群間で有意差はなく(不参加群30%vs継続群22%, NS)主観的妨げ要因を客観的データで確認することはできなかった(図2).

2) 運動は自分でできる

「運動は自分でできる」という要因に関しては,継続群の7%に対し,不参加群の48%が理由としていた(P<0.01).しかし,3カ月後の心肺運動負荷試験における最高酸素摂取量増加率は不参加群0%,継続群12%で,不参加群では運動耐容能の改善が認められず,「運動は自分でできる」との回答を客観的データで確認できなかった(図3).

3) 仕事や家事で多忙

「多忙」に関しては,継続群の18%に対し,不参加群では43%が主観的妨げとしていた.しかし,発症前の就労率(不参加群48%vs継続群60%, NS)および退院後1カ月以内の復職率(不参加群43%vs継続群28%, NS)には両群間で有意差はなく,ここでも主観的妨げ要因を客観的データで確認することはできなかった(図4).

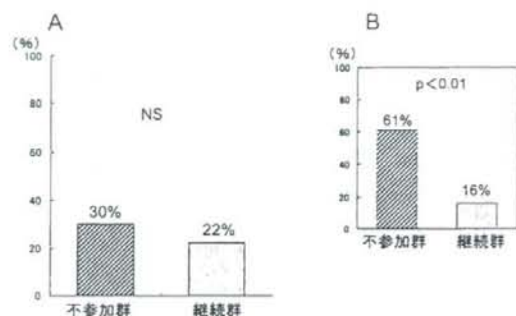

図2 妨げ要因としての「遠方」の検討
A:遠方居住の割合,B:主観的に遠方と回答した割合

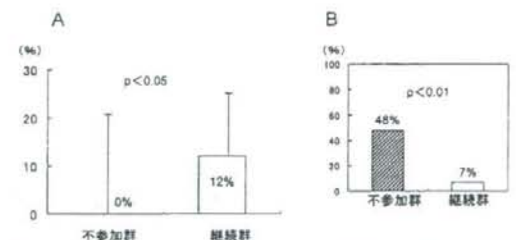

図3 妨げ要因としての「運動は自分でできる」の検討
A:最高酸素摂取量増加率,B:主観的に運動は自分でできると回答した割合

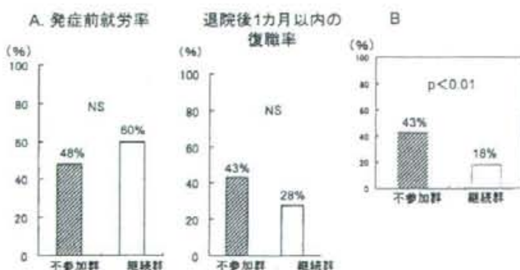

図4 妨げ要因としての「多忙」の検討
A:客観的データ,B:主観的に多忙と回答した割合

IV. 考 察

本研究において,外来心リハに参加しなかった不参加群は継続群と比較して,主観的には外来心リハへの妨げ要因(遠方,自分でできる,多忙)を明らかに多く保有するにも関わらず,それらに関連する客観的データには継続群との差が見られなかった.

1. 主観的要因と客観的データの不一致

1) 妨げ要因:自宅が遠方で通院が大変

主観的妨げ要因として「遠方居住」を挙げた割合は不参加群で有意に多かったが,実際の遠方居住者の割合は両群間で差がなかった.すなわち,不参加群は近隣在住であるにもかかわらず,「遠方」と回答したわけである.これまでの報告では,居住地が遠方という因子[7]のほか,通院所要時間が不参加要因である[8]とするものもある.本研究では交通手段・通院所要時間を調べていないため,近隣居住であっても実際の通院所要時間や交通手段の点で差がある可能性は残る.しかし一方で,心リハに対する意欲の欠如や面倒などの理由が「主観的に遠方」と自覚する背景に隠れている可能性も考えられる.

2) 妨げ要因:運動は自分でできる

主観的妨げ要因として「自分でできる」を挙げた割合は不参加群で有意に多かったが,最高酸素摂取量の増加率は継続群より有意に低く,増加は見られなかった.心リハ開始時の最高酸素摂取量には差がなかった(表1)ことから,不参加群は運動療法は自分でできるので心リハに参加する必要はないと考えているものの,実際のところ行えていなかったと推測される.

3) 妨げ要因:仕事や家事で多忙

主観的妨げ要因として「多忙」を挙げた割合は不参加群で有意に多かったが,発症前の就労率および退院後1カ月以内の復職率は両群間で有意差はなかった.心リハを継続できない理由として,仕事の多忙さがよく挙げられる[9].仕事の多忙さをどの指標で測定するかは問題であるが,今回は復職の有無で客観的に比較した.復職率に差がないにも関わらず,不参加群では主観的に多忙であるため心リハに参加できないと感じている点は,同程度の客観的妨げ要因でも不参加群と継続群とでは受け止め方が異なることを示唆している.

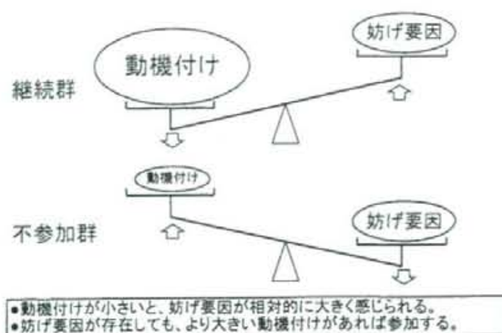

図5　心リハ参加への動機付けと妨げ要因とのバランスのモデル

2．心リハ参加への動機付けと妨げ要因のバランスのモデル

本研究の結果から，不参加群は継続群に比べて同程度の客観的妨げ要因でも主観的により大きな障害(barrier)として受け止めてしまうと考えられる．本研究の結果に基づき，心リハ参加への動機付けと妨げ要因とのバランスのモデルを提唱したい(図5)．すなわち，不参加群において妨げ要因の大きさには客観的に差がないにも関わらず主観的に大きく感じられたのは，心リハ参加への動機付けが小さかったからと考えられる．逆に継続群では，より大きい動機付けがあったために，不参加群と同じ大きさの妨げ要因が存在しても主観的には大きな障害と感じずに心リハ参加を継続したと考えられる．

患者側の要因とは別に，近年，AMI患者の心リハへの動機付けに関して，AMIに対する再灌流療法の普及により，合併症が減少し臥床期間が短縮した結果，患者の身体デコンディショニングが軽症化し，患者自身が退院や社会復帰のための心リハトレーニングを必要と感じなくなったこと，また，治療の進歩やクリティカルパスの導入により入院期間が大幅に短縮した結果，入院期間中に心リハや患者教育を実施する時間的余裕がなくなったことが指摘されている[10,11]．事実，われわれの施設における心リハ開始時の最高酸素摂取量は1993〜94年の73±13%から2005〜06年には79±15% ($p<0.01$)へと増加しデコンディショニングが軽減している一方，入院中の心リハ参加回数は10±6から6±4日($p<0.01$)へ減少しており(未発表データ)，上記の指摘が裏付けられる．すなわち歴史的に見ると，動機付けに促進的に働く要因が減弱しているといえる．もともと動機付けが弱い不参加群では，これらの促進要因の減弱がより強く作用した可能性があり，今後はこれまでに比べより迅速かつ強力に入院中の動機付け介入を行うことが必要かもしれない．

3．熱意・自己効力感について

前項および図5で述べた動機付けは，「熱意」や「自己効力感」に関連する．心リハの継続に関してAHCPRガイドライン"Cardiac Rehabilitation"[12]は，「最初の3カ月で20〜25%が中断，6〜1カ月の間に40〜50%が中断してしまう．確認はされていないが，この早期の脱落率は，治療の費用，保険償還の欠如，プログラムスケジュールの不便さや施設の不便さ，職場復帰ができなくなることや家族の非協力，あるいは単なる熱意の欠如に関係しているであろう」と述べている．また心リハ継続への妨げ要因を検討した過去の報告[7,13-15]においても，心リハへの不参加や脱落の要因として「遠方居住」,「雇用状態」,「女性」,「高齢」,「経済状態」などの客観的条件と並んで，「熱意の欠如」,「自己効力感の不足」,「自分の予後を自分で修正しうるという信念の欠如」といった患者の内面のあり方が挙げられている．

重要であるのは，この動機付けや自己効力感が医療スタッフからの働きかけにより修正可能とされている点である[7,14-16]．たとえばAdesら[17]は，種々の客観的・主観的因子を多変量解析した結果，AMIまたは冠動脈バイパス術後患者において，心リハ参加への最も強力な予測因子は担当医師の勧めであり，勧めが強力であるほど参加率が高かったと報告している．すなわち，図5における不参加群の動機付けの大きさを周囲からの働きかけで妨げ要因よりも大きくし，バランスを参加継続の方向へ変えることが可能ということである．

4．今後の方策

本研究の結果から，退院後の外来心リハ継続率を高めるための方策を考察する．退院後不参加群は，主観的な妨げ因子を多く保有し動機付けのレベルがいまだ低いと考えられることから，強力な動機付け介入が必要である．その際，患者に欠如している「熱意」,「自己効力感」,「自分の予後を自分で修正しうるという信念」を有効に高めることをめざして，動機付け介入の方法を工夫していくことが重要である．具体的には，心リハスタッフではなくすでに病棟で患者との信頼関係にある担当医や病棟看護師が直接患者に心リハの目的や必要性などを説明すること，単に口頭の説明だけでなくパンフレットなどの教育・啓蒙用教材を活用して，わかりやすくかつ繰り返し説明することなどが挙げられる．また，在院日数が短縮し患者教育の機会や時間が減っていることから，冠危険因子や退院後の生活方法などの全般的な教育・指導は退院後の心リハ継続期間中にじっくりと行うこととして，入院中にはまず退院後外来心リハ継続への動機付け教育を集中的かつ強力に推進していくことが重要と考えられる．

5．本研究の弱点

本研究は郵送法による質問紙調査であり，対面型質問方式に比べて精度が低い可能性がある．しかし退院後全く来院していない症例に対しては郵送質問紙法以外の適切な方法がなくやむを得なかった．また不参加理由として，先行研究で指摘されている学歴や収入などは今回の調査項目に

含めておらず，これらの未調査要因が間接的に今回の結果に影響した可能性は否定できない．また対象疾患はAMIのみであり，今回の結果を心臓術後や慢性心不全に直接当てはめることはできない．このような弱点はあるものの，主観的な妨げ要因を客観的な指標と対比して退院後不参加群では両者が必ずしも一致しないことを明らかにした意義は大きいと考える．

V．総　括

退院後の外来心リハ不参加群は，継続群と比較して客観的条件に差はないにも関わらず，主観的に大きな妨げ要因ととらえて，退院後外来心リハに参加していないことが明らかとなった．このことは，心リハ継続の動機付け不足が影響している可能性が大きいと考えられた．在院日数短縮化の中で，効果的に患者と関わり，退院後の外来心リハ継続に向けて強力な動機付け介入を行うことが今後の重要な課題である．

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Circ J 2008; **72:** 694–699

Mutation Site Dependent Variability of Cardiac Events in Japanese LQT2 Form of Congenital Long-QT Syndrome

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Background In the LQT2 form of long QT syndrome (LQTS), mutation sites are reported to correlate with clinical phenotypes in Caucasians, but the relationship in Asian patients remains unknown. The present study was designed to determine whether the location of *KCNH2* mutations would influence the arrhythmic risk in LQT2 patients.

Methods and Results In 118 genetically-confirmed LQT2 patients (69 families, 62 *KCNH2* mutations), the ECG parameters, Schwartz scores, and the incidence of cardiac events, defined as syncope, aborted cardiac arrest, and sudden cardiac death, were evaluated. To examine the effect of mutation sites, the participants were divided accordingly: pore (n=56) and non-pore (n=62) groups. The corrected QT_{end} interval was significantly greater in the pore than in the non-pore group (QTc; 522±63 ms vs 490±49 ms, p=0.002). In this study, the clinical course of each of the probands did not differ according to the mutation sites, whereas non-probands carrying the pore site mutation experienced their first cardiac events at significantly younger age than those with the non-pore site mutation (log-rank, p=0.0005).

Conclusions In a Japanese LQT2 cohort, family members with the pore site mutation were at higher arrhythmic risk than those with the non-pore site mutation. (*Circ J* 2008; **72:** 694–699)

Key Words: Arrhythmia; Long-QT syndrome; QTc interval; Risk factors; Torsade de pointes

The long QT syndrome (LQTS) is an inherited arrhythmogenic disease of the structurally normal heart that may cause sudden death. LQTS is characterized by an abnormality in myocardial repolarization that leads to prolongation of the QT interval, morphological changes in T waves and torsades-de-pointes (Tdp) type of ventricular tachycardia on surface ECGs[1,2] To date, 8 distinct genes responsible for LQTS have been identified, including those of Andersen (LQT7) and Timothy (LQT8) syndromes: on chromosome 11q15.5 (*KCNQ1*; LQT1), 7q35–36 (*KCNH2*; LQT2), 3p21 (*SCN5A*; LQT3), 4q25–27 (*ANKB*; LQT4), 21q22 (*KCNE1*; LQT5), 21q22 (*KCNE2*; LQT6), 17q23 (*KCNJ2*; LQT7) and 12p13.3 (*CACNIC*; LQT8)[3–10]

Moss et al[11] extensively examined the relationships between the site of mutation and clinical phenotype in approximately 44 different LQT2-related *KCNH2* mutations. They reported that subjects with causative mutations in the pore region (n=38, amino acid residues 550 through 650) had more severe clinical manifestations and experienced a higher frequency (74% vs 35%; p<0.001) of arrhythmia-related cardiac events occurring at younger age than did subjects with non-pore mutations (n=166).

In LQT1, based on the United States portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82), 600 patients with *KCNQ1* mutations were classified into 2 groups of patients with transmembrane and C-terminus mutations and their clinical phenotypes were examined[12] That study found that patients with transmembrane mutations were at increasing risk for cardiac events (hazard ratio, 2.06; p<0.001). Shimizu et al also studied the mutation site-dependent differences in 95 LQT1 patients from a multicenter Japanese population and also found that patients with transmembrane mutations were at higher risk of cardiac events and had longer QTc and $T_{peak\text{-}end}$ intervals[13]

In Japanese LQT2 patients, mutation site dependency is unclear, although this has been reported in Caucasian patients. Therefore, in the present study we aimed to compare the genotype and phenotype relationship, according to the classification adopted by Moss et al[11] in 118 Japanese LQT2 patients who were genetically identified in the 3 genetic centers in Japan and had no other mutations in LQTS-related genes (except LQT4 and 8).

(Received October 17, 2007; revised manuscript received December 11, 2007; accepted December 13, 2007)

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Table 1 *KCNH2* Mutations by Location, Amino-Acid Coding, Type of Mutation, and Reported Functional Effects

	No. of families	No. of subjects	Position	Exon	Type of mutation	Functional effect in expression studies
Pore regions						
A561T	1	1	S5	7	Missense	Trafficking defect (22)
A561V	1	1	S5	7	Missense	Dominant negative (23)
W563C*	1	1	Pore	7	Missense	
W563G*	1	2	Pore	7	Missense	
C566F*	1	1	Pore	7	Missense	
G572S	2	4	Pore	7	Missense	
M574V*	1	3	Pore	7	Missense	
R582L	1	2	Pore	7	Missense	
R582C	1	1	Pore	7	Missense	
G584C*	1	2	Pore	7	Missense	
G590V*	1	3	Pore	7	Missense	
I593V*	1	1	Pore	7	missense	
K595N*	1	2	Pore	7	Missense	
K595E*	1	1	Pore	7	Missense	
G601S	2	5	Pore	7	Missense	Trafficking defect (22, 24)
G604S	2	2	Pore	7	Missense	
S606P*	1	1	Pore	7	Missense	
T613M	2	3	Pore	7	Missense	Dominant negative (25)
A614V	4	6	Pore	7	Missense	Dominant negative (26)
T623I	1	1	Pore	7	Missense	Trafficking defect (22)
G628S	1	2	Pore	7	Missense	Trafficking defect (22)
N629K	1	1	Pore	7	Missense	Dominant negative (27)
N633S	1	1	Pore	7	Missense	
K638del	1	1	S6	7	Deletion	
F640del*	1	1	S6	7	Deletion	
S641F	1	3	S6	7	Missense	
V644F	1	4	S6	7	Missense	
Subtotal	34	56				
Non-pore regions						
N-terminal regions						
V41A*	1	1	N-term	2	Missense	
Y43D*	1	3	N-term	2	Missense	
E50fs+10X*	1	1	N-term	2	Deletion/frameshift	
G53S*	1	1	N-term	2	Missense	
82-84insIAQ	1	1	N-term	2	Insertion	
F106L*	1	1	N-term	3	Missense	
D111V*	1	1	N-term	3	Missense	
V115M*	1	1	N-term	3	Missense	
P151fs+179X	1	1	N-term	3	Insertion/frameshift	
G187-A190del*	1	3	N-term	4	Deletion	
R312-S318del*	1	2	N-term	5	Deletion	
S320L	1	1	N-term	5	Missense	
P334L	1	1	N-term	5	Missense	
K364fs+3X*	1	3	N-term	5	Insertion/deletion/frameshift	
K386fs+3X*	1	4	N-term	5	Insertion/frameshift	
Transmembrane domains other than pore regions						
Q391X	1	2	S1	6	Nonsense	
F471fs+50X*	1	1	S1-S2	6	Deletion/frameshift	
I489F*	1	1	S1-S2	6	Missense	
A490T	1	1	S1-S2	6	Missense	Current density↓ (28)
H492Y*	1	2	S1-S2	6	Missense	
W497X*	1	3	S3	6	Nonsense	
D501N	1	1	S3	6	Missense	
R534C	1	2	S4	7	Missense	Trafficking defect (22)
C-terminal region						
Q738X*	1	2	C-term	9	Nonsense	
G745-G749del, Fins/fs+56X*	1	1	C-term	9	Insertion/deletion/frameshift	
R752W	1	2	C-term	9	Missense	Trafficking defect (22)
S818L	1	1	C-term	10	Missense	Reduced I_{Kr} current (29)
P846T*	1	1	C-term	10	Missense	
W853fs+14X*	1	2	C-term	10	Deletion/frameshift	
R863X	1	2	C-term	10	Nonsense	
L911fs+6X*	1	3	C-term	12	Deletion/frameshift	
R912fs+63X*	1	2	C-term	12	Insertion/frameshift	
S1029fs+23X*	1	3	C-term	13	Deletion/frameshift	
P1034fs+23X*	1	3	C-term	13	Insertion/frameshift	
A1144T*	1	2	C-term	15	Missense	
Subtotal	35	62				

*Novel mutation.

del, deletion; ins, insertion; fs, first amino acid affected by a frameshift (number after fs is number of amino acids before termination); term, terminus.

Table 2 Clinical Characteristics of Pore and Non-Pore Mutations

	Pore (n=56)	Non-pore (n=62)	p value
Demographics			
Female gender (%)	33 (59%)	42 (68%)	0.344
Proband (%)	33 (59%)	34 (55%)	0.712
Age (years) at baseline ECG (range)	31±18 (7–74)	31±16 (2–71)	0.920
Age (years) at first event (range)	16±10 (5–48)	20±13 (5–71)	0.203
Diagnosis			
Schwartz score	5.3±1.6	4.5±1.8	0.017
Schwartz score ≥4 (%)	47 (84%)	41 (66%)	0.034
ECG measurements			
Heart rate (beats/min)	65±13	64±15	0.537
RR (ms)	953±188	975±186	0.510
QT_{end} (ms)	505±79	482±69	0.089
QT_{peak} (ms)	377±67	382±65	0.650
$T_{peak\text{-}end}$ (ms)	129±55	99±41	0.001
Corrected QT_{end} (ms)	522±63	490±49	0.002
Corrected QT_{peak} (ms)	389±62	388±47	0.927
Corrected $T_{peak\text{-}end}$ (ms)	134±52	101±42	<0.001
Torsade de pointes (%)	17 (30%)	18 (29%)	1.000
T-wave alternans (%)	7 (13%)	4 (7%)	0.346
Notched T wave (%)	43 (77%)	32 (52%)	0.007
Cardiac events			
All cardiac events (%)	38 (68%)	32 (52%)	0.092
Syncope (%)	36 (64%)	32 (52%)	0.194
Aborted cardiac arrest/SCD (%)	6 (11%)	2 (3%)	0.145
Therapy			
β-blocker therapy	26 (53%)	21 (36%)	0.117
Pacemaker (%)	1 (2%)	0	1.000
Sympathectomy (%)	0	0	1.000
Defibrillator (%)	1(2%)	2 (3%)	1.000

Data are mean value ± SD or number (%) of subjects.
ECG, electrocardiography; SCD, sudden cardiac death.

Methods

Study Population

The study population consisted of 118 patients from 69 unrelated Japanese LQT2 families enrolled from 3 institutes in Japan: National Cardiovascular Center, Kyoto University Graduate School of Medicine and Shiga University of Medical Science. The *KCNH2* mutations were confirmed in all patients by using standard genetic tests.[14–17] Screening for mutations in *KCNQ1*, *SCN5A*, *KCNE1*, *KCNE2*, and *KCNJ2* was also conducted, and patients with compound mutations of *KCNH2* and/or additional mutations in these LQTS-related genes were excluded from the analysis. Symptomatic patients were defined as *KCNH2* mutation carriers who experienced at least 1 episode of syncope (ie, complete loss of consciousness, or cardiac arrest requiring cardiac resuscitation), while asymptomatic patients were those without these events. Follow-up was censored at age 50 years to avoid the influence of coronary artery disease on cardiac events.

Genetic Analysis and Characterization

Genomic DNA was isolated from venous blood by use of the QIAamp DNA blood midikit (Qiagen, Hilden, Germany). The protocol for genetic analysis was approved by the institutional ethics committee and was performed under its guidelines. Established primer settings were used to amplify the entire coding regions of the known LQTS genes from genomic DNA.[14–17] Denaturing high-performance liquid chromatography (DHPLC) was used for screening. For aberrant conformers, direct sequencing techniques were performed as described elsewhere.[13] PCR products were denatured at 95°C for 5 min then analyzed by DHPLC. PCR fragments presenting abnormal signals in the DHPLC analysis were subsequently sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides in an ABI 3130 genetic analyzer (PE Applied Biosystems).

The pore region of the *KCNH2* channel was defined as the area extending from S5 to the mid-portion of S6 involving amino acid residues 550 through 650, according to a previous report.[11] The non-pore region included the N-terminus region, transmembrane domains other than the pore region and the C-terminus region.

Clinical Characterization

Routine demographic data and basal 12-lead ECGs were obtained for all subjects at the time of enrollment in each institute and there was at least yearly follow-up contact. All ECGs were taken before or without β-blocker medication. The ECG parameters measured from the basal recordings were the RR, QT_{end}, QT_{peak} and $T_{peak-end}$ (QT_{end}–QT_{peak}) intervals. The latter is thought to reflect the transmural dispersion of ventricular repolarization (TDR).[18–20] The rate-dependent QT intervals were corrected for heart rate by Bazett's method.[21] The QT_{peak} was defined as the time interval between QRS onset and the peak of the positive T wave or the nadir of the negative T wave. $T_{peak-end}$ was then obtained by calculating QT_{end} minus QT_{peak}.

These parameters were measured manually in lead V_5 averaged from 2 or 3 consecutive beats. Bifid T waves other than U waves were included in the QT measurements. If ECG recordings were obtained during a cardiac event, the patients were requested to undergo the examination again after improving. Measurements were performed by 3 investigators who were completely unaware of the patient's clinical and genetic status. There were no significant differences in the measured data between the investigators, and the

mean values were used for analysis. LQTS-related cardiac events were defined as syncope, aborted cardiac arrest, or unexpected sudden death.

Statistical Analyses

All data are expressed as the mean value ± SD. The Student's t-test was used to compare continuous data between mutations located in the pore region and those in the non-pore region. Differences in frequencies were analyzed by the chi-square test. Time to the first cardiac event (syncope, cardiac arrest, or sudden cardiac death) before initiation of β-blocker therapy and before age 50 years was determined by Kaplan-Meier cumulative estimates. Two-sided probability values <0.05 were considered statistically significant. Statistical calculations were performed with SPSS software (version 11.01J, Chicago, IL, USA).

Results

Genetic Characteristics

Table 1 lists the *KCNH2* mutations we identified, classified by location, number of patients with these causative mutations, coding effects (missense, insertion, deletion and frameshift) and functional outcomes. We identified 62 different *KCNH2* mutations among the 69 LQTS families: 42 missense, 16 deletion/insertion, 11 frameshift and 4 nonsense mutations. There were 27 (44%) mutations causing amino acid changes in the pore region and 35 (56%) mutations within the non-pore regions (15 in the N-terminus, 8 in the non-pore transmembrane, and 12 in the C-terminus). In the pore mutations there were 25 (93%) missense mutations and the remaining 2 were protein deletions (K638del and F640del).

In contrast, the non-pore mutations included more significantly complex mutations such as deletion, insertion, frameshift or nonsense mutations that resulted in truncation of channel proteins (15/35, 43%). Thirty-five mutations (56%, 11 in the pore region and 24 in the non-pore regions) were novel and indicated by asterisk in Table 1. Functional effects by cellular electrophysiologic tests have been reported in only 12 of the 62 mutations (19%);[22–29] however, all those previous reports indicated that the *KCNH2* mutations had loss-of-function effects and made the I_{Kr} current reduce or disappear. Four pore mutations had dominant-negative effects, 4 pore mutations and 2 non-pore mutations had trafficking defects, and 2 non-pore mutations reduced the I_{Kr} current.

Clinical Characteristics

Table 2 is a comparison of the clinical characteristics of the 56 patients with pore mutations and the 62 patients with non-pore mutations. There were no significant differences between the 2 groups regarding gender, the percentage of probands and the age at baseline ECG recording. Diagnostic LQTS scores of Schwartz et al[30] were noticeably greater in the pore group. RR and QT_{peak} intervals were comparable; however, corrected QT_{end} and $T_{peak-end}$ intervals were much longer in the pore than in the non-pore group. Although the incidence of TdP and T-wave alternans did not differ between groups, notched T waves were more frequently seen in the pore group (p=0.007 vs non-pore group). The incidence of cardiac events and the introduction of β-blocker therapy were not statistically different between the 2 groups.

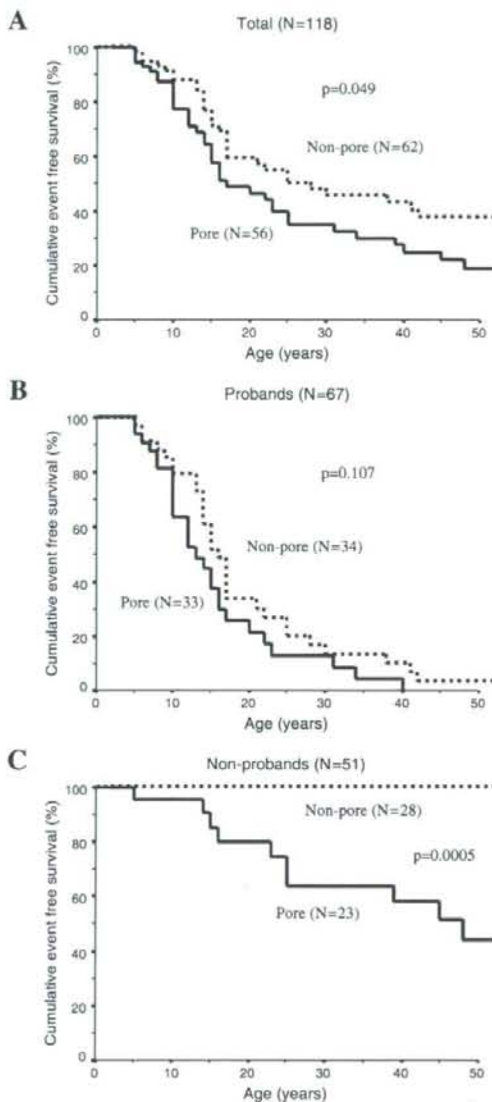

Fig 1. (A) Kaplan-Meier cumulative cardiac event-free survival curves from birth through to age 50 years for the total of 118 patients with *KCNH2* mutations located in the pore (n=56, smooth line) and non-pore (n=62, dotted line) regions. The pore group patients experienced their first cardiac event at a younger age than the non-pore group (log-rank, p=0.049). The difference was caused mainly by the high first-event rate in non-probands. Kaplan-Meier cumulative cardiac event-free survival curves for 67 probands (B) and 51 non-probands (C) with pore mutations (smooth line) and non-pore mutations (dotted line).

Clinical Course by Mutation Location

Fig 1A shows the Kaplan-Meier cumulative cardiac event-free survival curves from birth through to age 50 years for 118 patients (pore group, n=56; non-pore group, n=62). The pore-group patients experienced their first cardiac event at a younger age than the non-pore group (log-rank, p=

Table 3 Clinical Characteristics of Pore and Non-Pore Mutations in Non-Probands

	Pore (n=23)	Non-pore (n=28)	p value
Demographics			
Female gender (%)	14 (61%)	19 (68%)	0.769
Age (years) at baseline ECG (range)	42±20 (9–74)	33±20 (2–71)	0.124
Diagnosis			
Schwartz score	4.7±1.5	3.5±1.7	0.008
Schwartz score ≥4 (%)	18 (78%)	12 (43%)	0.021
ECG measurements			
Heart rate (beats/min)	65±15	70±17	0.251
RR (ms)	959±179	894±179	0.201
QT_{end} (ms)	480±51	441±54	0.0011
QT_{peak} (ms)	352±47	352±53	0.974
$T_{peak-end}$ (ms)	128±46	89±30	0.001
Corrected QT_{end} (ms)	494±45	470±40	0.044
Corrected QT_{peak} (ms)	364±49	374±40	0.423
Corrected $T_{peak-end}$ (ms)	131±43	96±32	0.002
Torsade de pointes (%)	1 (4%)	0	0.451
T-wave alternans (%)	0	0	–
Notched T wave (%)	17 (74%)	14 (50%)	0.095
Cardiac events			
All cardiac events (%)	11 (48%)	0	<0.001
Syncope (%)	10 (43%)	0	<0.001
Aborted cardiac arrest/SCD (%)	1 (4%)	0	0.451
Therapy			
β-blocker therapy	6 (26%)	0	0.006
Pacemaker (%)	0	0	–
Sympathectomy (%)	0	0	–
Defibrillator (%)	0	0	–

Data are mean value ± SD or number (%) of subjects.
Abbreviations see in Table 2.

0.049). We examined the clinical course of the 67 probands and 51 non-probands separately (Figs 1B,C). The clinical courses of the probands were not significantly different according to mutation site (Fig 1B), whereas in the non-pore group 28 non-probands remained asymptomatic and more than half had suffered from cardiac events by the age of 50 (Fig 1C). Therefore, the difference stemmed from markedly distinct prognoses among the non-probands.

Table 3 summarizes the clinical characteristics of the 51 non-probands. The absolute and corrected QT_{end} and $T_{peak-end}$ intervals were all significantly greater in the pore than in the non-pore group. In the non-probands, the incidence of all cardiac events, syncope, and β-blocker therapy were significantly greater in the pore group than in the non-pore group.

Discussion

This study demonstrates that the clinical features of 118 Japanese LQT2 patients who had 62 different *KCNH2* mutations correlated with the mutation sites, but only in non-probands. In probands, there was no significant relationship between mutation site and prognosis. Moss et al[11] reported approximately 179 LQT2 patients based on 44 different *KCNH2* mutations and those patients with pore mutations had significantly (p<0.0001) higher frequency of LQTS-related cardiac events and longer QTc intervals than those with non-pore mutations. In contrast to their results, in the present study the mutation-dependent difference in prognosis was relatively small, though significant (p=0.049), when analyzed in the total patient cohort (Fig 1A). Indeed, the beneficial outcome of the non-pore patients stemmed from their family members (Fig 1C), and probands showed virtually similar prognosis to that of pore mutation carriers. Although Moss et al did not report separate sub-analysis of probands and family members, the percentage of family members in their non-pore group was significantly larger than that of the pore group (84% vs 57%, p<0.001). The very good prognosis of the non-pore mutation group in their study may have reflected that large number of family members.

The character of the mutation per se may be important as another reason for the variance between these 2 studies, as both had a similar number of LQT2 patients. Compared with the study by Moss et al,[11] the type of mutation in the present study was quite different: in our non-pore group, there were significantly more complex mutations, such as nonsense or frameshift, that caused the truncation of channel proteins (15/35, 43%) than in the report of Moss et al (4/30, 13%). For example, nonsense-mediated mRNA decay (NMD) has recently been reported to play an important role in reducing dominant negative suppression effects.[31] Premature termination codon caused by either a deletion or insertion mutation would also cause NMD and thereby attenuate the severity of cardiac phenotypes. This different nature of the mutations may cause the apparently different prognosis of the non-pore mutation groups in each study.

In our pore site mutation group, there were only 2 in-frame deletions, but no frameshift mutations (Table 1). Although it was practically very difficult to conduct every functional assay for each novel *KCNH2* mutation identified here, some cellular electrophysiological effects are available in a small number of *KCNH2* mutations we found (Table 1). Several missense mutations in the pore region (such as A561V and T613M) have been shown to produce dominant negative suppression effects, a greater functional change predisposing to arrhythmic events. In contrast, functional assay of several missense mutations in the non-pore regions has revealed relatively smaller loss-of-function effects (such as with A490T or S818L) Greater functional disruption may also be reflected in the different prognosis

of family members in the pore and non-pore groups (Fig 1).

Previously we reported that LQT1 patients with *KCNQ1* mutations located in the transmembrane regions, including the pore region, are at a higher risk of congenital LQTS-related cardiac events and longer QTc and $T_{peak-end}$ intervals than are patients with C-terminal mutations.[13] In LQT2, we have also demonstrated that $T_{peak-end}$, representing transmural dispersion of ventricular repolarization,[19] is longer in pore patients than in non-pore patients (Table 2), supporting the finding that family members with pore mutations are more likely to suffer from LQTS-related cardiac events than those with a non-pore mutation.

Study Limitations

Cardiac events are not simply linked to the site of mutation in probands; there are other triggering factors such as modifier genes, including single nucleotide polymorphisms,[32] hypokalemia and bradycardia, which play significant roles in aggravating the symptoms of *KCNH2* mutation carriers. The influence of these factors could be interpreted in the similar occurrence of cardiac events in the probands irrespective of mutation site, because the presence of symptoms usually caused the patient to agree to undergo genetic testing.

Regarding each mutation, the number of study patients was relatively small (at most 5), and the location of the mutations was scattered, even in the same pore region. The coding effect was also so various that we had limited ability to show arrhythmic risk according to a specific mutation site. Our cohort contained 35 novel *KCNH2* mutations, and their functional outcomes were not available. Moreover, our study population included only Japanese, so more subjects per mutation and a greater spectrum of *KCNH2* mutations in a worldwide study are needed to evaluate the arrhythmic risks associated with these mutations.

Acknowledgments

Dr W. Shimizu was supported by the Uehara Memorial Foundation, and with Dr M. Horie by grants from Ministry of Education, Culture, Sports, Science and Technology Leading Project for Biosimulation, and health sciences research grants (H18-Research on Human Genome-002) from the Ministry of Health, Labour and Welfare, Japan.

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Age- and Genotype-Specific Triggers for Life-Threatening Arrhythmia in the Genotyped Long QT Syndrome

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Age and Long QT Syndrome. ***Introduction:*** **Patients with long QT syndrome (LQTS) become symptomatic in adolescence, but some become at age of ≥20 years. Since it remains unknown whether clinical features of symptomatic LQTS patients differ depending on the age of onset, we aimed to examine whether triggers for cardiac events are different depending on the age in genotyped and symptomatic LQTS patients.**

Methods and Results: **We identified 145 symptomatic LQTS patients, divided them into three groups according to the age of first onset of symptoms (young <20, intermediate 20–39, and older ≥40 years), and analyzed triggers of cardiac events (ventricular tachycardia, syncope, or cardiac arrest). The triggers were divided into three categories: (1) adrenergically mediated triggers: exercise, emotional stress, loud noise, and arousal; (2) vagally mediated triggers: rest/sleep; and (3) secondary triggers: drugs, hypokalemia, and atrioventricular (AV) block. In the young group, 78% of the cardiac events were initiated by adrenergically mediated triggers and 22% were vagally mediated, but none by secondary triggers. In contrast, the adrenergically mediated triggers were significantly lower in the intermediate group. The percentage of secondary triggers was significantly larger in the older group than in the other two groups (0% in young vs 23% in intermediate vs 72% in older; P < 0.0001). Concerning the subdivision of secondary triggers on the basis of genotype, hypokalemia was only observed in LQT1, drugs mainly in LQT2, and AV block only in LQT2.**

Conclusion: **Arrhythmic triggers in LQTS differ depending on the age of the patients, stressing the importance of age-related therapy for genotyped LQTS patients.** *(J Cardiovasc Electrophysiol, Vol. 19, pp. 794-799, August 2008)*

long QT syndrome, genetic test, age, triggers, drugs, hypokalemia, bradycardia

Introduction

The long QT syndrome (LQTS) is a disease entity characterized by an abnormality in the myocardial repolarization that leads to the prolongation of the QT interval, morphological changes in T waves, and torsade de pointes (TdP) type of ventricular tachycardia on surface electrocardiogram (ECG).[1] The prevalence of LQTS is reported as 1 per 5,000 and it induces syncope and sudden cardiac death usually among young people. Up to date, several different genes have been reported to cause the LQTS.[1-3]

Since the first description on two LQTS-related genes (*KCNH2* and *SCN5A*) in 1995,[1] a number of studies have been performed regarding the relationship between genotype and phenotype. In addition to the genetic background predisposing excessive QT prolongation and TdP, many triggers have been known to modify and aggravate the clinical features of LQTS.[4,5] They are, for example, gender (being female), exercise, emotional stress, loud noise, sudden arousal, drugs, hypokalemia, and bradycardia. Some of them are related to the autonomic nervous tone, and it is well known that LQT1 patients are at a higher risk of TdP during exercise and LQT2 patients, in sudden arousal and auditory stimuli.[5-7]

Although many LQTS patients develop symptoms during adolescence, some of them experience the first cardiac event in their adulthood. In order to study the age-related difference in the LQTS phenotype, we aimed to examine whether the above-mentioned triggers for cardiac events are different depending on the age in genotyped and symptomatic LQTS patients.

This work was supported by research grants from the Ministry of Education, Science, Sports and Culture of Japan and Health Sciences Research Grants (H18 – Research on Human Genome – 002) from the Ministry of Health, Labour and Welfare, Japan, to Drs. Shimizu and Horie.

The first two authors contributed equally to the original concept and to the authorship of this investigation.

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Manuscript received 3 October 2007; Revised manuscript received 28 January 2008; Accepted for publication 4 February 2008.

doi: 10.1111/j.1540-8167.2008.01138.x

Methods

Study Population

The study population consisted of consecutive 145 symptomatic patients (117 probands and 28 family members) of a known genotype (LQT1, LQT2, and LQT3) from 117 unrelated Japanese families out of 343 genotyped patients (185 probands and 158 family members). They were enrolled from three institutes in Japan – Shiga University of Medical Science, National Cardiovascular Center, and Kyoto University Graduate School of Medicine – between 1996 and 2007. Patients with LQT5, LQT6, LQT7 (Andersen-Tawil syndrome), and compound mutations were excluded from the present study. All of the patients experienced cardiac events, and they were associated with, or triggered by, well-defined conditions. LQTS-related cardiac events were defined as syncope (transient and complete loss of consciousness), documented TdP, aborted cardiac arrest, or unexpected sudden cardiac death without a known cause. We excluded the patients who were genotyped but remained asymptomatic. All subjects or their guardians provided informed consent for the genetic and clinical studies according to each institutional review board's guidelines.

The patients were classified into three groups according to the age of first onset of cardiac events: (1) young group ($n = 106$): patients who experienced their first cardiac event at age of less than 20 years; (2) intermediate group ($n = 20$): those who experienced their first cardiac event at age of 20-39 years; and (3) older group ($n = 19$): those who experienced their first cardiac event after age of 40 years.

Clinical Phenotyping

Routine clinical and electrocardiographic (ECG) parameters were acquired at the time of the first examination for the evaluation of LQTS. Measured parameters on the first recorded ECG included QT and R-R interval in milliseconds, with corrected QT interval (QTc) corrected for heart rate (HR) by Bazett's formula.[8] Measurement for ECG parameters was performed manually on lead V5 (if not available on leads II). A cumulative LQTS diagnostic "Schwartz" score (which is derived in part from the QTc, symptoms, and family history) was assigned.[9] In regard to the family history, we defined positive family history as subjects who have relatives with a Schwartz score of ≥ 4.

Genetic Analysis

Screening for mutations of *KCNQ1*, *KCNH2*, and *SCN5A* was performed using polymerase chain reaction (PCR)/single-strand conformation polymorphism (SSCP) or denatured high-performance liquid chromatography analyses (dHPLC, WAVE system; Transgenomic Inc., Omaha, NE, USA). For aberrant PCR products, DNA sequencing was conducted with a DNA sequencer (ABI 3130 DNA Sequencer; Perkin Elmer, Foster City, CA, USA).

Genetic mutations of amino acid sequence were characterized by a specific location and coding effect (missense, nonsense, splice site, frameshift, insertion, deletion, and intronic variant). The transmembrane regions of *KCNQ1*, *KCNH2*, and *SCN5A* were defined as six membrane segments (S1 to S6, amino acid residues 112 through 354 for *KCNQ1*, 397 through 666 for *KCNH2*, and 127 through 1771 for *SCN5A*, respectively). They, therefore, included cytoplasmic and extracellular linkers, as well as the pore region. As for LQT1 and LQT2, the pore region was defined as the area extending from S5 to the mid portion of S6 involving amino acid residues 262 through 354 for *KCNQ1* and 550 through 650 for *KCNH2*, respectively.[10-14]

Triggering Factors

We divided the triggers into three categories:(1) adrenergically mediated triggers: exercise, emotional stress, loud noise, and arousal; (2) vagally mediated triggers: rest/sleep; and (3) secondary triggers: drugs, hypokalemia, and AV block. There was a small number of undefined conditions associated with cardiac events, and they were classified as other triggers and excluded for analysis in Figures 1–3.

Statistical Analysis

Data are expressed as the mean value $\pm$ standard deviation (SD). The clinical characteristics of the study groups were compared with the chi-square test for categorical variables. For continuous variables, we analyzed the normally distributed data with one-way analysis of variance and non-normally distributed data with Kruskal-Wallis tests. For comparisons between two groups, the Student's *t*-test was used for parametric data and the Mann–Whitney's *U*-test for nonparametric data. Differences were accepted as significant for P value of <0.05.

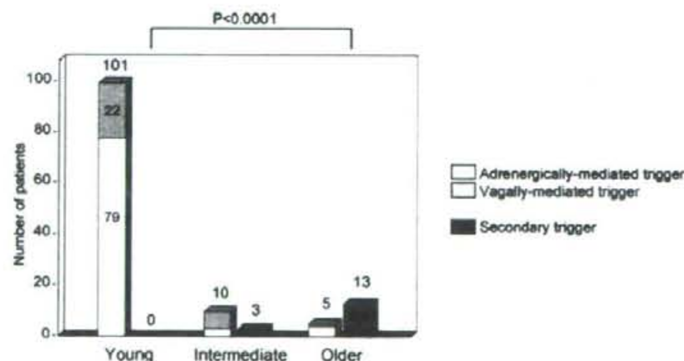
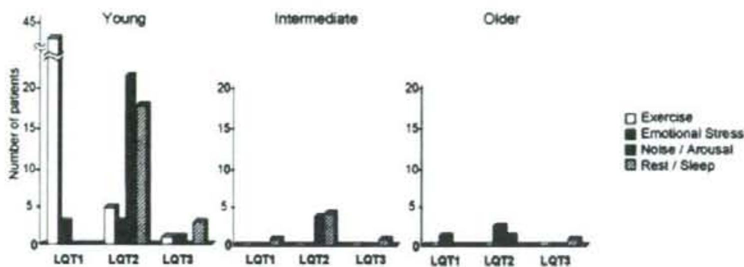


Figure 1. *Triggers for cardiac events in the young, intermediate, and older groups. Incidence of three categorical triggers. Bar graphs show the number of symptomatic patients and their triggers of the first cardiac events: open bars, adrenergically mediated; gray bars, vagally mediated; and black bars, secondary triggers. Other triggers for cardiac events that were undefined were excluded.*



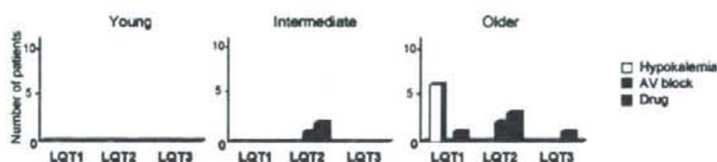


Figure 2. *Genotype-dependent difference of triggers for cardiac events in the young, intermediate, and older groups. A: autonomic triggers, B: secondary triggers; bar graphs indicate the number of patients and their patterns of a specific trigger as summarized in insets. Other triggers for cardiac events that were undefined were excluded.*

Results

Clinical Characteristics

Table 1 summarizes the clinical characteristics of the study subjects. The percentages of females, probands, and patients with positive family history were significantly different among the three groups. In the older group, the percentage of females and probands increased, but that of positive family history decreased. The intermediate group patients showed similar levels of QT prolongation, family history, and Schwartz scores as those of the young group patients. There were no significant differences in basal HR, QTc, and Schwartz scores among the three groups.

Genetic Characteristics

There were 58 LQT1, 75 LQT2, and 12 LQT3 patients (Table 1). In these genotyped patients, we identified 31 *KCNQ1*, 60 *KCNH2*, and 8 *SCN5A* mutations (total 99 different mutations). Among 58 LQT1 patients, most (48/58, 83%) of the first cardiac events occurred at young age. In contrast, first cardiac events occurred less at young age in LQT2 (51/75, 68%) and LQT3 (7/12, 58%) patients compared to the LQT1 patients ($P = 0.019$). The prevalence of transmembrane mutations in LQT1 and LQT3 patients and that of pore site mutations in LQT2 patients was evaluated, but no significant differences were observed among the three groups.

Triggers for Cardiac Events

Figure 1 illustrates the incidence of three categorical triggers in the three age groups. In Figure 1, left-sided bars indicate the number of patients in whom the event was induced by either adrenergically (open bar) or vagally (gray bar) mediated triggers. Right-sided black bars indicate those with secondary triggers. The vertical axis indicates the number of patients. In the young group, all 101 cardiac events were

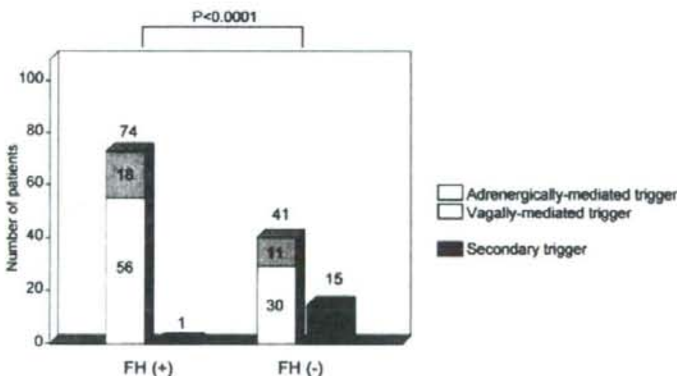


Figure 3. *Triggers for cardiac events in the patients with and without family history. Bar graphs show the number of symptomatic patients and their triggers to induce the cardiac events: open bars, adrenergically mediated; gray bars, vagally mediated; and black bars, secondary triggers. Other triggers for cardiac events that were undefined were excluded.*

TABLE 1

Clinical and Genetic Characteristics in the Three Groups

	Young (n = 106)	Intermediate (n = 20)	Older (n = 19)	P Value
Age at first cardiac event (years)	11.0 ± 0.4	28.0 ± 1.1	59.0 ± 3.5	
Female (%)	63 (59%)	16 (80%)	18 (95%)	0.004
Proband (%)	80 (75%)	18 (90%)	19 (100%)	0.035
Family history	68 (64%)	11 (55%)	3 (16%)	<0.001
HR (bpm)	65 ± 1.2	65 ± 2.8	65 ± 2.8	0.984
QTc (ms)	515 ± 5.7	523 ± 12	485 ± 10	0.084
Schwartz score	6.1 ± 0.2	6.2 ± 0.4	5.3 ± 0.5	0.335
Subtype				
LQT1 (n = 58)	48/58 (83%)	1/58 (2%)	9/58 (15%)	
LQT2 (n = 75)	51/75 (66%)	16/75 (21%)	8/75 (11%)	
LQT3 (n = 12)	7/12 (58%)	3/12 (25%)	2/12 (17%)	
Transmembrane mutation (LQT1)	39/48 (81%)	1/1 (100%)	7/9 (78%)	
Pore site mutation (LQT2)	25/51 (49%)	9/16 (56%)	1/8 (13%)	
Transmembrane mutation (LQT3)	6/7 (86%)	3/3 (100%)	1/2 (50%)	

Data are presented as the mean value ± SD or number (%) of subjects.
HR = heart rate; LQT1 = long QT syndrome caused by the *KCNQ1* potassium channel gene mutations; LQT2 = long QT syndrome caused by the *KCNH2* potassium channel gene mutations; LQT3 = long QT syndrome caused by the *SCN5A* sodium channel gene mutations; QTc = QT interval corrected by Bazett's formula.

associated with autonomic triggers, among which 79 (78%) events were adrenergically mediated and 22 (22%) were vagally mediated. On the other hand, only 5 of 18 cardiac events (28%) were associated with autonomic triggers in the older group, and secondary triggers induced cardiac events in the majority of the older group patients (13/18, 72%). The percentage of secondary triggers was significantly larger in the older group than in the other two groups (0% in young [0/101] vs 23% in intermediate [3/13] vs 72% in older [13/18]; P < 0.0001). Among the cardiac events triggered by autonomic factors, the percentage of the adrenergically mediated triggers was significantly lower in the intermediate group patients (79/101 [78%] in young vs 3/10 [30%] in intermediate vs 4/5 [80%] in older group; P < 0.001). Thus, triggering factors were significantly different among the three groups.

Figure 2A shows autonomic triggers in the three genotypes in each age group. There were also genotype-dependent differences in triggers for cardiac events in the young group, as previously reported:[5] in young LQT1 patients, 92% (44/48) of the cardiac events occurred during exercise (open bar), but none with noise/arousal (black bar) or rest/sleep (hatched bar). This is in sharp contrast with the pattern in young LQT2 patients: 37% (19/51) of the events occurred during rest/sleep and 43% (22/51) with noise/arousal. Irrespective of onset age, cardiac events triggered by noise/arousal were very specific and observed in only LQT2 patients (35%, 26 of 75 LQT2 patients). In contrast, 44% (3/7) of LQT3 patients experienced cardiac events during rest/sleep. In opposition to the young LQT1 patients, only ~20% of total LQT2 and LQT3 patients experienced cardiac events triggered by exercise or emotional stress.

Figure 2B depicts secondary triggers in the three genotypes in each age group. Hypokalemia (open bar), complete AV block (gray), and drugs (black) were associated with cardiac events in total of 6, 3, and 7 patients, respectively, in the intermediate and older groups. Interestingly, hypokalemia was associated with cardiac episodes in only older LQT1 patients. On the other hand, drugs and AV block triggered cardiac events mainly in LQT2 patients of >20 years. Responsible drugs were amphetamine, aprindine, cisapride (plus pirmenol), disopyramide, erythromycin, hydroxyzine, and procainamide.

TABLE 2

Clinical and Genetic Characteristics in Patients With or Without Family History

	FH (+) (n = 82)	FH (−) (n = 63)	P Value
Age at first cardiac event (years)	14.0 ± 1.1	26.0 ± 2.8	<0.001
Female (%)	55 (67%)	42 (67%)	0.960
Proband (%)	54 (66%)	63 (100%)	<0.001
HR (bpm)	65 ± 1.2	64 ± 1.4	0.728
QTc (ms)	512 ± 6.9	513 ± 7.0	0.890
Schwartz score	6.4 ± 0.2	5.6 ± 0.2	0.005
Subtype			
LQT1 (n = 58)	40/58 (69%)	18/58 (31%)	
LQT2 (n = 75)	36/75 (48%)	39/75 (52%)	
LQT3 (n = 12)	6/12 (50%)	6/12 (50%)	
Transmembrane mutation (LQT1)	35/40 (88%)	13/18 (72%)	
Pore site mutation (LQT2)	19/36 (53%)	16/39 (41%)	
Transmembrane mutation (LQT3)	6/6 (100%)	4/6 (67%)	

Data are presented as the mean value ± SD or number (%) of subjects.
FH = family history; HR = heart rate; LQT1 = long QT syndrome caused by the *KCNQ1* potassium channel gene mutations; LQT2 = long QT syndrome caused by the *KCNH2* potassium channel gene mutations; LQT3 = long QT syndrome caused by the *SCN5A* sodium channel gene mutations; QTc = QT interval corrected by Bazett's formula.

Family History

Comparison of clinical and genetic characteristics between patients with and without family history is shown in Table 2. The age at first cardiac event was significantly younger and Schwartz score was significantly higher in the patients with family history than in those without it. LQT1 patients appeared to have more family history compared to those of LQT2 and LQT3 genotypes. Figure 3 illustrates the incidence of three categorical triggers in patients with and without family history. Triggers for cardiac events were also significantly different between the two groups, and secondary trigger was seen in only 1 patient with family history and in 27% (15 of 56) of patients without family history.

Discussion

In the genotyped/symptomatic LQTS patients, the present study demonstrated that factors triggering cardiac events were different depending on the age of their first onset. In general, syncope and sudden death in LQTS are believed to be due to TdP-type of ventricular tachycardia and occur usually in the young.[15,16] However, pathophysiological properties of LQTS-related events were found to be even different among the three groups that were divided by age of less than 20, 20-39, and greater than 40 years. In the young group (<20 years), triggers were closely related to the autonomic nervous tone. In contrast, secondary triggers induced cardiac events in 72% of the older patients (>40 years), suggesting

that "double hit" by secondary trigger(s) appeared to aggravate the clinical phenotype, in addition to genetic variants in ion channel genes, in the older group. The intermediate group patients were at in-between risk in clinical characteristics and the triggers of cardiac events. Interestingly, regarding the triggers of cardiac events, the percentage of the adrenergically mediated trigger was lower in the intermediate group. This may reflect a relatively small number of LQT1 patients in the intermediate group.

Although there was no statistically significant difference in the QTc interval among the three age groups ($P = 0.084$), the older group showed shorter QTc interval compared to that in the other two groups. The QTc in the young group was even shorter than that in the intermediate group. This was probably due to the fact that the QTc in the LQT1 patients was significantly shorter than that in the LQT2 and LQT3 patients (LQT1: 490 ± 6.6, LQT2: 534 ± 8.4, LQT3: 555 ± 26; $P < 0.0001$) and the percentage of LQT1 patients was higher in the young group.

Our results in the young group are consistent with previous reports:[5,7] LQT1 patients experienced the majority of their cardiac events during exercise or emotional stress and only a few occurred during rest/sleep, in opposition to the pattern in LQT2 and LQT3 patients. Cardiac events in LQT2 patients in the young group were mainly associated with noise and sudden arousal and other adrenergic triggers. Cardiac events occurred during rest/sleep in half of the young LQT3 patients.

Among the secondary triggers, hypokalemia was associated with cardiac episodes in only LQT1 patients. Lower extracellular K^+ concentrations are known to reduce outward conductance of both rapid component of delayed rectifier potassium (I_{Kr}) and background inward rectifier potassium (I_{K1}) currents.[17-19] In LQT1, the slow component of delayed rectifier potassium current (I_{Ks}) is impaired, and the function of I_{Kr} and I_{K1} channels remains normal or even upregulated to compensate the total net outward K^+ conductance. Therefore, hypokalemia may unveil the potential repolarization disorder by reducing both "healthy" I_{Kr} and I_{K1}.

On the other hand, AV block and drug intake associated with cardiac events as secondary triggers were seen to be present in most of the intermediate and older LQT2 patients. Tan and colleagues[20] reported that pause-dependence of TdP onset was predominant in LQT2 but absent or rare in LQT1, suggesting that this disparity may reflect different mechanisms. Experimental studies have shown that I_{Ks} blockade (LQT1) causes delayed afterdepolarizations (DAD) but not early afterdepolarizations (EAD);[21] on the contrary, I_{Kr} blockade (LQT2) causes EADs, predominantly at slower HRs.[22] Extreme bradycardia due to AV block may lead to EAD as well as TdP through the postpausal prolongation of action potential plateau. Both a smaller I_{Kr} due to complete deactivation and an enhanced inward Na^+/Ca^{2+} exchanger at low HR may contribute to EAD formation by providing time for recovery and reactivation of L-type Ca^+ channel. In the presence of pathological bradycardia, therefore, I_{Kr} plays a more important role in abbreviating the repolarization and, thereby, keeping the appropriate QT interval because little accumulation of outward I_{Ks} occurs at lower HR.[23]

In this connection, drug-induced TdP has been shown to depend on intervals of preceding pauses.[24] The above-mentioned mechanism on the bradycardia-induced TdP may give an explanation of our result that most of the drug-induced events were observed in LQT2. Because responsible drugs are known to block cardiac I_{Kr} (except hydroxyzine), preexisting repolarization abnormality due to gene mutations may predispose the patients to fatal arrhythmias by further reducing the outward K^+ conductance.[25,26] In preliminary experiments of biophysical assay with heterologous expression systems, we found that these *KCNH2* mutations identified in drug-induced TdP patients produced mild loss-of-function of I_{Kr}.

We evaluated only "already-symptomatic" genotyped patients in this study. The percentage of "still-asymptomatic" patients was 58% of all genotyped patients (198 of 343). The average ages of asymptomatic patients were 19.0 ± 1.9 years (5–67 years) for probands and 34.0 ± 1.8 years (2–68 years) for family members. Asymptomatic probands were still young; therefore, some of them would be symptomatic in the future, being exposed to higher risk of lethal events. The results of our study again emphasize the importance of a careful approach to asymptomatic (preclinical) LQTS patients to decrease their arrhythmic risk, particularly in older patients ($\geq$20 years). Because of lack of apparent phenotypes, most of them were not diagnosed prior to the onset of symptoms. However, one of the most important missions of our genetic testing would be to achieve a preclinical diagnosis of LQTS, particularly in patients with forme-fruste phenotype. Because of low penetrance, inheriting a gene mutation per se does not always mean that the individual mutation carrier will present clinical manifestation,[27] but apparently "healthy" carriers have inherited the risk for developing the clinical phenotype. Once genetic information becomes available, we can introduce the timely beta-blocker therapy and conduct careful follow-up, including ECG recordings, lifestyle modifications (i.e., avoidance of QT-prolonging drugs), avoidance of hypokalemia, bradycardia, other alarming symptoms, and family education (home automatic electrical defibrillator, etc.).

Study Limitations

Intermediate and older group patients may have a higher possibility to use more drugs. We, therefore, could not exclude such an age-dependent risk accumulation affecting the results of trigger distribution. As for another issue, carriers of milder mutations may induce cardiac events more likely in association with secondary triggers. Our study included only subjects with three major genotypes, although they account for the majority of LQTS patients. Patients with compound mutations of LQT1-3 and 5–7 genotypes were all excluded from analysis. However, we failed to exclude compound mutation carriers with other (LQT4, 8–10) or unknown genotypes, which may result in a minor selection bias.

We evaluated only the Japanese population and there remains a concern about ethnic differences. However, the prevalent mutations found in more than 4 patients were A341V-KCNQ1, A344A/sp-KCNQ1, and A614V-HERG and were all popular in other ethnic cohorts. The genotype-specific triggers were also similar to those observed in previous studies from other countries.

Although syncope may result from diseases other than LQTS-related ventricular arrhythmia, we considered sudden onset/offset nature of loss of consciousness in a genotyped LQTS patient as syncope due to ventricular arrhythmias, if there was no evidence of another explanation, and included as a study subject. In this connection, very short duration of

TdP that did not cause syncope was underestimated if Holter ECG failed to detect it.

Conclusion

Triggers of cardiac events were closely related to the autonomic nervous tone with a higher incidence of family history in younger patients. In contrast, arrhythmic events in older patients were associated with secondary triggers, such as drugs, hypokalemia, and AV block, with genotype specificity. Thus, arrhythmic triggers in LQTS differ depending on the age of the patients, stressing the importance of age- and genotype-related therapy for genotyped LQTS.

Acknowledgments: We gratefully acknowledge the technical assistance of Arisa Ikeda, Tamami Hirata (Department of Cardiovascular Medicine, Shiga University of Medical Science, Otsu, Japan), Toshiko Shibata, Naotaka Ohta, and Toshiro Ura (Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan).

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Circulating CD34-Positive Cell Number Is Associated With Brain Natriuretic Peptide Level in Type 2 Diabetic Patients

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Patients with type 2 diabetes often suffer from asymptomatic left ventricular (LV) injury, including increased LV mass, without apparent myocardial ischemia. The mechanisms underlying diabetic LV injury remain unclear; however, it has been suggested that endothelial dysfunction plays a role. Accumulating evidence indicates that bone marrow–derived endothelial progenitor cells (EPCs) contribute to neovascularization of ischemic tissue and endothelialization of denuded endothelium. Recent studies have shown that circulating bone marrow–derived immature cells, including $CD34^+$ cells, contribute to the maintenance of the vasculature, both as a pool of EPCs and as the source of growth/angiogenesis factors (1). We hypothesized that circulating $CD34^+$ cells might be associated with LV dysfunction in patients with type 2 diabetes. Therefore, we studied the correlation between circulating $CD34^+$ cell levels and plasma brain natriuretic peptide (BNP) levels, an LV dysfunction marker, in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

The institutional review board of the National Cardiovascular Center approved this study, and all subjects provided informed consent. We examined 26 patients with type 2 diabetes (12 men and 14 women, duration of diabetes 16.1 ± 10.7 years) who were over 60 years of age (70.5 ± 6.4 years). Statin was given to nine subjects. ACE inhibitor or angiotensin receptor blocker was given to nine subjects, and thiazolidinedione was given to two subjects. Subjects were excluded from the study if they had known cardiovascular disease or chronic renal failure (defined as serum creatinine ≥180 μmol/l). No study subject showed hypokinesis by echocardiography or electrocardiogram change, indicating myocardial ischemia. Systolic (SBP) and diastolic (DBP) blood pressure and anthropometric parameters were determined. Blood samples were taken after 12-h fasting to measure circulating $CD34^+$ cells, plasma BNP, fasting plasma glucose (FPG), and A1C. Circulating $CD34^+$ cells were quantified by flow cytometry according to the manufacturer's protocol (ProCOUNT; Becton Dickinson Biosciences) as previously reported (2). BNP was quantified by enzyme immunoassay (Tohso, Tokyo, Japan). We further examined LV fractional shortening (LVFS), LV mass index (LVMI) (3), and peak flow velocity of the early filling wave (E), the late filling wave (A), and the E/A-wave ratio (E/A) by echocardiography. All echocardiograms were performed by several expert physicians who were blinded to $CD34^+$ cell level.

All statistical analyses were performed using JMP version 5.1.1 software (SAS Institute). Data are expressed as means ± SD. Comparisons of number of $CD34^+$ cells by sex were made using the two-tailed unpaired *t* test. Correlations between number of $CD34^+$ cells and clinical parameters were assessed by univariate liner regression analysis and multiple regression analysis. LVMI and plasma BNP concentrations were analyzed after logarithmic transformation.

RESULTS

FPG levels, A1C levels, and BMIs in the study subjects were measured to be 9.5 ± 2.6 mmol/l, 9.2 ± 1.8%, and 26.4 ± 4.3 kg/m^2, respectively. A total of 88% of the patients had hypertension (SBP 142 ± 18 mmHg, DBP 75.7 ± 13.5 mmHg). Plasma BNP levels were measured to be 95 ± 319 pg/ml. Although it has been reported that the level of BNP ≥100 pg/ml has a sensitivity of 90% of diagnosing congestive heart failure (CHF) in patients with CHF symptoms (4), none of the subjects in this study, including subjects with ≥100 pg/ml of BNP, showed symptoms of CHF. The level of circulating $CD34^+$ cells was measured to be 0.76 ± 0.39 cells/μl, and there was no significant difference between sexes. The range of LVMI was 73.3–340.2, and 11 subjects applied to the definition of LV hypertrophy (LVMI ≤131 in men and ≤100 in women) (3).

Plasma BNP levels had a significant inverse correlation with the number of circulating $CD34^+$ cells (Fig. 1*A*), whereas FPG, A1C, BMI, SBP, DBP, and age showed no significant correlations. There was a significant correlation between the number of circulating $CD34^+$ cells and LVMI by echocardiography (Fig. 1*B*). LVFS and E/A were not associated with circulating $CD34^+$ cell numbers (LVFS $r = -0.07$, $P = 0.72$; E/A $r = -0.11$, $P = 0.59$). There was also a significant correlation between BNP levels and LVMI ($r = 0.59$, $P = 0.001$).

In multiple regression analysis, the

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Received for publication 14 June 2007 and accepted in revised form 13 October 2007.

Published ahead of print at http://care.diabetesjournals.org on 24 October 2007. DOI: 10.2337/dc07-1125.

Abbreviations: BNP, brain natriuretic peptide; CHF, congestive heart failure; DBP, diastolic blood pressure; EPC, endothelial progenitor cell; FPG, fasting plasma glucose; LV, left ventricular; LVFS, LV fractional shortening; LVMI, LV mass index; SBP, systolic blood pressure.

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

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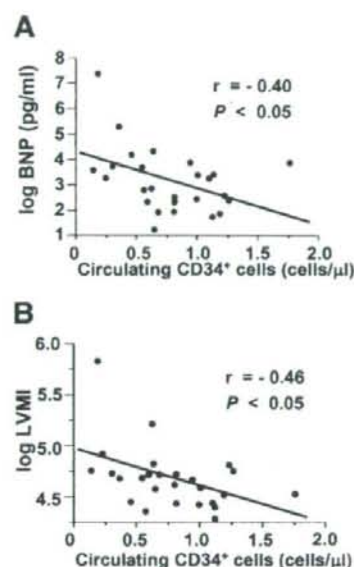

Figure 1—*Correlation between CD34⁺ cell numbers and plasma BNP levels (A) and correlation between CD34⁺ cell numbers and LVMI (B) in type 2 diabetic patients* (n = 26).

level of CD34⁺ cells was an independent correlate of both BNP ($\beta = -1.64$, $P = 0.017$) and LVMI ($\beta = -0.337$, $P = 0.031$) in the model including age, A1C, SBP, BMI, and medication (ACE inhibitor/angiotensin receptor blocker, statin, and thiazolidinedione).

CONCLUSIONS — In this study, circulating CD34⁺ cell number was found to significantly correlate with plasma BNP level, a marker of LV dysfunction. To the best of our knowledge, this is the first report that circulating bone marrow–derived cells are associated with diabetic LV abnormality. Circulating CD34⁺ cell numbers also significantly correlated with LVMI, whereas they did not correlate with LVFS (an LV systolic function marker) or E/A (an LV diastolic function marker). LV hypertrophy is a well-known predictor of cardiovascular events independent of coronary artery disease. The Framingham Heart Study identified an association between diabetes and increased LV wall thickness and mass (5). Although the precise mechanisms underlying the association between diabetes and LV hypertrophy remain unknown, our results suggest that reduced circulating CD34⁺ cell numbers may be involved in the progression of LV hypertrophy in diabetic patients. However, further investigations are necessary to demonstrate this hypothesis.

We measured the level of CD34⁺ cells in this study but not the levels of circulating CD34⁺/kinase insert domain receptor (KDR)⁺ cells that are regarded as EPCs. Circulating CD34⁺ cell levels are associated with ischemic stroke (6), and administration of CD34⁺ cells ameliorates cerebral ischemia in mice (7). This indicates that CD34⁺ cells may be involved in cardiovascular disease. Indeed, another recent report indicated that levels of circulating CD34⁺ cells are more strongly correlated with cardiovascular risk than levels of EPCs (8). Therefore, our results suggest that measurement of CD34⁺ cells may provide an indicator for diabetic LV hypertrophy.

Our study had several limitations. First, the study was performed only by cross-sectional analysis; therefore, a prospective study is needed to clarify whether circulating CD34⁺ cell numbers predict LV injury in diabetic patients. Second, although systemic blood pressure did not significantly associate with CD34⁺ cell numbers, further investigation of normotensive diabetic patients is needed to exclude the possible effects of hypertension on circulating CD34⁺ cell numbers, as most of the subjects in this study were hypertensive. Despite this caveat, these results may be of practical use in elderly patients with type 2 diabetes, as hypertension is a very common comorbid condition in this population.

In conclusion, reduced circulating CD34⁺ cell numbers are significantly associated with plasma BNP concentration and LVMI in elderly patients with type 2 diabetes. These results suggest that decreased circulating CD34⁺ cells may be involved in LV hypertrophy and that measurement of circulating CD34⁺ cell numbers may be useful for the identification of diabetic patients at high risk of LV injury.

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Role of modulator recognition factor 2 in adipogenesis and leptin expression in 3T3-L1 cells

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Received 3 November 2007

Abstract

The complex network of adipogenic transcription factors regulates adipocyte differentiation, obesity, and insulin resistance. Modulator recognition factor (Mrf) 2 knockout mice exhibit defects in fat accumulation and are protected from diet-induced obesity, suggesting that Mrf2 deficiency affects adipogenesis. Here, we report that the gene expressions of the 2 isoforms of the transcription factors Mrf2, Mrf2α, and Mrf2β, were induced upon adipogenesis in 3T3-L1 cells. Mrf2 mRNA expression was sensitive to stimulation by insulin, dexamethasone, and TNF-α in 3T3-L1 preadipocytes and differentiated adipocytes. Down-regulation of Mrf2α and Mrf2β gene expressions induced by small interfering RNAs increased the mRNA expression of leptin. These results indicate that Mrf2 can be a potential regulator of adipocyte differentiation and a potential repressor of leptin.
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Keywords: Adipocyte; Adipokine; Differentiation; Insulin; Leptin; Modulator recognition factor

Modulator recognition factor (Mrf2), which binds with high affinity to the target sequence AATA[T/C], is a member of the AT-rich interaction domain (ARID) family of transcription factors [1,2]. ARID family is critically involved in the regulation of differentiation of smooth muscle cells. Mrf2 has 2 isoforms of Mrf2α (3.0 kb) and Mrf2β (3.7 kb) that differ in the N-terminus but share the DNA-binding domain. Adult Mrf2 knockout mice are lean with significant reductions of lipid accumulation in brown and white adipose tissues, and are resistant to weight gain and obesity when they are maintained on high-fat diet [3]. In addition, they have less stored triglyceride per adipocyte and normal numbers of adipocytes. With differentiation both the size of adipocytes and the amount of stored triglyceride vary markedly, whereas the number of adipocytes is thought to be increased as a result of the proliferation of preadipocytes. Differentiation of adipocyte is regulated by a complex network of adipogenic transcription factors [4–6]. Matured adipocytes act as endocrine cells. The present study was designed to determine whether Mrf2 is involved in the regulation of adipocyte differentiation and affects secretion of adipokine(s).

Materials and methods

Materials. Murine 3T3-L1 cell line was purchased from American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and calf bovine serum, insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX) were from Sigma (St. Louis, MO). TRIZOL reagent was from Invitrogen (Carlsbad, CA). ExScript RT reagent and SYBR ExScript RT-PCR kit were from Takara (Kyoto, Japan). Synthetic short interfering RNAs (siRNA) were designed and provided by Ambion (Austin, TX). DeliverX

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doi:10.1016/j.bbrc.2007.12.002

Plus siRNA Transfection kit was from Panomics (Redwood City, CA). All other chemicals and reagents were from Sigma.

Cell culture and differentiation. 3T3-L1 preadipocytes were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2 under humidified condition. When the cells were grown for 2 days postconfluence (referred to as day 0), differentiation was induced with 10% FBS/DMEM containing 1.67 μM insulin, 1 μM dexamethasone, and 0.5 mM IBMX for 2 days. The cells were then incubated in 10% FBS/DMEM with insulin for 2 days and maintained thereafter with 10% FBS/DMEM to day 12. Medium was changed every 2 days. Fat droplets were seen in more than 90% of cells after day 10.

RNA preparation and quantitative real-time PCR. After the cells were washed with cold phosphate buffered saline (PBS) twice, total RNA from cells at various differentiated stages was isolated using TRIZOL reagent according to the manufacturer's instructions. Total RNA samples (400 ng) were reverse transcribed in 10 μl volume at 42 °C for 15 min with ExScript RT reagent. Quantitative real-time PCR analysis was performed in a 25 μl final reaction volume with ABI PRISM 7300 real-time PCR system (Applied Biosystems, Foster City, CA) using SYBR ExScript RT-PCR kit. The PCR thermal cycling program was as follows: 20 s at 95 °C for enzyme activation (allowing hot start), 40 cycles for 8 s at 95 °C, and 30 s at 60 °C for extension. The mRNA expressions were corrected for the ribosomal protein S3 (Rps3) mRNA expression for each time tested. Rps3 expression was stable throughout differentiation and under treatments administered to the cells. Primer sequences used were as follows: for mouse Rps3, upstream (CGGTGCAGATTTCCAAGAAG) and downstream (GGACAACTGCGGTCAACTC); for Mrf2α, upstream (TTCCA GTCGCCAAACATCT) and downstream (CTGAGCCTCCTCGCA GCT); for Mrf2β, upstream (GGATCCAGGATTAGCCATCG) and downstream (TTTTGCGTGGTCTGCCTTTA); for CCAAT/enhancer binding protein (C/EBP)α, upstream (CAAGAACAGCAACGAGTA CC) and downstream (GTCATTGTCACTGGTCAACTC); for C/EBPδ, upstream (CTCCCGCACACAACATACTG) and downstream (CTTC GGCAACCACCTAAAAG); for mouse leptin, upstream (GTGC CTATCCAGAAAGTCCA) and downstream (GACCTGTTGATA GACTGCCA); for adiponectin, upstream (GTTCCTCTTAATC CTGCCCA) and downstream (TCCAACATCTCCTGTCTCAC); for aP2, upstream (ATGAAAGAAGTGGGAGTGGG) and downstream (ATGCTCTTCACCTTCCTGTC); for peroxisome proliferators-activated receptor (PPAR)-γ2 (the adipocyte specific form of the two PPAR-γ transcriptional variants, PPAR-γ1 and PPAR-γ2), and upstream (GGAGTTCATGCTTGTGAAGG) and downstream (AAACCT GATGGCATTGTGAG). PCR melting curve was constructed to ensure that nonspecific products were eliminated. Then, the amount of mRNA was determined by comparison with the standard curve generated from serial dilutions of a T-vector containing cDNA of the gene.

SiRNA transfection. SiRNAs against murine Mrf2α and Mrf2β were designed and provided in the duplex-ready stable form. The sense and anti-sense strands of Mrf2α siRNAs (NM 023598, Exon V) were GCCGAUAUCCACACCAAGAtt and UCUUGGUGUGGAUAUCG GCtt. The sense and anti-sense strands of Mrf2β siRNAs (NM 023598, Exon III) were GGAUCCGAUUUGCAUAGCGtt and CGCUAUGC AAAUCGGAUCCtt, respectively. Negative control siRNA and β-actin siRNA were used as positive and negative controls. Preadipocytes were cultured to confluence in 10% calf bovine serum/DMEM. The cells were then incubated with 10% FBS/DMEM supplemented with insulin, dexamethasone, and IBMX for 48 h. Then, the medium was changed with 10% FBS/DMEM containing insulin for every 2 days. At day 8 fully differentiated adipocytes were washed with PBS and separated with trypsin and collagenase. Adipocytes (240,000 cells per well) were planted to each well of the 6-well plates with 2 ml 10% FBS/DMEM and incubated overnight. The cells were transfected with 30 nM siRNA using DeliverX Plus siRNA Transfection protocol specially designed for differentiated 3T3-L1 cells. Antibiotics were not used during the whole transfection procedures. Total RNA was extracted 24 h and 48 h after siRNA transfection.

Statistical analysis. All experiments were conducted in duplicate with independent separate cultures (n = numbers of independent experiments). Data are expressed as means ± SDs. Statistical comparison of control and treated groups was performed with Student's t-tests. The accepted level of significance was $P < 0.05$.

Results

Up-regulation of Mrf2α and Mrf2β gene expressions during adipocyte differentiation in 3T3-L1 cells

To determine whether transcription factor Mrf2 was regulated during adipocyte differentiation, we analyzed Mrf2α and Mrf2β gene expression during the conversion of 3T3-L1 fibroblasts to adipocytes by quantitative real-time PCR (Fig. 1). Both Mrf2α and Mrf2β mRNA expressions calibrated for each time tested by Rps3 mRNA expression were up-regulated after induction with insulin, dexamethasone, and IBMX. Thus, the mRNA expressions of Mrf2α and Mrf2β were maintained at high level. Compared with Mrf2β mRNA the increase of Mrf2α mRNA occurred early. The increase of Mrf2α mRNA expression could be observed at day 1 and peaked at day 4 (10.9 ± 1.2-fold).

Effects of insulin, dexamethasone, and IBMX on Mrf2α and Mrf2β gene expressions in preadipocytes

Insulin, dexamethasone, and IBMX represent a classic combination for the induction of adipocyte differentiation. Mrf2 gene expression in response to these inducers was investigated. As shown in Fig. 2A and B treatment of preadipocytes with insulin or dexamethasone alone up-regulated Mrf2α and Mrf2β mRNA expressions, respectively. The peak was observed at 1 h. Combination of insulin, dexamethasone, and IBMX further produced transient increase of Mrf2α and Mrf2β mRNA (Fig. 2C). After stimulation Mrf2α and Mrf2β mRNA levels were rapidly increased peaking at 1 h. The increased mRNA expressions returned to baseline values in 3 h. Mrf2α and Mrf2β

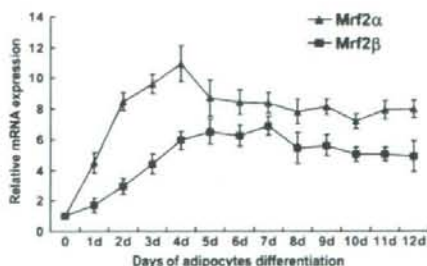

Fig. 1. Mrf2α and Mrf2β mRNA expressions during differentiation of 3T3-L1 cells. Proliferating 3T3-L1 cells were cultured in 10% FBS/DMEM until they reached confluence. Two days after reaching confluence the cells were induced to differentiate by insulin, dexamethasone, and IBMX (day 0). The cells were harvested and total RNA was isolated every day from day 0 to day 12. Mrf2α and Mrf2β mRNA expressions were measured using quantitative real-time PCR. Rps3 mRNA expression was used as an internal control. Values are means ± SD of the fold increase compared with control before differentiation ($n = 3$).

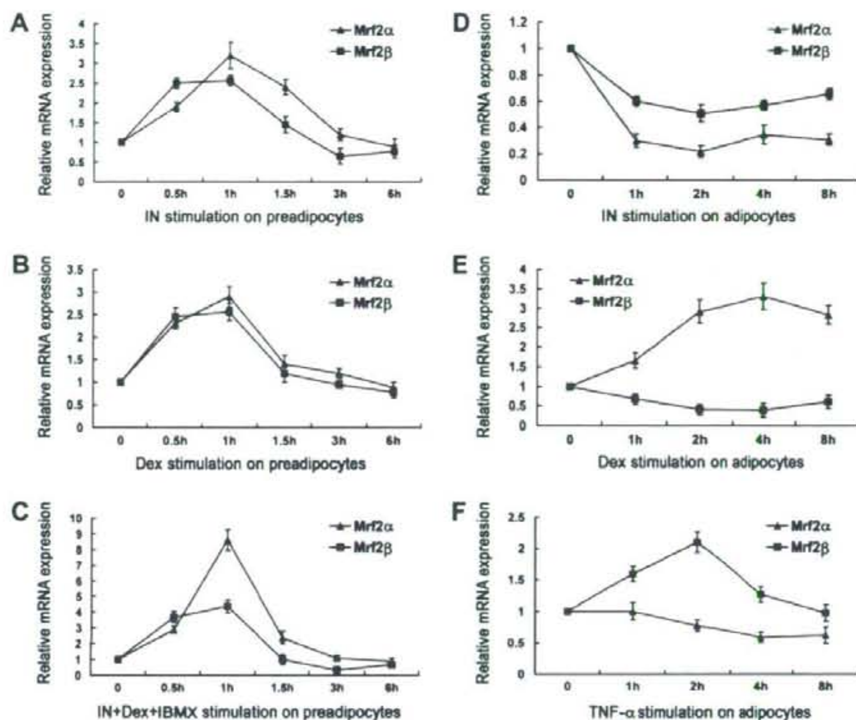

Fig. 2. Effects of insulin, dexamethasone, IBMX, and TNF-α on Mrf2α and Mrf2β mRNA expressions in preadipocytes and differentiated adipocytes. 3T3-L1 preadipocytes and differentiated adipocytes were starved in serum-free medium for 16 hours. Then, preadipocytes were stimulated by (A) insulin (IN, 1 μM), (B) dexamethasone (Dex, 1 μM), and (C) IN, Dex and isobutyl-1-methylxanthin (IBMX, 0.5 mM). Adipocytes were stimulated by (D) IN, (E) Dex, and (F) TNF-α for indicated periods. Total RNA was isolated and quantitative real-time PCR was performed. Rps3 mRNA expression was used as an internal control. Values are means ± SD of the fold increase compared with control without stimulation ($n = 3$).

mRNA expressions were increased again from 12 h (data not shown), and remained at high stable level until the cells became mature adipocytes (Fig. 1).

Effects of insulin, dexamethasone, and TNF-α on Mrf2α and Mrf2β gene expressions in differentiated adipocytes

In view of the well documented effects of insulin, dexamethasone, and TNF-α on adipose tissue metabolism and on the regulatory role of adipocyte transcriptional network, the effects of insulin, dexamethasone, and TNF-α on Mrf2 gene expressions were assessed in mature adipocytes. Mrf2α mRNA was decreased by insulin within 1 h of exposure and the maximal decrease was at 2 h (78.4 ± 4.8%), and Mrf2β mRNA was decreased by insulin at 2 h (50.0 ± 6.5%) (Fig. 2D). Different responses were observed with Mrf2α and Mrf2β mRNA expressions by stimulation with dexamethasone. Mrf2α mRNA was increased by 3.3 ± 0.4-fold at 4 h and Mrf2β mRNA was decreased maximally at 4 h by 58.0 ± 2.3% (Fig. 2E). Mrf2α mRNA was slightly up-regulated whereas Mrf2β mRNA was slightly down-regulated by TNF-α (Fig. 2F).

Effects of siRNA-mediated down-regulation of Mrf2α and Mrf2β gene expressions on adipogenesis transcription factors and adipokines

Mrf2α and Mrf2β, like other up-regulated transcription factors during adipocyte differentiation, were possibly involved in the regulation of adipogenesis transcriptional network and were possibly responsible for secretion of adipokines. To determine which transcription factors or adipokines were regulated by Mrf2, Mrf2α, and Mrf2β siRNAs were transfected into mature 3T3-L1 adipocytes. The cells were harvested 24 h and 48 h after transfection. RNA was isolated and quantitative real-time PCR was performed. When the β-actin siRNA was transfected, β-actin mRNA expression was decreased. When Mrf2α siRNA and Mrf2β siRNA were transfected, the expressions of Mrf2α mRNA and Mrf2β mRNA were decreased by 62.1% and 59.3%, respectively (Fig. 3A). In contrast, leptin mRNA expression was increased by 8.1 ± 1.8-fold at 24 h (Fig. 3B) and by 6.6 ± 1.8-fold at 48 h (Fig. 3C) after Mrf2α siRNA was transfected. For Mrf2β siRNA similar results were observed. Leptin mRNA expression was