after injury than the immature neonatal brain. In addition to regional differences, we should thus consider developmental age when trying to apply a regenerative therapy to developing brain.

Transplantation of neural stem cells

Zheng et al. (2006) extracted multipotent astrocytic stem cells from the subependymal zones of homozygous green fluorescence protein (GFP) transgenic neonatal mice at postnatal days 1–6. These cells are positive for glial fibrillary acidic protein and negative for beta-III tubulin and NeuN, markers of mature neurons (Laywell et al. 2000). The multipotent astrocytic stem cells were transplanted into Sprague-Dawley rat brains 24 h or 5 days after hypoxia-ischemia on postnatal day 7 (Zheng et al. (2006). GFP-positive cells successfully transformed into neurons positive for beta-III tubulin and NeuN and were located around the infarcted area, far from the injection site. This observation was however not quantitative, and furthermore, the authors did not describe any histological or behavioral improvements.

In a study by Park et al (2002b), the clonal multipotent neural precursor cell line (C17.2) was derived from the external germinal layer of the neonatal mouse cerebellum; these neural stem/progenitor cells were cultured with a polymer scaffold (polyglycolic acid, PGA), for 4 days, making a neural stem/progenitor cell-PGA complex. The neural stem/progenitor cell-PGA matrix was transplanted into the degenerated cavity of mouse brain, 7 days after hypoxia-ischemia on postnatal day 7. The transplanted neural stem cells not only differentiated into neurons, oligodendrocytes, and astrocytes, but also made neural networks with both donor-derived neurons and host-derived neurons. This neuronal network extended through the corpus callosum into the contralateral hemisphere. Although Park et al (2002b) reported that neurological function, as assessed by diminished unilateral rotation, seemed to be improved, they did not quantitatively analyze behavioral improvement.

Exogenous enrichment of the milieu around the injury

Another way to facilitate regeneration may be the enrichment of the milieu around the injured region. Neurotrophic factors play an important role in the development and maintenance of specific populations of neurons in the central and peripheral nervous system. Several neurotrophic factors have been reported to help rescue neurons and repair neuronal injury. These include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial-cell line-derived neurotrophic factor (GDNF), insulin-like growth factor, basic fibroblast growth factor (bFGF), transforming growth factor (TGF)-beta 3, neurotrophin-3 (NT-3), NT-4/5, and NT-6 (Gong et al. 1999; Hyman et al. 1994; Ikeda et al. 2000; Krieglstein et al. 1996; Zawada et al. 1998). Although these neurotrophic factors are also known to be upregulated after hypoxia-ischemia in the neonatal rat (Ikeda et al. 2002), this endogenous upregulation seems to be insufficient to support neural differentiation from progenitor cells.

To date, bFGF-2 seems to be the most promising candidate to facilitate neuroregeneration in neonatal bran damage (Wagner et al. 1999). Monfils et al. (2006, 2005) administered 50 ng/kg bFGF for 7 days after brain lesions to the motor cortex of a neonatal rat and observed significant neuronal recovery and synapse formation in the treated group. This was accompanied with improved forelimb movement. Although this injury is traumatic, bFGF could be applied after hypoxic-ischemic injury with or without another neurotrophic factor such ase EGF (Nakatomi et al. 2002).

Recently, Park et al. (2006b) transplanted a subclone of neural stem cells, transduced with a retrovirus encoding NT-3, into a site of unilateral hypoxic-ischemic brain damage in neonatal mice. They observed a greater than 80% increase in neurons derived from neural stem cells compared with those from non-transduced stem cells. Donor-derived stem cells were shown to differentiate successfully into cholinergic, gamma-aminobuytric-acid-ergic, or glutamatergic subtypes of neuron. These findings indicate the importance of adjusting the environment around the injured area for successful stem cell therapy.

Concluding remarks

Although considerable potential with respect to proliferation and migration has been revealed in the immature and developing brain, limitated terminal differentiation may hamper stem cell therapy. Aggressive efforts to adjust the environment of the damaged part in which tissue regeneration is occurring or more cautious stem cell transplantation will probably be required.

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Clinical Aspects of Type-1 Long-QT Syndrome by Location, Coding Type, and Biophysical Function of Mutations Involving the KCNQ1 Gene

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Background—Type-1 long-QT syndrome (LQTS) is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. We evaluated the effect of location, coding type, and biophysical function of KCNQ1 mutations on the clinical phenotype of this disorder.

Methods and Results—We investigated the clinical course in 600 patients with 77 different KCNQ1 mutations in 101 proband-identified families derived from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years. The clinical characteristics, distribution of mutations, and overall outcome event rates were similar in patients enrolled from the 3 geographic regions. Biophysical function of the mutations was categorized according to dominant-negative (>50%) or haploinsufficiency (≤50%) reduction in cardiac repolarizing I_{Ks} potassium channel current. Patients with transmembrane versus C-terminus mutations (hazard ratio, 2.06; $P<0.001$) and those with mutations having dominant-negative versus haploinsufficiency ion channel effects (hazard ratio, 2.26; $P<0.001$) were at increased risk for cardiac events, and these genetic risks were independent of traditional clinical risk factors.

Conclusions—This genotype–phenotype study indicates that in type-1 LQTS, mutations located in the transmembrane portion of the ion channel protein and the degree of ion channel dysfunction caused by the mutations are important independent risk factors influencing the clinical course of this disorder. (***Circulation.*** **2007;115:2481-2489.)**

Key Words: electrocardiography ■ genetics ■ long-QT syndrome

The hereditary long-QT syndrome (LQTS) is characterized by prolonged ventricular repolarization on the ECG and arrhythmia-related syncope and sudden death.[1] Mutations in 1 or more of several ion channel genes are known to cause this disorder,[2] with mutations in the KCNQ1 gene causing the type-1 long-QT syndrome.[3,4] The KCNQ1 gene codes for the potassium channel protein responsible for the slow component of the delayed rectifier repolarizing current (I_{Ks}). Mutations involving this gene result in reduction of the repolarizing I_{Ks} current and lengthening of the QT interval.[3]

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Functional I_{Ks} channels result from the coassembly of 4 subunits into a tetrameric protein channel that is transported to the myocyte membrane. Each subunit contains 6 membrane-spanning domains (S1 to S6) flanked by amino (N)- and carboxyl (C)-terminus regions. Two distinct biophysical mechanisms mediate the reduced I_{Ks} current in patients with KCNQ1 mutations: (1) coassembly or trafficking defects in which mutant subunits are not transported

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properly to the cell membrane and fail to incorporate into the tetrameric channel, with the net effect being a ≤50% reduction in channel function (haploinsufficiency)[5]; and (2) formation of defective channels involving mutant subunits with the altered channel protein transported to the cell membrane, resulting in a dysfunctional channel having >50% reduction in channel current (dominant-negative effect).[6]

Limited prior studies involving relatively small numbers of patients with type-1 LQTS have been reported with conflicting results on the relationship between various KCNQ1 mutations and the clinical outcome.[7,8] We hypothesized that the location, coding type, and functional effect of the channel mutation would have important influence on the phenotypic manifestations and clinical course of patients with this disorder. To test this hypothesis, we investigated the clinical aspects of a large cohort of subjects having a spectrum of KCNQ1 mutations categorized by their location, coding type, and type of biophysical ion channel dysfunction.

Methods

Study Population

The study population of 600 subjects with genetically confirmed KCNQ1 mutations was derived from 101 proband-identified families with the type-1 LQTS disorder. The proband in each family had QTc prolongation not due to a known cause. The subjects were drawn from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). All subjects or their guardians provided informed consent for the genetic and clinical studies.

Phenotype Characterization

Routine clinical and ECG parameters were acquired at the time of enrollment in each of the registries. Follow-up was censored at age 41 years to avoid the influence of coronary disease on cardiac events. Measured parameters on the first recorded ECG included QT and R-R intervals in milliseconds, with QT corrected for heart rate by Bazett's formula. The QTc interval was expressed in its continuous form and categorized into 3 levels: <500, 500 to 530, and >530 ms. Clinical data were collected on prospectively designed forms with information on demographic characteristics, personal and family medical history, ECG findings, therapy, and end points during long-term follow-up. LQTS-related cardiac events included syncope, aborted cardiac arrest, or unexpected sudden death without a known cause. Data common to all 3 LQTS registries involving genetically identified patients with type-1 genotype were electronically merged into a common database for the present study.

Genotype Characterization

The KCNQ1 mutations were identified with the use of standard genetic tests performed in academic molecular-genetic laboratories including the Functional Genomics Center, University of Rochester Medical Center, Rochester, NY; Baylor College of Medicine, Houston, Tex; Mayo Clinic College of Medicine, Rochester, Minn; Boston Children's Hospital, Boston, Mass; Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; and Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands.

Genetic alterations of the amino acid sequence were characterized by location and by the specific mutation (missense, splice site, in-frame insertions/deletions, nonsense, stop codon, and frameshift). The transmembrane region of the KCNQ1-encoded channel was defined as the coding sequence involving amino acid residues from 120 through 355 (S5-pore-S6 region 285 to 355), with the N-terminus region defined before residue 120 and the C-terminus region after residue 355. Nineteen study patients had intron mutations predicted to disrupt the canonical splice-site domains. Fifty-one subjects died of sudden cardiac death at a young age but did not have genotype studies. These 51 subjects were assumed to have the same KCNQ1 mutation as other affected members of their respective family. Twelve subjects had 2 mutations, one in the KCNQ1 gene and a second mutation in another LQTS ion channel gene; these 12 subjects are described separately and are not included in any of the tables or outcome analyses. Subjects with Jervell and Lange-Nielsen syndrome with deafness and 2 KCNQ1 mutations as well as those with 1 known KCNQ1 mutation and congenital deafness are not included in the present study.

The biophysical function of the mutant channels was classified as having dominant-negative effect (>50% reduction in function) or haploinsufficiency (≤50% reduction in function) on the basis of the following: (1) cellular expression studies for those with missense (n=21) and nonsense (n=2) mutations reported in the literature, with the functional information derived exclusively from heterologous expression studies; and (2) assumed loss of function for identified nonsense, splice site, in-frame deletion, and frameshift mutations (n=10) that have not yet been functionally characterized. Forty-one missense mutations and the 3 intron mutations that have not been functionally reported in cellular expression studies were categorized as unknown in terms of type of functional perturbation.

Statistical Analysis

Differences in the univariate characteristics by specific groupings were evaluated by standard statistical methods. The primary end point was time to syncope, aborted cardiac arrest, or sudden death, whichever occurred first. The cumulative probability of a first cardiac event was assessed by the Kaplan-Meier method with significance testing by the log-rank statistic. The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years.[9] Stratified and unstratified Cox regression models, allowing for time-dependent covariates, were fit to estimate the adjusted hazard ratio of each factor as a predictor of first cardiac events. We observed that sex was not proportional as a function of age with crossover in risk at age 13 years on univariate Kaplan-Meier analysis. To relax the assumption of proportional hazards for sex over the entire age range, separate nonparametric baseline hazard functions were allowed for male and female subjects via the stratified Cox model; then, to summarize the sex effect, sex was modeled in an unstratified Cox model as a time-dependent covariate (via an interaction with time), allowing for different hazard ratios by sex before and after age 13 years.

Because almost all the subjects were first- and second-degree relatives of probands, the effect of lack of independence between subjects was evaluated in the Cox model with grouped jackknife estimates for family membership.[10] All grouped jackknife standard errors for the covariate risk factors fell within 3% of those obtained from the unadjusted Cox model, and therefore only the Cox model findings are reported.

Patients who died suddenly at a young age from suspected LQTS and who did not have an ECG for QTc measurement were identified in the Cox models as "QTc missing." Prespecified covariate interactions were evaluated. The influence of time-dependent β-blocker therapy (the age at which β-blocker therapy was initiated) on outcome was determined by adding this variable to the final Cox model containing the various covariates.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Total Study Population

The spectrum and number of KCNQ1 mutations by location, type of mutation, and functional effect are presented in Table 1, with the location frequency of the mutations presented diagrammatically in Figure 1. A total of 77 different KCNQ1

TABLE 1. KCNQ1 Mutations by Location and Coding, Type of Mutation, and Functional Effect

Location and Coding*	No. of Subjects†	Type of Mutation	Functional Effect‡
N-terminus			
M1V	1	Missense	Unknown
G57V	1	Missense	Unknown
Transmembrane			
W120C	2	Missense	Unknown
T144A	7	Missense	Unknown
A150fs/133 [del CT 451-452]	2	Frameshift	Haploinsufficiency
E160K	3	Missense	Unknown
G168R	44	Missense	Unknown
Y171X [513 C>G]	6	Nonsense	Haploinsufficiency
R174H	2	Missense	Unknown
A178P	5	Missense	Dominant-negative effect (a)
Y184S	18	Missense	Unknown
G185S	10	Missense	Unknown
G189E	2	Missense	Unknown
G189R	4	Missense	Dominant-negative effect (b)
R190Q	4	Missense	Haploinsufficiency (b, c)
L191fs/90 [del TGCGC 572-576]	8	Frameshift	Haploinsufficiency
R195fs/40 [del G 585]	2	Frameshift	Haploinsufficiency
S225L	13	Missense	Dominant-negative effect (d)
A226V	3	Missense	Unknown
R237P	1	Missense	Unknown
D242N	3	Missense	Unknown
R243C	13	Missense	Haploinsufficiency (e)
V254 mol/L	59	Missense	Dominant-negative effect (b, f)
R258C	1	Missense	Haploinsufficiency
R259C	1	Missense	Haploinsufficiency (g)
L266P	15	Missense	Unknown
G269D	35	Missense	Dominant-negative effect (h)
G269S	25	Missense	Haploinsufficiency (i)
L273F	6	Missense	Dominant-negative effect (a)
I274V	1	Missense	Unknown
S277L	3	Missense	Unknown
Y278H	2	Missense	Unknown
E284K	2	Missense	Unknown
G292D	3	Missense	Unknown
F296S	2	Missense	Unknown
G306R	2	Missense	Dominant-negative effect (b, j)
V310I	1	Missense	Unknown
T312I	14	Missense	Dominant-negative effect (a)
G314S	8	Missense	Dominant-negative effect (h, k, l, m)
Y315C	10	Missense	Dominant-negative effect (d, n)
Y315S	1	Missense	Dominant-negative effect (h, m)
D317G	3	Missense	Unknown
P320H	1	Missense	Unknown
T322 mol/L	2	Missense	Unknown
G325R	3	Missense	Unknown
delF340 [del CTT 1017-1019]	7	In-frame deletion	Haploinsufficiency
A341E	9	Missense	Dominant-negative effect (b)
A341V	20	Missense	Dominant-negative effect (o)

TABLE 1. Continued

Location and Coding*	No. of Subjects†	Type of Mutation	Functional Effect‡
P343S	1	Missense	Dominant-negative effect (p)
A344A/sp [1032 G>A]	27	Splice site	Haploinsufficiency
A344V	17	Missense	Unknown
S349W	15	Missense	Unknown
L353P	4	Missense	Unknown
C-terminus			
Q357H	3	Missense	Unknown
R360G	3	Missense	Unknown
S373P	7	Missense	Unknown
K393N	10	Missense	Unknown
R397W	5	Missense	Unknown
P400fs/62 [ins C 1201-1022]	6	Frameshift	Haploinsufficiency
P448fs/13 [ins G 1344-1345]	11	Frameshift	Haploinsufficiency
I517T	3	Missense	Unknown
R518X [1552 C>T]	11	Nonsense	Haploinsufficiency (q)
M520R	3	Missense	Unknown
V524G	4	Missense	Unknown
Q530X [1588 C>T]	13	Nonsense	Haploinsufficiency (q)
R562 mol/L	2	Missense	Unknown
S566F	3	Missense	Unknown
I567S	6	Missense	Unknown
S571fs/20 [del C 1714]	3	Frameshift	Haploinsufficiency
R591C	5	Missense	Unknown
R591H	6	Missense	Haploinsufficiency (r)
R594Q	11	Missense	Haploinsufficiency (q)
D611Y	10	Missense	Haploinsufficiency (s)
A636fs/28 [del C 1909]	2	Frameshift	Haploinsufficiency
Intron			
IVS2+1 G>A	2	Splice site	Unknown
IVS4+5 G>A	2	Splice site	Unknown
IVS7+5 G>A	15	Splice site	Unknown

*The numbers and letters refer to the amino acid coding of the mutant channel protein. The brackets contain the nucleotide code for deletions, frameshift, splice site, and nonsense mutations.

†Included in this table are 52 subjects who died suddenly at a young age. These subjects were from families with a known KCNQ1 mutation and were assumed to have their respective family mutation.

‡Dominant-negative effect is associated with >50% reduction whereas haploinsufficiency is associated with <50% reduction in ion channel repolarizing current. See text for details. Letters in parentheses refer to references that are available in the online-only Data Supplement.

mutations were identified. A majority of the mutations were localized to the S1 to S6 transmembrane domains (66%), and missense (single amino acid substitutions) accounted for 81% of all the mutations.

The phenotypic characteristics of patients enrolled in each of the 3 registries and by location and type of mutation are presented in Table 2. The clinical characteristics of the patients were similar among the 3 registries except for QTc duration and frequency of β-blocker use. The QTc interval was longer and cardiac events and β-blocker use were more frequent in patients with mutations in the transmembrane than in the C-terminus location. β-Blockers were used less frequently in patients from the Japanese registry than in patients from the other 2 registries. The frequency of first cardiac events was higher in those with than without missense mutations. The clinical characteristics of the 19 subjects possessing intron mutations resembled those with transmembrane and missense mutations.

The QTc interval was significantly longer in the 12 patients with 2 mutations than in those with only single KCNQ1 mutations (570±70 versus 480±60 ms; $P<0.01$). All 12 patients with 2 mutations experienced at least 1 cardiac event.

The cumulative probabilities of first cardiac event by location and type of mutation are presented in Figure 2A and 2B, respectively. Significantly higher event rates were found in subjects with transmembrane than C-terminus mutations and in those with than without missense mutations, with the most rapid increase in event rates occurring during ages 7 to

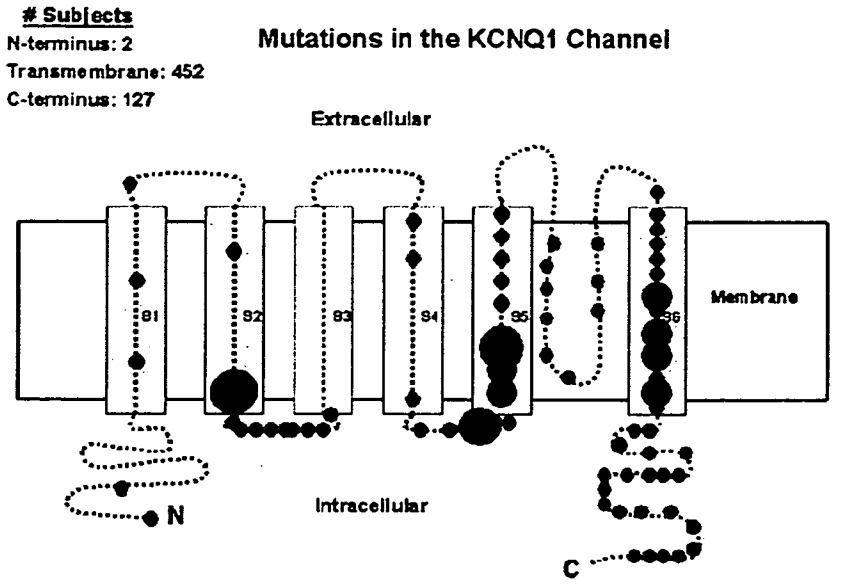

Figure 1. Frequency and location of 74 different mutations in the KCNQ1 potassium channel involving 581 subjects. The 19 subjects with 3 intron mutations are not included in this diagram. The α subunit involves the N-terminus (N), 6 membrane-spanning segments, and the C-terminus portion (C). The size of the circles reflect the number of subjects with mutations at the respective locations, with the small circles indicating <15, medium-sized circles 15 to 30, and large circles >30 subjects.

20 years. In patients with transmembrane-localized mutations, the event rates for patients with mutations localized to the pore region (S5-pore-S6) were nearly identical to those with nonpore mutations (data not shown).

The findings from the Cox regression analysis for location and type of mutation are presented in Table 3. The clinical risk factors associated with first cardiac events involved males before age 13 years, females after age 13

TABLE 2. Phenotypic Characteristics by Source of Subjects, Location of Mutation, and Type of Mutation

	Source of Subjects			Location of Mutation		Missense Mutation		
Characteristics	United States (n=425)	Netherlands (n=93)	Japan (n=82)	Trans Membrane (n=452)	C-Terminus (n=127)	Yes (n=483)	No (n=98)	Intron Mutation (n=19)
Female, %	57	53	54	57	51	54	62	63
ECG at enrollment								
QTc†‡, ms	488±58	450±45	472±46	485±53	460±61	481±59	471±38	478±60
Therapy, %								
β-Blockers†‡	45	34	26	45	28	42	38	37
Pacemaker	2.4	0	0	1.5	2.4	1.4	3.1	0
Sympathectomy	0.5	0	0	0.4	0	0.4	0	0
Defibrillator	6.4	3.2	0	5.8	3.1	5.2	5.1	0
First cardiac event*‡§, %	41	37	38	45	21	43	26	42
Syncope‡ (n=200)	35	31	29	38	17	36	21	32
Aborted cardiac arrest (n=15)	1.9	1.1	7.3	2.9	0.8	2.5	2.0	5.3
Death (n=23)	4.0	5.5	1.2	4.0	3.1	4.2	2.0	5.3
Ever cardiac event, %								
Syncope‡§	35	31	31	39	17	37	21	33
Aborted cardiac arrest†	2.4	15	8.8	5.3	3.2	5.4	2.0	11
Death	11	14	2.4	10	6.3	11	4.1	26

Plus-minus values are mean±SD. Percentages >10 are rounded to a whole number. The 600 subjects in this table include 51 subjects who died suddenly at a young age, were from families with known KCNQ1 mutation, and were assumed to have the family mutation. Patients with intron mutations are categorized separately and are not included in the location or missense categories. Seven subjects with transmembrane mutations and 1 with C-terminus mutations had missing data about the date of the first cardiac event. Eight subjects with missense mutations had missing data about the date of the first cardiac event. Numbers in parentheses indicate the total number of specific first cardiac events from the 3 sources of patients.

*First cardiac event was syncope, aborted cardiac arrest, or sudden death, whichever occurred first.

†$P<0.01$ for the comparison of characteristics among the 3 sources of subjects.

‡$P<0.01$ for the comparison of characteristics between the 2 locations of the mutations.

§$P<0.01$ for the comparison of characteristics between missense yes and no.

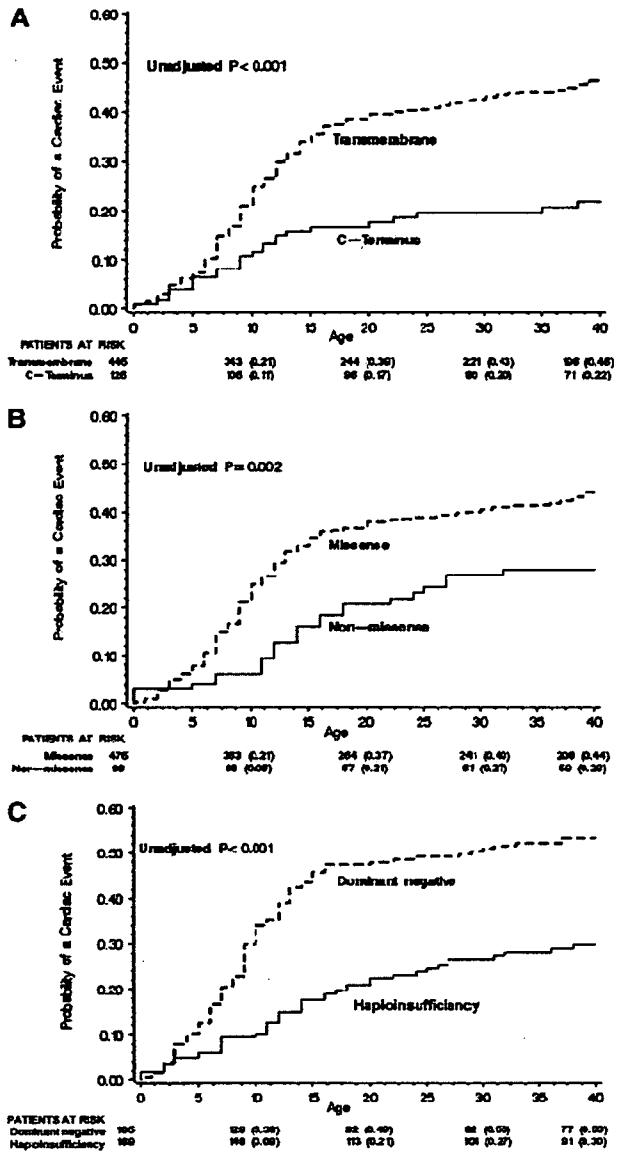

Figure 2. Kaplan-Meier estimate of the cumulative probability of a first cardiac event by location (A), type (B), and biophysical function of the mutation (C).

years, and longer QTc intervals. Mutations located in the transmembrane region of the channel made significant and independent contributions to the risk model, but missense mutations were not an independent risk factor. Three different intron mutations were present in 19 subjects from 4 families, and these intron mutations made a meaningful but nonsignificant contribution to the risk model. Prespecified interactions were investigated for their effect on cardiac events, and no significant interactions were found for transmembrane location by type of mutation, transmembrane location by QTc, or mutation type by QTc. Time-dependent β-blocker use was associated with a significant 74% reduction in the risk of first cardiac events ($P<0.001$).

TABLE 3. Cox Regression With Multiple Predictor Variables Including Location and Type of Mutations for First Cardiac Event

Variable	Hazard Ratio	95% CI	P
Netherlands:United States	1.15	0.74–1.78	0.55
Japan:United States	1.45	0.98–2.16	0.07
Male <13 y:female <13 y	1.72	1.25–2.38	<0.001
Female 13–40 y:male 13–40 y	2.27	1.30–3.96	<0.01
QTc 500–530 ms:QTc <500 ms	2.04	1.41–2.96	<0.001
QTc >530 ms:QTc <500 ms	3.25	2.25–4.69	<0.001
QTc missing*:QTC <500 ms	2.26	1.57–3.25	<0.001
Transmembrane:C-terminus	2.06	1.36–3.12	<0.001
Missense yes:no	1.33	0.86–2.05	0.20
Intron:C-terminus	2.45	0.98–6.11	0.06
Time-dependent β-blocker use	0.26	0.14–0.49	<0.001

The Cox analysis involved 592 subjects with 445 transmembrane, 126 C-terminus, 2 N-terminus, and 19 intron mutations; 8 subjects were not included in this Cox analysis because of missing data about the date of their first cardiac event.

*QTc missing category involves 47 subjects who died suddenly at a young age without a prior ECG.

Biophysical Function and Outcome

The clinical implications of disordered biophysical function of the mutant KCNQ1 channels were investigated in a subset of 356 subjects with known or suspected alteration in ion channel function (see Methods for functional categorization). The clinical characteristics of patients with dominant-negative and haploinsufficiency ion channel dysfunction are presented in Table 4. Patients with mutations having dominant-negative ion current effects had a longer QTc interval and a higher frequency of cardiac events than subjects with mutations resulting in haploinsufficiency. The cumulative probabilities of a first cardiac event by the biophysical function of the mutations are presented in Figure 2C. As shown in Table 5, patients with mutations having

TABLE 4. Phenotypic Characteristics by Biophysical Function of the KCNQ1 Mutations in 356 Subjects

Characteristics	Dominant-Negative Effect (n=187)	Haploinsufficiency (n=169)
Female, %	51	61
ECG at enrollment		
QTc,* ms	500±60	470±50
Therapy, %		
β-Blockers	47	37
Pacemaker	1.1	4.1
Sympathectomy	0.5	0
Defibrillator	4.8	7.7
First cardiac event*, %	53	27
Syncope	45	22
Aborted cardiac arrest	2.1	3.0
Death	5.3	2.4

Percentages >10 are rounded to a whole number. Two subjects had missing data about the date of their first cardiac event.

*$P<0.01$.

TABLE 5. Cox Regression With Multiple Predictor Variables Including Ion Channel Dysfunction for First Cardiac Events

Variable	Hazard Ratio	95% CI	P
Netherlands:United States	2.78	1.48-5.23	<0.01
Japan:United States	1.63	1.02-2.63	0.04
Male <13 y:female <13 y	1.94	1.29-2.91	<0.01
Female 13-40 y:male 13-40 y	1.95	0.99-3.87	0.06
QTc 500–530 ms:QTc <500 ms	1.88	1.18-2.99	<0.01
QTc >530 ms:QTc <500 ms	3.22	2.06-5.05	<0.001
QTc missing*:QTc <500 ms	2.07	1.29-3.33	<0.01
Dominant-negative:haploinsufficiency	2.26	1.56-3.25	<0.001
Time-dependent β-blocker use	0.21	0.09-0.48	<0.001

The analysis involved 354 subjects with known or suspected ion channel dysfunction; 2 subjects were not included because of missing data about the date of their first cardiac event.

*The QTc missing category involves 26 patients who died suddenly at a young age without a prior ECG.

dominant-negative functional effects experienced a significantly greater risk for cardiac events than those with haploinsufficiency (hazard ratio, 2.26; 95% CI, 1.56 to 3.25; $P<0.001$) after adjustment for relevant covariates including QTc and gender effects by age group. β-Blocker use was associated with a significant 79% reduction in first cardiac events in this subset of patients. Because substantial colinearity exists for transmembrane mutations, missense mutations, and mutations with dominant-negative biophysical function, the individual effects of these 3 mutation parameters could not be ascertained reliably in the same Cox model.

Discussion

The main results of the present study from 600 patients having a spectrum of KCNQ1 mutations derived from 3 LQTS registries are significantly higher cardiac event rates in patients with transmembrane mutations and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effect of these genetically determined factors is independent of traditional clinical risk factors and of β-blocker therapy.

Since 1995, when the first 2 genes responsible for LQTS were identified,[11,12] molecular genetic studies have revealed a total of 9 forms of congenital LQTS caused by mutations in genes involving potassium channel (LQT-1, -2, -5, -6, and -7), sodium channel (LQT-3, -9), and calcium channel proteins (LQT-8) as well as a membrane-adapter protein (LQT-4).[2,13] Genotype–phenotype studies have enabled us to stratify risk and to treat more specifically patients with LQT-1, LQT-2, and LQT-3 subtypes of this genetic disorder. LQT-1, the most common form of LQTS, accounts for ≈50% of genotyped patients[4,14] and has more variable expressivity and incomplete penetrance than the other forms.[15] Mutation location and knowledge of the functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for LQT-1, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

Mutations in KCNQ1 are responsible for defects in the slowly activating component of the delayed rectifier current I_{Ks}.[16] This current is the main repolarizing current at increased heart rate and is highly sensitive to catecholamines.[3] We speculate that I_{Ks} channels with transmembrane mutations might have reduced responsiveness to the regulatory β-adrenergic signaling of the ion-conduction pathway with more impairment of shortening of the QTc with exercise-related tachycardia than mutations in the C-terminus region.

Functional I_{Ks} channels result from the coassembly of 4 KCNQ1-encoded subunits. A mutated gene encodes a protein with aberrant function, and the presence of both normal and abnormal proteins in the ion channel contributes to a >50% reduction in ion channel function (dominant-negative effect). An alternative mechanism of reduced repolarizing KCNQ1 K^+ current is the inability of mutated subunits to coassemble with normal gene products, such as occurs with a trafficking defect, resulting in a ≤50% reduction in channel function (haploinsufficiency). With only 1 exception,[17] this is the case for all studied truncating mutations leading to incomplete proteins. Our assumption that truncated proteins (based on frameshift nonsense mutations) lead to haploinsufficiency seems justified. The biophysical effect of missense mutations is unpredictable, and both haploinsufficiency and dominant-negative effects have been described. In the absence of reported biophysical studies, missense mutations were classified as unknown.

Previous attempts to identify a genotype–phenotype relationship for KCNQ1 mutations failed to reach consensus on the clinical outcome of the type and site of mutations.[7,8] Relatively small numbers and different ethnic background of the previously reported patients with the LQT-1 genotype might be responsible for the discrepant results. The present larger study allows us to demonstrate for the first time that the biophysical effect clearly affects the clinical outcome (ie, dominant-negative mutations are associated with a more severe phenotype than are mutations conferring haploinsufficiency [Figure 2C], even after adjustment for relevant covariates [Table 5]). The risk observed in 19 subjects with 3 different intron mutations was not quite significant ($P=0.06$), possibly because of small numbers, but the magnitude of the risk effect was similar to the risk accompanying transmembrane mutations. Although these intron mutations produced splice-site alterations predicted to affect the transmembrane portion of the ion channel, we used a separate categorization of intron mutations in view of the limited understanding of the structural alterations and functional effects resulting from these exon-skipping intron mutations.

A few additional findings from this large genotype–phenotype study of type-1 LQTS patients emphasize high risk for first cardiac events during adolescence, a crossover in risk by sex at approximately age 13 years, and a lower rate of first cardiac events in the adult years than in the younger years. These findings are not especially new,[18,19] but the present study highlights their presence in type-1 LQTS.

Study Limitations

The present study used the biophysical function of mutations reported in the literature in only a portion of the mutations

that were included (see references associated with Table 1 in the online-only Data Supplement). The published studies were from many different laboratories with the use of different cellular heterologous expression systems involving *Xenopus* oocytes and other cells at both room and physiological temperatures. Although such nonuniform testing may have contributed to some inconsistency in the categorized biophysical function, the finding of a significantly higher event rate in mutations with dominant-negative than with haploinsufficient effects (hazard ratio, 2.26; $P<0.001$) is unlikely to have resulted from the nonuniform testing. Unfortunately, we did not have the resources to perform such uniform testing in all 77 mutations presented in the present study.

Once a mutation was identified in KCNQ1, thorough genetic sequencing was not performed routinely in all the ion channel genes to look for second mutations. Thus, some of the patients included in the analysis may have had a second mutation in addition to the identified KCNQ1 mutation. It is estimated that ≈10% of genotype LQTS patients may carry a second mutation, and those with >1 mutation could contribute to some of the findings in our study. In addition, it is possible that some of the reported mutations (Table 1) are simply uncommon sequence mutations, but this is relatively unlikely because all the subjects in the present study were derived from families in which the proband had QTc prolongation not due to a known cause.

The outcome analyses included subjects from families with a known KCNQ1 mutation who died suddenly and unexpectedly at a young age and were classified as LQTS-related death with the same mutation that was present in the family. It is possible that a few of these subjects could have died from a non-LQTS cause or had an LQTS mutation different from the family mutation, but we think the error rate is likely to be small. The number of deaths and aborted cardiac arrest events is small, and there is insufficient power to evaluate the risk association of the genotype characteristics with these endpoint events in a multivariate time-dependent model.

Conclusions

The present study confirms that in patients with type-1 LQTS, longer QTc intervals are associated with higher cardiac event rates and that male patients are generally younger than female patients at first cardiac events.[20,21] The new findings from the present study are that transmembrane mutations and mutations with dominant-negative functional effect adversely influence the outcome of this disorder independent of traditional clinical risk factors and β-blocker therapy. The present study was not designed to assess the effectiveness of different therapies in patients with KCNQ1 mutations. The findings presented do not provide justification for using specific genotype characteristics to identify patients for implanted defibrillator therapy.

Note Added in Proof

After this article was accepted for publication, we noted the recent article by Tsuji et al, in which the A344A/sp [1032G>A] mutation that we categorized as haploinsufficient (Table 1) was reported to have a weak dominant-negative effect.[22] We reran the KCNQ1 data recategorizing the 27 A344A/sp [1032 G>A] mutations as dominant-negative. Negligible changes occurred in the results as presented in Table 5 and Figure 2C; the hazard ratio for dominant-negative:haploinsufficiency (Table 5) was unchanged at 2.26 ($P<0.001$).

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Disclosures

Dr Ackerman is a consultant for Clinical Data (formerly Genaissance Pharmaceuticals) with respect to the FAMILION genetic test for cardiac ion channel mutations. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Type-1 long-QT syndrome is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. In the present study involving 600 patients having a spectrum of KCNQ1 mutations derived from 3 long-QT syndrome registries, we found that cardiac event rates are increased significantly in patients with mutations located in the transmembrane region of the potassium channel and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effects of these genetically determined factors are independent of traditional clinical risk factors and of β-blocker therapy. Mutation location and knowledge of functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for type-1 long-QT syndrome, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

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Impaired flow-mediated vasodilatation and insulin resistance in type 2 diabetic patients with albuminuria

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ABSTRACT

An elevated urinary albumin excretion is associated with an increased risk of cardiovascular disease due to atherosclerosis, but the pathophysiological mechanism underlying this association is poorly understood. We studied 217 diabetic patients, that is, 121 normoalbuminuric patients, 71 microalbuminuric patients, and 25 macroalbuminuric patients. We evaluated flow-mediated dilatation of brachial artery (%FMD, one endothelial function marker associated with endogenous NO production), von Willebrand factor (vWF, endothelial activation marker), high-sensitive CRP (hsCRP, a low-grade inflammation marker), asymmetric dimethyl arginine (ADMA, an endogenous inhibitor of NO synthesis), and insulin sensitivity by steady-state plasma glucose method. %FMD was apparently decreased in microalbuminuric and macroalbuminuric patients compared with normoalbuminuric patients ($p < 0.001$). Moreover, %FMD was significantly correlated with the degree of albuminuria ($r = -0.38$, $p < 0.05$). On the other hand, vWF and hsCRP did not show significant difference between normoalbuminuric patients and microalbuminuric patients. In diabetic patients with macroalbuminuria, ADMA was significantly elevated compared to those with normoalbuminuria. Insulin sensitivity was significantly associated with urinary albumin excretion rate. These results suggested that endothelial dysfunction which may be due to impaired NO production and insulin resistance underlie the association between diabetic nephropathy and atherosclerosis in diabetic patients.

1. Introduction

Elevated urinary albumin excretion rate (UAER) is strongly associated with an increased risk of cardiovascular diseases, which is independent of conventional risk factors including hypertension, hyperlipidemia, and smoking, among individuals with and without type 2 diabetes [1,2]. This suggests that elevated UAER may be associated with atherosclerosis by the unidentified mechanism.

The endothelium plays a crucial role in the maintenance of vascular tone and structure, and endothelial dysfunction is a key feature of atherosclerosis. Nitric oxide (NO) is one of the important endothelium-derived vasoactive mediators. NO is involved in a wide variety of regulatory mechanisms of cardiovascular system, including vascular tone and vascular structure [3].

Flow-mediated endothelium-dependent vasodilatation (FMD) method is based on the endothelial stimulus of increased shear stress (the tangential force on the vessel wall exerted by flowing blood). Increased shear stress is caused by post-ischemic hyperemia and elicits a slow Ca^+-independent two to threefold increase in NO production [4,5]. Indeed,

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Celemajer et al. reported that flow mediate vasodilatation was mainly blocked by N-monomethyl-L-arginine (an inhibitor of endothelial NO synthetase) [6].

To clarify the contribution of impaired NO production in vascular endothelium to the association between atherosclerotic disease and diabetic nephropathy, we examined FMD by ultrasonography. In addition, we measured asymmetric dimethyl arginine (ADMA), an endogenous NO synthesis inhibitor [3]. Since low-grade inflammation is another key feature of the pathophysiology of atherosclerosis [7], we further examined high-sensitive CRP, which is an inflammation marker, to investigate whether this feature is involved in the association between atherosclerotic disease and diabetic nephropathy.

It has recently been indicated that microalbuminuria and atherosclerosis are closely associated with insulin resistance [8–10], implying that insulin resistance may underlie these pathophysiological conditions although the causative relationship remains unknown. In the present study, we further examined insulin sensitivity in the type 2 diabetic patients with different stage of albuminuria and analyzed the correlation between insulin sensitivity and FMD, to investigate whether elevated UAER and endothelial dysfunction may be associated with insulin resistance.

2. Methods

2.1. Study subjects

We studied 217 patients with type 2 diabetes who were <75 years of age. Patients with a current acute illness (including clinically significant infectious disease) were excluded from this study. Twenty-four-hour urine collections were performed for two consecutive days to determine the stage of diabetic nephropathy. Creatinine clearance (Ccr) was calculated from the 24-h urine sample and serum creatinine levels. The patients were divided into three groups according to the UAER, as follows: normoalbuminuria (UAER <30 mg/day), microalbuminuria (30 ≤ UAER < 100 mg/day) and macroalbuminuria (UAER ≥ 300 mg/day). To exclude diabetic patients with nondiabetic kidney disease, we excluded patients with hematuria or abnormal urinary sediments. This study was conducted with the approval of National Cardiovascular Center Trust Ethics Committee, and patients gave written informed consent before participation.

2.2. Brachial artery flow-mediated dilatation

Using ultrasonography, arterial endothelium and smooth muscle function were measured by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli. Ultrasoundsonographic measurements were carried out according to the method described by Celemajer et al. [6]. Brachial artery diameter was measured from B-mode ultrasound images using 10-MHz liner array transducer (ProSound SSD-5500; Aloka, Japan) while an ECG trace was simultaneously recorded. The right brachial artery was scanned in longitudinal sections 1–10 cm above elbow, after at least 15 min of rest in the supine position, the skin surface was marked and the arm was kept in the same position during the study.

Baseline measurements of the diameter were carried out. Endothelium-dependent vasodilatation (flow-mediated dilatation) was determined by scans during reactive hyperemia. A pneumatic cuff placed around the forearm was inflated to 220 mmHg and was deflated after 4.5 min. The diameter of the brachial artery was scanned and recorded after dilation. After 10 min rest, the second control scan of the diameter was recorded. Then, sublingual glyceryl trinitrate spray (300 μg) was administered and 3.5 min later a final scan of the diameter was recorded.

Measurements of the vessel diameter were taken from the anterior to posterior "m" line (interface between the media and adventitia) at end-diastole, coincident with the R wave on a continuously recorded ECG. The diameters at four cardiac cycles were measured for each scan, and these results were averaged. Determinations of the FMD were carried out 45–60 s after the cuff release to measure a maximal diameter. Vasodilatation by reactive hyperemia or glyceryl trinitrate (NTG) was expressed as the percent change in diameter compared with the baseline values.

2.3. Insulin sensitivity test

Glucose utilization in response to insulin was evaluated with a newly modified steady-state plasma glucose (SSPG) method with octreotide acetate (Sandostatin; Novartis) after an overnight fasting period of 12 h [11]. Sandostatin (9.8-pmol bolus followed by a constant infusion of 73.5 pmol/h) and Humulin R insulin (45 pmol/kg bolus followed by a constant infusion at a rate of 4.62 pmol/(kg min); Eli Lilly) were infused intravenously for 120 min. Glucose in a final 12% solution containing KCl (0.5 μmol/(kg min)) was infused at a rate of 0.033 mmol/(kg min) (6 mg/(kg min)) through an antecubital vein via a constant infusion pump. Blood samples were drawn routinely at 0 and 120 min (9:00 and 11:00 a.m.) for the determination of glucose, insulin, and lipids. The value of glucose at 120 min (SSPG) was used as a marker of insulin sensitivity to glucose utilization. High SSPG levels showed peripheral insulin resistance.

Another marker of insulin resistance (IR) was estimated by calculating homeostasis model assessment (HOMA-IR) index ((fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l))/22.5) [12].

2.4. Measurement of vWF, hsCRP, and ADMA

vWF was determined in citrated plasma using a homemade enzyme-linked immunosorbent assay. Data are given as the percentage of pooled human plasma (set at 100%). Serum hsCRP concentration was determined by latex nephelometry method (SRL, Tokyo, Japan). Serum ADMA concentration was determined by high-performance liquid chromatography method (SRL, Tokyo, Japan).

2.5. Statistical analysis

Values are expressed as means ± S.D. Statistical analysis was performed by use of ANOVA followed by Scheffes' test. The

Table 1 – Characteristics of diabetic patients with normoalbuminuria, microalbuminuria, and overt nephropathy

Parameter	Stage of nephropathy		
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
n	121	71	25
Age (years)	62 ± 9	65 ± 8	66 ± 7
Men/women	76/45	34/37	12/13
Duration of diabetes (years)	12 ± 8	14 ± 8	18 ± 8*
BMI (kg/m^2)	25.0 ± 3.7	25.1 ± 3.7	25.1 ± 3.9
SBP (mmHg)	128 ± 13	133 ± 15	141 ± 19*
DBP (mmHg)	74 ± 10	73 ± 9	76 ± 10
FBS (mmol/l)	7.4 ± 1.4	7.5 ± 1.5	7.5 ± 1.9
HbA1c (%)	8.3 ± 1.5	8.9 ± 1.7*	8.8 ± 1.4
HOMA-IR	1.62 ± 0.98	1.71 ± 2.06	2.29 ± 1.47
Total cholesteroyl (mmol/l)	4.86 ± 0.90	4.86 ± 0.90	4.73 ± 0.75
Serum creatinine (μmol/l)	70 ± 20	60 ± 20	110 ± 40
Urinary albumin (mg/day)	10 ± 7	85 ± 79**	583 ± 576**
Creatinine clearance (ml/s)	1.43 ± 0.52	1.50 ± 0.63	0.73 ± 0.43**
ACEI or ARB (yes/no)	36/85	24/47	11/14*
Statin (yes/no)	45/76	25/46	10/15
Current smoker (yes/no)	11/110	7/64	6/19

*$p < 0.05$, **$p < 0.01$ vs. normoalbuminuria, mean ± S.D.

strength of correlation between variables was tested by linear correlation and multiple regression analysis. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Patients characteristics

Table 1 shows the clinical characteristics of three groups. There was no significant difference in age, gender, BMI, FBS and total cholesterol among the three groups. HbA1c of diabetic patients with microalbuminuric patients was significantly higher than normoalbuminuric patients. Systolic blood pressure of macroalbuminuric patients was significantly higher than normo- and micro-albuminuric patients. Creatinine clearance was significantly decreased in macroalbuminuric patients compared with normo- and micro-albuminuric patients. There is no significant difference in rate of patients taking ACE/ARB between normo- and micro-albuminuric patients whereas the rate of patients taking ACE/ARB of macroalbuminuric patients were significantly large compared with other two groups. On the other hand, there is no significant difference in rate of patients taking statin among three groups.

3.2. %FMD of diabetic patients

We studied the endothelial function by FMD using brachial artery echography. %FMD (Δhyperemia) of diabetic patients with microalbuminuria (4.5 ± 3.7%) and macroalbuminuria (4.2 ± 2.4%) was apparently decreased compared with those of diabetic patients with normoalbuminuria (6.6 ± 3.7%) (Fig. 1A). Moreover, %FMD was significantly correlated with UAER in normo- and micro-albuminuric patients independent of age, HbA1c, and systolic blood pressure by multiple regression analysis ($r = -0.38$, $p < 0.05$) (Fig. 2). Dilatation of brachial artery by NTG (ΔNTG) showed no difference among three groups (Fig. 1B).

3.3. vWF, hsCRP, and ADMA of diabetic patients

We studied other atherosclerotic markers, that is, vWF, hsCRP, and ADMA. There was no significant difference of the levels of vWF and hsCRP between normoalbuminuric and microalbu-

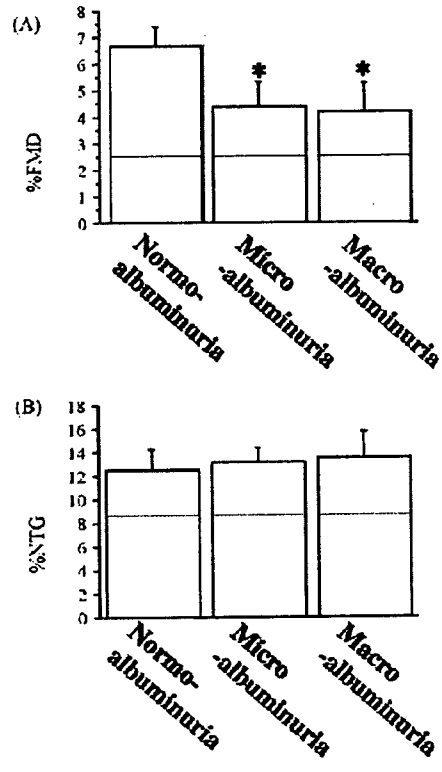

Fig. 1 – %FMD (A) and %NTG (B) in diabetic patients with normoalbuminuria, microalbuminuria and macroalbuminuria. Each value means (means ± S.D.), *$p < 0.001$.

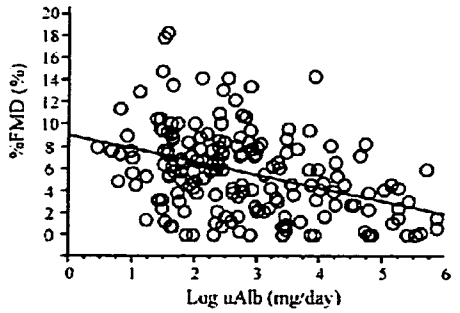

Fig. 2 – Correlation between degree of UAE and %FMD in normo- and micro-albuminuric diabetic patients. There was a significant correlation between both variables ($r = -0.38$, $p < 0.05$, $n = 192$).

minuric patients (Table 2). Although the levels of ADMA in microalbuminuric patients did not show significant difference compared with normoalbuminuric patients (Table 2), the levels of ADMA in macroalbuminuric patients were significantly elevated compared with normoalbuminuric patients (Table 2).

3.4. *Insulin sensitivity of diabetic patients*

We studied the insulin sensitivity by SSPG method. The levels of SSPG had weak but significant correlation with both %FMD ($r = -0.175$, $p < 0.05$) and UAER ($r = 0.181$, $p < 0.05$) independent of age, HbA1c, and systolic blood pressure (Fig. 3A, B).

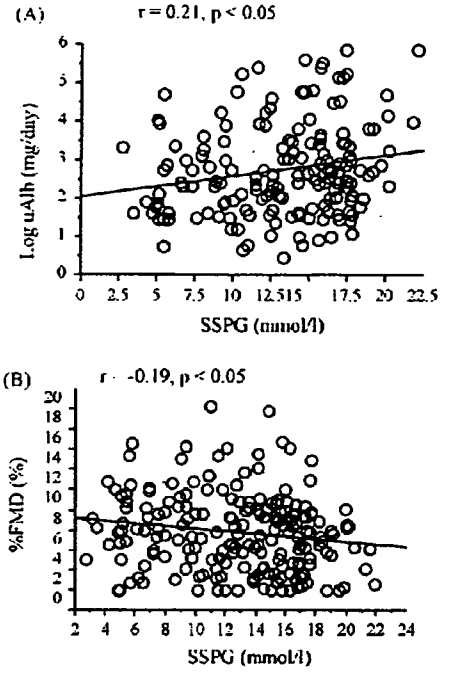

Fig. 3 – Correlation between SSPG and UAE (A), and correlation between SSPG and %FMD (B) in normo- and micro-albuminuric patients.

4. Discussions

There were two main findings from this investigation in type 2 diabetic patients. First, diabetic micro- and macro-albuminuric patients showed significant reduction of %FMD compared with normoalbuminuric patients. This finding suggests that the endothelial dysfunction may account for the association between atherosclerosis and albuminuria in diabetic patients. Second, the level of SSPG was significantly associated with both UAER and %FMD. This finding suggests that insulin resistance may play a role in both atherosclerosis and nephropathy in type 2 diabetic patients.

In diabetic patients, %FMD is decreased compared with healthy control [13,14]. These reports indicated that diabetes mellitus is associated with endothelial dysfunction due to impaired NO production. However the involvement of endothelial dysfunction in diabetic nephropathy has been unclarified. We demonstrated that microalbuminuric and macroalbuminuric patients showed significant decreased %FMD compared with normoalbuminuric patients. In contrast, there was no significant difference of vWF between normoalbuminuric patients and microalbuminuric patients. vWF is a product of vascular endothelial cell, and induces coagulation and platelet aggregation [15]. These findings suggest that endothelial dysfunction due to impaired NO production is specifically induced in micro- and macroalbuminuric patients. One recent report showed that coronary endothelium-dependent dilatation was impaired in a rat model of spontaneous albuminuria [16] supporting this hypothesis. It has been reported that renal NO production was decreased in rodent diabetic model [17]. This report suggests that decrease of NO production may play a role in the

Table 2 – Parameters of atherosclerosis in diabetic patients with normoalbuminuria, microalbuminuria, and overt nephropathy

Parameter	Stage of nephropathy		
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
von Willebrand factor (%)	147 ± 44	146 ± 44	143 ± 41
High-sensitive CRP (ng/ml)	976 ± 1401	951 ± 1110	1113 ± 1187
ADMA (nmol/ml)	0.45 ± 0.06	0.47 ± 0.07	0.55 ± 0.11*

*$p < 0.001$ vs. normoalbuminuria, mean ± S.D.

progression of diabetic nephropathy as well as atherosclerosis. We investigated serum ADMA levels in diabetic patients. There was no significant difference of ADMA levels between normo- and micro-albuminuric patients, suggesting that the reduction of %FMD in microalbuminuric patients might not be resulted from the elevation of ADMA. However, in macroalbuminuric patients, ADMA level was significantly higher than normoalbuminuric patients. Vallance et al. reported that the level of ADMA was elevated in patients with chronic renal failure and suggested the involvement of this in coronary artery disease [18]. They indicate that the elevation of ADMA might be associated with atherosclerosis in patients with chronic renal disease [18]. Thus, this finding suggests that the elevation of ADMA might be associated with atherosclerotic change in diabetic patients with macroalbuminuria.

An association between chronic low-grade inflammation and development of atherosclerotic disease has been observed in basic and clinical studies [7,19–21]. Furthermore, diabetic patients have higher CRP levels than normal subjects, suggesting that chronic inflammation may contribute diabetic atherosclerotic complication [22]. An association between micro- and macro-albuminuria and inflammation has also been reported [23,24]. However, several other studies showed that inflammatory molecules were not associated with micro- and macro-albuminuria [25–27]. Thus the knowledge of this association is still controversial. Also we could not demonstrate the association between CRP and development of microalbuminuria in this study. Our data suggested that chronic low-grade inflammation might not be involved in the association between atherosclerosis and microalbuminuria. However, since this study was performed by cross-sectional analysis and other inflammatory marker was not measured, further study is necessary for demonstrating this hypothesis.

Insulin resistance has been reported to play an important role in the development and progression of atherosclerotic coronary disease [8,9]. Recently the association between insulin resistance and microalbuminuria was also reported [10]. Nakamura et al. demonstrated that administration of pioglitazone to diabetic patients attenuated UAER [28]. In this study, we showed that both the UAER and %FMD were significantly correlated to the level of SSPG. These findings suggest that insulin resistance may be involved in both the elevated urinary albumin excretion and endothelial dysfunction due to impaired NO production. However, HOMA-IR, another insulin sensitivity marker which reflects insulin sensitivity in both the liver and the periphery, did not show significant difference among three groups, suggesting that particularly peripheral insulin resistance may be important for the pathogenesis of atherosclerosis and diabetic nephropathy.

In summary, we showed that %FMD of micro- and macro-albuminuric patients was decreased compared with those of normoalbuminuric patients, without showing significant difference in other various atherosclerotic markers. Furthermore, the level of SSPG was significantly correlated to UAER and %FMD. These findings suggest that endothelial dysfunction which may be due to impaired NO production underlies the mechanism of association between elevated urinary albumin excretion and atherosclerosis in diabetic patients, and that peripheral insulin resistance might be possibly involved in both diabetic nephropathy and atherosclerosis.

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