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Original Article

Analysis of Beta3 Adrenergic Receptor Gene Polymorphism Using Noninvasive Samples Obtained at Scheduled Infant Health Checkups.

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Abstract

Obesity is a risk factor for life-style-related diseases, and is based on three factors: genetic, environmental, and life-style. In adults, it is difficult to achieve and maintain normal body weight, so it is more effective to intervene from infancy to establish weight control. Legally required health checkups in infants of 18 and 36 months present important opportunities for obesity prevention. We consider genetic analysis to be a very important factor for obesity prevention in infancy. However, since health checkups don't involve the collection of blood, genetic analysis is considered difficult. In this study, we attempted the typing of beta3 adrenergic receptor gene polymorphism as a genetic factor from non-invasively obtained samples, buccal mucosa, hair and cerumen in 96 infants at their 18- and 36-month health checkups. Sampling buccal mucosa, hair and cerumen instead of blood caused almost no anxiety to the child or parent, so 94.1% cooperation with sampling was obtained. From buccal mucosa, about 76% of the samples could be used for the typing of polymorphism (81% by enzyme method, 59% by kit method). From hair, about 44% of the samples permitted typing of polymorphism, but from cerumen only about 4% of the samples could be used. Results from buccal mucosa and hair typed about 90% of infant polymorphism. These results suggest that this method would be practical at periodic health checkups, and would probably be applicable to mass screenings for genetic factor analysis for other diseases.

Key words: infant, beta3 adrenergic receptor gene polymorphism, noninvasive health checkups

Introduction

The prevalence of obesity is increasing in children due to changes in dietary habits, a decrease in exercise, and psychological concerns [1]. Obesity is an important risk factor for life-style-related diseases such as hypertension, heart disease, fatty liver, hyperlipoma, hyperglycemia, and some forms of cancer in adults [2]. It is also reported that obesity increases the risk of death from any cause and particularly from cardiovascular disease in adults between 30 and 74 years of age [3]. An individual's body weight and body composition are determined by interactions between the environment and genetics. The environment and genetics influence life-style, which may then act to either enhance or ameliorate obesity [4-6]. Obesity is understood to be a complex phenotype resulting from the combined effects of three factors: genetic, environmental and life-style [7]. Once obesity has been established in adulthood, the probability of successfully achieving and keeping an ideal body weight through voluntary weight loss is low [4, 8], but it may be easier for children to lose and maintain weight [9]. Long-term studies have suggested that there is a relationship between childhood obesity and adult obesity, especially from about three years of age, and that childhood obesity is significantly correlated with adult obesity [10-15]. Obesity in children under three years of age is considered low risk for obesity in adulthood and is called "benign" obesity. Treatment for weight reduction is probably inappropriate in this group [16], so it is most effective to start treatment for preventing obesity from around three years of age. In Japan, health checkups for one and a half-year-old (18-month) and three-year-old (36-month) infants are required by law, presenting a useful occasion to

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promote obesity prevention from infancy. However, in this period, obesity treatment classified by body type may be ineffective and cause a misunderstanding of factors affecting obesity [17]. Our understanding of the genetic influences on obesity has increased at a tremendous rate in recent years, with findings that 40 to 70 percent of variations in obesity-related phenotypes in humans is heritable [5]. Total evaluation involving genetic, environmental and life-style factors is therefore essential.

Generally, genetic analysis is made from blood samples. However, investigation of genetic factors in infants is made more difficult by the need for painfully invasive blood sampling. Thus, the mandated checkups in infancy don't involve blood screening, but a noninvasive screening method would prove useful at these health checks.

The purpose of this study was to evaluate methods of sampling and purification of DNA for genetic factor analysis, without causing pain and anxiety. The samples used in this study were buccal mucosa, hair and cerumen. Each sample could be obtained merely by scraping or external collection, and effective methods for the purification of DNA are already in existence. To analyze environmental and life-style factors, we used questionnaires.

Materials and Methods

Ⅰ. Subjects and sampling materials

The subjects were 96 infants who underwent health checkups at 18- and 36-months of age in Kagawa Pref., and whose parents agreed to participate in this study. Details are shown in Table 1. The obesity-related genetic factor checked in this study was beta 3 adrenergic receptor gene polymorphism, a mutation frequently found in Japanese. Samples obtained for the isolation of DNA were buccal mucosa, hair and cerumen (cerumen sampled from 27 infants only). Buccal mucosa was scraped from the oral cavity with a plastic spoon. To sample hair, a few hairs were plucked and each hair cut to 5mm length, being sure to preserve the root of the hair. Cerumen was obtained by scraping the external auditory canal with the blunt end of a toothpick. We checked the genetic factor, beta3 adrenergic receptor polymorphism, using PCR-RFLP.

Ⅱ. Genetic factor analysis

1. Purification of DNA

Genomic DNA was isolated from buccal mucosa according to the enzyme method described by Ohhashi et al. [18]. DNA from the buccal mucosa was also isolated using QIAamp (QIAGEN, Hilden, Germany).

DNA from hair was isolated using a DNA purification kit, ISOHAIR (NIPPONGENE, Tokyo, Japan).

DNA from cerumen was isolated by the boiling method as follows.

(1) Boiling method

Cerumen, solubilized in 100 μl Tris-EDTA buffer in an Eppendorf type micro-tube, was heated for 10min in boiling water, then chilled for 2min in shaved ice and centrifuged briefly. The supernatants were used as PCR templates.

(2) Enzyme method

Buccal mucosa was incubated in 50 μl of lysis solution containing 5% Chelex-100 (Bio-Rad Lab Inc., Hercules, CA, U.S.A.), 0.4% SDS, and 20 μg proteinaseK at 65℃ for 30min, then kept at 37℃ overnight. DNA was extracted with phenol/chloroform and the aqueous phase was transferred to a clean tube. Then DNA was precipitated with ethanol, and finally dissolved in 100 μl of TE.

(3) ISOHAIR method

The quantity of DNA in the hair, and especially the root of hair is small [19] and keratin is hard to dissolve. Thus we used ISOHAIR, DNA extraction kits for hair. Finally, DNA was dissolved in 20 μl TE buffer.

(4) QIAamp

Twenty-two buccal mucosa samples were isolated using QIAamp. Buccal mucosa was dissolved in PBS and the sample was treated according to the QIAamp protocol. Finally, DNA was dissolved in 100 μl AE buffer (packaged in QIAamp).

2. PCR method

To examine the Trp64Arg polymorphism in exon 1 of the beta3 adrenergic receptor gene, exon 1 was amplified using Polymerase Chain Reaction (PCR) with primers BATNUP (5'CGCCCAATACCGCCAACAC) and BSTNDOWN (5'CCACCAGGAGTCCCATCACC) as described by E. Widén et al. [20]. PCR amplification was conducted in a 50 μl volume.

A 10 μl sample of DNA template from the boiling method was mixed with 5 μl of X10 buffer (Pharmacia Biotech, Piscataway, NJ, U.S.A.), 2 μl of 2.5mM dNTP mixture (Pharmacia Biotech), 1 μl of each primer at 20 μM (Sawady Technology, Tokyo, Japan), 0.25 μl of 5U/μl Taq polymerase (Pharmacia Biotech), and 30.75 μl of distilled water.

A 1 μl sample of DNA template from the enzyme method was mixed with 5 μl of X10 buffer, 2 μl of 2.5mM dNTP mixture, 39.75 μl distilled water, 1 μl of each primer at 20 μM, and 0.25 μl of 5U/μl Taq polymerase.

Samples of 5 μl of DNA template from ISOHAIR or QIAamp were mixed with 5 μl of X10 buffer, 2 μl of 2.5mM dNTP mixture, 1 μl of each primer at 20 μM, 0.25 μl of 5U/μl Taq polymerase, and 35.75 μl of distilled water.

The PCR conditions were denaturation at 94℃ for 3min, followed by 35 cycles of denaturation at 94℃ for 30sec, annealing at 63℃ for 30sec, and extension at 72℃ for 30sec, with a final extension at 72℃ for 4min.

3. Restriction enzyme reaction

Ten μl of PCR product, 1 μl of 10U/μl Mva Ⅰ (TAKARA, Tokyo, Japan), 2 μl of X10 buffer, and 7 μl of distilled water was incubated at 37℃ for 2 hours and the enzyme then inactivated by heating at 70℃ for 10 min.

Table 1 Health checkup participation

	male	female	height (cm)	weight (kg)
A town: one and a half-year-olds	6	3	81.66 ± 0.75	10.59 ± 0.22
three-year-olds	6	12	93.98 ± 2.70	14.16 ± 0.15
B town: one and a half-year-olds	11	10	78.42 ± 2.71	10.00 ± 0.90
three-year-olds	25	23	96.60 ± 3.31	15.16 ± 2.36

Height and weight are presented as mean ± SD.

4. Electrophoresis

The samples were electrophoresed on a MULTIGEL15/25 (DAIICHI PURE CHEMICALS, Tokyo, Japan) apparatus for 3 hours at 100V, and then stained with ethidium bromide and analyzed under ultraviolet light. Thus, the wild type has two bands (99 and 62 bp), the hetero mutation has three bands (161, 99 and 62), and the homo mutation has only one band (161 bp) (Fig. 1).

Ⅲ. Analyzes of environmental and life-style factors

We used a questionnaire to analyze environmental and life-style factors. It consisted of questions about the nurturing environment, eating habits, play habits, and the infants' personality (Fig. 2). In this study we examined anthropometric data and the responses to questions about "activity" and "likes and dislikes" and questions like "Is one or both of the parents obese?", "Who usually prepares meals?", "Who mainly influences the menu?", "Who usually gives the child snacks?", "Who do you usually consult?", "What concerns do you have about your child's dietary habits?", and compared them with the findings about the beta3-adrenergic receptor gene polymorphism.

Result

1. Participation

A total of 102 families received an explanation of the purpose of this study by a public health nurse. There were 5 families who initially consented but failed to appear at the sampling room. Since in this study obesity probability was not going to be revealed, one family declined permission for sampling. Thus, 96 families consented to sampling (only 27 families for cerumen). From 5 infants, hair could not be obtained (hair too short to plucking), and from 2 infants buccal mucosa could not be obtained (subjects too upset), while from 1 infant cerumen could not be sampled due to middle otitis (Table 2).

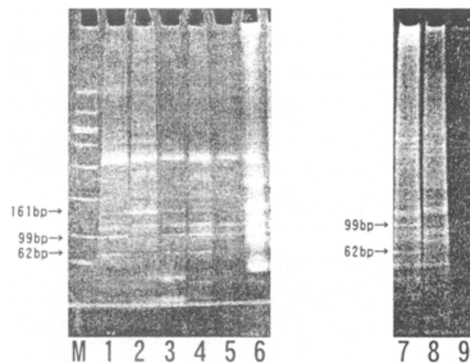

Fig. 1 Electrophoresis
Lane M: Gel Marker (Research Genetics)
Bands are 50, 100, 200, 300, 400, 500, 525, 700, and 1000 bp
Lanes 1, 5 and 7 show wild type (99 and 62 bp)
Lanes 3 and 4 show hetero mutation type (161 and 99 and 62 bp)
Lane 2 shows homo mutation type (161 bp)
Lanes 6 and 8 show non-specific bands only
Lane 9 shows no band

Fig. 1 The single base replacement of T by C in codon 64 in exon 1 predicted an amino acid change from tryptophan(TGG) to arginine(CGG). The amplified exon 1 fragments were digested with Mva Ⅰ, which is specific for the sequence CC(A/T)GG, so the enzyme did not digest fragments with the mutation but did digest fragment without the mutation. In the presence of the mutation, the restriction site for Mva Ⅰ is lost, and only one fragment 161 bp in length is observed under our conditions. In the absence of the mutation, a restriction site for Mva Ⅰ exists, and fragments of 99 and 66 bp in length appear.

Table 2 Sampling cooperation rate

	Cerumen		Hair		Buccal mucosa	
Consent	26	96.3%	91	94.8%	94	97.9%
Decline	1	3.7%	5	5.2%	2	2.1%
Total	27		96		96	

About family:
・Is one or both of the parents obese?
(yes (father ・ mother) ・no)
・Do both parents work outside the home?
(yes ・ no)
Are there any siblings?
(yes ・ no)

About dietary habits:
・Who usually prepares meals?
( )
・Who mainly influences the menu?
( )
・Is your child made to eat a set volume?
(yes ・ no)
・How fast does your child eat?
(fast ・ slow)
・Write down any of the child's likes and dislikes.
Likes[ ]Dislikes[ ]

About snacks:
・How many and when in a day?
( )
・Who usually gives the child snacks?
( )
・What kinds of snack (and drinks) ?
( )

About play habits:
・Where does the child play usually?
( )
・How long does the child play outdoors per day?
About ( ) hours or minutes
・How does the child play inside the home?
( )

About child growth:
・What concerns do you have about your child's growth?
( )
・Who do you usually consult?
( ) ・nobody in particular

About the child's personality:
・Activity
(active ・ inactive)
・Likes and dislikes
(clear ・ not clear)
・Child's physique
(obese ・ rather obese ・ rather thin ・ thin)
・Who does the child most resemble?
( )
・What concerns do you have about your child's dietary habits?
(Check all that apply)
glutton ・ light eater ・ unbalanced diet ・ fast eater ・ slow eater ・ eats while watching TV ・ late supper ・ irregular meal times ・ others ( )

Fig. 2 Questionnaire to analyze environmental and life-style factors

Table 3 Bands

	Cerumen		Hair		Buccal mucosa					
					enzyme method		QIAamp method		total	
No band	25	92.6%	31	32.3%	9	12.2%	1	4.5%	10	10.4%
Non-specific band only	0	0.0%	18	18.8%	3	4.1%	8	36.4%	11	11.5%
Specific band	1	3.7%	42	43.8%	60	81.1%	13	59.1%	73	76.0%
No sample	1	3.7%	5	5.2%	2	2.7%	0	0.0%	2	2.1%
Total	27		96		74		22		96	

Table 4 Beta3 adrenergic receptor gene polymorphism type

		Wild type	Hetero mutation	Homo mutation	No band	Non-specific band only	Total
Sex	Female	28	11	4	1	4	48
	Male	31	11	2	1	3	48
Health checkups	One and a half-year-olds	16	11	1	0	2	30
	Three-year-olds	43	11	5	2	5	66
	Total	59	22	6	2	7	96

2. DNA isolation

From cerumen, 25 samples showed no band, no sample showed only non-specific bands, and 1 sample showed specific bands after PCR-RFLP.

From hair, 31 samples showed no band, 18 samples showed only non-specific bands, and 42 samples showed specific bands after PCR-RFLP.

From buccal mucosa, 10 samples showed no band, 11 samples showed only non-specific bands, and 73 samples showed specific bands after PCR-RFLP (including 1 sample with no band, 8 samples with only non-specific band, and 13 samples with specific bands isolated using the QIAamp kit method) (Table 3).

3. Beta3 adrenergic receptor typing

From the results involving each of the 3 kinds of samples, 87 out of 96 individuals could be typed as beta3 adrenergic receptor. Samples from 2 individuals showed no band. Samples from 7 individuals showed only non-specific bands. The various samples from the same individual all showed the same beta3 adrenergic receptor type.

Of the 87 individuals typed, 59 were classified as the wild type (67.8%), 22 as the hetero mutation type (25.3%) and 6 as the homo mutation type (6.9%). The overall frequency of the beta3 adrenergic receptor mutation was 0.195 (Table 4).

4. Correlation between the beta3 adrenergic receptor typing and questionnaire results.

There was no correlation between the beta3 adrenergic receptor type and anthropometric data (Table 5).

In this study, 96 individuals participated. However, the number was still too small to analyze each questionnaire item, because there were too few individuals in some sections. It is therefore hard to interpret the questionnaire results statistically (Table 6).

Discussion

1. Participation rate

The degree of cooperation with this study was 96 out of 102 families consenting to sampling (94.1%). There were 5 families who did not decline sampling when the public health nurse explained the study, but who did not appear at the sampling room. This may reflect subsequent rejection of sampling by the infant or parent, or may simply be the result of forgetfulness while attending the many other health checks. In order not to represent this sampling as a kind of health check, the sampling room was some distance from the health checkroom. One family declined the sampling because they felt there would be no benefit for them if we would not later explain the probability of obesity from the sampling. However, the main purpose of this study was to devise a sampling method for determining genetic factors. The importance of genetic factors has not yet been determined, so we explained to subjects that we could not inform them of the probability of obesity as a result of the sampling.

The results showed that few families declined this sampling at the 18- and 36-month health checkups. Sometimes too much effort was needed to take samples of short infant hair. Two infants became so upset at buccal mucosa sampling that the parent refused further efforts, resulting in failure to obtain these buccal mucosa samples. Technically, it is possible to take buccal mucosa samples, even from an upset infant. Using a plastic spoon gave parents comfort. A cerumen sample could not be obtained from one infant with middle otitis. In the 26 infants sampled, the DNA detection rate was so low (3.8%), that we decided to abandon cerumen sampling. A cerumen sample may possibly be used for DNA detection but more efficient sampling tools are needed. The results of the samplings are shown in Table 7.

Fig. 3 shows the process of sampling and isolation. Public health officials were cooperative because sampling buccal mucosa, hair, and cerumen caused little disruption in the health checkup.

2. DNA isolation

DNA isolation from a buccal mucosa sample could be completed in two days. Over 80% of the samples showed specific bands. Approximately 12% of the samples showed no band, and in these samples no DNA likely to be appropriate for PCR was yielded. About 4% of the samples showed only non-specific bands, indicating the presence of a PCR inhibitor, such as intraoral bacteria or other substances. QIAamp is advertised as applicable for buccal swabs, so we tried it for 22 buccal mucosa samples. This method needed only a short time (about 3 hours), showed 4.5% no-band samples and was an effective method for obtaining a good DNA recovery rate. However, 36% of the samples showed only non-specific bands indicating a low isolation rate. Because of its very simple protocol, QIAamp may

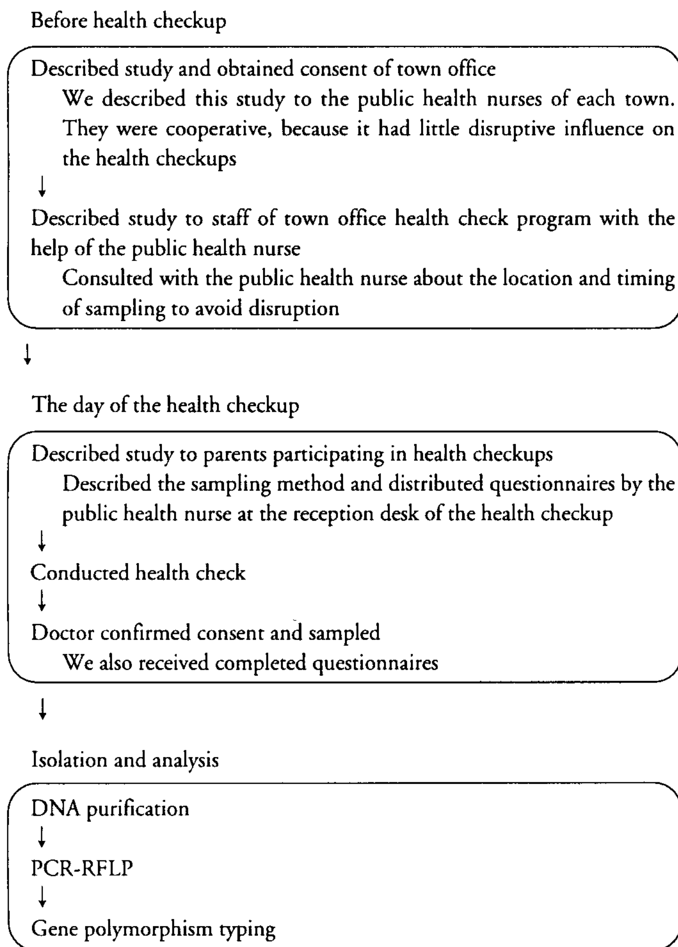

Fig. 3 Process of sampling and isolation

not eliminate contamination by some inhibitors. Originally QIAamp was developed for blood samples, and it seems less effective for buccal samples. An easy-to-use kit would be very useful for mass sample treatment, so we should consider the development of an appropriate kit for buccal mucosa.

Among hair samples, 32% showed no bands, a relatively high proportion. Use of a DNA absorbent like centricon might improve the no-band ratio, but would need more effort. Eighteen percent of the samples showed non-specific bands. Melanin in hair was suspected of inhibiting the PCR reaction. It is possible that the addition of T4 gene protein would improve the non-specific band ratio.

Currently, the PCR-RFLP method is often used to detect gene point mutations. In a given individual, the beta3 adrenergic receptor typing was the same among the different kinds of samples, suggesting the reliability of PCR-RFLP. It may be possible to improve the reliability and power of typing by using another method, like pin-point sequencing [21].

3. Beta3 adrenergic receptor typing

By using 3 kinds of samples (cerumen, buccal mucosa, and hair), the beta3 adrenergic receptor type could be identified in 87 (90.6%) of 96 samples. By using only 2 kinds of samples (buccal mucosa and hair), the beta3 adrenergic receptor type could be identified in 86 (89.6%) of 96 samples. No individual kind of sample showed a sufficient detection ratio, but the buccal mucosa by enzyme method was the most useful. The 7 samples that showed only non-specific bands indicated that some improvement in the process of isolating DNA and identifying polymorphism is needed, but nevertheless the 90% detection rate from 2 kinds of samples suggested that this is a useful method for first screening.

When we had identified beta3 adrenergic receptor gene polymorphism by using both a blood sample and a buccal mucosa sample from nine adults, we confirmed that PCR-RFLP from the different samples from the same individual showed the same type.

Mutation of the beta3 adrenergic receptor gene was present with an allelic frequency of 0.195. These data are consistent with previous reports on Japanese subjects of 0.21 (Yuan X et al., 1997 [22]), 0.225 (H. Kim-Motoyama et al., 1997 [23]), and 0.22 (T. Yoshida et al., 1995 [24]).

4. Correlation of the beta3 adrenergic receptor type and questionnaire results.

In this study there was no significant correlation between the beta3 adrenergic receptor type and any item on the questionnaire. But the hetero type was heavier than the wild type, and the homo type was heaviest, in birth weight, weight and Kaup index.

The primary role of beta3 adrenergic receptor is thought to be the regulation of the metabolic rate and lipolysis. In Japanese people, it has been reported that individuals who have beta3 adrenergic receptor mutations find it hard to lose weight [25], easy to gain weight [26] experience difficulty in losing weight by diet or exercise treatment [24], have a low basal metabolic rate [27], insulin resistance, high body mass index and a high ratio of visceral to subcutaneous fat area [17, 23, 28]. There are some other obesity-related genes similar to beta3 adrenergic receptor (for example alpha TNF factor) [6, 29-33], each of which may have variable effects on obesity or metabolism. Some gene polymorphisms like those involving leptin have a very low mutation ratio [34], while other gene polymorphisms have only a low correlation or no correlation with obesity. A beta3 adrenergic receptor mutation that has been reported to correlate well with insulin resistance has also been reported to have a negative or only a slight relationship with insulin resistance [35, 36]. Thus, it is still difficult currently to evaluate obesity-related gene polymorphisms.

The reasons we used beta3 adrenergic receptor polymorphism to indicate obesity-related gene polymorphism are as follows: the mutation ratio is high in Japanese, the mutation can be detected by PCR-RFLP, the detected band is around 100 bp, a size which is not easily influenced by DNA nucleases, and this polymorphism is related to the obesity risk, mutation does not always result in obesity, or a tendency to obesity.

In this study we were not able to demonstrate a characteristic relationship between beta3 adrenergic receptor gene polymorphism and obesity, so it may be that there were too few extra-obese infants, or the beta3 adrenergic receptor may actually have little influence on infant obesity. In a much larger sample such as thousands, a significant correlation between beta3 adrenergic receptor gene polymorphism and these obesity indexes may be shown. In the future we hope to evaluate beta3 adrenergic receptor gene polymorphism with regard to one's life-style and one's growth. So to evaluate the role and impact of the genetic factor, we think it is important to follow the gene analyzed group for decades.

A genetic factor for obesity is understood to be complex and resulting from the combined effects of many kinds of gene. For further research into new obesity-related gene polymorphisms,

the DNA detection method used in this study can be applied. Using the boiling method with cerumen, DNA solution could be purified about 10 times for one PCR. Using the enzyme method with buccal mucosa, DNA solution could be purified about 100 times for one PCR. Using the ISOHAIR method with hair, DNA solution could be purified about 4 times for one PCR. Using the QIAamp method with buccal mucosa, DNA solution could be purified about 20 times for one PCR.

Wilson and Jungner developed guidelines for screening [37], which should be applied to the method suggested by this study.

We have suggested a noninvasive method for investigation of genetic factors, and a method which has possible practical applications in health checkups for 18- and 36-month old infants. Further studies using more general evaluations involving genetic, life-style and environmental factors will provide a definitive guide for obesity prevention of obesity.

Table 5 Beta3 adrenergic receptor gene polymorphism type and anthropometric data

	Wild type	Hetero mutation	Homo mutation	No band	Non-specific bands only	Total	P value
Birth weight (g)	3038.6	3019.8	3339.0	3139.0	3051.7		
	434.5	407.5	381.6	55.2	448.8		0.616
Started walking (months)	12.1	12.6	12.0	12.5	13.1		
	2.6	2.2	1.7	0.7	2.2		0.468
Outdoor play (minutes)	105.6	118.4	186.0	60.0	62.5		
	61.6	63.6	124.4	0.0	6.1		0.113
One and a half-year olds							
Number total	16	11	1	0	2	30	
Male	9	6	1	0	1	17	
Female	7	5	0	0	1	13	
Weight (kg)	9.95	10.47	11.10	-	10.00		
	0.92	0.58	-	-	0.21		0.234
Male(kg)	10.26	10.65	11.10	-	10.15		
	0.93	0.43	-	-	-		0.482
Female(kg)	9.55	10.26	-	-	9.85		
	0.78	0.72	-	-	-		0.379
Height (cm)	78.92	80.68	80.5	-	75.60		
	2.78	2.04	-	-	2.69		0.117
Male(cm)	79.28	80.50	80.5	-	77.5		
	2.92	1.45	-	-	-		0.477
Female(cm)	78.46	80.90	-	-	73.7		
	2.75	2.76	-	-	-		0.477
Kaup index	15.96	16.12	17.1	-	17.50		
	0.75	1.04	-	-	0.85		0.117
Chest circumference (cm)	46.36	46.70	47.5	-	46.40		
	1.21	1.17	-	-	0.57		0.809
Head circumference (cm)	46.24	46.45	47.2	-	47.40		
	1.80	2.16	-	-	0.14		0.994
Three years old							
Number	43	11	5	2	5	66	
	22	5	1	1	2	31	
	21	6	4	1	3	35	
Weight (kg)	14.70	15.67	16.12	14.98	13.51		
	1.43	4.18	2.40	0.04	1.09		0.249
Male(kg)	15.15	17.01	18.05	14.95	14.63		
	1.28	5.77	-	-	0.04		0.569
Female(kg)	14.23	14.55	15.64	15.00	12.77		
	1.47	2.26	2.48	-	0.55		0.353
Height (cm)	95.93	96.11	97.52	95.85	93.38		
	3.43	2.91	3.59	6.43	1.88		0.420
Male(cm)	97.10	96.80	99.1	100.4	94.70		
	3.19	2.48	-	-	0.99		0.385
Female(cm)	94.71	95.53	97.13	91.3	92.50		
	3.31	3.33	4.02	-	1.92		0.294
Kaup index	15.97	16.84	16.96	15.70	15.50		
	0.91	3.73	1.76	3.25	1.11		0.775
Chest circumference (cm)	49.37	51.41	51.40	51.10	48.92		
	1.73	4.08	2.33	1.27	1.66		0.108
Head circumference (cm)	49.68	49.86	49.62	50.25	49.60		
	1.88	2.05	2.20	2.47	2.43		0.994

Upper number: average
Lower number: standard deviation
Statistical analysis by non-parametric method (Kruskal-Wallis Test)

Table 6 Beta3 adrenergic receptor gene polymorphism typing and questionnaire results

		Wild type	Hetero mutation	Homo mutation	No band	Non-specific bands only	total
Activity	Active	44	16	5	2	5	72
	Inactive	12	5	1	0	2	20
Likes and dislikes	Clear	40	15	5	1	4	65
	Not clear	17	6	1	1	3	28
Is one or both of parents obese?	Father	15	7	0	0	4	26
	Mother	24	4	2	1	2	33
	None	26	12	4	1	2	45
Who prepares meals mainly?	Mother	56	20	6	1	6	89
	Grandmother	7	6	1	1	2	17
	Institutional lunch	0	0	1	0	0	1
Who influences the menu mainly?	Father	22	10	2	0	0	34
	Mother	21	6	2	1	3	33
	Child	13	7	0	1	4	25
	Grandmother	2	0	1	1	2	6
	Grandfather	1	0	1	0	0	2
	Others	2	1	1	0	0	4
	None	6	1	1	0	0	8
Who usually gives the child snacks?	Mother	45	14	5	1	5	70
	Institution	13	1	2	0	2	18
	Grandmother	7	6	1	1	1	16
	Father	2	0	0	0	0	2
	Grandfather	0	1	0	0	0	1
	Great-grandmother	0	1	0	0	1	2
Who do you usually consult?	Spouse	16	7	1	0	2	26
	Grandmother	15	5	4	0	2	26
	Friend	16	4	2	0	3	25
	Sibling	2	0	0	0	1	3
	Doctor	1	1	0	0	0	2
	Nobody in particular	15	6	1	1	1	24
What concerns do you have about your child's dietary habits?	Glutton	2	2	0	1	0	5
	Light eater	13	6	2	1	4	26
	Unbalanced meal	13	7	3	1	2	26
	Fast eater	19	5	2	1	3	30
	Slow eater	2	0	0	0	0	2
	Eats while watching TV	18	7	2	1	2	30
	Late supper	2	0	1	1	1	5
	Irregular meal times	0	1	0	1	1	3
	Others	10	5	1	0	1	17

Table 7 Feature of sampling

Sample	Sampling method	Reaction of infant	Ease of sampling (sampling rate)	Ease of typing
Buccal mucosa	Scrape with spoon.	A few infants become too upset when putting spoon in mouth.	Some difficulty inserting spoon in mouth. (97.9 %)	Required two days. Use of kit made typing faster but lower detection rate.
Hair	Plucked out by parent.	Some pain when pulling out hair. Some infants upset.	Some parental protest. Impossible for hair too short. (94.8 %)	Required half a day using the kit.
Cerumen	Scrape with blunt end of tooth pick.	Fewer infant protests than with buccal, but poor yield. Strong scraping probably painful. Abandoned procedure.	Some difficulty in sampling. Infant with middle ear or external otitis could not be sampled. Risk of external otitis. (96.3 %)	Required about 20 minutes for the boiling method.

Conclusion

We studied methods of sampling and purification of DNA for genetic factor analysis with infants (in the presence of parents) during mandated health checkups for 18- and 36-month old infants. Results were as follows:

1. We took noninvasive samples such as buccal mucosa, hair, and cerumen from 96 infants at health checkups with informed consent. The sampling method was so simple that it placed little stress on the participants and parents as well as on the staff at the health checkups and it easily achieved a high 94% sampling cooperation rate.

2. We could identify 90.6% of the infants' beta3 adrenergic receptor gene polymorphism by using the noninvasive samples which was taken. The frequency of mutation was 0.195.

3. The DNA isolation kits for hair and buccal mucosa were easy to use but had a low identification rate and the boiling method for cerumen product was too low for identification. The enzyme method with buccal mucosa required the most steps during isolation but gave the best identification rate (81.1%). But the matching of buccal mucosa and hair samples was even more effective for typing polymorphism.

4. We concluded that noninvasive samples are useful in analyzing gene polymorphism in infants and that buccal mucosa is especially useful as a sample.

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