表1. 百寿者の生化学所見

| 項目 | 百寿者 (f=199, m=72) | 若年対照群 (f=1316, m=2382) |
|---|---|---|
| 年齢 | 100.9±1.5 | 55.4±10.8 |
| BMI | 19.2±3.3 | 23.0±2.9 |
| Albumin | 3.6±0.4 | 4.7±0.7 |
| Cholesterol | 164.0±32.8 | 212.1±22.7 |
| Hb | 11.1±1.6 | 14.4±1.5 |
| HbA1C | 5.4±0.7 | <5.8 |
| Homocysteine | 15.0±5.7 | 3-14 |
| TAT | 9.0±9.0 | <3 |
| CRP | 0.64±1.54 | <0.3 |

Hirose et al.: Tokyo centenarian study: aging inflammation hypothesis. GGI 4: S182-S185: 2004, Blackwell publishing

意に負の影響を及ぼすことが判明した．超高齢期の自立度を高めるためには脳血管障害，骨折を予防する必要があることが示唆された．

## 5. 動脈硬化[4]

百寿者の動脈硬化の頻度を調べるために頸動脈エコー検査を行った（図）．図の如くIMT（内中膜肥厚度）は年齢と共に厚くなった．一方プラークは50歳代より出現し90歳で最も頻度が高くなった．百寿者での頻度は80歳代と同等であった．動脈硬化の退縮は起こりにくいことを考えると，動脈硬化の強い人は90から100歳になる間に死亡してしまうと考えられる．この10年間に100歳になるためのハードルがひとつあると考えられる．

## 6. 血液生化学所見[5]

百寿者の血液生化学所見を表1に示す．百寿者の特徴としては，1)BMI，アルブミン値が低く低栄養である，2)CRPが高く炎症反応が亢進している，3）凝固系の亢進，4）ホモシステインの高値，5)貧血傾向が挙げられ，加齢による所見と考えられた．これらの所見の関連を調べるために百寿者をアルブミン値により2群に分類し（栄養良好群：アルブミン>=3.6g/dl，栄養不良群：アルブミン<3.6g/dl）各種パラメーターを比較した（表2）．栄養良好群では炎症反応が低値で，コレステロールも高く貧血も軽度であった．さらにADL，認知機能も高かった．栄養状態は超高齢期のADL，認知機能に影響を与えることが示された．即ち超高齢期では栄養を保つことが健康長寿につながると考えられる．

## 7. 仮説の提唱—老化炎症仮説，防御因子の存在—[5]

以上より二つの仮説を提唱したい．一つは“老化炎症仮説”である．炎症の亢進が栄養状態を低下させることが知られている（cytokine induced malnutrition)[6]．加齢に伴い炎症反応が亢進する事が老化の基本的な現象であり，これに続いて低栄養が起こり，さらにADL，認知機能の低下を引き起こすという筋道である．イタリアのFranceschiらは百寿者の免疫能の検討を基にinflamm-aging hypothesisを提唱した[7]．我々は栄養状態の検討から，Franceschiらは免疫の検討から加齢に伴い炎症反応が亢進すると考えており興味深い．もしこの仮説が妥当なら，過剰の炎症反応を抑えることにより超高齢期における自立度を保持することに有益であると考えられる．

100歳まで生きるためには1)誰でも生活習慣病にならなければ可能であるとする考えと，2)障害を阻止する，または死なないようにするという特殊な防御因子が必要であるという考えがある．我々は障害があるにもかかわらず元気に生活する百寿者を見る度に，強力な防御因子がなければ100歳までは生きられないと考えている．どちらが正しいかは今後遺伝素因の検討により明らかになろう．4節で述べたように100歳まで大病をしていない人が30%いたが，60%以上の方は大病をして生き残った方々である．

表2. 栄養の影響

| | unit | n (f/m) | well nourished | malnourished | p value |
|---|---|---|---|---|---|
| Nutritional parameters | | | | | |
| BMI | | 29/38 | 20.3±10.0 | 18.7±10.3 | 0.047 |
| Albumin | g/dl | 144/103 | 3.91±0.23 | 3.18±0.27 | <0.001 |
| Lipid parameter | | | | | |
| total cholesterol | mg/dl | 144/103 | 174.3±31.6 | 152.4±30.0 | <0.001 |
| HDL cholesterol | mg/dl | 144/103 | 56.4±14.5 | 45.2±10.9 | <0.001 |
| Inflammatory parameter | | | | | |
| CRP | mg/dl | 109/59 | 0.29±0.47 | 0.89±1.46 | <0.001 |
| IL-6 | ng/ml | 15/34 | 4.4±2.8 | 11.8±11.5 | 0.018 |
| Peripheral Blood | | | | | |
| RBC | ×106/μl | 144/99 | 369±48 | 339±51 | <0.001 |
| Hb | g/dl | 144/99 | 11.5±1.6 | 10.5±1.6 | <0.001 |
| ADL (Barthel Index) | | 70/30 | 54.6±33.4 | 31.8±28.5 | 0.02 |
| cognitive function (MMSE) | | 74/30 | 16.1±7.0 | 12.5±6.0 | 0.002 |

表3. 女性百寿者のアディポサイトカイン濃度

| | 百寿者 (n = 66) | 対照群 (n = 66) |
|---|---|---|
| BMI (kg/m2) | 19.5±3.1 | 19.5±2.3 |
| Adiponectin (μg/ml) | 20.3±7.4 ** | 10.8±3.9 |
| Leptin (ng/ml) | 4.7±3.8 ** | 8.2±5.8 |
| TNF-α (pg/ml) | 1.52±0.66 ** | 0.62±0.32 |
| LPL (ng/ml) | 99±30 ** | 71±24 |
| IL-6 (pg/ml) | 5.9±6.4 ** | 1.9±0.4 |

** : p < 0.01

Arai Y, et al. : High adiponectin concentration and its role for longevity in Japanese female centenarians. GGI (in press) Blackwell publishing

病気を乗り越えて長寿を達成していることは，防御因子が必要という考えを支持するものと考える．

## 8. 脂肪組織と長寿

7節では“防御因子仮説”について解説したが実際にはどんなものが考えられるだろうか？我々はアディポカインの一種であるアディポネクチンに注目した．アディポネクチンはインスリン抵抗性を改善し，抗糖尿病作用，抗炎症作用，抗動脈硬化作用を示す[8]．百寿者では糖尿病が少ないこと(4節)，動脈硬化が少ないこと(5節)，炎症反応が低い方が状態がよいこと(6節)を述べた．女性百寿者66名とBMIをマッチさせた若年女性でアディポネクチンを測定したところ百寿者ではアディポネクチンは2倍高かった（表3)．さらにアディポネクチン濃度はCRP，HbA1Cと有意の逆相関を示した．即ち百寿者ではアディポネクチンは抗炎症作用，抗糖尿病作用を持つ可能性があり，アディポネクチンは防御因子のひとつである事が示唆された[9]．

脂肪組織の萎縮が原因で発症するLipodystrophyではインスリン抵抗性が高まり動脈硬化をおこしやすいことが知られている．肥満に伴い脂肪細胞は肥大しインスリン抵抗性を増強するアディポカインを分泌するようになることも示された．基礎老化科学の結果ではカロリー制限が唯一の哺乳類の寿命を延長出来る方法である．カロリー制限がなぜ寿命の延長につながるのかは不明であるが脂肪組織を介する可能性も考えられる．これらを考慮すると機能の良い脂肪組織は抗老化組織である可能性がある．脂肪組織の分化，増殖を制御する因子と長寿の関係を検討することは興味深い[10,11]．

## 9. 100歳老人は人の長寿モデルか？—超百寿者調査—[12]

2節で述べたように百寿者の増加は著しく，様々な状態の百寿者がいることが判明した．100歳老人はヒト健康長寿モデルだろうか？2002年には105歳（超百寿者）は約1,000名生存していた．全人口あたり10万人に1人で北では低く南では高い．105歳到達率は3,000名に1人であった（100歳到達率は400名に1人）．これらのデータより我々は100歳ではなく105歳がヒトの長寿モデルと考え，2002年に全国超百寿者調査を行った．その結果，1）アルブミン値の低下，CRPの上昇など100歳で見られる生化学所見が強調されること，2）アポE genotypeが100歳に比較してもE2が多くE4が低下しており，100歳で観察される遺伝素因がより強調されること，3）ほとんどが100歳の時には元気で自立していたことなどが分かった．即ち超百寿者は究極の加齢現象を観察し，長寿遺伝子の同定に最適の年齢群であることが示唆された．

## 10. 長寿，遺伝，抗老化—将来展望—[13]

平均寿命達成に寄与する遺伝素因の強さは25%という報告がある（デンマーク双子調査）．超高齢者まで到達する遺伝素因の強さはもっと強いと考える研究者が多い．鈴木らは沖縄百寿者の調査から百寿者家系と非百寿者家系の平均寿命，80歳，90歳到達率を調べた．百寿者家系ではいずれの到達率も有意に高かった．現在長寿遺伝子同定の研究は百寿者のDNAを利用して活発に行われている．長寿遺伝子同定の戦略に関しては，1）極端な長寿者を対象にする，2）遺伝素因の強い長寿者を対象にする，3）長寿同胞解析による連鎖解析と併せて，4）超百寿者を対象とする高密度SNPを用いた関連解析を行う，5）3と4の結果を重ねあわせて長寿遺伝子を同定する，6）超百寿者を対象に候補遺伝子について関連解析を行うということが考えられる．我々の研究班でもこの戦略に従い解析を行っている．長寿遺伝子があるか，あるとすればどの様な作用を持つかなど誰でも知りたい事が数年で明らかになることを期待している．このような解析を通して科学的に根拠のある抗老化医療の確立が可能となることを希望したい．

## まとめ

百寿者調査，超百寿者調査の結果について解説した．本論文では百寿者の性格，介護などについては割愛したが，psychosocialな因子がwell-beingの高い長寿に寄与していることは疑いない．これらの検討も今後の研究テーマとなろう．抗老化または健康長寿達成にどの様な介入が出来るかについては，現在様々な試みがされている．今後，防御因子の同定，長寿遺伝子の同定などを通して健康長寿達成に有効な介入方法の開発に努めたい．

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超百寿者調査，90歳以上の健康同胞調査は現在も進行中です．調査協力していただける方をご存じでしたら，是非著者までご連絡下さい．

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# Contribution of an affect-associated gene to human longevity: Prevalence of the long-allele genotype of the serotonin transporter-linked gene in Japanese centenarians

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**Abstract**

Negative affect such as depression and anxiety has been reported to be associated with morbidity and mortality, and polymorphisms of the serotonin transporter (5HTT) gene may be associated with such affect disorders. Hypothesizing that 5HTT gene polymorphisms could influence human longevity via negative affect; we compared the polymorphic variation of the 5HTT gene between 265 Japanese centenarians and control subjects. In addition, we evaluated the relationships between the 5HTT genotype and the physical, cognitive, and biologic status of centenarians, as indicated by the Barthel Index, the Mini-Mental State Examination, and serum albumin concentration, respectively. The frequency of the l/l genotype and the l allele was significantly greater in centenarians than in younger control subjects, particularly women. A significant effect of the 5HTT genotype on serum albumin concentration was observed in both sexes. Although, there was sex optionality, the l allele may carry a longevity advantage possibly through behavioral mechanisms.


*Keywords:* Serotonin transporter gene-linked polymorphism region; Longevity; Centenarians; Gender difference; Serum albumin

## 1. Introduction

Recent progress in genome studies has indicated that gene polymorphisms contribute to human longevity. These polymorphisms are thought to influence longevity through several potential mechanisms including the immune system (Takata et al., 1987; Bonafe et al., 2001) and the metabolic system (Schachter et al., 1994; Geesaman et al., 2003). Although psychobehavioral systems are also important factors in longevity, little is known about them, and little interest has been paid to polymorphisms of candidate genes that might influence human behavior. Recently, relationships between gene polymorphisms and human emotion, affect, or behavior have been reported (Lesch et al., 1996). We describe here an investigation of the contribution of the affect-related serotonin transporter (5-hydroxytryptamine, 5HTT) gene-linked polymorphism region (5HTT-LPR) to longevity in a cohort taken from the Japanese population.

The adverse impact of negative affect on health has been reported in many papers (Kiecolt-Glaser et al., 2002). Major depression or depressed mood has been associated with increased risk of myocardial infarction (Pratt et al., 1996), decreased physical function (Rantanen et al., 2000), and higher cancer risk (Penninx et al., 1998), as well as mortality (Herrmann et al., 1998). Anxiety has been linked to heart disease, including myocardial infarction (Eaker et al., 1992; Kawachi et al., 1994).

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The 5HTT-LPR has been linked with negative affect and personality (Lesch et al., 1996). The two most common alleles are a 44-bp insertion (l allele) or deletion (s allele) in its promoter region. The s allele reduces the efficiency of transcription of the gene, resulting in decreased serotonin transporter expression and serotonin uptake by lymphoblasts (Lesch et al., 1996). Possession of the s allele has been associated with higher neuroticism, higher harm avoidance, and depression (Lesch et al., 1996), including geriatric depressive symptoms (Steffens et al., 2002). For healthy adults, the 5HTT showed strongest association to personality trait among personality-associated genes (Munafo et al., 2003).

A recent report has also indicated that gene-environment interactions exist in relation to the 5HTT gene polymorphism and an individual's emotional state. Decreased serotonergic function, which is associated with impulsiveness, aggressive behavior, and low sociality, is observed in rhesus monkeys with the s allele if they are not reared by their mothers (Bennett et al., 2002). In addition, in humans the susceptibility to becoming depressed after experiencing negative life events is higher in individuals with s alleles than in those with l alleles (Caspi et al., 2003). In women, carriers of the s allele tend to complain of psychological and somatic symptoms if they are not employed or have a chronic disease (Grabe et al., 2004). In a recent functional neuroimaging study it was reported that activation of the right amygdala in response to angry and fearful facial expressions was stronger in subjects with, than in those without the s alleles (Hariri et al., 2002).

The frequency of occurrence of the l/l genotype and l allele is far less common in Japanese people than in Caucasians (Nakamura et al., 1997; Arinami et al., 1999; Ishiguro et al., 1999; Ishikawa et al., 1999; Katsuragi et al., 1999; Kumakiri et al., 1999; Murakami et al., 1999; Kunugi et al., 2000; Umekage et al., 2003). The reported effects of 5HTT polymorphism on negative affect have not been consistent. While no relationship has been observed between this polymorphism and either bipolar affective disorder (Kunugi et al., 1997) or anxiety-related personality (Umekage et al., 2003), Murakami et al. (1999) and Katsuragi et al. (1999) reported an association with anxiety-related personality. Another study has also revealed an association with anxiety-related personality trait, but this finding could not be confirmed statistically because of the low number of l/l genotype subjects studied (Nakamura et al., 1997).

Negative influences of the l allele on health in Japanese people have also been reported. Possession of the l allele was correlated with the early onset of alcohol dependence (Ishiguro et al., 1999), habitual smoking (Ishikawa et al., 1999), and coronary heart disease among smokers (Arinami et al., 1999). However, these reports included either only men or only few women as participants. These inconstant results may be attributable not only to ethnic differences, but also to gender differences and to the small sample sizes used in these previous Japanese studies.

Even though the findings regarding the effect of 5HTT gene polymorphism on negative emotion or health in Japanese subjects are equivocal, they do suggest that it would be valuable to explore the influences of 5HTT on human health and mortality. We hypothesized that 5HTT gene polymorphism influences human longevity via a negative impact on the affective state as a result of stressful life experiences. The frequency of the l/l genotype and l allele would therefore be higher among long-lived individuals than in the younger population. We also hypothesized that if a particular gene polymorphism has a positive impact on longevity, the functional status, such as basic activities of daily living (ADL), or cognitive function among people who possess this genotype would be better maintained, even in the final stages of their life. In the study presented here, we compared the distribution of 5HTT polymorphisms in a large number of Japanese centenarians with that in younger control subjects. We also explored the effects of 5HTT polymorphisms on physical, cognitive, and biological function in the centenarians.

## 2. Materials and methods

### 2.1. Subjects

#### 2.1.1. Centenarian group

A total of 304 Japanese centenarians (66 men, 238 women) living in the 23 wards of metropolitan Tokyo participated in a survey in which they were visited by Tokyo Centenarian Study staff between July 2000 and May 2002. We randomly chose centenarians from the residential list and sent a letter inviting participation to 1194 centenarians, accounting for 66.9% of an estimated 1785 centenarians living in this area in the study period. Five hundred thirteen (43.0%) agreed to participation. Three hundred four represented 25.5% of the letter recipients, participated in the visit survey. Women outnumbered men in our sample by 1:3.5, which was not significantly different from the ratio for the total centenarian population in this area (1:3.9). Among our participants, 206 were living at home and 98 were institutionalized; eight of those living at home were living alone. A medical doctor, a psychologist, and a nurse conducted the survey in a visit to the centenarians' residences. After a medical checkup and blood sampling, the psychologist conducted cognitive assessment and psychological testing. The ADL were evaluated using the Barthel Index (Collin et al., 1988). Psychologists assessed cognitive function using the Mini-Mental State Examination (MMSE; Folstein et al., 1975) at the time of the visit. Anxiety-related personality traits were evaluated by NEO-FFI (Costa and McCrae, 1985) Japanese version (Shimonaka et al., 1997) answered by proxy who knows participants well. The Barthel Index and the MMSE are used commonly in centenarian studies to evaluate physical function and cognitive status, respectively. Serum albumin concentration

was measured as a biologic indicator of health status that has been linked to physical and cognitive function as well as the mortality risk of centenarians (Shimizu et al., 2001). Of the 304 participants, 265 (men 59, women 206) blood samples were suitable for analysis of DNA polymorphism. Table 1 details the characteristics of the centenarians studied.

### 2.1.2. *Younger control subjects*

Data for younger controls were derived from two sources. We recruited 225 adults (131 women, 94 men; mean age, 37.9 ± 11.5 years; range 19–67 years). They were recruited from the university students, co-medical personnel, and bank clerk as volunteers. However, the frequency of the l/l genotype among Japanese is low, precluding accurate estimation of genotype distribution based on the small sample size. Therefore, we combined our control 5HTT genotype distribution results with genotype distributions reported in previous studies of Japanese populations (Arinami et al., 1999; Ishiguro et al., 1999; Ishikawa et al., 1999; Katsuragi et al., 1999; Kumakiri et al., 1999; Murakami et al., 1999; Kunugi et al., 2000; Nakamura et al., 1997; Umekage et al., 2003). Participants' age range of those studies was 19–77 and they were composed of the volunteers of medical checkup patients, healthy general public and medical staff. The details of those participants were shown in Table 2.

The genotype distribution by gender is shown in Table 2. We compared our results in centenarians with genotype in this combined younger control group. Written informed consent was obtained from all participants or their families. The ethics committee of Keio University School of Medicine approved this study.

## 2.2. *DNA polymorphism*

Genomic DNA was obtained from peripheral blood mononuclear cells by phenol extraction. Polymorphisms of the 5HTT gene were determined by a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method, as previously described (Lesch et al., 1996).

## 2.3. *Statistical analysis*

Statistical analysis was carried out using SPSS software version 11.5J (SPSS, Japan). The $\chi^2$-test was performed to

Table 1
Mean (and S.D.) of background characteristics in centenarians

| | Men | Women | Total |
|---|---|---|---|
| *N* | 59 | 206 | 265 |
| Mean age (years) | 101.1 (1.8) | 101.2 (1.8) | 101.2 (1.8) |
| Physical and cognitive functions | | | |
| Barthel Index | 61.9 (33.9) | 42.0 (33.5) | 46.4 (34.5) |
| MMSE | 17.0 (8.3) | 12.7 (8.3) | 13.7 (8.2) |
| Albumin (g/dl) | 3.7 (0.4) | 3.6 (0.4) | 3.6 (0.4) |

MMSE, Mini-Mental State Examination.

Table 2
Distribution of serotonin transporter (5HTT)polymorphism in previous studies of Japanese subjects and our own non-centenarians

| Authors | Men | | | Women | | | Combined | | | Participants characteristics | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | l/l | l/s | s/s | l/l | l/s | s/s | l/l | l/s | s/s | Sample | Mean age (S.D.) | Range |
| Arinami et al. | 10 (4.5%) | 64 | (28.8%) 148 | (66.7%) – | – | – | – | – | – | Medical checkup patient | 55.1(7.4) | 47–77 |
| Ishiguro et al. | – – | – | – – | – | – | – | 8 (2.8%) | 81 | (27.9%) 201 | (69.3%) Healthy volunteers | 51.7(8.2) | – |
| Ishikawa et al. | 15 (3.9%) | 104 | (26.9%) 268 | (69.3%) – | – | – | – – | – | – – | Medical checkup patient | Group A46.8(–) group B 52.6(–) | 37–59 46–65 |
| Katsuragi et al. | – – | – | – – | – | – | – | 4 (4.0%) | 31 | (30.7%) 66 | (65.3%) Medical stuffs | 25.0(3.6) | – |
| Kumakiri et al. | – – | – | – – | – | – | – | 11 (5.8%) | 60 | (31.4%) 120 | (62.8%) Medical stuffs | *144 were between 20 and 35 | 20–62 |
| Murakami et al. | – – | – | – – | – | – | – | 16 (4.0%) | 159 | (31.7%) 326 | (65.1%) Healthy volunteers | 47.9(10.4) | 19–81 |
| Kunugi et al. | – – | – | – – | – | – | – | 15 (4.6%) | 97 | (29.8%) 214 | (65.6%) Medical stuffs and patients | Staff 32(14) patients 57(8) | – |
| Nakamura et al. | – – | – | – – | – | – | 3 (1.6%) | – – | – | – – | Collage students | 19.6(2.2) | – |
| Umekage et al. | 2 (3.7%) | 15 | (27.8%) 37 | (68.5%) 11 | (5.8%) | 55 (28.9%) | 13 (5.3%) | 70 | (28.7%) 161 | (66.0%) Medical stuffs | 37.7(11.9) | – |
| Our sample | 1 (1.1%) | 30 | (31.9%) 63 | (67.0%) 6 | (4.6%) | 39 (29.8%) | 7 (3.1%) | 69 | (30.7%) 149 | (66.2%) University students co-medical personal bank clerk. | 37.9(11.5) | 19–67 |
| Overall | 28 (3.7%) | 213 | (28.1%) 516 | (68.2%) 20 | (3.9%) | 149 (29.4%) | 74 (3.9%) | 567 | (30.2%) 1237 | (65.9%) | | |

Table 3
Comparison of serotonin transporter genotype and allele frequency between centenarians and younger controls

| | Men | | Women | | Combined | |
|---|---|---|---|---|---|---|
| | Centenarians | Young control | Centenarians | Young control | Centenarians | Young control |
| Genotype frequency | | | | | | |
| l/l | 3 (5.1%) | 28 (3.7%) | 19 (9.2%) | 20 (3.9%) | 22 (8.3%) | 58 (4.2%) |
| l/s | 22 (37.3%) | 213 (28.1%) | 61 (29.6%) | 149 (29.4%) | 83 (31.3%) | 408 (29.6%) |
| s/s | 34 (57.6%) | 516 (68.2%) | 126 (61.2%) | 338 (66.7%) | 160 (60.4%) | 911 (66.2%) |
| $\chi^2$ value | | | | | | |
| l/l vs. l/s and s/s | | 0.3 n.s. | | 6.1* | | 7.8** |
| l/l and l/s vs. s/s | | 2.8 n.s. | | 2.5 n.s. | | 3.8 n.s. |
| Allele frequency | | | | | | |
| L allele | 28 (23.7%) | 269 (17.8%) | 99 (24.0%) | 189 (18.6%) | 127 (24.0%) | 524 (19.0%) |
| S allele | 90 (76.3%) | 1245 (82.2%) | 313 (76.0%) | 825 (81.4%) | 403 (76.0%) | 2230 (81.0%) |
| $\chi^2$ value | | 2.6 n.s. | | 5.3* | | 7.2** |

* Indicates $p < 0.05$.
** Indicates $p < 0.01$.

evaluate distribution differences in genotype and allele frequency. Genotype × sex analysis of variance (ANOVA) was performed to evaluate associations between 5HTT genotype, personality traits and Barthel Index, MMSE score, and serum albumin concentrations. Multiple comparisons were performed by the Tukey HSD method. Differences were considered significant at $p < 0.05$. Statistical power was calculated by Sample power version 2 (SPSS, Japan).

## 3. Results

### 3.1. Distribution and allele frequency of the 5HTT genotype

The distribution of genotype and allele frequency for 5HTT in centenarians and control subjects is presented in Table 3. The genotype distributions matched with expectations for a Hardy–Weinberg equilibrium, in younger control. The l/l genotype was uncommon in both centenarians (8.3%) and control subjects (3.9%). The distribution of genotypes between centenarians and young control was compared. Statistically significant difference was observed (l/l versus l/s and s/s, $\chi^2 = 7.8, p < 0.01$). When an analysis was performed according to gender, a significant distribution difference was observed for women ($\chi^2 = 6.1$, $p < 0.05$) but not for men. However, significant difference was not observed between l allele-positive (l/l and l/s, combined) and s/s participants. The frequency of the l allele was significantly higher in centenarians (24.0% versus 19.0%; $\chi^2 = 7.1$, $p < 0.01$); this effect was observed only for women (24.0% versus 18.6%; $\chi^2 = 5.3$, $p < 0.05$). This null effect in men was not derived from low statistical power to detect a significant difference ($\alpha = 0.05$, power = 10%, 2-tailed) in this sample.

### 3.2. Association between the 5HTT genotype and physical, cognitive, and biological status

The effects of the 5HTT genotype on NEO-FFI, Barthel Index, MMSE, and serum albumin were analyzed in centenarians (Table 4). No significant effect of genotype was observed for Barthel Index or MMSE and any of NEO-FFI dimensions. A significant main effect of genotype was observed for serum albumin concentration ($F = 4.2$, $p < 0.05$). Further, multiple comparison indicated that serum albumin concentrations were higher in association with the l/l genotype than with the s/s genotype ($p < 0.01$). Neither a significant gender main effect nor a gender-genotype interaction was observed. We also conducted the same analysis on serum albumin concentration for our

Table 4
Mean and (S.D.) of NEO-FFI, Barthel Index, Mini-Mental State Examination and serum albumin concentration by 5HTT genotypes in centenarians

| | Men | | | | | | Women | | | | | | Statistics |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | l/l | | l/s | | s/s | | l/l | | l/s | | s/s | | |
| NEO-FFI dimensions | | | | | | | | | | | | | |
| Neuroticism | 21.5 | (6.4) | 15.9 | (8.8) | 17.4 | (5.8) | 22.2 | (6.4) | 19.3 | (6.2) | 19.4 | (5.8) | n.s.. |
| Extravesion | 23.0 | (5.7) | 26.7 | (6.2) | 25.8 | (5.7) | 27.9 | (7.9) | 27.0 | (6.9) | 26.0 | (6.4) | n.s.. |
| Openness | 25.0 | (4.2) | 22.2 | (6.1) | 23.2 | (6.0) | 21.9 | (4.4) | 22.4 | (5.0) | 22.4 | (4.8) | n.s.. |
| Agreeableness | 30.0 | (0.0) | 31.5 | (6.4) | 31.5 | (8.1) | 30.4 | (7.8) | 30.8 | (7.8) | 31.3 | (7.8) | n.s.. |
| Conscientiousness | 35.5 | (3.5) | 32.3 | (9.2) | 33.9 | (7.7) | 31.7 | (6.6) | 31.4 | (7.2) | 31.8 | (5.8) | n.s.. |
| Barthel Index | 73.3 | (46.2) | 73.3 | (32.7) | 73.3 | (34.1) | 48.7 | (37.7) | 44.9 | (32.9) | 39.5 | (33.1) | Sex $p < 0.01$ |
| MMSE | 20.3 | (5.5) | 16.3 | (8.7) | 17.1 | (8.4) | 13.9 | (8.5) | 13.2 | (7.7) | 12.3 | (8.1) | Sex $p < 0.05$. |
| Albumin (g/dl) | 4.1 | (0.2) | 3.7 | (0.4) | 3.6 | (0.4) | 3.8 | (0.4) | 3.6 | (0.4) | 3.6 | (0.4) | Genotype $p < 0.05$ |

younger control subjects. However, no significant effect of 5HTT genotype was observed (data not shown).

## 4. Discussion

The influence of 5HTT polymorphism on longevity in the Japanese population was investigated. We hypothesized that 5HTT polymorphism could mediate the human affective state, which influences health and longevity. First, we found a significant difference in genotype distribution and allele frequency for 5HTT polymorphism between centenarians and younger control subjects, particularly women. Second, we found a statistically significant but weak association between the 5HTT genotype and serum albumin concentration, which is a biological marker of health status among centenarians.

There is much psychological stress in human life. Centenarians living in Tokyo have experienced catastrophic life events including a catastrophic earthquake, two world wars, and personal life events such as bereavement resulting from the deaths of spouses and children. The negative impact of these life events on the affective state is thought to be greater in individuals with the s allele (Caspi et al., 2003; Grabe et al., 2004). We found that the distribution of both the l/l genotype and the l allele, which is a protective factor against stressful experiences, was higher in centenarians than in young control subjects. Even though we could not examine the actual responses to the stressful situations of centenarians during their long lives, this finding suggests that this affect-related gene polymorphism may have a positive impact upon human longevity. The mechanism is unknown but perhaps acts through immune (Takata et al., 1987; Bonafe et al., 2001) or metabolic pathways (Schachter et al., 1994; Geesaman et al., 2003) that are also linked to longevity.

However, no association was found between the 5HTT genotype and anxiety-related personality traits among the centenarians. Our previous report indicated that centenarians are more neurotic than young controls (Masui et al., 2002). This higher degree of neuroticism contributes to increased health-seeking behavior, such as frequently consulting a doctor (Jerram and Coleman, 1999), which is assumed to be a helpful coping mechanism for survival. Such a confounding factor might influence the null effect of 5HTT polymorphism on the behavioral aspect of centenarians. In addition, a recent functional neuroimaging study has shown that activation of the right amygdala, which processes angry and fearful facial expressions, was stronger in the *s*-allele-positive group than in the *s*-allele-negative group, even though there was no difference in anxiety-related personality traits between these groups (Hariri et al., 2002). According to this finding, we might hypothesize that even if we could find no difference in personality phenotype among the different genotypes, the biological response, is functioning and may contribute to survival.

The frequency of the l/l genotype and l allele was prominent only for female centenarians. We hypothesize that this gender difference could be explained by the biological and lifestyle differences between men and women. Several studies have suggested the existence of gender-genotype interactions in the effects of the serotonergic system. Whole blood serotonin level is higher in men (Weiss et al., 2005). The rate of serotonin synthesis in the central spinal fluid is lower in women than in men (Nishizawa et al., 1997), and it has been reported that the effect of the 5HTT genotype on serotonin turnover differs between the genders (Williams et al., 2003). The effect of treatment with a serotonin reuptake inhibitor on emotional disorders is greater in women throughout the life cycle (Yonkers, 2003). A higher frequency of suicide attempts was reported only in women with the s/s genotype (Baca-Garcia et al., 2002). The higher ratio of the l/l genotype in women, theoretically results in increased sensitivity of their serotoninergic system.

Lifestyle differences may also influence gender differences in genotype distribution. Several papers have reported adverse effects of the l allele on longevity. Relationships have been found in Japanese men between possession of the l allele and a smoking habit (Ishikawa et al., 1999), earlier onset of alcoholism (Ishiguro et al., 1999), and a higher prevalence of coronary heart disease among smokers (Arinami et al., 1999). Both alcohol drinking and smoking habits are more prevalent in men than in women in the Japanese population. The positive effect on survival of possessing the l allele might thus be obscured in men because of a lifestyle-genotype interaction.

A gender difference in the gene effect has been reported in several centenarian studies. Among Italian centenarians, an interferon-γ allele polymorphism difference was observed only in women (Lio et al., 2002a), while a −1082G homozygosity difference was observed only in men (Lio et al., 2002b). The G allele at the −174C/G locus, which influences plasma interleukin (IL)-6 concentration, was decreased only in male centenarians (Bonafe et al., 2001). These authors explained the gender difference in terms of hormonal regulation. With regard to the gender difference in the effect of 5HTT polymorphism, it was considered that this might be influenced by the difference of lifestyle and/or hormonal status, between men and women.

We were able to analyze the data from 25.2% of all recruited centenarians. This might have resulted in a selection bias such that those centenarians with low anxiety levels who possess the l allele and high ADL healthier centenarians tended to participate in this study. With regard to the anxiety level, because of the prevalence of dementia among our sample of centenarians (65% of all participants), in the majority of cases agreement to participate was obtained from a family member or by proxy, rather than from the centenarians themselves. Thus, it is unlikely that the anxiety level of the centenarians influenced the decision to participate. We also compared the NEO-FFI score between those mail-survey participants ($n = 209$) who did not agree to be visited by our research members and those

who did; no significant difference between the two groups was observed for any of the personality dimensions. Thus, we believe that the gene-related anxiety level did not influence participation in the study.

On the other hand, though the ADL level of whole participants of our study was deteriorated, Barthel Index score in visit survey participants (mean = 44.1, S.D. = 34.8) were significantly higher ($p < 0.01$) than in mail-survey participants (mean = 34.4, S.D. = 32.7). This implies that the healthier centenarians were likely to participate in our study. To avoid the sampling bias, non-invasive sampling method (e.g. postal buccal washes) will be need in future study. However, in terms of exploring the long live and healthy aging gene polymorphisms, the purpose of our study, observed result here could be situated as to find a clue of contribution of affect-associated gene to the longevity.

In terms of sampling bias, we also have to mention the young control data. Because of the low frequency of l/l genotype, and not population based standardized value, we used pooled values as control. This method has advantage to stabilized the scattered genotype distribution among small sample studies. However, we could not avoid the publication bias, that not all conducted data were published and difficult to control the participants back ground. Standardized distribution of 5HTT polymorphism in various age groups with representative sample must be compared to conform and replicate this finding.

We hypothesized that 5HTT gene polymorphism would influence not only longevity, but also functional status among centenarians. As expected, we found that l/l individuals exhibited a significant but slightly higher serum albumin concentration, which is a strong marker of physical function, cognitive function, and mortality in centenarians (Shimizu et al., 2001), than s/s individuals. The biological mechanism linking the gene polymorphism and serum albumin level has not yet been characterized.

Finally, the frequency of the l allele is low in the Japanese population, and the l/l genotype is relatively rare (about 5%, from our review of the literature). It is paradoxical that the frequency of the l/l genotype, which may contribute to longevity, is lower among ethnic groups with the longest average lifespan. However, serotonergic function may vary with both ethnicity and gender (Kunugi et al., 1997; Gelernter et al., 1998). The lifetime morbidity rate for affective disorders is lower in Asian populations than among Caucasians (Weissman et al., 1996). Some of this difference may be artifact due to differences in the way different cultures measure affective disorders. For example, despite a lower prevalence of affective disorders in Asia suicide among the elderly is markedly higher than in the U.S. and many Western countries (Rockett and Smith, 1989). Nevertheless, a positive effect of the l allele upon longevity or biological functions might not apply equally to all ethnic groups. In addition, we did not confirm any association between age and serum albumin concentration by multi-cohort or longitudinal study. Further study is required to investigate these associations using a lager sample and different design, as well as to confirm the associations in different ethnic groups and genders.

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ARTICLE

# A combination of three common inherited mitochondrial DNA polymorphisms promotes longevity in Finnish and Japanese subjects

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**Mitochondrial DNA (mtDNA) coding region polymorphisms, as well as the 150T polymorphism in the noncoding region, have been associated with longevity. We have studied here the association of 150T with longevity further and assessed differences in this association between various mtDNA haplogroups. We analysed a sample of 321 very old subjects and 489 middle-aged controls from Finland and Japan. 150T was more frequent among the very old than among the controls in both the Finnish and Japanese subjects. Interestingly, the association was not similar in all haplogroups, and a stratified analysis revealed that two additional common polymorphisms, 489C and 10398G, modified the association between 150T and longevity. These findings suggest that longevity is partly determined by epistatic interactions involving these three mtDNA loci.**
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**Keywords:** mitochondrial DNA; longevity; control region; epistasis; association study; phylogenetic analysis; haplogroup

## Introduction

Mitochondrial DNA (mtDNA) is a maternally inherited genome that encodes 22 tRNAs, two rRNAs and 13 subunits of the respiratory chain complexes and ATP synthase. These complexes catalyse the reactions of oxidative phosphorylation that produce ATP and also contribute to oxygen free radicals, which are thought to play a role in the aging process.[1] Interestingly, longevity shows maternal inheritance.[2] Uniparental inheritance and high mutation rate have led to mtDNA lineages (haplogroups), which are defined by ancient polymorphisms and characterized by considerable variation. The European population is almost exclusively distributed among the nine haplogroups designated as H, I, J, K, T, U, V, W and X, whereas haplogroups A, B, C, D, F, G and certain subclusters of macrohaplogroups M and N are characteristic to Asian populations, haplogroups A, B, C and D to native Americans and haplogroups L0, L1, L2 and L3 to African populations.[3–5]

There is evidence that certain coding region polymorphisms specific to mtDNA haplogroups may be relevant for common diseases and traits.[6] Longevity has been found to be associated with mtDNA coding region polymorphisms

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such as 5178A (characterizing haplogroup D) in the Japanese[7] and 9055A (characterizing haplogroup K) in the French[8] and Irish,[9] and mtDNA haplogroup J in the Italians[10] and the Finns.[11] Furthermore, the 150T polymorphism within a 1.1 kb noncoding control region of mtDNA has been reported to be more prevalent in centenarians than in controls.[12] Interestingly, 150T is present in several haplogroups among the global population including haplogroups D and J.[13] In this study we identified carriers of 150T among 810 very old subjects and middle-aged controls from Finland and Japan in order to examine the association of this polymorphism with longevity further. We also assessed the possibility that the association between 150T and longevity is modified by other control region sequence variation or haplogroup-specific coding region variation.

## Subjects and methods

The group of very old subjects comprised 225 Finns (age 90 or 91 years) and 96 Japanese (age 100–104 years), and the middle-aged controls 393 Finns and 96 Japanese. The controls were from the same geographical regions as the very old subjects. The allele status at position 150 was determined by direct sequencing of the Japanese samples and by allele-specific amplification of the Finnish samples. DNA extracted from blood was amplified in the presence of an oligonucleotide containing an allele-specific Locked Nucleic Acid® (LNA) (Proligo LLC, Paris, France). Samples harbouring 150C could be amplified in the presence of a forward primer 5′-CTGTCTTTGATTCCTGCCTCATC (LNA underlined), and samples harbouring 150T in the presence of a forward primer 5′-CTGTCTTTGATTCCTGCCTCATT. Each sample was amplified in both reactions with 5′-CTGTTAAAAGTGCATACCGCCAA as the reverse primer. The amplified 302-bp fragment was visualized by agarose gel electrophoresis. Sequencing of selected samples was used to verify the reliability of the results obtained by allele-specific amplification.

Established haplogroup-defining polymorphisms in the coding region were determined by direct sequencing or by restriction fragment analysis[14] in the carriers of 150T in order to assign the samples to mtDNA haplogroups.[3,4] The mtDNA control region spanning the nucleotides 16024-00576 was sequenced in the samples harbouring 150T.

One-tailed Fisher's exact test was used to assess the hypotheses that the frequency of 150T was higher and the frequencies of haplogroups harbouring 150T were higher among the very old subjects than among the controls. The frequencies of the very old subjects and the controls in each haplogroup were represented as $2 \times 2$ tables, and heterogeneity among these was evaluated by using RelRisk 2.33.[15] Associations between alleles at polymorphic sites and longevity were measured by the $\chi^2$ statistic. An estimate of the significance of the highest observed chi-square ($\chi^2_{max}$) was determined in a randomization test, where the labels 'case' and 'control' were permuted 100 000 times and the global *P*-value was estimated by the proportion of permutation samples with $\chi^2_{max}$ equal to or higher than that in the observed data.

## Results

We found 65 very old subjects and 66 controls with 150T (Table 1), implying a significantly higher frequency among the very old than among the controls in both the Finns and the Japanese. The control region sequence was available for all 150T carriers, except for one U5 control subject who was therefore excluded from subsequent analyses. A total of 88 polymorphic sites were identified, and the strongest association to longevity among these control region sequences was found for 489C ($\chi^2 = 14.099$; permutation test: $P = 0.00165$). When the position 489 was excluded, a permutation test revealed that no other control region variants were significantly associated with longevity among the 150T carriers ($\chi^2_{max} = 7.075$; permutation test: $P = 0.126$). A phylogenetic analysis then revealed that 150T and 489C were both present in subhaplogroups J2, D5 and M7b, whereas 150T, but not 489C, was present in T2, U5 and N9a (Figure 1). In addition, the coding region polymorphism 10398G was found to co-segregate with 489C.

A contingency table analysis of the frequencies of the very old subjects and the controls in subhaplogroups J2, D5, M7b, T2, U5 and N9a revealed a significant heterogeneity ($\chi^2 = 17.604$, df = 5, $P = 0.0035$). Subhaplogroups J2, D5 and M7b were more common among the very old, and the combined frequency of these subhaplogroups was significantly higher among the very old subjects than among the controls (Table 1).

## Discussion

Secondary origins of heavy strand replication of mtDNA (nucleotide positions 146–151) are located in the vicinity of nucleotide position 150, and it has been suggested that 150T alters the location of the origin of heavy strand replication and that 150T is related to the regulation of mtDNA replication,[12] for instance by providing a replicative advantage to a genome that harbours the mutation. The proportion of 150T relative to 150C in fibroblasts has been found to increase with advanced age.[12] Other polymorphisms in this region could have a similar effect, and 152T>C and 195T>C have also been shown to accumulate in the fibroblasts of aged individuals[16] and 189A>G in the muscle.[17] We found that the majority of the samples belonging to J2 harboured at least four mutations close to the origins of replication of mtDNA, whereas similar pattern was not found for D5, M7b, N9a, T2, or U5. Therefore, polymorphisms near the origin of the

**Table 1** Frequency of subjects with the 150T allele among the very old subjects and middle-aged controls

| | *Very old* | | *Controls* | | |
|---|---|---|---|---|---|
| | *N* | *%* | *N* | *%* | *P-value* |
| *Finns and Japanese* | | | | | |
| All haplogroups | 65 | 20.2 | 66 | 13.5 | 0.007* |
| Subhaplogroups J2, D5, M7b | 22 | 6.9 | 5 | 1.0 | $7.8 \times 10^{-6}$* |
| Subhaplogroups T2, U5, N9a | 37 | 11.5 | 56 | 11.5 | 0.53 |
| Other haplogroups | 6 | 1.8 | 5 | 1.0 | 0.24 |
| *Finns* | | | | | |
| All haplogroups | 46 | 20.4 | 57 | 14.5 | 0.037* |
| Subhaplogroup J2 | 8 | 3.6 | 3 | 0.8 | 0.015* |
| Subhaplogroup T2 | 1 | 0.4 | 4 | 1.0 | 0.90 |
| Subhaplogroup U5 | 34 | 15.1 | 48 | 12.2 | 0.18 |
| Other haplogroups | 3 | 1.3 | 2 | 0.5 | 0.26 |
| *Japanese* | | | | | |
| All haplogroups | 19 | 19.8 | 9 | 9.4 | 0.032* |
| Subhaplogroup D5 | 7 | 7.3 | 1 | 1.0 | 0.032* |
| Subhaplogroup M7b | 7 | 7.3 | 1 | 1.0 | 0.032* |
| Subhaplogroup N9a | 2 | 2.1 | 4 | 4.2 | 0.89 |
| Other haplogroups | 3 | 3.1 | 3 | 3.1 | 0.66 |

Other haplogroups include G, M7a and B4 among very old Japanese subjects, G, B4 and D4 among the Japanese controls, H in two very old Finnish subjects, an undefined haplogroup in one very old Finnish subject and H and U in the Finnish controls. An asterisk denotes significance at the 95% confidence level ($P<0.05$), unadjusted for multiple comparisons.

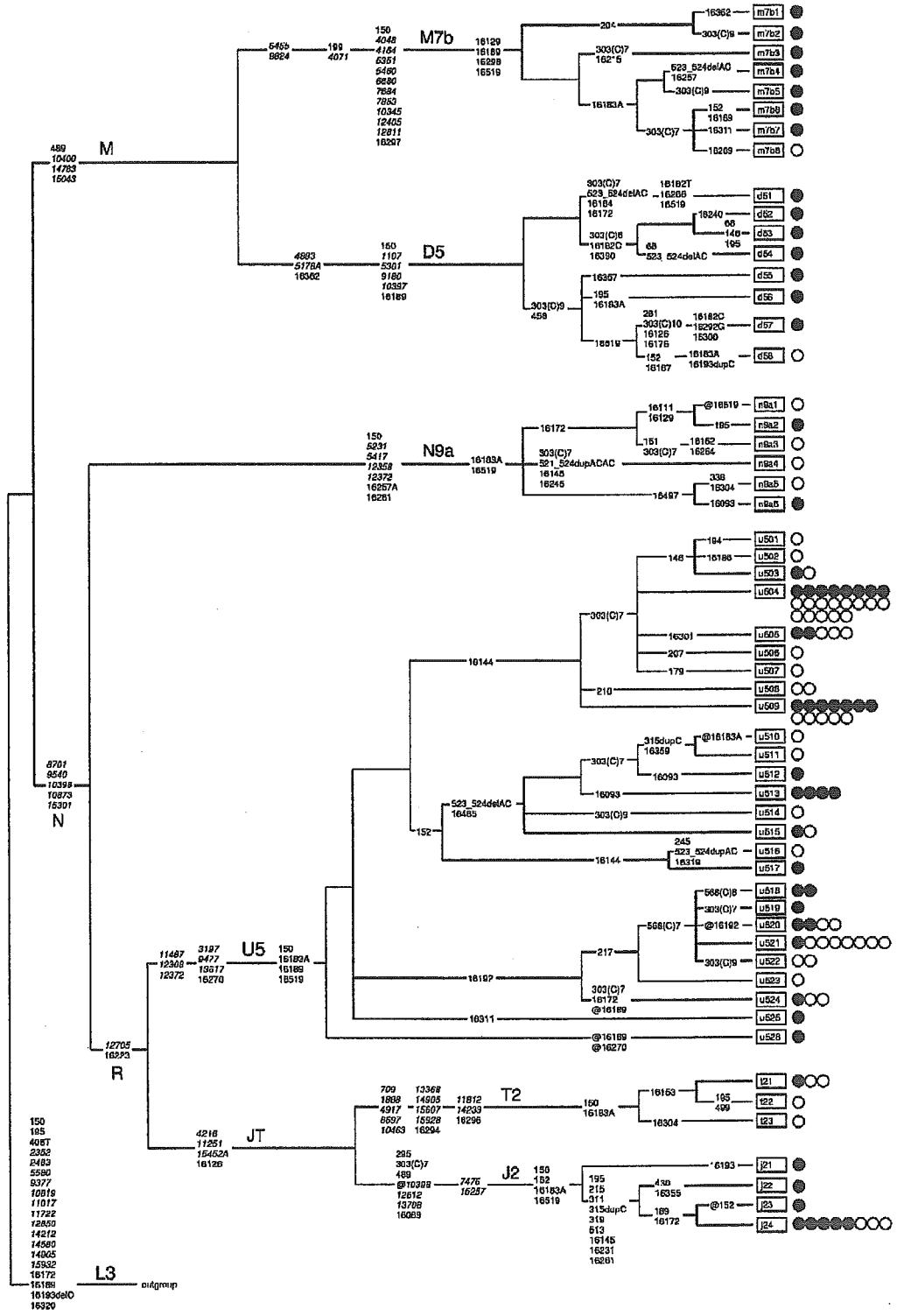
heavy strand replication could explain the association between longevity and subhaplogroup J2, but not the association between longevity and D5 and M7b.

150C>T polymorphism emerged separately in the early evolution of the European subhaplogroups J2, T2 and U5, and of the Asian subhaplogroups D5, M7b and N9a, but has only occasionally been noted elsewhere in the mtDNA phylogeny. Subhaplogroups D5 and M7b of the Japanese belong to mtDNA macrohaplogroup M, which has diverged from African haplogroup L3 and from macrohaplogroup N some 60 000 years ago.[18] On the other hand, N9a of the Japanese and J2, T2 and U5 of the Finns belong to macrohaplogroup N. Most of the haplogroups in macrohaplogroup N harbour an ancient 10398G>A mutation, which alters the amino acid 114 in the MTND3 gene, but haplogroup J has experienced a back-mutation at this site resulting in the 10398G allele in common with macrohaplogroup M and, therefore, common with D5 and M7b. In addition, haplogroup J harbours the control region mutation 489T>C, which also occurred early in the evolution of macrohaplogroup M. Our data thus showed that 150T is associated with longevity in subhaplogroups J2, D5 and M7b that harbour 10398G and 489C, but not in subhaplogroups T2, U5 and N9a that lack the latter two polymorphisms. The association between a combination of these three mtDNA variants and longevity provides the first epidemiological support for the assumption that the pathogenic or adaptive nature of a variant is influenced by interactions with other loci in mtDNA.[19]

Our previous study on Finnish nonagenarians has suggested an association between haplogroups J and U and longevity, respectively, and a negative association between haplogroup H and longevity.[11] The absence of 150T in haplogroup H coincides with the lower frequency of this haplogroup among the very old and, similarly, the higher frequency of haplogroup J among the very old coincides with the presence of 150T within haplogroup J2. However, there is discrepancy between the higher frequency of haplogroup U among the very old and the lack of association between 150T and longevity within subhaplogroup U5, suggesting that other yet unidentified polymorphisms in mtDNA contribute to the association between haplogroup U and longevity.

Longevity is a complex trait in which variation depends on epistatic interactions between several genetic loci and

**Figure 1** Phylogenetic tree depicting the origin and evolution of mtDNA subhaplogroups harbouring 150C>T (M7b, D5, N9a, U5, T2 and J2). Polymorphisms detected in mtDNA control region spanning nucleotides 16024-00576 in the very old subjects and the controls are shown in normal font, and established haplogroup-defining polymorphisms[3,4] are shown in italics. Letter suffixes indicate transversions. Insertions, deletions and repeat length polymorphisms are shown according to the recommended nomenclature. @=back-mutation; outgroup=the African reference sequence for human mtDNA (Genbank NC_001807.4, GI:17981852). The root of the phylogeny includes the transition at position 150, because the outgroup sequence coincidentally contains a parallel 150T allele. Each solid circle represents one very old subject and each open circle represents a control subject.


M
M7b
D5
N9a
N
R
U5
JT
T2
J2
L3
outGroup
m7b1
m7b2
m7b3
m7b4
m7b5
m7b8
m7b7
m7b6
d51
d52
d53
d54
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d56
d57
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n9a1
n9a2
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on environmental exposures during life.[20] Previous analyses of association between mtDNA variation and longevity[7,8] have focused on single loci and not taken into account nucleotide variation elsewhere in the genome. If several loci play a greater role in determining trait variability than do single loci, a combination of alleles may show a stronger association with a trait than any single locus. This is what we found here for the combination of the 150T, 489C and 10398G alleles, suggesting that mtDNA variation promotes longevity through epistatic effects. The possibility of epistasis also implies that attempts to assess the role of mtDNA variation in complex diseases or traits by single-loci association analyses may overlook loci whose contribution is revealed only when considered in combination with others.

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第47回日本老年医学会学術集会記録
〈ミニレビュー〉

# 百寿者の遺伝背景—長寿遺伝子同定の戦略—

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**Key words**：Familiar longevity, Sporadic longevity, Longevity gene, Whole genome scan, Genetics of healthy aging
（日老医誌　2005；42：664—665）

## 初めに

超高齢社会を迎えて，健康長寿をいかに達成するかが今までになく注目されている．この為健康長寿のモデルと考えられる百寿者の遺伝素因の検討が注目されている．このレビューでは，百寿者の遺伝素因に関して今後の研究方向を中心に解説を試みる．

## 百寿者の人口動態

百寿者の数は急速に増加しており，昭和25年には全国で100人弱だったとされている．しかし現在では2万人を超えておりなおも急増している．男女比は1対4で女性が多い．百寿者の急増，女性が多いことは各国で報告されており日本に特有の現象ではない．全人口当たりの割合では昭和25年では85万人に一人であったが，平成15年では6,000人に一人となった．

## 百寿者の遺伝素因を調べて何が分かるか

この様な長寿者の遺伝素因を調べることにより，1)長寿遺伝子の同定，2）老化制御遺伝子の同定，3）疾患遺伝子の対照群として有用であると考えられる．一方で百歳まで生き残るにはただ単に疾患の危険因子がなければ可能で特別な長寿遺伝子は存在しないという考えもある．現時点では長寿遺伝子が存在するかどうかは確定されていない．今後数年で解答がでることが予想される．

## 長寿は遺伝か環境か

長生きは家系かどうかという質問をよく尋ねられる．デンマークの双子調査では平均寿命到達率は20から30%の遺伝関与があると報告されている[1)]．一方沖縄百寿者調査を行っている鈴木らは沖縄百寿者家系の調査を行い百寿者家系と非百寿者家系の寿命の長さを検討した[2)]．百寿者家系では，平均寿命到達率，80歳到達率，90歳到達率などは非百寿者家系に比較して有意に高かった．しかし実際に百寿者家系を調べると，図1の如く長寿家系もあり，図2の如く長寿家系とはいえないものもある．長寿家系の百寿者と非長寿家系の百寿者がいることが分かる．つまり長寿に遺伝が関与するかという質問は単純すぎることが示唆された．我々は長寿家系出身の百寿者を家族性長寿(familiar longevity)，非長寿家系の百寿者を弧発性長寿（sporadic longevity）として別のカテゴリーに属するものとして，別々に解析をすることが重要であろうと考えている．

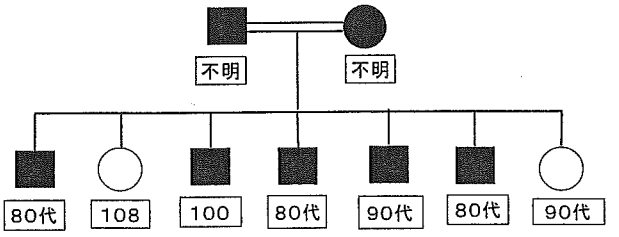


**図1**　家系図1
長寿家系—Familiar longevity—

## 今までの長寿遺伝子同定のための方法

従来特定のphenotype（この場合は長寿）を決定するためには，1）候補遺伝子解析，2）同胞罹患解析が行われている．候補遺伝子は長寿に関係あると思われる遺伝子を対象として長寿者と若年対照群で比較することが行われている．長寿者の特徴から考えられる遺伝素因，基礎老化から得られた遺伝素因が候補遺伝子として検討されている．Sib解析は長寿の同胞を集めて解析するもので，長寿ではsib解析は出来ないと考えられていた（familiarとsporadic longevityがあることより）．2001年アメリカのPerlsらは初めてsib解析を行った．その結

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Genetics of centenarian
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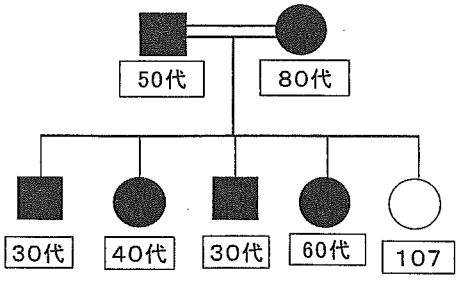


図2　家系図2
非長寿家系—sporadic longevity—

果第4染色体に長寿遺伝子の場があることを報告した[3]．この発表は老化科学研究者に衝撃を与えた．引き続いて彼らは2003年に長寿遺伝子はMTP（microsomal transfer protein）であるという報告をした[4]．実際にsib解析が長寿遺伝子を同定できるということが判明した．このために長寿同胞を収集する調査が行われつつある．ただしMTPはアメリカの長寿者では有意の結果を示したが，フランス，日本では確認できなかった．

Whole genome scanが様々な疾患原因遺伝子同定のために行われている．マイクロサテライトを用いる方法が一般的であったが，最近多数のSNPを用いた高密度SNPによるwhole genome scanが可能となってきた．例えば400マイクロサテライトを用いてスキャンすると10cM（センチモルガン）までしか確定できないが，50万SNPを用いると3Kベースまで詰めることが可能となる．このために遺伝子の同定が格段に容易くなる．Whole genome scanは候補遺伝子法と異なり全genomeを調べるために取りこぼしが少なく，思いがけない遺伝子がでてくる可能性もある．

## どのような対象を解析するか，対照群の設定をどうするか

長寿遺伝子を同定するためには極端な長寿者を調べることが成功する可能性が高いと予想される．100歳以上者は全人口あたり6,000人に一人であり（百歳率）extreme longevityの代表と考えられるが，同じ年に生まれた人が何人100歳まで到達（百歳達成率）できるかを調べると約260人に一人（男性770人，女性155人）であった．百歳達成率は急速に高くなっており近い将来には100歳者は長寿モデルでなくなる可能性もあること，また対照群の設定が困難になる事も予想される（多くの人が100歳まで到達すると仮定すると，一般の若い人を対照群とするとその中に100歳まで到達する人がでてくるため）．我々は長寿のハードルを高くして105歳としこの年代を対象とすることを計画した．

2002年での105歳以上者（超百寿者）は全国で850名であり100万人あたり7人という割合であった．同じ年に生まれた人が105歳まで到達できる確率は3,000人に一人である．この数字は非常に希な長寿であること，対照群の設定が容易であることを示す．さらにTokyo centenarian studyでの調査参加率は約15%であることから130名の方の調査が予想された．この数字は統計解析を行う際に十分ではないが必要な数を満たしていると考えられる（実際に我々の調査では130名の方の参加を得た）．

もう一つの対象群は長寿同胞である．長寿である同胞はfamiliar longevityの遺伝素因解析に有用であろう．2004年よりEU全体で2,800組の長寿同胞を集め健康長寿の遺伝解析をするgenetics of healthy aging（GEHA）調査がスタートした．日本でも健康長寿調査を行うことを準備している．

## 将来の展望

extreme longevityを達成した方が増えていること，遺伝子工学が急速に進歩している現在では，長寿遺伝子があるかどうかの議論を行うよりもextreme longevityを集め，genome解析を行う方がより確実な結果を得ることが出来る．超百寿者を対象に高密度SNPを用いたwhole genome scanを行い，ついで遺伝素因の強い健康長寿同胞のsib解析を行い，さらに二つの結果を重ねることで長寿遺伝子があるかどうか，あればどんなものかが確定できるものと考えられる．

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*Original Article*

# Association of Dopamine β-Hydroxylase Polymorphism with Hypertension through Interaction with Fasting Plasma Glucose in Japanese

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**Dopamine-β-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine and is released from sympathetic neurons into the circulation. Several lines of evidence, including the finding of elevated plasma DBH activity in essential hypertension, suggest an important role of DBH in hypertension. Recently, a novel polymorphism (-1021C/T) in the 5′ flanking region of the DBH gene has been shown to account for 35–52% of the variation in plasma DBH activity. We therefore investigated the possible association between the DBH -1021C/T polymorphism and hypertension in a large Japanese population. Moreover, because the development of hypertension is considered to be due at least partly to gene-environmental interactions, we also investigated the possible interactions between the DBH -1021C/T polymorphism and environmental factors. Consequently, we found a significant interaction between the DBH -1021C/T polymorphism and fasting plasma glucose (FPG) in the association with hypertension. CC homozygotes showed a steeper increase in probability of hypertension with FPG than T allele carriers. We also found a marginally significant trend suggesting the presence of an interaction between the DBH -1021C/T polymorphism and FPG in the association with blood pressure. Consistent with the presence of the interaction, we found that a 19 bp sequence containing the DBH -1021C/T polymorphism includes two palindromic non-canonical E boxes separated by 5 bps, and closely resembles the glucose response element of the L-type pyruvate kinase gene. These findings could be helpful in conducting further molecular and biological studies on the relationship among glucose metabolism, the sympathetic nervous system, and hypertension. (*Hypertens Res* 2005; 28: 215–221)**

*Key Words*: **dopamine-β-hydroxylase, essential hypertension, genetics, polymorphism, glucose**

## Introduction

Hypertension is considered to be a complex trait to which genetic, environmental, and demographic factors contribute interactively (*1–5*). Dopamine-β-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine and is released from sympathetic neurons into the circulation. Because the sympathetic nervous system is intimately involved in both the origin and the perpetuation of a hypertensive state (*6, 7*), DBH may play an important role in the pathogenesis of essential hypertension. Indeed, neonates with DBH deficiency show episodic hypotension (*8*). DBH activity, derived largely from sympathetic nerves, can be measured

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**Table 1. Characteristics of Participants According to Hypertension Status**

| Variable | Normotensive ($n$=547) | Hypertensive ($n$=275) |
|---|---|---|
| Sex (male %) | 78.8 | 89.1 |
| Age (years) | 52.7±8.6 | 57.3±8.5 |
| Body mass index (kg/m$^2$) | 22.6±2.8 | 23.8±2.9 |
| SBP (mmHg) | 112.6±10.7 | 143.2±17.4 |
| DBP (mmHg) | 72.0±9.1 | 89.1±9.9 |
| Total cholesterol (mg/dl) | 198.0±30.6 | 202.4±37.2 |
| HDL cholesterol (mg/dl) | 54.2±14.5 | 51.9±14.0 |
| Triglyceride (mg/dl) | 116.7±81.7 | 150.9±127.7 |
| Fasting plasma glucose (mg/dl) | 101.2±17.3 | 106.0±19.2 |

Data are mean±SD. Blood pressure readings before the start of antihypertensive medication were not available for 118 hypertensive subjects whose values were measured under treatment. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein.

in human plasma (*9*, *10*), and elevated plasma DBH activity has also been shown in essential hypertension (*11*, *12*), although the conclusions have not been completely consistent (*13*). Moreover, DBH inhibitors have been shown to produce a dose-dependent decrease in mean arterial blood pressure (*14*, *15*).

The DBH gene, approximately 23 kb in length, is composed of 12 exons (*16*). Recently, a novel polymorphism (−1021C/T) in the 5′ flanking region of the DBH gene has been shown to account for 35–52% of the variation in plasma DBH activity in several ethnically different populations, including Japanese (*17*). The strong association of the DBH −1021C/T polymorphism with plasma DBH activity has also been replicated in a native Western European population (*18*). Thus, considering several lines of evidence for the relation between DBH and blood pressure, the DBH −1021C/T polymorphism appears to be an attractive candidate variable contributing to hypertension. Nevertheless, there have been few reports investigating the possible association between the DBH gene and hypertension. We therefore investigated the possible association between the DBH −1021C/T polymorphism and hypertension. Moreover, because the development of hypertension is considered to be due at least partly to gene-environmental interactions, we also investigated the possible interactions between the DBH −1021C/T polymorphism and environmental factors.

## Methods

### Subjects

According to the criteria described below, 275 hypertensive subjects and 547 normotensive subjects were selected from a population in the Hyogo region of Japan (Table 1) (*19*). All subjects were Japanese urban residents. They had participated in a medical check-up, and the mean values of variables in their personal health records were used in the analyses. All subjects gave their informed consent. The ethics committee of Ehime University approved the study.

### Diagnostic Categories

Each subject was assigned to one of the blood pressure diagnostic categories defined by the following criteria. Hypertensive subjects had a previous diagnosis of hypertension and were being treated with antihypertensive medication, or their systolic/diastolic blood pressure (SBP/DBP) was ≥140/90 mmHg. Normotensive subjects had never been treated with medication for hypertension, and their SBP/DBP was <140/90 mmHg.

Subjects were considered to have impaired fasting glycemia (IFG) if their fasting plasma glucose (FPG) concentration was ≥110 mg/dl. Subjects were considered to have diabetes mellitus (DM) if their FPG was ≥126 mg/dl.

### DNA Analysis

The TaqMan chemical method, which is an established and frequently used method (*20–23*), was used to detect the DBH −1021C/T polymorphism. The forward primer was 5′-GGATCAAGCAGAATGTCCTGAAG-3′, the reverse primer was 5′-GGCACCTCTCCCTCCTGTC-3′, the T-allele specific probe was 5′-Fam-CTCTCCCACAAGTAGA-MGB-3′, and the C-allele specific probe was 5′-Vic-CTC CCGCAAGTAGA-MGB-3′. The person who assessed the genotype was blinded to the clinical data of the subjects from whom the samples originated.

### Statistical Methods

Statistical analysis was performed with SPSS statistical software. Comparisons of categorical variables were performed using the $\chi^2$ test. Analysis of variance was used to assess differences in means and variances of continuous variables. Logarithmically transformed plasma triglyceride (TG) and FPG values were used in the analysis. Logistic regression models were used to assess whether the DBH −1021C/T polymorphism made a statistically significant contribution to prediction of hypertension, with consideration of interactions between the polymorphism and confounding factors. General linear regression models were used to assess whether the DBH −1021C/T polymorphism made a statistically significant contribution to prediction of blood pressure, with consideration of interactions between the polymorphism and confounding factors. $p$ values less than 0.05 were considered statistically significant.