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Minireview

Programmed cell death triggered by nucleotide pool damage and its prevention by MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase

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ABSTRACT

Accumulation of oxidized bases such as 8-oxoguanine in either nuclear or mitochondrial DNA triggers various cellular dysfunctions including mutagenesis, and programmed cell death or senescence. Recent studies have revealed that oxidized nucleoside triphosphates such as 8-oxo-dGTP in the nucleotide pool are the main source of oxidized bases accumulating in the DNA of cells under oxidative stress. To counteract such deleterious effects of nucleotide pool damage, mammalian cells possess MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase and related enzymes, thus minimizing the accumulation of oxidized bases in cellular DNA. Depletion or increased expression of the MTH1 protein have revealed its significant roles in avoiding programmed cell death or senescence as well as mutagenesis, and accumulating evidences indicate that MTH1 is involved in suppression of degenerative disorders such as neurodegeneration.

Contents

1. Introduction

Cellular components such as lipids, proteins and nucleic acids are at high risk of being oxidized by reactive oxygen species (ROS). ROS are inevitable byproducts of electron transport in the mitochondria or other normal metabolic pathways and are also generated as useful products for various biological processes such as host defense, neurotransmission, vasodilation and signal transduction. Their production is markedly enhanced by various environmental exposures. Such oxidative damage is considered to be a major cause for various types of cellular dysfunction resulting in cell death or mutagenesis, which may in turn cause degenerative disorders and neoplasms [1].

Organisms are equipped with defense mechanisms to minimize the accumulation of ROS. For example, superoxide dismutases convert superoxide to oxygen and hydrogen peroxide and the latter is further detoxified by peroxidases or catalases. Mice lacking the *SOD2* gene encoding mitochondrial superoxide dismutase have severe abnormalities in development and growth, including cardiomyopathy and neurodegeneration [2]. Once excessive ROS

Abbreviations: 8-oxoG, 8-oxoguanine; 8-oxo-dGTP, 8-oxo-2′-deoxyguanosine triphosphate; 2-OH-A, 2-hydroxyadenine; 2-OH-dATP, 2-hydroxy-2′-deoxyadenosine triphosphate; AIF, apoptosis-inducing factor; BER, base excision repair; NO, nitric oxide; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; SOD, superoxide dismutase; SSBs, single strand breaks.

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accumulates in the cells, these cells can no longer avoid severe oxidative damage. Even in the presence of functional superoxide dismutases, accumulation of oxidized macromolecules in human tissues gradually occurs during normal aging; hence, oxidative damage has been implicated in aging and degenerative disorders and may well be the major cause of these disorders [1].

Among the various types of oxidative damage to cellular macromolecules, damage to nucleic acids is particularly hazardous because of the genetic information present in cellular DNAs (nuclear and mitochondrial), can be altered. Furthermore, oxidized nucleotides can disturb various cellular processes. Such oxidative damage accumulating in cells often results not only in mutagenesis, but also in programmed cell death. The former can initiate carcinogenesis in somatic cells, and mutations fixed in germ lines cause genetic polymorphisms or cause hereditary diseases with a malfunction of the gene(s), while the latter often causes degenerative diseases [3–6].

There are two pathways for the accumulation of oxidized bases in cellular DNA or RNA: one is a result of the incorporation of oxidized nucleotides generated in nucleotide pools while the other is a result of the direct oxidation of bases in DNA or RNA [7]. Recent progress in studies of the sanitization of nucleotide pools, as well as DNA repair, has revealed that the impact of oxidation of free nucleotides is unexpectedly large, in comparison with the direct oxidation of DNA [8]. In this review, we focus on the programmed cell death induced when oxidized purine nucleoside triphosphates are accumulated in the nucleotide pools and how their sanitizing enzyme MTH1 prevents such biological consequence.

2. Oxidation of purine nucleotides and their incorporation into cellular DNA

Among the nucleobases, guanine is known to be the most susceptible to oxidation and its simple oxidized form, 8-oxoguanine (8-oxoG), is one of the major oxidation products in DNA or nucleotides [9]. *In vitro* exposure of the guanine base to H_2O_2 and ascorbic acid or to $Fe(II)^{-}$-EDTA generates 8–9 times more 8-oxoG residues in the nucleotide dGTP than in DNA. Interestingly, the C-8 position of dATP is not oxidized in the treatments; instead, the C-2 position of dATP is oxidized, thus yielding 2-hydroxy-2′-deoxyadenosine triphosphate (2-OH-dATP). However, treatment with $Fe(II)^{-}$-EDTA generates 2-hydroxyadenine (2-OH-A) residues in DNA to as little as 1.5% of the level of 2-OH-A residues that are formed from dATP [10]. Free nucleotides are thus more susceptible to oxidation by ROS than is DNA.

These *in vitro* studies indicated that dGTP is likely to be most susceptible to oxidation by *in vivo* generated ROS, thus generating 8-oxo-dGTP. Although there have been few reports measuring the *in vivo* concentration of 8-oxo-dGTP in the nucleotide pool, it has recently been reported that 8-oxo-dGTP is present at 0.2–2 μM range in the mitochondrial dNTP pools of several rat tissues under normal conditions [11].

It has been established that 8-oxo-dGTP and 2-OH-dATP are frequently misinserted opposite template adenine or guanine, respectively, in DNA by various DNA polymerases for bacterial genomes, and in the nuclear and mitochondrial DNA in mammals, because of their altered base pairing properties [11–18] (Fig. 1A). 8-OxoG pairs with adenine and cytosine at equal efficiency because it prefers the *syn*-form compared with guanine, which takes mostly an *anti*-form and exclusively pairs with cytosine. However, 2-OH-A also can pair with guanine in a *syn*-form in addition to thymine. It has been shown that these oxidized nucleotides indeed increased certain mutations when they were introduced into *Escherichia coli* or mammalian cells [19,20].

As summarized in Fig. 1B, 8-oxo-dGTP is misinserted opposite template adenine as well as cytosine in DNA, thus causing mainly an A:T to C:G transversion mutation after two rounds of replication. 2-OH-dATP tends to be misinserted opposite guanine mostly, thus inducing mainly G:C to T:A transversion mutation.

3. MTH1 is a major oxidized purine nucleoside triphosphatase in mammals

E. coli mutT mutants exhibit the strongest mutator phenotype among all known *E. coli* mutator mutants and the spontaneous occurrence of A:T to C:G transversion mutation increases 1000-fold compared with wild-type. Maki and Sekiguchi demonstrated that the MutT protein hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate, thus sanitizing the nucleotide pool [12]. The MutT protein also efficiently hydrolyzes 8-oxo-GTP and *mutT* mutants accumulate 8-oxoG in DNA and mRNA; 8-oxoG in the latter also results in the production of mutant proteins [21]. The *E. coli* Orf135 protein hydrolyzes 2-OH-dATP [22] and its mutants exhibit a 2-fold increase in the spontaneous occurrence of A:T to C:G transversion. The introduction of 2-OH-dATP, but not 8-oxo-dGTP or other nucleotides, into Orf135 mutants, specifically increases the mutation frequency compared with wild-type [23]. MutT and Orf135 proteins share the nudix (nucleoside diphosphate linked moiety X) motif corresponding to the 23 residues from Gly37 to Gly59 of *E. coli* MutT, which constitute the phosphohydrolase module for hydrolysis of phosphate bonds of the substrates [24,25].

We have identified a human homolog of the MutT protein and designated it as MTH1 (MutT homolog-1) [26–28]. However, it is now referred to as NUDT1 because it is the first identified protein with the nudix motif in eukaryotes. In contrast to MutT, MTH1 efficiently hydrolyzes two forms of oxidized dATP, 2-OH-dATP and 8-oxo-dATP, as well as 8-oxo-dGTP. It also hydrolyzes the corresponding ribonucleotides, 2-OH-ATP, 8-oxo-GTP and 8-oxo-ATP. Among these, MTH1 has the highest affinity to 2-OH-ATP (K_m = 4.3 μM), while the highest catalytic efficiency was observed in 2-OH-dATP (k_{cat}/K_m = 1.68 s^{-1} μM^{-1}) [29,30]. We determined the solution structure of MTH1 by multi-dimensional heteronuclear NMR spectroscopy [31]. The protein adopts a highly similar folding pattern to *E. coli* MutT, despite the low sequence similarity outside the conserved nudix motif [32]. The substrate binding pockets are dissimilar, which might account for the different substrate specificities observed for the two enzymes [33]. Based on the arrangement of the pocket-forming residues, combined with the mutagenesis data, we generated models for the substrate recognition of MTH1 in which Asn-33 and Asp-119 play pivotal roles in discriminating the oxidized form of the purine, namely 8-oxoG and 2-OH-A, while Trp-117 is important for determining the affinity with purine rings [34,35]. Among known proteins with the nudix motif, two other mammalian proteins, MTH2 (NUDT15) and NUDT5, were identified with the potential to hydrolyze either 8-oxo-dGTP or 8-oxo-(d)GDP to 8-oxo-(d)GMP, respectively [36–38]. NUDT5 also hydrolyzes 8-oxo-dADP and to a lesser extent 2-OH-dADP [39]. The discovery of NUDT5 with 8-oxo-(d)GDPase activity, further revealed that MTH1 and MutT can both hydrolyze 8-oxo-GDP [38,40]. MTH1 also recognizes oxidized forms of dATP and ATP as mentioned above. Therefore, we expect that their diphosphate forms can be hydrolyzed by MTH1, suggesting that MTH1 is the most powerful enzyme for the sanitization of nucleotide pools [8] (Fig. 1B). Gene knockdown experiments for MTH1, MTH2 and NUDT5 in cultured human cells revealed that MTH1 deficiency induced an increased occurrence of A:T to C:G transversion mutations when 8-oxo-dGTP was introduced into cells [41].

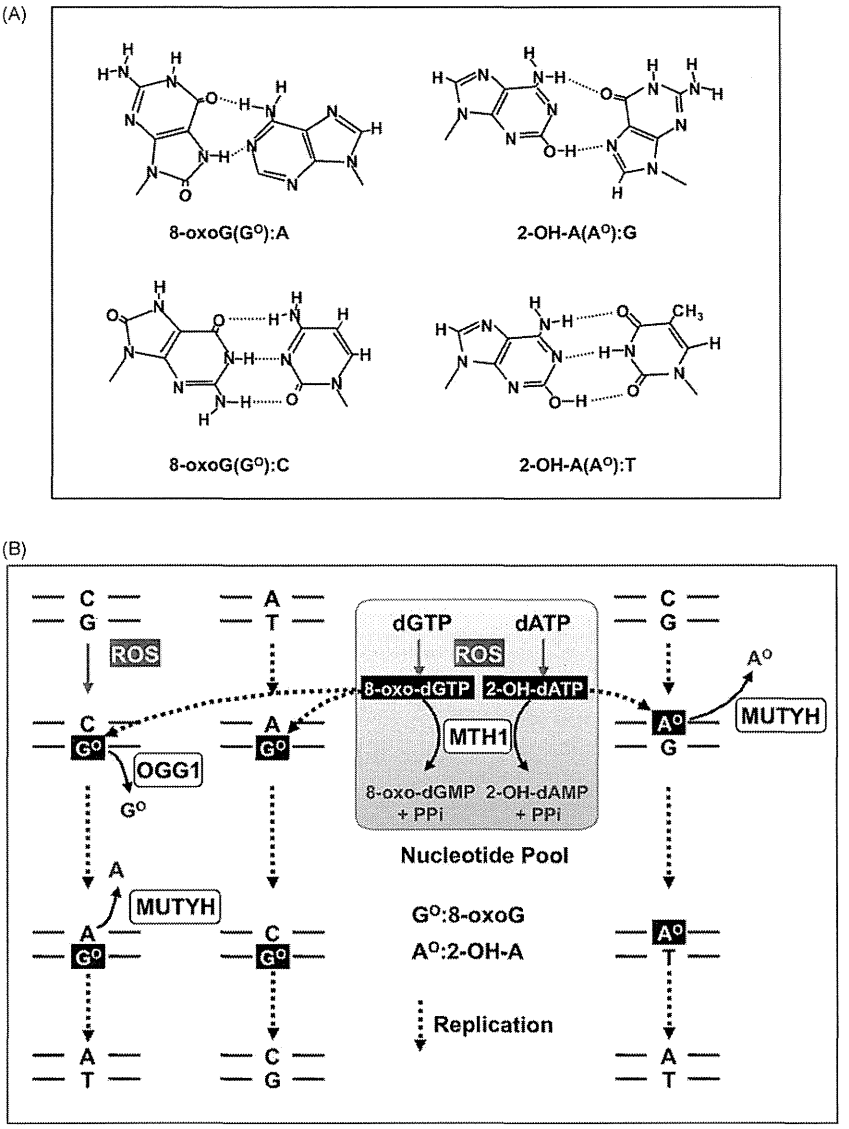

Fig. 1. Altered base pairing and mutagenesis caused by the oxidation of nucleic acids, and defense mechanisms in mammals. (A) Altered base pairing of 8-oxoguanine and 2-hydroxyadenine. During DNA replication, 8-oxoG (G^O) and 2-OH-A (A^O) can pair with adenine (A) and guanine (G) as well as with cytosine (C) or thymine (T), respectively. (B) Mutagenesis caused by 8-oxoG and 2-OH-A. 8-OxoG accumulates in DNA as a result of the incorporation of 8-oxo-dGTP from nucleotide pools or because of the direct oxidation of guanine in DNA. This buildup increases the likelihood of an A:T to C:G or G:C to T:A transversion. On the other hand, 2-OH-A is derived mainly from the incorporation of 2-OH-dATP from nucleotide pools. The accumulation of 8-oxoG or 2-OH-A in DNA is minimized through the coordinated actions of MTH1, OGG1 and MUTYH. See text for details (modified from Ref. [6] with permission).

4. MTH1 deficiency increases susceptibility to cellular dysfunction caused by ROS

We reported that lung adenomas/carcinomas developed spontaneously in 8-oxoG DNA glycosylase 1 (OGG1)-null mice at about 1.5 years after birth, and that 8-oxoG was highly accumulated in their genomes because of the lack of excision repair of 8-oxoG [42]. In that study, we found that no tumor was formed in the lungs of mice lacking both the OGG1 and MTH1 proteins, despite an increased accumulation of 8-oxoG in these mice. This observation suggests that *Mth1* gene disruption resulted in a suppression of the tumorigenesis caused by an OGG1 deficiency. If cell death is caused by the accumulation of a large amount of oxidized purine nucleoside triphosphates in nucleotide pools with MTH1 deficiency, in addition to the accumulation of 8-oxoG in cellular DNA because of the OGG1 deficiency, then cells with premutagenic lesions might not survive to produce precancerous cells with mutations in either proto-oncogenes or tumor suppressor genes. This might be why carcinogenesis is suppressed in mice lacking both the OGG1 and MTH1 proteins [43].

We have demonstrated that MTH1-null mouse embryo fibroblasts (MEF) are highly susceptible to cell dysfunction and death caused by exposure to H_2O_2, with condensed nuclei and degenerated mitochondria in which electron dense deposits were seen in place of intact cristae [44]. The cell death observed was not dependent on either poly(ADP-ribose) polymerase or caspases. A continuous accumulation of 8-oxoG, both in the nuclear and mitochondrial DNA, was observed after exposure to H_2O_2. All of the H_2O_2-induced alterations observed in MTH1-null MEFs were effectively suppressed by the expression of wild-type human MTH1 (hMTH1), while they were only partially suppressed by the expression of mutant hMTH1 which possessed either only 8-oxo-dGTPase

or 2-OH-dATPase activity. MTH1 thus protects the cells from H_2O_2-induced cell dysfunction and death by hydrolyzing oxidized purine nucleotides.

It has been shown that hMTH1 depletion in p53-proficient human cancer-derived or SV40-transformed cell lines promotes H_2O_2-induced apoptosis through a Noxa- and caspase-3/7-mediated signaling pathway [45]. In contrast, hMTH1 depletion in primary human cells results in rapid cellular senescence with an increased accumulation of 8-oxoG in genomic DNA and upregulation of tumor suppressor genes including *p53*, especially under high oxygen tension (20%) [46]. In both cases, nuclear accumulation of γ-H2AX immunoreactivity was observed, suggesting that incorporation of 8-oxoG into nuclear DNA results in double-strand breaks, thus inducing p53-dependent responses. These results indicate that the nucleotide pool is a critical target of intracellular ROS and that oxidized nucleotides, unless continuously eliminated, can rapidly induce programmed cell death or senescence [8].

5. Two distinct pathways of cell death are triggered by 8-oxoG accumulating in nuclear and mitochondrial DNAs

Under oxidative stress conditions, generation of 8-oxo-dGTP in the nucleotide pool as well as direct oxidation of guanine in DNA results in the increased accumulation of 8-oxoG in nuclear and mitochondrial DNAs [44,47], thus inducing programmed cell death or senescence (Fig. 2). However, it is not clear which form of DNA is involved—nuclear or mitochondrial—or how such programmed processes are executed. To distinguish the biological effects of 8-oxoG accumulation in nuclear or mitochondrial DNA, we established cells that accumulate 8-oxoG selectively in either type of DNA by expression of a nuclear or mitochondrial form of human OGG1 proteins. These selectively excise 8-oxoG opposite cytosines in DNA in OGG1-null mouse cells [48,49]. The increased accumulation of 8-oxoG in nuclear DNA caused poly(ADP-ribose) polymerase (PARP)-dependent nuclear translocation of apoptosis-inducing factor (AIF). On the other hand, the increased accumulation of 8-oxoG in mitochondrial DNA caused mitochondrial dysfunction followed by Ca^{2+} efflux and activation of calpains. Both types of cell death were accompanied by increased accumulation of single strand breaks (SSBs) in the respective DNAs. These were suppressed by knockdown of MUTYH that excises adenine inserted opposite 8-oxoG in DNA during replication, thus initiating base excision repair (BER). Recently, it has been shown that DNA polymerase λ efficiently insert cytosine opposite 8-oxoG after adenine excision by MUTYH, thus ensuring the faithful repair of A:8-oxoG mispairs [50]. Under increased accumulation of 8-oxoG in template DNA, however, MUTYH might induce futile BER because an adenine can be reinserted opposite an 8-oxoG during BER, thus causing accumulation of SSBs in the nascent strand [51] (Fig. 2A). Knockdown of MUTYH resulted in escape from both types of cell death, indicating that MUTYH functions as a molecular switch for the two types of programmed cell death when 8-oxoG accumulates in either nuclear or mitochondrial DNA. These results indicate that MUTYH-dependent excision of adenines paired with 8-oxoGs lead to the accumulation of SSBs in each type of DNA [48]. SSBs accumulating in nuclear DNA activate PARP followed by nuclear translocation of AIF, thus executing cell death [52,53] (Fig. 2B). In contrast, SSBs accumulating in mitochondrial DNA results in their degradation, and in mitochondrial dysfunctions such as ATP depletion and opening the membrane permeability transition pore. These lead to Ca^{2+} efflux from mitochondria causing activation of the Ca^{2+}-dependent proteases, calpains, in the cytoplasm. Activated calpains induce lysosomal rupture and cell death [54,55] (Fig. 2C).

We recently found that mice lacking MUTYH, OGG1 and MTH1 proteins are highly susceptible to the rapid development of various types of spontaneous tumors (our unpublished data), thus demonstrating that MUTYH-dependent programmed cell death is why mice lacking both OGG1 and MTH1 proteins do not develop the lung tumors observed in mice lacking only the OGG1 protein.

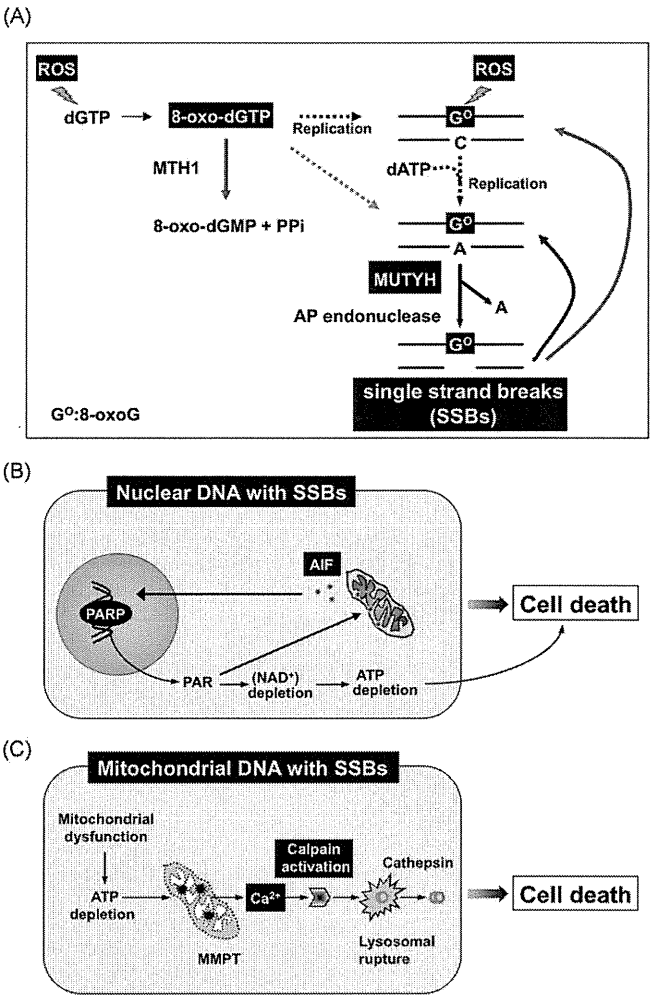

Fig. 2. MUTYH-dependent programmed cell death triggered by accumulation of 8-oxoguanine in nuclear and mitochondrial DNA. (A) Reactive oxygen species (ROS) oxidize dGTP in the nucleotide pool and, to a lesser extent, guanine in DNA. 8-Oxo-dGTP escaping from hydrolysis by MTH1 is utilized by DNA polymerases as a substrate for DNA synthesis, thus increasing the accumulation of 8-oxoG (G^O) in DNA. During the next round of replication, adenine (A) can be inserted opposite 8-oxoG in DNA, MUTYH excises the adenine in the nascent strand and AP endonucleases incise the abasic sites. Cytosine (C) or adenine may be inserted opposite 8-oxoG during repair replication; however, insertion of adenine causes futile cycle of the base excision repair (BER), thus accumulating single strand breaks (SSBs) in the nascent strand when 8-oxoG accumulates to a large extent in the template DNA. (B) When 8-oxoG accumulates highly in nuclear DNA, poly(ADP-ribose) polymerase (PARP) binds the SSBs generated by MUTYH-initiated BER, thus increasing poly(ADP-ribosyl)ation (PAR) resulting in nuclear translocation of apoptosis-inducing factor (AIF) in mitochondria. AIF executes apoptotic cell death with large chromosomal DNA fragmentation. (C) 8-OxoG accumulated highly in mitochondrial DNA causes degradation of mitochondrial DNA through MUTYH-initiated BER, thus causing mitochondrial dysfunction. Mitochondrial membrane permeability transition (MMPT) initiated by ATP depletion causes Ca^{2+} efflux from mitochondria, thus an increased Ca^{2+} in the cytoplasm activates calpains, which in turn cause lysosomal rupture to execute cell death (modified from Ref. [48] with permission).

6. Oxidation of the nucleotide pool for mitochondrial DNA causes MUTYH-dependent cell death

We reported that both 8-oxoG accumulation and the expression levels of MTH1 are highly increased in the cardiovascular tissues of a rat model of genetic hypertension compared with control rats,

suggesting that the oxidation of nucleotide pools may play a role in the development of hypertension [56]. Cardiovascular tissues are constitutively exposed to nitric oxide (NO), a vasodilator and neurotransmitter, which produces peroxynitrite in the presence of superoxide [1]. Peroxynitrite itself produces the hydroxyl radical, which is known to vigorously oxidize nucleic acids *in vitro*; however, it has not been clear whether or how NO participates in the oxidation of nucleic acids *in vivo* [57].

We examined whether hMTH1 would prevent cellular dysfunction induced by sodium nitroprusside, a spontaneous NO donor [58]. Exposure caused 8-oxoG accumulation in the DNA of proliferating MTH1-null cells, which underwent mitochondrial degeneration and subsequently died. Quiescent MTH1-null cells also died with the 8-oxoG accumulation but only when it affected mitochondrial and not nuclear DNA. In both proliferative and quiescent conditions, the accumulation of 8-oxoG in DNA and the consequent cell death were effectively prevented by hMTH1 treatment. Knockdown of MUTYH in quiescent MTH1-null cells significantly reduced cell death, suggesting that 8-oxoG incorporated into mitochondrial DNA is a main cause of this form of cell death. To verify this possibility, an artificially modified hMTH1 with a mitochondrial targeting peptide (mTP), namely mTP-EGFP-hMTH1, which localizes exclusively in mitochondria, was expressed in MTH1-null cells [58]. mTP-EGFP-hMTH1 selectively prevented the accumulation of 8-oxoG in mitochondrial, but not nuclear DNA, after exposure of proliferating cells to NO and also efficiently prevented cell death. We thus conclude that exposure of cells to NO causes oxidation of mitochondrial deoxynucleotide pools and that the buildup of oxidized bases in mitochondrial DNA initiates cell death.

It is likely that the accumulation of 8-oxoG in nuclear DNA by the incorporation of 8-oxo-dGTP from the nucleotide pools does not induce acute cell death [58]. The MUTYH protein in mammalian cells functions in a replication-coupled manner by association with proliferating cell nuclear antigen (PCNA), replication protein A (RPA) and MutS homolog 6 (MSH6) in the nucleus [59–61] and the levels of MUTYH in the nucleus increased 3- to 4-fold during progression of the cell cycle and reached maximum levels in S phase compared with levels in early G1 and that MUTYH was localized at the site of DNA replication [62]. Therefore, MUTYH in nuclei selectively recognizes and excises adenine inserted into the nascent strand opposite template 8-oxoG in DNA, but not the template adenine that pairs with 8-oxoG in nascent strand derived from 8-oxo-dGTP in the nucleotide pool. Thus, 8-oxoG derived from nucleotide pool may not result in accumulation of SSBs through MUTYH-initiated BER. It is likely that mismatch repair might recognize 8-oxoG inserted opposite template adenine in DNA [63] and OGG1 also excises 8-oxoG inserted opposite template cytosine in DNA [64,65]. However, these processes are not so efficient because 8-oxoG level in nuclear DNA in the absence of MTH1 is still high 24 h after exposure to NO, which might cause delayed cell death through further replication (Fig. 2A and B).

In mitochondria, MUTYH might function independently of replication because mitochondria lack replication coupling factors such as PCNA [43]. It has been shown that the bacterial MutY protein can excise an adenine opposite an 8-oxoG regardless of the origin of the adenine base; the template adenine that pairs with an 8-oxoG in the nascent strand derived from 8-oxo-dGTP in the nucleotide pool (Fig. 2A: gray dotted line), or adenine inserted into the nascent strand opposite template 8-oxoG [66]. Therefore, in mitochondria, MUTYH can excise adenine opposite 8-oxoG regardless of their origin, as does bacterial MutY. We thus suggest that the accumulation of 8-oxoG in mitochondrial DNA in the absence of MTH1 results in excess formation of SSBs in both strands of DNA through MUTYH-initiated BER. This would cause double-strand breaks and thereby induce mitochondrial degeneration followed by cell death (Fig. 2A and C), particularly when cells are exposed to excess NO under conditions of inflammation or excitotoxicity [58,67].

7. Neuronal accumulation of 8-oxoG causes neurodegeneration, which can be suppressed by MTH1

Oxidatively damaged bases, such as 8-oxoG accumulates in both nuclear and mitochondrial DNAs during aging [44,68,69] and such accumulation appears to increase dramatically in patients with various neurodegenerative diseases, such as Parkinson's disease (PD) [70,71], Alzheimer's disease (AD) [72,73] or amyotrophic lateral sclerosis (ALS) [74,75]. We have shown that a significant increase of 8-oxoG in mitochondrial DNA was accompanied by an elevated expression of MTH1 [71], the mitochondrial form of OGG1 (OGG1-2a) [76] and an N-terminally truncated form of MUTYH encoded by an alternatively spliced *MUTYH* mRNA in the substantia nigra neurons of patients with PD [77]. In postmortem tissue specimens from patients with AD, the expression levels of MTH1 in the entorhinal cortex were also elevated, whilst the levels of MTH1 apparently decreased in the stratum lucidum at CA3, corresponding to mossy fiber synapses, where MTH1 was highly expressed in the control subjects [78]. In contrast, expression level of OGG1-2a was found to decrease in the orbitofrontal gyrus and the entorhinal cortex in patients with AD compared with control subjects [79]. The accumulation of 8-oxoG was increased in most of the large motor neurons in patients with ALS, with a decreased expression of OGG1-2a but not MTH1. It is thus likely that OGG1-2a is indeed unstable under increased oxidative stress, compared with MTH1 [75].

We reported that the levels of 8-oxoG in cellular DNA and RNA increased in the mouse nigrostriatal system during tyrosine hydroxylase (TH)-positive dopamine neuron loss induced by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [80]. In contrast to wild-type mice, MTH1-null mice exhibited a greater accumulation of 8-oxoG in mitochondrial DNA, accompanied by a more significant decrease in TH- and dopamine transporter-positive fibers in the striatum after MPTP administration [80]. We thus demonstrated that MTH1 indeed protects the dopaminergic neurons from oxidative damage in nucleotide pools. This was especially effected by preventing 8-oxoG accumulation in the mitochondrial DNA of striatal nerve terminals of dopaminergic neurons [81], which is likely to cause mitochondrial dysfunction through the MUTYH-initiated BER as shown in Fig. 2A and C.

Recently, a transgenic mouse has been established in which the human MTH1 is expressed [82]. Wild-type mice exposed to 3-nitropropionic acid, an inhibitor for mitochondrial succinate dehydrogenase, develop neuropathological and behavioral symptoms that resemble those of Huntington's disease, with an increased 8-oxoG accumulation in medium spiny neurons in striatum. hMTH1 transgene expression conferred a dramatic protection against these Huntington's disease-like symptoms, including weight loss, dystonia and gait abnormalities, striatal degeneration and death [82]. The findings indicate that oxidized nucleoside triphosphates such as 8-oxo-dGTP accumulating in nucleotide pools in medium spiny neurons have a significant contribution to their degeneration.

Enhanced oxidative stress has been implicated in the excitotoxicity of the central nervous system and 8-oxoG was reported to be accumulated in the rat hippocampus after administration of kainate, an excitotoxin for glutamate receptors [83]. We reported that the 8-oxoG levels in mitochondrial DNA and cellular RNA increased significantly in the CA3 subregion of the mouse hippocampus 6–12 h after kainate administration but returned to basal levels within a few days [67]. 8-OxoG accumulation in mitochondrial DNA was remarkable in CA3 microglia, whereas that in nuclear DNA or cellular RNA was also detected in the CA3 pyrami-

dal cells and astrocytes. MTH1-null and wild-type mice exhibited a similar degree of CA3 neuron loss after kainate administration; however, the 8-oxoG levels that accumulated in mitochondrial DNA and cellular RNA in the CA3 microglia increased significantly in the MTH1-null mice in comparison with wild-type mice [67]. This demonstrated that MTH1 efficiently suppresses the accumulation of 8-oxoG in both cellular DNA and RNA in the hippocampus—especially in microglia—caused by the excitotoxicity that plays a major role during neurodegeneration [84].

We examined the expression levels of MTH1 and OGG1 in the mouse hippocampus after kainate administration. The *Mth1* mRNA level decreased soon after kainate administration and then quickly recovered beyond the basal level. A continuously raised MTH1 protein level was observed, whereas the *Ogg1* mRNA level remained constant [67]. These results may indicate that oxidative stress in brain induces expression of MTH1 especially in microglia, thus avoiding cellular dysfunction.

8. Future perspectives

Oxidative DNA damage has been considered as one of major threats for organisms, causing mutagenesis and carcinogenesis [5]. Because bases of free nucleotides in the nucleotide pools are more susceptible to oxidation by ROS, compared with those in DNA, oxidized nucleotides generated in the nucleotide pools have greater impact as causes for mutagenesis through their incorporation into DNA. Beyond mutagenesis, the incorporation of oxidized nucleotides into nuclear or mitochondrial DNA from the damaged nucleotide pools triggers programmed processes resulting in cell death or senescence. Such programmed processes are involved in tumor suppression or neurodegeneration in animal models [67,80,82]. MTH1, a major sanitizing enzyme for oxidized nucleotide pools plays a crucial role by suppressing their accumulation in cellular DNA. In addition to oxidized purine deoxyribonucleoside triphosphates, MTH1 efficiently hydrolyzes oxidized purine ribonucleoside triphosphates such as 2-OH-ATP, 8-oxo-ATP and, to a lesser extent, 8-oxo-GTP. As a result, cellular dysfunction may also be caused by their incorporation into RNA. Alternatively, such oxidized purine ribonucleoside triphosphates might interfere with various pathways of signal transduction or metabolisms in which ATP or GTP function as essential mediators of co-factors, thus suggesting that free forms of oxidized purine nucleotides might themselves exert a certain degree of cytotoxicity.

Conflict of interest statement

There is no conflicting interest.

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Glucose tolerance status and risk of dementia in the community

The Hisayama Study

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ABSTRACT

Objective: We investigated the association between glucose tolerance status defined by a 75-g oral glucose tolerance test (OGTT) and the development of dementia.

Methods: A total of 1,017 community-dwelling dementia-free subjects aged ≥60 years who underwent the OGTT were followed up for 15 years. Outcome measure was clinically diagnosed dementia.

Results: The age- and sex-adjusted incidence of all-cause dementia, Alzheimer disease (AD), and vascular dementia (VaD) were significantly higher in subjects with diabetes than in those with normal glucose tolerance. These associations remained robust even after adjustment for confounding factors for all-cause dementia and AD, but not for VaD (all-cause dementia: adjusted hazard ratio [HR] = 1.74, 95% confidence interval [CI] = 1.19 to 2.53, $p = 0.004$; AD: adjusted HR = 2.05, 95% CI = 1.18 to 3.57, $p = 0.01$; VaD: adjusted HR = 1.82, 95% CI = 0.89 to 3.71, $p = 0.09$). Moreover, the risks of developing all-cause dementia, AD, and VaD significantly increased with elevated 2-hour postload glucose (PG) levels even after adjustment for covariates, but no such associations were observed for fasting plasma glucose (FPG) levels: compared with those with 2-hour PG levels of <6.7 mmol/L, the multivariable-adjusted HRs of all-cause dementia and AD significantly increased in subjects with 2-hour PG levels of 7.8 to 11.0 mmol/L or over, and the risk of VaD was significantly higher in subjects with levels of ≥11.1 mmol/L.

Conclusions: Our findings suggest that diabetes is a significant risk factor for all-cause dementia, AD, and probably VaD. Moreover, 2-hour PG levels, but not FPG levels, are closely associated with increased risk of all-cause dementia, AD, and VaD. ***Neurology*® 2011;77:1126–1134**

GLOSSARY

AD = Alzheimer disease; **CI** = confidence interval; ***DSM-III-R*** = *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised; **FPG** = fasting plasma glucose; **HR** = hazard ratio; **IFG** = impaired fasting glycemia; **IGT** = impaired glucose tolerance; **NGT** = normal glucose tolerance; **OGTT** = oral glucose tolerance test; **PG** = postload glucose; **VaD** = vascular dementia.

Diabetes mellitus is one of the most common metabolic disorders, and its prevalence has risen globally in recent years. Some epidemiologic studies have reported that diabetes is independently implicated in the development of dementia.[1–3] However, these findings are inconsistent for its subtypes; one study found an association between diabetes and the risk of both Alzheimer disease (AD) and vascular dementia (VaD),[1] whereas other studies found an association with only AD[2,3] or only VaD,[4–7] and still others showed no association between diabetes and either condition.[8,9] These conflicting results may have been related to differences in the study designs, including the defined criteria for diabetes and dementia subtypes, as well as in the regional characteristics and ethnicities of the settings and subjects. Thus, accurate definitions of diabetes and dementia subtypes are needed to ascertain the true associations between the two, and a 75-g oral glucose tolerance test (OGTT) and morphologic examination of the brain may meet this requirement. However, to date, very few cohort studies have had enough quality data to allow reliable diagnosis using these methods.

Supplemental data at www.neurology.org

Supplemental Data

From the Departments of Environmental Medicine (T.O., Y.D., T.N., Y.H., J.H., Y.K.), Neuropsychiatry (T.O., S.K.), Medicine and Clinical Science (Y.D., T.N., Y.H., J.H.), and Neuropathology (T.I.), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

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To resolve these issues, we performed a prospective cohort study of dementia in a Japanese community-dwelling population, all members of which underwent the OGTT. The most important feature of this study is that the subtypes of dementia were verified by detailed neurologic and morphologic examination, including neuroimaging and autopsy. Using data from this cohort study, we investigated the association between glucose tolerance levels defined by the OGTT and the development of dementia and its subtypes.

METHODS

Study population. A population-based prospective study of cerebro-cardiovascular diseases was begun in 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area of Kyushu Island in Japan. In addition, comprehensive surveys of cognitive impairment in the elderly of this town have been conducted since 1985. In 1988, a total of 1,228 residents aged ≥60 years (91.1% of the total population in this age group) participated in a screening examination for the present study. After exclusion of 33 subjects who had dementia, 90 who had already had breakfast, 5 who were on insulin therapy, and 81 who could not complete the OGTT, a total of 1,019 subjects without dementia underwent the OGTT. From a total of 1,019 subjects, 2 who died before starting follow-up were excluded, and the remaining 1,017 subjects (437 men and 580 women) were enrolled in this study.

Follow-up survey. The subjects were followed up prospectively for 15 years, from December 1988 to November 2003 (mean 10.9 years; SD 4.1 years). A complete description of the follow-up survey is provided in appendix e-1 on the *Neurology*® Web site at www.neurology.org.

Diagnosis of dementia. The diagnosis of dementia was made based on the guidelines of the *DSM-III-R*.[10] Subjects diagnosed with AD met the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria[11] and subjects diagnosed with VaD met the National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria.[12] Possible or probable dementia subtypes were diagnosed with clinical information including neuroimaging. Definite dementia subtypes were also determined on the basis of clinical and neuropathologic information. The diagnostic procedure for autopsy cases was reported previously.[13] A neuropathologic diagnosis of AD was made following the National Institute on Aging–Reagan Institute criteria,[14] where the frequency of neuritic plaques and neurofibrillary tangles was evaluated using the Consortium to Establish a Registry for Alzheimer's Disease criteria[15] and Braak stage.[16] Definite VaD cases were confirmed with causative stroke or cerebrovascular change and no neuropathologic evidence of other forms of dementia. Every dementia case was adjudicated by expert psychiatrists.

During the follow-up, 232 subjects (79 men and 153 women) developed dementia. Of these, 201 (86.6%) were evaluated by brain imaging, and 118 (50.9%) underwent brain autopsy; in 110, both were performed. Thus, 209 subjects in all (90.1%) had some kind of morphologic examination. Among the 118 autopsy cases, the clinical diagnosis of 42 cases (35.6%) was changed by the neuropathologic findings. Among all dementia cases, 18 AD cases and 11 VaD cases had other coexisting subtypes of dementia. These cases were counted as events in the analysis for other dementia. In all, 105 cases were categorized as AD, 65 as VaD, and 62 as other dementia.

Risk factor measurement. At the baseline examination, we performed the OGTT after an at least 12-hour overnight fast. Plasma glucose levels were determined by the glucose-oxidase method. Glucose tolerance status was defined by the 1998 WHO criteria[17]: normal glucose tolerance (NGT), fasting plasma glucose (FPG) <6.1 and 2-hour postload glucose (PG) <7.8; impaired fasting glycemia (IFG), FPG 6.1 to 6.9 and 2-hour PG <7.8; impaired glucose tolerance (IGT), FPG <7.0 and 2-hour PG 7.8 to 11.0; and diabetes, FPG ≥7.0 mmol/L or 2-hour PG ≥11.1 mmol/L. Each of the FPG and 2-hour PG level was also divided into 4 categories (FPG: <5.6, 5.6 to 6.0, 6.1 to 6.9, and ≥7.0 mmol/L; 2-hour PG: <6.7, 6.7 to 7.7, 7.8 to 11.0, and ≥11.1 mmol/L).

In order to assess the independent effects of glucose tolerance levels on dementia occurrence, the following baseline factors in addition to age and sex were used as confounding factors: 1) information on smoking habits, alcohol intake, and physical activity was obtained by means of a questionnaire administered to each subject; 2) a low education level was defined as ≤6 years of formal education; 3) history of stroke was determined on the basis of all clinical data available in the Hisayama Study; 4) hypertension was defined as blood pressure levels ≥140/90 mm Hg or current treatment with antihypertensive agents; 5) EKG abnormalities were defined as left ventricular hypertrophy (Minnesota Code 3-1), ST depression (4-1, 2, or 3) or atrial fibrillation (8-3); 6) serum total cholesterol levels were measured enzymatically; and 7) body mass index (kg/m^2) and waist to hip ratio were used as indicators of obesity.

Statistical analysis. The SAS software package, version 9.2 (SAS Institute, Cary, NC), was used to perform all statistical analyses. Age- and sex-adjusted mean values of possible risk factors were calculated by the analysis of covariance method. Frequencies of risk factors were adjusted for age and sex by the direct method. The differences in the mean values and frequencies of risk factors between NGT and other glucose tolerance levels were tested using Fisher least significant difference method and logistic regression analysis, respectively. The incidence of dementia was calculated by the person-years method and was adjusted for age and sex by the direct method using 5-year age groups of the overall study population; the differences among glucose tolerance levels and trends across FPG and 2-hour PG levels were tested using Cox proportional hazards model. The adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs) were also calculated using the Cox proportional hazards model. Missing values of waist to hip ratio (n = 27) and education (n = 12) were replaced with the means in the multivariate analysis. The population attributable fraction of combined category of IGT and diabetes for dementia was calculated using the following equation with the observed multivariate-adjusted HR of the combined category and its frequency in event cases (Pe)[18]:

$$PAF = Pe\,(HR - 1)/HR$$

Two-sided $p < 0.05$ was considered statistically significant in all analyses.

Standard protocol approvals, registrations, and patient consents. This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Re-

Table 1 Age- and sex-adjusted mean values or frequencies of potential risk factors for dementia according to the 1998 WHO criteria: The Hisayama Study, 1988[a]

	Normal glucose tolerance (n = 559)	Impaired fasting glycemia (n = 73)	Impaired glucose tolerance (n = 235)	Diabetes (n = 150)	No. of missing values
Age, y, mean (SD)	68 (6)	70 (6)[b]	69 (6)	69 (6)	0
Men, %	40.8	52.1	43.8	45.3	0
Fasting plasma glucose, mmol/L, mean (SD)	5.3 (0.9)	6.4 (0.9)[c]	5.8 (0.9)[c]	7.7 (0.9)[c]	0
Two-hour postload glucose, mmol/L, mean (SD)	5.9 (2.2)	5.9 (2.2)	8.9 (2.2)[c]	14.9 (2.2)[c]	0
Systolic blood pressure, mm Hg, mean (SD)	133 (21)	141 (21)[c]	143 (21)[c]	145 (21)[c]	0
Diastolic blood pressure, mm Hg, mean (SD)	75 (10)	76 (10)	78 (10)[c]	77 (10)[b]	0
Hypertension, %[d]	43.8	66.7[c]	63.2[c]	62.2[c]	0
Electrocardiogram abnormalities, %	20.6	31.7	18.8	21.6	0
Body mass index, kg/m^2, mean (SD)	21.8 (3.0)	22.2 (3.0)	23.2 (3.0)[c]	23.2 (3.0)[c]	0
Waist to hip ratio, cm/cm, mean (SD)	0.91 (0.07)	0.93 (0.07)[b]	0.93 (0.07)[c]	0.94 (0.07)[c]	27
Total cholesterol, mmol/L, mean (SD)	5.3 (1.1)	5.5 (1.1)	5.4 (1.1)	5.7 (1.1)[c]	0
History of stroke at entry, %	3.3	3.5	5.9	6.3	0
Education ≤6 y, %	10.3	12.5	13.9	11.3	12
Smoking, %	23.5	23.8	23.5	22.7	0
Alcohol intake, %	23.4	29.0	27.7	34.8[c]	0
Physical activity, %	20.2	22.8	16.8	14.7	0

[a] Mean age was sex adjusted. Percentage of men was age adjusted. Electrocardiogram abnormalities were defined as Minnesota Code 3-1, 4-1, 4-2, 4-3, or 8-3.
[b] $p < 0.05$ vs normal glucose tolerance.
[c] $p < 0.01$ vs normal glucose tolerance.
[d] Hypertension: blood pressure ≥140/90 mm Hg or current use of antihypertensive agents.

search, and written informed consent was obtained from the participants.

RESULTS Table 1 shows the age- and sex-adjusted mean values or frequencies of risk factors for dementia by the WHO criteria at baseline. Compared with those with NGT, the mean values of systolic and diastolic blood pressures, body mass index, waist to hip ratio, and total cholesterol, and the frequencies of hypertension and alcohol intake, were higher in subjects with IFG, IGT; or diabetes.

The age- and sex-adjusted incidences and adjusted HRs of all-cause dementia and its subtypes according to glucose tolerance status defined by the WHO criteria are shown in table 2. Compared with those with NGT, the age- and sex-adjusted incidence and HR of all-cause dementia were significantly higher in subjects with IGT as well as those with diabetes. This association remained unchanged in subjects with diabetes even after adjustment for age, sex, hypertension, EKG abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity. In regard to subtypes of dementia, the age- and sex-adjusted incidence and adjusted HRs of AD were significantly higher in subjects with diabetes than in those with NGT. The age- and sex-adjusted incidence and HR of VaD were significantly increased in subjects with IGT or diabetes compared with those with NGT; however, these associations were not significant after multivariable adjustment. No significant associations were observed between glucose tolerance levels and the risk of other dementia. When IGT and diabetes were brought together in one category, this category also had the significantly higher risks of all-cause dementia, AD, and VaD in the age- and sex-adjusted analysis, and these associations remained significant for all-cause dementia and AD even after adjustment for other possible risk factors. The population attributable fraction of this combined category was 14.6% for all-cause dementia, 20.1% for AD, and 17.0% for VaD.

Table 3 presents the associations between FPG levels and adjusted risks of all-cause dementia and its subtypes. The age- and sex-adjusted incidences and HRs of all-cause dementia and any of the dementia subtypes did not differ among FPG levels. This tendency was unchanged even in the multivariate analysis. Conversely, as shown in table 4, the age- and

Table 2 Age- and sex-adjusted incidence and adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes according to glucose tolerance status defined by WHO criteria

Glucose tolerance level	Person-years at risk, n	No. of events, n	Age- and sex-adjusted incidence	Crude HR (95% CI)	p	Age- and sex-adjusted HR (95% CI)	p	Multivariable-adjusted[a] HR (95% CI)	p
All-cause dementia									
Normal	6,658	115	20.1	1 (referent)		1 (referent)		1 (referent)	
IFG	854	13	16.0	0.89 (0.50-1.58)	0.70	0.74 (0.42-1.31)	0.30	0.63 (0.35-1.13)	0.12
IGT	2,611	63	24.9	1.46 (1.07-1.99)	0.02	1.40 (1.03-1.91)	0.03	1.35 (0.98-1.86)	0.07
DM	1,544	41	29.3	1.62 (1.14-2.32)	0.008	1.71 (1.19-2.44)	0.003	1.74 (1.19-2.53)	0.004
IGT + DM	4,155	104	26.3	1.52 (1.17-1.98)	0.002	1.51 (1.16-1.97)	0.002	1.46 (1.10-1.92)	0.008
Alzheimer disease									
Normal	6,658	51	8.6	1 (referent)		1 (referent)		1 (referent)	
IFG	854	5	6.6	0.77 (0.31-1.94)	0.58	0.63 (0.25-1.57)	0.32	0.61 (0.24-1.55)	0.29
IGT	2,611	29	11.7	1.53 (0.97-2.41)	0.07	1.46 (0.92-2.30)	0.11	1.60 (0.99-2.59)	0.05
DM	1,544	20	14.2	1.81 (1.08-3.03)	0.03	1.94 (1.16-3.26)	0.01	2.05 (1.18-3.57)	0.01
IGT + DM	4,155	49	12.5	1.63 (1.10-2.41)	0.01	1.62 (1.10-2.40)	0.02	1.73 (1.15-2.60)	0.009
Vascular dementia									
Normal	6,658	27	5.1	1 (referent)		1 (referent)		1 (referent)	
IFG	854	6	7.1	1.76 (0.73-4.26)	0.21	1.40 (0.58-3.41)	0.46	1.01 (0.41-2.52)	0.98
IGT	2,611	20	7.8	1.95 (1.09-3.47)	0.02	1.86 (1.05-3.32)	0.04	1.39 (0.76-2.54)	0.29
DM	1,544	12	8.7	2.00 (1.01-3.95)	0.04	2.07 (1.05-4.09)	0.04	1.82 (0.89-3.71)	0.09
IGT + DM	4,155	32	7.9	1.97 (1.18-3.29)	0.01	1.94 (1.16-3.23)	0.01	1.54 (0.90-2.63)	0.11
Other dementia									
Normal	6,658	37	6.4	1 (referent)		1 (referent)		1 (referent)	
IFG	854	2	2.2	0.42 (0.10-1.75)	0.23	0.36 (0.09-1.51)	0.16	0.34 (0.08-1.44)	0.14
IGT	2,611	14	5.5	0.99 (0.54-1.84)	0.99	0.96 (0.52-1.78)	0.90	0.94 (0.49-1.78)	0.84
DM	1,544	9	6.5	1.08 (0.52-2.24)	0.83	1.10 (0.53-2.28)	0.80	1.19 (0.56-2.52)	0.66
IGT + DM	4,155	23	5.8	1.03 (0.61-1.73)	0.92	1.01 (0.60-1.70)	0.97	0.97 (0.57-1.67)	0.91

Abbreviations: CI = confidence interval; DM = diabetes mellitus; HR = hazard ratio; IFG = impaired fasting glycemia; IGT = impaired glucose tolerance.

[a] Multivariate adjustment was made for age, sex, hypertension, electrocardiogram abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity.

sex-adjusted incidences and HRs of all-cause dementia, AD, and VaD significantly increased with rising 2-hour PG levels. Compared with those with 2-hour PG levels of <6.7 mmol/L, the age- and sex-adjusted incidences and HRs of all-cause dementia, AD, and VaD were marginally or significantly higher in subjects with 2-hour PG levels of 7.8 to 11.0 mmol/L and significantly higher in subjects with 2-hour PG levels of ≥11.1 mmol/L. These associations remained robust even after multivariable adjustment; the risks of all-cause dementia and AD were significantly increased in subjects with 2-hour PG levels of 7.8 to 11.0 mmol/L and over, and the risk of VaD was significantly higher in those with 2-hour PG levels of ≥11.1 mmol/L.

Sensitivity analysis in which only definite cases of dementia determined by brain autopsy were used as event cases did not make any material difference in these findings, except with respect to VaD, for which the significant association disappeared, probably due to the few event cases (table 5). When only clinical diagnoses were used for cases with both clinical and neuropathologic diagnoses, the findings were substantially unchanged, though the HRs became slightly lower probably due to the decreased accuracy of diagnosis (tables e-1, e-2, and e-3).

DISCUSSION In a long-term prospective study of an elderly Japanese population, we demonstrated that diabetes that was assessed 15 years earlier was a significant risk factor for the development of all-cause dementia, AD, and VaD. Moreover, the risks of developing all-cause dementia and its sub-

Table 3 Age- and sex-adjusted incidence and adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes according to fasting plasma glucose levels

Fasting plasma glucose levels	Person-years at risk, n	No. of events, n	Age- and sex-adjusted incidence	Crude HR (95% CI)	p	Age- and sex-adjusted HR (95% CI)	p	Multivariable-adjusted[a] HR (95% CI)	p
All-cause dementia									
<5.6	5,589	101	20.7	1 (referent)		1 (referent)		1 (referent)	
5.6-6.0	3,286	71	25.1	1.24 (0.91-1.68)	0.17	1.21 (0.89-1.64)	0.22	1.18 (0.86-1.61)	0.31
6.1-6.9	1,724	39	21.6	1.13 (0.91-1.91)	0.14	1.13 (0.78-1.64)	0.52	0.96 (0.65-1.41)	0.82
≥7.0	1,067	21	22.3	1.21 (0.70-1.79)	0.64	1.14 (0.71-1.82)	0.60	1.21 (0.75-1.96)	0.44
				p for trend: 0.23		*p* for trend: 0.42		*p* for trend: 0.63	
Alzheimer disease									
<5.6	5,589	48	10.1	1 (referent)		1 (referent)		1 (referent)	
5.6-6.0	3,286	30	10.3	1.11 (0.70-1.74)	0.67	1.14 (0.72-1.80)	0.58	1.11 (0.69-1.77)	0.68
6.1-6.9	1,724	16	9.1	1.15 (0.65-2.02)	0.64	1.00 (0.57-1.77)	0.99	0.99 (0.49-1.64)	0.72
≥7.0	1,067	11	11.9	1.23 (0.64-2.37)	0.53	1.29 (0.67-2.48)	0.45	1.41 (0.72-2.76)	0.32
				p for trend: 0.47		*p* for trend: 0.56		*p* for trend: 0.58	
Vascular dementia									
<5.6	5,589	24	4.9	1 (referent)		1 (referent)		1 (referent)	
5.6-6.0	3,286	19	6.7	1.38 (0.76-2.52)	0.29	1.29 (0.71-2.36)	0.41	1.19 (0.64-2.19)	0.58
6.1-6.9	1,724	17	8.7	2.40 (1.29-4.47)	0.006	1.93 (1.03-3.61)	0.04	1.48 (0.77-2.84)	0.24
≥7.0	1,067	5	5.2	1.12 (0.43-2.93)	0.82	1.10 (0.42-2.89)	0.84	0.99 (0.37-2.69)	0.99
				p for trend: 0.10		*p* for trend: 0.19		*p* for trend: 0.49	
Other dementia									
<5.6	5,589	29	5.7	1 (referent)		1 (referent)		1 (referent)	
5.6-6.0	3,286	22	8.1	1.33 (0.76-2.31)	0.32	1.27 (0.73-2.21)	0.40	1.21 (0.68-2.16)	0.51
6.1-6.9	1,724	6	3.8	0.69 (0.29-1.67)	0.42	0.60 (0.25-1.45)	0.26	0.53 (0.22-1.31)	0.17
≥7.0	1,067	5	5.2	0.92 (0.36-2.37)	0.86	0.91 (0.35-2.36)	0.85	1.02 (0.39-2.67)	0.97
				p for trend: 0.68		*p* for trend: 0.53		*p* for trend: 0.52	

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a] Multivariate adjustment was made for age, sex, hypertension, electrocardiogram abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity.

types progressively increased with elevating 2-hour PG levels.

In prior prospective epidemiologic studies, there have been conflicting results regarding the associations between diabetes and incidences of all-cause dementia and AD, while the influence of diabetes on the risk of VaD has been positive in most studies.[1,4–7] Cohort studies in which diabetes was defined by nonfasting blood glucose levels or clinical information did not reveal clear associations of diabetes with the development of all-cause dementia and AD,[4–8] while the risks of dementia and its subtypes significantly increased in diabetes in some studies, most of which defined diabetes using the OGTT.[1–3] The latter findings were in accord with ours. This fact suggests that differences in the methods used to define diabetes lead to a discrepancy in the association between diabetes and the risk of dementia, especially AD, and that an OGTT is essential for the definition of diabetes in epidemiologic studies on the diabetes–dementia association.

In our study, the incidence of VaD was significantly higher in subjects with IGT or diabetes than in those with NGT, but this association disappeared after adjustment for other covariates. This might occur due to the few VaD cases. In addition, since other known cardiovascular risk factors, such as hypertension, obesity, and dyslipidemia, accumulate under a prediabetic or diabetic state, as shown in our data (table 1), IGT and diabetes seem to increase the risk of VaD through mediation of these risk factors, especially hypertension.

In the present study, increased 2-hour PG levels including a prediabetic range were significantly

Table 4 Age- and sex-adjusted incidence and adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes according to 2-hour postload glucose levels

2-Hour postload glucose levels	Person-years at risk, n	No. of events, n	Age- and sex-adjusted incidence	Crude HR (95% CI)	p	Age- and sex-adjusted HR (95% CI)	p	Multivariable-adjusted[a] HR (95% CI)	p
All-cause dementia									
<6.7	5,354	85	17.6	1 (referent)		1 (referent)		1 (referent)	
6.7–7.7	2,277	44	20.9	1.20 (0.84–1.73)	0.32	1.25 (0.87–1.80)	0.24	1.16 (0.78–1.71)	0.47
7.8–11.0	2,844	67	24.7	1.53 (1.11–2.11)	0.009	1.54 (1.12–2.12)	0.009	1.50 (1.07–2.11)	0.02
≥11.1	1,192	36	32.8	2.08 (1.41–3.07)	<0.001	2.32 (1.57–3.44)	<0.001	2.47 (1.62–3.77)	<0.001
				p for trend: <0.001		*p* for trend: <0.001		*p* for trend: <0.001	
Alzheimer disease									
<6.7	5,354	37	7.6	1 (referent)		1 (referent)		1 (referent)	
6.7–7.7	2,277	20	8.8	1.25 (0.73–2.16)	0.41	1.23 (0.71–2.12)	0.46	1.49 (0.83–2.67)	0.17
7.8–11.0	2,844	30	11.3	1.59 (0.98–2.57)	0.06	1.56 (0.96–2.53)	0.07	1.87 (1.13–3.12)	0.02
≥11.1	1,192	18	15.8	2.44 (1.39–4.29)	0.002	2.75 (1.56–4.85)	<0.001	3.42 (1.83–6.40)	<0.001
				p for trend: 0.002		*p* for trend: <0.001		*p* for trend: <0.001	
Vascular dementia									
<6.7	5,354	21	4.6	1 (referent)		1 (referent)		1 (referent)	
6.7–7.7	2,277	12	6.3	1.33 (0.65–2.70)	0.43	1.49 (0.73–3.04)	0.27	1.14 (0.54–2.41)	0.73
7.8–11.0	2,844	20	7.2	1.83 (0.99–3.38)	0.05	1.87 (1.01–3.45)	0.04	1.38 (0.72–2.64)	0.34
≥11.1	1,192	12	11.2	2.75 (1.35–5.60)	0.005	3.15 (1.55–6.43)	0.002	2.66 (1.24–5.70)	0.01
				p for trend: 0.004		*p* for trend: 0.002		*p* for trend: 0.02	
Other dementia									
<6.7	5,354	27	5.4	1 (referent)		1 (referent)		1 (referent)	
6.7–7.7	2,277	12	5.8	1.04 (0.52–2.04)	0.92	1.08 (0.55–2.15)	0.82	0.86 (0.40–1.84)	0.70
7.8–11.0	2,844	17	6.2	1.21 (0.66–2.23)	0.53	1.21 (0.66–2.23)	0.53	1.14 (0.60–2.16)	0.69
≥11.1	1,192	6	5.8	1.05 (0.44–2.55)	0.91	1.12 (0.46–2.71)	0.81	1.21 (0.48–3.04)	0.69
				p for trend: 0.65		*p* for trend: 0.59		*p* for trend: 0.59	

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a] Multivariate adjustment was made for age, sex, hypertension, electrocardiogram abnormalities, body mass index, waist to hip ratio, total cholesterol, history of stroke at entry, education, smoking habits, alcohol intake, and physical activity.

linked to elevated risks of all-cause dementia, AD, and VaD, but no such associations were observed for FPG. The epidemiologic evidence from Asia has also indicated that 2-hour PG levels are better in detecting prediabetes and diabetes compared with FPG levels.[19] However, very few prospective studies have investigated the associations between FPG as well as 2-hour PG levels and the risks of dementia and its subtypes. Only the Uppsala Longitudinal Study of Adult Men evaluated the associations of FPG levels with the risks of developing AD and VaD,[20,21] and this study concluded that increased FPG levels were not risk factors for these subtypes of dementia. This is in good agreement with our findings. The Uppsala Study[21] and the Honolulu-Asia Aging Study[1] also found no clear associations between 2-hour PG levels and the risks of AD and VaD. These findings are inconsistent with ours. Our recent clinicopathologic study of deceased Hisayama residents revealed that higher levels of 2-hour PG but not of FPG were clearly associated with increased risk for formation of neuritic plaques even after adjustment for confounding factors.[22] This evidence together with the findings of the present study suggests that elevated 2-hour PG levels play an important role in the formation of neuritic plaques, and thereby in the development of AD. Meanwhile, it is well known that increased 2-hour PG levels are closely associated with the development of stroke, which is well established as a main cause of VaD. Thus, it is reasonable to postulate a close association between 2-hour PG levels and the risk of VaD.

Possible pathophysiologic mechanisms through which diabetes or elevated blood glucose levels might

Table 5 Age- and sex-adjusted hazard ratios and their 95% confidence intervals for the development of all-cause dementia and its subtypes determined by autopsy according to 2-hour postload glucose levels

2-Hour postload glucose levels	Person-years at risk, n	No. of events, n	Crude HR (95% CI)	p	Age- and sex-adjusted HR (95% CI)	p
All-cause dementia						
<6.7	5,354	47	1 (referent)		1 (referent)	
6.7–7.7	2,277	23	1.14 (0.69–1.88)	0.61	1.24 (0.75–2.05)	0.39
7.8–11.0	2,844	29	1.19 (0.75–1.89)	0.47	1.20 (0.76–1.91)	0.44
≥11.1	1,192	19	1.94 (1.14–3.31)	0.01	2.24 (1.31–3.83)	0.003
			p for trend: 0.04		*p* for trend: 0.02	
Alzheimer disease						
<6.7	5,354	12	1 (referent)		1 (referent)	
6.7–7.7	2,277	7	1.35 (0.53–3.44)	0.53	1.40 (0.55–3.56)	0.48
7.8–11.0	2,844	12	1.94 (0.87–4.33)	0.10	1.92 (0.86–4.26)	0.11
≥11.1	1,225	8	3.27 (1.34–8.00)	0.009	3.88 (1.58–9.53)	0.003
			p for trend: 0.009		*p* for trend: 0.005	
Vascular dementia						
<6.7	5,354	17	1 (referent)		1 (referent)	
6.7–7.7	2,277	8	1.09 (0.47–2.54)	0.83	1.23 (0.53–2.86)	0.63
7.8–11.0	2,844	8	0.90 (0.39–2.09)	0.81	0.92 (0.40–2.12)	0.84
≥11.1	1,192	7	1.98 (0.82–4.77)	0.13	2.32 (0.96–5.61)	0.06
			p for trend: 0.36		*p* for trend: 0.26	
Other dementia						
<6.7	5,354	18	1 (referent)		1 (referent)	
6.7–7.7	2,277	8	1.04 (0.45–2.39)	0.93	1.17 (0.51–2.70)	0.72
7.8–11.0	2,844	9	0.96 (0.43–2.14)	0.92	0.98 (0.44–2.19)	0.97
≥11.1	1,192	4	1.04 (0.35–3.07)	0.95	1.16 (0.39–3.43)	0.79
			p for trend: 0.99		*p* for trend: 0.88	

Abbreviations: CI = confidence interval; HR = hazard ratio.

affect the initiation and promotion of dementia have been extensively discussed in a number of studies.[23] A recent review summarized 4 major pathways for hyperglycemia-induced dementia: namely, atherosclerosis, microvascular disease, glucose toxicity leading to the accumulation of advanced protein glycation and increased oxidative stress, and changes in insulin metabolism resulting in an insulin-resistant state and distorted amyloid metabolism in the brain.[23] The former 2 pathways are considered to be involved in the development of VaD, while the latter 2 pathways may mainly contribute to the development of AD. Additionally, recent evidence has emerged to imply that vascular factors may be involved in AD.[23] It is reported that 2-hour PG values can be a good marker of oxidative stress levels arising from hyperglycemia[24,25] and correlate with insulin resistance.[26] Higher oxidative stress and insulin resistance may precede the accumulation of amyloid-β peptide and neurofibrillary tangles[23,27] and accelerate arteriosclerosis in the brain,[28] resulting in increased risk of AD and VaD. It is known that Asians have lower levels of insulin secretion compared with other ethnic groups[29] and can develop diabetes, insulin resistance, and metabolic syndrome with lower body mass index levels.[30] These findings suggest that hyperglycemia plays a larger role in the development of dementia compared with insulin resistance in Asians including Japanese. Further studies are needed to elucidate the pathogenesis of hyperglycemia and diabetes in the development of dementia.

The strengths of our study include its longitudinal population-based study design, use of OGTT for determination of glucose tolerance levels in all subjects, long duration of follow-up, perfect follow-up of subjects, and morphologic examination of the brains of most dementia cases with autopsy and neuroimaging. Several limitations of our study should be noted. First, the diagnosis of glucose tolerance status was based on a single measurement of glucose levels at baseline, as was the case in most other epidemiologic studies. During the follow-up, risk factor levels were changed due to modifications in lifestyle or medication especially in subjects with diabetes, and

misclassification of glucose tolerance categories was possible. This could have weakened the association found in this study, biasing the results toward the null hypothesis. Therefore, the true association may be stronger than that shown here. Second, some subjects (n = 33 to 65) did not participate in the follow-up surveys of cognitive function performed in 1992, 1998, and 2005, and their cognitive conditions were evaluated only by mail or telephone. This might have resulted in failure to detect dementia cases. However, we also collected information on the development of dementia in another way, namely through the daily monitoring system established in the town. Thus, we believe that we detected almost all dementia cases, and this bias did not affect our findings. Third, the diagnosis of dementia was verified by autopsy only in 50.9% of dementia cases, resulting in a certain degree of subtype misclassification; agreement rate between clinical diagnosis and neuropathologic diagnosis was not high (64.4%) in our autopsy cases of dementia. However, a sensitivity analysis using only definite cases of dementia determined by brain autopsy did not make any material difference in our findings.

Our findings emphasize the need to consider diabetes as a potential risk factor for all-cause dementia, AD, and probably VaD. The other main finding, that elevated 2-hour PG levels are closely associated with increased risks of all-cause dementia and its subtypes, supports the view that postprandial glucose regulation is critical to prevent future dementia. Further investigations are required to clarify the associations between 2-hour PG levels by the OGTT and subtypes of dementia in other ethnic populations.

AUTHOR CONTRIBUTIONS

Tomoyuki Ohara contributed to the study concept, design, data collection, endpoint adjudication, interpretation of data, statistical analysis, and writing the manuscript. Yasufumi Doi contributed to the study concept, design, interpretation of data, statistical analysis, and writing the manuscript. Toshiharu Ninomiya contributed to the data collection, endpoint adjudication, interpretation of data, and statistical analysis. Yoichiro Hirakawa and Jun Hata contributed to data collection and interpretation of data. Toru Iwaki and Shigenobu Kanba contributed to endpoint adjudication and interpretation of data. Yutaka Kiyohara is a study coordinator and contributed to the study performance, obtaining supporting sources, study concept, design, endpoint adjudication, interpretation of data, and writing of manuscript. All authors critically reviewed the manuscript and approved final version.

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DISCLOSURE

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Midlife and Late-Life Blood Pressure and Dementia in Japanese Elderly

The Hisayama Study

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Abstract—The associations between blood pressure and dementia have been inconclusive. We followed up a total of 668 community-dwelling Japanese individuals without dementia, aged 65 to 79 years, for 17 years and examined the associations of late-life and midlife hypertension with the risk of vascular dementia and Alzheimer disease using the Cox proportional hazards model. During the follow-up, 76 subjects experienced vascular dementia and 123 developed Alzheimer disease. The age- and sex-adjusted incidence of vascular dementia significantly increased with elevated late-life blood pressure levels (normal: 2.3, prehypertension: 8.4, stage 1 hypertension: 12.6, and stage 2 hypertension: 18.9 per 1000 person-years; $P_{trend}<0.001$), whereas no such association was observed for Alzheimer disease ($P_{trend}=0.88$). After adjusting for potential confounding factors, subjects with prehypertension and stage 1 or stage 2 hypertension had 3.0-fold, 4.5-fold, and 5.6-fold greater risk of vascular dementia, respectively, compared with subjects with normal blood pressure. Likewise, there was a positive association of midlife blood pressure levels with the risk of vascular dementia but not with the risk of Alzheimer disease. Compared with those without hypertension in both midlife and late life, subjects with midlife hypertension had an ≈5-fold greater risk of vascular dementia, regardless of late-life blood pressure levels. Our findings suggest that midlife hypertension and late-life hypertension are significant risk factors for the late-life onset of vascular dementia but not for that of Alzheimer disease in a general Japanese population. Midlife hypertension is especially strongly associated with a greater risk of vascular dementia, regardless of late-life blood pressure levels. (***Hypertension.*** **2011;58:22-28.)** • **Online Data Supplement**

Key Words: prospective studies ■ aged ■ hypertension ■ vascular dementia ■ Alzheimer disease

Vascular dementia (VaD) has been acknowledged to be more prevalent in Japan as compared with Western countries.[1–5] We reported previously that the prevalence of VaD did not apparently decrease over the past 2 decades,[2] despite the fact that the incidence of stroke significantly decreased because of the improvement of blood pressure (BP) lowering therapy since the 1970s.[6] In addition, the incidence of Alzheimer disease (AD) in Japanese studies is greater to almost same degree as that of Western studies in recent years,[1,4,5] resulting in a drastic increase in the burden of dementia in Japan.[2]

Hypertension and dementia are common disorders in the elderly.[7,8] Because hypertension has been shown to be a major risk factor for cerebrovascular disease, higher BP was likely to be strongly associated with a greater risk of VaD.[9] In addition, recent evidence has emerged to imply that vascular factors may be involved in AD, which has traditionally been considered a primarily neurodegenerative disorder.[10,11] However, the results of observational longitudinal studies showing the effects of BP on the risks of dementia and its subtypes are inconsistent.[12,13] In particular, some studies have suggested that midlife hypertension is a risk factor for late-life dementia, whereas lower diastolic BP in late life may be related to increased risks of dementia and AD.[12] These facts raise the possibilities that the effects of hypertension on the development of dementia may be different between midlife and late life, possibly because the longitudinal changes related to hypertension in the brain may begin earlier in the adult life span.[12,13] However, few studies have compared the effects of midlife and late-life hypertension on the development of dementia in an identical population.

The purposes of this study were to investigate the association of BP levels in midlife and late life with the development of dementia and its subtypes in a general Japanese population and to elucidate whether the effects of hypertension on the risk of dementia are different between midlife and late life in an identical population. The findings from this study are expected to provide clear evidence of the diverse

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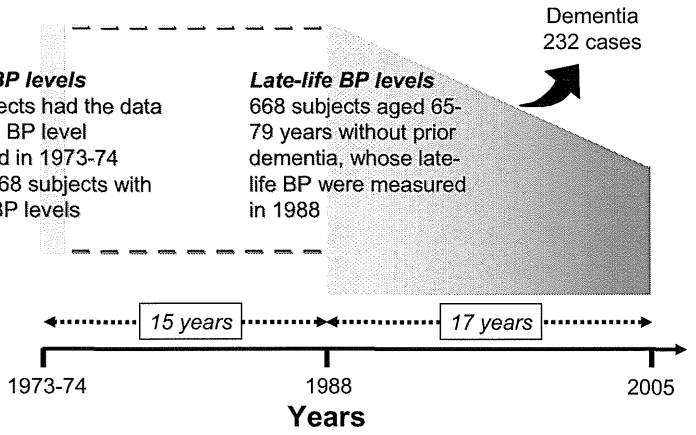

Figure. Diagram of the study design. BP indicates blood pressure.

associations of BP levels with the risk of dementia in midlife and late life.

Methods

Study Population

A population-based prospective study of cerebro-cardiovascular diseases was established in 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. Full community surveys of health status and neurological conditions of the residents aged ≥40 years have been repeated since 1961.[6] In addition, comprehensive surveys of cognitive function in the elderly, including neuropsychological tests, have been conducted every 6 or 7 years since 1985.[2]

In 1988, a total of 682 residents aged 65 to 79 years (90.5% of the total population in this age group) participated in a health checkup. After excluding 12 subjects with dementia at baseline and 2 subjects who died before starting the follow-up, the remaining 668 subjects (266 men and 402 women) were enrolled in this study to investigate the association between late-life BP and the risk of dementia. Among them, 534 subjects (210 men and 324 women) had also participated in a health checkup conducted in 1973–1974 and were included in the analysis of the effects of midlife BP on the risk of late-life dementia (Figure). Written, informed consent was obtained from the participants at baseline in 1988. This study was conducted with the approval of the ethics committee of the Kyushu University Faculty of Medicine.

BP Categories

Sitting BP was measured with a sphygmomanometer 3 times at the right upper arm after ≥5 minutes of rest, and the mean of the 3 measurements was used in the analysis. BP levels measured in 1973–1974 and 1988 were classified into 4 categories according to the criteria of the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7).[14]

The extended Materials and Methods section provides detailed information on the follow-up survey, diagnosis of dementia, other risk factors, and statistical analysis. Please see the online Data Supplement at http://hyper.ahajournals.org.

Results

The clinical characteristics of the study population according to BP levels defined by the criteria of JNC-7 in late life (1988) are summarized in the top part of Table 1. The mean age of the overall population was 72±4 years old. Subjects with higher BP levels were likely to be older. The proportion of subjects using antihypertensive agents and the proportion with diabetes mellitus increased gradually with higher BP levels. There were no associations of BP levels with the mean serum total cholesterol levels and the proportion of women, educational status, or smoking habit. Likewise, the clinical characteristics according to BP levels at midlife (1973–1974) are shown in the bottom part of Table 1. The mean age at midlife was 57±4 years old. The mean values of age, serum total cholesterol, and body mass index and the proportion of the use of antihypertensive agents increased with higher midlife BP levels.

During the 17-year follow-up period, 232 subjects developed dementia of some kind. Of these, 199 (85.8%) underwent evaluation with neuroimaging, and 115 (49.6%) received a general autopsy examination; in 106 cases, both were performed. Thus, 208 subjects in all (89.7%) had some kind of morphological examination. Among dementia cases, 15 AD cases and 13 VaD cases had other coexisting subtypes of dementia, of which 8 cases were a mixed type of AD and VaD. These cases were counted as events in the analysis for each subtype. In all, 123 subjects developed AD, and 76 developed VaD.

First, we estimated the associations between late-life BP levels defined by the criteria of JNC-7 and the risk of dementia (Table 2 and Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org). The age- and sex-adjusted incidence of all-cause dementia showed an increasing linear trend with the rise of late-life BP levels (P_{trend}=0.07). In regard to subtypes of dementia, the age- and sex-adjusted incidence of VaD significantly increased with elevated late-life BP levels (P_{trend}<0.001). Meanwhile, there were no significant associations between late-life BP levels and the age- and sex-adjusted incidence of AD (P_{trend}=0.88). Table 2 also shows the multivariate-adjusted hazard ratios of all-cause dementia and its subtypes across late-life BP levels defined by the criteria of JNC-7. After adjusting for potential confounding factors (age, sex, education level, use of antihypertensive agents, diabetes mellitus, chronic kidney disease, serum total cholesterol, body mass index, history of stroke, smoking habits, and alcohol intake), the risk of VaD increased progressively with elevated BP levels. This relationship was not altered substantially after adjusting for the above-mentioned confounding factors and serum homocysteine. No clear associations were observed between BP levels and the risks of all-cause dementia and AD. There was no evidence of heterogeneity in these associations between the