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Phenotypic Overlap of Lethal Arrhythmias Associated with Cardiac Sodium Mutations. Individual-specific or mutation-specific?

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ABSTRACT

Mutations in cardiac sodium channel gene *SCN5A* are responsible for a spectrum of hereditary arrhythmias including type-3 long QT syndrome (LQT3), Brugada syndrome (BrS), conduction disturbance and sinus node dysfunction. These syndromes were originally regarded as independent entities with distinct clinical manifestations and biophysical properties. However, recent evidence shows considerable clinical overlap among these disorders, implicating a new disease entity referred to as an overlap syndrome of cardiac sodium channelopathy. Class IC sodium channel blockers often induced BrS phenotype in some patients with LQT3. Furthermore, recent genetic studies have revealed that E1784K is the most prevalent *SCN5A* mutation responsible not only for LQT3 but BrS, confirming the clinical and genetic overlap of LQT3 and BrS. Here, we show evidence that the clinical manifestations of *SCN5A* mutations are most likely determined by the biophysical and pharmacological properties of the mutations. We also provide an overview of current knowledge on the clinical features, prevalence, and molecular and biophysical mechanisms underlying overlap syndrome to gain more insight into this complex issue and generate better therapeutic strategies for patient management.

1. Biochemistry and biophysics of cardiac sodium channels

Voltage-gated sodium channel is responsible for the rapid upstroke of the action potential in most excitable tissues, and plays a pivotal role in the initiation, propagation, and maintenance of normal cardiac rhythm in the heart. Cardiac sodium channel consists of the most prevalent pore-forming α subunit (Nav1.5) encoded by the gene *SCN5A* located on chromosome 3p21 and auxiliary β subunits (Navβ1-Navβ4) encoded by the genes *SCN1B-SCN4B*, respectively. The α subunits comprise a 4-fold symmetry macromolecule consisting of structurally homologous domains (D1–D4) each containing 6 membrane-spanning segments (S1–S6) and a region (S5-S6 loop) controlling ion selectivity and permeation. The positively charged S4 segment of each domain functions as a voltage sensor (1,2).

Na channels switch between 3 functional states (closed, open, inactivated) depending on the membrane potential. A membrane depolarization causes a rapid rise in local Na permeability due to the opening (activation) of Na channels from their resting closed state. Normally, activation of Na channels is transient owing to inactivation, another gating process mediated by structures located on the cytoplasmic face of the channel protein (mainly the D3–D4 linker). Na channels cannot reopen until the membrane is repolarized and they undergo recovery from inactivation. Membrane repolarization is achieved by fast inactivation of Na channels and is augmented by activation of voltage-gated K channels. Activation, inactivation, and recovery from inactivation occur within a few milliseconds. In addition to these rapid gating transitions, Na channels are also susceptible to slower inactivating processes (slow inactivation) if the membrane remains depolarized for a longer time. These slower events may contribute to determining the availability of active channels under various physiological conditions.

2. Genetics of cardiac sodium channelopathies (Table 1)

Mutations of *SCN5A* are responsible for a spectrum of hereditary arrhythmias including type-3 congenial long QT syndrome (LQTS; LQT3) (3), acquired LQTS (4), Brugada syndrome (BrS; BrS1)(5), cardiac conduction disturbance (CCD) (6), congenital sick sinus syndrome (SSS) (7), atrial standstill(8-10), AV block (11), sudden infant death syndrome (SIDS)(12-14), and familial atrial fibrillation (FAF)(15-17) (table 1). In addition to these primary electrical diseases which usually lack structural abnormalities, *SCN5A* mutations have also been reported in patients with dilated cardiomyopathy(15,18). Moreover, recent genetic studies have indicated that mutations in Na channel beta subunit genes, *SCN1B* and *SCN4B*, were associated with type-5 BrS complicated with CCD (BrS5)(19) and type-10 LQTS (LQT10), respectively.

3. Biophysical properties of cardiac sodium channel channelopathies

Congenital LQTS is characterized by the prolongation of the QT interval on surface ECGs and an increased risk of potentially fatal ventricular arrhythmias, especially torsade de pointes (20). QT interval is determined by the cardiac action potential which is orchestrated by a fine balance between inward and outward currents expressed in myocardial cells. After the first identification of the *SCN5A* mutation ΔKPQ, comprising a deletion of three conserved amino acids 1505-1507 in the cytoplasmic D3-D4 linker in 1995 (21), more than 100 distinct *SCN5A* mutations responsible for

LQT3 have been reported. The common *in vitro* consequence of most of these mutations is a persistent Na current during the action potential plateau due to destabilized fast Na channel inactivation (3). This failure of fast inactivation shifts the ionic balance during the plateau phase toward inward current (gain-of-function) and delays repolarization, thus increasing action potential duration and the corresponding QT interval. Na channel blockers such as mexiletine (class IB) or flecainide (class IC) shorten QT in patients with LQT3 due to block of this persistent current (22-24), therefore are theoretically useful in the management of affected patients.

BrS is another primary electrical disorder without underlying structural heart diseases characterized by the coved-type ST elevation in the right precordial leads (25,26). It predisposes affected individuals to ventricular fibrillation (VF). Mutations in *SCN5A* are identified in 20-30% patients with BrS, and most of the heterologously expressed mutant Na channels exhibit biophysical abnormalities resulting in reduced cardiac Na current (loss-of-function) (27). Reduced Na current is thought to exaggerate differences in action potential duration between the inner (endocardium) and outer (epicardium) layers of ventricular muscle, thereby favoring a substrate promoting reentrant arrhythmias. Loss-of-function of cardiac Na channels is either owing to 1) haploinsufficiency due to non-functional mutations, 2) impaired altered channel gating properties including enhanced inactivation, disruption of activation and impaired recovery from inactivation, or 3) impaired intracellular trafficking and decreased membrane surface expression of the channel molecules.

4. Clinical overlap of cardiac sodium channel channelopathies

SCN5A mutations with loss-of-function properties have also been identified in patients with CCD (6,28), SSS(7), and atrial standstill(8), and numbers of reports have shown that the mutation carriers tend to exhibit overlapping clinical properties of these syndromes (29,30) (table 2). Importantly, such loss-of-function properties are apparently opposite to those described in LQT3 (gain-of-function), and different *SCN5A* mutations were initially linked to separate arrhythmias syndromes. Surprisingly, some LQT3 patients display ECG findings characteristic of BrS, suggesting that a single mutation can be associated with a wide spectrum of disease phenotypes. Such phenotypic overlap between LQT3 and BrS was first reported in a large multigenerational Dutch family with an insertion mutation 1795insD, in which the mutation carriers showed ECG features of both LQT3 and BrS, and sinus node dysfunction (SND) (31,32). Importantly, sodium channel block in the overlap phenotype shortens QT but exacerbated the ST segment elevation BrS phenotype, and thus enhances arrhythmia risk (32). Biophysical studies demonstrated that the mutant channels displayed enhancement of both closed-state inactivation and slow inactivation which was thought to sensitize carriers to the BrS phenotype during flecainide therapy (33), in addition to the persistent Na current, a hallmark Na channel property of LQT3.

The overlap between the LQT3 and BrS phenotypes was also reported in other *SCN5A* mutations such as ΔKPQ (34,35), E1784K (34) and ΔK1500 (36). Priori *et al.* showed the additional evidence for the elusive link between these two clinical syndromes by the fact that the class IC sodium channel blocker flecainide induced ST-segment elevation in the right precordial leads not only in patients with BrS but also with LQT3(34). Out of 13 patients with 7 LQT3 families (*SCN5A* mutations of V411M, T1304M, ΔK1500, ΔKPQ, R1626P, E1784K, P2006A), 6 patients showed flecainide-induced ST elevation. However, they failed to identify the determinants of flecainide-induced ST elevation in patients with LQT3. In fact, they assumed that the clinical overlap appeared to be individual-specific, rather than gene-specific or mutation-specific, most likely because the size of their patients was rather small. Nonetheless, these observations raise a concern about the safety of class IC drug therapy in LQT3 patients and questions about the underlying mechanisms.

Phenotypic variability in LQT3 has thus far been reported sporadically or only within a single kindred(31,32). Therefore, it is not clear whether development of the BrS phenotype in a patient with LQT3 is solely determined by the biophysical properties of the mutant channel, or by co-inherited genetic variations, gender, ethnicity, or other environmental factors. One approach to dissect such phenotypic variability is to perform a clinical assessment of individuals with multiple pedigrees from genetically heterogeneous populations with the same mutation. Recent multicenter large-scale genetic screening of *SCN5A* mutations revealed that the E1784K is the most prevalent in both LQT3 (12%, 3/25)(37) and BrS (4.8%, 14/293)(38)

5. Clinical phenotypes in 15 LQT3 families with *SCN5A*-E1784K

We enrolled 44 genotyped LQT3 families with different ethnic backgrounds (Asian 20, Caucasian 24) ascertained in 7 institutions of Japan, Italy, Germany, UK and the US. In 44 LQT3 families, E1784K was the most prevalent *SCN5A* mutation, identified in 15 families (34%). Two probands died suddenly, and 66 out of 93 surviving members underwent genetic testing. There were 41 mutation carriers and 25 non carriers, and QTc was significantly prolonged in carriers.

Spontaneous ST elevation in the right precordial leads was observed in 5/41 mutation carriers (shown with * in Fig 1; coved-type: n=1, saddle-back type: n=4, Fig 2A). Nine mutation carriers without diagnostic ST elevation at baseline underwent provocation with flecainide, ajmaline, or pilsicainide, and the test was positive (coved-type ST elevation, Fig 2B) in 5 (shown with + in Fig 1). Thus, the diagnosis of BrS was established in 9/41 mutation carriers (one individual, A;II:1, showed spontaneous saddle-back ST elevation which was converted to coved-type by ajmaline).

SND was common in the cohort, presenting in 16/41 mutation carriers (Fig 2C), and 4 of these 16 carriers with SND also exhibited the BrS phenotype (Fig 2B, 2D). Moreover, one carrier (A;III:5) showed SND without manifesting QT prolongation or ST elevation. Four patients received a permanent pacemaker and three received an implantable cardioverter defibrillator.

6. Biophysical properties and membrane trafficking of E1784K

Whole-cell patch clamp recording showed that E1784K has the following biophysical abnormalities: 1) significantly smaller peak current density, 2) persistent Na current, 3) significantly faster macroscopic current decay, 4) hyperpolarizing shift of the steady-state inactivation, 5) significant depolarizing shift in the voltage-dependence of activation, and 6) normal recovery from inactivation. Furthermore, using Na channel plasmid construct with an extracellular FLAG epitope, membrane trafficking of E1784K determined by a confocal laser scanning microscopy was comparable to wild type. These observations provide strong evidence that the loss-of-function properties displayed by

E1784K are most likely attributable to the aforementioned changes in gating properties rather than a change in channel density.

7. Molecular mechanisms of enhanced flecainide sensitivity

Class IC drug challenge test was positive in 56% patients with E1784K. We therefore investigated tonic block and use-dependent block by flecainide in WT and E1784K channels, and compared with those of T1304M, a mutation that did not show ST elevation during the flecainide challenge test (34). Cells transfected with WT, E1784K, or T1304M were depolarized by 2 Hz pulse trains in the absence or presence of 10 μM flecainide. During exposure to flecainide, peak currents normalized to predrug baseline were progressively reduced by the repetitive pulses (Fig 4A, B). There was a remarkable difference in the extent of first pulse (tonic) block that was only 4.5 ± 4.0% for WT, and 7.1 ± 2.7% for T1304M, compared to substantial tonic block in E1784K (43.7 ± 8.0%, $p<0.001$). Conversely, use-dependent block, determined by the difference in peak current values between the 1st and 100th test pulses relative to the 1st pulse, was slightly attenuated in E1784K. The net effect of flecainide after a train of 100 pulses was significantly greater in E1784K than WT but not in T1304M. Moreover, dose-response curves for flecainide block measured at a holding potential of -150 mV (thus representing drug affinity for the resting state) shows that the E1784K channels were 7.5-fold more sensitive to resting-state block by flecainide than were the WT channels (IC_{50}: WT= 150.3 μM, E1784K= 20.4 μM) (Fig 4C,D). These results indicate that the E1784K channels are much more sensitive to block by flecainide than are the WT and T1304M channels, and that this augmented sensitivity is attributable to enhanced tonic block rather than a change in use-dependent block.

8. Functional determinants of LQT3 associated with BrS and SND

To explore the functional determinants for the phenotypic overlap of BrS in LQT3 patients, we compared the biophysical and pharmacological properties of reported LQT3 mutations, and sought features commonly and specifically observed in those manifesting a BrS phenotype (Table 3). The overlapping phenotype (LQT3 and BrS) has been previously reported for 1795insD (31,32), ΔKPQ (34,35), ΔK1500 (36), and E1784K (34). In contrast, a carrier of T1304M did not show ST elevation during a flecainide test (34). Similarly, SND has been reported in carriers of the same *SCN5A* mutations, 1795insD (39), ΔKPQ (40), ΔK1500 (36), E1784K (41), and D1790G (23), but not in other *SCN5A* mutations, including T1304M. Thus, it is plausible to speculate that the biophysical characteristics common to these mutations but not found in T1304M are channel properties responsible for evoking mixed phenotypes of BrS and SND in patients with LQT3. To this end, Table 3 compares the functional properties of E1784K, and those reported for 1795insD, ΔKPQ, ΔK1500, E1784K, and T1304M (12). Among the biophysical properties listed in Table 3, we found that both the negative-shift in steady-state inactivation, and the enhanced tonic block by flecainide are common to 1795insD, ΔKPQ, ΔK1500, and E1784K, but not T1304M. This negative shift of inactivation will reduce the availability of the channels at the resting membrane potential, and increase the proportion of inactivated channels in both the open and closed state, reducing Na current and increasing the sensitivity to Na-channel blockers. A positive shift in activation is another "loss-of-function" property evident in all the mutants including T1304M, making it less likely that this specific channel property underlies mixed clinical phenotypes in LQT3. Other channel properties such as current decay, recovery from inactivation, slow inactivation, or use-dependent block were not common among 1795insD, ΔKPQ, ΔK1500, and E1784K.

A negative shift in inactivation is observed in E1784K, 1795insD, ΔKPQ, and ΔK1500, and may play a role in the overlap of the LQT3 clinical phenotype with BrS and SND in the mutation carriers, although the number of LQT3 mutations that have been evaluated in this detail is still small, biophysical and pharmacological properties presented in a cultured cell line may not necessarily reflect the situation *in vivo*, and the effects of the mutation may be different in ventricular myocytes vs sinus node cells. Further studies that combine clinical and *in vitro* phenotyping in LQT3 mutations with and without overlapping clinical phenotypes will be required to confirm the findings of the present study. Nevertheless, a negative shift in inactivation and enhanced tonic block are common biophysical properties observed among *SCN5A* mutations with the LQT3/BrS overlapping phenotype. These findings suggest that prophylactic class IC drugs should be avoided in LQT3 mutations displaying these biophysical properties *in vitro*.

CONCLUSIONS

E1784K is the most common LQT3 mutation. In patients with this and other LQT3 mutations, overlap with BrS and SND is relatively common. *In vitro* studies with E1784K and previous reports in LQT3 mutations with and without this clinical overlap syndrome implicate a negative shift in inactivation and enhanced tonic block by drugs as underlying mechanisms. These data suggest that patients with LQT3 mutations displaying these characteristics *in vitro* should not receive class IC drugs. Furthermore, the present findings reinforce the general concept that *in vitro* characterization of the function of ion channel variants is a key component in generating specific therapeutic strategies for patient management.

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FIGURE LEGENDS

Figure 1. Pedigrees of E1784K families

Pedigrees of 15 LQT3 families (A-O) carrying E1784K are shown. Probands are indicated by an arrow. Ten symptomatic mutation carriers, shown by the filled symbols, had episodes of syncope (n=9) and unexplained palpitations (n=1, B;II:2). Asymptomatic mutation carriers (n=31) are shown as symbols with a dot, and shaded symbols are the individuals with QT prolongation who declined genetic testing or sudden cardiac death victims (SCD; A;II:6 and C;III:1). Individuals exhibiting ST elevation in the right precordial leads are depicted with an asterisk. Values for QTc intervals are given beneath each symbol. The Na-channel provocation test was positive in individuals with + (A;II:1, A;III:8, A;III:9, E;II:3, K;II:1), and negative in the individuals with – (A;II:3, E;I:2, E;II:2, N;II:1). Individuals with a positive and negative Na-channel blocker provocation test are shown with + and -, respectively.

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Figure 2. ECG characteristics of E1784K mutation carriers

(A) QT prolongation (QTc=470 ms) and spontaneous saddle-back type ST elevation observed in the right precordial leads in a carrier, A;II:1.

(B) ECG recordings before and after the Na-channel blocker provocation test. Pilsicainide (left, patient K;II:1) induced coved-type ST elevation in V1 and the QTc was concomitantly shortened (QTc: control 495 ms, pilsicainide 459 ms). Ajmaline (right, patient A;III:9) also induced coved-type ST elevation in V1 and V2 and QTc shortening (control 501 ms, ajmaline 490 ms).

(C) SND demonstrated by a 3.9 sec sinus arrest in a carrier, A;I:1.

(D) A Venn diagram representing electrophysiological manifestation of 41 *SCN5A*-E1784K mutation carriers. Thirty eight carriers exhibited an abnormally long QTc; 3 individuals had a normal QTc, and one exhibited SND (SND) only. SND and BrS were observed in 15 and 9 individuals, respectively, with 4 displaying both phenotypes.

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Figure 3. Properties of E1784K whole-cell current

(A) Representative whole-cell current traces obtained from tsA-201 cells transfected with either WT or E1784K Na channels; all studies were conducted in cells co-transfected with human sodium channel β_1 subunits. Currents were recorded from a holding potential of -120 mV and stepped from -90 mV to +90 mV for 20 ms in 10 mV increments.

(B) Current-voltage relationship. Current was normalized to cell capacitance to give a measure of Na current density.

(C) Na currents were recorded with a test pulse potential of -20 mV from a holding potential of -120 mV showed prominent tetrodotoxin (TTX)-sensitive late Na current (shown with arrows) and the faster decay in E1784K.

(D) Steady-state availability for fast inactivation and the conductance-voltage relationship were measured with standard pulse protocols shown in the inset. Curves were fit with the Boltzmann equation. The voltage-dependence of steady-state fast inactivation and activation were significantly shifted in the hyperpolarizing (-15.0 mV) and depolarizing (+12.5 mV) directions, respectively.

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Figure 4. Tonic block, use-dependent block, and the dose-dependence of flecainide

(A) Representative current traces of WT, E1784K, and T1304M before and after 10 μM flecainide. A train of 100 pulses (to -20 mV for 20 ms) was applied at 2 Hz from a holding potential of -120 mV. Numbers indicate the 1st (1), 30th (30), and 100th (100) pulse of the train.

(B) Time course of the peak current levels after application of 10 μM flecainide. Peak current levels recorded with each pulse were normalized to the baseline prior to flecainide.

(C) Representative steady-state current traces of WT and E1784K before and after flecainide (10 and 100 μM). Cells were depolarized by -20 mV from a holding potential of -150 mV.

(D) Concentration-response curve for flecainide-induced tonic block in WT and E1784K. The normalized peak currents were fit to the Hill equation. The IC_{50} values, representing dissociation constants for resting state were: WT, 150.3 μM; E1784K, 20.4 μM. Thus, the mutant channel was far more sensitive to tonic block by flecainide.

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Figure 1

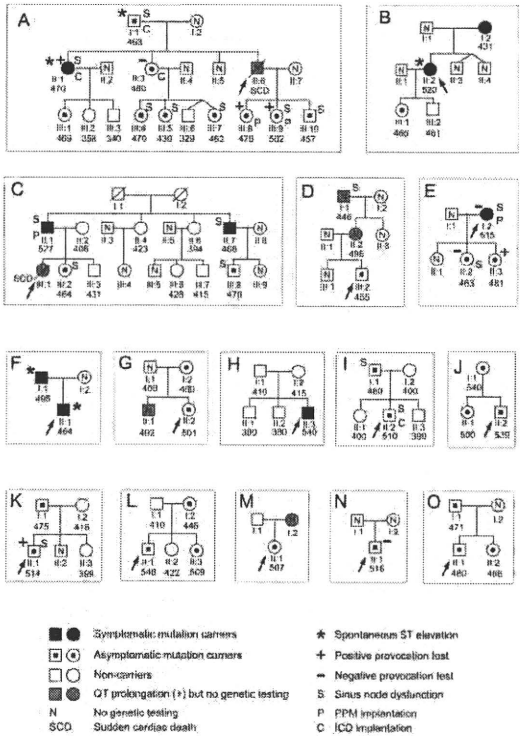

Figure 2

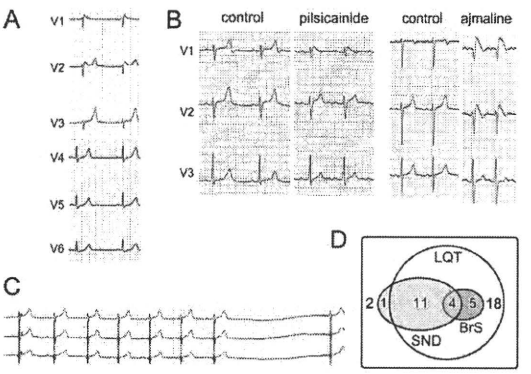

Figure 3

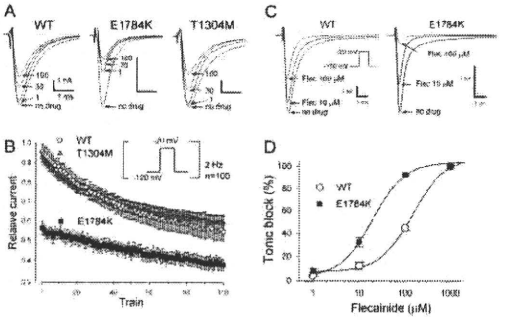

Figure 4

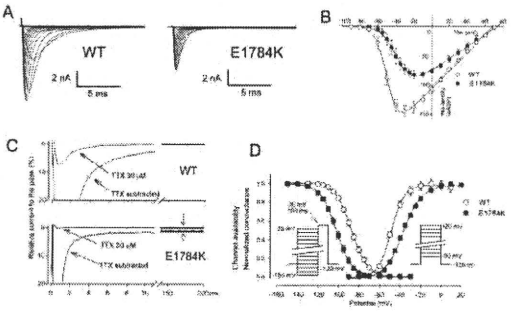

Table 1

Inherited cardiac sodium channelopathies

1. Cardiac Na channel α subunit (*SCN5A*)
   - Congenital long QT syndrome (LQT3)
   - Acquired long QT syndrome
   - Brugada syndrome (BrS1)
   - Cardiac conduction disturbance (CCD)
   - Congenital sick sinus syndrome (SSS1)
   - Atrial standstill
   - AV block
   - Sudden infant death syndrome (SIDS)
   - Familial atrial fibrillation (FAF)
   - Dilated cardiomyopathy (DCM)

2. Sodium channel β1 subunit (*SCN1B*)
   - Brugada syndrome with CCD (BrS5)

3. Sodium channel β4 subunit (*SCN4B*)
   - Congenital long QT syndrome (LQT10)

Table 2. Mutations in overlapped cardiac sodium channelopathies

Clinical manifestation	*SCN5A* and *SCN1B* (*) mutations	References
BrS+LQT3 (+/- CCD)	V411M, D1114N, W1191X, I1350T, ΔK1500, ΔKPQ, R1612P, P2006A	(21,34,36,43-47)
BrS+LQT3+SSS (+/- CCD)	G1262S, ΔF1617, E1784K, 1795insD	(7,31,32,34,41,42,48,49)
BrS+SSS	E161K, T187I, E1225K, ΔK1479, K1578fs. R1623X	(30,50,51)
BrS+AS	R367H	(10)
BrS+CCD	P336L, D356N, R376H, N406S, G752R, F861fs951X, E867X, G1319V	(51-55)
	G1406R, I1660V, S1710L, S1812X, E87Q*, Y179X*	(19,29,51,52,56-58)
DCM (+/- CCD+SSS+AF)	T220I, R814W, F851fs, D1275N, D1595H	(8,15,18)
CCD+SSS/AS	G1408R	(7)
AS+AF	L212P	(9)
LQT3+CCD	P1332L, V1763M, M1766L, V1777M, P2005A	(59-66)

BrS: Brugada syndrome

LQT3: type-3 long QT syndrome
CCD: cardiac conduction disturbance
AS: atrial standstill
AF: Atrial fibrillation
DCM: dilated cardiomyopathy

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ORIGINAL ARTICLE

Lifetime risk of stroke and impact of hypertension: estimates from the adult health study in Hiroshima and Nagasaki

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Very few reports have been published on lifetime risk (LTR) of stroke by blood pressure (BP) group. This study included participants in the Radiation Effects Research Foundation Adult Health Study who have been followed up by biennial health examinations since 1958. We calculated the LTR of stroke for various BP-based groups among 7847 subjects who had not been diagnosed with stroke before the index age of 55 years using cumulative incidence analysis adjusting for competing risks. By 2003, 868 subjects had suffered stroke (512 (58.9%) were women and 542 (62.4%) experienced ischemic stroke). BP was a significant factor in determining risk of stroke for men and women, with distributions of cumulative risk for stroke significantly different across BP groups. The LTR of all-stroke for normotension (systolic BP/diastolic BP < 120/80 mm Hg), prehypertension (120–139/80–89 mm Hg), stage1 hypertension (140–159/90–99 mm Hg) and stage 2 hypertension (> 160/100 mm Hg) were 13.8–16.9–25.8–25.8% in men and 16.0–19.9–24.0–30.5% in women, respectively ($P<0.001$ among BP groups in both sexes). The estimates did not differ significantly ($P=0.16$) between normotensive and prehypertensive subjects. One in five Japanese atomic bomb survivor subjects experienced stroke over their lifetime from the age of 55 years. Long-term stroke risks were elevated in those with hypertension (> 140/90 mm Hg) at any of the index ages of 45, 55, 65 and 75 years.
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Keywords: blood pressure; epidemiology; lifetime risk; stroke

INTRODUCTION

Stroke mortality is higher in Japan than in other developed countries,[1] and the incidence of stroke among Japanese subjects has remained high in recent years, especially in the elderly.[2] With aging of the population, stroke has become an ever more important health burden in Japan, and thus activities aiming at stroke prevention require urgent attention. Elevated blood pressure (BP) has emerged as one of the prominent risk factors for stroke.[3]

Lifetime risk (LTR) estimates, which represent risk of the disease of interest and adjust for competing risk of death from other causes, provide a simple conceptual basis for estimating absolute risk of developing a disease during the remainder of one's life.[4] Estimating the LTR in relation to known risk factors for stroke, such as hypertension, can help to highlight the magnitude and influence of these risk factors and the public health burden associated with them. Past estimates of the LTR of stroke in relation to known risk factors and the corresponding burden on the population, however, are limited.[4–6] The objectives of this study were to determine the LTR of stroke and to clarify long-term effects of BP levels at midlife in a Japanese population from the Adult Health Study (AHS) of atomic bomb survivors.

METHODS

Study population

The Radiation Effects Research Foundation (RERF; formerly the Atomic Bomb Casualty Commission or ABCC) established a longitudinal Life Span Study cohort consisting of 120 321 Japanese atomic bomb survivors in 1950[7,8] and its subcohort, AHS, consisting of 19 961 survivors in 1958. The AHS includes individuals exposed to a wide range of radiation doses; about half were within 2 km of the hypocenter (proximal exposure), a quarter were at distances of more than 3 km (distal exposure) and a quarter were not in the city at the time of the bombings. The AHS includes biennial health examinations and complete follow-up with respect to death. Of all subjects, more than 70% have continued to participate in each 2-year examination cycle.

To parallel methodologies used in other studies of the LTR, our primary interest was to evaluate risk of stroke starting at an index age of about 55 years. Because of the biennial nature of our clinical health examinations, and to be

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able to utilize BP measures at the index age, we defined index ages using 2-year intervals (for example, 55.0–56.9 years old (y.o.)). In addition to an index age of 55 y.o., we looked at similar index age intervals for the following: 45 y.o. (45.0–46.9), 65 y.o. (65.0–66.9) and 75 y.o. (75.0–76.9). As subjects needed to have an exam with BP measurements and be stroke free and alive at an index age, the number of subjects varied for corresponding index age-associated cohorts. For the 55 y.o. index age cohort, 7487 participants were identified as being stroke free and alive at the index age, and had BP measurements at the corresponding index age-associated clinical examination during the study period between 1 July 1958 and 31 December 2003. Participants were monitored until they developed their first-ever stroke, which was identified at the examination or by death certificate, died, or until their most recent RERF evaluation before December 2003, whichever came first. Informed consent was obtained from all participants, and the Ethics Committee at RERF approved this study.

To complete our surveillance of non-fatal and fatal events, diagnosis at each examination and underlying and contributing causes of deaths based on death certificates during the study periods were coded according to the International Classification of Disease (ICD) codes in the RERF database. The ICD codes of stroke-related disease are 330–332, 334, 352 and 435 (ICD-7), 333, 430–434, 436 and 438 (ICD-8), 430, 431 and 433–438 (ICD-9), and G45, I60, I61, I63–66 and I69 (excluding I698; ICD-10). All potential non-fatal stroke cases with these codes were systematically reviewed by one researcher (IT) regarding experience of stroke events, such as rapidly developed clinical signs of focal disturbance in cerebral function lasting more than 24 h (unless interrupted by surgery or death) without apparent cause other than vascular origin (for example, blood disease, brain tumor, or brain metastases). We did not consider the following symptoms as sole evidence of focal dysfunction: dizziness, vertigo, localized headache, blurred vision, dysarthria, impaired cognitive function (including confusion) or seizures according to the definitions of typical/atypical stroke symptoms in the World Health Organization (WHO)-initiated Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Projects.[9,10] Identified stroke cases were further classified by subtype of stroke based on participants' accepted diagnostic information (autopsy, computed tomography (CT) or magnetic resonance imaging (MRI) scan conducted outside of RERF, death certificate and clinical information centrally collected by one neurologist[11]). In addition, an external collaborating neurologist (TO) oversaw this process.

Baseline examination

Sitting BP was measured in the left arm after an adequate sedentary period. BP groups were defined using systolic BP (SBP) and diastolic BP (DBP) in accordance with criteria from the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) classification:[12] normal, SBP < 120 mm Hg and DBP < 80 mm Hg; prehypertension, SBP 120–139 mm Hg and/or DBP 80–89 mm Hg; stage 1 hypertension, SBP 140–159 mm Hg and/or DBP 90–99 mm Hg; and stage 2 hypertension, SBP > 160 mm Hg and/or DBP > 100 mm Hg. Non-fasting serum cholesterol levels were measured at the biennial examinations by Abell–Kendall method during 1958–1965, automated methods (Technicon Autoanalyzer, Technicon Instruments, Tarrytown, NY, USA) during 1965–1967 and a 7050 analyzer (Hitachi, Tokyo, Japan) from 1986 afterward. Measurements of height and weight were made at each examination. Profile of possible risk factors was shown in Table 1.

Statistical analysis

Gender-specific analyses of LTR of stroke and its subtypes (hemorrhagic *vs.* ischemic stroke) in the various index age cohorts were carried out using cumulative incidence analysis, adjusting for the competing risk of non-stroke death. Estimates and their associated estimated standard errors derived for the risk of stroke at 10, 20, 30 and 40 years after the index age as well as the LTR were generated using the cmprsk, add-on package in R.[13] This is one of the recommended tools for cumulative incidence analyses that can take into account competing events and also compare the cumulative incidence of a particular type of event among different factors or groups.[14–16] Stroke incidence was analyzed by 3 different periods to address potential diagnostic differences as well as potential cultural changes in lifestyle: 1950–1977 *vs.* 1978–1989 *vs.* 1990 or later.[17] Descriptive summary statistics and graphical analyses were used to assess each of the variables across all subjects as well as by gender. Differences in continuous variables between groups were assessed using two-sample *t*-test when assumptions did not hold and sufficient transformations were not possible. The χ^2-tests were used to assess relationships between categorical variables. All statistical analyses were performed using the statistical software R program (R version 2.11.1).[13]

RESULTS

Subject characteristics

The characteristics of the study cohort used in the analyses in terms of BP categories, serum total cholesterol levels and body mass index are shown in Table 1 for each of the index age cohorts. As our primary interest was evaluating LTR of stroke estimates from an index age of about 55 y.o. in order to be analogous to other similar studies, results presented here focus on that index age cohort unless indicated otherwise. Only 24.5% of subjects were classified as normotensive using the JNC 7 BP categories, and 40.7% had stage 1 or stage 2 hypertension. There were statistically significant differences in distribution of BP factor levels between men and women ($P<0.001$), with a higher percentage of men classified as having stage 1 or 2 hypertension (45.5 *vs.* 38.0% in women). Women had significantly higher serum total cholesterol levels and body mass index than men ($P<0.001$ for both comparisons).The median follow-up time for event-free subjects was 16.1 years (range: 1 day–42.1 years), with 3954 subjects experiencing either stroke or death event. A total of 868 strokes (38% cases were identified using only information from death certificates) and 3086 cases of non-stroke-related death occurred as of December 2003.

Stroke subject characteristics

Of the 868 subjects with valid documented stroke event, 512 (58.9%) were women. Most strokes (62.4%) were ischemic, with 26.6% (231 participants; 146 women) developing hemorrhagic stroke. A total of 38 subarachnoid hemorrhage events (28 women) were included in the overall category of hemorrhagic stroke. The subjects experienced stroke not otherwise specified, and these cases often had insufficient information to classify the stroke as either hemorrhagic or ischemic. Of the 868 strokes, 332 were verified by death certificate alone, 319 were diagnosed by neuroimaging outside of RERF, 26 were verified by autopsy, 147 were verified by medical records and 44 were verified through earlier identification by one neurologist.[11] The median age of subjects experiencing stroke was 72.0 years (range: 55.4–98.4 years). There were significant differences in age of first stroke by stroke subtype ($P<0.001$). The median age of first event was 69.6 years (range: 56.2–96.4 years) for hemorrhagic stroke, 71.7 years (range: 55.6–93.2 years) for stroke not otherwise specified and 73.5 years (range: 55.4–98.4 years) for ischemic stroke.

LTR of stroke

The LTR for both sexes reached similar levels at the 40-year risk estimate: one in five persons (20%; Table 2). Significant gender differences were observed for all-stroke incidence ($P<0.001$). When we evaluated strokes by subtype, however, gender differences were only observed for ischemic stroke ($P<0.001$). At the ages of 65 and 75 years, risks of each stroke subtype were higher for men, but the LTR were higher for women.

We stratified subjects into groups who turned 55 years of age in the first (1958–1977), second (1978–1990) or third periods (1991+) in men. The LTR of all-stroke was significantly different by time period

Table 1 Characteristics by index age cohort

		Index age (years)			
Sex	*Variables*	*45*	*55*	*65*	*75*
Men	*BP group*[a] *(n)*				
	Normal	595	530	454	203
	PreHT	758	913	871	429
	Stage 1 HT	366	698	747	409
	Stage 2 HT	282	507	651	391
	DBP (mm Hg)				
	Median (range)	80 (45–152)	84 (20–160)	82 (30–140)	80 (30–134)
	SBP (mm Hg)				
	Median (range)	120 (80–244)	130 (80–260)	138 (80–240)	140 (70–260)
	BMI (kg m^{-2})				
	Median (range)	21.6 (12.6–34.9)	21.8 (12.9–38.3)	22.4 (12.1–37.2)	21 (12.7–33.4)
	Total cholesterol (mg dl^{-1})				
	Median (range)	174 (74–361)	179 (75–415)	179 (76–354)	179 (80–324)
	Index age time period (n)				
	1950–1977	1377	1152	1188	474
	1978–1989	570	1088	528	516
	1990–2003	54	408	1007	442
	Strokes (n)				
	All strokes	221	356	344	181
	Hemorrhagic stroke	59	85	73	40
	Ischemic stroke	146	238	227	124
	Stroke NOS	16	33	44	17
	Non-stroke death	866	1303	1596	1012
	Alive and stroke free	914	989	783	239
	Follow-up from index age (years)				
	Median (range)	24 (0.01–44.8)	14.4 (0.01–36.2)	6.3 (0.01–28)	1.8 (0.01–21.1)
	Person-years	41306.9	38061.5	25127.2	8128.6
Women	*BP group*[a] *(n)*				
	Normal	1603	1306	963	379
	PreHT	1324	1690	1654	949
	Stage 1 HT	570	1081	1267	928
	Stage 2 HT	289	762	1024	822
	DBP				
	Median (range)	78 (40–150)	80 (20–180)	80 (8–160)	80 (30–160)
	SBP				
	Median (range)	120 (78–262)	128 (80–280)	134 (76–260)	142 (86–260)
	BMI (kg m^{-2})				
	Median (range)	22.4 (15–42.7)	22.6 (14.3–42.7)	22.4 (12.7–42)	22.3 (11.8–40.2)
	Total cholesterol (mg dl^{-1})				
	Median (range)	172 (85–361)	199 (94–426)	191 (90–536)	202 (80–409)
	Index age time period (n)				
	1950–1977	2921	2051	1727	743
	1978–1989	796	2218	1309	896
	1990–2003	69	570	1872	1439

Table 1 (Continued)

Sex	Variables	Index age (years) 45	55	65	75
	Strokes (n)				
	All strokes	317	512	570	335
	Hemorrhagic stroke	105	146	159	96
	Ischemic stroke	179	304	330	203
	Stroke NOS	33	62	81	36
	Non-stroke death	1109	1783	2140	1622
	Alive and stroke free	2360	2544	2198	1121
	Follow-up from index age (years)				
	Median (range)	26.1 (0.01–44.7)	17.9 (0.01–42.1)	9.3 (0.01–38.6)	3.2 (0.01–26.9)
	Person-years	90155.7	82763.7	54732.6	18635.8

Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic BP; HT, hypertension; JNC 7, Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; SBP, systolic blood pressure; stroke NOS, stroke not otherwise specified.
[a]BP groups were defined using SBP and DBP, and followed criteria from the JNC 7 classification:[12] normal, SBP<120 mm Hg and DBP<80 mm Hg; preHT, SBP 120–139 mm Hg and/or DBP 80–89 mm Hg; stage 1 HT, SBP 140–159 mm Hg and/or DBP 90–99 mm Hg; and stage 2 HT, SBP≥160 mm Hg and/or DBP≥100 mm Hg.

Table 2 Age- and sex-specific short-, intermediate-term and LTR estimates for stroke and its subtypes: adult health study, 45-year follow-up, 1958–2003, Japan

Stroke type	Sex	Index age (years)	Short- and intermediate-term risk estimate (s.d.) (%) 10 year	20 year	30 year	40 year	LTR estimate (s.d.) (%)
All-stroke	Men	45	1.7 (0.3)	6.3 (0.6)	12.2 (0.9)	17.7 (1.3)	19.6 (1.5)
		55	5.9 (0.5)	12.4 (0.7)	18.4 (1.0)	20.4 (1.1)	20.5 (1.1)
		65	8.3 (0.6)	14.9 (0.8)	17.4 (0.9)	—	17.6 (0.9)
		75	10.8 (1.4)	15.3 (1.1)	—	—	15.6 (1.1)
	Women	45	1.0 (0.2)	3.4 (0.3)	8.2 (0.5)	15.9 (1.0)	18.0 (1.3)
		55	2.2 (0.2)	7.9 (0.4)	16.3 (0.8)	21.6 (1.0)	22.2 (1.1)
		65	5.6 (0.4)	14.6 (0.7)	20.2 (0.8)	—	21.3 (0.9)
		75	10.2 (0.6)	16.8 (0.9)	—	—	17.6 (0.9)
Ischemic stroke	Men	45	0.9 (0.2)	3.7 (0.4)	7.9 (0.7)	12.6 (1.1)	13.9 (1.4)
		55	3.6 (0.4)	8.0 (0.6)	12.6 (0.8)	14.1 (0.9)	14.2 (0.9)
		65	5.4 (0.5)	10.1 (0.7)	11.9 (0.8)	—	11.9 (0.8)
		75	7.3 (0.7)	10.4 (0.9)	—	—	10.7 (0.9)
	Women	45	0.4 (0.1)	1.6 (0.2)	4.7 (0.4)	9.4 (0.8)	10.6 (1.0)
		55	1.1 (0.2)	4.3 (0.3)	9.9 (0.6)	13.8 (0.9)	14.4 (1.0)
		65	2.8 (0.3)	8.4 (0.5)	12.6 (0.7)	—	13.5 (0.8)
		75	8.6 (0.5)	10.3 (0.7)	—	—	11.0 (0.8)
Hemorrhagic stroke	Men	45	0.6 (0.2)	2.0 (0.3)	3.3 (0.5)	4.1 (0.6)	4.6 (0.6)
		55	1.7 (0.3)	3.2 (0.4)	4.0 (0.5)	4.5 (0.5)	4.5 (0.5)
		65	1.9 (0.3)	3.1 (0.4)	3.7 (0.4)	—	3.8 (0.5)
		75	2.3 (0.3)	3.5 (0.5)	—	—	3.5 (0.5)
	Women	45	0.4 (0.1)	1.4 (0.2)	2.6 (0.3)	5.1 (0.6)	5.7 (0.8)
		55	0.9 (0.1)	2.4 (0.2)	4.3 (0.4)	5.5 (0.5)	5.5 (0.5)
		65	1.8 (0.2)	4.4 (0.4)	5.4 (0.5)	—	5.6 (0.5)
		75	3.3 (0.4)	4.8 (0.5)	—	—	4.9 (0.5)

Abbreviation: LTR, lifetime risk.

of baseline age (P=0.02), but when separated into stroke subtype, none was statistically significant. In contrast, we found that the LTR of all-stroke was not significantly different by time period in women (P=0.21), but when data were assessed by stroke subtype, there were significant differences in the risk for ischemic stroke (P=0.006).

LTRs of all-stroke by BP group

The JNC 7 BP group at index age exam was significantly associated with the LTR of all-stroke for men and women (P<0.001; Table 3). Stages 1 and 2 hypertension status at baseline resulted in significantly higher risk of all-stroke in both men and women. In the stroke

Table 3 Sex-specific short-, intermediate-term and LTR estimates for stroke and its subtypes beyond 55 years of age by BP groups: adult health study, 45-year follow-up, 1958–2003, Japan

			Short- and intermediate-term risk estimate (s.d.) (%)				*LTR estimate*
Stroke type	*Sex*	*BP group*[a]	*10 year*	*20 year*	*30 year*	*40 year*	*(s.d.) (%)*
All-stroke	Men	Normal	2.6 (0.6)	7.8 (1.3)	13.1 (1.9)	—	13.8 (2.0)
		PreHT	3.3 (0.6)	8.4 (1.1)	14.1 (1.5)	16.9 (1.7)	16.9 (1.7)
		Stage 1 HT	6.1 (0.9)	14.3 (1.5)	23.1 (2.2)	25.8 (2.4)	25.8 (2.4)
		Stage 2 HT	13.1 (1.5)	21.3 (1.9)	25.3 (2.2)	—	25.8 (2.3)
	Women	Normal	1.2 (0.3)	5.3 (0.7)	11.3 (1.3)	16.0 (1.7)	16.0 (1.7)
		PreHT	1.1 (0.3)	5.0 (0.6)	12.6 (1.2)	19.4 (1.8)	19.9 (1.8)
		Stage 1 HT	2.8 (0.5)	9.3 (1.0)	20.0 (1.8)	24.0 (2.3)	24.0 (2.3)
		Stage 2 HT	5.5 (0.8)	15.7 (1.4)	26.4 (2.1)	30.5 (2.4)	30.5 (2.4)
Ischemic stroke	Men	Normal	1.6 (0.6)	6.6 (1.2)	10.6 (1.7)	—	11.2 (1.8)
		PreHT	1.8 (1.7)	4.7 (2.6)	8.7 (1.2)	10.7 (1.5)	10.7 (1.5)
		Stage 1 HT	3.6 (0.7)	9.2 (1.3)	16.2 (1.9)	18.3 (2.2)	18.3 (2.2)
		Stage 2 HT	8.5 (1.3)	13.5 (1.6)	16.8 (1.9)	—	17.3 (2.0)
	Women	Normal	1.0 (0.3)	3.2 (0.6)	7.7 (1.1)	11.3 (1.5)	11.3 (1.5)
		PreHT	0.5 (0.2)	2.3 (0.4)	7.2 (1.0)	12.6 (1.5)	13.1 (1.6)
		Stage 1 HT	1.3 (0.4)	5.2 (0.8)	11.7 (1.5)	14.5 (2.0)	14.5 (2.0)
		Stage 2 HT	2.2 (0.5)	8.6 (1.1)	16.2 (1.8)	18.4 (2.0)	18.4 (2.0)
Hemorrhagic stroke	Men	Normal	0.6 (0.3)	0.6 (0.3)	1.5 (0.7)	—	1.5 (0.7)
		PreHT	1.0 (0.3)	2.6 (0.6)	3.5 (0.8)	4.2 (0.9)	4.2 (0.9)
		Stage 1 HT	2.2 (0.6)	4.1 (0.9)	4.8 (1.0)	5.4 (1.2)	5.4 (1.2)
		Stage 2 HT	3.7 (0.8)	6.0 (1.1)	6.4 (1.2)	—	6.4 (1.2)
	Women	Normal	0.1 (0.08)	1.3 (0.4)	2.6 (0.7)	3.7 (0.9)	3.7 (0.9)
		PreHT	0.4 (0.17)	1.6 (0.3)	3.6 (0.7)	4.7 (0.8)	4.7 (0.8)
		Stage 1 HT	1.3 (0.3)	2.9 (0.6)	5.3 (0.9)	6.2 (1.1)	6.2 (1.1)
		Stage 2 HT	2.6 (0.6)	5.5 (0.9)	6.9 (1.0)	8.9 (1.4)	8.9 (1.4)

Abbreviations: BP, blood pressure; DBP, diastolic BP; HT, hypertension; JNC 7, Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; LTR, lifetime risk; SBP, systolic BP.
[a]BP groups were defined using SBP and DBP, and followed criteria from the JNC 7 classification:[12] normal, SBP<120 mm Hg and DBP<80 mm Hg; preHT, SBP 120–139 mm Hg and/or DBP 80–89 mm Hg; stage 1 HT, SBP 140–159 mm Hg and/or DBP 90–99 mm Hg; and stage 2 HT, SBP≥160 mm Hg and/or DBP≥100 mm Hg.

subtype-specific analyses, there were significant differences in risk between men and women for hemorrhagic stroke ($P<0.001$ between each BP group) and ischemic stroke ($P<0.001$ between each BP group). Similar results were also seen for the other index age cohorts.

DISCUSSION

Our findings showed the mortality-adjusted residual LTR of stroke beyond 55 years of age was one in five involving a sample of 7487 middle-aged Japanese who have been followed up for 45 years; female stroke LTR was slightly higher compared with male LTR (20.5% for men *vs.* 22.2% for women). Separated into stroke subtypes, the observed probabilities were higher for ischemic stroke (one in seven for men and women) than for hemorrhagic stroke (one in 20 for both sexes). The LTR for all-stroke and stroke subtypes were similar across the other index ages. In addition, we stratified subjects into groups who turned 55 years of age in the first (1958–1977), second (1978–1990) or third periods (1991+), because our study observation period corresponded to rapid westernization in Japan and drastic changes in risk-factor profiles due to widespread use of antihypertensive drug therapy.[18] We found differences in risk for stroke and stroke subtype by gender and time group, which may support a past report indicating that risks of any stroke subtype have leveled off during the last few decades.[19] However, we were unable to draw conclusions on different risks by time group because of the inadequate number of cases available for analysis, especially for men.

A few reports have been published on the LTR of stroke from the Rotterdam Study (6-year follow-up),[5] the Framingham Study (>50-year follow-up)[4] and the Suita Study (17-year follow-up);[6] and the LTR of stroke in our study was similar to the reported results from the these studies (about 20% for both men and women of 55 years of age). Male gender has been considered an important risk factor for stroke,[20] but the higher LTR in women of 55 years of age in the AHS has been attributed to the longer life span of women. There may be, however, other potential reasons that women are at greater risk than men for atrial fibrillation-related thromboembolic events.[21,22] Elderly women (above age 85 years) were observed to have higher stroke incidence than that in men.[23–25] Our LTR estimates emphasized that the LTR remains a major threat and public health burden for Japan's aging society, especially for women.

Our longitudinal study using LTR estimates indicated that hypertension in midlife (40s, 50s) remained a risk factor for stroke compared with normotensive subjects, which supports a past report indicating that elevated BP in the 40s is suggested to have the so-called 'carryover' effects on stroke incidence for subjects when they reach their 60s.[26] Moreover, our findings suggested that stroke risk of subjects with prehypertension in their 50s, but not for such subjects in their 40s, was similar to that of normotensive subjects. Recent studies about relationship between elevated BP and PAF for stroke incidence of Japanese in the Japan Public Health Centre study[27] and the Circulatory Risk in Communities Study[28] suggested that the

increase in population attributable fraction (PAF) when hypertension progressed from high normal to mild becomes more pronounced, indicating that BP levels lower than 140/90 mm Hg may not necessarily represent stroke risk, which were supportive for us. On the other hand, Kokubo *et al.* reported significantly elevated risk of stroke according to BP category comparing normal and high-normal BPs in a general urban Japanese men.[29] Those controversial issues regarding prehypertension impact on stroke events could be because of white coat hypertension and the potential impact of fluctuations in BP levels after midlife that may affect stroke incidence. Further analysis of the relation between BP fluctuation and stroke could shed light on the issue whether early BP control over normal levels at midlife could be beneficial for prevention of stroke incidence.

Our study has several strengths, including a large population not preselected for existing disease or occupational fitness, a 45-year follow-up with biennial health examinations and virtually complete mortality ascertainment. In addition, we believe medical surveillance bias to be minimal, as the entire cohort is eligible for free, special medical care. Our estimates were based on simultaneously gathered data on both stroke incidence and other-cause mortality attributable to the competing risk of death in the same cohort. During recent decades in Japan, ischemic stroke has been reported to be the dominant subtype as a proportion of all strokes, being three to four times more frequent than cerebral hemorrhage, which is similar to our findings.[30,31]

The study also has several limitations and uncertainties. The possible stroke cases with stroke-related ICD codes were reviewed retrospectively. Ascertainment of stroke events from death certificates or cases before the widespread use of imaging tests (beginning around the 1970s) is of limited diagnostic accuracy and represents only a fraction of cases of incident disease. We did not consider the BP-lowering effects of antihypertensive agents because of unavailable medication information before 1990. Finally, atomic bomb survivors exposed to radiation were included in this analysis, and thus our results may not accurately reflect the general population.

In conclusion, our study suggests that hypertension at midlife among Japanese subjects confers a LTR for stroke. It is important to address this risk early when hypertension is identified to ensure that proper therapy can be initiated and stroke risk lowered.

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Association of human T lymphotropic virus type I with Sjögren syndrome

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Association of human T lymphotropic virus type I with Sjögren syndrome

Sjögren syndrome (SS) is an autoimmune disease caused by a combination of genetic and environmental factors. The most important environmental factor is viral infection. The retrovirus human T lymphotropic virus type I (HTLV-I) is deemed as an SS pathogen, because anti-HTLV-I antibodies were positive in 23% of patients with SS but only in 3.4% of control subjects (blood donors).[1] The patients with SS in that study, however, were limited to those who visited the hospital, and the control is not screened for SS, a bias may have been present. Thus, in the present study, we measured anti-HTLV-I antibodies in 852 Nagasaki atomic bomb survivors who had previously been screened for SS.

Between November 2002 and October 2004, 1008 Nagasaki atomic bomb survivors who had been followed biennially since 1958 at the Radiation Effects Research Foundation (RERF),[2] answered a questionnaire concerning ocular and oral symptoms and were screened for anti-SS-A/Ro, anti-SS-B/La antibodies and rheumatoid factor. We then examined them for SS using American–European Consensus Group criteria[3] and its modifications, including the tear flow test (Schirmer-I test), salivary flow test (Saxon test), cornea and conjunctiva staining test, salivary ultrasonography and salivary MRI.[4] We found 23 SS cases, a prevalence of 2.3%.[4] From April 2006 to June 2008, 852 participants (18 with SS, 335 men and 517 women, average age 71.1 years) underwent HTLV-I antibody measurements. RERF's Human Investigation Committee reviewed and approved the study protocol, and all participants provided written informed consent.

Of the 852 participants, 75 (8.8%) were anti-HTLV-I antibody positive by chemiluminescent enzyme immunoassay (Fujirebio, Tokyo, Japan) and western blotting (BML, Tokyo, Japan). A total of 5 (6.7%) of the seropositive subjects and 13 (1.7%) of the seronegative subjects were diagnosed as having SS. In all, 5 (27.8%) of the 18 SS participants and 70 (8.4%) of the 834 non-SS participants had anti-HTLV-I antibodies (p=0.016, Fisher exact test). Prevalence of women (57/75, 76%, 460/777, 59%, p=0.005) and positive anti-SS-A/Ro antibodies (7/75, 9.3%, 23/777, 3.4%, p=0.020) and titre of rheumatoid factor (9.8 U/ml, 7.7 U/ml, p=0.038) were also significantly higher among HTLV-I seropositive group than seronegative group. The finding that HTLV-I infection was predominant in women may partly explain the predominance of SS in women. The prevalence of SS-B/La antibodies was similar for the two groups (1/75, 1.3%, 7/777, 0.9%). An age-adjusted and sex-adjusted OR of having SS for those in the HTLV-I seropositive group was 3.68 (95% CI, 1.26 to 10.75, p=0.014) by a linear logistic model. That suggests a possible association between HTLV-I infection and SS as reported in previous immunological studies.[5–7]

Table 1 Characteristics of Sjögren syndrome (SS) according to anti-human T lymphotropic virus type I (HTLV-I) antibody status

	HTLV-I positive SS (n=5)	HTLV-I negative SS (n=13)
Mean±SD age, years	71.4±5.8	71.7±5.6
Sex (male:female)	0:5	3:10
Sicca symptoms, N (%)	4 (80)	9 (69)
Dry eye signs, N (%)	5 (100)	9/9* (100)
Dry mouth signs, N (%)	4 (80)	10 (77)
Anti-SS-A Ab, N (%)	4 (80)	10 (77)
Anti-SS-B Ab, N (%)	1 (20)	3 (23)
Anticentromere Ab, N (%)	0 (0)	2/12* (17)
Extraglandular manifestations, N (%)	3 (60)	2 (15)
Secondary SS, N (%)	1 (20)	2 (15)
RF positive, N (%)	0 (0)	4 (31)

*Number was reduced because not all the participants agreed to take full examinations.
Ab, antibodies; RF, rheumatoid factor.

The prevalence of sicca symptoms, signs and positive autoantibodies was similar between the HTLV-I positive and negative SS groups (table 1). The frequency of extraglandular manifestations tended to be higher in HTLV-I seropositive group than in HTLV-I seronegative group (table 1), which supports the previous report.[8]

Our results suggest that the association between HTLV-I and SS is mediated through anti-SS-A/Ro antibodies, because the association between being anti-HTLV-I antibody positive and SS disappeared when anti-SS-A/Ro antibodies were incorporated in the analysis (data not shown). While genetic and environmental factors interact in the development of SS, HTLV-I may be an immune-activating pathogen for SS through anti-SS-A/Ro antibody production. Further studies are needed to confirm this.

All participants in the present study were atomic bomb survivors, but no association has been reported between radiation dose and either SS[4] or HTLV-I infection,[9] nor did we find a significant association between radiation dose by DS02[10] and HTLV-I in the present study. Thus, our data should be generalisable to the Japanese population.

In this first epidemiological study of measuring anti-HTLV-I antibodies in SS and non-SS participants, we confirmed the

association between HTLV-I infection and SS or anti-SS-A/Ro antibodies.

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Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Human Investigation Committee in Radiation Effects Research Foundation.

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Competing Interests None.

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ORIGINAL ARTICLE

Metabolic cardiovascular disease risk factors and their clustering in subclinical hypothyroidism

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Summary

Objective A possible association between subclinical hypothyroidism and cardiovascular disease (CVD) has been reported. Monitoring of atomic-bomb survivors for late effects of radiation exposure at the Radiation Effects Research Foundation has provided the opportunity to examine associations between subclinical hypothyroidism and metabolic CVD risk factors. The objective of the study was to evaluate associations between subclinical hypothyroidism and metabolic CVD risk factors, and a cluster of these factors.

Design and participants This was a cross-sectional study of 3549 subjects (mean age 70 years; 1221 men and 2328 women) between 2000 and 2003 comprising 306 subjects with subclinical hypothyroidism and 3243 control euthyroid subjects in Japan.

Measurements We investigated associations between subclinical hypothyroidism and metabolic CVD risk factors such as hypertension, diabetes mellitus, dyslipidaemia and hyperuricaemia, and a cluster of these factors.

Results Subclinical hypothyroidism was not significantly associated with either hypertension, diabetes mellitus or hyperuricaemia defined by taking into account the use of medications in both men and women, but in men it was associated with dyslipidaemia ($P = 0{\cdot}02$). We observed a significantly increased odds ratio (OR) for the presence of three or more metabolic CVD risk factors in men with subclinical hypothyroidism after adjusting for age, body mass index (BMI), and smoking status [OR: 1·83, 95% confidence interval (CI): 1·13–2·94, $P = 0{\cdot}01$]. The significant associations remained after an additional adjustment for atomic-bomb radiation dose.

Conclusions There appears to be a significant increase in a cluster of metabolic CVD risk factors among people with subclinical hypothyroidism.

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Introduction

The association between cardiovascular disease (CVD) and subclinical hypothyroidism, as defined by elevated TSH levels along with normal free T4 levels, has been studied for a number of decades.[1] A meta-analysis of 10 observational studies indicates that subclinical hypothyroidism may be associated with a modestly increased risk of coronary heart disease,[2] although there is a study showing no association in older adults.[3]

CVD risk factors have been intensively investigated in people with subclinical hypothyroidism, particularly lipid profiles. Several studies have found that individuals with subclinical hypothyroidism have higher total cholesterol and low-density lipoprotein (LDL) cholesterol levels than euthyroid subjects,[4–7] but other studies did not confirm the association.[8–10] The inconsistency of the results in these studies may in part be due to the following reasons: (1) whether the association was tested against the lipid levels (continuous data) or the prevalence of subjects with abnormal levels (binary data); (2) whether lipid-lowering medication was taken into consideration; and (3) the difference of the analytical methods used. Evaluating total cholesterol levels or LDL-cholesterol levels as continuous variables is not adequate if the analysis does not take into account the use of medications. Excluding people with lipid-lowering medication can result in overlooking the severe condition of subclinical hypothyroidism. Therefore, analyses based on the prevalence of risk factors, taking into consideration of both the metabolic parameters and medical treatment, are required to accurately evaluate the relationship between subclinical hypothyroidism and CVD risk factors.

The Radiation Effects Research Foundation (RERF, formerly the Atomic Bomb Casualty Commission, ABCC) has been conducting biennial health examinations of atomic-bomb survivors since 1958.

Using that cohort, we previously demonstrated that subclinical hypothyroidism was associated with CVD when the mean age of the cohort was 58·5 years.[11] In the present study, we defined metabolic CVD risk factors based on clearly defined criteria and investigated possible associations between subclinical hypothyroidism and the risk factors using a cohort with a mean age of 70 years.

Methods

Participants

The Adult Health Study (AHS) is a clinical programme established in 1958 by ABCC, now RERF, comprising Hiroshima and Nagasaki atomic-bomb survivors. The AHS biennial health examinations presented clinical information complementary to death and tumour registry data. A detailed description of this programme has been published elsewhere,[12,13] A total of 4552 ambulant AHS cohort members visited RERF for biennial health examinations between March 2000 and February 2003 with no knowledge of the thyroid disease study. We asked them to participate in the thyroid disease study at the time of the examinations, and 4091 subjects (89·9%, mean age: 70 years, 1352 men and 2739 women) agreed to participate and completed thyroid examinations. The results of the relationship between thyroid diseases and atomic-bomb radiation have been previously published.[14] Among the 4091 participants, those with a history of treatment of thyroid disease with such methods as surgery, radiation and thyroid hormone or antithyroid medication were excluded from our study subjects. Subjects with TSH levels >4·5 mU/l and normal free T4 levels [9·1–19·6 pmol/l (0·71–1·52 ng/dl)] were defined as having subclinical hypothyroidism (n = 306). Subjects with TSH levels of 0·45–4·5 mU/l and normal free T4 levels were treated as controls (n = 3243). Therefore, our study consisted of 3549 subjects. Among 306 subjects with subclinical hypothyroidism, 294 had TSH levels of <10 mU/l and 12 had TSH levels of 10 mU/l or more. The Dosimetry System 2002 (DS02) was used in estimating the atomic-bomb radiation doses of individual subjects.[15] This study was reviewed and approved by an RERF Institutional Ethical Committee, the Human Investigation Committee and written informed consent was obtained from all participants.

Clinical examination and laboratory methods

Participants visited RERF's Hiroshima and Nagasaki Laboratories for clinical examination every 2 years. A trained nurse recorded information on current and past disease and medications every time the participant visited during the period of this study. Current and past smokers were both categorized as smokers. Body mass index (BMI in kg/m^2) was determined as the body weight divided by the square of the standing height. Sitting blood pressure (in mmHg) was measured on the left arm after an adequate sedentary period. A blood sample was drawn between 9:00 am and 10:30 am for the half of the participants examined in the morning and between 1:00 pm and 2:30 pm for the remaining participants who were examined in the afternoon in order to perform a biochemical test and to measure thyroid function and antithyroid antibodies. Free T4 and TSH levels were determined in a single serum sample for each participant with a Lumipulse 1200 analyser using an immunometric technique based on chemiluminescence (Fujirebio Inc. Tokyo, Japan). Antithyroid peroxidase antibody (TPOAb) and antithyroglobulin antibody (TgAb) were measured by ELISA (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Subjects were classified as positive for antithyroid antibodies if their serum concentration of either TPOAb or TgAb was 10 IU/ml or more. Serum total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose and uric acid levels were measured by an automated procedure (Hitachi 7050; Hitachi, Ltd., Tokyo, Japan), with quality control monitored as recommended by the College of American Pathologists (Northfield, MN, USA). Hemoglobin (Hb) A1c was measured by HPLC using an automated analyser (HA-8150, Arkray, Tokyo, Japan). A measurement of HbA1c level had been routinely performed before the beginning of this study in Hiroshima, but in Nagasaki it was started in July 2000. Measurement of the LDL-cholesterol level was started in September 2001 in both Hiroshima and Nagasaki. Therefore, the HbA1c and LDL-cholesterol levels were not available for all study subjects.

Diagnostic criteria for metabolic CVD risk factors

Hypertension was defined as either a systolic blood pressure of 140 mmHg or more, a diastolic blood pressure of 90 mmHg or more, or antihypertensive medication use. Diabetes mellitus was defined as either fasting glucose levels of 7·0 mmol/l (126 mg/dl) or more, casual glucose levels of 11·1 mmol/l (200 mg/dl) or more, 2-h postprandial plasma glucose levels of 11·1 mmol/l (200 mg/dl) or more after a 75 g glucose load, HbA_{1c} levels of 6·5% or more, the use of insulin, or oral hypoglycaemic medication use. Dyslipidaemia was defined as either total cholesterol levels of 5·70 mmol/l (220 mg/dl) or more, HDL cholesterol levels lower than 1·04 mmol/l (40 mg/dl) in men and lower than 1·30 mmol/l (50 mg/dl) in women, fasting triglyceride levels of 1·695 mmol/l (150 mg/dl) or more, or lipid-lowering medication use. Hyperuricaemia was defined as uric acid levels of 416 μmol/l (7.0 mg/dl) or more, or antiuricaemic medication use. Diagnoses were made without knowledge of the thyroid status.

Statistical analysis

To compare variables between the two groups, we employed the χ^2 and Wilcoxon rank sum tests and then applied multiple regression analysis with adjustment for age. Multivariate logistic regression analysis was used to evaluate the association between subclinical hypothyroidism and metabolic CVD risk factors adjusting for age, BMI and smoking status. Multivariate logistic regression analysis was used to determine if there was an association between subclinical hypothyroidism and a number of accompanied metabolic CVD risk factors. All statistical analyses were conducted separately in men and women. All significance tests were two-sided and P values of <0·05 were considered significant. In the analysis of metabolic CVD risk factor clustering in subclinical hypothyroidism, we set the significance of P as 0·017 (≒0·05/3), i.e., over-all significance P