rhythymic risk even among women who carry lower-risk (nonpore) mutations in the *KCNH2* channel. In contrast, the protective effects of testosterone on I_{Kr} and ventricular repolarization in postadolescent male subjects result in a reduction in the risk of arrhytmic events among carriers of low-risk mutations, with a possible remaining residual risk in men who harbor higher-risk mutations in the functionally more important pore-loop region.

In a prior study, Priori et al.[16] proposed a risk stratification scheme for LQTS patients that is based on the LQTS genotype, QTc, and gender. This study, however, assessed a composite end point of cardiac events of any type, comprising mostly nonfatal symcopal episodes,[16] whereas the large sample size of genotyped LQT2 patients in the present study facilitated for the first time the development of a risk stratification scheme for the end point of life-threatening cardiac events within the LQT2 population. We show that combined assessment of clinical and genetic data, related to mutation location, can be used to identify risk groups of LQT2 patients with a significantly different risk of ACA or SCD and with a pronounced difference in the rate of ACA or SCD during follow-up. These findings suggest that risk stratification in LQTS should combine clinical and mutation-related risk factors that are specific for each of the 3 main LQTS genotypes.

Prior data suggest that LQT2 patients expereince a relatively high rate of cardiac events during β-blocker therapy.[17] In the present study, medical therapy with β-blockers was associated with a pronounced 61% reduction in the risk of ACA or SCD in the total LQT2 population. However, the present findings also suggest that careful follow-up, with consideration of ICD therapy for primary prevention, is warranted in high- and very-high-risk LQT2 patients. These patient subsets were shown to experience 3.5 to 5.3 events per 100 patient years (which corresponds to a high rate of 1.5 to 2.1 life-threatening cardiac events per patient from birth through age 40 years) despite frequent usage of β-blocker therapy (>80%).

Study limitations

We did not carry out expression studies to assess the effects of estrogen and testosterone on ion channel mutations by their location. Therefore, further studies are necessary to evaluate the mechanism related to the observed gender-specific risk related to mutation location.

Because of sample size limitations, we did not carry out comprehensive analysis of the relation between all function regions of the *KCNH2* channel (including the PAS, CNBD, and other C-terminus and and N-terminus domains) and gender-specific risk. However, the results from the secondary analysis in which non–pore-loop mutations were further subcategorized into mutations in the transmembrane and C/N-terminus regions support the consistency of our findings.

Conclusions and clinical implications

The present study shows a distinct association between mutation characteristics and time-dependent differences in the clinical course of LQT2 patients. We have shown that after the onset of adolescence, women with and without high-risk mutations show increased risk for life-threatening cardiac events, whereas the risk of ACA or SCD in men is increased only among carriers of the higher-risk pore-loop mutations. Thus, a comprehensive approach that combines clinical and genetic data should be used for risk assessment and management of LQTS patients.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2011.03.049.

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Journal of the American College of Cardiology.
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Published by Elsevier Inc.

Vol. 57, No. 22, 2011
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.01.026

Flecainide Therapy Reduces Exercise-Induced Ventricular Arrhythmias in Patients With Catecholaminergic Polymorphic Ventricular Tachycardia

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Objectives This study evaluated the efficacy and safety of flecainide in addition to conventional drug therapy in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT).

Background CPVT is an inherited arrhythmia syndrome caused by gene mutations that destabilize cardiac ryanodine receptor Ca^{2+} release channels. Sudden cardiac death is incompletely prevented by conventional drug therapy with β-blockers with or without Ca^{2+} channel blockers. The antiarrhythmic agent flecainide directly targets the molecular defect in CPVT by inhibiting premature Ca^{2+} release and triggered beats in vitro.

Methods We collected data from every consecutive genotype-positive CPVT patient started on flecainide at 8 international centers before December 2009. The primary outcome measure was the reduction of ventricular arrhythmias during exercise testing.

Results Thirty-three patients received flecainide because of exercise-induced ventricular arrhythmias despite conventional (for different reasons, not always optimal) therapy (median age 25 years; range 7 to 68 years; 73% female). Exercise tests comparing flecainide in addition to conventional therapy with conventional therapy alone were available for 29 patients. Twenty-two patients (76%) had either partial ($n = 8$) or complete ($n = 14$) suppression of exercise-induced ventricular arrhythmias with flecainide ($p < 0.001$). No patient experienced worsening of exercise-induced ventricular arrhythmias. The median daily flecainide dose in responders was 150 mg (range 100 to 300 mg). During a median follow-up of 20 months (range 12 to 40 months), 1 patient experienced implantable cardioverter-defibrillator shocks for polymorphic ventricular arrhythmias, which were associated with a low serum flecainide level. In 1 patient, flecainide successfully suppressed exercise-induced ventricular arrhythmias for 29 years.

Conclusions Flecainide reduced exercise-induced ventricular arrhythmias in patients with CPVT not controlled by conventional drug therapy. (J Am Coll Cardiol 2011;57:2244–54) © 2011 by the American College of Cardiology Foundation

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a malignant inherited arrhythmia syndrome characterized by physical or emotional stress-induced bidirectional or polymorphic ventricular tachycardia (VT) in structurally

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normal hearts, with a high fatal event rate in untreated patients (1–3). Approximately 60% of CPVT patients have mutations in genes encoding the cardiac ryanodine receptor Ca^{2+} release channel (RyR2) or cardiac calsequestrin (4–6), and these cause spontaneous RyR2 channel openings (7,8). The resulting increase in cytosolic Ca^{2+} triggers delayed afterdepolarizations, ventricular premature beats (VPBs), and ventricular tachycardia, especially under conditions of β-adrenergic stimulation (9,10).

Hence, β-blockers are considered first-line therapy, but unfortunately they are not completely effective in preventing life-threatening arrhythmias (1–3,11–16). An implantable cardioverter-defibrillator (ICD) is often used in patients who continue to have ventricular arrhythmias despite β-blocker therapy. However, ICDs are not fully protective and can be proarrhythmic in CPVT patients because both appropriate and inappropriate ICD shocks can trigger catecholamine release, subsequently resulting in multiple shocks (arrhythmic storm), and death (17,18). Thus, additional therapy is desired for CPVT. Small case series show that left cardiac sympathetic denervation is effective in patients who are insufficiently protected by β-blocker therapy and/or experiencing too many ICD shocks (19–22).

Recently, we discovered that the antiarrhythmic agent flecainide directly blocks RyR2 channels, prevents RyR2-mediated premature Ca^{2+} release, and suppresses triggered beats in myocytes isolated from mouse hearts lacking calsequestrin, an animal model of CPVT (23). This effect is not mediated by Na^{+}-channel block, the conventional mode of action thought to underlie flecainide activity, but rather can be attributed to open state block of RyR2 channels (that is, flecainide directly targets the molecular defect responsible for the arrhythmogenic Ca^{2+} waves that trigger CPVT in vivo) (24). In preliminary work, flecainide also appeared to be effective in 2 highly symptomatic CPVT patients (23).

Here we collate the data from every CPVT patient started on flecainide at 8 international centers and report on the efficacy and safety of flecainide treatment in CPVT.

Methods

Participants and study design. To better understand the efficacy and safety of flecainide in CPVT, we reviewed the chart of each consecutive CPVT patient in whom flecainide was started at 8 tertiary referral centers in the Netherlands, Canada, France, Israel, Japan, and the United States before December 2009. All patients had a clinical diagnosis of CPVT (based on exercise-induced bidirectional or polymorphic VT in the absence of structural cardiac disease) and a putative pathogenic mutation in the gene encoding RyR2 or cardiac calsequestrin. Determination of flecainide starting dose and dosing increases were made by the treating physician as part of specialized clinical care. Data collection and analysis were done retrospectively by chart review and were approved by the institutional review board at each participating institution.

Abbreviations and Acronyms

CPVT = catecholaminergic polymorphic ventricular tachycardia

ICD = implantable cardioverter-defibrillator

NSVT = nonsustained ventricular tachycardia

RyR2 = cardiac ryanodine receptor Ca^{2+} release channel

VPB = ventricular premature beat

VT = ventricular tachycardia

Primary and secondary outcome measures. Couplets or VT during exercise are significantly associated with future arrhythmic events in CPVT (2). Because all patients were monitored by repeat exercise testing as part of routine clinical care, we used the reduction of ventricular arrhythmias during exercise testing as the primary outcome measure. The effect of flecainide was quantified by comparing the ventricular arrhythmia score (see later text) of the last exercise test on conventional therapy with the ventricular arrhythmia score of the first exercise test after a minimum of 5 days on the stable flecainide dose. Only patients on an unchanged or lower β-blocker dose during flecainide treatment were included in the primary analysis. Depending on the site, exercise testing was performed using a treadmill (standard or modified Bruce protocols) or bicycle ergometer.

Secondary outcome measures were the incidence of arrhythmic events (defined as syncope, aborted cardiac arrest, appropriate ICD shocks, and sudden cardiac death), assessment of well-being and side effects of flecainide, and monitoring of proarrhythmic effects of flecainide, in particular QRS duration during exercise and increase in the ventricular arrhythmia burden (25,26).

Definitions of ventricular arrhythmia. Exercise testing was analyzed and scored using the following pre-defined parameters (modified from Rosso et al. [27]): 1) ventricular arrhythmia score, defined by the worst ventricular arrhythmia (1, no or isolated VPBs; 2, bigeminal VPBs and/or frequent VPBs [>10 per min]; 3, couplet; and 4, nonsustained ventricular tachycardia [NSVT], ≥3 successive VPBs); 2) the presence of either of the parameters of the ventricular arrhythmia score or the presence of bidirectional VT (>3 successive VPBs with a beat-to-beat alternating right and left QRS axis); 3) sinus rate at the onset of ventricular arrhythmias, most often an isolated VPB; 4) maximum number of VPBs during a 10-s period; and 5)

supported by ZorgOnderzoek Nederland Medische Wetenschappen (ZonMW, grant 120610013 to Drs. van der Werf and Wilde), by the U.S. National Institutes of Health (grants HL88635, HL71670 to Dr. Knollmann and HL076264 to Dr. Kannankeril), by a grant from the Heart and Stroke Foundation of Ontario (grant NA3397 to Dr. Krahn), by a grant from the French national government named Programme Hospitalier de Recherche Clinique (grant AOR04070, P040411 to Dr. Leenhardt), by a Research Grant for Cardiovascular Diseases (21C-8) from the Ministry of Health, Labor, and Welfare, Japan (to Dr. Shimizu), by the American Heart Association Established Investigator Award (grant 0840071N to Dr. Knollmann), and by the Fondation Leducq Trans-Atlantic Network of Excellence, Preventing Sudden Death (grant 05-CVD-01 to Dr. Wilde). Dr. Leenhardt is a consultant to Sanofi-Aventis and MedaPharma. Dr. Wilde is on the Advisory Board of PGX Health. All other authors have reported that they have no relationships to disclose.

Manuscript received November 27, 2010; revised manuscript received December 17, 2010, accepted January 3, 2011.

Table 1 Baseline Characteristics and Flecainide Therapy Parameters

Patient #	Sex	Mutation*	Age at First Symptom, yrs	Proband or Relative	Presenting Symptom	Age at Diagnosis, yrs	Aborted Cardiac Arrest	ICD	Age at Baseline, yrs	Drug Therapy at Baseline, mg (mg/kg body weight)	Indication for Starting Flecainide Treatment	Daily Starting/Stable Flecainide Dose, mg (mg/kg body weight)†	Follow-Up, months	Response to Flecainide Treatment	Side Effects of Flecainide
1‡	F	A4091T	5	Proband	Seizure	6	Yes	Yes	13	Nadolol 160 (2.4), verapamil 180 (2.7)§	NSVT (on Holter recordings)	300 (4.5)	25	Complete	None
2	F	R2401H	6	Proband	Syncope	6	No	No	7	Nadolol 15 (0.9)	NSVT (on Holter recordings)	96 (5.6)/120 (7.1)	22	None	None
3‡	M	CASQ2: 532+ 1G>A	NA	Relative	None	3	No	Yes	12	Metoprolol 125 (2.3), verapamil 120 (2.2)§	NSVT (on ICD recordings) + frequent ICD shocks	100 (1.9)/150 (2.8)	28	Complete	None
4‡	F	E4076K	28	Relative	Syncope	31	No	No	37	Metoprolol 100 (1.6)	Couplets + side effects	100 (1.6)/150 (2.4)	23	Partial	None
5	F	S4124G	NA	Relative	None	31	No	No	36	Bisoprolol 5 (0.08), verapamil 240 (3.7)§	NSVT + side effects	100 (1.5)/150 (2.3)	28	Partial	None
6	F	S4124G	45	Proband	Syncope	50	No	No	68	Bisoprolol 2.5 (0.04)	NSVT + side effects	75 (1.2)/150 (2.4)	13	Partial	Sinus arrest and dizziness
7	F	S4124G	26	Relative	Aborted cardiac arrest	26	Yes	No	41	None‖	NSVT	150 (2.2)	22	Partial	Dizziness
8‡	M	S4124G	8	Relative	Syncope	8	No	No	10	Metoprolol 50 (1.9)	Couplets	50 (1.9)/100 (3.7)	22	Partial	None
9‡	M	E4187Q	NA	Proband	None (detected by cardiological examination after SCD of his son)	47	No	No	53	Metoprolol 200 (2.4)	NSVT + side effects	150 (1.7)	20	Partial	None
10‡	M	E4187Q	NA	Relative	None	19	No	Yes	25	Metoprolol 200 (2.7)	NSVT	150 (2.0)	20	None	None
11‡	F	E4187Q	NA	Relative	None	14	No	Yes	20	Metoprolol 150 (2.6)	NSVT	100 (1.8)	20	Complete	None
12‡	M	E4187Q	NA	Relative	None	11	No	Yes	17	Metoprolol 100 (1.6)	NSVT	100 (1.6)/300 (4.8)	20	Partial	None
13	F	E1724K	13	Relative	Syncope	13	No	No	25	Metoprolol 25 (0.4)	Couplets	100 (1.3)¶#	NA#	NA#	Fatigue, dizziness, chest pain
14	F	E1724K	9	Proband	Syncope	15	No	No	50	Sotalol 160 (2.1)	Bigeminy/frequent VPBs + side effects	100 (1.3)	20	None	None
15‡	M	R420W	NA	Relative	None	38	No	No	49	Metoprolol 100 (1.3)	Couplets	150 (1.9)/300 (3.9)	19	Complete	None
16‡	M	R420W	NA	Relative	None	12	No	No	16	Metoprolol 100 (1.7)	NSVT	100 (1.7)	19	Complete	None
17	F	Y4962C	NA	Relative	None	41	No	No	45	Atenolol 25 (0.4)	NSVT	150 (2.5)	12	Complete	None
18‡	F	M2605V, A4510T, 14757-6_7CT>TA	NA	Proband	None (detected by exercise testing at pre-participation screening)	40	No	No	40	Metoprolol 100 (1.4)	Couplets	200 (2.9)	18	Partial	None

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Table 1 Continued

Patient #	Sex	Mutation*	Age at First Symptom, yrs	Proband or Relative	Presenting Symptom	Age at Diagnosis, yrs	Aborted Cardiac Arrest	ICD	Age at Baseline, yrs	Drug Therapy at Baseline, mg (mg/kg body weight)	Indication for Starting Flecainide Treatment	Daily Starting/Stable Flecainide Dose, mg (mg/kg body weight)†	Follow-Up, months	Response to Flecainide Treatment	Side Effects of Flecainide
19	F	R420W	33	Proband	Syncope	33	No	Yes	36	Bisoprolol 5 (0.08)	Bigeminy/frequent VPBs	100 (1.5)	17	Complete	None
20	M	R420W	NA	Relative	None	11	No	No	12	Atenolol 25 (0.7)	Couplets	100 (2.6)	23	Complete	None
21‡	F	G3946S	14	Proband	Syncope	15	No	No	34	Nadolol 160 (2.7)	Couplets	200 (3.3)	18	Complete	None
22	F	R420Q	14	Proband	Syncope	15	No	Yes	20	Bisoprolol 1.25 (0.03)	Couplets	200 (4.0)	17	None	None
23‡	F	R2474G	1	Proband	Convulsion without fever	11	No	Yes	18	Atenolol 100 (2.1), verapamil 120 (2.6)	NSVT	150 (3.2)	20	Complete	None
24	F	R420W	NA	Relative	None	20	Yes	No	24	Metoprolol 25 (0.4)#	Bigeminy/frequent VPBs + side effects	100 (1.8)	17	Complete	None
25	F	E1724K	10	Proband	Syncope	31	No	No	39	Carvedilol 2.5 (0.05)	NSVT	100 (2.2)	14	Partial	None
26‡	F	F2215L	5	Proband	Cardiac arrest	10	Yes	No	24	Propranolol 140 (2.8)	NSVT (on Holter recordings) + syncope + palpitations	100 (2.0)	13	None	None
27	F	R4157H	56	Relative	Palpitations	57	No	Yes	57	Bisoprolol 5 (0.08)**	NSVT	150 (2.3)	31	NA**	None
28	F	M3978I	14	Relative	Syncope	15	No	Yes	25	Nadolol 40 (0.7)	Frequent VPBs + syncope	150 (2.5)	31	Complete	Nausea and dizziness
29	F	M3978I	14	Proband	Syncope	14	No	Yes	26	Bisoprolol 5 (0.06)††	Bigeminy/frequent VPBs	150 (3.1)	32	None	None
30	F	M3978I	13	Relative	Syncope	32	No	No	45	None‡‡	Bigeminy/frequent VPBs	150 (2.3)	NA§§	Partial	Nausea and dizziness
31	F	M3978I	13	Relative	Syncope	38	No	No	50	Bisoprolol 5 (0.09)	VPBs + palpitations	100 (1.8)	NA‖‖	None	Nausea and dizziness
32	M	V4771I	4	Proband	Syncope with seizure	18	No	No	18	Sotalol 240 (3.2)	NSVT	200 (2.7)	29 yrs¶¶	Complete	None
33‡	F	R2401H	9	Proband	Syncope	9	No	Yes	17	Nadolol 160 (2.5)	Syncope with VF and arrhythmic storm (recorded on ICD log)	150 (2.3)	40	Complete	None
Total	F: 24 (73%)	*RyR2*: 32 (97%)	Median: 13 (range 1–56)	Probands: 15 (45%)	Symptoms: 21 (64%)	Median: 18 (range 3–57)	Yes: 4 (12%)	Yes: 12 (36%)	Median: 25 (range 7–68)	β-blocker: 31 (94%); Ca^{2+} channel blocker: 4 (12%)	Severe ventricular arrhythmia: 26 (79%); symptoms: 5 (15%)	Median: 100 (range 50–300)/150 (range 100–300)	Median: 20 (range 12–40)	Complete: 14/31 (45%); partial: 10/31 (32%)	Yes: 6 (18%)

*_RYR2_ mutations unless otherwise indicated. †Stable dose was identical to starting dose when only 1 dose is displayed. ‡Patients who were treated with a first-line β-blocker at an optimal dose (n = 15). §Verapamil was discontinued when flecainide was started. ‖This patient discontinued β-blocker therapy during 3 consecutive pregnancies, and thereafter agreed with her treating cardiologist to permanently discontinue β-blocker therapy and avoid exercise. ¶Flecainide was discontinued within a few days and before exercise testing on flecainide could be performed. #Metoprolol was discontinued and flecainide was started in this patient because of intolerable side effects. **This patient was not included in the primary analysis because the bisoprolol dose was also increased. ††This patient discontinued β-blocker therapy on her own initiative after flecainide treatment was started and before an exercise test on combined therapy could be performed. The ventricular arrhythmia score on flecainide monotherapy did not change compared with that on the baseline exercise test while taking a β-blocker. ‡‡This patient discontinued β-blocker therapy because of side effects. §§This patient discontinued flecainide and restarted β-blocker therapy on her own initiative. ‖‖This patient discontinued flecainide because of side effects after exercise testing while taking a β-blocker and flecainide was performed. ¶¶This patient was excluded from the follow-up calculation.

ICD = implantable cardioverter defibrillator; NA = not applicable; NSVT = nonsustained ventricular tachycardia; SCD = sudden cardiac death; VF = ventricular fibrillation; VPB = ventricular premature beat.

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ratio of VPBs to sinus beats during the 10-s period with the maximum number of VPBs.

Reaching a ventricular arrhythmia score of 1 was considered complete suppression of ventricular arrhythmias. Other ventricular arrhythmia score improvements were considered partial suppression.

Statistical analysis. Continuous data are presented as mean ± SD or median (range), and categorical variables as number (percentage). Related samples were compared using the paired Wilcoxon signed-rank test for continuous and ordinal variables and the McNemar test for dichotomous variables. Independent continuous variables were compared by means of the Mann-Whitney *U* test. A 2-tailed p value <0.05 was considered statistically significant. Statistical analysis was performed with SPSS software package, version 15.0 (SPSS, Inc., Chicago, Illinois).

Results

Patient characteristics. A total of 33 genotype-positive CPVT patients from 21 families were started on flecainide at 8 tertiary care centers (Table 1). All patients had persistent physical or emotional stress-induced ventricular arrhythmias documented by exercise testing, Holter recordings, or ICD interrogation and/or persistent symptoms of palpitations, syncope, aborted cardiac arrest, or appropriate ICD shocks, while taking β-blockers with or without Ca^{2+}-channel blockers. Twenty-four of the patients (73%) were female. The median age at the start of flecainide therapy was 25 years (range 7 to 68 years). Thirty-one patients (94%) were treated with β-blockers, and 4 (12%) of them also received Ca^{2+}-channel blockers (Table 1).

In 1 patient (Patient #13), flecainide was stopped because of side effects before exercise testing could be repeated; in another patient (Patient #27) the β-blocker dose was increased during flecainide treatment; and 2 patients (Patients #7 and #30) did not receive β-blocker therapy when flecainide was started (Table 1). In the remaining 29 patients, exercise tests on combination therapy of flecainide with conventional drugs at unchanged or lower doses were available for analysis. In 17 patients (59%), baseline exercise testing was performed <48 h before flecainide initiation.

Flecainide therapy reduces exercise-induced ventricular arrhythmias. Flecainide treatment improved the ventricular arrhythmia score in 22 patients (76%) ($p < 0.001$) (Fig. 1A). Fourteen patients (48%) had complete suppression of ventricular arrhythmias (including 7 patients without any VPBs), and 8 (28%) had partial suppression. None of the patients experienced significant (i.e., couplet or VT) worsening of the exercise-induced ventricular arrhythmia score.

Flecainide treatment also significantly improved all other predefined parameters of exercise-induced ventricular arrhythmia (Table 2). For example, patients receiving flecainide therapy achieved significantly higher heart rates before ventricular arrhythmias occurred. Independently, flecainide caused a significant reduction in maximum sinus rate during exercise, even though a higher mean workload was achieved. As expected (28), flecainide prolonged the PR interval (149 ± 21 ms vs. 160 ± 24 ms; $p = 0.003$), and the QRS duration (83 ± 9 ms vs. 89 ± 11 ms; $p = 0.005$), but did not change the QTc interval (399 ± 26 ms vs. 405 ± 19 ms; $p = 0.171$) at rest. These parameters remained within the normal range at rest and during peak exercise in all patients, except for a slightly prolonged resting PR interval (220 ms) in 1 patient (Patient #20).

We next assessed the reproducibility of exercise testing as a measure of the ventricular arrhythmia burden in CPVT. Although not available for all patients, a subset of patients underwent repeated exercise testing either at the same dose of conventional therapy (n = 14) or at the same flecainide dose (n = 16). In both cases, the ventricular arrhythmia score of the second exercise test was not statistically different from that on the first exercise test (Fig. 2). Similarly, all other predefined parameters of exercise-induced ventricular arrhythmia also did not change significantly (e.g., the maximum number of VPBs during a 10-s period was 5 ± 5 on the first exercise test at the stable flecainide dose and 6 ± 6 on the second exercise test at the same flecainide dose [$p = 0.556$]), suggesting that ventricular arrhythmia scores obtained from exercise testing are reproducible measures of drug efficacy in CPVT and that tachyphylaxis was not present.

We found that 14 of the 29 patients included in the primary analysis received drug therapy that could be considered suboptimal (i.e., an unusual β-blocker for CPVT [bisoprolol, carvedilol, or sotalol]) or a relatively low β-blocker dose (atenolol, metoprolol, or nadolol <1 mg/kg body weight daily) (2). These patients had either side effects on other β-blockers and/or a higher β-blocker dose, or nadolol was not available in their country. To assess whether flecainide was also effective in CPVT patients on optimal conventional therapy, we next analyzed the 15 patients who were treated with a first-line β-blocker at an optimal dose (Table 1). Flecainide significantly improved the ventricular arrhythmia score ($p = 0.003$) (Fig. 1B), and all other pre-defined arrhythmia parameters in this subgroup to a similar extent as in the primary analysis.

The ventricular arrhythmia score in the 2 patients (Patients #7 and #30) who did not receive β-blocker therapy when flecainide was started improved from NSVT to couplet and from NSVT to bigeminal VPBs and frequent VPBs, respectively.

Flecainide dose in CPVT. To estimate the optimal dosing of flecainide in CPVT, we analyzed the relationship between starting dose and VT suppression during the first exercise test on flecainide. Patients without suppression of exercise-induced ventricular arrhythmias on the starting flecainide dose received a significantly lower dose (113 ± 39 mg, n = 13; $p = 0.038$) compared with patients with either partial (142 ± 38 mg, n = 6) or complete ventricular arrhythmia suppression (150 ± 60 mg, n = 12). Eight

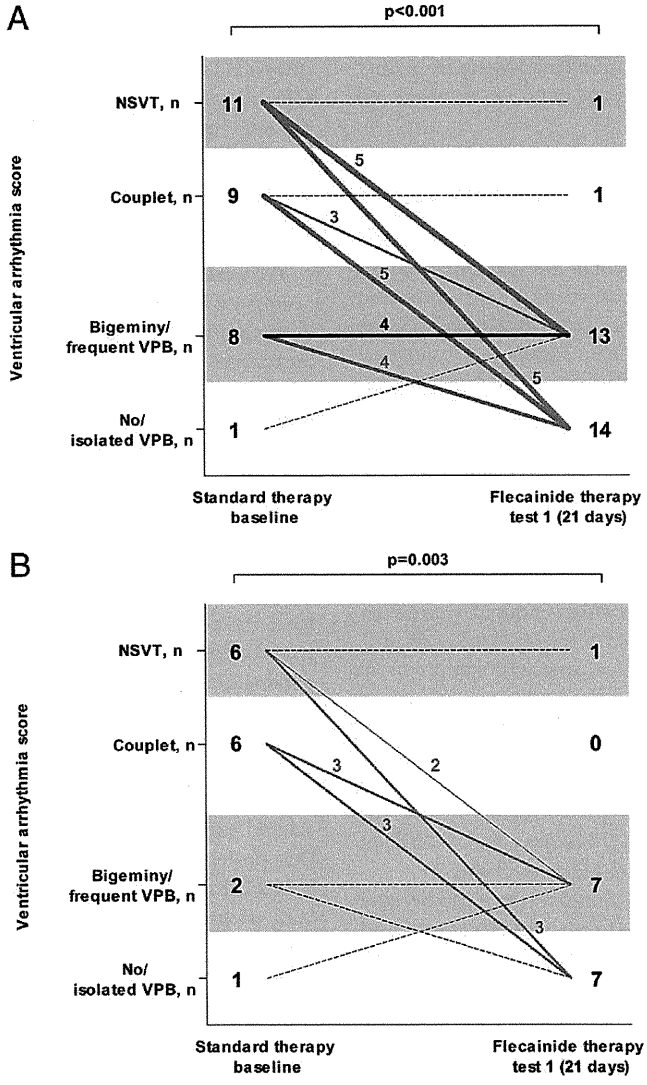

Figure 1 Ventricular Arrhythmia Score on Standard Therapy and on Flecainide

Ventricular arrhythmia score per patient on the baseline exercise test on standard therapy and on the first exercise test on the final (stable) flecainide dose in the entire cohort (n = 29) **(A)** and in the patients who were treated with a first-line β-blocker at an optimal dose (n = 15) **(B)**. The number of patients in each ventricular arrhythmia category and change in ventricular arrhythmia category are shown. The **line thickness** indicates the number of patients, and a **dotted line** represents 1 patient. The median time interval between the 2 tests is shown. All exercise tests were performed on patients receiving an unchanged β-blocker dose. NSVT = nonsustained ventricular tachycardia; VPB = ventricular premature beats.

patients (24%) received an increased flecainide dose after the initial exercise test (Table 1). The dose increased from an average daily dose of 96 ± 28 mg to 178 ± 78 mg (range 100 to 300 mg), which resulted in a significant improvement in the ventricular arrhythmia score (Fig. 3).

Clinical follow-up. Three patients (Patients #13, #30, and #31) discontinued flecainide with <6 months of follow-up due to side effects. One patient (Patient #6) required a pacemaker because flecainide exacerbated pre-existing sinus node dysfunction. Flecainide was resumed after pacemaker implantation, and this patient was included in the study. In 2 patients (Patients #7 and #28), the stable flecainide dose was decreased because of dizziness. All other patients tolerated flecainide well without severe side effects. The β-blocker dose was decreased in 5 patients (Patients #4, #5, #6, #9, and #12) who had a partial suppression of ventricular arrhythmias on flecainide and experienced side effects of β-blocker therapy (in particular, fatigue) before flecainide

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Table 2 Exercise Test Results of the Baseline Exercise Test on Standard Therapy and on the First Exercise Test on the Final (Stable) Flecainide Dose

	Standard Therapy Baseline (n = 29)	First Exercise Test on Stable Flecainide Dose (n = 29)	p Value
Time after start flecainide, days	—	21 (5–363)	—
Sinus rate at baseline, beats/min	57 ± 10	59 ± 9	0.061
Sinus rate at maximal exercise, beats/min	145 ± 23	133 ± 18	0.002
Maximum workload attained, METs	11 ± 3	12 ± 4	0.042
Sinus rate at onset of ventricular arrhythmias, beats/min	113 ± 19	118 ± 19	0.046*
Maximum no. of VPBs during a 10-s period†	12 ± 5	5 ± 5	<0.001
Ratio of VPBs to sinus beats during the 10-s period with the maximum no. of VPBs†	1.2 ± 0.8	0.4 ± 0.4	<0.001
Isolated VPB	29 (100)	22 (76)	0.016
Bigeminal VPBs	28 (97)	13 (45)	<0.001
Frequent VPBs (>10/min)	27 (93)	14 (48)	0.001
Couplet	20 (69)	2 (7)	<0.001
Nonsustained ventricular tachycardia	11 (38)	1 (3)	0.002
Longest ventricular salvo, VPBs†	5 (3–9)	4	—
Bidirectional NSVT	4 (36)	—	—

Data are mean ± SD, median (range), or n (%). *Only the 22 patients who still had ventricular arrhythmias on the first exercise test at the stable flecainide dose were included in this analysis. †Data were available for 28 patients (not available for Patient #32).

MET = metabolic equivalent; NSVT = nonsustained ventricular tachycardia; VPB = ventricular premature beat.

was started. One patient (Patient #29) refused to take β-blockers during follow-up, with no worsening of exercise-induced ventricular arrhythmias on flecainide monotherapy.

Thus, 30 of 33 patients (91%) continued to receive flecainide and were included in the further analysis of the incidence of arrhythmic events. During a median follow-up of 20 months (range 12 to 40 months, excluding Patient #32), VT recurred in only 1 patient (Patient #1) who experienced several appropriate ICD shocks for polymorphic VT after 8 months of flecainide treatment. Her serum flecainide level was low (0.34 μg/ml) at the time of the event compared with levels obtained previously (0.75 to 0.82 μg/ml), suggesting noncompliance. She was hospitalized for 48 h, nadolol and flecainide were resumed at their previous doses, and no further ventricular arrhythmias occurred during a further follow-up of 17 months. The other 29 patients remained free of arrhythmic events during follow-up. The longest follow-up of 29 years was achieved in Patient #32, who presented with exercise-induced VT in 1981. After unsuccessful trials of multiple antiarrhythmic drugs (including mexilitine, amiodarone, propranolol, sotalol, and Ca^{2+}-channel blockers), flecainide (200 mg/day) was added to sotalol (160 mg/day), which resulted in complete suppression of ventricular arrhythmia during exercise testing. In 2008, an exercise test 48 h after stopping flecainide and sotalol showed NSVT. After restarting the combined therapy, a subsequent exercise test only showed isolated VPBs, but no VT. Subsequent genotyping revealed a mutation in the gene encoding RyR2. In Patient #33, flecainide 150 mg/day was started in 2007 because of 2 episodes of syncope with ventricular fibrillation on the ICD interrogation despite nadolol 240 mg/day. Exercise testing showed complete suppression of ventricular arrhythmias, and she has been free of arrhythmic events on flecainide for 40 months.

Discussion

Main findings. Our study demonstrates that flecainide reduces or prevents exercise-induced ventricular arrhythmias in the majority of CPVT patients receiving conventional drug therapy. These findings are important because several studies have demonstrated a significant failure rate of current drug therapy (1,3,11–16), including potentially fatal arrhythmic events in 11% of CPVT patients over an 8-year period (2). Based on our clinical experience reported here, flecainide in addition to β-blocker therapy should be considered for CPVT patients who otherwise have few alternative therapeutic options. The optimal dose appears to be between 150 and 200 mg/day (range 100 to 300 mg/day). Daily doses <100 mg were associated with a lack of therapeutic response.

Rationale for use of flecainide. CPVT is caused by mutations in the genes encoding RyR2 and cardiac calsequestrin (4,5), 2 proteins that control Ca^{2+} release from the sarcoplasmic reticulum. As a result of the mutations, Ca^{2+} is released prematurely and excessively into the cytosol under conditions of catecholaminergic stimulation, generating repetitive spontaneous Ca^{2+} waves (9,29). The increase in intracellular Ca^{2+} in turn activates the electrogenic Na^+/Ca^{2+} exchanger, which produces a transient inward current (I_{Ti}). I_{Ti} generates delayed afterdepolarizations, which can lead to triggered activity, and the initiation of ventricular arrhythmias (30). Flecainide directly targets the molecular defect in CPVT by inhibiting RyR2 channels and preventing arrhythmogenic Ca^{2+} waves (23,24). Flecain-

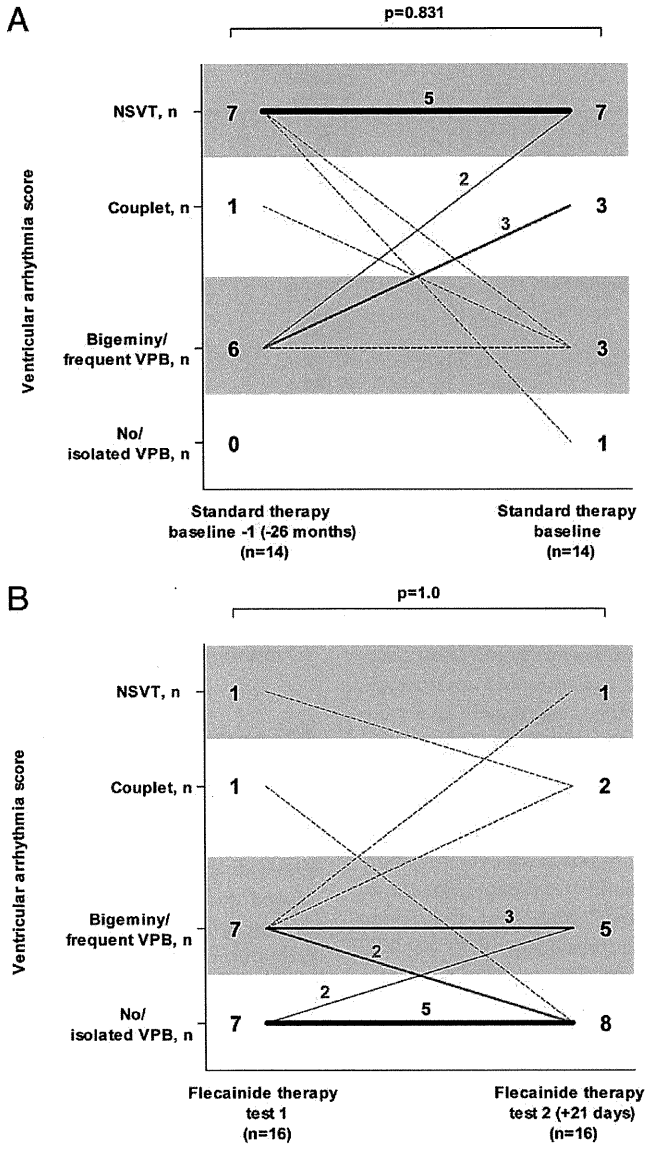

Figure 2 **Reproducibility of Ventricular Arrhythmia Score on Exercise Testing**

Ventricular arrhythmia score per patient on the baseline exercise test and on the previous exercise test at the same standard therapy dose **(A)** and on the first and second exercise tests at the final (stable) flecainide dose **(B)**. The number of patients in each ventricular arrhythmia category and change of ventricular arrhythmia category are shown. The **line thickness** indicates the number of patients, and a **dotted line** represents 1 patient. The median time interval between the 2 tests is shown. The standard therapy exercise tests were performed on patients receiving the same β-blocker dose with or without Ca^{2+}-channel blocker. All exercise tests on patients receiving flecainide were at the same stable flecainide dose in combination with an unchanged or lower β-blocker dose. The sinus rates at maximal exercise on the first and second exercise tests on flecainide were not significantly different (140 ± 19 vs. 144 ± 20; p = 0.245). However, the 2 patients with a ventricular arrhythmia score of 4 and 3 on the second exercise test did reach a significantly higher maximum sinus rate compared with the first exercise test (increase of 32 and 19 beats/min, respectively). Abbreviations as in Figure 1.

ide's Na^{+}-channel blockade further reduces the rate of triggered beats (23,24). This dual action could explain why flecainide is so effective in severe CPVT and provides a rationale for combination therapy with β-blockers.

RyR2-mediated sarcoplasmic reticulum Ca^{2+} release importantly regulates the beating rate of sinoatrial nodal cells (31), especially in response to catecholamines (32), and flecainide reduces the rate of spontaneous sarcoplasmic

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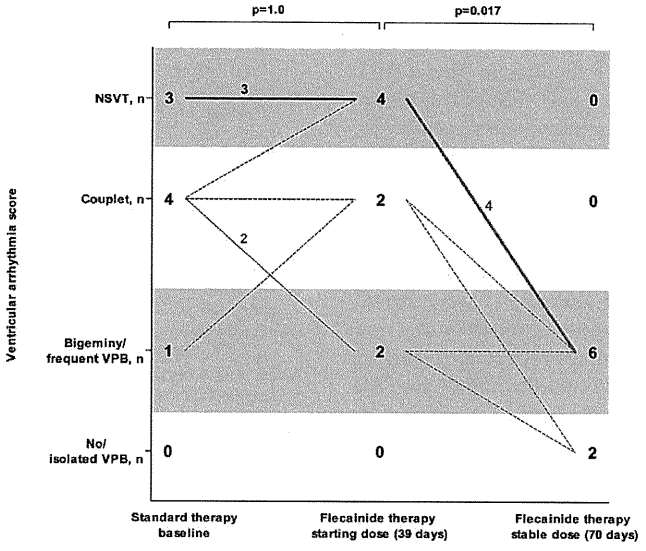

Figure 3 **Dose Dependence of Flecainide in 8 CPVT Patients Who Had an Increase in Flecainide Dose**

The number of patients in each ventricular arrhythmia category and change in ventricular arrhythmia category on the last exercise test at the flecainide starting dose (96 ± 28 mg; range 50 to 150 mg) and on the first exercise test at the final (stable) flecainide dose (178 ± 78 mg; range 100 to 300 mg) is shown. The **line thickness** indicates the number of patients, and a **dotted line** represents 1 patient. The median time interval from the start of flecainide therapy is shown. All exercise tests were performed with the patients receiving an unchanged β-blocker dose. Abbreviations as in Figure 1.

reticulum Ca^{2+} release in myocytes (24). This mechanism may explain why maximum hearts rates were significantly lower in flecainide-treated patients even though workloads were higher compared with baseline exercise testing (Table 2). The reduction in sinus rate during exercise may further contribute to flecainide's efficacy in CPVT.

Clinical implications. Given the high fatality rate of untreated CPVT patients (1,2), adequate treatment is mandatory and potentially life-saving. β-blockers are considered first-line therapy. In the largest published series of patients with CPVT, the risk of cardiac arrest (defined as aborted cardiac arrest, appropriate ICD shocks, and sudden cardiac death), despite β-blocker therapy during a mean follow-up period of 8 years, was 11% (2). Others have reported very diverse fatal or near-fatal event rates despite β-blocker therapy (1,3,11–16), although the highest event rates may be explained by the predominance of (symptomatic) probands and underdosing of β-blockers. An ICD was recommended for CPVT patients who were survivors of cardiac arrest, or when syncope or sustained VT persisted despite maximum tolerable β-blockade (33). Yet, ICDs have a potentially harmful effect in CPVT patients (17,18). Moreover, many CPVT patients are children, in whom ICD implantation can lead to significant complications (34). Thus, to avoid ICD implantation and prevent ICD shocks in patients with ICDs, controlling ventricular arrhythmias is of great clinical importance. Alternative therapies are needed for CPVT patients.

Left cardiac sympathetic denervation is an effective alternative when symptoms persist despite β-blockade, but requires surgery, is not universally available, and has only been tested in small cohorts (19–22). The use of Ca^{2+}-channel blockers in addition to β-blockade has been reported to decrease ventricular ectopy in CPVT patients with continuous symptoms and/or exercise-induced ventricular arrhythmias (12,27,35), but is not effective in all patients (27,35,36). From the original 6 patients treated with verapamil and β-blockers after failure of β-blockers alone, reported by Rosso et al. (27) in 2007, 3 had clinically significant ventricular arrhythmias during 37 ± 6 months of follow-up (36). Other pharmacological agents, including Na^{+}-channel blockers, amiodarone, and magnesium, lack of efficacy in CPVT patients (1,12).

In this analysis of all consecutive patients started on flecainide at 8 international centers, adding flecainide to standard therapy was effective in further reducing exercise-induced VT and preventing arrhythmic events CPVT patients. To suppress CPVT, adequate dosing of flecainide seems critical. An increased dose may be effective when the initial dose of flecainide fails to suppress VT. Based on these results, flecainide could be added to β-blocker therapy when symptoms or either spontaneous or exercise-induced ventricular arrhythmias persist despite β-blocker.

In our young patient population with no structural heart disease, the proarrhythmic effect of flecainide as documented in patients with ischemia and impaired left ventric-

ular function (37) may not be applicable. Consistent with this hypothesis, flecainide did not cause arrhythmic events during a median follow-up of 20 months, which is longer than the mean follow-up of 10 months in the CAST (Cardiac Arrhythmia Suppression Trial). The only arrhythmic event was associated with low flecainide serum levels, suggesting that the event was due to the underdosing and not toxicity.

Study limitations. This study reports on our experience of using flecainide in a clinical setting. The number of patients is relatively small because CPVT is a rare condition and only patients without other treatment alternatives were started on flecainide. However, it is the largest evaluation of a new therapeutic strategy in CPVT patients refractory to current drug therapy, with a median of 20 months follow-up. One patient has received flecainide for 29 years with continuous VT suppression on unchanged doses, and another severely symptomatic patient has been free of arrhythmic events on flecainide for 40 months. Nevertheless, long-term follow-up in more patients would further support the clinical utility of flecainide in CPVT.

Another potential limitation is that we only quantified the effect of flecainide on exercise-induced ventricular arrhythmias, which may not accurately predict fatal arrhythmic events. However, exercise testing is clinically used to guide therapy in CPVT. In a previous study including 70 CPVT patients, exercise-induced couplets or more successive VPBs were significantly associated with future arrhythmic events (sensitivity, 0.62; specificity, 0.67) (2).

Furthermore, we cannot exclude potential bias introduced by the variability of exercise test results on unchanged treatment, as illustrated in Figure 2. Finally, in 14 patients, conventional therapy may be considered suboptimal because they received an unusual β-blocker for CPVT or a low β-blocker dose for reasons previously outlined. However, flecainide was equally effective in the subgroup of CPVT patients who were treated with a first-choice β-blocker at an adequate dose (Fig. 1B).

Conclusions

Our results suggest that flecainide is a safe and effective therapy to reduce ventricular arrhythmias in the majority of CPVT patients who have exercise-induced ventricular arrhythmias despite conventional therapy.

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Key Words: antiarrhythmia agents ■ catecholaminergic polymorphic ventricular tachycardia ■ ventricular arrhythmia.

Altered Metabolism of Low-Density Lipoprotein and Very-Low-Density Lipoprotein Remnant in Autosomal Recessive Hypercholesterolemia

Results From Stable Isotope Kinetic Study In Vivo

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Background—Autosomal recessive hypercholesterolemia (ARH) exhibits different responsiveness to statins compared with that in homozygous familial hypercholesterolemia (FH). However, few data exist regarding lipoprotein metabolism of ARH. Therefore, we aimed to clarify lipoprotein metabolism, especially the remnant lipoprotein fractions of ARH before and after statin therapy.

Methods and Results—We performed a lipoprotein kinetic study in an ARH patient and 7 normal control subjects, using stable isotope methodology (10 mg/kg of [$^2H_3$]-leucine). These studies were performed at baseline and after the 20 mg daily dose of atorvastatin. Tracer/tracee ratio of apolipoprotein B (apoB) was determined by gas chromatography/mass spectrometry and fractional catabolic rates (FCR) were determined by multicompartmental modeling, including remnant lipoprotein fractions. FCR of low-density lipoprotein (LDL) apoB of ARH was significantly lower than those of control subjects (0.109 versus 0.450±0.122 1/day). In contrast, the direct removal of very-low-density lipoprotein remnant was significantly greater in ARH than those in control subjects (47.5 versus 2±2%). Interestingly, FCR of LDL apoB in ARH dramatically increased to 0.464 1/day, accompanying reduction of LDL cholesterol levels from 8.63 to 4.22 mmol/L after treatment with atorvastatin of 20 mg/d for 3 months.

Conclusions—These results demonstrate that ARH exhibits decreased LDL clearance associated with decreased FCR of LDL apoB and increased clearance for very-low-density lipoprotein remnant. We suggest that increased clearance of remnant lipoprotein fractions could contribute to the great responsiveness to statins, providing new insights into the lipoprotein metabolism of ARH and the novel pharmacological target for LDLRAP1. (***Circ Cardiovasc Genet.* 2012;5:35-41.**)

Key Words: lipoproteins ■ ARH ■ genetics ■ metabolism ■ LDLRAP1

Familial hypercholesterolemia (FH) is a common inherited disorder of plasma lipoprotein metabolism, characterized by an elevated level of low-density lipoprotein cholesterol (LDL-C), tendon xanthomas, and premature coronary artery disease.[1] Genetic causes of FH involve gene mutations such as LDL receptor (LDLR), apolipoprotein B-100 (apoB-100), and proprotein convertase subtilisin/kexin type 9 (PCSK9).[2] In contrast, there was a report of autosomal recessive inherited cases, who showed elevation of LDL-C, large xanthomas, and premature coronary artery disease typical of homozygous FH but in whom the fibroblasts had normal LDLR function.[3] Subsequently, Garcia et al[4] showed that this disorder was caused by a recessive form of null mutations in the LDLR adaptor protein 1 (LDLRAP1).

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Since then, evidence has been accumulating that it was not linked to mutations in the LDLR gene.[5,6] The N-terminal domain of LDLRAP1 contains a phosphotyrosine-binding (PTB) domain, which binds to the internalization sequence (FDNPVY) in the cytoplasmic tail of the LDLR.[7] LDLRAP1 protein serves as an adaptor for LDLR endocytosis in the liver

Received December 1, 2009; accepted November 28, 2011.

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The online-only Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.111.960948/-/DC1.

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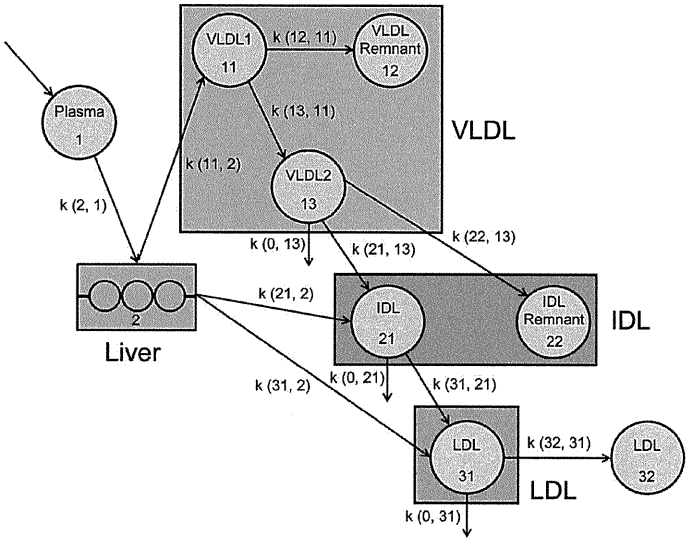

Figure 1. Multicompartmental model for apolipoprotein B–containing lipoproteins. Compartment 1 represents the intracellular amino acid pool and compartment 2 represents a delay for synthesis of lipoproteins. Very-low-density lipoprotein (VLDL) comprises 3 compartments: VLDL1, VLDL2, and VLDL remnant. Two compartments were allocated for intermediate-density lipoprotein (IDL): IDL and IDL remnant. LDL had intravascular and extravascular pools.

and a deficiency in this protein results in the decline of LDL-C catabolism, as seen with homozygous FH.[8] However, ARH differs from homozygous FH in the severity of the clinical phenotype and response to statins, the cause of which still remains unclear.[5]

One of the possible mechanisms of great responsiveness to statins was elucidated by a metabolic study using LDLRAP1 knockout mice that showed preserved ability for LDLR-dependent VLDL clearance.[9] However, few data exist regarding the metabolic basis of LDLRAP1 in clinical settings, especially, the metabolism of remnant lipoprotein fractions. Therefore, we examined lipoprotein kinetics in the homozygous ARH patient, using a stable isotope methodology with kinetic modeling including several remnant lipoprotein fractions, before and after atorvastatin therapy.

Methods

Study Subjects

This study was approved by the Ethics Committee of Kanazawa University, Suzu General Hospital, for the ARH patient and Jikei University School of Medicine for the control subjects. All study subjects gave their written informed consent to participate. We examined 8 subjects including 1 patient with suspected ARH without any evidence of chronic disease or malignancy and 7 normal control subjects (all men; age, 41±8 years). All lipid-lowering therapy had been strictly suspended for 3 months until the baseline study. We checked the lipid level of the patient suspected ARH 1 month before the baseline study as well as 1 week before the baseline study to confirm that his cholesterol level was appropriately elevated and reached plateau. Next, we reexamined ARH patient after treatment with atorvastatin of 20 mg/d for 3 months.

Genetic Studies

Genomic DNA was isolated from peripheral blood white blood cells according to standard procedures and was used for PCR. We analyzed the coding regions of LDLR, PCSK9, and LDLRAP1 genes. Primers for the study were as used previously.[10,11] PCR products were purified by Microcon (Millipore Corp, Bedford, MA) and used as templates for direct sequencing. DNA sequencing was carried out according to the manufacturer's instructions, using a dye terminator method (ABI PRISM 310 Genetic Analyzer (PerkinElmer Biosystems, Waltham, MA).

Biochemical Analysis and LDLR Activity

Serum concentrations of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined enzymatically. LDL-C concentrations were derived by means of the Friedewald formula. Apolipoprotein E (apoE) phenotype was separated by isoelectric focusing and detected by Western blot with apoE polyclonal antibody (phenotyping apoE IEF system, JOKOH, Tokyo, Japan). Lipoprotein lipase (LPL) mass in postheparin plasma was measured according to the method we previously reported.[12]

LDLR activity was measured by 2 methods, both of which used peripheral lymphocytes; The first was commercially available binding assay and the second was our original assay, which was described in detail elsewhere.[13] Briefly, we could measure accurate LDLR activity by using heparin to exclude the overestimation signals only bound at the surfaces of lymphocytes, even in the case with internalization defective type of disease.

Lipoprotein Kinetic Study

After an overnight fast, the study subjects were given a bolus injection (10 mg/kg) of [$^2H_3$]-leucine (Cambridge Isotope Laboratories, Woburn, MA). Blood samples were drawn periodically for 48 hours after the bolus injection.

Determination of Isotopic Enrichment

Samples were prepared for GC-MS analysis as reported previously.[14,15] For detailed determination of isotopic enrichment, please see online-only Data Supplement Method I.

Kinetic Modeling

Figure 1 shows the multicompartmental model used in this study, which was built using an interactive computer program (SAAM II, version 1.1; SAAM Institute Inc) to determine apoB kinetic parameters.[16,17] For detailed kinetic modeling, please see online-only Data Supplement Method 2.

Changes in Lipoprotein Subfractions

Lipoproteins of ARH were separated by the method based on those sizes using HPLC (LipoSEARCH, Skylight Biotech, Akita, Japan).[18] Changes in cholesterol, triglyceride, free cholesterol, and phospholipids in each lipoprotein subfraction was assessed by HPLC.

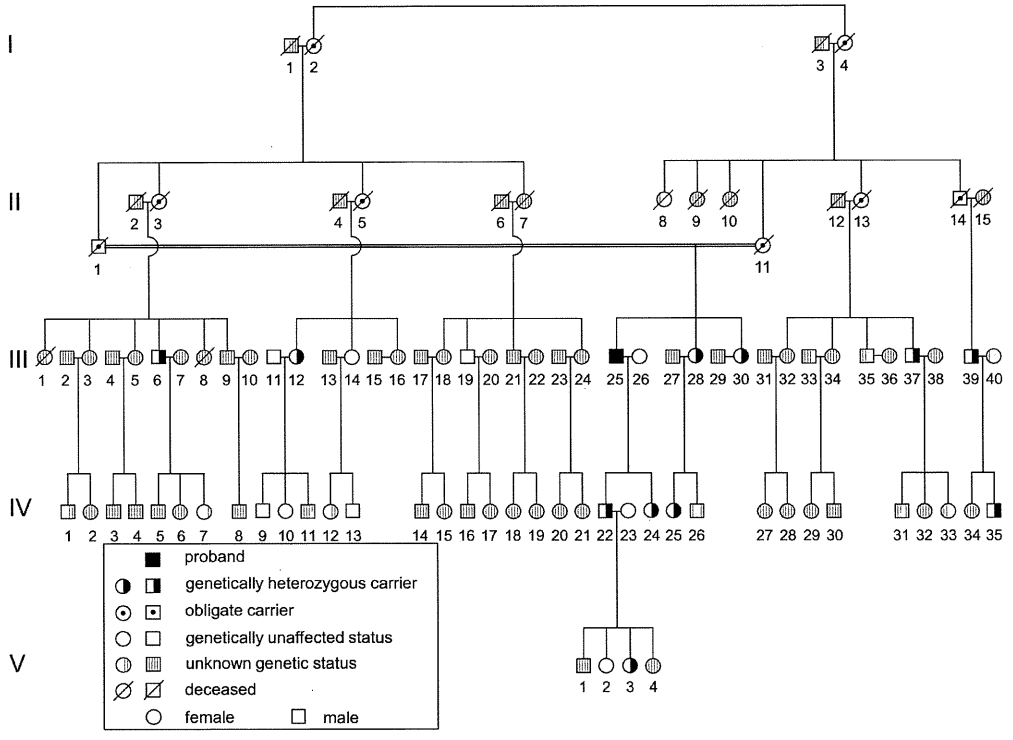

Figure 2. Pedigree of the autosomal recessive hypercholesterolemia patient. The proband was born to consanguineous parents (first cousins). The clinical data of the relatives, who were investigated further, are listed in online-only Data Supplement Table.

Results

Identification of ARH

A 68-year-old Japanese man presented at Kanazawa University Hospital for further examination of his hypercholesterolemia and severe tendon xanthomas (online-only Data Supplement Figure IA and IB). The proband was born to consanguineous parents (first cousins); neither parent had any signs of hypercholesterolemia or xanthomas. Large cutaneous and tendon xanthomas were identified on his fingers and foot, which had developed around 10 years of age. The thickness of his Achilles tendons reached 26 mm (online-only Data Supplement Figure IC). Initial serum TC and TG concentrations were high: 13.27 mmol/L and 3.39 mmol/L and were decreased to 5 mmol/L and 0.5 mmol/L after statin treatment for 8 years, respectively (online-only Data Supplement Figure ID). Several severe stenotic lesions including total occlusion of right common carotid artery were observed. Angiogram revealed total occlusion of bilateral external iliac arteries as well as left anterior descending artery (online-only Data Supplement Figure IE and IG). Bypass surgeries were conducted for both lesions (online-only Data Supplement Figure IF and IG). An abdominal aortic aneurysm, 33 mm in diameter, was observed. These extents of atherosclerosis are considered to be compatible with his high LDL-C level. Microscopic analysis revealed no specific findings in his liver (online-only Data Supplement Figure IH). Apo E phenotype of the ARH patient was E2/E3 in contrast to the result that those of control subjects were all E3/E3.

Although there was no mutation detected in LDLR and PCSK9 genes, homozygous mutation of an extra cytosine inserted into the region of the LDLRAP1 gene was found (c.606dup, previously described as ins C_{599}) in our proband (online-only Data Supplement Figure II), which is completely identical to that found in the first Japanese family identified with ARH.[19] An investigation, which extended back over 5 generations, failed to show any relationship between these 2 families, whose geographical origin were completely different. Using genetic analysis, we diagnosed 11 ARH heterozygous subjects and 6 normal subjects in the proband's family (Figure 2). Their lipid data and major clinical findings including the presence of coronary artery disease are listed in the online-only Data Supplement Table. As for LDLR activity, we found extremely accelerated LDLR activity (as much as 160% of normal control subjects) measured by the binding assay, using the measurement of 3,3′-dioctadecylindocarbocyanin (DiI)-labeled LDL uptake in blood peripheral lymphocytes (BML, Tokyo, Japan). In contrast, the value measured by our internalization assay using heparin showed that the activity was reduced to 14% of normal control subjects.

Lipoprotein Kinetic Study

At the time of the kinetic study (Table 1), the ARH patient showed higher serum TC levels (10.26 versus 4.87±0.58 mmol/L) and higher LDL-C levels (8.63 versus 2.95±0.49 mmol/L) than those of the control subjects.

The VLDL apoB, IDL apoB, and LDL apoB tracer/tracee ratio curves at baseline and after atorvastatin therapy, as well

Table 1. Characteristics of ARH Patients and Control Subjects

Subjects	Sex	Age, y	BMI, kg/m^2	TC, mmol/L	TG, mmol/L	LDL-C, mmol/L	HDL-C, mmol/L	ApoB, g/L	ApoB/LDL-C	Lathosterol, μg/mL	LPL, ng/mL
Baseline	Male	68	26	10.26	1.26	8.63	1.06	1.90	0.56	6.3	324
After statin therapy		68	26	6.02	1.06	4.22	1.32	1.13	0.69	1.2	401
Control subjects (n=7)	All male	41±8	22±1	4.87±0.58	1.08±0.24	2.95±0.49	1.38±0.13	0.89±0.12	0.78±0.24	n.d.	n.d.

Values of control subjects are shown as mean±SD.

ARH indicates autosomal recessive hypercholesterolemia; BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; apoB, apolipoprotein B; LPL, lipoprotein lipase; n.d., not determined.

as those for the mean of the control subjects, are shown in Figure 3. Kinetic parameter of apoB within each lipoprotein fraction is shown in Table 2. Fractional catabolic rates (FCRs) of VLDL, IDL and LDL apoB were markedly slower in the ARH patient at baseline (3.153 1/day for VLDL, 1.414 1/day for IDL, 0.109 1/day for LDL) compared with those of the control subjects (8.408±2.697 1/day for VLDL, 8.326±3.467 1/day for IDL, 0.450±0.122 1/day for LDL). Production rates (PRs) of the ARH patient of the 3 fractions were within the mean value ±2 SD of those of control subjects. Therefore, the markedly increased concentrations of IDL and LDL apoB were primarily due to the decreased catabolism rate in the ARH patient.

Surprisingly, the FCR of LDL apoB significantly increased to within the normal range after statin therapy in the ARH patient (0.109–0.464 1/day), resulting in a 70% reduction of LDL apoB concentration. This result was completely different from that seen with homozygous FH, where the FCR of LDL apoB was reported to be unchanged after statin therapy.[20] In addition to the response observed in FCR of LDL apoB, those of VLDL and IDL apoB also increased by the statin therapy in the ARH patient (3.153–7.881 1/day for VLDL, 1.414–2.525 1/day for IDL).

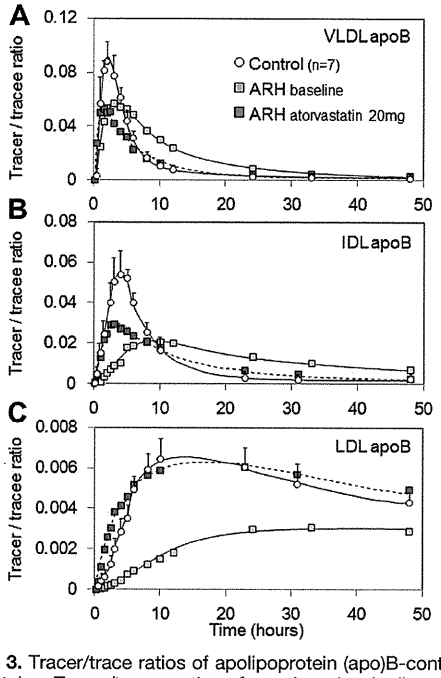

Figure 3. Tracer/trace ratios of apolipoprotein (apo)B-containing lipoproteins. Tracer/tracee ratios of very-low-density lipoprotein VLDL apoB (**A**), intermediate-density lipoprotein IDL apoB (**B**), and LDL apoB (**C**) in the autosomal recessive hypercholesterolemia patient at baseline (**blue squares**), on atorvastatin treatment (**pink squares with dotted line**), and in control subjects (**open circles**). Data were fitted by multicompartmental modeling using SAAMII. Bars represent standard error of the means.

Remnant Fractions

Next, we investigated detailed metabolic channeling in the ARH patient (the results are summarized in Table 3). In the control subjects, the liver primarily secrets VLDL (87.0±11.0%), most of which (85.5±18.7%) was, in turn, converted to IDL by lipoprotein-mediated delipidation, thus leaving the VLDL remnant as a minor fraction (12.0±11.8% of total VLDL mass). Some crucial differences between the ARH patient and the control subjects were noted in VLDL metabolism. In the ARH patient: (1) only half of VLDL was converted to IDL (52.5%); (2) VLDL remnant mass comprised as much as 60.2%, resulting from an alteration in metabolic channeling in favor of the conversion to VLDL remnant (47.5% versus 1.8±2.1%, ARH versus control, respectively): (3) removal rate of VLDL remnant (k[0,12]) was increased (4.3 1/day) compared with that of the control subjects (1.3±0.9 1/day); and (4) direct removal of VLDL, including VLDL remnant, was much higher compared with that of the control subjects (47.5% versus 14.5±18.7%), a finding mirroring the decreased conversion to IDL as noted above. Furthermore, these tendencies were more pronounced after atorvastatin therapy. As shown in the middle panel of Table 3, most IDL was derived from VLDL and exclusively converted to LDL (97.8±3.1%) in the control subjects. In the ARH patient, however, about one-quarter of IDL was directly secreted from the liver and more IDL fractions were directed into remnant, again resulting in the increased remnant mass. These tendencies remained unchanged by atorvastatin therapy. Finally, the only notable difference in LDL metabolism was higher direct secretion of LDL with atorvastatin therapy, a finding consistent to higher tracer/tracee ratios during early time points (pink squares with dotted line in Figure 3C).

Changes in Lipoprotein Subfractions

As shown in online-only Data Supplement Figure III, relatively wide range of apoB-containing lipoproteins, including large VLDL, could be reduced by atorvastatin therapy in all fractions of lipids (cholesterol, triglyceride, free-cholesterol, and phospholipids) in the ARH patient.

Discussion

In this study, we performed an in vivo lipoprotein kinetic study, allowing us to assess detailed metabolic behavior of

Table 2. Kinetic Parameters of ApoB in the Study Subjects

	VLDL			IDL			LDL		
Subjects	Conc, mmol/L	FCR, 1/Day	PR, mg/kg per Day	Conc, mmol/L	FCR, 1/Day	PR, mg/kg per Day	Conc, mmol/L	FCR, 1/Day	PR, mg/kg per Day
Baseline	0.340	3.153	9.180	0.657	1.414	9.560	7.730	0.109	6.980
After statin therapy	0.248	7.881	3.026	0.341	2.525	13.335	2.333	0.464	16.756
Control subjects (n=7)	0.104±0.033	8.408±2.697	13.172±4.664	0.091±0.052	8.320±3.467	10.562±5.194	2.037±0.315	0.450±0.122	13.947±3.636

Values of control subjects are shown as mean±SD.
Apo indicates apolipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; Conc, concentration; FCR, fractional catabolic rate; PR, production rate.

apoB-containing lipoproteins in ARH. Our results demonstrated that in ARH there existed reduced LDL catabolism, which could be normalized by statin therapy and dramatically increased clearance of VLDL remnant as well as other remnant lipoprotein fractions in spite of the fact that our ARH patient has apoE2 isoform which could cause the disturbance in remnant clearance.[21] These unique metabolism of apoB-containing lipoprotein fractions, including VLDL and its remnant fractions were completely different from those reported in heterozygous/homozygous FH patients.[17,22]

One of the possible explanations for the paradoxical acceleration of remnant lipoprotein fractions in ARH is the existence of another pathway, which is independent from the FDNPVY internalization for VLDL and its remnants and does not require LDLRAP1 protein.[23] In addition, Altenburg et al[24] demonstrated that deficiency in the molecule which enhanced the affinity between ligands such as VLDL remnant and LDLR could accelerate the internalization of the remnants. This is consistent with the notion that remnants are passed from one cell surface molecule to the other before internalization.[24] If LDLRAP1 served as an anchor between VLDL remnant and LDLR, deficiency in this protein could result in the increased catabolism of VLDL remnant in ARH. Another possibility is that unknown pathways may exist that are inactivated in the presence of LDLRAP1. This hypothesis seems to be supported by the fact that the LDLR can transfer such remnants to an additional receptor for uptake by the liver when its internalization is impaired. These pathways are not always through LDLR, LDLR-related protein (LRP), and heparan sulfate proteoglycan.[25]

In contrast to homozygous FH patients, the ARH patient responded to statin therapy by an increasing rate of LDL apoB catabolism, resulting in about 70% reduction of LDL apoB pool size. Statin therapy also modulated LDL synthesis in favor of more direct secretion from the liver (11% at baseline to 16% with the treatment versus a mean of 7% for the control subjects). The rate of LDL catabolism is a function of LDLR activity or/and LDL particle affinity to the LDLR. Thus, our results indicate that atorvastatin upregulate LDLR activity in the absence of LDLRAP1. Another possibility for the increasing rate of LDL apoB catabolism seen in ARH is that directly secreted LDL may have a higher affinity for LDLR compared with LDL-processed delipidation/remodeling. Different ratio of apoB/LDL-C between the ARH patient and the control subjects suggest that different LDL processing occurred through delipidation/remodeling of LDL particles under the condition of the absence of this adaptor protein. We also provide additional information for the impact of atorvastatin on the distribution of lipoprotein subfractions in ARH. Relatively wide range of apoB-containing lipoproteins, including large VLDL, could be reduced by atorvastatin therapy. This may be explained by the statin-induced upregulation of possible pathway which could accelerate the clearance of remnant lipoprotein fractions in ARH.

As for the dramatic decrease in PR of VLDL apoB under atorvastatin therapy, one of the possible explanations is the upregulated activity of HMG-CoA reductase suggested by the relatively high level of lathosterol at baseline (Table 1). On

Table 3. Metabolic Channeling of ApoB in the Study Subjects

VLDL	Conversion to IDL, %	VLDL Direct Removal, %	Removal From Remnant, %	Remnant Mass, %
Baseline	52.5	47.5	47.5	60.2
After statin therapy	28.1	71.9	42.8	82.3
Control subjects (n=7)	85.5±18.7	14.5±18.7	1.8±2.1	12.0±11.8

IDL	Direct Production, %	Conversion to LDL, %	IDL Direct Removal, %	Removal From Remnant, %	Remnant Mass, %
Baseline	8.6	56.4	77.2	77.2	80.8
After statin therapy	12.2	85.5	17.0	17.0	29.6
Control subjects (n=7)	5.9±7.7	97.8±3.1	2.3±3.3	2.3±3.3	17.4±14.4

LDL	Direct Production, %	Via IDL, %
Baseline	11.0	90.0
After statin therapy	16.2	85.8
Control subjects (n=7)	7.3±6.1	92.3±6.1

Values of control subjects are shown as mean±SD.
Apo indicates apolipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; and LDL, low-density lipoprotein.

the other hand, the increase in the PR of LDL apoB during atorvastatin therapy could be partially explained by the elevation of LPL mass (Table 1), in accordance with the previous report.[26] Also, another study has shown that atorvastatin therapy is associated with an increase in LPL activity.[27] These data suggest that atorvastatin treatment may cause an increase in the conversion of VLDL to LDL.

Limitations

Our study has several limitations. First, only 1 ARH patient was included in this study because of the rarity of this disease, making it difficult to compare the results statistically. Also, the age of the control subjects were younger than the ARH patient, although all were male. Second, we did not measure apoE FCR in the ARH patient and thus could not draw any conclusion regarding the possibility of the clearance through VLDL receptor. However, the fact that the ARH patient has apoE2 isoform, which could cause the disturbance in remnant clearance, indicates the less influence of the apoE pathway on the catabolism of these lipoproteins. In this study, as much as 30% increase in HDL-C was achieved through atorvastatin therapy. Another kinetic study targeting apoA-I for the ARH patient may reveal the metabolic aspects about the increase in HDL-C.

Finally, it would be worthwhile to compare lipoprotein kinetics of ARH with that of FH directly. Although we cited previously published data on the apoB kinetics in FH patients to discuss the comparison between the kinetics of ARH and FH, further kinetic study comparing ARH and FH directly is needed to confirm this matter.

Conclusion

In summary, the first detailed lipoprotein kinetic study including remnant lipoprotein fractions in ARH before and after statin therapy revealed 2 important aspects of the lipoprotein metabolic basis of this disease. First, FCR of LDL apoB in ARH was decreased by about 76% that of normal control subjects at baseline; however, the catabolic parameter was elevated to normal range after statin therapy (atorvastatin 20 mg). Second, and possibly the major finding from this investigation, is that the clearance of the VLDL remnant as well as other remnant fractions were dramatically increased compared with normal control subjects. We suggest that these results will provide new insights into the lipoprotein metabolism of ARH and the novel pharmacological target for LDLRAP1.

Acknowledgments

We express our special thanks to the following: Mihoko Mizuno, Kazuko Honda, Misae Omoto, and Sachio Yamamoto (all staff of Kanazawa University); Yae Inoue (staff of National Defense Medical College) for their outstanding technical assistance; and to Dr Kenkoh Hasatani and Dr Hiroshi Masuya, both of Suzu General Hospital, for their help in this kinetic study.

Sources of Funding

This work has been supported by a scientific research grant from the Ministry of Education, Science, and Culture of Japan (No. 21591160), a grant from the Mitsubishi Pharma Research Foundation and Japan Heart Foundation, and Astellas/Pfizer Grant for Research on Atherosclerosis Update.

Disclosures

None.

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CLINICAL PERSPECTIVE

Autosomal recessive hypercholesterolemia (ARH), which is due to mutations in an adaptor protein involved in low-density lipoprotein receptor internalization (LDLRAP1), is an extremely rare disorder, with only about 50 cases described in the literature. This defect appears to be a phenocopy of homozygous familial hypercholesterolemia; however, the clinical phenotype of ARH appears to be less severe and more responsive to statins—the mechanism for this observation still remains unknown. One of the possible mechanisms of great responsiveness of ARH to statins was elucidated by a metabolic study using LDLRAP1 knockout mice that showed a preserved ability for LDLR-dependent very-low-density lipoprotein (VLDL) clearance. However, few data exist regarding the metabolic basis of LDLRAP1 in clinical settings, especially the metabolism of remnant lipoprotein fractions. Therefore, we examined lipoprotein kinetics in the ARH patient by using a stable isotope methodology with kinetic modeling including several remnant lipoprotein fractions, before and after atorvastatin therapy. We demonstrate that ARH exhibits decreased LDL clearance associated with decreased fractional catabolic rates of LDL apoB and increased clearance for VLDL remnant; this observation indicates the lack of LDLRAP1-dependent modulation of VLDL metabolism, activating an alternate pathway that can remove VLDL remnant paradoxically. This preferred pathway could potentially contribute to the greater responsiveness of ARH to statins. Our results will provide new insights into the lipoprotein metabolism in ARH.

Efficacy and Safety of Coadministration of *Rosuvastatin*, *Ezetimibe*, and *Colestimide* in Heterozygous Familial Hypercholesterolemia

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Aggressive low-density lipoprotein (LDL) cholesterol-lowering therapy is important for high-risk patients. However, sparse data exist on the impact of combined aggressive LDL cholesterol-lowering therapy in familial hypercholesterolemia (FH), particularly on side effects to changes in plasma coenzyme Q10 and proprotein convertase subtilisin/kexin type 9 levels. We enrolled 17 Japanese patients with heterozygous FH (12 men, 63.9 ± 7.4 years old) with single LDL receptor gene mutations in a prospective open randomized study. Permitted maximum doses of rosuvastatin (20 mg/day), ezetimibe (10 mg/day), and granulated colestimide (3.62 g/day) were introduced sequentially. Serum levels of LDL cholesterol decreased significantly by −66.4% ($p < 0.001$) and 44% of participants achieved LDL cholesterol levels <100 mg/dl. There were no serious side effects or abnormal laboratory data that would have required the protocol to have been terminated except for 1 patient with myalgia. Coadministration of ezetimibe and granulated colestimide further lowered serum LDL cholesterol more than rosuvastatin alone without changing plasma coenzyme Q10 and proprotein convertase subtilisin/kexin type 9 levels. In conclusion, adequate introduction of this aggressive cholesterol-lowering regimen can improve the lipid profile of FH. © 2012 Elsevier Inc. All rights reserved. (Am J Cardiol 2012;109:364–369)

Familial hypercholesterolemia (FH) is the most severe monogenic hypercholesterolemia owing to a genetic defect or mutation of the low-density lipoprotein (LDL) receptor, apolipoprotein B, or proprotein convertase subtilisin/kexin type 9 (PCSK9) gene.[1] PCSK9 binds to the epidermal growth factor-like repeat A domain of the LDL receptor, inducing LDL receptor degradation. Because FH is highly refractory to cholesterol-lowering medical therapy, monotherapy using a strong statin may not be enough to achieve the target LDL cholesterol level. Thus, a combination drug therapy with a different mechanism is needed.[2,3] However, additional use of ezetimibe and/or resins may result in a depletion of other products downstream of the mevalonate pathway such as coenzyme Q10 (CoQ10)[4] or inactivation of the sterol regulatory element binding protein 2 associated with the induction of PCSK9.[5] We investigated the efficacy and safety of coadministration of maximum permitted doses of rosuvastatin, ezetimibe, and granulated colestimide in Japanese patients with heterozygous FH.

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0002-9149/12/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.amjcard.2011.09.019

Methods

The study population consisted of 17 patients (12 men, mean ± SD 63.9 ± 7.4 years old) with heterozygous FH. All 17 subjects were heterozygous with a confirmed LDL receptor gene mutation and fulfilled our clinical diagnostic criteria for heterozygous FH: patients with primary hyper-LDL cholesterolemia (>160 mg/dl) with tendon xanthoma or those with first-degree relatives with previously diagnosed heterozygous FH showing primary hyper-LDL cholesterolemia (>160 mg/dl).[6] Exclusion criteria of the present study were FH patients with a homozygous gene mutation, patients under LDL apheresis therapy or any immunomodulatory medication, patients with fasting serum triglyceride levels >500 mg/dl, patients with hepatic disease, or patients within 12 weeks after the onset of an acute myocardial infarction or stroke. Written informed consent to participate in the present study was obtained from each patient before entry in the study. Ethical committees of Kanazawa University Hospital and KKR Hokuriku Hospital approved the study protocol.

This study was conducted as a prospective open randomized study to investigate the efficacy and safety of coadministration of rosuvastatin (20 mg/day), ezetimibe (10 mg/day), and granulated colestimide (3.62 g/day) at the maximum doses permitted in Japan. All patients were outpatients at the beginning of the study. Any lipid-lowering agents had been washed out ≥4 weeks before entry in the present study. All study patients were placed on the following diet therapy: 25 to 30 kcal for ideal body weight, fat restriction <20% of total oral intake, cholesterol