation Score	QTc_RAS±SD	From total sample (n=3966), n
0	412.7±13.4	79
1	415.5±16.9	462
2	416.6±16.9	1021
3	418.3±17.8	1132
4	419.3±16.9	641
5	423.2±19.4	135

図 12-8(続き)
B. 再分極感受性SNPのQT時間に対する相加的効果を示す。*KCNQ 1*と*KCNH 2*の3つの再分極感受性SNPの遺伝型を数量化(QT prolongation score)し，各群間でQTc_RASを比較すると，scoreの上昇とともにQTc_RASが延長していることがわかる。

QTを1.9 msec短縮するアミノ酸置換型のSNP (rs1805123; K 897T)[12]と，1.5 msec延長するSNP (rs 3815459)を同定した。この3つのSNPの遺伝型を点数化してそれぞれのQTc_RAS値を比較すると，3つのSNPは単独ではQT時間に与える影響は小さいが，それらが組み合わさることによって相加的に効果が増強することがわかる(**図12-8 B**)。また，心筋再分極はイオンチャネル遺伝子多型に影響される複雑な遺伝的素因であるということができる。

3) ゲノムワイド集団相関解析

心筋の再分極はLQTS原因遺伝子の変異や多型ばかりではなく，さまざまな環境要因や遺伝的要因による影響を受けるが，その規定因子を特定しようとする際に，既知の心筋活動電位関連遺伝子の候補アプローチにはおのずと限界がある。Arkingらは，KORA S4コホート3,966名の心電図とゲノムDNAを用いた集団関連研究によって心筋再分極を修飾する遺伝的要因を同定した[28]。第1段階は，全コホートのうちQT_RAS時間の最も長い女性100名と短い女性100名に対し，115,000個のSNPを用いた全ゲノムタイピングを行い，QT_RAS時間と関連の強い10個のSNPを同定した。また，心筋再分極に関与する45個の候補遺伝子についてもSNPタイピングを行い，関連のある10個のSNPを同定した。この一次スクリーニングで陽性になったSNPについて，両群とも200名ずつ増やした二次スクリーニングを行い，陽性遺伝子を8個に絞り込み，最終的に残り全員による三次スクリーニングで，再分極の修飾遺伝子として*NOS 1AP*を同定した(**図12-9**)。さらに，KORA F3，Framingham Heart Studyコホートのゲノム解析でも，同様の結果が得られた。

*NOS 1AP*は，神経組織に強く発現するNOS 1 (nNOS)の修飾蛋白CAPONの遺伝子である。CAPONはNOS 1のC末端にあるPZDドメインに結合する蛋白で，NMDA受容体との共役を修飾することが知られている[29]。心臓における機能は十分に解明されていないが，NOS 1は筋小胞体に発現しているので[30]，CAPONはCa遊離を介して心筋の再分極過程に影響を与える可能性がある。最近，モルモットの心臓にCAPONをアデノウイルスで*in vivo*遺伝子導入すると，L型Caチャネルの抑制を介して心筋活動電位が短縮することが判明し，CAPONは心筋再分極の修飾因子であることが示された[31]。しかし，*NOS 1 AP*遺

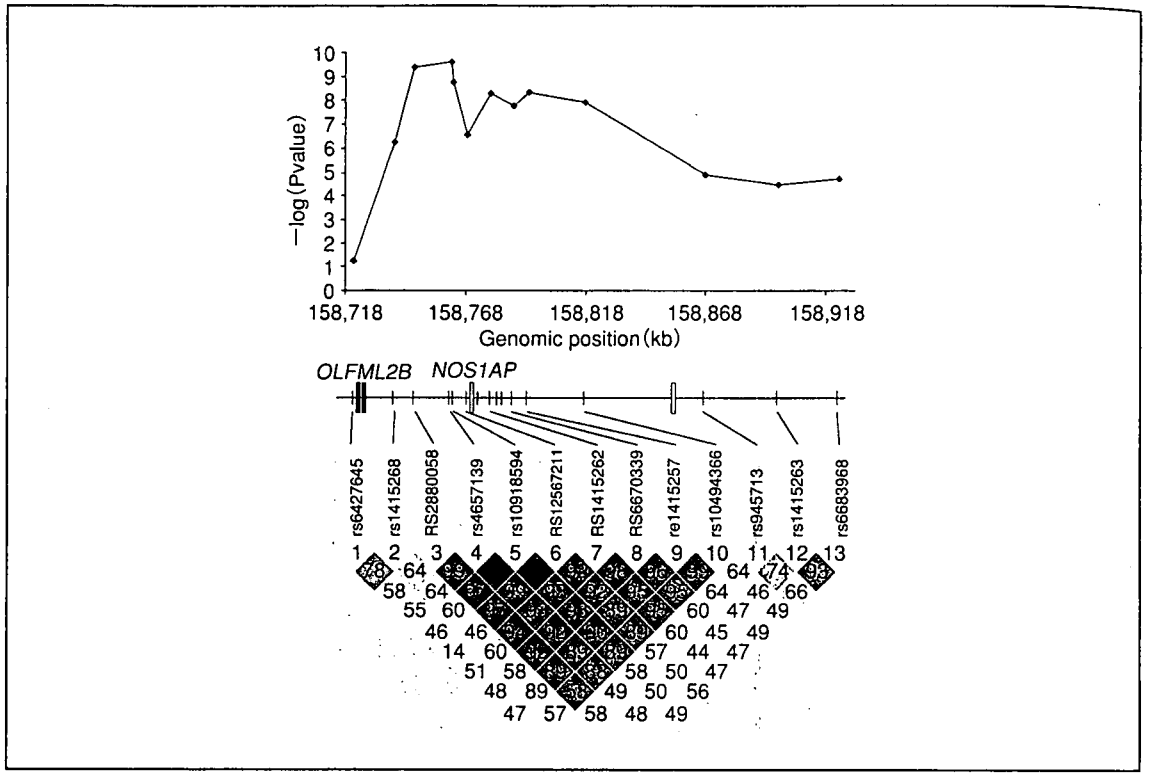

図 12-9 ゲノムワイド集団相関解析で明らかになった再分極修飾遺伝子 *NOS1AP*(文献 28 より引用，を改変)
中段に *NOS 1AP* の 2 つのエクソン(ピンク)とさらに上流に存在する遺伝子 *OLFML 2B*，および相関解析に用いた SNP(|)のゲノム上の位置が示されている。下段はそれぞれの SNP のペア間の LD 尺度(D')が示され，上段にはそれぞれの SNP の p 値が対数表示されている。*NOS 1 AP* のエクソン 1 周辺(とくに rs4657139 周辺の 5'よりの部分)の p 値が非常に高く，QTc_RAS との相関が高いことがわかる。

伝子内には QT 時間を左右する変異や多型は特定されていない。今後，国際 HapMap プロジェクトの推進によってさらに高密度の SNP マップが完成すれば，心筋再分極を規定する定量的形質遺伝子座(quantitative trait locus ; QTL)が明らかになると期待される。

おわりに

後天性 LQTS の少なくとも一部には，なんらかの遺伝子バリエーションや異常をもつ症例があることが明らかになってきた。また一部の疾患特異的な SNP は今後の薬剤治療にも影響を与える可能性がある。これらの遺伝子変異や SNP は通常はその機能異常が顕性化せず，薬剤など QT を延長させるリスク(トリガー)を受けたときにはじめて QT 延長が顕性化する。したがって，新薬の開発で個々の薬剤に QT 延長作用があるか否かの検討は重要だが，QT を延長させる遺伝子基盤をもつ者にはそのようなリスクを回避させる詳細な情報を与えるなど，患者側の要件を考慮することも今後重要な課題になるであろう。将来的には，薬剤の投与に先立ってこれら QT 延長遺伝子基盤を迅速にスクリーニングする技術の確立が望まれる。

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キーワード解説

遺伝子多型・連鎖解析・ハプロタイプ・連鎖不均衡

ヒトゲノムは約30億の塩基対によって構成され，約25,000の遺伝子が存在する。2006年現在，単一遺伝子の異常によるメンデル遺伝病は約1,700種類確認されている。自分と他人のゲノムを比較すると0.1%に違いがあることがわかっており，このバリエーションが個人差や疾患の原因となっている。塩基配列のバリエーションのうち，マイナー対立遺伝子の頻度が1%以下のものを通常「**遺伝子変異**」と呼び，1%以上のものを「**遺伝子多型**」と呼ぶ。約20種類存在する血液型は典型的な遺伝子多型の1つで，そのほかにも，制限酵素によるDNA消化パターンによって見分けられる制限酵素断片長多型(RFLP)やマイクロサテライトと呼ばれる遺伝子多型などがゲノムに10万個以上存在している。また塩基が1個置き換わる遺伝子多型はSNP(single nucleotide polymorphism)と呼ばれ，約1,000塩基に1個の割合で300～400万個がゲノムに広く分布している。SNPは，遺伝子のどこに存在しているか(翻訳領域にあるのか，非翻訳領域にあるのか，プロモータなどの調節領域にあるのか)，翻訳領域にある場合は蛋白配列を変えるかどうか(変えるもの：non-synonymous，変えないもの：synonymous)によってその意味合いが異なる。これらの遺伝子多型の一部は，わずかながら表現型に差異をもたらし，個体差の原因となる。またそれを利用して各個人に適した医療の提供を目ざすのが個別化医療の理念である。

一方，遺伝子多型のなかには個体の表現型とは直接関係はないが，未知の遺伝子の部位を同定する際に染色体上の物理的なマーカーとして役立つものもある。マイクロサテライトと呼ばれるマーカーは，例えば**図1A**のCAリピートのような繰り返し数が異なる直列配列で，ゲノム上に10^5個ほど存在するといわれる。繰り返し配列の外側の塩基配列は比較的保たれているので，そこにプライマーを設計してPCRで増幅しゲル電気泳動すると，CAの繰り返しの数はPCRの産物の長さとして認識される。あらかじめ異なる長さのPCR産物に番号をつけておけば，各個人の対立遺伝子の遺伝子型を番号表示することができる。

父方と母方染色体は減数分裂の際に相同組換えを起こす。一般に，ある特定の遺伝子Aと多型マーカーBの物理的距離が近ければ近いほどにその間に組換えが入る可能性は低くなる。したがって，ある遺伝病の家系内で，未知の遺伝子Aの異常によってもたらされる表現型(病気)と一致して伝達される既知のマーカーBが見つかれば，遺伝子Aが直接同定できなくても，遺伝子上でAはBの近傍に存在すると推測することができる。相同組換えの際に1つの集まりとして家系を通じて伝達される対立遺伝子の集まりを**ハプロタイプ**と呼ぶ。遺伝病の家系において，このような多型マーカーのタイピングを全染色体に対して行い，表現型と連鎖するマーカーの組合わせ(ハプロタイプ)を同定し，疾患原因遺伝子の染色体上の部位を特定するのが**連鎖解析(ポジショナルクローニング)**である。**図1B**は先天性QT延長症候群(LQTS)の原因遺伝子*KCNH2*(HERG)を最初に同定した連鎖解析の論文である[1]。このような連鎖解析を中心とした手法を用いて，LQTSをはじめとするまれで単一遺伝子性不整脈の原因遺伝子が次々と明らかになった。

ハプロタイプは，このような単一遺伝子疾患ばかりではなく，心房細動をはじめとするさまざまな多因子遺伝子疾患の研究アプローチとしても用いられている。例えばレニン，アンジオ

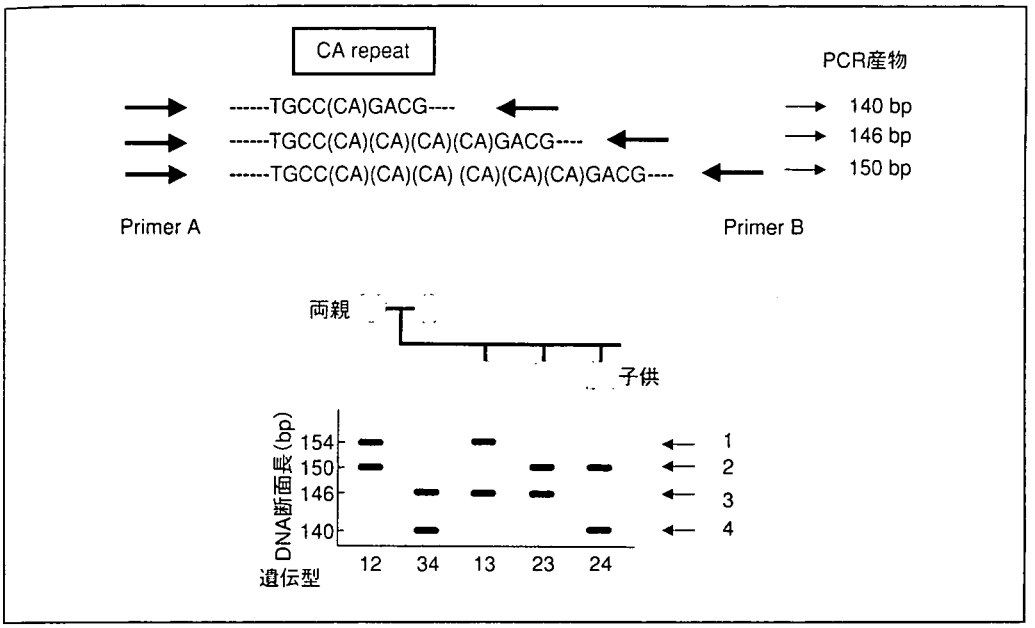


図 1A　CA リピートの多型タイピング
ここには繰り返し数が異なる 3 つの CA 直列配列が示されている。CA の繰り返し数は上から 1 回，4 回，6 回である。その両側の配列は同一であるため，そこにプライマー A, B を設計して PCR すると，PCR 産物の長さはそれぞれ 140 bp, 146 bp, 150 bp と CA 繰り返し配列の分だけ異なる。PCR 産物はゲル電気泳動で長さを見分けられるので，例えば 140 bp, 146 bp, 150 bp, 154 bp をそれぞれ 1, 2, 3, 4 と番号づけをすると，家系内の個人の遺伝型は 2 つの対立遺伝子の CA リピートの長さによって，左から 12, 34, 13, 23, 24 とタイピングすることができる。

テンシン系(RAS)は心房細動の遺伝子基盤の 1 つと推測されるが，250 名の正常人，250 名の心房細動患者のゲノム DNA を用いて，アンジオテンシノーゲン(AGT)を含めた RAS 遺伝子の SNP 関連解析が行われた[2]。**図 2** に示すように，AGT には，翻訳領域にアミノ酸置換型の SNP が 2 つ(G 6A, G2 17 A)と 5'非翻訳領域に SNP が 4 つある。減数分裂の際にもしこれらの SNP の間にランダムに相同組換えが入るとすれば，ハプロタイプの種類は理論的には 2^6 (= 64)になる。しかし，この遺伝子領域はなかばひとかたまりになって組換えが起こる(**連鎖不均衡**：linkage disequilibrium ; LD)傾向が強いため，12 種類のハプロタイプしかなかった。それぞれのハプロタイプの出現頻度を正常群・心房細動群で比較すると，心房細動群における出現頻度が低いものと(→)逆に高いもの(*)があった。これらの研究から，RAS が心房細動の疾患関連因子であることが証明された。

このような知見をさらに拡げると，全ゲノムの SNP をタイピングすれば，理論的には，疾患にかかわる未知の遺伝的要因を網羅的に検索し同定することができると考えられる。相同組換えがゲノム上でランダムかつ均等に起こっていると仮定すると，300 万個の全ゲノムの SNP を数千人規模の患者と対照群で解析することになり，100 億の SNP タイピングという非現実的な作業が必要となる。しかし，前述したように，相同組換えは染色体上で均一に起こっているのではなく，ゲノムには，組換えが高頻度に起こる部位(組換えホットスポット)と連鎖不均衡の強い部分(LD ブロック)による分節構造をとることが最近の研究から明らかになった[3]。したがって，組換えがほとんど起こらない LD ブロック内に存在する SNP の組合わせ(ハプロタイプ)を慎重に選ん

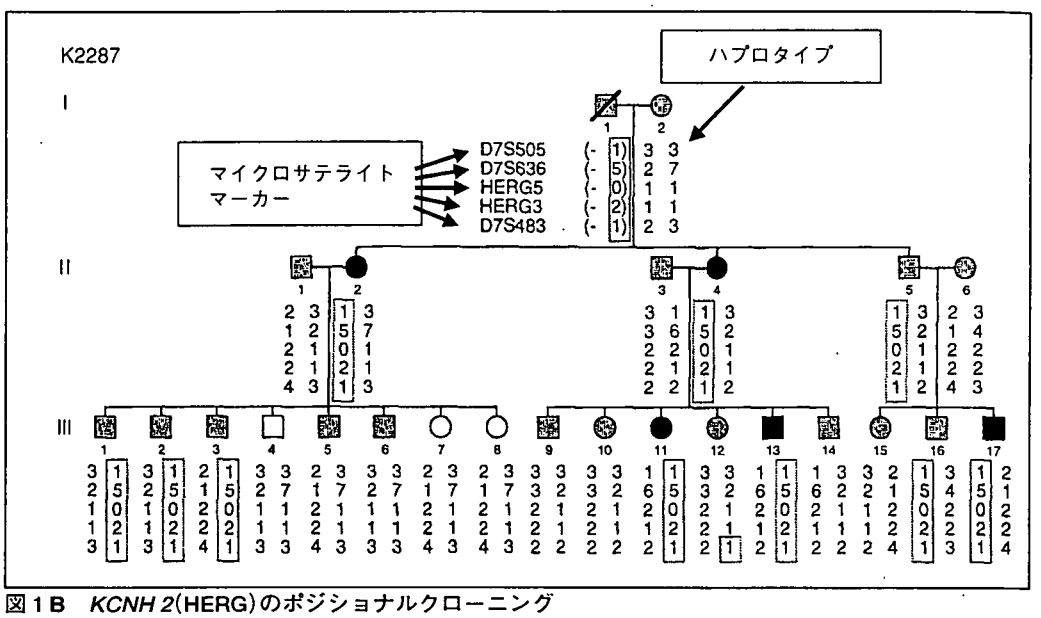
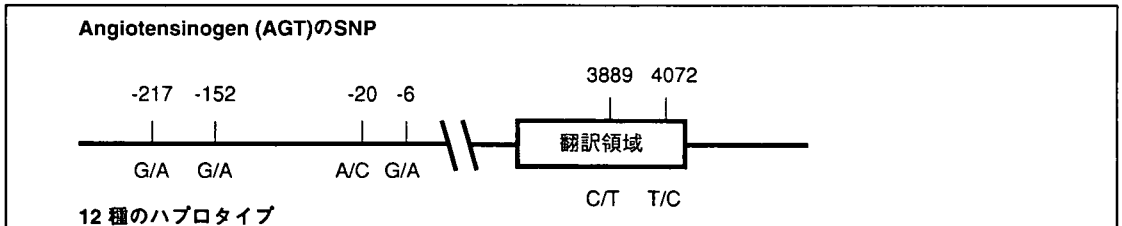


図 1 B　*KCNH 2*(HERG)のポジショナルクローニング
LQTS の患者・正常人をそれぞれ黒・白で，判定困難または臨床像が不明なものは灰色で示している。D 7S505 や HERG 5 などは第 7 染色体に存在するマイクロサテライトマーカーの名前で，その遺伝型が数字で示されている。15021 というマーカーの組合わせ（ハプロタイプ）をもつ者が高率に QT 延長を示しており，本家系の QT 延長原因遺伝子はこれらのマーカー近傍にあると推測される。



12 種のハプロタイプ

	Haplotype*						Overall	AF	Controls		
	-217	-152	-20	-6	3889†	4072†	(n=500)	(n=250)	(n=250)	OR	P‡
	G	G	A	A	C	C	0.562	0.545	0.586	1.2	0.108
→	A	G	A	A	C	C	0.136	0.112	0.154	1.4	0.047
→	A	A	A	A	C	C	0.011	0.000	0.020	22.4	0.017
	G	G	C	A	C	C	0.016	0.015	0.012	0.8	0.705
	G	A	C	A	C	C	0.014	0.008	0.020	2.5	0.171
	G	G	A	G	C	C	0.022	0.029	0.013	0.4	0.080
	G	G	A	A	T	C	0.043	0.041	0.047	1.1	0.744
	A	G	A	A	T	C	0.011	0.005	0.016	3.3	0.231
	G	G	C	A	T	C	0.046	0.058	0.034	0.6	0.083
*	G	G	A	A	C	T	0.042	0.069	0.016	0.2	0.0002
	G	G	A	G	C	T	0.060	0.069	0.053	0.8	0.328
	G	G	A	G	T	T	0.011	0.015	0.004	0.3	0.107
	Log likelihoods						-1432.8	-737.1	-664.4	62.5§	0.0002

図 2　アンジオテンシノーゲン(AGT)の SNP を用いた心房細動の関連解析

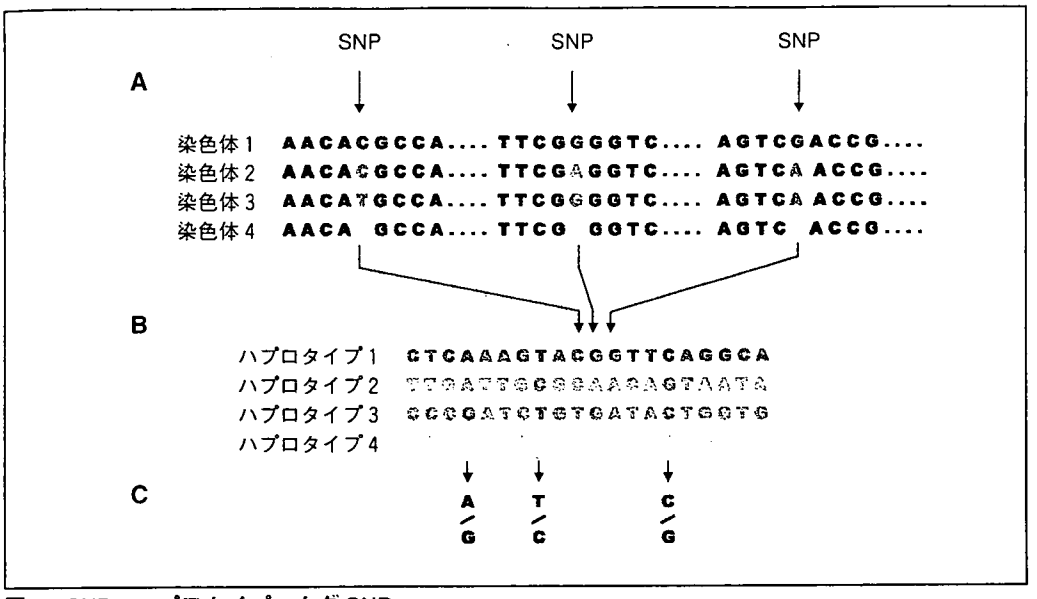


図3　SNP，ハプロタイプ，タグSNP

A. SNP：4名の同じ染色体領域における4種類のDNA断片が例示されている。これらの染色体では，DNA塩基配列の大部分は同一だが，多型の存在する塩基部位が3個示されている。それぞれのSNPでは，2種類の対立遺伝子のいずれかになる可能性がある。最初のSNPには対立遺伝子CとTがある。

B. ハプロタイプ：複数の近傍のSNPにおける対立遺伝子の組合わせによって構成されている。ここでは，6,000塩基長のDNAに存在する20個のSNPについて観察された遺伝子型が示されている。多型塩基のみが示されており，図A.に示された3個のSNPも含まれている。この染色体領域の場合には，集団調査で解析された染色体の大部分がハプロタイプ1～4のいずれかに分類されることが判明している。

C. タグSNPs：SNP20個のSNPのうち，わずか3個のタグSNPについて遺伝子型を判定できれば，これらの4つのハプロタイプを一意に特定できる。例えば，これらの3つのタグSNPがA-T-Cというパターンの染色体は，ハプロタイプ1のパターンと一致する。個々の集団では，共通のハプロタイプを含む染色体の数が多いことがわかる。

で遺伝子型を判定すれば，この領域で高頻度に存在するハプロタイプの特定に必要なタグSNPはわずかな数ですむ(**図3**　http：//www.natureasia.com/campaign/hapmap/HapMap-J.pdf日本語訳監修中村祐輔の抜粋)。このように，ゲノムのLD構造と代表的なSNPタイピングに関する体系的，網羅的なデータが得られれば，多因子遺伝子疾患の疾患感受性遺伝子をゲノムワイドで効率良く探索することが可能になる。この目的のために，2002年に「国際**HapMap**プロジェクト」が発足し，アフリカ，アジアとヨーロッパを起源とする269名のDNAサンプルを用いて100万カ所以上の一般的なSNPのタイピングが行われ，ハップマップ(HapMap；ハプロタイプ地図の略称)が作成された。その結果は2005年，ヒトゲノムに存在する頻度の高い一般的な多型の公共データベースとして最初のデータが公開された(http：//www.hapmap.org/index.html.ja)[4]。

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読者と一緒に考えるQ&A

Question **なぜ人により後天的に Tdp を発症したりしなかったりするのか。それは遺伝子だけの問題か**

もし自分が発症したら子孫にどの程度伝わるのか。それを知る方法はあるのか

Answer ：後天性 LQTS は多因子疾患であり，その発症は年齢・性・基礎心疾患の有無と程度・肝腎機能・電解質・薬物代謝酵素活性・併用薬剤などさまざまな要因が複雑に絡み合って決定づけられる。Tdp に対する最後の砦ともいうべき心筋再分極予備能にも個人差があるため，失神などの症状や家族歴，心電図の QT 延長がなければ，Tdp を発症前から予測するのはきわめて困難である。しかし，後天性 LQTS の少なくとも一部は先天性 LQTS の無症候性キャリアであり，発端者の遺伝子異常が同定されていれば，それは 50%の確率で子孫に伝わるということがいえる。心筋再分極は遺伝子異常の程度や上記のさまざまな要因の影響を大きく受けるため，キャリアが必ず発症するとはかぎらないが，少なくとも経過観察は必要であり，QT 延長薬剤の使用や低カリウム血症を回避させるなどの生活指導も重要である。一方，発端者の遺伝子異常が不明の場合，子孫が発症するか否かを判断する方法は，現時点では心電図をはじめとする臨床検査のみである。

Sex Hormone and Gender Difference—Role of Testosterone on Male Predominance in Brugada Syndrome

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Testosterone in Brugada Syndrome. ***Introduction:*** **The clinical phenotype is 8 to 10 times more prevalent in males than in females in patients with Brugada syndrome. Brugada syndrome has been reported to be thinner than asymptomatic normal controls. We tested the hypothesis that higher testosterone level associated with lower visceral fat may relate to Brugada phenotype and male predominance.**

Methods and Results: **We measured body-mass index (BMI), body fat percentage (BF%), and several hormonal levels, including testosterone, in 48 Brugada males and compared with those in 96 age-matched control males. Brugada males had significantly higher testosterone (631 ± 176 vs 537 ± 158 ng/dL; P = 0.002), serum sodium, potassium, and chloride levels than those in control males by univariate analysis, and even after adjusting for age, exercise, stress, smoking, and medication of hypertension, diabetes, and hyperlipidemia, whereas there were no significant differences in other sex and thyroid hormonal levels. Brugada males had significantly lower BMI (22.1 ± 2.9 vs 24.6 ± 2.6 kg/m^2; P < 0.001) and BF% (19.6 ± 4.9 vs 23.1 ± 4.7%; P < 0.001) than control males. Testosterone level was inversely correlated with BMI and BF% in both groups, even after adjusting for the confounding variables. Conditional logistic regression models analysis showed significant positive and inverse association between Brugada syndrome and hypertestosteronemia (OR:3.11, 95%CI:1.22–7.93, P = 0.017) and BMI (OR:0.72, 95%CI:0.61–0.85, P < 0.001), respectively.**

Conclusions: **Higher testosterone level associated with lower visceral fat may have a significant role in the Brugada phenotype and male predominance in Brugada syndrome.** *(J Cardiovasc Electrophysiol, Vol. 18, pp. 415-421, April 2007)*

Brugada syndrome, gender, sex hormones, testosterone, body mass index

Introduction

Brugada syndrome is characterized by coved-type ST-segment elevation in the right precordial electrocardiographic (ECG) leads (V1–V3) and an episode of ventricular fibrillation (VF) in the absence of structural heart disease.[1-5] The prevalence of the disease is estimated to be up to 5 per 10,000 inhabitants and is one of the important causes of sudden cardiac death of middle-aged males, particularly in Asian countries including Japan.[4]

More than eight dozen distinct mutations in *SCN5A*, the gene encoding the α subunit of the sodium channel, have been so far identified in patients with Brugada syndrome and all mutations display an autosomal-dominant mode of transmission.[6,7] Therefore, males and females are expected to inherit the defective gene equally. However, more than 80% of patients in Western countries and more than 90% of patients in Asian countries affected with Brugada syndrome are males.[8] Recent experimental studies have unveiled the cellular mechanism of Brugada phenotype. The male predominance in the Brugada syndrome is suggested to be due, at least in part, to intrinsic differences in ventricular action potential (AP) between males and females.[9]

A male hormone, testosterone is reported to increase net outward currents[10-12] and is expected to accentuate Brugada phenotype, such as ST-segment elevation and subsequent episodes of VF in patients with Brugada syndrome. Testosterone is also known to decrease visceral fat.[13-15] Since patients with Brugada syndrome have been reported to be

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thinner than asymptomatic normal controls by Matsuo et al.,[16] we speculated that higher testosterone level associated with lower visceral fat may modulate Brugada phenotype and may relate to male predominance in patients with Brugada syndrome.

Methods

Patient Population and Data Collection

The study population consisted of 48 males with Brugada syndrome who agreed to participate in this study and showed Type 1 "coved" ST-segment elevation in V1–V3 leads[17] ranging in age from 30 to 69 years with a mean age of 50 ± 11 years (mean ± SD). Brugada males who were less than 30 years old and more than 70 years old were excluded from this study to minimize the influence of age on the basal sex hormonal levels including testosterone. Forty of the forty-eight Brugada males have been included in our previous clinical studies.[18-20] In all patients, physical examination, chest roentgenogram, laboratory values, echocardiography with wall motion analysis, and Doppler screening excluded structural heart diseases. The clinical, electrocardiographic, and electrophysiologic characteristics of the 48 Brugada males are shown in Table 1. Average age of the 48 Brugada males at diagnosis was 47 ± 12 years old. Aborted cardiac arrest or VF was documented in 21 males (44%), syncope alone in 11 males (23%), and 16 males (33%) were asymptomatic. Family history of sudden cardiac death (SCD) was observed in eight males (17%). An *SCN5A* coding region mutation was identified in seven (17%) of 42 males in whom genetic screening was conducted. Implantable cardioverter defibrillator (ICD) was implanted in all 32 symptomatic males with documented VF and/or syncope. ICD was also implanted in nine of 16 asymptomatic males due to induction of VF during the electrophysiologic study. Type 1 ST-segment elevation was recorded spontaneously in 43 males (90%) and was induced by sodium channel blockers in five males (10%). Complete right bundle branch block was observed in three males (6%). Late potential was recorded by a signal-average ECG system in 27 (59%) of 46 males. During the electrophysiologic study, VF requiring direct cardioversion for termination was induced in 32 (73%) of 44 males. Average HV interval was 46 ± 11 msec.

TABLE 1
Clinical, Electrocardiographic, and Electrophysiologic Characteristics in the 48 Brugada Males

Clinical characteristics	
Age at diagnosis (years)	47 ± 12
Aborted cardiac arrest or VF (%)	21/48 (44%)
Syncope alone (%)	11/48 (23%)
Asymptomatic (%)	16/48 (33%)
Family history of SCD	8/48 (17%)
SCN5A mutation	7/42 (17%)
ICD implantation	41/48 (85%)
Follow-up period (month)	41 ± 2
Arrhythmic event (%)	9/48 (19%)
Electrocardiographic characteristics	
Spontaneous coved-type ST elevation	43/48 (90%)
CRBBB (%)	3/48 (6%)
RR (msec)	939 ± 113
PQ interval (II) (msec)	186 ± 34
QRS duration (V2) (msec)	104 ± 18
Corrected QT interval (V5) (msec)	394 ± 27
ST amplitude at J point (V2) (mV)	0.32 ± 0.16
Late potential (%)	27/46 (59%)
Electrophysiologic characteristics	
Induction of VF	32/44 (73%)
Mode (Triple/Double/Single)	16/15/1
HV interval (msec)	46 ± 11

CRBBB = complete right bundle branch block; ICD = implantable cardioverter defibrillator; SCD = sudden cardiac death; VF = ventricular fibrillation.

We first obtained data, such as the hormonal levels, visceral fat parameters, and ECG parameters in the 48 Brugada males prospectively between January and July in 2003, mainly at regular outpatient clinics for checking ICD. Only a Brugada male refused to participate during the recruitment of the case.

Thereafter, age-matched control males were randomly selected from the municipal population registry in Suita City. The hormonal and visceral fat data were collected sequentially between August and December in 2003. The municipal population registry in Suita City included 5,846 control subjects, among whom 1,052 males were age-matched to the 48 Brugada males. The 96 control males with a mean age of 50 ± 11 years were sequentially recruited from the age-matched 1,052 males. None of the recruited 96 control males refused to participate in this study. There were no significant differences in the clinical characteristics between the 96 control males and the remaining 956 age-matched males. Therefore, we had no way of knowing the body weight of the individuals who were selected to serve as controls from a very large database. Although K. Matsuo is a co-author of this study, none of the Brugada males and control males who appeared in the article by Matsuo[16] are included in the present study population.

All protocols were approved by the Ethical Review Committee in the National Cardiovascular Center. Written informed consent was obtained from all subjects.

Sex and Thyroid Hormonal Levels and Serum Electrolytes

Blood samples for analysis of basal hormone levels and serum electrolytes were obtained between 8:00 and 9:00 AM after an overnight fast. Plasma sex hormonal levels including testosterone, estradiol, DHEA-S, LH, and FSH were measured using commercially prepared immunoassay kits (testosterone, LH, and FSH: Chemiluminescent immunoassay [Bayer HealthCare, New York, NY, USA]; estradiol: Electrochemiluminescent immunoassay [Roche Diagnostics GmbH, Mannheim, Germany]; DHEA-S: Radioimmunoassay [Diagnostic Products Corporation, Los Angeles, CA, USA]). Thyroid hormonal levels including free T3, T4, and TSH, and serum electrolyte levels including sodium, potassium, and chloride were also measured.

Body Mass Index and Body Fat Percentage

Body weight (BW) was measured to the nearest 0.1 kg and height to the nearest cm. Body-mass index (BMI) was calculated as weight/height^2 (kg/m^2) as a parameter of visceral fat. We also measured body-fat percentage (BF%) by using body composition analyzer (Biospace Co., Ltd. Tokyo, Japan). These visceral fat parameters were measured just after blood sampling. In the 32 symptomatic Brugada males who had had documented VF and/or syncope, the BW and BMI were also measured within 48 hours after their clinical events during admission in our hospital or other emergent hospitals.

ECG Parameters

In the 48 males with Brugada syndrome, 12-lead ECG was recorded just before blood sampling, and ECG parameters were assessed by an investigator (WS) blinded to clinical information. The ECG parameters included RR interval, PQ interval measured in lead II, QRS interval measured in lead V2, QT interval, corrected QT (QTc) interval measured in leads V5, and ST amplitude at J point measured in lead V2.

Statistical Analysis

We first conducted univariate analysis by using unpaired *t*-test to compare each data between the Brugada males and the control males. Since several confounding variables, such as age, exercise (none, sometimes, regularly), stress (none, sometimes, regularly), current smoking (no, yes), and medication (no, yes) of hypertension, diabetes, and hyperlipidemia may affect the hormonal levels including testosterone level and the visceral fat parameters, analysis of covariance (ANCOVA) was used to compare least square mean values between the Brugada males and the control males adjusting for these confounding variables. Pearson's correlation coefficients were calculated between the testosterone level and the visceral fat parameters. Partial correlation coefficients were calculated between the testosterone level and the visceral fat parameters after adjusting for age, exercise, stress, current smoking, and medication. Moreover, conditional logistic regression models were used to calculate odds ratios and 95% confidence intervals adjusting for age, BMI, exercise, stress, current smoking, hypertension, diabetes, and hyperlipidemia. Hypertestosteronemia was defined as serum testosterone levels $\geq$700 ng/dL, which is 75 percentiles of testosterone levels among case and control combined groups. In the 32 Brugada males with documented VF and/or syncope, a paired *t*-test was used to compare the visceral fat parameters at the clinical cardiac events and at the measurement of hormonal and visceral fat data. A two-sided P value below 0.05 was considered to indicate significance. All statistical analyses were performed by using SAS software, Ver 8.2.

Results

Hormonal Levels, Serum Electrolytes, and Visceral Fat

Table 2 illustrates univariate analysis for comparing sex and thyroid hormonal levels, serum electrolytes, and visceral fat parameters between the two groups. Testosterone level was significantly higher in the Brugada males than in the control males, whereas there were no significant differences in other sex hormonal levels; estradiol, DHEA-S, LH, FSH, and thyroid hormonal levels; T3, T4, and TSH. Serum sodium, potassium, and chloride levels were all significantly higher in the Brugada males than in the control males. BMI, BF%, and BW were all significantly lower in the Brugada males than in the control males. All variables followed normal distribution, both in the 48 Brugada and 96 control males.

The comparison of the confounding variables that may affect the hormonal levels and the visceral fat parameters between the 48 Brugada males and the 96 control males was shown in Table 3. Even after adjusting for age, exercise, stress, current smoking, and medication (hypertension, diabetes, and hyperlipidemia), the testosterone level, serum sodium, potassium, and chloride levels were all significantly higher, and the visceral fat parameters were significantly lower in the 48 Brugada males than in the 96 control males (Table 4). There were also significant differences in these parameters between the 24 definite Brugada males with documented VF and/or *SCN5A* mutations and the 96 control males after adjusting for the confounding variables (Table 4).

Correlation between Testosterone, Visceral Fat, and Serum Electrolytes

Testosterone level was inversely correlated with all visceral fat parameters, BMI, BF%, or BW in both the Brugada males and the control males, even after adjusting for age,

TABLE 2

Sex and Thyroid Hormonal Levels, Serum Electrolytes, and Visceral Fat Parameters in the 48 Brugada Males and the 96 Age-Matched Control Males

	Brugada Males (n = 48)	Control Males (n = 96)	P Value
Sex hormones			
Testosterone (ng/dL)	631 ± 176	537 ± 158	0.002
Estradiol (pg/mL)	28.9 ± 7.6	31.1 ± 12.6	0.263
DHEA-S (ng/mL)	1,901 ± 850	1,966 ± 861	0.668
LH (mIU/mL)	4.6 ± 2.6	3.9 ± 2.0	0.073
FSH (mIU/mL)	6.2 ± 4.9	5.0 ± 2.9	0.066
Thyroid hormones			
Free T3 (pg/mL)	3.3 ± 0.4	3.4 ± 0.3	0.360
Free T4 (ng/dL)	1.3 ± 0.1	1.3 ± 0.2	0.089
TSH (μIU/mL)	1.9 ± 1.4	1.7 ± 1.4	0.619
Serum electrolytes			
Sodium (mEq/L)	143.7 ± 2.0	142.6 ± 2.0	0.003
Potassium (mEq/L)	4.6 ± 0.3	4.3 ± 0.3	<0.001
Chloride (mEq/L)	105.1 ± 2.1	103.6 ± 2.1	<0.001
Viceral fat			
BMI (kg/m^2)	22.1 ± 2.9	24.6 ± 2.6	<0.001
BF% (%)	19.6 ± 4.9	23.1 ± 4.7	<0.001
BW (kg)	62.9 ± 9.7	70.0 ± 8.6	<0.001

Values are mean ± SD where indicated.
BMI = body-mass index; BF% = body-fat percentage; BW = body weight.

TABLE 3

Comparison of the Confounding Variables Between the 48 Brugada Males and the 96 Age-Matched Control Males

	Brugada Males (n = 48)	Control Males (n = 96)	P Value
Exercise			
None (%)	39.6	44.8	
Sometimes (%)	41.6	43.8	
Regularly (%)	18.8	11.5	0.482
Stress			
None (%)	27.1	21.9	
Sometimes (%)	54.2	54.2	
Regularly (%)	18.8	24.0	0.684
Current smoking (%)	25.0	27.1	0.789
Medication			
Hypertension (%)	20.8	19.8	0.883
Diabetes (%)	2.1	13.5	0.028
Hyperlipidemia (%)	10.4	5.2	0.246

TABLE 4

Testosterone, Serum Electrolytes, and Visceral Fat Parameters in the Brugada Males and the 96 Age-Matched Control Males after Adjusting for Confounding Variables

	Brugada Males	Control Males (n = 96)	P Value
ALL Case (n = 48)			
Testosterone (ng/dL)	631 ± 44	538 ± 40	0.003
Sodium (mEq/L)	144.2 ± 0.5	143.2 ± 0.5	0.007
Potassium (mEq/L)	4.6 ± 0.1	4.3 ± 0.1	<0.001
Chloride (mEq/L)	105.5 ± 0.5	103.9 ± 0.5	<0.001
BMI (kg/m^2)	22.3 ± 0.7	24.9 ± 0.7	<0.001
BF% (%)	20.0 ± 1.3	23.9 ± 1.1	<0.001
BW (kg)	63.4 ± 2.4	70.1 ± 2.1	0.001
Definite Brugada case with VF and/or *SCN5A* (n = 24)			
Testosterone (ng/dL)	656 ± 59	550 ± 48	0.009
Sodium (mEq/L)	143.9 ± 0.7	142.9 ± 0.6	0.042
Potassium (mEq/L)	4.7 ± 0.1	4.4 ± 0.1	<0.001
Chloride (mEq/L)	105.2 ± 0.7	103.9 ± 0.6	0.006
BMI (kg/m^2)	21.5 ± 1.0	24.5 ± 0.8	<0.001
BF% (%)	19.9 ± 1.7	24.1 ± 1.4	<0.001
BW (kg)	60.5 ± 3.1	69.2 ± 2.5	0.001

Values are mean ± SE adjusted for age, exercise, stress, current smoking, and medication of hypertension, diabetes and hyperlipidemia. BMI = body-mass index; BF% = body-fat percentage; BW = body weight; VF = ventricular fibrillation.

exercise, stress, current smoking, and medication (Brugada: BMI, r = –0.394, P = 0.011; BF%, r = –0.390, P = 0.012; BW, r = –0.335, P = 0.032; Control: BMI, r = –0.333, p = 0.002; BF%, r = –0.333, P = 0.001; BW, r = –0.305, P = 0.004), suggesting that Brugada males had higher testosterone level associated with lower visceral fat compared with control males (Fig. 1). No significant correlations were observed between other serum electrolytes and testosterone level or visceral fat parameters. Testosterone level was not correlated with age, even after adjusting for exercise, stress, current smoking, and medication (r = 0.007, P = 0.947).

Conditional Logistic Regression Models Analysis

Conditional logistic regression models analysis showed significant positive and inverse association between Brugada syndrome, hypertestosteronemia (Odd Ratio (OR): 3.11, 95%CI: 1.22–7.93, P = 0.017), and BMI (OR: 0.72, 95%CI: 0.61–0.85, P < 0.001), respectively (Table 5). Other variables did not significantly increase or decrease risks of Brugada syndrome (Table 5).

Visceral Fat at Clinical Cardiac Events in Brugada Males

In the 32 symptomatic Brugada males with documented VF and/or syncope, the time-span between the clinical cardiac events and the measurement of hormonal and the visceral fat data was 42 ± 32 months (mean ± SD, 1–99 months). The BMI and BW at the clinical cardiac events (VF or syncope) were significantly lower than those at the measurement of hormonal and visceral fat data (BMI, 21.0 ± 2.6 vs 22.1 ± 2.9 kg/m^2; BW, 60.0 ± 8.9 vs 62.9 ± 9.7 kg: P < 0.001, respectively).

Testosterone versus ECG Parameters, Symptoms or SCN5A Mutation in Brugada Males

Baseline electrocardiographic data of the 48 Brugada males are shown in Table 1. No significant correlations were observed between testosterone level and ECG parameters, including ST amplitude (r = –0.123, P = 0.406) and QTc interval (r = –0206, P = 0.160), in the 48 Brugada males. There was no significant difference in testosterone level between 32 symptomatic and 16 asymptomatic Brugada males (649 ± 185 vs 593 ± 157 ng/dL: P = 0.298). No significant difference was observed in testosterone level between 43 Brugada males with spontaneous Type 1 ST-segment elevation and five Brugada males with sodium channel blocker-induced Type 1 ST-segment elevation (624 ± 171 vs 688 ± 230 ng/dL: P = 0.448). Testosterone level was also no different between seven Brugada males with *SCN5A* mutation and 41 Brugada males without *SCN5A* mutation (700 ± 198 vs 619 ± 172 ng/dL: P = 0.261).

Follow-Up

Arrhythmic events occurred in nine (19%) of 48 Brugada males during average follow-up periods of 41 ± 2 months after blood sampling for the present study (Table 1). In more detail, arrhythmic events appeared in eight (38%) of 21 Brugada males with a history of aborted cardiac arrest or VF, in one (9%) of 11 Brugada males with syncope alone, but did not appear in any (0%) of 16 asymptomatic Brugada males.

Discussion

The major findings of the present study were: (1) Brugada males had significantly higher testosterone level, serum sodium, potassium, and chloride level, and significantly lower BMI, BF%, and BW than those in control males by univariate analysis, even after adjusting for age, exercise, stress, current smoking, and medications related to hypertension, diabetes and hyperlipidemia. (2) Testosterone level was inversely correlated with the BMI, BF%, and BW in both Brugada males and control males, even after adjusting for the confounding variables. (3) Conditional logistic regression models analysis showed strong positive association between Brugada syndrome and higher testosterone level (hypertestosteronemia) and strong inverse association between Brugada syndrome and BMI.

Testosterone in Brugada Phenotype and Male Predominance

For the past decade, numerous clinical, experimental, and molecular genetic studies have elucidated Brugada syndrome as a distinct clinical entity.[1-5,17] However, several problems remain unresolved, such as genetic heterogeneity, ethnic difference, and gender difference.[7] Di Diego and Antzelevitch recently suggested the cellular basis for male predominance in Brugada syndrome by using arterially perfused canine right ventricular wedge preparations.[9] Transient outward current (I_{to})-mediated phase 1 AP notch was larger in male dogs than in female dogs in the right ventricular epicardium, but not in the left ventricular epicardium, responsible for the male predominance in the Brugada phenotype. Recent clinical studies suggested that male hormone testosterone might be attributable to gender difference of the prevalence in this

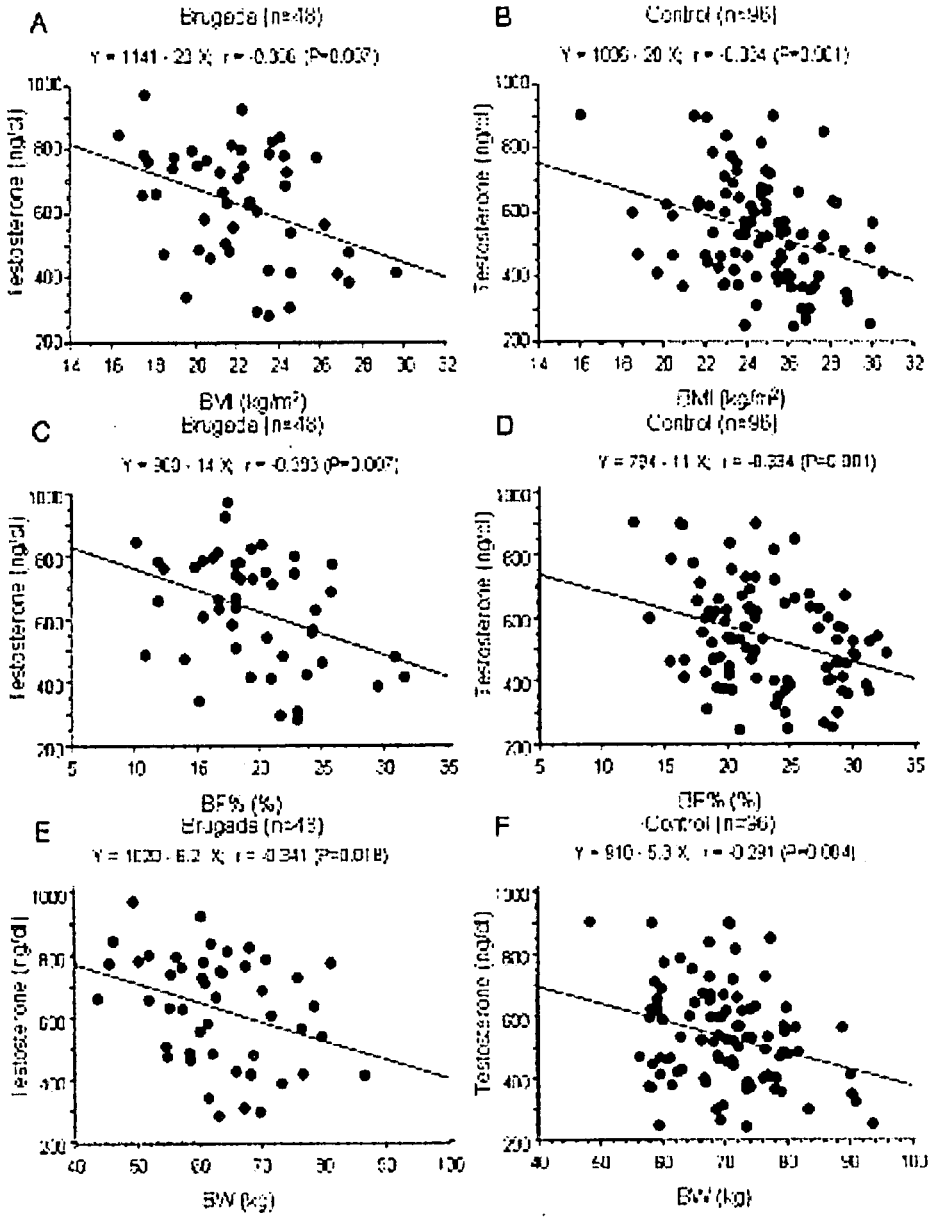

Figure 1. *Correlation between testosterone level and visceral fat parameters; body mass index (BMI) (A and B), body fat percentage (BF%) (C and D), and body weight (BW) (E and F) in the 48 Brugada males and the 96 age-matched control males. Testosterone level was inversely correlated with the BMI, BF%, or BW in both Brugada males and control males.*

syndrome. Matsuo et al. reported two cases of asymptomatic Brugada syndrome in whom typical coved ST-segment elevation disappeared following orchiectomy as therapy for prostate cancer,[21] indicating that testosterone may contribute to the Brugada phenotype in these two cases. Several experimental studies reported that testosterone increased outward potassium currents, such as the rapidly activating component (I_{Kr})[10,11] and the slowly activating component (I_{Ks})[12] of the delayed rectifier potassium current, and the inward rectifier potassium current (I_{K1}),[11] or decreased inward L-type calcium current (I_{Ca-L}).[12] Since the maintenance of the AP dome is determined by the fine balance of currents active at the end of phase 1 of the AP (principally I_{to} and I_{Ca-L}),[22,23] any agents that increase outward currents or decrease inward currents can increase the magnitude of the AP notch, leading to loss of the AP dome (all-or-none repolarization) in the epicardium, but not in the endocardium, contributing to a significant voltage gradient across the ventricular wall during ventricular activation, thus augmenting ST-segment elevation, the Brugada phenotype.[24] Therefore, testosterone would be expected to accentuate the Brugada phenotype. In the present study, males with Brugada syndrome had significantly higher testosterone level than age-matched control males, even after adjusting for age, exercise, stress, current smoking, and medication (hypertension, diabetes, and hyperlipidemia), which may affect the testosterone level. Moreover, conditional logistic regression models analysis showed strong positive association between Brugada syndrome and higher testosterone level (OR: 3.11). Our data suggest a significant role of testosterone, male hormone, in the Brugada phenotype. The

TABLE 5
Odds Ratios of Presence of Hypertestosteronemia and Confounding Risk Factors for Brugada Syndrome in Males

Variable	Odd Ratio	95% Confidence Interval	P Value
Hypertestosteronemia	3.11	1.22–7.93	0.017
Age	0.99	0.95–1.03	0.637
BMI	0.72	0.61–0.85	<0.001
Exercise	1.57	0.87–2.83	0.135
Stress	0.69	0.35–1.35	0.277
Current smoking	0.71	0.26–1.90	0.493
Hypertension	3.12	0.85–11.45	0.087
Diabetes	0.13	0.01–1.27	0.079
Hyperlipidemia	2.14	0.44–10.49	0.348

Hypertestosteronemia was defined as serum testosterone levels ≥ 700 ng/dL.

data also indicate that the male predominance in the Brugada phenotype is at least in part due to testosterone, which is present only in males.

Lower Visceral Fat May Be a Predictor for Brugada Phenotype

Matsuo et al. recently reported in their epidemiologic study that cases with the Brugada-type ECG had significantly lower BMI than that in control subjects.[16] Similarly, in the present study, males with Brugada syndrome had significantly lower visceral fat parameters, BMI, BF%, and BW than those in age-matched control males, even after adjusting for several confounding variables. Moreover, conditional logistic regression models analysis showed strong inverse association between Brugada syndrome and BMI (OR: 0.72). All of the visceral fat parameters were inversely correlated with testosterone level in both Brugada and control males, even after adjusting for the confounding variables. It has been well demonstrated that testosterone level in obese males is decreased compared to normal males of similar age.[13] Tsai et al. reported that lower baseline total testosterone level independently predicted an increase in visceral fat in the Japanese-American male cohort for 7.5 years.[15] Reversely, Marin et al. reported that testosterone treatment of middle-aged abdominally obese males was followed by a decrease of visceral fat mass measured by computerized tomography.[14] These data suggest that primarily higher level of testosterone in Brugada males compared to that in control males may result in lower visceral fat in Brugada males, which would be an "innocent bystander" sign of Brugada phenotype. In reverse, if primary lower visceral fat (body weight loss) would result in higher testosterone level, the weight loss could be a trigger for Brugada phenotype, just like fever is.[25] It is noteworthy that the visceral fat parameters at the clinical cardiac events (VF or syncope) in the 32 symptomatic Brugada males were significantly lower than those at the time of blood sampling for this study. This indicates that testosterone level is expected to be additively higher at the clinical cardiac events, which may contribute to spontaneous episodes of VF or syncope.

Other Hormonal Levels and Serum Electrolytes

Estradiol, female hormone, is reported to reduce the expression of Kv4.3 channels, which are important molecular components of I_{to} currents.[26] However, in contrast to testosterone, other sex hormonal levels including estradiol were not different between the Brugada males and the control males in the present study. Although thyroid hormones are also demonstrated to alter membrane currents, such as I_{to} and I_{Ca-L},[27,28] no significant differences were observed in the thyroid hormonal levels between the two groups in the present study.

On the other hand, serum sodium, potassium, and chloride levels were all significantly higher in the Brugada males than in the control males, even after adjusting for several confounding variables. Recently, many agents and conditions that cause an outward shift in current activity at the end of phase 1 AP have been known to unmask ST-segment elevation, as found in the Brugada syndrome, leading to the acquired form of this disorder.[4,29] Electrolyte abnormalities, such as hyperkalemia, are reported to amplify ST-segment elevation like that in Brugada syndrome.[30] The lower visceral fat found in the Brugada males is expected to decrease serum level of insulin, leptine, a novel adipocyte-derived hormone, or ghrelin, a novel growth hormone-releasing peptide, suppressing β-adrenergic receptor or plasma norepinephrine level, resulting in an increase of serum potassium level.[31,32] Further studies including measurement of levels of insulin, leptine, and ghrelin will be required to elucidate the precise mechanism.

Study Limitations

Although the testosterone level was significantly higher in the Brugada males than in the control males, no statistically significant correlations were observed between the testosterone level and the ST amplitude in the Brugada males. The degree of the ST-segment elevation is variable between Brugada patients because it is influenced by several factors other than sex hormonal levels or electrolytes levels, such as basal autonomic tone, presence of *SCN5A* mutation, or probably intrinsic current density of I_{to}, etc., in the right ventricular epicardial cells. The threshold of ST-segment elevation for spontaneous induction of VF also varies between Brugada patients. Therefore, the Brugada phenotype, such as ST-segment elevation or spontaneous induction of VF, may correlate with the testosterone level day to day individually (intra-personally) in each Brugada male, but may not correlate among the pooled data obtained from many Brugada males, probably due to inter-person difference of the ST-segment elevation.

There were no significant differences in testosterone level between symptomatic and asymptomatic Brugada males, between Brugada males with spontaneous ST elevation and those with sodium channel blocker-induced ST elevation, or between Brugada males with and without *SCN5A* mutation, all of which are probably due to a relatively small number of Brugada males in the present study. Further evaluation with increasing number of Brugada males will be required.

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Mechanism and New Findings in Brugada Syndrome

Wataru Shimizu, MD; Takeshi Aiba, MD; Shiro Kamakura, MD

Brugada syndrome is a clinical entity characterized by coved type ST-segment elevation in the right precordial electrocardiographic leads (V_{1-3}) and an episode of ventricular fibrillation in the absence of structural heart disease. Although a number of clinical and experimental reports have elucidated the electrocardiographic, electrophysiologic, cellular, and molecular aspects, several problems remain unsolved. Recently developed high-resolution optical mapping techniques in arterially-perfused wedge preparations enable recording of transmembrane action potentials from 256 sites simultaneously at the epicardial surface, thus providing further advances in the understanding of the cellular mechanism of the specific ST-segment elevation and subsequent ventricular arrhythmias. In this review article, new findings relating to several unresolved problems such as gender difference (male predominance) and ethnic difference (higher incidence in Asian population) are also presented. (*Circ J* 2007; **Suppl A:** A-32–A-39)

Key Words: Brugada syndrome; Ethnicity; Gender; Genetics; Mutation; Polymorphism; ST-segment; Ventricular fibrillation

Brugada syndrome (BS) is characterized by coved-type ST-segment elevation in the right precordial electrocardiography (ECG) leads (V_{1-3}) and an episode of ventricular fibrillation (VF) in the absence of acute ischemia, electrolyte abnormalities or structural heart disease![1–8] A type-1 ST-segment elevation, which is defined as a coved ST-segment elevation of ≥0.2 mV at the J point with or without a terminal negative T wave, is required to diagnose BS, regardless of the absence or presence of sodium-channel blockers (Figs 1A,B)[7] A type-1 ST-segment elevation recorded only in the higher V_{1-2} leads (ie, 3rd and 2nd intercostal spaces) has been suggested to show similar prognostic value for subsequent cardiac events as that recorded in the standard V_{1-2} leads (Fig 1C)[7,9,10] A type-2 saddle-back ST-segment elevation alone is not diagnostic for BS (Fig 1B). The prevalence of this syndrome is estimated to be 5 per 10,000 inhabitants, and is one of the important causes of sudden cardiac death of middle-aged males in Asian countries particularly![11,12] BS usually manifests during adulthood, with a mean age of sudden death of 41±15 years, and child cases are rare[7] A family history of unexplained sudden death is present in approximately 20–40% of the population in Western countries, and less (15–20%) in Japan[4,7,13,14] A significant male predominance in BS has long been reported, and more than 80% of patients in Western countries and more than 90% of patients in Asian countries affected with BS are men![15] Since Brugada and Brugada described 8 patients with a history of aborted sudden cardiac death caused by VF as a distinct clinical entity in 1992[1] a number of clinical and experimental reports from around the world have demonstrated the clinical, electrocardiographic, electrophysiologic, cellular, ionic, genetic and molecular features of BS![2–14] However, several problems remain unsolved, such as genetic heterogeneity, late onset of first cardiac events, and gender and ethnic differences[8] In this review article, we present our recent data relating to the cellular and molecular mechanism of BS, the late onset of its clinical manifestation, male predominance, and higher incidence in Asian populations.

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Genetic and Molecular Aspects

Advances in molecular genetics in the past decade have established a link between several inherited cardiac arrhythmias, including BS and long QT syndrome, and mutations in genes encoding ion channels, membrane components or receptors![16] In 1998, the first mutation linked to BS was identified by Chen et al in *SCN5A*![17] the gene encoding the α subunit of the sodium channel. Thereafter, a large family of BS was reported to link to a second locus on chromosome 3, which is close to but different from the *SCN5A* locus;[18] however, specific gene or genes other than *SCN5A* have not yet been identified on chromosome 3. *SCN5A* mutations are reported to account for 18–30% of clinically diagnosed BS patients at present[7] Antzelevitch et al have recently reported that 3 probands associated with a BS-like ST-segment elevation and a short QT interval were linked to mutations in *CACNA1C* (A39V and G490R) or *CACNB2* (S481L), the gene encoding the α1 or β2b subunit of the L-type calcium channel, respectively![19] Their genetic and heterologous expression studies revealed loss of function of the L-type calcium channel current ($I_{Ca\text{-}L}$). However, approximately two-thirds of BS patients have not been yet genotyped, suggesting the presence of genetic heterogeneity[8] Other candidate genes for the Brugada phenotype include those encoding the transient outward current (I_{to}) and the delayed rectifier potassium current (I_K), or those coding the adrenergic receptors, cholinergic receptors, ion-channel-interacting protein, promoters, transcriptional factors, neurotransmitters, or transporters[7,8]

Among the approximately 100 mutations in *SCN5A* linked to BS, some of them have been studied in expression systems, and have been shown to result in loss of function of the sodium channel current (I_{Na}) by several mechanisms[20]

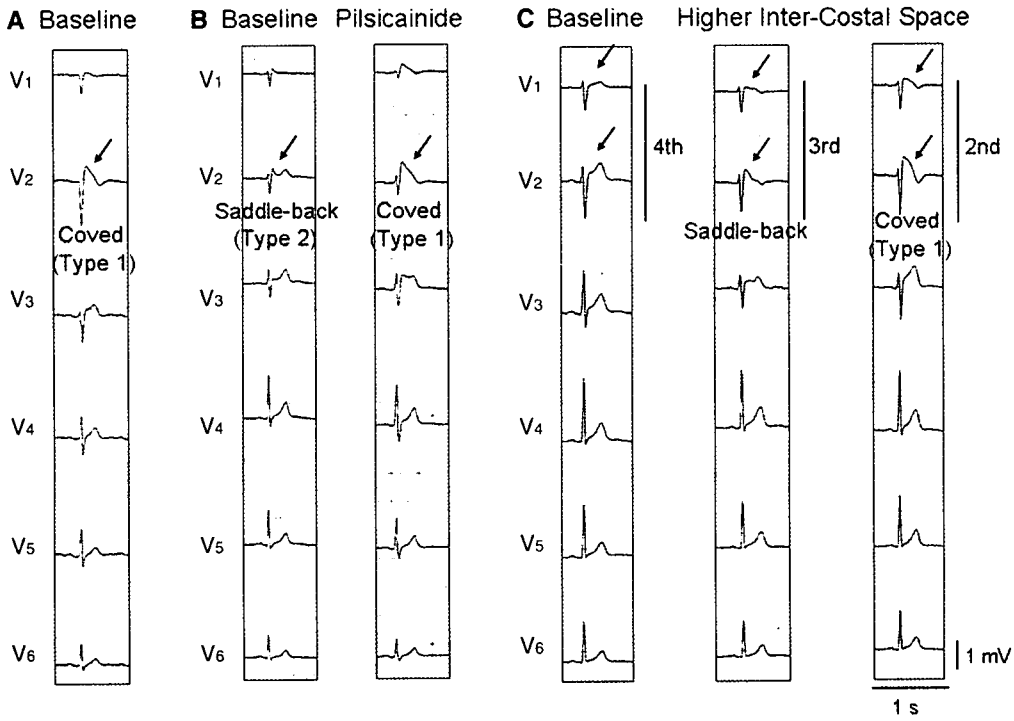

Fig 1. (A) Spontaneous type 1 coved type ST-segment elevation (arrow). (B) Unmasking of ST-segment elevation by a class IC sodium-channel blocker, pilsicainide. Under baseline conditions, type 2 saddle-back type ST-segment elevation is recorded in lead V_2 (Left, arrow). Pilsicainide injection (30 mg) unmasks the type 1 coved type ST-segment elevation in lead V_2 (Right, arrow). (C) Unmasking of the type 1 electrocardiogram (ECG) by recording the right precordial (V_{1-2}) leads at the 3rd and 2nd intercostal spaces. No significant ST-segment elevation is observed in leads V_1 and V_2 of the standard 12-lead ECG (4th intercostal space) (Left, arrow), whereas saddle-back type (Middle, arrow) and type 1 coved type (Right, arrow) ST-segment elevation are unmasked in leads V_1 and V_2 recorded from the 3rd and 2nd intercostal spaces, respectively.

These functional effects include: (1) lack of expression of the sodium channel; (2) a shift in the voltage-dependence and time-dependence of I_{Na} activation, inactivation or reactivation; (3) entry of the sodium channel into an intermediate state of inactivation from which it recovers more slowly; (4) accelerated inactivation of the sodium channel; and (5) a trafficking defect. Some common *SCN5A* polymorphisms are reported to modulate the functional consequences of primary *SCN5A* mutations. Baroudi et al first suggested that the interaction of *SCN5A* polymorphisms and *SCN5A* mutations may affect the consequence of the functional effects. They reported that a common polymorphism (R1232W) of *SCN5A* affected protein trafficking when it was co-expressed with a T1620M mutation, although the T1620M mutation alone produced only gating abnormalities in the I_{Na}[21] On the other hand, another common polymorphism (H558R) of *SCN5A* was reported by Ye et al to rescue normal trafficking and normal I_{Na} for the M1766L mutant protein[22] These effects of common *SCN5A* polymorphisms on modifying the functional consequence of *SCN5A* mutations may make the clinical phenotype more complex.

Cellular Mechanism of Brugada Phenotype

The I_{to}-mediated phase 1 notch of the action potential (AP) has been reported to be larger in the epicardium than in the endocardium in many species, including humans[23] Because the maintenance of the AP dome is determined by the fine balance of currents active at the end of phase 1 of the AP (principally I_{to} and $I_{Ca\text{-}L}$), any interventions that cause a net outward shift in the current active at the end of phase 1 can increase the magnitude of the AP notch, leading to loss of the AP dome (all-or-none repolarization) in the epicardium, but not in the endocardium, contributing to a significant voltage gradient across the ventricular wall during ventricular activation[23] The heterogeneous loss of the AP dome in the epicardium has been shown to produce premature beats via a mechanism of phase 2 reentry in experimental studies using isolated sheets of canine right ventricle[24] Therefore, these mechanism of all-or-none repolarization in the epicardial cells and phase 2 reentry-induced premature beat between the adjacent epicardial cells were expected to be responsible for the clinical phenotype in BS.

In the late 1990s, Antzelevitch's group developed an experimental model of BS using arterially perfused canine right ventricular (RV) wedge preparations, in which transmembrane APs and pseudo-ECGs were simultaneously recorded. These experimental studies have provided significant insights of the cellular mechanism of the Brugada phenotype, ST-segment elevation and subsequent VF[25,26] The I_{to}-mediated AP notch and the loss of the AP dome in the epicardial cells, but not in the endocardial cells, of the right ventricle gives rise to a transmural voltage gradient, producing ST-segment elevation in the ECG in the wedge preparations. Fig 2 shows transmembrane APs simultaneously recorded from 2 epicardial (Epi) and 1 endocardial sites, together with a transmural ECG in a Brugada model using the RV wedge preparation. Under control conditions, a small J wave coincides with the small notch observed in the epicardial cells, but not in the endocardial cells (Fig 2A). Combined administration of terfenadine ($I_{Ca\text{-}L}$ block) and pilsicainide (I_{Na} block) produces a loss of the AP dome in

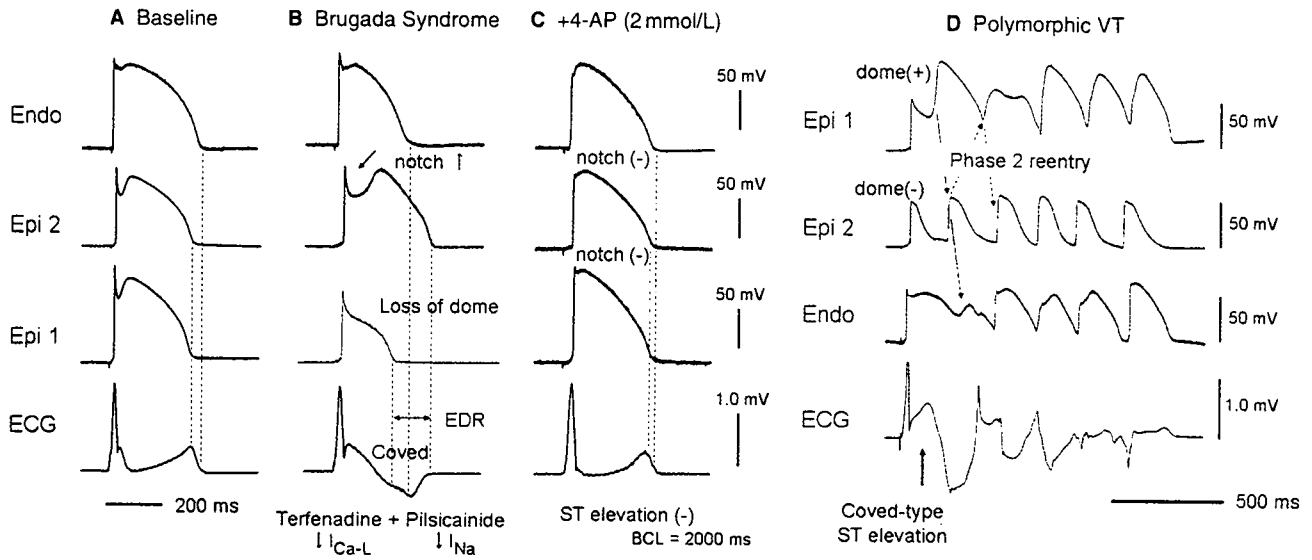

Fig 2. Type 1 coved type ST-segment elevation and non-sustained polymorphic ventricular tachycardia (VT) via phase 2 reentry induced in a Brugada model using an arterially perfused canine right ventricular wedge preparation. Shown are transmembrane action potentials (APs) simultaneously recorded from 2 epicardial sites (Epi 1 and Epi 2) and 1 endocardial site (Endo) together with a transmural ECG (basic cycle length (BCL)=2,000 ms). (A) Under baseline conditions, phase 1 AP notch in Epi, but not in Endo, is associated with a J wave in the ECG. (B) Combined administration of terfenadine (5 μmol/L) and pilsicainide (5 μmol/L) produces a loss of AP dome in Epi 1, but not in Epi 2, resulting in a marked epicardial dispersion of repolarization (EDR), and a coved-type ST segment elevation and a negative T wave in the ECG. (C) 4-aminopyridine (4-AP), a selective blocker of the transient outward current (I_{to}) (2 mmol/L), restores the AP dome, decreases the phase 1 AP notch, and normalizes the ST-segment elevation. (D) In the setting of heterogeneous loss of the AP dome (coexistence of loss of dome regions and restored dome regions) in the epicardium and a remarkable coved type ST-segment elevation in the ECG with combined administration with terfenadine and pilsicainide, electrotonic propagation from the site where the dome is restored (Epi 1) to the site where it is lost (Epi 2) results in development of a premature beat induced by phase 2 reentry, triggering spontaneous polymorphic VT (Modified from *Nat Clin Pract Cardiovasc Med* 2005; **2:** 408–414 with permission).

Epi 1, but not in Epi 2, resulting in a marked epicardial dispersion of repolarization (EDR), and a coved-type ST segment elevation and negative T wave in the ECG (Fig 2B). A selective I_{to} blocker, 4-aminopyridine, restores the AP dome, decreases the phase 1 AP notch, and normalizes the ST-segment elevation (Fig 2C). Fig 2D shows non-sustained polymorphic ventricular tachycardia (VT) via phase 2 reentry induced in a Brugada model using the wedge preparation. In the setting of remarkable coved type ST-segment elevation with combined administration of terfenadine and pilsicainide, heterogeneous loss of the AP dome (coexistence of loss of dome regions and restored dome regions) in the epicardium creates a marked EDR, giving rise to premature beats caused by phase 2 reentry, which precipitates non-sustained polymorphic VT.

Optical Mapping Study

The AP data in the Brugada model using arterially perfused canine RV wedge preparations strongly supported the hypothesis that episodes of VF in BS are triggered by premature beats between the adjacent epicardial cells via the mechanism of phase 2 reentry. However, the precise mechanism of the initial premature beats and the maintenance of non-sustained polymorphic VT or VF remain unsolved, because the number of AP recording sites available for floating microelectrodes is small in the wedge preparations. To overcome this limitation, we recently developed high-resolution (256×256) optical mapping techniques that allowed us to record transmembrane APs from 256 sites simultaneously at the epicardial or endocardial surface of the wedge preparations (Figs 3–5)[8,27] Fig 3 shows the mechanism of phase 2 reentry-induced premature beats (P2R-extrasystoles) under Brugada-ECG conditions. A steep repolarization gradient between the loss of dome region and the restored dome region in the epicardium, but not in the endocardium, develops the initial P2R-extrasystole. We then recorded spontaneous episodes of P2R-extrasystoles and subsequent non-sustained polymorphic VT or VF under these conditions, and analyzed the epicardial AP duration (APD) and conduction velocity (Figs 4,5). Once again, most of the P2R-extrasystoles originated from the area showing the steepest (maximum) gradient of repolarization (GR_{max}) between the loss of dome site and the restored dome site in the epicardium (Figs 4C,5C, arrows), leading to non-sustained polymorphic VT or VF. These data also indicate that a steep repolarization gradient between the loss of dome region and the restored dome region in the epicardium is essential to produce the P2R-extrasystoles that precipitate polymorphic VT or VF. On the other hand, the epicardial GR_{max} does not differ between episodes of polymorphic VT and those of VF. Figs 4D,E and 5D,E show the mechanism underlying the difference between polymorphic VT and VF. Just before inducing the episodes of polymorphic VT or VF, the epicardial depolarization map paced from the endocardium at the basic cycle length of 2,000 ms shows a remarkable conduction delay in the episode of VF (Fig 5D) compared with that of polymorphic VT (Fig 4D). The conduction parameters, such as QRS duration and interval between the stimulus and the earliest epicardial activation, are significantly longer in the episodes of VF than in those of polymorphic VT. Figs 4A,B

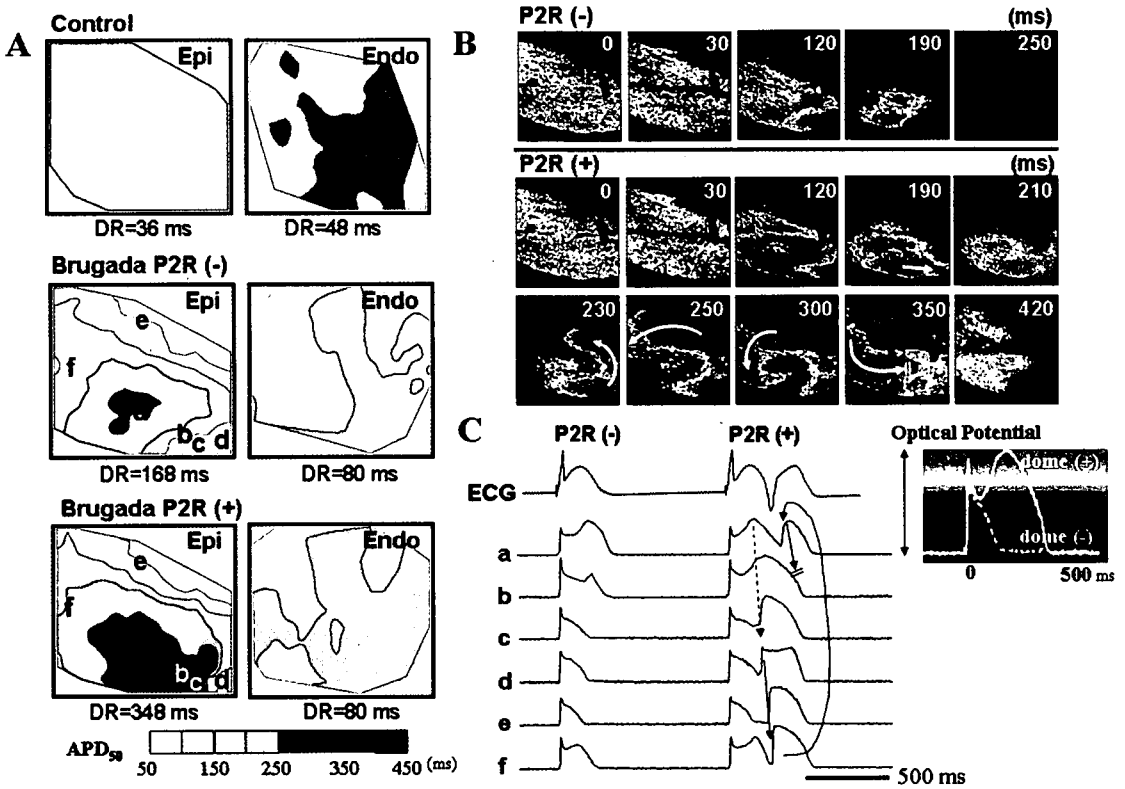

Fig 3. Mechanism of the phase 2 reentry-induced premature beats (P2R-extrasystoles) under the condition of Brugada-ECG in a model using a wedge preparation combined with high-resolution (256×256) optical mapping techniques. (A) Representative action potential duration measured at 50% (APD_{50}) contour map on the right ventricular epicardium (Epi) and endocardium (Endo) in the control, in the ST-segment elevation (Brugada-ECG) without phase 2 reentrant extrasystoles (P2R (–)) and in the Brugada-ECG just before P2R extrasystoles (P2R (+)). (B) Snapshots of an optical isopotential movie on the Epi surface during P2R(–) and P2R(+) in the Brugada-ECG. (C) Optical action potentials (APs) at each site (a–f) on the Epi surface and transmural ECG. Under the Brugada-ECG, the AP morphology in Epi, but not Endo, changes to heterogeneous because of the combination of abbreviated (loss-of-dome; site d,e) and prolonged (restore-of-dome; site a,b) APs, resulting in increasing dispersion of repolarization (DR) in Epi (168 ms) rather than in Endo (80 ms). Further prolongation of the AP in the Epi area (site b) is closely adjacent to the loss-of-dome APs (site d), thus producing a repolarization mismatch within a small area (DR = 348 ms) and developing a P2R-extrasystole at the loss-of-dome site (site d). Thus, a steep repolarization gradient in Epi, but not in Endo, develops the initial P2R-extrasystole in the Brugada-ECG (Modified from *J Am Coll Cardiol* 2006; **47:** 2074–2085 with permission).

represents a phase map and the optical APs during the P2R-induced polymorphic VT, showing that reentry is initiated from the epicardial GR_{max} area and rotates mainly in the epicardium without wave-break. In contrast, Figs 5A,B represents these during P2R-induced VF, showing that the development of the initial P2R is similar to that of polymorphic VT, but that the first P2R-wave is broken up into multiple wavelets, resulting in degeneration of VT into VF. The phase singularity points during the first P2R-wave almost coincide with the sites of delayed conduction (Fig 5D). Wave-break during the first P2R-extrasystole produces multiple wavelets in the episodes of VF, whereas no wave-break or wave-break followed by wave collision and termination occurs in the episodes of polymorphic VT. Figs 4E and 5E are histograms of the epicardial APD measured at 50% (APD_{50}) during the first P2R-wave. There is a large variety of APD_{50} in the epicardium during the first P2R-wave in the episodes of VF, whereas only slight variety in the APD_{50} is observed in the episodes of polymorphic VT. These data suggest that both conduction delay and dispersion of repolarization play significant roles in the perpetuation of VF episodes.

Late Onset of Clinical Manifestation

Because BS is a primary electrical disease, and at least one-third of the patients have mutations in ion channel genes (*SCN5A*, *CACNA1C*, *CACNB2*), clinical manifestation during childhood would be expected. However, BS usually manifests in middle age, at 40–50 years of age? Frustaci et al recently reported a significant myocytes apotosis in both the right and left ventricular myocardium in a histological study of BS patients with *SCN5A* mutations, and suggested that abnormal function of the sodium channels may lead to a sufficient degree of cellular damage, attributing to the arrhythmic event?[28] We recently analyzed several ECG parameters recorded during long-term follow-up of BS patients with and without the *SCN5A* mutation?[29] In both patient groups, the depolarization parameters, including P wave, QRS, S wave duration and PQ interval, increased with age, especially in patients with the *SCN5A* mutation. Taken together with the experimental data?[27] the findings suggest that depolarization abnormalities (conduction slowing) are required for the maintenance of VF in BS, although the initiating premature beats are caused by a phase 2 reentry mechanism.

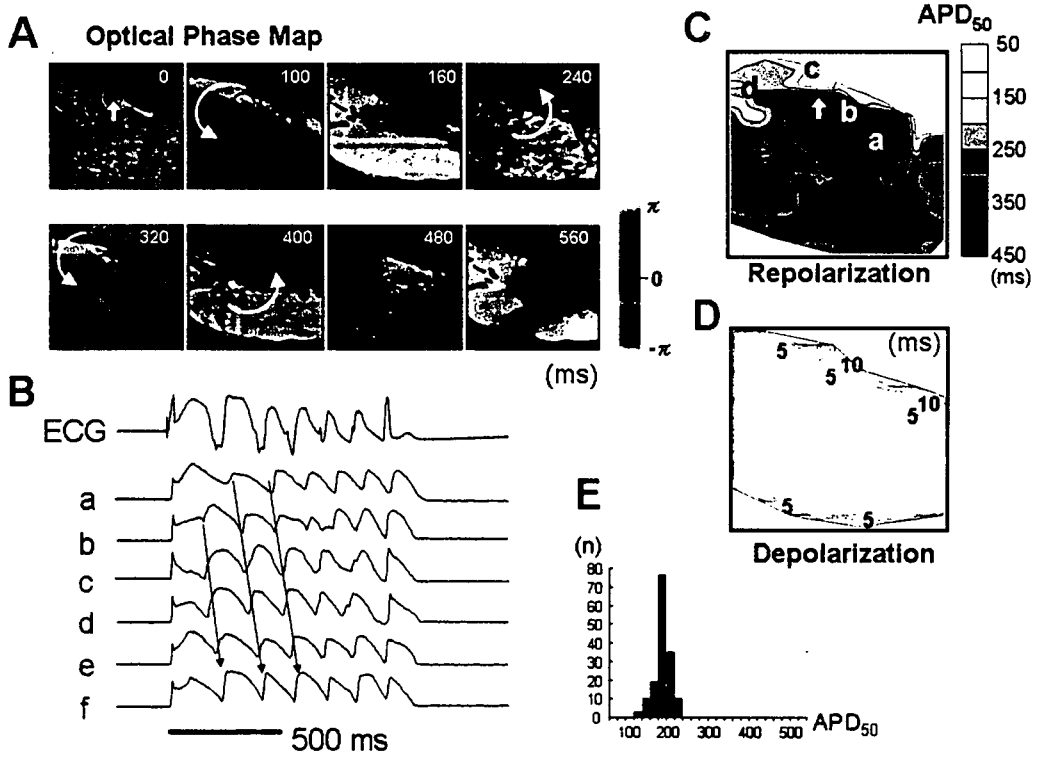

Fig4. Mechanism underlying non-sustained polymorphic ventricular tachycardia (VT) in a Brugada model using a wedge preparation combined with high-resolution (256×256) optical mapping techniques. (A) Representative snapshots from a phase movie during polymorphic VT originating from epicardial (Epi) phase 2 reentry (P2R). (B) Optical action potentials at each site (a–f), together with a transmural ECG. (C, D) Repolarization and depolarization maps on the Epi surface in the condition of Brugada-ECG just before polymorphic VT. (E) Epi action potential duration at 50% repolarization (APD_{50}) histogram during the first P2R-wave. Reentry is initiated from the steepest (maximum) repolarization gradient site in Epi (arrow in A and C) and rotates mainly in Epi without wave-break. The Epi depolarization map paced from Endo shows no conduction delay (D). There is a little variety of APD in Epi during the first P2R-wave (E). Open circles mark phase singularity points (Modified from *J Am Coll Cardiol* 2006; **47**: 2074–2085 with permission).

Male Predominance

Because all mutations so far identified in *SCN5A* display an autosomal dominant mode of transmission in BS, males and females would be expected to inherit the defective gene equally. However, an apparent male predominance is observed in patients with BS[15] Di Diego et al suggested the cellular basis for male predominance in BS while using arterially-perfused canine RV wedge preparations[30] They reported that the I_{to}-mediated phase 1 AP notch in the RV epicardium was larger in male dogs than in female dogs was responsible for the male predominance in the Brugada phenotype. On the other hand, the male hormone, testosterone, has been reported to increase the outward potassium currents (the rapidly [I_{Kr}][31,32] and the slowly [I_{Ks}][33] activating component of I_K, and the inward rectifier potassium current [I_{K1}][32]) or decrease the inward currents ($I_{Ca\text{-}L}$)[33] Therefore, testosterone would be expected to accentuate the Brugada phenotype. Clinically, Matsuo et al report 2 cases of asymptomatic BS in which typical coved ST-segment elevation disappeared following orchiectomy as therapy for prostate cancer[34] supporting the expectation for testosterone. Moreover, testosterone is also known to decrease visceral fat[35] and patients with BS are thinner than the normal population[36] On the basis of these clinical and experimental findings, we directly measured the testosterone level in male patients with BS and compared them with age-matched normal males[37] The testosterone level was significantly higher and body mass index (BMI) significantly lower in the Brugada males than in the controls after adjusting for several confounding variables influencing testosterone level or BMI (eg, age, exercise, stress, smoking, and medication). Interestingly, testosterone level was inversely correlated with BMI in both Brugada and control males even after adjusting for confounding variables, suggesting that Brugada males have a higher testosterone level associated with lower visceral fat (Fig6). Moreover, conditional logistic regression model analysis showed that both higher testosterone level and lower BMI independently increase the risk of BS. These data suggest that the male predominance in the Brugada phenotype is at least in part related to testosterone, which is present only in males.

Higher Incidence in Asian Population

The incidence of BS is higher in Asian countries, including Thailand and Japan, than in Western countries[11,12,38] It has been reported that common polymorphisms might modulate the activity of the primary disease-causing mutation or influence susceptibility to arrhythmia, even in the general population[39] The common polymorphisms may attribute to ethnic differences in the clinical phenotype in inherited cardiac arrhythmias, including BS, because some common polymorphisms are ethnically dependent. Pfeufer et al reported that polymorphisms in the *SCN5A* promoter were associated with a widening of QRS duration in a cen-