Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths

*Prospective Studies Collaboration**

Summary

Background Age, sex, and blood pressure could modify the associations of total cholesterol (and its main two fractions, HDL and LDL cholesterol) with vascular mortality. This meta-analysis combined prospective studies of vascular mortality that recorded both blood pressure and total cholesterol at baseline, to determine the joint relevance of these two risk factors.

Methods Information was obtained from 61 prospective observational studies, mostly in western Europe or North America, consisting of almost 900 000 adults without previous disease and with baseline measurements of total cholesterol and blood pressure. During nearly 12 million person years at risk between the ages of 40 and 89 years, there were more than 55 000 vascular deaths (34 000 ischaemic heart disease [IHD], 12 000 stroke, 10 000 other). Information about HDL cholesterol was available for 150 000 participants, among whom there were 5000 vascular deaths (3000 IHD, 1000 stroke, 1000 other). Reported associations are with usual cholesterol levels (ie, corrected for the regression dilution bias).

Findings 1 mmol/L lower total cholesterol was associated with about a half (hazard ratio 0·44 [95% CI 0·42–0·48]), a third (0·66 [0·65–0·68]), and a sixth (0·83 [0·81–0·85]) lower IHD mortality in both sexes at ages 40–49, 50–69, and 70–89 years, respectively, throughout the main range of cholesterol in most developed countries, with no apparent threshold. The proportional risk reduction decreased with increasing blood pressure, since the absolute effects of cholesterol and blood pressure were approximately additive. Of various simple indices involving HDL cholesterol, the ratio total/HDL cholesterol was the strongest predictor of IHD mortality (40% more informative than non-HDL cholesterol and more than twice as informative as total cholesterol). Total cholesterol was weakly positively related to ischaemic and total stroke mortality in early middle age (40–59 years), but this finding could be largely or wholly accounted for by the association of cholesterol with blood pressure. Moreover, a positive relation was seen only in middle age and only in those with below-average blood pressure; at older ages (70–89 years) and, particularly, for those with systolic blood pressure over about 145 mm Hg, total cholesterol was negatively related to haemorrhagic and total stroke mortality. The results for other vascular mortality were intermediate between those for IHD and stroke.

Interpretation Total cholesterol was positively associated with IHD mortality in both middle and old age and at all blood pressure levels. The absence of an independent positive association of cholesterol with stroke mortality, especially at older ages or higher blood pressures, is unexplained, and invites further research. Nevertheless, there is conclusive evidence from randomised trials that statins substantially reduce not only coronary event rates but also total stroke rates in patients with a wide range of ages and blood pressures.

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Introduction

The effects of other vascular risk factors——particularly blood pressure—on the epidemiological associations of cholesterol with ischaemic heart disease (IHD) and stroke remain uncertain. Although blood levels of total cholesterol are used widely to predict IHD, the relative risk per unit change in cholesterol decreases with age[1,2] and, perhaps, blood pressure,[3,4] and it is unclear whether an importantly positive association persists into old age. Furthermore, total cholesterol consists largely of the cholesterol in low-density lipoprotein particles (LDL cholesterol) plus the cholesterol in high-density lipoprotein particles (HDL cholesterol), which have opposite associations with IHD risk. Results from randomised trials have shown that treatment with a statin, which lowers LDL cholesterol, substantially reduces the incidence of IHD.[5] These trials have also shown a substantial reduction in the incidence of ischaemic stroke (without any apparent increase in haemorrhagic stroke).[5] The definite reduction in total stroke in the statin trials contrasts strongly with the weakness of the epidemiological association between blood cholesterol and stroke,[1,6–13] and that epidemiological association needs further exploration.

The results from retrospective epidemiological studies of IHD or stroke can be distorted by reverse causality (since vascular disease can itself directly or indirectly affect both blood cholesterol and blood pressure). In people with no previous history of vascular disease, however, prospective epidemiological studies have to be very large to assess reliably the extent to which one risk factor affects the relevance of another. The Prospective Studies Collab-

oration (PSC) has brought together evidence from many individual prospective studies of vascular mortality that recorded both blood pressure and total cholesterol at baseline, to undertake collaborative meta-analyses of the joint relevance of these two risk factors.

The present collaboration differs from previous meta-analyses in several ways that increase its reliability and precision: it is large, involving 55 262 vascular deaths in 892 337 apparently healthy adults in 61 cohorts (and, additionally, provides parallel analyses of the Multiple Risk Factor Intervention Trial [MRFIT] observational study that involve a further 34 242 vascular deaths); HDL cholesterol measurements at baseline are available for 153 798 of these participants, in whom there were 4966 vascular deaths (but, HDL cholesterol was not measured at baseline in MRFIT); and individual records are available for every participant in every study (except MRFIT), allowing detailed analyses of cause-specific mortality with respect to age, sex, blood pressure, and some other factors. Moreover, repeat measurements of HDL cholesterol in 40 313 participants allow quantitative correction for the regression dilution bias.[14] Results for blood pressure have already been published,[15] and the present report characterises, with greater precision and better control of some biases than has previously been possible, the age-specific relevance of total and HDL cholesterol to vascular mortality, and the extent to which this relation is modified by sex, blood pressure, and other risk factors.

Methods

Study design

Details of study selection, data collection, and statistical methods have all been described previously,[15,16] and are available in full in the webappendix (which includes webtables 1–6 and webfigures 1–11).

See Online for webappendix

For downloadable slides of all PSC results see http://www.ctsu.ox.ac.uk/projects/psc/

Cause-specific mortality was sought in the greatest detail available, using a three-digit International Classification of Diseases coding (ICD-6 to ICD-10), with vascular causes categorised as before[15] (webtable 1). In most studies the cause of death was initially obtained from the death certificate, but in many studies confirmation was then sought from medical records, autopsy findings, or other sources.

Associations between cause-specific mortality and cholesterol levels were estimated by Cox regression, adjusted for age (within whatever range of age at risk was being considered), sex, and study. The resulting hazard ratios (HR) are presented as floating absolute risks, which does not alter their values: it merely means that an appropriate 95% CI is ascribed to the log of the HR in every group (including even the reference group, with HR 1).[17,18]

To avoid a few potentially unreliable measurements unduly affecting the results, the few individuals with total cholesterol greater than 12 mmol/L, HDL cholesterol greater than 3·5 mmol/L, or the ratio total/HDL cholesterol greater than 12 were excluded from the main analyses (but, webtable 4 and webfigure 1 show analyses that included them). For the analyses of IHD, the participants were divided by baseline measurements into six categories of total cholesterol (<4·5, 4·5–5·4, 5·5–6·4, 6·5–7·4, 7·5–8·4, 8·5–12 mmol/L) or four categories of HDL cholesterol (<1, 1–1·24, 1·25–1·49, 1·5–3·5 mmol/L), non-HDL cholesterol (<4, 4–4·9, 5–5·9, ≥6 mmol/L), or total/HDL cholesterol (<4·25, 4·25–5·49, 5·5–6·74, 6·75–12).

The HRs in these categories were plotted against the mean usual values rather than the mean measured values.[15] To obtain the mean usual values in all six categories of total cholesterol, the means of the measured values in those six categories were shrunk towards the overall mean by the regression dilution ratio of 0·65. (Hence, the range of usual cholesterol values from the lowest to the highest cholesterol category is only about two-thirds as wide as the range of measured values; therefore, graphs of risk versus usual cholesterol are, appropriately, about 50% steeper than uncorrected graphs of risk versus measured cholesterol would have been.) The corresponding shrinkage factors (ie, regression dilution ratios) for HDL, non-HDL, and total/HDL cholesterol were 0·73, 0·70, and 0·68, respectively. The four regression dilution ratios are the correlation coefficients between a baseline measurement and a remeasurement some time later of the same factor on the same person (webappendix, webtables 2 and 3). 175 000 participants had not only a baseline measurement of total cholesterol but also at least one remeasurement of it some years later (37 000 had more than one); of these participants, 40 000 had a baseline measurement of HDL cholesterol and at least one remeasurement of it (and 3000 had more than one).

To facilitate direct comparisons of the strengths of different associations, HRs are presented for differences that are in approximate proportion to the standard deviation (SD) of the measured value of each factor: 1 mmol/L for total cholesterol and non-HDL cholesterol, 0·33 mmol/L for HDL cholesterol, and 1·33 for the ratio total/HDL cholesterol. The informativeness (ie, predictive

	Person-years (thousands)	IHD	Ischaemic stroke	Haemorrhagic stroke	Total stroke*	Other vascular
40–49 years	3442	1309	47	154	412	388
50–59 years	4140	5561	178	466	1370	1387
60–69 years	2751	10 419	540	743	2938	2590
70–79 years	1056	10 829	850	915	4311	3234
80–89 years	222	5626	519	422	2632	2256
Total (40–89 years)	11 611	33 744	2134	2700	11 663	9855
Subtotal with HDL cholesterol	1496	3020	145	231	914	1032
MRFIT (40–89 years)	8058	21 243	480	945	3596	9403

*Includes 869 deaths from subarachnoid haemorrhage (not included with haemorrhagic stroke) and 5960 deaths in which the type of stroke was unknown or not reported.

Table 1: **Person-years and numbers of deaths attributed to IHD, stroke, and other vascular causes, by age at risk**

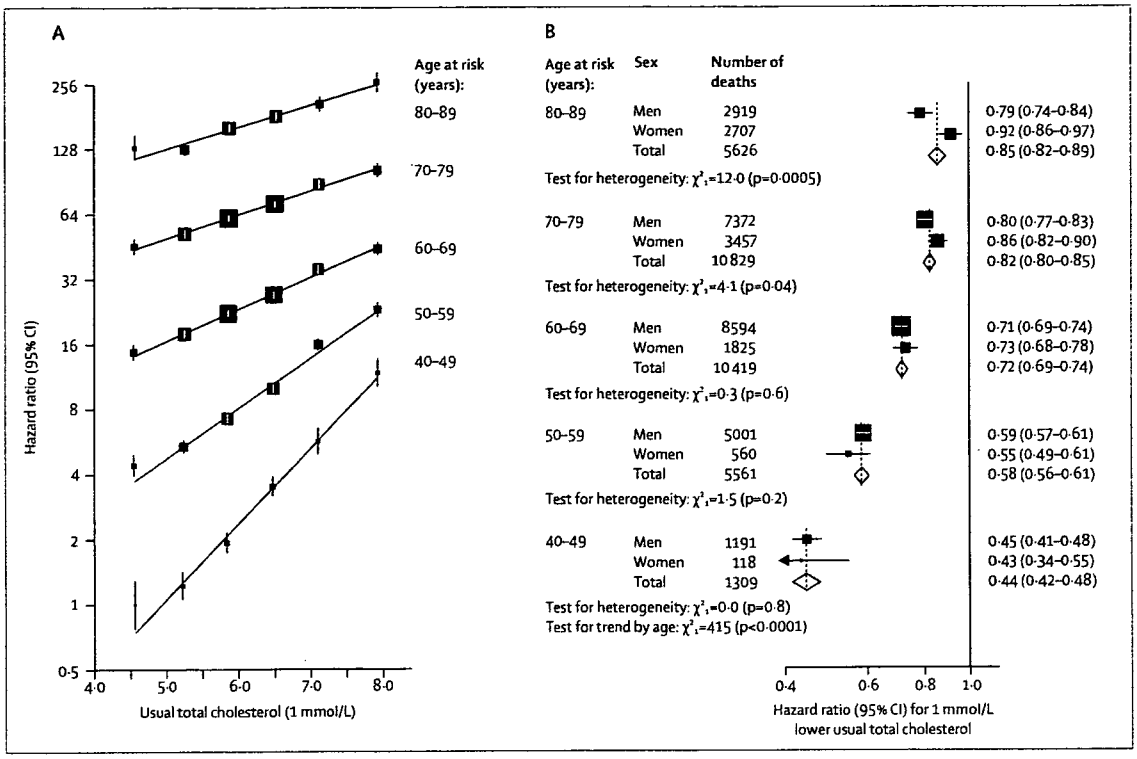

Figure 1: **IHD mortality (33744 deaths) versus usual total cholesterol**
(A) Age-specific associations. (B) Age-specific and sex-specific hazard ratios for 1 mmol/L lower usual total cholesterol. Hazard ratios on the left are plotted on a floating absolute scale of risk (so each log hazard ratio has an appropriate variance assigned to it). The slopes of the age-specific lines on the left are given on the right, subdivided by sex. Each square (left or right) has an area inversely proportional to the variance of the log of the hazard ratio that it represents.

ability) of the measured values of each of these four factors was taken to be directly proportional to the χ^2 statistic (twice the change in the log-likelihood) on addition of the baseline measurement of that factor into a regression model containing just baseline age, sex, and study.[19] On comparison of the predictive abilities of total, HDL, non-HDL, and the ratio total/HDL cholesterol, these χ^2 statistics were calculated only for people with complete data for all four factors.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. SL, PS, and GW had full access to the data, and the writing committee had final responsibility for the decision to submit for publication.

Results

Individual records for all 892337 eligible participants (without previous vascular disease recorded) in 61 studies were included in this meta-analysis: 70% from Europe, 20% from the USA or Australia, and 10% from Japan or China (webtable 5). During 11·6 million person-years at risk between the ages of 40 and 89 years (mean follow-up 13 [SD 6] years; mean time to death in those who died was 12 [7] years), there were 33744 deaths attributed to IHD, 11663 to stroke, and 9855 to other vascular causes (table 1). For about half (5960) of the stroke deaths the type of stroke was not recorded; when it was recorded, slightly more deaths were attributed to haemorrhage than to ischaemia (2700 *vs* 2134; excluding the 869 attributed to subarachnoid haemorrhage). In the 153798 individuals with baseline data for HDL cholesterol there were 3020 IHD deaths, 914 stroke deaths, and 1032 deaths attributed to other vascular causes during 1·5 million person-years of follow-up (table 1). Although individual records were not available from the additional 347681 men in the MRFIT observational study—which, over 25 years of follow-up, involved 34242 vascular deaths between the ages of 40 and 89 years (table 1)—the results from parallel analyses with identical methods are also provided.

Overall, the mean total cholesterol measurement at baseline was 5·8 (SD 1·3) mmol/L, and was similar in the subset with HDL cholesterol measurements (webtable 5). Mean total cholesterol levels tended to be lower in Japan and China than elsewhere. Overall, the mean HDL cholesterol was 1·4 (0·4) mmol/L, that for non-HDL cholesterol (ie, total minus HDL cholesterol, which is mostly LDL cholesterol) was 4·4 (1·2) mmol/L,

	Hazard ratio* (95% CI)		
	IHD	Stroke	Other vascular
40–49 years	0·45 (0·42–0·47)	0·87 (0·76–1·00)	0·62 (0·55–0·69)
50–59 years	0·57 (0·55–0·58)	0·91 (0·85–0·97)	0·75 (0·71–0·79)
60–69 years	0·68 (0·66–0·69)	0·93 (0·89–0·97)	0·83 (0·80–0·86)
70–79 years	0·79 (0·78–0·81)	1·02 (0·97–1·06)	0·89 (0·85–0·92)
80–89 years	0·85 (0·82–0·89)	1·05 (0·98–1·11)	1·02 (0·96–1·09)

*Log hazard ratios are inverse-variance-weighted mean of MRFIT (male), PSC male, and PSC female log hazard ratios adjusted for age at risk (in 5-year groups), study, and regression dilution (from the diamonds in webfigures 3, 7, and 10).

Table 2: **Combined PSC and MRFIT hazard ratios for IHD, stroke, and other vascular mortality for 1 mmol/L lower usual total cholesterol**

and that for the ratio total/HDL cholesterol was 4·6 (1·6). Throughout the range of cholesterol values studied (webtables 2–4), systolic blood pressure was about 2·4 mm Hg higher per 1 mmol/L higher usual total cholesterol (and about 1·7 mm Hg higher per 1·33 higher usual total/HDL cholesterol), after adjustment for age at measurement, sex, and study.

Figure 1 shows the age-specific relation between usual total cholesterol and IHD mortality, plotted on a doubling (ie, logarithmic) scale. On this scale, relations were approximately linear (ie, log-linear) within each decade of age at risk. In both men and women, a prolonged difference of 1 mmol/L lower total cholesterol was associated with an IHD mortality that was about a half lower in early middle age (40–49 years), about a third lower in later middle age (50–69 years), and about a sixth lower in old age (70–89 years) (figure 1). These findings were only slightly attenuated by adjustment for systolic blood pressure and were unaltered by adjustment for smoking (webtable 6). For participants dying at age 70–89 years, the mean time between the baseline cholesterol measurement and death from IHD was 14 (SD 6) years (webtable 3), which is slightly longer than the mean of 12 (7) years for all IHD deaths. Allowance for this mean time being 2 years longer would, however, make little difference to the estimated relevance of the usual cholesterol to IHD mortality in old age (perhaps, for example, changing the relative risk of 0·85 at ages 80–89 years into 0·84; webtables 2–4).

Although the relative risks per 1 mmol/L were less extreme at older ages, IHD was much more common in old age than in middle age so the absolute difference in annual IHD mortality per 1 mmol/L increased with age (figure 1). Combined analyses of all IHD deaths allowed reasonably reliable subdivision of the bottom group of baseline total cholesterol measurements into less than 3·5 and 3·5–4·5 mmol/L (corresponding to mean usual levels of about 3·9 and 4·5 mmol/L; webtable 4) and of the top group into 8·5–9·5 and 9·5–12 mmol/L (corresponding to mean usual levels of about 7·8 and 8·4 mmol/L). Throughout this range, lower cholesterol was associated with lower IHD mortality, and the few people excluded because their baseline cholesterol measurement was regarded as too high to be reliable (>12·0 mmol/L) had the highest IHD mortality of all

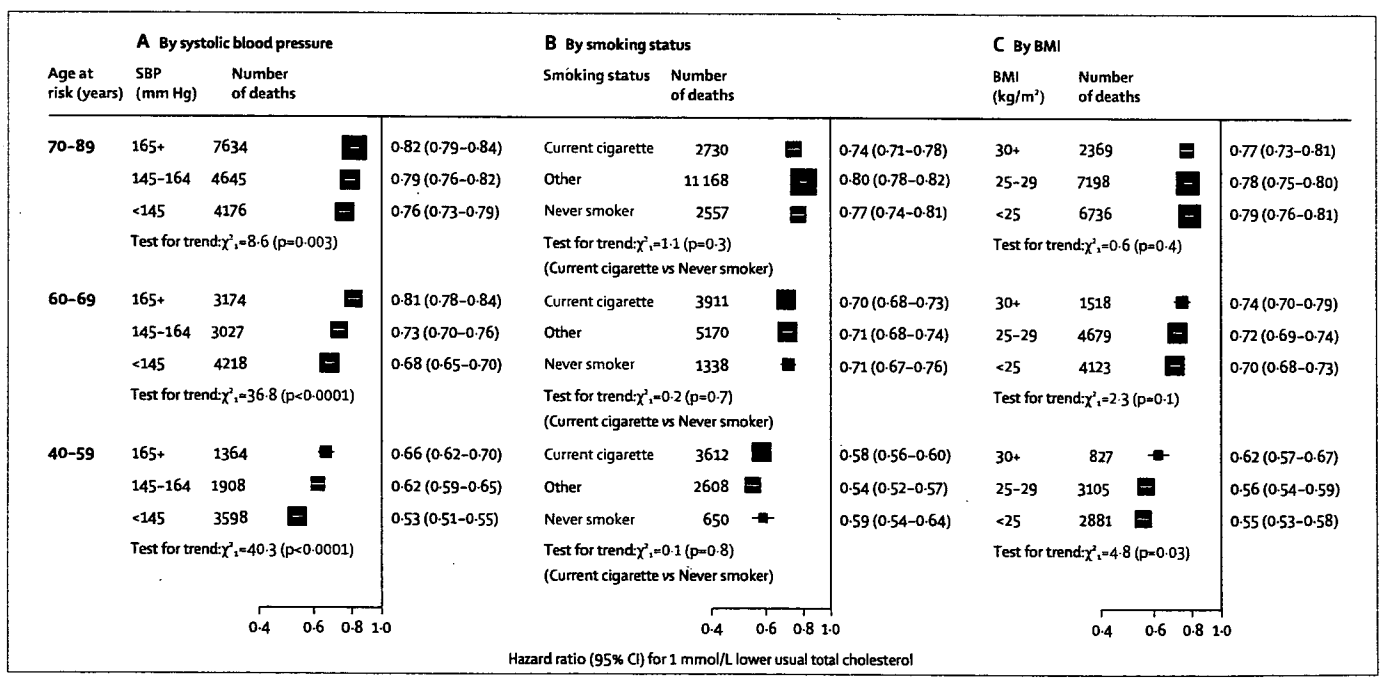

Figure 2: **IHD mortality (33 744 deaths) versus usual total cholesterol by: (A) systolic blood pressure (SBP); (B) smoking status; and (C) body-mass index (BMI)**
Conventions as in figure 1. (The BMI analysis involved just 33 436 deaths because of missing BMI values for 308 people who died of IHD.)

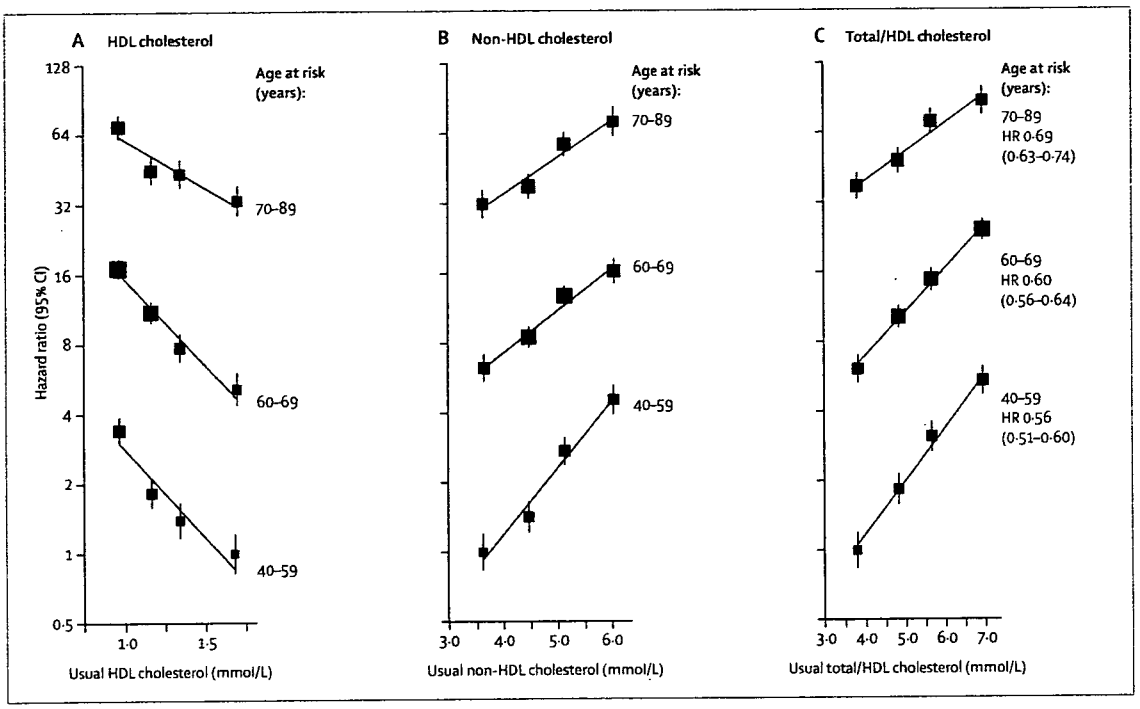

Figure 3: **IHD mortality (3020 deaths) versus usual (A) HDL cholesterol; (B) non-HDL cholesterol; and (C) total/HDL cholesterol**
Age-specific associations. Conventions as in figure 1. HR denotes the hazard ratio (95% CI) per 1·33 lower total/HDL cholesterol (see also webfigure 5).

(webfigure 1). Given age, the IHD hazard ratios per 1 mmol/L were not significantly different for Europe or USA/Australia (webfigure 2; there were too few IHD deaths in east Asia for reliable comparison). Although the age-specific results were generally somewhat stronger in the MRFIT study than in these PSC analyses, the PSC results were not materially different from the combined PSC and MRFIT results (webfigure 3). Table 2 shows these age-specific combined relative risks.

There were approximately log-linear associations (as in figure 1) between usual total cholesterol and IHD mortality at different levels of systolic blood pressure, in different categories of smoking status and at different levels of body-mass index (BMI). Figure 2 shows the strengths of the age-specific associations between IHD mortality and total cholesterol in every category of baseline systolic blood pressure, smoking status, and BMI. For systolic blood pressure, the proportional effects of cholesterol on IHD mortality within each age group decreased somewhat with increasing levels of systolic blood pressure. But, since IHD was more common at higher levels of systolic blood pressure than at lower levels, the absolute difference in IHD mortality associated with a given difference in total cholesterol was similar at each level of systolic blood pressure (ie, the absolute effects of cholesterol and blood pressure were approximately additive; webfigure 4). By contrast, smoking status and BMI were of little relevance to the proportional effects of cholesterol on IHD mortality within each age group, so the absolute difference in IHD mortality associated with a given difference in total cholesterol was somewhat greater for those who were smokers or more obese. There were too few deaths in participants with HDL cholesterol measurements to investigate such interactions reliably.

Figure 3 shows the age-specific relations between IHD mortality and usual HDL cholesterol, usual non-HDL cholesterol, and usual total/HDL cholesterol. For HDL cholesterol, there was a strong negative (ie, inverse) association with IHD mortality in every age group, with no evidence of a threshold (at least within the range studied) beyond which higher HDL cholesterol was no longer associated with lower IHD mortality. For non-HDL cholesterol and for the ratio total/HDL cholesterol, there were strong positive associations with IHD mortality at all ages, with no evidence of thresholds within the ranges studied. On average, 1 mmol/L lower non-HDL cholesterol, 0·33 mmol/L higher HDL cholesterol, and 1·33 lower total/HDL cholesterol were each associated with about a third lower IHD mortality. Within every age group, the strengths of these associations were comparable for men and women (webfigure 5). On comparison of different age groups, the proportional (but not the absolute) associations of these cholesterol fractions with IHD mortality were less extreme in old age than in middle age (figure 3; webfigure 5). After standardising for age, the effects of HDL and non-HDL cholesterol on IHD mortality were largely independent

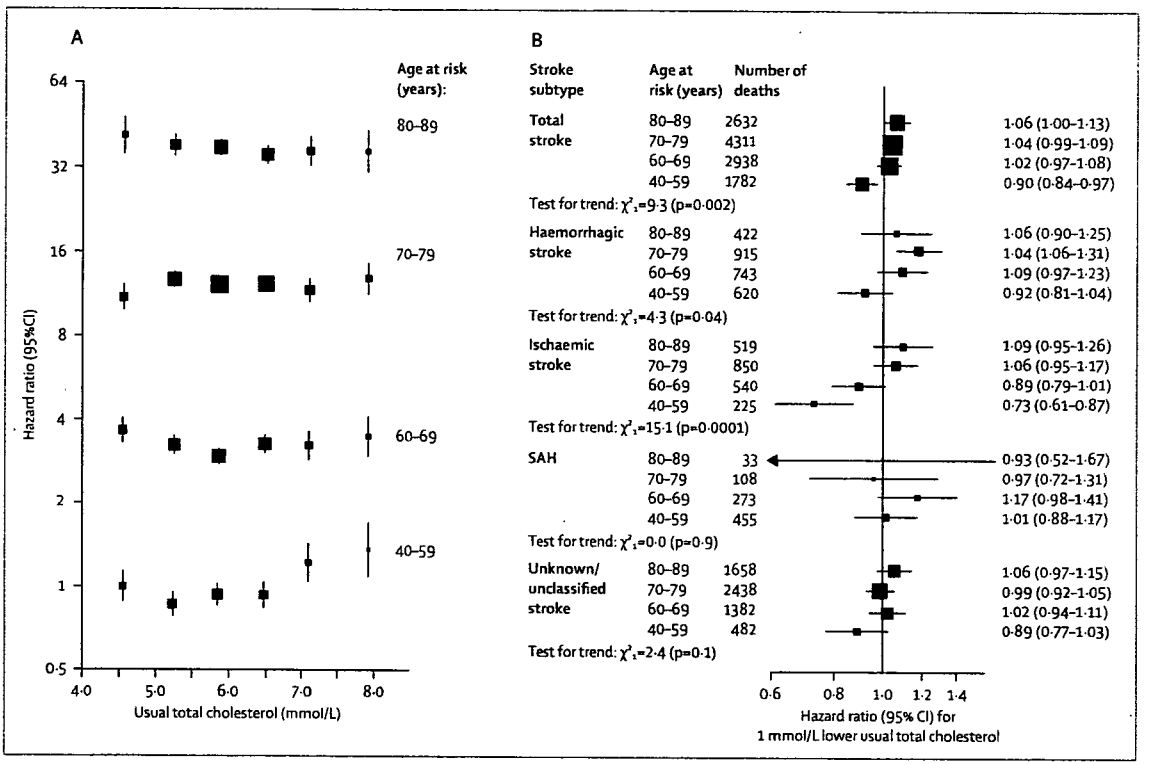

Figure 4: **Stroke mortality (11 663 deaths) versus usual total cholesterol**
(A) age-specific associations for total stroke; and (B) subtype-specific and age-specific hazard ratios for 1 mmol/L lower usual total cholesterol. Conventions as in figure 1. SAH=subarachnoid haemorrhage.

of each other (χ^2_1 for interaction=6·3, which is small compared with the χ^2_1 values of 286 for HDL and 438 for non-HDL cholesterol), so a specific difference in non-HDL cholesterol was associated with a similar HR irrespective of the level of HDL cholesterol, and vice versa (webfigure 6). Hence, the ratio of non-HDL cholesterol to HDL cholesterol is substantially more informative than either alone. (Knowledge of this ratio is, of course, exactly equivalent to knowledge of the simple ratio of total to HDL cholesterol.)

Of the simple indices considered that involve HDL cholesterol, the most informative on its own as a predictor of IHD mortality was the ratio total/HDL cholesterol (χ^2_1 relating IHD mortality to a single baseline measurement=627), which was more than twice as informative as total cholesterol (χ^2_1=277). By comparison, non-HDL cholesterol was only about 60% more informative (χ^2_1=438) than total cholesterol, and the ratio total/HDL cholesterol was 40% more informative than non-HDL cholesterol. Hence, the ratio total/HDL cholesterol is substantially (and highly significantly) more informative about IHD risk than is non-HDL cholesterol (ie, total minus HDL cholesterol), and offers one of the best ways of using measurements of total and of HDL cholesterol to predict risk. Although an optimal linear combination of non-HDL cholesterol and 1/HDL cholesterol can be obtained by entering the two terms jointly into the regression model (yielding χ^2_2=641), the ratio total/HDL cholesterol provides 98% of the informativeness of this combination. Likewise, within each separate age range, the ratio total/HDL cholesterol was the most informative of the simple indices studied, and total cholesterol was the least informative.

There was a weak positive association between total cholesterol and total stroke mortality at ages 40–59 years, but little association at older ages (figure 4). Overall, the type of stroke was reported for only about half of these deaths, with the remainder being strokes of unknown type (either recorded as unknown for a particular individual or from a study that did not record the type at all). There was a weak positive association between total cholesterol and ischaemic stroke in middle age (40–59 years and perhaps also 60–69 years), whereas at older ages there was little or no association of total cholesterol with ischaemic stroke, and a negative association with haemorrhagic stroke. When the MRFIT and PSC findings were combined (table 2, webfigure 7), there was a weak positive association with total stroke at ages 40–69 years but not 70 years and older (and the positive association in middle age can be largely or wholly accounted for by the association of total cholesterol with systolic blood pressure; see discussion). The MRFIT study attributed only 480 deaths to ischaemic

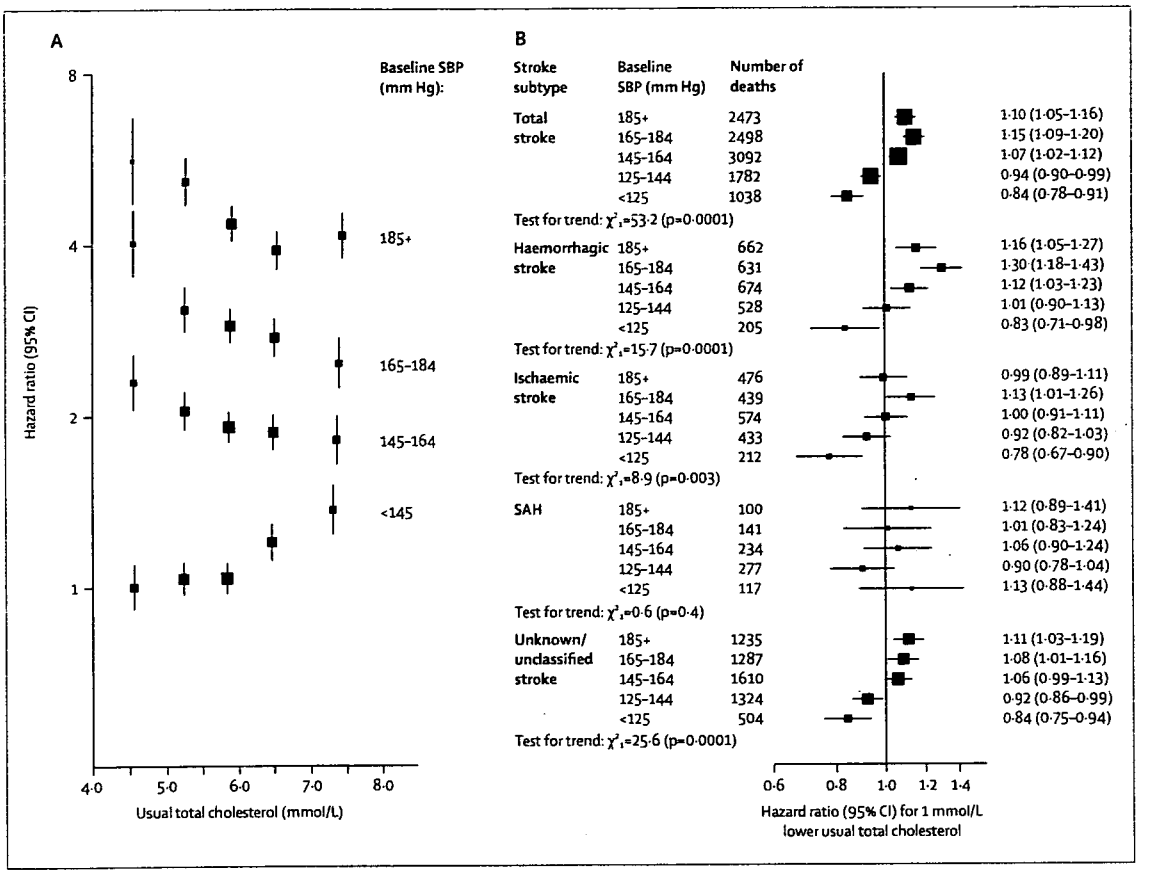

Figure 5: **Stroke mortality (11 663 deaths) versus usual total cholesterol**
(A) SBP-specific associations for total stroke; and (B) subtype-specific and SBP-specific hazard ratios for 1 mmol/L lower usual total cholesterol. Conventions as in figures 1 and 4.

stroke and 945 to haemorrhagic stroke. Webfigure 8 compares the age-standardised associations of cholesterol with mortality for ischaemic and for haemorrhagic stroke in the PSC and in the MRFIT study—for each outcome, the combined results in PSC and MRFIT together are similar to those in PSC alone.

Figure 5 shows the associations in the PSC between stroke mortality and total cholesterol at different levels of baseline systolic blood pressure. In these analyses, there was a positive association between total cholesterol and total stroke only in people with a baseline systolic blood pressure measurement below 145 mm Hg, above which there was a negative association. The negative associations at higher blood pressure levels were especially striking for haemorrhagic stroke (figure 5). These negative associations were not materially altered by exclusion of the first 5 years of follow-up (to minimise reverse causality) or by additional adjustment for smoking or for finer subcategories of systolic blood pressure. When the MRFIT findings were combined with the PSC findings, the number of stroke deaths increased by only 15% in those with systolic blood pressure of 145 mm Hg or greater and, within this systolic blood pressure range, the slope of the graph of total stroke mortality versus cholesterol category was not materially altered (HR per 1 mmol/L lower total cholesterol: 1·08 [95% CI 1·05–1·11] in PSC *vs* 1·07 [1·04–1·10] in both studies together). Similarly, when the MRFIT and PSC findings were combined for those with systolic blood pressure below 145 mm Hg, the hazard ratios for total stroke were not materially altered (HR per 1 mmol/L lower total cholesterol: 0·91 [0·87–0·95] in PSC *vs* 0·90 [0·87–0·94] in both studies together).

Figure 6 shows the age-specific relations between stroke mortality and HDL cholesterol-related variables. There was a weak positive association of stroke mortality with the ratio total/HDL cholesterol at ages 40–69 years (HR 0·86 [0·74–0·99] per 1·33 lower total/HDL cholesterol), but no evidence of an association at older ages, and no statistically significant association in either age range for HDL cholesterol or non-HDL cholesterol considered separately (each trend: $\chi^2_1<2·8$, $p\geq0·1$). For participants in the PSC with data on HDL cholesterol, there were only 145 deaths attributed to ischaemic stroke,

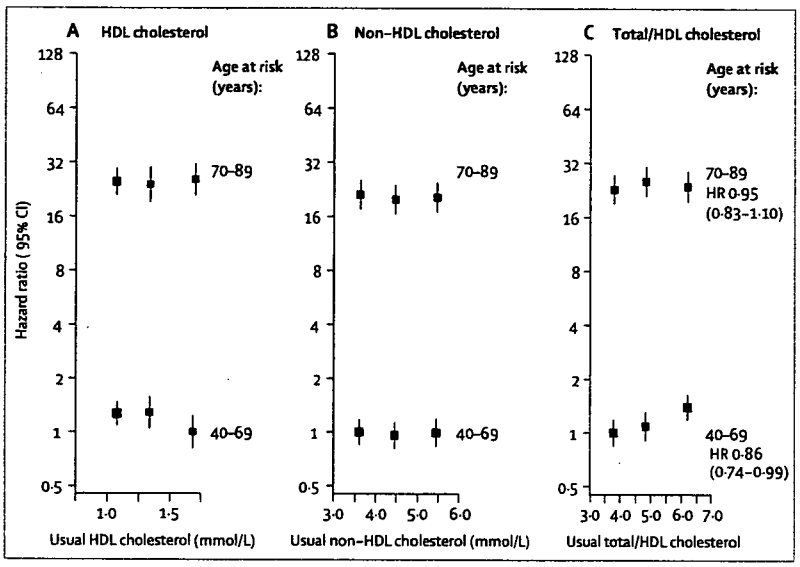

Figure 6: **Stroke mortality (914 deaths) versus usual (A) HDL cholesterol; (B) non-HDL cholesterol; and (C) total/HDL cholesterol**

Age-specific associations. Conventions as in figures 1 and 3. HR denotes the hazard ratio per 1·33 lower total/HDL cholesterol.

231 to haemorrhagic stroke, and 61 to subarachnoid haemorrhage. No material differences between the results for particular stroke subtypes were apparent (data not shown), but the numbers of deaths are too small for these findings to be informative.

Many of the vascular deaths that were not attributed to IHD or stroke may in fact have been directly or indirectly due to one or both of these diseases, and for the aggregate of all other vascular deaths the relative risks in each age range were intermediate between those for IHD and stroke. Taking the combined data from PSC and MRFIT, total cholesterol was positively associated with other vascular mortality in every 10-year age range up to age 80 years, but not at ages 80–89 years (table 2). Further details are shown in webfigures 9–11. A slightly J-shaped association before 70 years of age (webfigure 9) almost disappeared after excluding the first 5 years of follow-up (data not shown), suggesting that it may have been largely or wholly due to reverse causality. Further adjustment for smoking and systolic blood pressure did not materially alter the shapes or strengths of these associations. Parallel analyses of the MRFIT study yielded somewhat stronger associations, but again did not materially alter the age-specific findings (webfigure 10). In men, other vascular mortality was positively associated with total cholesterol in each age range (and the association was highly significant in every range except 80–89 years). In women older than 60 years, however, no positive association was apparent, despite the strongly positive association of female IHD mortality with total cholesterol (webfigure 3). In participants with data for HDL cholesterol, there were 1032 deaths from vascular causes other than IHD or stroke, and there was a positive association with the ratio total/HDL cholesterol that appeared to be steeper in middle age than in old age (webfigure 11).

For the aggregate of all non-vascular causes of death there was a negative association between total cholesterol and mortality (HR 1·10 [1·08–1·11] per 1 mmol/L lower total cholesterol; 42 865 deaths at ages 40–89 years). However, this negative association might be largely or wholly non-causal (eg, a consequence of confounding or of specific non-vascular diseases lowering total cholesterol), since it is weakened when the first 5 years of follow-up are excluded (data not shown), and the randomised trials of statins (which substantially lower total cholesterol) show no adverse effect on cancer or other non-vascular mortality.[5]

For both IHD and stroke, there was statistically significant heterogeneity between the HRs for individual studies (both $p<0·00001$) that could not be accounted for by differences in age, sex, smoking, the type of blood sample collected (fasting or non-fasting; serum or plasma), the method of outcome ascertainment, or the year of baseline survey (data not shown). There was a small trend for the age-specific associations of baseline cholesterol with mortality to be weaker in the studies with longer follow-up and in those that had relied on death certificates alone to code deaths, but allowance for this trend did not materially reduce the heterogeneity. There was, however, little evidence that extreme results from a few atypical studies had appreciably biased the overall results—after excluding successively the studies that contributed most to the heterogeneity (until, for IHD and for stroke, the p values for heterogeneity in the remaining studies were greater than 0·1), the estimated HRs were unaltered to two decimal places.

Discussion

This collaborative meta-analysis of almost 900 000 individuals in 61 prospective observational studies, with 55 000 vascular deaths during nearly 12 million person-years of follow-up, has characterised reliably the age-specific associations of total cholesterol with IHD, stroke, and other vascular mortality, and has assessed the quantitative and qualitative relevance of other risk factors to these associations. For IHD mortality, age and blood pressure substantially affected the strength of the proportional difference in risk associated with a specific difference in cholesterol, but did not affect its direction. For stroke mortality, however, age and blood pressure affected not only the strength but also the direction of the association. (Results for other vascular mortality are intermediate between those for IHD and stroke.) This collaboration has also, on the basis of only 3000 IHD deaths, assessed the independent relevance of HDL cholesterol, showing that the ratio of total to HDL cholesterol is a substantially more informative predictor of IHD mortality than are total cholesterol, HDL cholesterol, or non-HDL cholesterol.

Age substantially attenuates the proportional relation of IHD mortality with cholesterol (table 2, figure 1). However, total cholesterol is a strongly positive risk factor for IHD mortality throughout the main range of measured cholesterol values (about 3·5–9·5 mmol/L, which corresponds to a range of usual cholesterol values of about 4–8 mmol/L) not only in early middle age (when each mmol/L lower usual total cholesterol is associated with a halving of IHD mortality) but also in old age (when it is associated with a sixth lower IHD mortality). Little of the strength of the relation with IHD mortality (table 2) can be accounted for by the association of cholesterol with systolic blood pressure, since a difference of 1 mmol/L in usual cholesterol was associated with a difference of only about 2 mm Hg systolic blood pressure. At ages 60–69 years, for example, 2 mm Hg lower usual systolic blood pressure would be associated with an IHD HR of about 0·94,[15] and allowance for this systolic blood pressure would change the IHD HR of 0·68 in table 2 into 0·72 (ie, 0·68/0·94).

Although the proportional differences in risk decrease with age, the absolute effects of cholesterol on annual IHD mortality rates are much greater at older than at younger ages (see figure 1). For example, the absolute difference in the annual risk of IHD death for a 1 mmol/L difference in total cholesterol was about ten times greater at 80–89 years than at 40–49 years of age. Furthermore, the absolute effects at a specific age were somewhat greater for smokers than for non-smokers, and somewhat greater for obese than for non-obese individuals (since the hazard ratios for these factors were approximately multiplicative with those for cholesterol). At a specific age, however, the absolute effects on IHD mortality of cholesterol and of blood pressure were approximately independent of each other (ie, the absolute effects of cholesterol and blood pressure were roughly additive rather than multiplicative), so blood pressure somewhat attenuated the proportional effects of blood cholesterol on IHD mortality.

HDL cholesterol added greatly to the predictive ability of total cholesterol. (This result differs from the findings of a smaller meta-analysis[20] which suggested no additional advantage in measuring HDL cholesterol.) Higher HDL cholesterol and lower non-HDL cholesterol levels were approximately independently associated with lower IHD mortality, so the ratio of total/HDL cholesterol was substantially more informative about IHD mortality than either, and was more than twice as informative as total cholesterol. Because higher non-HDL cholesterol levels predicted similar relative risks at both above-average and below-average HDL cholesterol levels, the absolute relevance of LDL cholesterol is likely to be greater if HDL cholesterol levels are low. Other lipid-related measurements (eg, of apo B and apo A,[21,22] or of small dense LDL cholesterol particles[22]) may add more predictive power, and more detailed measurements of lipoprotein particles of many different sizes or types could well prove even more informative than any of these measurements.

Although haemorrhage accounts for a substantial proportion of fatal strokes (table 1), for many of the strokes in these studies the type (haemorrhagic or ischaemic) was not verified by a CT or MRI scan or by any other reliable method. Hence, misclassification may have attenuated any real associations with specific types of stroke, particularly at older ages when death certificates become less reliable. (Since all analyses are standardised for age, the relative risks in old age should not be materially biased by selective mortality at earlier ages.) Nevertheless, total stroke mortality and ischaemic stroke mortality in the present analyses were positively associated with total cholesterol only in middle age (table 2, figure 4) and only in those with lower blood pressure (baseline systolic blood pressure less than about 145 mm Hg; figure 5). Moreover, even in middle age, the positive association with stroke mortality was not strong (mortality ratio of 0·93 at ages 60–69 years: table 2), and can be approximately accounted for by the association of each 1 mmol/L usual total cholesterol with about 2 mm Hg systolic blood pressure (since 2 mm Hg lower usual systolic blood pressure would be associated with a stroke hazard ratio of about 0·92 at ages 60–69 years).[15] Even before allowance for systolic blood pressure, total cholesterol was not positively associated with ischaemic stroke mortality at older ages (or at higher levels of blood pressure), and was negatively associated with haemorrhagic and with total stroke mortality in these subgroups. Other observational studies have also suggested that total cholesterol is negatively associated with haemorrhagic stroke in people with high blood pressure.[3,8,23]

By contrast, meta-analyses of the randomised trials of just a few years of statin therapy to lower cholesterol, which greatly reduces the number of circulating LDL particles, have shown that regimens that reduce LDL cholesterol by about 1·5 mmol/L reduce by about a third the incidence not only of IHD but also of ischaemic stroke, approximately independently of age, blood pressure, or prerandomisation blood lipid concentrations (while appearing not to increase the incidence of haemorrhagic stroke).[5] The contrast between the statistically reliable results from randomised trials[5] for stroke and the present statistically reliable observational epidemiological results for stroke is substantial. Further investigation of exactly how lipoprotein particles affect stroke risks might help to explain this striking discrepancy.

Randomised trials of cholesterol-lowering statin therapy in a wide range of patient populations have shown substantial reductions in the incidence of IHD and of stroke.[5,24] In the PSC, the continuous positive relations observed at all ages between total cholesterol and IHD mortality, irrespective of the level of blood pressure, are in keeping with these randomised trial results, and with strategies to lower population levels of LDL cholesterol in all age groups.[25] The absence of any

independently positive association between total cholesterol and stroke mortality in middle age (after allowing for systolic blood pressure) or in those with systolic blood pressure below 145 mm Hg, and the negative association of cholesterol with stroke mortality at older ages or at higher blood pressures, are unexplained, and invite research. Irrespective of the explanation, however, treatment should be guided principally by the definitive evidence from randomised trials,[5] that statins substantially reduce not only coronary event rates but also total stroke rates in patients with a wide range of ages and blood pressures.

Contributors

All members of the writing committee contributed to the collection and analysis of the data, and to the preparation of the report. All collaborators had an opportunity to contribute to the interpretation of the results and to the redrafting of the report. The writing committee accepts full responsibility for the content of this paper.

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Conflict of interest statement

All of the writing committee (except NQ) work in the CTSU, which has a policy of staff not accepting fees, honoraria, or consultancies. The CTSU is, however, involved in clinical trials of cholesterol modification therapy with funding from the MRC, BHF, and/or various companies (Merck, Schering, Solvay) as research grants to (and administered by) Oxford University. NQ works in Oxon Clinical Epidemiology Limited, and has stock options in Glaxo Smith Kline.

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The Presence of *BRAF* Point Mutation in Adult Papillary Thyroid Carcinomas From Atomic Bomb Survivors Correlates With Radiation Dose

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In papillary thyroid carcinogenesis, the constitutively activated mitogen-activated protein (MAP) kinase signaling pathway caused by a genetic alteration such as *RET/PTC* rearrangement or mutation of *RAS* and *BRAF* genes, is thought to be a major early event. Among these, the recently identified $BRAF^{V600E}$ mutation has been found at high frequency in adult patients with papillary thyroid carcinoma (PTC). However, the association between this mutation and radiation exposure in adult PTC is still unknown. In this study, we examined the $BRAF^{V600E}$ mutation in 64 PTCs among adult atomic bomb survivors in Hiroshima, Japan, comprising 17 nonexposed (0 mGy) and 47 exposed patients who developed the carcinoma after the bombing, and assessed the association of $BRAF^{V600E}$ mutation with clinicopathological and epidemiological variables. The median radiation dose in PTCs with the $BRAF^{V600E}$ mutation was significantly lower than that without the mutation (18.5 vs.156.9 mGy, Wilcoxon rank-sum test, $P=0.022$). A significant difference was found in the median latency period (years elapsed from atomic bombing to diagnosis) between exposed patients with and without $BRAF^{V600E}$ mutation (29 vs. 21 yr, Wilcoxon rank-sum test, $P=0.014$). These findings were further confirmed by logistic regression analysis with $BRAF^{V600E}$ mutation status as a dependent variable and taking into account possible interactions between the variables. We found that the log-transformed radiation dose and latency period were independently associated with the $BRAF^{V600E}$ mutation ($P=0.039$ and $P=0.010$, respectively). These results suggest that involvement of *BRAF* mutation in thyroid carcinogenesis in exposed people may differ from that in the nonexposed people.

Key words: $BRAF^{V600E}$ mutation; radiation dose; latency period; thyroid carcinogenesis

INTRODUCTION

Thyroid cancer is well-known to be associated with exposure to external or internal ionizing radiation, such as from the atomic bomb (A-bomb) or the Chernobyl accident. The excess relative risk of thyroid cancer per Sv was 1.15 in the Life Span Study of A-bomb survivors [1], and a strong relationship between thyroid cancer and radiation dose was indicated from the Chernobyl accident [2].

In papillary thyroid carcinogenesis, constitutive activation of the MAP kinase signaling pathway caused by a genetic alteration, including rearrangements of *RET/PTC* or mutation of *RAS* and *BRAF* genes, is thought to be a major early event [3–5]. Among these alterations, the *BRAF* gene mutation in the pathogenesis of papillary thyroid carcinoma (PTC) has recently gained considerable attention.

The *BRAF* gene encodes a serine/threonine kinase responsible for the transduction of signals in the MAP kinase cascade, which leads to the regulation of transcription factors, cytoskeletal elements, and other protein kinases that control cell proliferation [6]. *BRAF* somatic mutations were first discovered in several types of human cancers, including malignant melanomas, and colorectal and ovarian cancers [7].

Abbreviations: MAP kinase, mitogen-activated protein kinase; PTC, papillary thyroid carcinoma; A-bomb, atomic bomb; mGy, milli gray.

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Except for very rare instances, the *BRAF* mutation identified in thyroid cancer so far is almost exclusively the thymine-to-adenine transversion at nucleotide 1799, resulting in the substitution of glutamate for valine at residue 600 (V600E) [8]. The V600E (formerly called V599E) substitution is thought to convert BRAF inactive conformation into its active form by disrupting the residue–residue interaction between the activation loop and the ATP binding site [9]. Recent data on the frequent prevalence of $BRAF^{V600E}$ mutation in thyroid microcarcinomas support the hypothesis that $BRAF^{V600E}$ mutation is an early event in PTC pathogenesis [10], along with the induction of goiter or invasive PTC in $BRAF^{V600E}$ transgenic mice [11].

$BRAF^{V600E}$ mutation has so far been described as occurring with a frequency ranging from 29 to 83% in PTC from adult patients [8]. In order to clarify the role of the $BRAF^{V600E}$ mutation in PTC, associations of the $BRAF^{V600E}$ mutation and clinico-pathological and epidemiological factors in PTC have been examined [8]. Among the common subtypes of PTC, prevalence of the $BRAF^{V600E}$ mutation showed a clear association with histological subtype. The highest frequency was in tall cell PTC (overall 77%), the second highest in conventional PTC (overall 60%), and the lowest in follicular variant PTC (overall 12%) [8], implying the possible role of $BRAF^{V600E}$ mutation in the determination of histology of PTC. The relationship between $BRAF^{V600E}$ mutation frequency and age at diagnosis in adult patients is still controversial. Both a significant correlation [12,13] and no correlation [14–17] have been reported. Thus, presence or absence of $BRAF^{V600E}$ mutation may be a key event for characterization of PTC, as is the case in colon cancer [18].

With regard to the relation to radiation exposure, the $BRAF^{V600E}$ gene mutation was studied in a type of radiation-related PTC, postChernobyl PTC, which is believed to have developed in those exposed to radiation as children. Very low frequencies of $BRAF^{V600E}$ mutation in this PTC have been reported (range: 0–12%) [19–23]. However, the prevalence of $BRAF^{V600E}$ mutation was also low (range: 0–6%) in PTC among children and adolescents who were not exposed to radiation [19,20,23]. A low prevalence of the $BRAF^{V600E}$ mutation has thus been observed in childhood PTC regardless of the presence or absence of past radiation exposure.

On the other hand, the association of the $BRAF^{V600E}$ mutation with radiation exposure has not been studied in adult PTC with history of radiation exposure. Because the prevalence of $BRAF^{V600E}$ mutation has been reported to be high in adult PTC [8], analysis of the association of $BRAF^{V600E}$ mutation with radiation exposure in adult PTCs is particularly important.

In this study, we compared clinico-pathological and epidemiological characteristics of adult PTC among A-bomb survivor patients by $BRAF^{V600E}$ mutation status.

MATERIALS AND METHODS

Tissue Specimens

Study subjects comprised 64 cases of adult PTC found among A-bomb survivors in Hiroshima. Classification of histology was done according to histopathological typing of the World Health Organization [24]. Study materials were formalin-fixed and paraffin-embedded thyroid tissue specimens obtained from the subjects between 2003 and 2005 under approval of the Human Investigation Committee and the Ethics Committee for Genome Research at the Radiation Effects Research Foundation (RERF).

DNA Preparation and Determination of $BRAF^{V600E}$ Mutation

Five-micrometer tissue sections were deparaffinized, stained with Methyl Green (Sigma-Aldrich, St. Louis, MO) and dissected manually or using laser microdissection system Leica AS LMD (Leica, Wetzler, Germany). DNA was extracted from the microdissected noncancerous or cancerous regions using QIAamp DNA Micro kit (QIAGEN, Hilden, Germany). Polymerase chain reaction (PCR) was performed in a 25 μL mixture containing 10 pmoles of each primer, 200 μM of each dNTP, 0.5 U of FastStart High Fidelity DNA polymerase (Roche, Basel, Switzerland), 20–50 ng of genomic DNA, and 1× reaction buffer supplied by the manufacturer. PCR conditions consisted of initial denaturation (95°C for 2 min), followed by 40 cycles (denaturation at 94°C for 30 s, annealing at 54°C for 60 s, extension at 72°C for 30 s). Primers used were 5′-tcatgaagacctcacagtaaaaat-3′ and 5′-tggatccagacaactgttcaa-3′. $BRAF^{V600E}$ mutation was initially screened by restriction fragment length polymorphism (RFLP) using restriction enzyme *Tsp*RI (New England Biolabs, Ipswich, MA) and was confirmed by direct sequencing using DNA sequencer CEQ8000 (Beckman Coulter, Inc., Fullerton, CA).

Statistical Analysis

Univariate analysis for comparison of clinico-pathological and epidemiological variables by radiation exposure or $BRAF^{V600E}$ mutation status was conducted using nonparametric tests (Wilcoxon rank-sum test) for continuous variables, because the distribution of radiation dose and latency period could not be assumed to be symmetrical. Fisher's exact was used for categorical variables. Logistic regression analysis was carried out among A-bomb survivor patients who were exposed to atomic

radiation, and we assessed the relationship between $BRAF^{V600E}$ mutation status and clinico-pathological and epidemiological variables including log- transformed radiation dose, latency period, histology, gender, and age at the time of A-bombing (or age at diagnosis). All statistical analyses were performed with SPSS software (version 12.0).

Radiation Dose

A-bomb radiation doses used in this analysis were estimated by the recently implemented DS02 system [25].

RESULTS

Noting that almost all PTC in A-bomb survivor patients occurred in adults, we examined the $BRAF^{V600E}$ mutation in 64 adult PTC cases among A-bomb survivor patients (cohort members of the Life Span Study) in Hiroshima, Japan, comprising 17 nonexposed (0 mGy) and 47 exposed patients (median dose: 150.7 mGy) who developed carcinoma after the bombing. All thyroid cancer tissue samples used were formalin-fixed, paraffin-embedded surgical specimens resected during 1956–1993. Patient characteristics such as gender, age at the time of A-bombing, age at diagnosis, latency period (years from A-bombing to diagnosis, being defined only for exposed patients), and histological subtypes are summarized in Table 1. All patients analyzed in this study were diagnosed at the age of 20 yr or older. All tumors were well-differentiated PTC including three cases of follicular variant; none was a solid variant.

DNA samples were extracted from microdissected specimens of cancerous or noncancerous tissue. We first conducted screening of $BRAF^{V600E}$ mutation by RFLP, followed by direct sequencing of the fragments to confirm the mutation (Figure 1). The frequency of $BRAF^{V600E}$ mutation in nonexposed patients (71%) was in good agreement with that reported for conventional adult PTC (Table 1) [8].

We then examined whether $BRAF^{V600E}$ mutation status in PTC in all A-bomb survivor patients consisting of both the nonexposed and the exposed people is related to any clinico-pathological or epidemiological characteristics, including radiation dose, age at the time of A-bombing, and age at diagnosis. The relationship between $BRAF^{V600E}$ mutation status and each factor is summarized in Table 2. The median radiation dose in PTC with $BRAF^{V600E}$ mutation was significantly lower than that in PTC without $BRAF^{V600E}$ mutation (Table 2, $P=0.022$). Furthermore, a marginally significant association was found between $BRAF^{V600E}$ mutation status and histological subtype (Table 2, $P=0.062$); all three cases of follicular variant harbored wild-type *BRAF*. This was in agreement with the previous observation of a low prevalence (12%) of $BRAF^{V600E}$ mutation in follicular variant PTC [8]. On the other hand, age at the time of A-bombing, age at diagnosis, and gender did not evidence significant association with $BRAF^{V600E}$ mutation status in the univariate analysis.

Additionally, an analysis on clinico-pathological or epidemiological characteristics including latency period that can be defined only for exposed patients was also undertaken in relation to $BRAF^{V600E}$ mutation with only the 47 exposed patients (Table 2). In addition to the characteristics found to be associated with $BRAF^{V600E}$ mutation status in all patients,

Table 1. Clinico-Pathological and Epidemiological Characteristics of Patients by Radiation Exposure Status

	Nonexposed patients[a] ($n=17$)	Exposed patients ($n=47$)	*P*-value
$BRAF^{V600E}$ mutation			
Present (*n*)	12	26	0.4[c]
Absent (*n*)	5	21	
Frequency (%)	71	55	
Median radiation dose (mGy, range)	0	150.7 (0.4–2758)	
Median latency period[b] (yr, range)	—	26.0 (11–46)	
Median age at the time of atomic-bombing (yr, range)	21.0 (5–52)	25.0 (1–49)	0.4[d]
Median age at diagnosis (yr, range)	48.0 (34–84)	54.0 (20–89)	0.6[d]
Histology			
Conventional PTC (*n*)	17	44	0.6[c]
Follicular variant (*n*)	0	3	
Gender			
Male (*n*)	1	5	1.0[c]
Female (*n*)	16	42	

[a]The nonexposed patients were either those with radiation dose estimated to be 0 mGy or those who were not in the city of Hiroshima at the time of bombing.
[b]Latency period: years from A-bombing to diagnosis.
[c]Fisher's exact test.
[d]Wilcoxon rank-sum test.

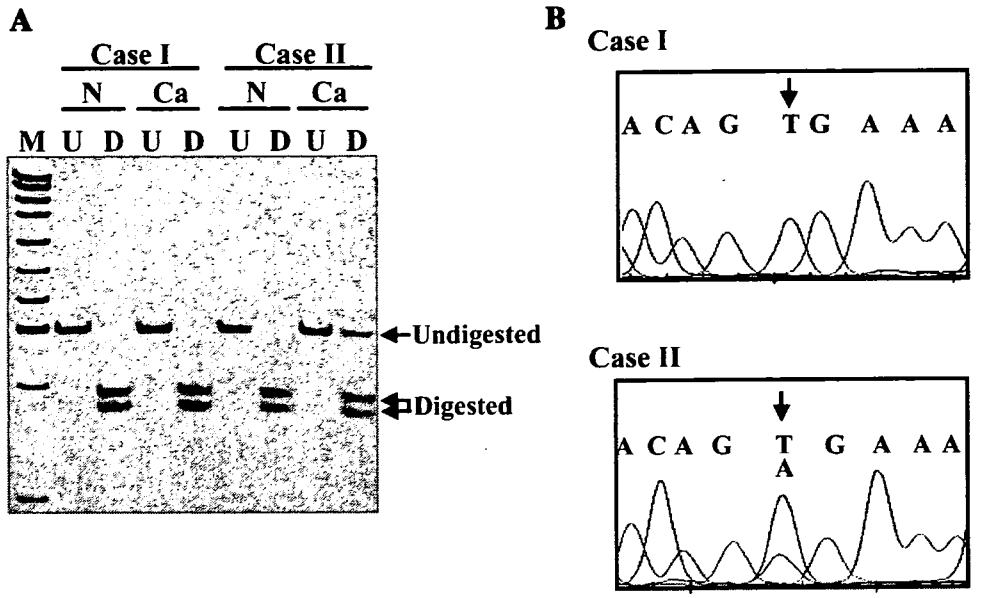

Figure 1. Detection of *BRAF*^{V600E} mutation. (A) RFLP analysis of *BRAF*^{V600E} mutation. DNA fragments containing nucleotide position 1799 were amplified and subsequently digested with restriction enzyme *Tsp*RI, as described in Materials and Methods. Representative results of gel electrophoresis of two samples are shown. N indicates noncancer; Ca, cancer; U and D, undigested and digested with *Tsp*RI; and M, molecular weight marker, respectively. Horizontal arrows indicate positions of undigested- or digested-bands. *Tsp*RI digests wild-type fragments, but not mutated ones. (B) Direct sequencing of PCR fragments. Sequences of the fragments amplified using DNA from tissue specimens of cases I and II are shown. Vertical arrow indicates nucleotide positions 1799. Heterozygous signal of T and A was detected for case II.

latency period also showed a statistically significant association with the mutation; the median latency period in PTC with *BRAF*^{V600E} mutation was significantly longer than that in PTC without the mutation (Table 2, $P=0.014$).

Because these clinico-pathological and epidemiological variables may be interrelated, we further performed multivariate logistic regression analysis for the 47 exposed patients, with *BRAF*^{V600E} mutation status as the dependent variable (Table 3). Log-transformed radiation dose was used as an explanatory variable, because the distribution of log-transformed radiation dose could be assumed to be nearly symmetrical, whereas that of nontransformed radiation dose could not. Log-transformed radiation dose showed a significant inverse association with *BRAF*^{V600E} mutation ($P=0.039$), and latency period revealed a significant positive association ($P=0.010$), confirming the results of univariate analyses shown above. The same result was obtained when age at the time of A-bombing was substituted for age at diagnosis as a variable in the regression analysis.

DISCUSSION

In papillary thyroid carcinogenesis, constitutive activation of the MAP kinase signaling pathway, namely *RET* and *NTRK* tyrosine kinase receptor rearrangements and *RAS* and *BRAF* oncogene activation, seems to be required for transformation [26]. Interestingly, mutual exclusion of these genetic events in the MAP kinase signaling pathway was reported between *BRAF* mutation and *RET/PTC* rearrangements, and between *BRAF* and *RAS* mutations [3–5,21,27–30]. Furthermore, a recently identified *AKAP9-BRAF* rearrangement was not shared with *BRAF* mutation in radiation-associated PTC [22]. Thus, no PTC case possessed more than one of the following mutational events: *BRAF*^{V600E}, *NTRK1* or *RET/PTC* rearrangements [5]. These data suggest that a single genetic event in the MAP kinase signaling pathway may be sufficient for thyroid cell transformation and tumorigenesis. Recent in vitro and in vivo experiments have also demonstrated the requirement of activation of the RET/PTC-RAS-BRAF-MAPK pathway in thyroid tumorigenesis [34–36].

In our study, we found that 71% (12/17) of PTC among nonexposed PTC patients had *BRAF*^{V600E} mutation, indicating that among the several events in the RET/PTC-RAS-BRAF-MAPK pathway, *BRAF*^{V600E} mutation is the most common for non-exposed adult Japanese PTC patients. On the other hand, *BRAF*^{V600E} mutation accounted for only 17% (2/12) of the adult PTC patients who were exposed to radiation dose greater than 500 mGy. These findings suggest that *BRAF*^{V600E} mutation is not a major event in the development of radiation-associated PTCs in adult patients, such as A-bomb survivors with high radiation exposure.

On the other hand, *RET/PTC* rearrangements have been shown to be particularly prevalent in PTCs from

Table 2. Clinico-Pathological and Epidemiological Characteristics of Patients by $BRAF^{V600E}$ Mutation Status

	All patients†			Exposed patients		
$BRAF^{V600E}$ mutation status	Present ($n=38$)	Absent ($n=26$)	*P*-value	Present ($n=26$)	Absent ($n=21$)	*P*-value
Median radiation dose (mGy, range)	18.5 (0–2,758)	156.9 (0–2,304)	0.022*	104.9 (0.4–2,758)	333.4 (0.7–2,304)	0.025*
Median age at the time of atomic-bombing (yr, range)	24 (1–52)	22.5 (3–49)	0.8*	29.5 (1–47)	24 (3–49)	0.9*
Median age at diagnosis (yr, range)	54 (20–89)	51 (29–70)	0.2*	54 (20–89)	51 (29–70)	0.2*
Latency period (yr, range)	—	—		29 (15–46)	21 (11–36)	0.014*
Histology						
Conventional PTC (*n*)	38	23	0.062‡	26	18	0.082‡
Follicular variant (*n*)	0	3		0	3	
Gender						
Male (*n*)	3	3	0.7‡	2	3	0.6‡
Female (*n*)	35	23		24	18	

†Patients consist of 17 nonexposed and 47 exposed patients.
*Wilcoxon rank-sum test.
‡Fisher's exact test.

Table 3. Logistic Regression Analysis of $BRAF^{V600E}$ Mutation Status*

	β[a]	*P*-value
Radiation dose (log_{10} transformed)	−0.979	0.039
Latency period	0.124	0.010
Age at the time of atomic-bombing	0.031	0.4
Gender	−0.685	0.7
Histology	−23.948	1.0

*Analysis was performed only for the 47 exposed patients.
[a]Regression coefficients in the logistic regression model.

post-Chenobyl children and from patients with a history of radiation therapy [31–33]. In addition, *AKAP9-BRAF* rearrangement was recently found in PTCs in post-Chernobyl children [22]. Moreover, in vitro and in vivo experiments have revealed that external X-ray irradiation can induce rearrangement of *RET/PTC1* and *RET/PTC3* in tumor cell lines and human normal thyroid tissue transplanted in scid mice [37–39]. These findings suggest that chromosomal rearrangements may be important in the development of radiation-associated papillary thyroid cancer. Thus, we hypothesize that radiation exposure may influence the selection of an early genetic event; a genetic event other than $BRAF^{V600E}$ mutation in the MAP kinase signaling pathway including chromosomal rearrangements may be involved in the development of PTCs among A-bomb survivors, specifically those exposed to high radiation dose.

We found a significant association of $BRAF^{V600E}$ mutation with latency period (years from A-bombing to diagnosis) among exposed patients (Table 2). Notably, latency period was positively associated with $BRAF^{V600E}$ mutation in logistic regression analysis including age at the time of A-bombing or age at diagnosis as a covariable (Table 3). The low frequency of $BRAF^{V600E}$ mutation in PTCs with short latency period also suggests that a molecular event other than $BRAF^{V600E}$mutation in the MAP kinase signaling pathway may play a major role in the development of PTC among A-bomb survivors.

This study has several limitations: PTC specimens resected before 1956 are unavailable, rendering it impossible to assess PTC developed within 10 yr after the A-bombing. Other molecular events, specifically *RET/PTC* rearrangement and *RAS* mutations, need to be analyzed with an increased number of study subjects. Our findings in this study therefore argue for the need to do further studies to clarify the mechanisms of radiation-associated PTC.

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Prognosis in patients with hepatocellular carcinoma correlates to mutations of *p53* and/or *hMSH2* genes

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ABSTRACT

Association of gene alterations and prognosis has not fully been elucidated in hepatocellular carcinoma (HCC). To clarify the relationship between *p53* and *hMSH2* mutations and prognosis, we analysed these mutations in 83 HCC cases and assessed their association with various clinicopathological factors. The 3-year disease-free survival (DFS) or overall survival (OS) rates in HCC patients with *p53* mutation and *p53* wild/*hMSH2* mutation significantly decreased compared with those without these mutations (14.3% and 37.5% versus 67.5% for DFS; 35.7% and 50.0% versus 96.4% for OS, respectively). In the multivariate analysis, categories by *p53* and *hMSH2* mutation status, and liver cirrhosis demonstrated statistical significances for DFS and OS. Moreover, the frequency of patients with *p53* and/or *hMSH2* mutations in intrahepatic metastasis (75.0%) was significantly higher than that in multicentric occurrence (14.3%). Thus, *p53* and *hMSH2* mutations will be useful for identifying subsets of HCC patients with poor prognosis.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, especially in Asia and Africa. Hepatocarcinogenesis seems to be a multi-step process where normal hepatocyte is transformed through hepatitis, cirrhosis and adenomatous hyperplasia into malignant tumour and then clinical liver cancer.[1] The various risk factors associated with the development of HCC are well known. They mainly include chronic HCV and HBV infection, heavy alcohol intake, prolonged exposure to aflatoxin B1 (AFB1) and metabolic liver diseases such as hemochromatosis. HCC development is closely associated with cirrhosis, and 80–90% of HCC are found in a chronic hepatitis or a cirrhotic liver.

The HCC, as well as precursor benign lesions, have been extensively studied in terms of genetic alteration in the past 10 years. As in other solid tumours, genetic abnormalities including genomic instability, gene alterations and aberrant expression of genes are accumulated during the carcinogenesis process. Indeed, chromosomal aberrations with loss of heterozygosity have been found in many cirrhotic livers and dysplastic nodules as well as HCC.[1–3] Furthermore, genetic alterations including *p53* family, *Wnt* pathways and DNA mismatch repair genes have been detected in the cirrhotic and dyplastic nodules, and HCC.[1,4–7]

P53 behaves as a multifunctional transcription factor involved in the control of cell cycle, programmed cell death, senescence, differentiation, DNA replication, DNA repair

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