表1　急性期，回復期，維持期および第Ⅰ～Ⅲ相の定義（文献1より引用改変）

<table>
<tr><td rowspan="2">急性期</td><td rowspan="2">発症（手術）当日から
ICU/CCUに在室
観血的モニタや点滴・注射薬による治療
ベッド上でのリハビリテーション
離床するまで
病態が不安定
発症から2週間
病棟内のリハビリテーション
急性期病院を退院するまで</td><td>急性期</td><td>第Ⅰ相
(Phase Ⅰ)</td></tr>
<tr><td rowspan="3">前期回復期</td><td rowspan="3">第Ⅱ相
(Phase Ⅱ)</td></tr>
<tr><td>回復期</td><td>急性期が終了してから
運動負荷試験を実施
運動療法室でのリハビリテーション
一般病棟を退院する
外来通院可能となる
リハビリ開始から6(5)カ月まで
社会復帰する
明らかな回復が見込まれる</td></tr>
<tr><td>後期回復期</td></tr>
<tr><td>維持期
(慢性期)</td><td>回復期が終わってから
復職・復学，社会復帰
6(5)カ月（保険期間終了）～終生</td><td>維持期</td><td>第Ⅲ相
(Phase Ⅲ)</td></tr>
</table>

「急性期」は早期離床，合併症予防．　　「第Ⅲ相」は社会復帰後．
「回復期」は治療としての包括的介入．身体機能の回復・改善．
「維持期」は身体機能維持と二次予防（Secondary prevention）．
「第Ⅰ相」は離床まで．
「第Ⅱ相」は社会復帰まで．

表2　身体的脱調節[2)]

1. 運動能力の低下
2. 心拍数反応の増加
3. 血圧調節の障害
4. 骨格筋量，筋力の低下
5. 呼吸機能の低下
6. 窒素・カルシウムの負バランス
7. 循環血液量・血清蛋白の減少

推奨されました．しかし，最近では軽症から重症のAMIをすべて平均しても入院期間は約3週間であり，通常のAMIは2週間以内で退院するようになってきています．国立循環器病センターでは再灌流療法に成功したKillip Ⅰ型で合併症がなく，血中クレアチンキナーゼ（CK）最高値が1,500 IU/*l*以上では14日間のクリニカルパスを（表3）[1)]，また中でもCK 1,500 IU/*l*未満の場合は10日間パスを採用していると報告しています．実際には急性期心臓リハビリプログラムは，絶対安静から受動座位，能動座位，端座位，起立，歩行のように血圧や脈拍，胸痛の有無等をチェックしながら進行し，それに伴って病棟での活動範囲や自分でできる範囲が広がっていきます．

表3　急性心筋梗塞症14日間クリニカルパス（国立循環器病センター）

病日	PCI後 1日目	2日目	3日目	4日目	5日目	6日目	7日目	8日目	9日目	10日目	11日目	12日目	13日目	14日目
達成目標	・急性心筋梗塞およびカテーテル検査に伴う合併症を防ぐ	・急性心筋梗塞およびカテーテル検査に伴う合併症を防ぐ	・急性心筋梗塞に伴う合併症を防ぐ	・心筋虚血が起きない	・心筋虚血が起きない ・服薬自己管理ができる ・退院後の日常生活の注意点について知ることができる			・心筋虚血が起きない ・退院後の日常生活の注意点について理解ができる			・亜最大負荷で虚血がない ・退院後の日常生活の注意点について言える			退院
負荷検査・リハビリ	・圧迫帯除去，創部消毒 ・室内排便負荷	・尿カテーテル抜去	・末梢ライン抜去 ・トイレ排泄負荷	・200 m歩行負荷試験 ・合格後200 m歩行練習1日3回 ・栄養指導依頼	・心臓リハビリ依頼 ・心臓リハビリ開始日の確認	・心臓リハビリ室でエントリーテスト ・心リハ非エントリー例では500 m歩行負荷試験		・心臓リハビリ室で運動療法（心臓リハビリ非エントリー例では，マスターシングル試験または入浴負荷試験）						
安静度	・圧迫帯除去後床上自由	・室内自由	・負荷後トイレまで歩行可	・200 m病棟内自由		・亜最大負荷試験合格後は入浴可および院内自由								
食事	・循環器疾患普通食（1,600 kcal，塩分6 g） ・飲水量指示			・循環器疾患普通食（1,600 kcal，塩分6 g） ・飲水制限なし										
排泄	・尿留置カテーテル ・排便：ポータブル便器	・尿留置カテーテル ・排便：ポータブル便器	・排尿・排便：トイレ使用											
清潔	・洗面ベッド上 ・全身清拭，背・足介助	・洗面：洗面台使用 ・全身清拭，背・足介助		・洗面：洗面台使用 ・清拭：背部のみ介助		・洗面：洗面台使用 ・患者の希望に合わせて清拭		・洗面：洗面台使用 ・患者の希望に合わせて入浴						

院内のリハビリが終了する頃には，退院に向けてのチェックを開始します．この時期の運動負荷試験は，退院後の生活における運動の許容範囲を決定する上で重要です．われわれの施設では，呼気ガス分析を併用した心肺運動負荷試験を施行し，精密な運動許容範囲の指導を行っています．

3 患者教育と第Ⅱ相リハビリへの導入

急性期リハビリに並行して，患者教育を行うことも必要です．患者本人が自分の病態について理解することは，その後の生活指導や再発予防のための冠危険因子の管理に役立つばかりでなく，リハビリへの意欲を持たせることにもつながるからです．具体的には，患者教室のようにいくつかのテーマについて医師や看護師，栄養士，臨床心理士などの多職種により行います．

前述のように，最近ではAMIの入院期間が短縮しており，これには患者の金銭的負担が減り，平均在院日数が短縮できるというメリットもあります．しかし，以前は1カ月近くの入院が2週間未満まで短くなり，いままでは十分な患者教育や退院後の回復期リハビリ参加への動機付けが可能であったものがなかなか困難な状況になっており，われわれ心臓リハビリに関わる医療者にとっては重要な課題となっています．

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1) 循環器病の診断と治療に関するガイドライン（2006年度合同研究班報告）心疾患におけるリハビリテーションに関するガイドライン（2007年改訂版）（班長：野原隆司）：日本循環器学会ホームページ版　http://www.j-circ.or.jp/guideline/pdf/JCS2007_nohara_h.pdf
2) 齋藤宗靖．心臓リハビリテーションの概念と歴史的変遷．In：木全心一，監修．狭心症・心筋梗塞のリハビリテーション．改訂第4版．東京：南江堂；2009. p. 3-10.

〈大宮一人〉

Q2 回復期(第Ⅱ相)リハビリの内容について具体的に教えてください．また，急性期リハビリのみで終わった時との効果の違いについて教えてください．

Answer

回復期（第Ⅱ相）リハビリは，離床から退院までの前期回復期と社会復帰までの後期回復期に分けられ，発症後3〜5カ月までと考えられます．昨今のAMI入院期間の短縮により身体的脱調節を有する患者が減り，さらに経皮的冠動脈インターベンションが超急性期に行われ再灌流がなされることから心筋のダメージが少なくなり，退院後の回復期リハビリへのエントリーのための動機付けもなかなか困難になってきています．しかし，多くの有益な効果が知られており，いかに回復期リハビリへ導入するかが重要な課題になっています．

1 退院時の生活指導

AMIにて入院，急性期リハビリを終了し退院を許可された場合，患者の最大の関心事は職業への復帰を含めた社会生活への復帰の度合いと考えられます．重症度や職業の違い，日常の活動度は患者個々で異なり，その指導はケースごとに変えなければなりません．特に問題となるのが肉体労働者や職業的ドライバーに対する職場復帰の際の許可条件と考えられます．喫煙者の場合は入院中に禁煙指導を行い，必ず禁煙をさせるようにします．また，糖尿病や脂質異常症，高尿酸血症などの食事・栄養によるものは家族とともに栄養指導を行い，理解を深めることが重要です．

この時期は，特に退院してから社会復帰までの期間が大きい位置を占めます．患者は脱調節を改善し，日常生活に戻っていくわけですが，この時期の患者個々の運動能力には個人差があり，リハビリのゴールも異なります．すなわち，この時期のリハビリは，患者個々のライフスタイルにより適応するような運動能力の増加をはかり，円滑な職場復帰などの社会復帰を促進することが目的となります．最近の傾向として，前述のようにさまざまな改善により，早期退院が可能な状況になり，社会復帰も早くなっています．社会情勢の違いも関係し，たとえばドイツのように急性期病院から回復期リハビリ病院に転院してゆっくりと心臓リハビリを行うような社会基盤には日本の場合まだなっていないのが現状です．われわれの施設でも，回復期リハビリに参加する患者数は減少傾向にあり，これをいかに増やしていくかが今後の課題となっています．

2 運動療法を含む回復期リハビリの効果

運動療法の効果としては，日本循環器学会のガイドライン（表1）[1]にも記載されているように，運動耐容能や心筋虚血閾値，骨格筋のミトコンドリア濃度や毛細管密度などは増加し，最大下同一

表1　運動療法の身体効果[1)]

項目	内容	ランク
運動耐容能	最高酸素摂取量増加	A
	嫌気性代謝閾値増加	A
症　状	心筋虚血閾値の上昇による狭心症発作の軽減	A
	同一労作時の心不全症状の軽減	A
呼　吸	最大下同一負荷強度での換気量減少	A
心　臓	最大下同一負荷強度での心拍数減少	A
	最大下同一負荷強度での心仕事量（心臓二重積）減少	A
	左室リモデリングの抑制	A
	左室収縮機能を増悪せず	A
	左室拡張機能改善	B
	心筋代謝改善	B
冠動脈	冠狭窄病変の進展抑制	A
	心筋灌流の改善	B
	冠動脈血管内皮依存性，非依存性拡張反応の改善	B
中心循環	最大動静脈酸素較差の増大	B
末梢循環	安静時，運動時の総末梢血管抵抗減少	B
	末梢動脈血管内皮機能の改善	B
炎症性指標	CRP，炎症性サイトカインの減少	B
骨格筋	ミトコンドリアの増加	B
	骨格筋酸化酵素活性の増大	B
	骨格筋毛細管密度の増加	B
	Ⅱ型からⅠ型への筋線維型の変換	B
冠危険因子	収縮期血圧の低下	A
	HDL コレステロール増加，中性脂肪減少	A
	喫煙率減少	A
自律神経	交感神経緊張の低下	A
	副交感神経機能亢進	B
	圧受容体反射感受性の改善	B
血　液	血小板凝集能低下	B
	血液凝固能低下	B
予　後	冠動脈性事故発生率の減少	A
	心不全増悪による入院の減少	A（CAD）
	生命予後の改善（全死亡，心臓死の減少）	A（CAD）

A：証拠が充分であるもの，B：報告の質は高いが報告数が充分でないもの，CAD：冠動脈疾患

負荷での心拍数，心筋酸素消費量の指標となる二重積は減少します．すなわち，心筋への負荷を減らし，同一負荷での心筋酸素消費量が低下することで結果的に運動能力を向上させるというメリットがあります．また，動脈硬化の予防という観点から，冠危険因子の是正にも有効です．すなわち，動脈硬化に抑制的に働くHDLコレステロールが増加し，中性脂肪，肥満に対する是正効果が

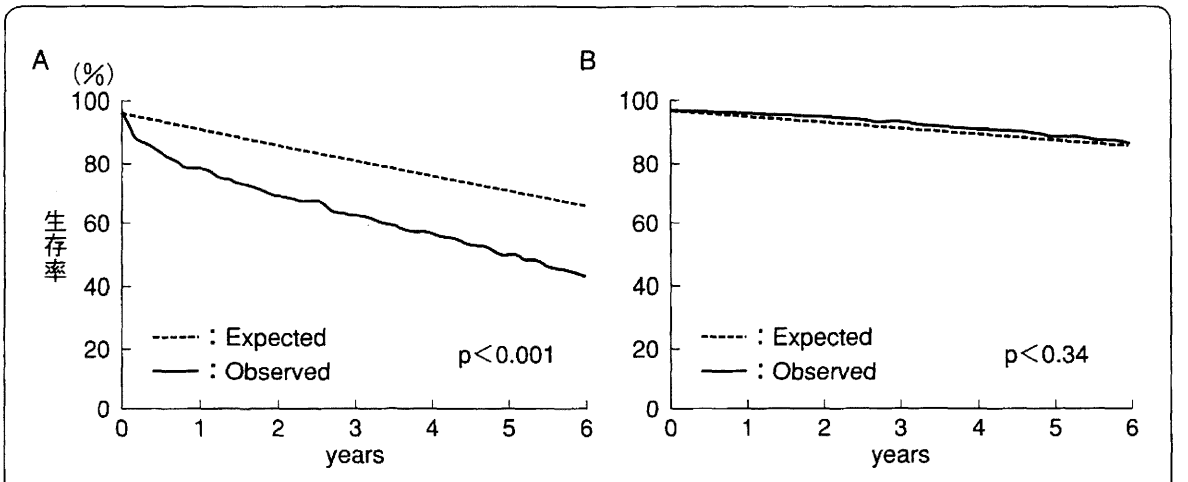

図1 心筋梗塞後の心臓リハビリテーションの効果[2)]

A：心リハ非施行群，B：心リハ施行群．

あります．これらは特に冠動脈疾患患者においてその病態を改善させる可能性があることを示すと思われます．われわれの施設のデータでも，回復期リハビリに参加しない群は運動耐容能や嫌気性代謝閾値などの運動生理面のパラメータの改善が参加群に比して低く，さらに冠危険因子の改善も得られにくいことが明らかになっています．

3 地域における心臓リハビリの生命予後に関する効果

AMIに対する心臓リハビリが比較的広い地域における患者群の生命予後を改善するという興味深い報告が最近されています[2)]．米国ミネソタ州Olmsted郡のAMI患者1,821例を対象に平均6.6年観察したところ，774例が死亡し，493例が心筋梗塞を再発しました．3年生存率は非心臓リハビリ群64％に対して心臓リハビリ群95％と心臓リハビリ群で死亡およびAMI再発がそれぞれ56％，28％減少しました．さらに退院後3年以内の死亡の48％が心臓リハビリ不参加が原因で起こったことが示されました．さらに図1[2)]のごとく非心臓リハビリ群では同州の予測生存曲線よりも明らかに予後不良ですが，心臓リハビリ群ではその地域の予測生存曲線とほぼ一致したことから，心臓リハビリが比較的広い地域における患者の予後を改善することが示されました．わが国ではAMI他の心疾患の予後は欧米に比して良好であることがよく知られていますが，生活習慣の欧米化がみられる昨今では今後回復期リハビリが重要になってくることが予想されます．つまり，回復期リハビリを行わないことは，これらの有益な状況が得られないことになるため，AMI二次予防のためには可能な限り回復期リハビリに参加することが重要であると思われます．

4 健康関連QOLに対する効果

MI後患者124例を対象とし，回復期における8週間の有酸素運動と中等度の強度の上下肢の筋力トレーニングを併用した運動療法を主体とする心臓リハビリの影響について検討し，運動療法参加群は非参加群に比べて，握力や膝伸展筋力および酸素摂取量などを含む運動能力と健康関連QOL指標のSF-36のうち特に身体的側面に関する下位尺度が有意に改善することが示されました(図2)[3)]．さらに，急性心筋梗塞発症後6カ月間の通院監視型心臓リハビリが終了した患者の運動継続，身体活動量および健康関連QOLを，発症後1年以上経過した時点で検討しました．その結

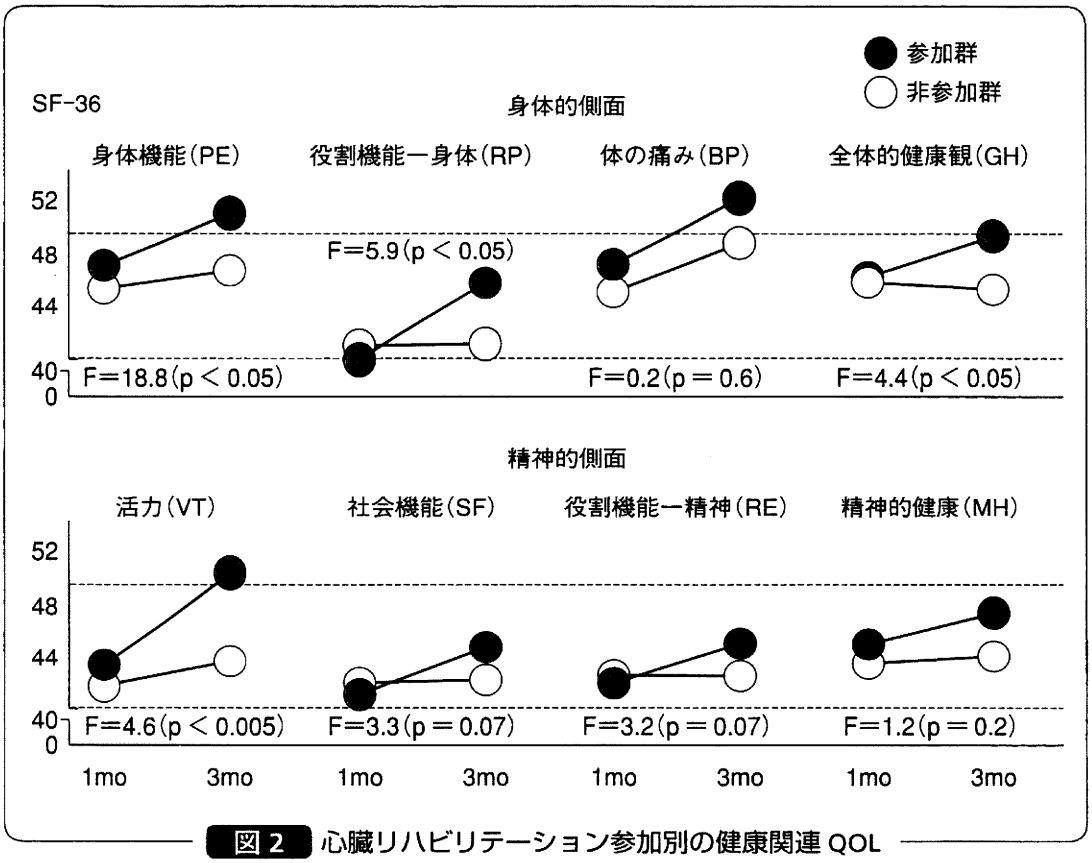

図 2 心臓リハビリテーション参加別の健康関連 QOL

果，心臓リハビリ終了後も運動を継続していた群の身体活動量と健康関連 QOL 得点は，運動を継続していなかった群より高く，運動継続群の SF-36 各下位尺度得点は国民標準値に到達していました[4]．これらのことから，心臓リハビリ患者の健康関連 QOL を維持・向上させるためには，運動習慣をいかに定着させるかが重要であることが伺えます．

■文献

1) 循環器病の診断と治療に関するガイドライン（2006年度合同研究班報告）心疾患におけるリハビリテーションに関するガイドライン（2007年改訂版）（班長：野原隆司）：日本循環器学会ホームページ版 http://www.j-circ.or.jp/guideline/pdf/JCS2007_nohara_h.pdf

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〈大宮一人〉

Q3 維持期(第Ⅲ相)リハビリの内容について具体的に教えてください．またメディックスクラブについて教えてください．

Answer

心筋梗塞後第Ⅲ相リハビリは，第Ⅱ相リハビリで社会復帰を果たした後の維持期リハビリであり，特に期間は決められずに一生涯続くものと考えられます．この相では社会復帰を果たした後であることの安心感，仕事や家事に忙殺されることなどからリハビリ継続は容易ではありません．さらに，どこでどのように運動を継続するかが問題となります．ドイツ型の Ambulante Herzgruppe という形態を模してメディックスクラブという組織が活動しており，それについても紹介します．

1 AMI 第Ⅲ相リハビリについて

維持期（第Ⅲ相）リハビリは，社会復帰をめざした第Ⅱ相終了後から一生涯継続される心臓リハビリの時期とされます．一度 AMI という大きな疾患にかかったとしても，その後のケアや管理がよければ逆に疾患のない人と同様かそれ以上の健康が保てることがよくあります．多くの研究で，二次予防のための運動習慣，食事管理，禁煙の継続などの重要性が述べられており，この時期は重要ですが，特に第Ⅰ相，Ⅱ相でどれだけの理解と実践が可能であったかが重要であると考えられます．

しかし，この時期のリハビリ継続は様々な理由から困難な場合が多く見られます．患者側としては，程度の差こそあれ社会復帰を果たしたことへの安心感が芽生えたり，職場復帰後の仕事や家事に忙殺されたりして徐々に管理が甘くなることがよくみられます．禁煙に一度成功したものの再度喫煙してしまったり，糖尿病や脂質異常症の悪化，運動不足への逆戻りなどがよく経験されます．また，現在の心大血管リハビリテーションのくくりの中では，特例はあるもののリハビリ期間は5カ月とされていることから特に運動療法の継続はなかなか難しいものがあります．また第Ⅱ相の中で運動習慣の動機付けができていないとそれ以後の継続は困難です．

2 心臓リハビリを長期に継続することのメリットについて

Hambrecht ら[1] は，70歳以下の安定狭心症患者101例を，PCI 群50例と12カ月の運動 training 群51例に無作為に割り付け予後を検討しました．すると，training 群で event-free survival rate が88％と PCI 群の70％に比べて有意に高値でした（図1）[1]．つまり，PCI よりも継続した運動 training のほうが予後改善効果に優れていたことになります．さらに，当然ですが医療費も PCI 群より training 群で有意に安価でした．この101例を2年後の時点まで追跡した結果に

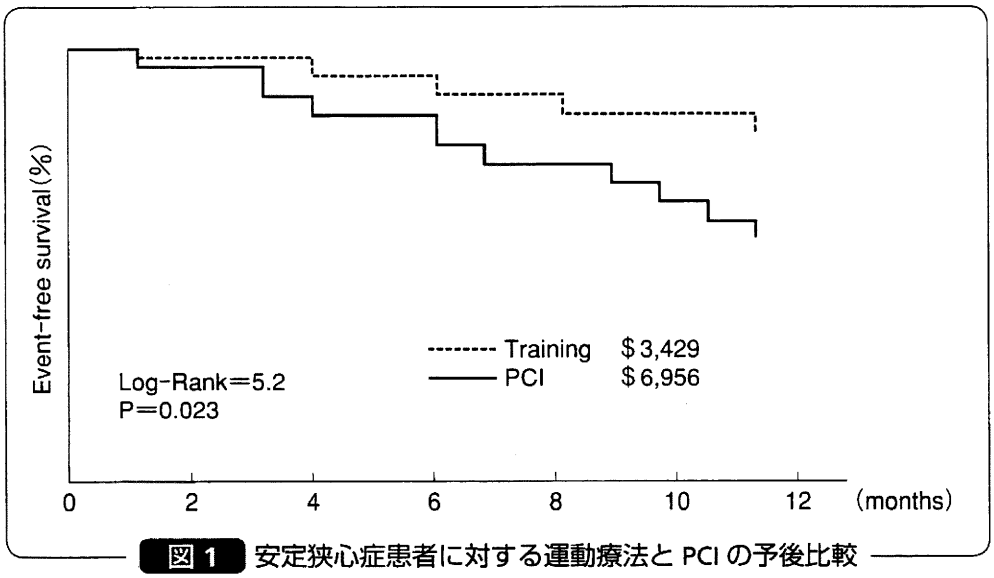

図 1 安定狭心症患者に対する運動療法と PCI の予後比較

ついても報告されています[2]．慢性的な血管の炎症が ACS の引き金となるのではないかという仮説のもと，炎症性サイトカインである interleukin（IL）-6, tumor necrosis factor（TNF）-α，インターフェロンγなどの血液データが検討されました．症例は同様に 2 年間の定期的な運動 training 継続群および PCI 群において検討されました．各群ともに 2 年間の経過の中で AMI 発症や再狭窄，運動療法群の中でも ACS 発症が少数見られています．12 カ月と同様に，24 カ月時点においても training 群において有意に event free survival rate が良好でした（$p<0.05$）．検査データでは HDL コレステロールが運動療法群で有意に増加，高感度 CRP 値が減少しました．炎症性サイトカインの中では，IL-6 のみが運動療法群で低下したが，他のサイトカインは両群とも同等でした．継続的な運動療法は長期間においても PCI に勝るという非常にシンプルではありますが示唆に富む結果と思われます．

3 メディックスクラブについて

長期間の運動継続が有効であることは議論の余地はありませんが，いつ，どこで，どのように行うのかという問題点があります．第Ⅲ相の心臓リハビリの一つの形態として，ドイツ式の地域密着型の運動グループの存在があり，わが国ではジャパンハートクラブという NPO 法人が立ち上がり，メディックスクラブという会員組織による第Ⅲ相リハビリが行われています．ドイツでは，たとえば AMI においては急性期病院を数日で終え，郊外の療養型の心臓リハビリ病院に転院し，比較的ゆっくりとしたリハビリを受けます．そこを退院した後は第Ⅲ相のリハビリに移行するわけですが，それは国や自治体の補助を受けた Ambulante Herzgruppe というグループがドイツ国内だけで約 5,500 以上あり稼働しています．このグループは 1 人の運動指導員と 10～15 人の患者で構成されており，週 2～3 回，1 回 90 分の運動を，無料で借りられる学校の体育館や運動場などで行うものです．

わが国でも，NPO 法人ジャパンハートクラブ（谷口興一理事長）がこの Ambulante Herzgruppe を模してメディックスクラブという第Ⅲ相リハビリグループを組織して会員を募り活動しています．詳細は web（http://www.npo-jhc.org/）を参照してください．図 2 にその組織図を記します[3]．

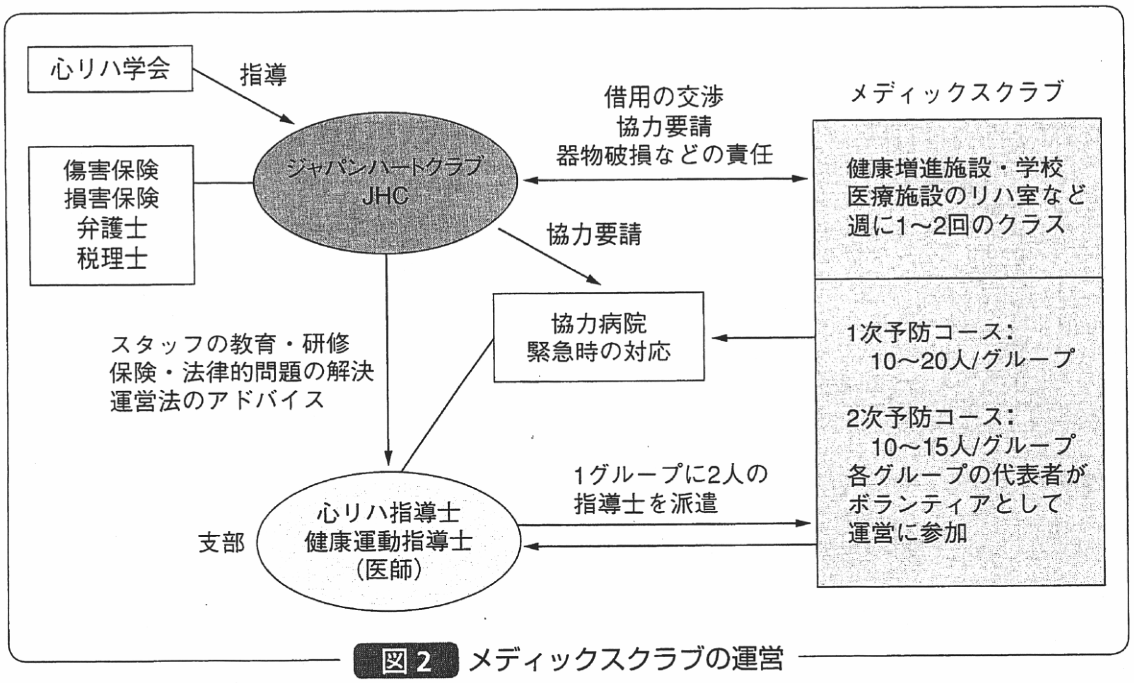

図2 メディックスクラブの運営

2008年度は延べ5,000人以上の会員が参加して運動療法を中心に取り組んでいます．支部は今後も増加していくことが予想され，日本における第Ⅲ相リハビリの主な受け皿となることが期待されています．

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〈大宮一人〉

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Brief Communications

Plasma MicroRNA 499 as a Biomarker of Acute Myocardial Infarction

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BACKGROUND: MicroRNAs (miRNAs) are endogenous small RNAs 21–25 nucleotides in length. Recently, we reported that miRNA 208 (miR-208) is produced exclusively in the rat myocardium and that plasma miR-208 is a biomarker of myocardial injury in rats. In the present study, we assessed the hypothesis that plasma concentrations of myocardial-specific miRNAs can be used to diagnose myocardial injury in humans.

METHODS: We used array analysis of miRNA production in various human tissues to identify heart-specific miRNAs. We assessed the plasma concentrations of miR-499 in 14 individuals with acute coronary syndromes, 15 individuals with congestive heart failure, and 10 individuals without cardiovascular diseases. Plasma miR-499 concentrations were measured with a real-time reverse-transcription PCR method that used an artificial small RNA as an internal calibrator.

RESULTS: The miRNA array analysis of various human tissues indicated that miR-499 was produced almost exclusively in the heart. Plasma miR-499 concentrations were measurably increased in all individuals with acute myocardial infarction but were below the limit of detection for all individuals in the other patient groups.

CONCLUSIONS: The plasma concentration of miR-499 may be a useful biomarker of myocardial infarction in humans.

MicroRNAs (miRNAs),[3] endogenous small RNAs 21–25 nucleotides in length, can pair with the 3′ untranslated region sites in mRNAs of protein-coding genes to downregulate their expression *(1)*, and they play important roles in various physiological and pathologic processes *(2, 3)*. More than 500 human miRNAs have been identified *(4)*, and most human protein-coding genes appear to be targeted by these miRNAs *(5, 6)*. miRNAs appear to function as rheostats to fine-tune adjustments in the protein output *(7, 8)*.

The presence of miRNAs in various body fluids has recently been reported *(9–11)*, and we recently reported that the plasma concentration of miRNA 208 (miR-208), a myocardial-specific miRNA in rats, is a useful biomarker of myocardial injury *(12)*. Other groups have also reported that plasma miRNAs are sensitive and specific biomarkers of various tissue injuries *(13, 14)*. In the present study, we examined which human tissues produced miR-499 and assessed whether the plasma concentration of miR-499 is a useful biomarker of myocardial injury in humans.

We collected blood samples from 29 inpatients and 10 healthy asymptomatic outpatients at the National Cardiovascular Center Hospital after obtaining their written informed consent. This study was approved by the Ethics Committee of the National Cardiovascular Center.

The acute coronary syndromes group consisted of 9 patients with acute myocardial infarction (AMI) and 5 patients with unstable angina pectoris. All acute coronary syndrome patients underwent coronary angiography and percutaneous coronary intervention. The blood samples from the acute coronary syndrome patients were obtained within 48 h of the last onset of chest pain. We also obtained blood samples from AMI patients before their final discharge when their clinical status was stable. The congestive heart failure (CHF) group consisted of 8 patients with old myocardial infarction [New York Heart Association (NYHA) class III], 4 patients with dilated cardiomyopathy (NYHA class II), and 3 patients with valvular diseases (1 patient in NYHA class III and 2 in NYHA class II). The blood samples of patients in the CHF group were obtained while they were in NYHA functional class II or III. The control individuals consisted of asymptomatic healthy and/or borderline hypertensive outpatients who were visiting the hospital for regular health checkups. Creatine kinase MB was increased in the patients with AMI and not in the patients with unstable angina pectoris (Table 1).

We isolated total plasma RNA with the mirVana™ PARIS Kit (Ambion) according to the manufacturer's protocol. Before purification, we added a fixed amount of a small synthetic RNA to the plasma samples for a dual assay to verify the RNA-purification procedures. Details of the procedure are described in the Supplemental Data file available in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol56/issue7.

[3] Nonstandard abbreviations: miRNA, microRNA; miR-208, miRNA 208; AMI, acute myocardial infarction; CHF, congestive heart failure; NYHA, New York Heart Association.

Table 1. Patient characteristics.[a]

	AMI[b] (n = 9)	UAP (n = 5)	CHF_III (n = 9)	CHF_II (n = 6)	Normal (n = 10)
F/M sex, n	3/6	2/3	2/7	2/4	5/5
Age, years	66.8 (9.28)	70.2 (16.2)	71.6 (6.6)	61.5 (16.4)	41.5 (8.0)
CKMB, U/L[c]	122.2 (124.9)	18.9 (6.6)	ND	ND	ND
BNP, ng/L[c]	ND	ND	674 (341)	175 (142)	ND
Log miR-499 copies/100 μL	4.19 (0.24)	<2.38	<2.38	<2.38	<2.38

[a] Data are expressed as the mean (SD) where indicated.
[b] AMI, acute myocardial infarction; UAP, unstable angina pectoris; CHF_III, congestive heart failure in NYHA class III; CHF_II, congestive heart failure in NYHA class II; Normal, healthy control individuals; CKMB, creatine kinase MB; BNP, brain natriuretic peptide; ND, not determined.
[c] CKMB (reference interval, 0–23 U/L) and BNP (reference interval, <18.4 ng/L) were measured in the AMI groups (AMI and UAP) and the CHF groups, respectively.

To identify myocardial-specific miRNAs, we used the ABI TaqMan MicroRNA Array kit (Applied Biosystems) according to the manufacturer's protocol for profiling the production of miRNAs in various human tissues and cultured cells.

To measure miR-499 concentrations, we used a TaqMan microRNA real-time RT-PCR kit (Applied Biosystems) *(15)* according to the manufacturer's protocol. We simultaneously assessed the concentration of the internal reference small RNA in a single tube. The limit of detection for miR-499 was 240 copies/100 μL. All assays were performed in duplicate. Calibration assays with various amounts of synthetic miR-499 were performed on each assay plate. Details of the statistical analyses are described in the Supplemental Data file in the online Data Supplement.

The miRNA array analyses of 671 species of miRNAs in various tissues and cells indicated that miR-499 is produced almost exclusively in the human heart (see Supplemental Table in the online Data Supplement). miR-208a and miR-208b concentrations appear to be very low in the human heart (see Supplemental Table in the online Data Supplement), and these 2 miRNAs appear not to be useful as plasma biomarkers.

Fig. 1 summarizes the data for plasma miR-499 concentrations in the study population. Plasma miR-499 concentrations were below the limit of detection in the control and CHF groups; however, plasma miR-499 concentrations were measurably increased in patients with AMI in the acute phase (within 48 h of the last onset of chest pain) and became undetectable before hospital discharge, whereas this miRNA was not detected in the plasma of patients with unstable angina pectoris. The large variation in the plasma miR-499 concentration in AMI patients was most likely related to variation in the time of blood collection. Our preliminary investigation indicated that the peak plasma miR-499 concentration occurred between 6 h and 12 h of the onset of myocardial infarction (data not shown). A positive correlation between creatine kinase MB activity and plasma miR-499 concentration was clearly

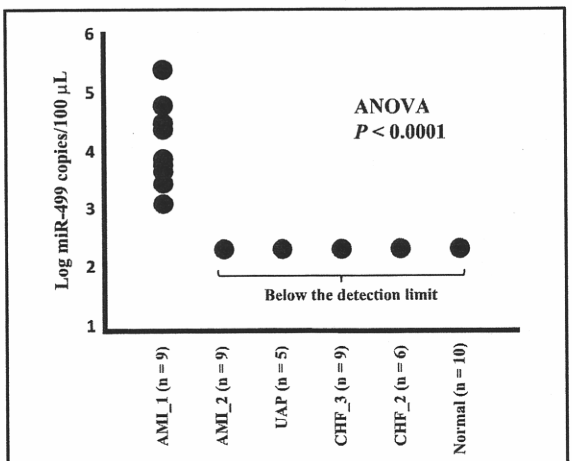

Fig. 1. Plasma concentrations of miR-499 in the study population.

Plasma miR-499 concentrations were assessed by real-time reverse-transcription PCR with a synthetic miRNA included as an internal calibrator. Values are expressed as log miR-499 copies/100 μL. Concentrations were measured in patients with AMI [repeatedly measured in samples obtained within 48 h (AMI_1) and at just before hospital discharge (AMI_2)], in patients with unstable angina pectoris (UAP), in CHF patients in NYHA class III (CHF_3), in CHF patients in NYHA class II (CHF_2), and in healthy control individuals (Normal). An ANOVA indicated that the mean miR-499 values were significantly different among the groups ($P < 0.0001$). The subsequent Dunnett test indicated that values in the AMI_1 group were significantly higher than those of the other groups ($P < 0.0001$ for all comparisons).

observed in individuals with AMI (see Supplemental Data in the online Data Supplement).

The present study is the first to confirm that a cardiac-specific miRNA, miR-499, can be a biomarker of myocardial infarction in humans. The next question is whether this assessment of the plasma miR-499 concentration has any clinical significance. We expected the PCR-based assay of plasma miR-499 to detect possible myocardial micronecrosis in CHF. In fact, our study showed that this method could not detect plasma miR-499 concentrations reliably in CHF patients. A more sensitive assay to detect plasma miR-499 can be developed, however, and it might establish miR-499 as a new biomarker of cardiovascular diseases in the same way that the recently developed high-sensitivity assays for troponins have become very useful for evaluating patients with cardiovascular diseases *(16)*.

Accumulating evidence suggests the usefulness of circulating miRNAs as stable blood-based biomarkers for various diseases *(9–11)*. The present study has confirmed, for the first time, that the plasma miR-499 concentration may be a biomarker of myocardial infarction in humans. Our array data indicate other intriguing candidates for clinical applications, including miR-124a for the central nervous system, miR-122 for the liver, and miR-133a for skeletal muscle. These observations await further clinical investigations.

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EDITORIAL

Exercise Training in Post-CABG Patients at Low Prognostic Risk

– Beyond Recovery From Surgery –

Yoichi Goto, MD, PhD

Cardiac rehabilitation with exercise training has been shown to improve exercise capacity, coronary risk factors, and health-related quality of life (QOL), to retard the progression of atherosclerosis, and to decrease morbidity and mortality in patients with coronary artery disease (CAD).[1] Based on these lines of evidence, the American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommend cardiac rehabilitation for all eligible patients with CAD, including those after coronary artery bypass grafting (CABG).[2] However, among studies focusing exclusively on a CABG population, the existing evidence of the efficacy of exercise training is limited to improvements in exercise tolerance and psychological sense of well-being.[2,3] In this issue of the Journal, Bilinska et al report the effects of exercise training on hemodynamic and neurohumoral responses to static (handgrip) exercise and on inflammatory markers in patients after CABG.[4] Their study is unique in the following 3 aspects.

Article p2598

Sympathetic and Metabolic Control of Cardiovascular Response to Exercise

The first of these is that the authors assessed the effects of dynamic exercise training on the response to static exercise. Static exercise is known to elicit a greater increase in systolic blood pressure (BP) than dynamic exercise, but the effects of exercise training on the hemodynamic and neurohumoral responses to static exercise have not been well understood. The finding that the increases in heart rate, systolic BP, total peripheral resistance and plasma norepinephrine concentration during handgrip exercise were attenuated after 6-week exercise training were anticipated, but the finding of the greater increase in the nitric oxide (NO) level in response to handgrip exercise after exercise training is intriguing. Recent studies suggest that not only the sympathetic nerve system but also NO-mediated metabolic regulation significantly contribute to the control of the cardiovascular response to acute exercise or mental stress.[5–7] Therefore, hemodynamic changes, such as increases in BP and vascular resistance during static exercise, are the composite result of interaction between 2 regulatory systems, that is, the sympathoexcitatory α-adrenergic and sympathoinhibitory NO systems.

Sugawara et al reported that, after exercise training, increased NO-mediated vasodilatation is counterbalanced by enhanced α-adrenergic vasoconstriction, resulting in an unchanged basal limb blood flow.[8] Additionally, the Bilinska study demonstrated that both attenuated norepinephrine release and enhanced NO release may be involved in the attenuated increases in systolic BP and peripheral vascular resistance during handgrip exercise after exercise training.[4] These findings may be important for explaining the mechanism of the beneficial cardiovascular effects of exercise training, because there is a view that high levels of baseline sympathetic outflow are not dangerous per se, but that high levels of sympathetic outflow in conjunction with endothelial dysfunction may have synergistic and detrimental effect in terms of cardiovascular risk.[9] If so, a plausible scenario is that the vicious cycle of autonomic dysfunction and endothelial dysfunction can be prevented or ameliorated by regular exercise training.

Effect of Exercise Training on Systemic Inflammation

Secondly, Bilinska et al demonstrate that exercise training results in a significant reduction in inflammatory markers in post-CABG patients. Although previous studies have reported a reduction in inflammatory markers after exercise training in CAD patients,[10,11] this is the first report in post-CABG patients. It is conceivable that, in post-CABG patients, even after active myocardial ischemia is extinguished, the remaining atherosclerotic plaques at the original sites may continue to be a source of chronic inflammation.

The precise mechanisms by which exercise training ameliorates systemic inflammation is unclear, but Handschin and Spiegelman proposed peroxisome proliferative-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) as a key factor in the beneficial effect of exercise.[12] PGC-1α is a critical coordinator of the activation of metabolic genes controlling substrate use and mitochondrial biogenesis, and according to Handschin and Spiegelman, regular exercise induces PGC-1α in skeletal muscles, which in turn suppresses the production of proinflammatory cytokines such as interleukin-6 or tumor-necrosis factor-α in muscles.[12] Conversely, a sedentary lifestyle would decrease PGC-1α expression in skeletal muscles, resulting in elevation of proinflammatory cytokines and hence, chronic systemic inflammation.

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Interval vs Endurance Mode of Exercise Training

Thirdly, the study being discussed is unique because the investigators used interval training rather than endurance (continuous) training. Recent studies have demonstrated that high-intensity interval training is more effective than continuous moderate exercise training in enhancing exercise capacity, PGC-1α level, and endothelial function in patients with metabolic syndrome or chronic heart failure.[13,14] If interval training proves to be more effective than endurance training in gaining cardiovascular benefits, the mode of exercise training, and hence, the style of contemporary cardiac rehabilitation, will be greatly changed.

Remaining Issues

The study population was highly selected, young male patients after off-pump CABG with preserved left ventricular function and without myocardial ischemia, uncontrolled coronary risk factors, or comorbidities; that is, the patients were at very low prognostic risk, which means it is not easy to confirm that the observed beneficial effects will translate into meaningful clinical outcome, because the long-term event rate in this population should be very low. In addition, it remains unknown whether the presented findings obtained in a highly selected population can be generalized to real-world patients with multiple risk factors and comorbidities.

Lastly, despite the established and additional potential benefits, the use of outpatient exercise training/cardiac rehabilitation remains very low in Japan.[15] Considering the significant impact of exercise training on both the NO and PGC-1α systems that regulate fundamental cardiovascular pathophysiology, this important therapeutic modality warrants more widespread application.

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ORIGINAL ARTICLE
Molecular Cardiology

Association of the Functional Variant in the 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase Gene With Low-Density Lipoprotein-Cholesterol in Japanese

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Background: The association between single nucleotide polymorphisms (SNPs) at 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*) and low-density lipoprotein-cholesterol (LDL-C) levels has been well replicated in genome-wide association studies (GWAS) of white populations. Recently, the common intronic SNP of *HMGCR* (rs3846662) has been reported to be a functional variant, influencing the alternative splicing of exon 13. The aim of this study was to examine the association between rs3846662 of *HMGCR* and the level of LDL-C in Japanese.

Methods and Results: Significant differences in LDL-C levels were observed among the genotypes of rs3846662 (P=0.0002 (n=2,686) and P=0.004 (n=2,110)) for the Suita and Ehime samples, respectively. The G allele of rs3846662 was associated with higher LDL-C levels (β, 3.56; $P=4.91\times10^{-5}$). Consistent with this observation, the risk G allele at rs3846662 was more prevalent in subjects with myocardial infarction (MI) (n=701) than in subjects without MI (n=3,118); 0.559 and 0.511 in MI cases and controls, respectively (nominal P=0.0038). The odds ratio adjusted for age, sex, diabetes, hypertension, and drinking and smoking habits was 1.15 (95% confidence interval 1.04–1.28; P=0.0075).

Conclusions: The previously reported association of rs3846662 with LDL-C levels was replicated in the present Suita and Ehime samples. The LDL-associated SNP, rs3846662, appears to confer susceptibility to MI in Japanese. (*Circ J* 2010; **74:** 518–522)

Key Words: Genetics; Lipids; Myocardial infarction; Polymorphism

As outlined in the 2007 edition of the Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese,[1] elevated levels of low-density lipoprotein-cholesterol (LDL-C) are an important risk factor. LDL-C is known to be determined by both genetic and environmental factors. Substantial progress has been made toward detecting genes influencing circulating levels of LDL-C. In a recently published genome-wide association study (GWAS, n=19,840) with subsequent replication in 20,623 individuals,[2] 7 previously reported loci (*APOE/C1/C4/C2*, *APOB*, *HMGCR*, *LDLR*, *PCSK9*, *CELSR2/PSRC1/SORT1*, *CILP2/PBX4*),[3–8] as well as 4 novel loci (*ABCG8*, *TIMD4/HAVCR1*, *MAFB*, *HNF1A*) have shown genome-wide significant association with LDL-C levels. Although GWAS of lipid and lipoprotein levels have been predominantly conducted in populations of European ancestry, there have been only a few replication studies conducted in non-European populations.[4,9–11]

The association between single nucleotide polymorphisms (SNPs) of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGCR*) and LDL-C levels has been well replicated in GWAS of white populations.[3,4,12] HMGCR is the rate-limiting enzyme in cholesterol synthesis, and inhibitors of HMGCR have been widely used as cholesterol-lowering drugs.[13] Recently, the common SNP in intron 13 of *HMGCR* (rs3846662) has been reported to be a functional variant, influencing the alternative splicing of exon 13.[14] In that study, lymphoblastoid cells from subjects homozygous for the major A allele showed higher levels of an alternatively spliced isoform missing exon 13 compared with those from

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Table 1. Clinical Characteristics of the Study Populations

	Suita sample		Ehime sample	
	Men	Women	Men	Women
No. of subjects	1,468	1,760	1,062	1,319
Age (years)	66.0±10.7	63.8±10.5	58.6±15.3	62.1±13.2
BMI (kg/m^2)	23.4±2.9	22.4±3.2	23.5±3.0	23.3±3.3
Total cholesterol (mg/dl)*	198.7±31.6	217.4±32.5	190.6±34.6	208.1±33.5
HDL-C (mg/dl)*	54.8±14.3	64.6±15.0	58.1±14.8	64.0±15.6
LDL-C (mg/dl)*	121.2±28.5	134.3±30.4	130.4±102.1	123.2±30.2
Triglycerides (mg/dl)*	119.0±84.8	93.0±55.6	59.8±7.3	103.7±55.5
% Medication for dyslipidemia	11.0	18.5	3.7	6.7
% Smokers	28.8	5.3	38.2	2.1
% Drinkers	67.2	27.3	85.0	33.9

Continuous variables are mean±standard deviation.
*Subjects with lipid-lowering medication were excluded.
BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

homozygotes for the minor G allele. The alternatively spliced isoform of HMGCR has been detected in various human tissues, including kidney, liver, heart, spleen, lung, placenta, skeletal muscle, ovary, peripheral blood leukocytes, small intestine, bone marrow, brain, spinal cord, testes, thyroid gland, and uterus.[14,15] The proportion of the alternative splicing variant to the total HMGCR mRNA has been suggested to be tissue-specific.[14] In a recent pharmacogenetic study, in vitro upregulation of alternative splicing induced by statin treatment was inversely associated with the in vivo statin response and was partly determined by the genotypes of rs3846662.[16] Given the difference in allele frequencies and linkage disequilibrium (LD) patterns across the populations, it remains to be determined whether the previously reported functional variant, rs3846662, in *HMGCR* is associated with LDL-C levels in a Japanese population.

Methods

Study Populations

Suita Sample The study design of the Suita Study has been described previously.[17–24] In brief, the sample consisted of 14,200 men and women (30–79 years of age at enrollment), stratified by sex and 10-year age groups (10 groups and 1,420 subjects in each group) who had been randomly selected from the municipal population registry. They were all invited by letter to attend regular cycles of follow-up examination (every 2 years). Subjects were asked to estimate the amount and frequency of their alcohol intake per week, expressed as ethanol (g) per day.

To investigate the association of a genetic variation determining the LDL-C level with the risk of myocardial infarction (MI), genotyping of rs3846662 was carried out in 701 patients with MI randomly selected from in- and outpatients with documented MI and who were enrolled in the Division of Cardiology at the National Cardiovascular Center between May 2001 and April 2003. Those who were free from MI (n=3,118) served as controls.

Only those who gave written informed consent were included for the study. The study protocol was approved by the Institutional Ethics Committee and the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center.

Ehime Sample Ehime sample comprised subjects from the Nomura study of Ehime University, which is a longitudinal epidemiological study based on the Nomura Town residents.[25] Subjects were recruited through a community-based annual medical check-up process. Anthropometric and biochemical parameters were obtained from personal health records evaluated during the annual medical check-up. Information on smoking and drinking habits was obtained by interview. Subjects were asked to estimate average alcohol consumption per occasion expressed as 'gou', equivalent to 22.5 g of ethanol. All the study procedures were approved by the Ethics Committee of the Ehime University Graduate School of Medicine. Informed consent was given by each participating subject.

Genotyping Assays

Genotyping was performed by TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Deviation from Hardy-Weinberg equilibrium and the degree of LD were analyzed using HaploView 4.0 (http://www.broad.mit.edu/mpg/haploview/).[26]

The exons 1–20 of *HMGCR* were sequenced in 48 subjects with low or high LDL-C levels using a 3730 DNA analyzer (Applied Biosystems) according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean±standard deviation. Continuous variables were tested for normality of distribution, and logarithmic transformation was applied to those with skewed distributions. Residuals, defined as the observed minus predicted values on the basis of confounding factors, were used for the genotype–phenotype association analysis by 1-way analysis of variance (ANOVA) tests. Covariates included in the model were derived from multiple logistic regression analysis and used to calculate a residual value for each variable. Genotype frequencies between control and MI cases were compared by chi-square test. Odds ratio (OR) and 95% confidence interval (CI) for the risk allele were estimated by logistic regression analysis with adjustment for covariates. Statistical analysis was performed using a JMP statistical package 7.0 (SAS Institute, Cary, NC, USA).

Results

Clinical characteristics of the study populations are shown in

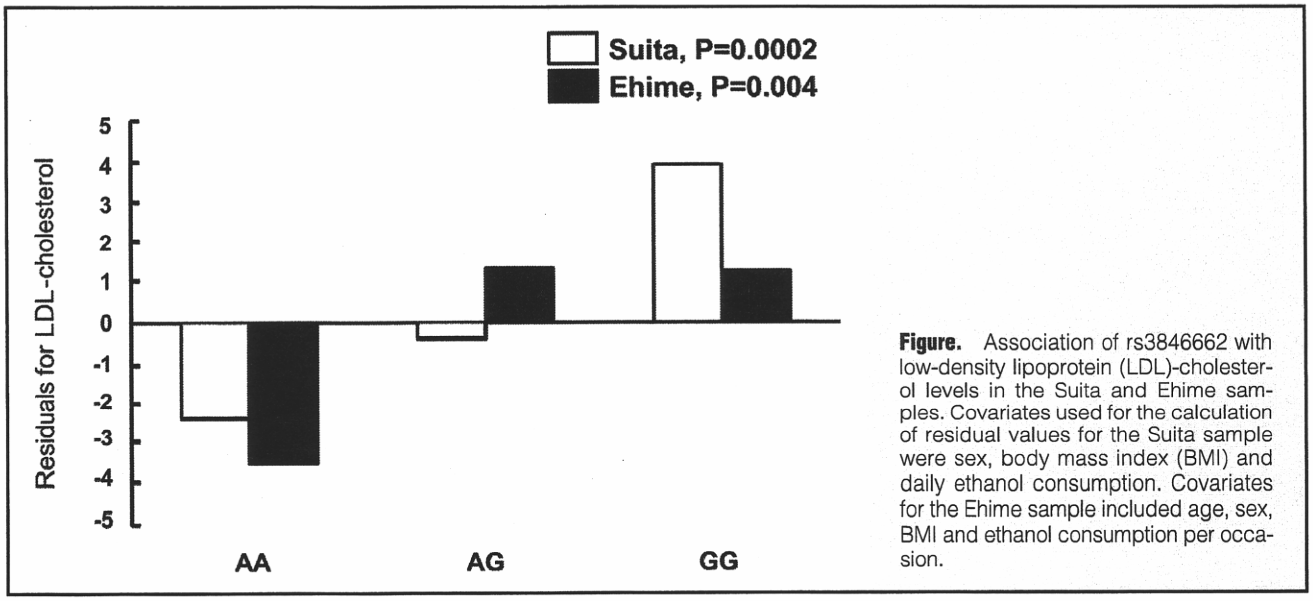

Figure. Association of rs3846662 with low-density lipoprotein (LDL)-cholesterol levels in the Suita and Ehime samples. Covariates used for the calculation of residual values for the Suita sample were sex, body mass index (BMI) and daily ethanol consumption. Covariates for the Ehime sample included age, sex, BMI and ethanol consumption per occasion.

Table 2. Logistic Regression Analysis of MI

rs3846662	Risk allele frequency	Genotype frequency			P value*	HWE†	OR‡ (95%CI)	P value
		AA	AG	GG				
Control (n=3,118)	0.511	0.232	0.514	0.254	0.0038	0.119	1.15 (1.04–1.28)	0.0075
MI cases (n=701)	0.559	0.193	0.496	0.311		0.905		

*Genotype frequencies between control and MI cases were compared by chi-square test.
†Deviation from HWE was analyzed by an exact test and P values are presented.
‡OR and 95%CI for the risk allele were estimated by logistic regression analysis with adjustment for age, sex, diabetes, hypertension, and drinking and smoking habits. BMI and the presence of hyperlipidemia were not significant predictors for MI and not included in the model.
MI, myocardial infarction; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval; BMI, body mass index.

Table 1 and **Figure** summarizes the association of rs3846662 genotypes with LDL-C levels in the Suita and Ehime samples. Significant differences in residual values of LDL-C were observed among the genotypes of rs3846662 (P=0.0002 (n=2,686) and P=0.004 (n=2,110)) for the Suita and Ehime samples, respectively. In accordance with the previous report,[12] the G allele of rs3846662 was associated with higher LDL-C levels in the Suita sample (β, 3.56, P=4.91×10^{-5} with adjustment for sex, body mass index (BMI) and ethanol consumption). Although the association was in the same direction in both the Ehime and Suita samples, the frequency of the risk G allele was more common in the Suita than in the Ehime sample (0.511 among the Suita sample, 0.495 among the Ehime sample). In the Ehime sample, homozygotes for the A allele had significantly lower levels of LDL-C (β, −3.22, P=0.001 with adjustment for age, sex, BMI and ethanol consumption).

To examine the association between rs3846662 and the risk of MI, genotype frequencies were compared between patients with MI (n=701) and those free from MI (**Table 2**). The risk G allele of rs3846662 was more prevalent in subjects with MI than in subjects without MI (0.559 and 0.511 in MI cases and controls, respectively; nominal P=0.0038). The OR adjusted for age, sex, diabetes, hypertension, and smoking and drinking habits was 1.15 (95%CI 1.04–1.28; P=0.0075).

In order to assess whether a functional rare variant of *HMGCR* with a large effect is involved in influencing the variation in LDL-C levels in Japanese, we sequenced the exon regions of *HMGCR* in 48 subjects with low (n=18; residual LDL-C adjusted for sex, BMI and daily ethanol consumption: −71.76 to −4.25 mg/dl) or high (n=30; residual LDL-C adjusted for sex, BMI and daily ethanol consumption: 54.05–135.87 mg/dl) LDL-C levels. The sequencing analysis revealed 1 synonymous mutation on exon 17 (Thr758Thr) and 2 non-synonymous mutations on exon 9 (Tyr311Ser) and 19 (Gln824Lys). The minor allele frequency (MAF) for Thr758Thr, Tyr311Ser and Gln824Lys were 0.01, 0.03 and 0.01, respectively. Exons 11–20 are known to encode a catalytic domain.[27] Because only 1 subject with low LDL-C (uncorrected LDL-C: 46 mg/dl; residual LDL-C adjusted for sex, BMI and daily ethanol consumption: −71.8 mg/dl) had Gln824Lys, further genotyping of Gln824Lys on exon 19 was carried out in 192 subjects. However, we did not find any other subject with this mutation. Overall MAF (n=240) for Gln824Lys was 0.002.

Discussion

We have replicated the previously reported association of rs3846662 within intron 13 of *HMGCR* with LDL-C level in 2 independent Japanese populations: the Suita and Ehime samples. Furthermore, rs3846662 was found to be associated with the risk of MI. The risk allele frequency for rs3846662 was more common in patients with MI than in those without MI. The OR adjusted for age, sex, diabetes, hypertension,

and smoking and drinking habits was 1.15 (95%CI 1.04–1.28, P=0.0075).

Results of our GWAS[24] conducted in 900 Japanese men and women using the Illumina Sentrix HumanHap550 BeadChip (Illumina Inc, San Diego, CA, USA) are also in line with our current observation (see Supplement for more details). Among the 368,274 SNPs with a call rate >90% and MAF >0.1, rs3846662 of *HMGCR* was 1 of the top 38 SNPs exceeding the arbitrary threshold of $-\log_{10}P > 4.0$. Of 38 top-ranked SNPs, 20 were genotyped in the remaining Suita sample (n=1,000–1,500) for validation of the associations detected in the initial subpopulation (n=900). Although the strength of the association for the 20 SNPs genotyped in the additional Suita sample was weakened by increasing the sample size, the strongest association for LDL-C was observed for rs3846662, indicating this SNP as a good candidate for replication. Although it is possible that unrecognized genes or loci influencing LDL-C levels could be newly identified by increasing the sample size of the initial screening, the observation that none of the markers (n=368,274) achieved genome-wide significance after Bonferroni correction suggests that there is no master gene involved in determining LDL-C levels.

Because it can be speculated that multiple rare alleles with a much greater effect may contribute to variations in LDL-C levels in Japanese, we sequenced the 20 exons of *HMGCR* in 48 subjects with high or low LDL-C levels. Despite our anticipation, we failed to identify any unrecognized SNP with a larger effect.

One of the limitations of the current study is the use of the Friedewald formula to estimate LDL-C levels.[28] However, a recent study conducted in 27,331 women[29] demonstrated a significant correlation between the fasting LDL-C concentration by Friedewald equation and the direct method. Nearly identical results were obtained for fasting LDL-C levels by the 2 methods in terms of the ability to predict cardiovascular disease, questioning the advantage of the direct method over the Friedewald formula. Therefore, it is unlikely that the use of the Friedewald formula altered the outcome of the results significantly.

We have replicated the association of rs3846662 with LDL-C in 2 independent Japanese populations. In contrast to the remarkable effect of HMGCR inhibitors as a cholesterol-lowering drug, the effect of rs3846662 on LDL-C is rather small, explaining only a fraction. The physiological and functional importance of a gene may not necessarily be reflected by an effect size of a commonly occurring genetic mutation. There have been many reports investigating the effect of genetic polymorphisms on MI using a candidate gene approach.[30,31] Although our findings need to be tested in a larger sample, the LDL-associated functional SNP, rs3846662, identified through GWAS appears to confer susceptibility to MI in Japanese. The GWAS approach is a powerful tool for identifying genes involved in pathogenic pathways and will provide new clues to fundamental strategies for disease prevention and therapy. The possible candidate for future validation may be found in the GWAS data included in the Supplement.

In conclusion, the previously reported association of rs3846662 with LDL-C levels was replicated in Japanese populations.

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