

人類生態班 B

ラオス人民民主共和国サバナケット県ソンコン郡における長寿のゲノム栄養学的研究

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Nutrigenomics studies on longevity of people living in Songkohn District, Lao PDR

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要旨：共同研究者の門司（長崎大学熱帯医学研究所）らにより、ラオス国サバナケット県ソンコン郡ラハナム村、ターカムニア村、ドンバング村のセンサス（人口調査）が行われ、老齢人口の割合などが得られた。このセンサスを基に現地で行われた歯科検診の際、文書による同意書を得た被験者について遺伝子解析のための口腔粘膜の採取を行った（2004年12月）。得られた試料は63歳以上の高齢者95名（うち最高齢101歳）、20～30代の若者133名、合計228名であった。

持ち帰ったラオスの試料について、儉約遺伝子として知られているβ3アドレナリン受容体（BAR-3）遺伝子及び2型脂肪酸結合蛋白質（FABP2）遺伝子のコーディングSNP、日本人百寿者の研究において長寿と関係があるとされているミトコンドリアハプログループDに位置づけられるND2（Met237-allele）を、それぞれPCR-RFLP法とTaqMan PCR法により測定し、若者群と高齢者群との間で各遺伝子のアリル頻度を詳細に解析した。

BAR-3は儉約タイプと言われているArg64-allele頻度が高齢者群で0.021、若者群で0.004であり生殖年齢以降の余命との関係が示唆された。また文献的研究から他の民族に比べても低頻度であった。FABP2遺伝子のThr54-allele頻度は高齢者群（0.100）、若者群（0.109）で長寿との関連は見られなかったが他民族に比べ低値を示した。母性遺伝するND2遺伝子のMet237-alleleは高齢者群（0.011）、若者群（0.015）でハプログループDに属する日本人百寿者群（0.56-0.6）、一般群（0.4-0.45）とは異なる結果を示した。

Introduction

After completion of “Human Genome Project (HGP)” , we are now in an epoch of post-genomic research. HGP revealed that we have approximately 22,000 genes and more than 5 million sites of single-nucleotide polymorphisms (SNPs) in our genomes. Some of these SNPs can be utilized to explain the individual difference in drug response or its side effects, indicating a feasibility of “personalized medicine” in the near future. In the field of nutrition and health sciences, we are also hoping that nutrigenomics studies will pave a concrete way for “personalized nutrition” . This personalized nutrition now becomes extremely important especially in developing countries more than in developed countries. Because in most of the developing countries, people had kept their traditional food habit for a long time in the 20th century and after a start of the present century they are rapidly changing their foods to more westernized foods such as fast foods or high-calorie foods, they are now facing to a drastic change in quality and quantity of nutrients uptaken. These behavioral changes may cause novel health problems in public and produce a potential selection pressure, while infectious disease was a major selection pressure for a long time in the 20th century because of nation’ s poor public health service and under-nutrition.

People living in the rural area of Lao PDR may have acquired and succeeded a certain phenotype resistant against both infectious diseases and under-nutrition through a natural selection during the last century. For example, the phenotype of susceptibility to infectious diseases and nutrient deficiency might have been deleted from their genomes. On the other hand, they may be inherently or genetically unarmed against massive exposure of high-calorie foods or over-nutrition. The genotype of resistance to under-nutrition may be not in agreement with the genotype of resistance to over-nutrition. In most of the developed countries, many people are now struggling against life-style related diseases such as obesity, diabetes mellitus, hypertension, hyperlipidemia, atherosclerosis predisposed by over-nutrition or high-calorie diet, even though Caucasian, compared with the native Americans in general, have been reported to show less allelic frequency of so-called “thrifty genes” including beta-3 adrenergic receptor (BAR3), fatty acid binding protein-2 (FABP2), NADH dehydrogenase subunit-2 (ND2), uncoupling protein-1 (UCP1) and so on. Therefore, it is of crucial importance to survey allelic frequencies of the thrifty genes in the developing country prior to drastic changes in their eating habit.

In the present study, we aimed to measure the allelic frequencies of the above-mentioned thrifty genes of the general population in Songkohn District, Lao PDR in comparison with the Japanese counterpart, in order to estimate any phenotypic linkage between traditional food habit and genetic background.

The ultimate goal of this study is to shed light on the possible relationship of traditional food habit and genetic background of the people living in Lao PDR, comparing with other eastern Asian countries. We already have several hundreds samples of the genomic DNAs from the Bangladesh and Japanese populations. In comparison with these samples, the allelic frequencies of several thrifty genes will be measured with Laotian samples. Consequently some regional (or natural environmental) and ethnic (cultural environmental) factors may be identified for nation or county officers to assess and predict health status in a given population, and to make an effective plan for health promotion program in individual level.

Methods:

Subjects

Using a random sampling method employing resident dental health examinations, we recruited 133 young

people (100 females and 33 males), age 20-30 years old in the Lahanam villages of Songkohn District, Lao PDR in December 8th to 15th, 2004, and all of the elderly people (95 in total, 44 females and 51 males), from 63 to 101 years old, living in the Lahanam villages in December 8th to 15th, 2004 were also recruited.

DNA samples and SNP analyses

During the occasion of the dental health checkup, the written consents of the subjects were taken who had been sufficiently informed about the experiment. Their buccal mucosal cells were collected by scrubbing the inside of the subject's cheek pouch with a polyester fiber-tipped applicator swab (Nippon Becton Dickinson, Tokyo). After dried in air, each swabs were individually packaged in a paper bag which was printed with a consecutive number and age and gender of each subjects.

A preparation of genomic and mitochondrial DNAs and SNP analyses were all conducted in a Laboratory of Molecular & Cellular Biology, Siebold University of Nagasaki. DNA samples were prepared by using QIAamp DNA Blood Mini kit (Qiagen, Tokyo). Nonsynonymous coding SNPs of the BAR3 gene (Trp64Arg), the FABP2 gene (Ala54Thr) and the mitochondrial ND2 gene (Leu237Met) were detected by PCR-RFLP (polymerase chain reaction based restriction fragment length polymorphism) method or PCR TaqMan probe method with allele-specific double-colored fluorescent probes. Furthermore, SNPs of the UCP1, SIRT1, and PGC1alpha genes, and the mitochondrial ATPase and cytochrome b genes were also analyzed by the same methods.

Statistical analysis

First of all, the allele frequencies were calculated in each SNP and were compared with those of the Japanese population or other ethnic groups reported in the world in order to characterize a profile of the thrifty genes in the Laotian population. Each SNP was analyzed independently to find out the SNPs potentially linked to longevity or aging. In order to elucidate the ratio of the odds of becoming elderly with a targeted genotype (dominant allele) relative to the untargeted individuals, we used an odds ratio as an indicator of the strength of association between the thrifty genes and longevity.

Ethical considerations

All DNA samples were anonymous and unlinked to specific individual information such as name, address, or any health status except for age and gender. The entire protocol of the present study was approved by the human genome research ethics review committee of Siebold University of Nagasaki.

Results and Discussion

Trp64Arg allelic frequency of the BAR3 gene

First of all, we measured the Arg64-allele frequency of the most famous thrifty gene, the β 3-adrenergic receptor (BAR3) gene. A carrier with the Arg-allele of the BAR3 gene is a poorer responder to blood noradrenalin for lipolysis in adipose tissues, resulting in a phenotype of sparing fat deposit in the body. In this context, we firstly expected a higher frequency of the Arg-allele in the Laotians population than in the Japanese population from bases of their traditional food habit and cultural environment. However, our speculation was wrong. As shown in Table 1, only 5 heterozygotes were detected with 223 Trp64Trp homozygotes, indicating that the frequency of the Arg64-allele was 0.0105. No Arg64Arg homozygote was found in these samples.

The Arg64-allele frequency (0.021) of the old age group was 5-fold higher than that (0.004) of the young age group. Although a statistical analysis is certainly required with more large population of Laotians,

Table 1. Allele frequency and genotype frequency of the *BAR3* gene in Songkohn District, Lao PDR

age groups	Allele		Genotype		
	Trp64-allele	Arg64-allele	Trp64Trp	Trp64Arg	Arg64Arg
total	451	5	223	5	0
(%)	(98.9)	(1.1)	(97.8)	(2.2)	(0)
young	265	1	132	1	0
(%)	(99.6)	(0.4)	(99.2)	(0.8)	(0)
old	186	4	91	4	0
(%)	(97.9)	(2.1)	(95.8)	(4.2)	(0)

Young: ages from 20 to 30. Old: from 63 to 101.

this finding may suggest that the Arg64-allele of the *BAR3* gene is contributing toward a long-lasting life by protecting its carrier from under-nutrition after reproduction period. As mentioned above, the Arg64-allele is a well-known thrifty gene and this allele has been well established to be linked to life-style related diseases under over-nutrition condition in the developed countries. Even though the Laotian' s eating habit will shift from a low-calorie diet to high-calorie diet in the near future, a few Arg64-allele carriers may suffer from life-style related diseases.

International comparison of the Arg64-allele frequency of the BAR3 gene.

The Arg64-allele frequency of the *BAR3* gene was extremely low, when it was compared with those of other ethnic groups previously reported elsewhere (Table 2).

As shown in Table 2, the higher frequency groups include Alaskan Eskimos, Mexican and Andes Indians, Bolivians, and Finnish, suggesting that this allele may be selected for acclimation toward cold climate. On the other hand, Turkish and Nauruans have been reported to carry no Arg64-allele of the *BAR3* gene and Sardinians and Samoans show low frequency of the allele. These groups may be adapted for tropical climate by removing or negatively selecting the Arg-allele of the *BAR3* gene. In other words, the Arg-allele might have negatively influenced the success of biological adaptation to the hot tropical environment of Lao. In this context, one can reasonably explain why the Laotians population showed extremely low frequency of the Arg-allele.

Ala54Thr allele frequency of the FABP2 gene

Secondly, we obtained some results with the allele frequency of the thrifty *FABP2* gene. The Thr-allele, a missense variant of codon 54 from the Ala-allele of the *FABP2* gene, has been reported to be associated with elevated fasting insulin levels and insulin resistance in Pima Indians and this variant-type *FABP2* was shown in vitro to be involved in 2-fold higher absorption rate of fatty acids than Ala54-*FABP2*. Although the variant allele has been reported widely spread in various ethnic groups, a number of the Thr-allele carrier was only 37 including 26 Ala54Thr and 11 Thr54Thr genotypes out of 228 samples. The frequency of the Thr-allele was the same between young (0.11) and old (0.10) age groups (Table 3). We failed to find any association between the Thr-allele of the *FABP2* gene and longevity. Although longevity is not directly related to a single gene, a certain profile of the thrifty genes may determine the individual' s life expectancy which is clearly influenced by environmental factors.

Table 2. International comparison of the Arg64-allele frequency of the *BAR3* gene

Country	Ethnic Group (Nationality)	subgroup		Arg-allele (BAR3)
USA	Americans	Alaskan Eskimos		0.38
Brazil	Brazilians	Parkataje Indians	Amazon	0.33
USA	American	Pima Indians		0.31
Japan	Japanese	SUN students		0.26
Bolivia	Bolivian			0.18
China	Chinese	hypertensive men		0.18
Japan	American	Japanese		0.18
USA	American	Mexicans		0.18
China	Chinese	hypertensive women		0.16
Czech	Czeck	hypertensive		0.15
Taiwan	Chinese	Taiwanese		0.15
Thailand	Thais			0.15
USA	American	Mexicans		0.13
Finland	Finnish			0.13
Chile	Chilean	Aymara natives		0.13
Sweden	Swedish			0.13
USA	American	Blacks		0.12
Italy	Italians	Southern Italy	working white men	0.11
USA	Americans	Caucasian	postmenopausal	0.10
Canada	Canadian	Blacks		0.10
USA	Americans	Africans		0.10
Jamaica	Jamaicans	women		0.10
Germany	German			0.09
USA	American	Whites		0.08
Poland	Polish	obese		0.08
Australia	Australians	Whites	women	0.08
Canada	Canadian	Whites		0.08
Greece	Greek	pregnant		0.07
Spain	Spanish	men		0.07
Italy	Italians	Sardinian	hypertensive	0.07
Czech	Czeck	normotensive		0.07
UK	British	50's male		0.07
Austria	Austrians	Caucasian	obese men	0.07
USA	Samoans			0.07
Spain	Spanish	women		0.06
Netherlands	Dutch	Caucasian		0.06
Italy	Italians	Sardinian	normotensive	0.03
France	Turkish	obese men		0.00
USA	Nauruans			0.00

International comparison of the Thr64-allele frequency of the FABP2 gene.

Table 3. Allele frequency and genotype frequency of the *FABP2* gene in Songkohn District, Lao PDR

age groups	Allele		Genotype		
	Ala54-allele	Thr54-allele	Ala54Ala	Ala54Thr	Thr54Thr
total	408	48	191	26	11
(%)	(89.5)	(10.5)	(83.8)	(11.4)	(4.8)
young	237	29	111	15	7
(%)	(89.1)	(10.9)	(83.4)	(11.3)	(5.3)
old	171	19	80	11	4
(%)	(90.0)	(10.0)	(84.2)	(11.6)	(4.2)

As mentioned above, the variant Thr-allele of the FABP2 gene has been frequently reported in various ethnic groups and its frequency is relatively high from 0.5 in Indians and Chilean to 0.2 in Canadians and African Americans (Table 4). The frequency of 0.010 in the Laotians is the lowest in the world and is close to that in the Tongan. We have so far no reasonable explanation why the Laotians carry a few The-allele of the FABP2 gene, but it is noteworthy to mention that Tonga is geographically close to Lao PDR.

Leu237Met allele frequency of the mitochondrial ND2 gene

Table 4. International comparison of the Arg64-allele frequency of the *FABP2* gene

Country	Ethnic Group (Nationality)	subgroup	Thr-allele (FABP2)	reference
India	Indians	South India	0.53	Guettier, 2005
Chile	Chilean	obese women	0.47	Albala, 2004
Japan	Japanese		0.36	Ito, 1999
Korea	Korean	young men	0.34	Kim, 2001
Japan	Japanese	men	0.34	Yamada, 1997
Japan	Japanese		0.34	Hayakawa, 1999
Japan	Japanese	women	0.33	Takakura, 2005
Chile	Chilean	women	0.32	Albala, 2004
USA	Americans	Caucasians	0.32	Chiu, 2001
USA	American	Pima Indians	0.29	Baier, 1995
China	Chinese		0.29	Xiang, 1999
Finland	Finnish		0.28	Erkkila, 2002
Sweden	Swedish		0.28	Wanby, 2004
Finland	Finnish		0.27	Tahvanainen, 2000
Canada	Canadians	French	0.26	Stan, 2005
USA	Americans	Caucasians	0.24	Lara-Castro, 2005
USA	Americans	Africans	0.22	Lei, 1999
USA	Americans	Africans	0.19	Lara-Castro, 2005
Canada	Canadians	normal fetus	0.17	Levy, 2001
Australia	Tongan		0.12	Duarte, 2003

Table 5. Allele frequency of the *ND2* gene

The mitochondrial Met237-allele of the ND2 gene characterizes mitochondrial DNA (mtDNA) haplogroup D. The mitochondrial haplogroup D has been proposed to be associated with aging and longevity in the Japanese, especially in the Japanese centenarians. Only 3 samples of mtDNA haplogroup D were detected out of 228 mtDNA samples (Table 5). Furthermore, no apparent association between haplogroup D and longevity was suggested.

age group	Leu237-allele	Met237-allele
total	225	3
(%)	(98.7)	(1.3)
young	131	2
(%)	(98.5)	(1.5)
old	94	1
(%)	(98.9)	(1.1)

International comparison of Leu237Met allele frequency of the mitochondrial ND2 gene

The mitochondrial genome is inherited only from a mother and no recombination occurs during meiosis

of germ cells. Therefore the frequency of the mitochondrial SNP is dependent mainly on spontaneous mutation and inter-group movement of women. However, a natural selection might have also happened upon a given missense variation of the mitochondrial gene. For example, it has been considered that a mother of mtDNA haplogroup D was born in Yakutsk, Siberia and its carrier propagated from Siberia to North- and South-America continents through the Bering Strait. In fact, haplogroup D was recently reported in the Aleuts, the Eskimos, and the Na-Dene. These people are living in so cold climate that mtDNA haplogroup D could be beneficial to a low-temperature environment. In the Japanese population, the frequency of haplogroup D is 0.4 ~ 0.45 in the general population and 0.55 ~ 0.6 in the centenarians. But, we found that the frequency of haplogroup D in Okinawa's elderly group was 0.44 (unpublished observation), suggesting that haplogroup D may not be beneficial to longevity in sub-tropical region of Japan.

According to the previous reports, haplogroups M (0.57), A (0.12), C (0.21), B (0.04), and F (0.04) are predominant besides D (0.14) in Asian people. At present, we have no information available about mtDNA haplogroup frequency in the Laotians except for 3 of haplogroup D in 228 mtDNA samples (0.013). This number also says that haplogroup D may not be favorable in a hot environment.

Now we are in the middle of searching for other haplogroups than haplogroup D in the Laotians mtDNA samples, with special reference to sub-haplogroup M8a1, which is susceptible to diabetes mellitus through impairment of insulin secretion upon high blood glucose.

Finally, we have to discuss why the Laotians showed a scanty incidence of the so-called thrifty genes. In other words, they had quite homogenous SNP profile of 2 nuclear and 1 mitochondrial genes tested in the present study. Generally speaking, the higher infant-death rate is, the stronger a natural selection pressure is. Consequently, minor alleles of each SNP may be always in a minimum size. If this is true, drastic environmental changes such as westernization of life style including high-calorie diet and low energy-expenditure will influence Laotians for ill-health. On the other hand, we cannot exclude a possibility that a cultural tradition of endogamy may cause a peculiar profile of SNPs in these areas.

We think the "longevity" is offered to a person who has not suffered from life-style related diseases and has had life style best fitted to his genotype. By surveying the Laotians genotype more in detail, we would like to propose possible life styles for the Laotians best fitted to their individual genotypes.