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A Cotwin Control Study of Smoking and Risk Factors of Metabolic Syndrome

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Abstract

Background: Smoking effects are relatively well-documented, especially on cancers and cardiovascular diseases. However, the direction and magnitude of association between smoking and obesity remain unclear. Conflicting results so far are thought to stem from the multiple confounding structure of smoking and other obesogenic life style characteristics.

Methods: Cotwin control study is a genomic epidemiology design, in which the other twin (=cotwin) serves as a control of the twin. Cotwin control study, discordant for smoking habits can provide powerful evidence of association between smoking and obesity by completely matching genomic information, intrauterine environment, and almost all environmental factors. We selected 3,697 like-sex twin pairs (2,762 male and 935 female pairs) out of 63,666 pairs of adult twins in the existing Korea Twin and Family Register, whose smoking habits are discordant. We used the information of obesity as body mass index (BMI, kg/m^2), blood pressure, and blood cholesterol level at the time or later than the smoking information. Paired t-test was done to compare the smoking effects.

Results: Lifetime smoking rate was 80.1% (47.9 current smoker) for men and 10% (1.7% current smoker) for women. Among 2,762 and 935 male and female like-sex twin pairs, 363 male pairs and 20 female pairs correspond to the definition of smoker-nonsmoker pair. The male smokers demonstrated increase in BMI by 0.47, while female smokers show slight decrease (by 0.13), which were not statistically significant. Diastolic and systolic blood pressure, and cholesterol level were slightly increased among smokers by 1.85 mmHg,0.62 mmHg, and 1.28 mg/dl for men. For women, the results show increase in diastolic blood pressure (3.42mmHg) and cholesterol level (1.25 mg/dl), and systolic pressure (8.17 mmHg).

Conclusion: The results refute the possibility that smoking can reduce BMI. Considering the direct adverse effect of smoking, it should be emphasized that smoking do not decrease obesity and thus increase overall metabolic syndrome.

Keywords: genomic epidemiology, twin study, smoking, metabolic syndrome, cotwin control study

Introduction

Monozygotic twins share all the genomic information, as well as intrauterine, development, and almost other environments. If a pair is discordant for certain risk factors such as smoking, any health status of the pair can be easily interpreted to stem from the discordant risk factor. The study design using the twins, particularly monozygotic twins discordant for risk factors or health outcomes is called cotwin control study (Duffy, 2000). The cotwin is the ideal control and no other controls can come close to it, no matter how well-matched it would be. The cotwin control study design is particularly powerful in examining environmental factors of common complex human diseases, where multiple genes and environments contribute to the etiology of diseases. Consider an example of orchestra, when the background harmony is very strong, the only way to hear the pure tune of piccolo is cancelling the background sounds by complete matching. The perfect matching of twins can offer this same effect, to detect even smaller difference between the cotwins.

The health effects of smoking are relatively well documented. However, the smoking effects on the obesity has reported conflicting findings, which reported that smoking was decreasing the weight (Nichter *et al.*, 2004), that the countries where smoking rate decrease show

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increase in obesity prevalence (Gruber *et al.*, 2005), and that the smoking the social groups which have higher smoking rate also showed higher obesity prevalence (Zimlichman *et al.*, 2005). Recently studies focus on various risk factors of metabolic syndrome or specific sub-type of obesity such as central or visceral obesity (Canoy *et al.*, 2005; Mizuno *et al.*, 2005). The discrepancy between studies may stem from 1) the different measurements of obesity, 2) temporal relationship between smoking and obesity-related traits, 3) socioeconomic variables control, 4) complex and sometimes unpredictable inter-correlation between smoking and the risks of obesity, such as physical activity, diet, and so on. In addition the various risk factors of obesity are rapidly changing as is the smoking rate across the populations.

Obesity is determined by a range of life style, multiple genetic factors, and intrauterine environments (Dabelea *et al.*, 2000; Breier *et al.*, 2001). Such complicated risk factors can not be easily controlled to see the purer effects of smoking. We attempted to examine the common belief that smoking may reduce the weight through twin study. In addition, we would like to examine the effect of smoking on other risk factors of metabolic syndrome.

Methods

Subjects

This study used the Korean Twin Registry. We selected twins who are discordant for smoking habits among the adult twins of the registry (127,332 persons, 63,666 pairs). The twins in the registry are representing Korean population, and further description was reported in previous paper (Sung *et al.,* 2002). We limited the like-sex twins who have taken health examinations more than twice between the 1986 and 2004, and also have the information on smoking habits. In addition we further selected for those twin pairs who took health examination with less than 2 years difference between the cotwins, to insure the minimal age effects. Because we did not have genomic information regarding the exact zygosity of the subjects, we excluded twin pairs whose height difference was more than 3 centimeter in the twin pair. The limitation of height difference can control not only the dizygotic twins, but also for monozygotic twins who had distinct differences in the intrauterine environments. If there are twin-to-twin transfusion syndromes or other conditions which may result in more than 15% of birth weight difference, the twins might have different physique, which cannot be attributable to the smoking effects.

Smoking habits

In the biennial health examination provided by Korean National Health Insurance, the subjects are asked brief questionnaire including two questions about their smoking habits. We only determined the smoking habits when the individual took health examination more than two times, and more than two answers for the smoking habits. Smoking habits were classified into three categories 1) if the responses were all non-smoking from more than two questionnaires, one was classified as "never-smoker" 2) if the information of the last health examination was put as smoker, one was classified as "current smoker", 3) if the information of the last health examination was put as quitter, or the last information is put as non-smoking but previous records suggested to be a smoker, one was classified "past-smoker". If there is any ambiguity or conflicts between the information of an individual about smoking habits, it was excluded from analysis. All the smoking habits were measured within two years difference within each pair

Obesity and other risk factors

The data source of obesity and risk factors of metabolic syndrome were same with that of smoking. Obesity was measured as body mass index (BMI, kg/m^2), calculated by the body weight (kilogram) divided by the square of height (meter). Among the longitudinal data, the data at the moment when last smoking information was selected for systolic and diastolic blood pressure (mmHg) and total serum cholesterol level (mg/dl), along with BMI. The details of the methods used to measure blood pressure, BMI, and cholesterol level were reported in previous study (Song *et al.,* 2003).

Analysis

Paired t-test was used to compare the effect of smoking between smoker and non-smoker cotwins by the sex. Only the current smoker and never smoker categories were used for this analysis to see the effect of smoking, not mixed with the effect of cessation. We also limited the analysis for those pairs whose height difference is less than 3 centimeters. No specific adjustment was considered for age or other covariates. In the analysis of intrapair difference, any adjustment modifies the pairwise variation, rather than the absolute mean levels, different from what we intended. We examined whether the intrapair difference showed any residual association with other covariates.

Results

There were 3,697 adult like-sex twin pairs (2,762 male and 935 female) who met the selection criteria both cotwins took health examination more than 2 times and

Table 1. Number of like-sex twin pairs by the smoking status *of the co-twins, whose height differences are less than 3 cm.* (number before excluding by height difference)

	current smoker	ex-smoker	Non-smoker	marginal sum
male twin				
current smoker	594 (781)	432 (736)	219 (363)	651 (1,880)
ex-smoker		223 (333)	207 (350)	430 (683)
Non-smoker			144 (199)	144 (199)
Sum	**594 (781)**	**655 (1,069)**	**570 (912)**	**(2,762)**
female twin				
current smoker	2 (2)	5 (8)	12 (20)	19 (30)
ex-smoker		7 (13)	77 (119)	84 (132)
Non-smoker			682 (773)	682 (773)
Sum	**2 (2)**	**12 (21)**	**771 (912)**	**(935)**

have longitudinal smoking information, and the intrapair difference of age for the last health examination should be less than 2 year. Among them for men, 80.1% experienced smoking during lifetime, and 47.9% of them were classified as current smoker for women 10% experienced smoking and 1.7% of them were current smokers. There were 363 current smokers - never smoker pairs for men and 20 current smokers - never smoker pairs for women. The smoking discordant rate for men, from three by three tables, categorized by current smoker, quitter, and never smoker, was 52.5%. If we exclude quitters to make two by two tables of current - never smoker, the discordant rate fell to 13.1% for men. On the other hand, the smoking discordant rate for women was much lower 15.7% in the three by three table, and 2.1% in two by two table. (Table 1) When we limited the twin pairs by the difference in the height (<3 cm), there were 219 male pairs and 12 female pairs discordant for their smoking habits (current smoker and never smoker). The average age was 37.5 (standard deviation, SD 7.7) for men and 32.7 (SD 13.5) for women among those 219/12 pairs.

Intrapair difference of BMI does not show significant correlation with the increase of age or height, which confirmed that further adjustment for age or other covariates are not necessary (Fig. 1).

When we compared the body mass index between current smoker and never smoker, for the male twins, smoker demonstrated significantly increased BMI by 0.46 (p=0.01), while for female twins smoker showed small decrease of 0.12 (BMI, p=0.81), which was not statistically significant.

For blood pressure and cholesterol level, smoker cotwins show uniform increase for all risk factors in both sexes systolic blood pressure 1.8, diastolic blood pressure 0.6 mmHg, and total cholesterol lever 1.3 mg/dl for men systolic blood pressure 3.5, diastolic blood

Table 2. Mean increase in body mass index (BMI, kg/m^2), systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), and total serum cholesterol level (mg/dl) among smoker like-sex twins compared with their non-smoking co-twins. (by paired t-test)

Risks of Metabolic Syndrome	Increase in smokers among male like-sex twins n=219 (95% CI)	Increase in smokers among female like-sex twins n=12 (95% CI)
BMI	0.47 (0.10 ~ 0.83)*	-0.13 (-1.14 ~ 0.89)
SBP	1.85 (-0.28 ~ 3.98)	3.42 (-7.19 ~ 14.0)
DBP	0.62 (-1.02 ~ 2.25)	1.25 (-6.39 ~ 8.89)
cholesterol	1.28 (-3.89 ~ 6.45)	8.17 (-9.72 ~ 26.1)

*statistically significant (p=0.01)

pressure 8.1 mmHg, and total cholesterol lever 1.3 mg/dl for women (Table 2). However the blood pressure and cholesterol results were not statistically significant.

Discussion

Cotwin control study design

The study analyzed like-sex twin pairs discordant for smoking. Original suggestion of cotwin control study was intended for monozygotic twins rather than like-sex twins. Since monozygotic twins split from one fertilized ovum, all the genomic information is shared. For monozygotic twins, additive genetic effects, as well as dominant genetic effects are shared 100%. Also monozygotic twins share intrauterine, maternal, and developmental environments. On the other hand, dizygotic twins share the same amount of genetic constitution as the ordinary siblings do. Dizygotic twins share 50% of additive genetic effects and only 25% of dominant genetic effects. Since we did not have exact zygosity information of the like-sex twins, there could be certain amount of dizygotic twins, which can be predicted by the number of opposite-sex twins. The study highly selected the subjects to see the discordant effects. This selection made it impossible to project or model the zygosity structure based on population level like-sex and opposite-sex twin pairs, using the subtraction rule of Weinberg (Sung *et al.*, 2002). The only way we could further exclude the possible dizygotic twins were to exclude those pairs whose height differences were greater than 3 cm. The height shows higher heritability than weight, ranging 60-80% of genetic contribution (Silventoinen *et al.*, 2003). If we apply this heritability value, the intrapair correlation of heights for monozygotic twins should higher than that of dizygotic twins by 30-40%. The maximal height difference in the first 363/20 pairs were as large as 19 cm. We expect that applying the

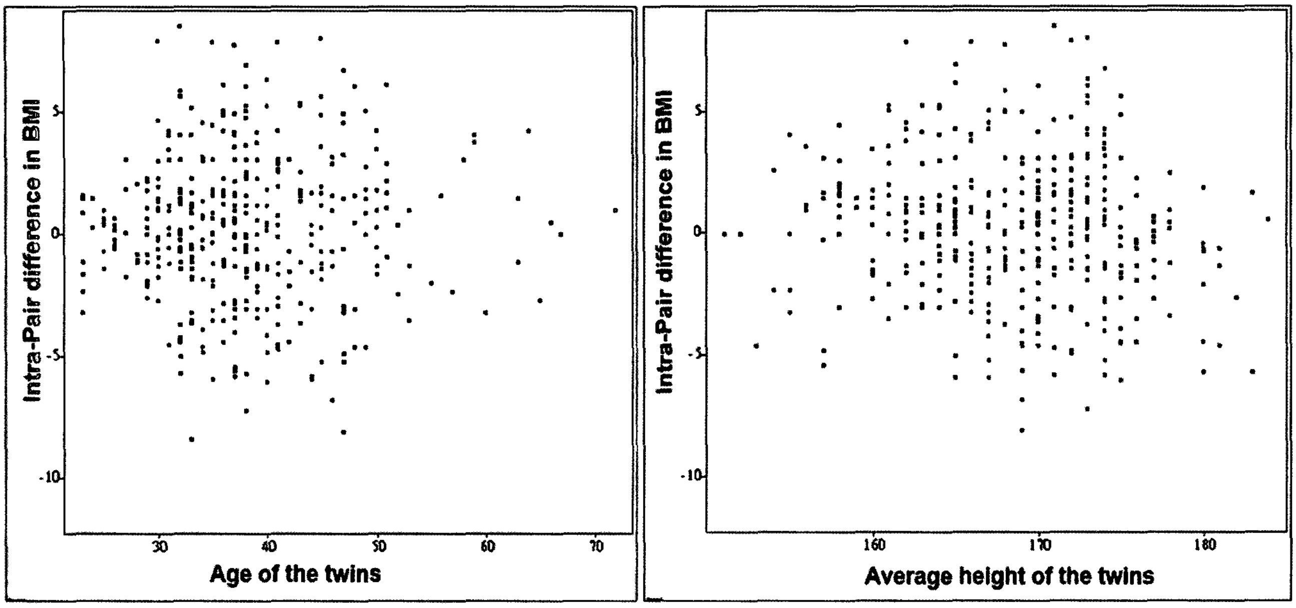

Fig. 1. PIntrapair difference in body mass index (BMI, kg/m2) with the increase of age (year) and average height (cm). There was not significant correlation between them. (r2=0.08 (p=0.56), r2=-0.05 (p=0.62) respectively)

limitation for height difference should have excluded some dizygotic twin pairs. Also the height difference could have excluded a rare but influential monozygotic pairs, who had twin-to-twin transfusion syndrome or whose birth weight differed greatly with out the twin-to-twin transfusion syndrome. There were several reports that even among monozygotic twins, there were about 2-5% of twin pairs whose birth weight difference exceeds 15% (Machin, 1996). The large birth weight gap usually can not caught up along the growth, and the shorter cotwin have higher risk of metabolic syndrome, coronary heart disease and stroke (McMillen and Robinson, 2005). Thus, applying height difference limits can not only reduce possible dizygotic twins, but monozygotic twins who had large birth weight gap, thus reducing the noise from intrauterine effects, to see the purer effect of smoking habits.

As we described in methods, the study did not discriminate monozygotes and dizygotes by standardized test using 7-8 short tandem repeated markers. Even if we only selected like-sex twins and applied height difference limit, there may be certain amount of dizygotic twins mixed together. However the misclassification can not be differential in terms of obesity or smoking status. We think the study results were not drawn from the residuals of random misclassification.

Measurements

The study used data sources which were collected for other purposes. We could not directly confirm the validity or reliability of the data sources. We only used the longitudinal data, not sporadic data, for smoking habits. The average number of health examination for each individual was 4.7 times during the study period. We excluded any ambiguous or conflicting information across longitudinal information. The Korean National Health Service data has been successfully reported several new findings, which were highly compatible with other important studies (Mizuno *et al.*, 2005). We believe the validity and reliability of measures used in the study is as good as other studies.

However, in the study, since we could not accurately evaluate the starting point of smoking, we only used the information of smoker or not. We did not analyze the obesity according to cumulative amount of smoking such as pack-year, or the first age at smoking, etc. The relatively small difference in blood pressure and cholesterol level could have been stem from the loss of the cumulative smoking amount.

Similarly, although we could classify the "quitters," it was not possible to detect exact time when they quit smoking. We did not analyzed the effect of smoking cessation which is another important issue regarding smoking. The effect of smoking cessation may need to be elucidated with longer follow-up data.

Sex difference in smoking discordant rate

The effective sample size showed severe sex disequilibrium

219 male and 12 female pairs. The overall like-sex twin pairs who had smoking information were 2,762 male and 935 female (less than 3 sex ratio). The simple discordant rate show big difference between two sexes 13.1% male, and 2.1% female. The big difference came from the large difference in the smoking rate (45.7% and 1.7%). When we calculated Cohen's kappa value which can adjust the difference in smoking rate, male pairs showed Cohen' kappa value of +0.24 (95% CI 0.22-0.26), while female showed kappa value of +017 (95% CI 0.11-0.24). The kappa value results show that most of the discordant rate can be explained by the difference in smoking rate. There is nosex difference in other aspects, and the results of the study can be compared between the sexes.

The smoking rate in the study was rather lower than the general populations, which show 64.5% for men and 4.7% for women (KIHASA, 2002a). Even if we count in the decreasing smoking rate and 2-year time gap between the report and the study, 10-15% of difference in smoking rate can not be explained. We think the difference was made from the abundance of public service workers in the cohort, rather than the fact that twins show lower smoking rate than general populations. The smoking rate of public servants were much lower than that of general population there was a report in 2002, saying 43.3% and 0.8% of men and women are smokers among the public servants (KIHASA, 2002b). Considering that the smoking rate in the study is between the smoking rate of general population and that of public servants, we do not believe the smoking rates in the study were underestimated.

Sex difference

The study shows sex difference in the smoking effect on obesity. Men showed significant BMI increase among the smokers, while women showed insignificant but small decrease. It is impossible to decide whether this was true sex difference or the effect for women was less reliable owing to large confidence intervals. Over all effects showed good concordance between men and women, especially for blood pressure and cholesterol levels. The possible sex difference in the smoking effects on obesity should be followed-up to see the authenticity of the results. Given the low smoking rate among women, it should be supported by other study designs such as cohort studies.

Strengths and limitations

The study used a large twin registry, which represents Korean population. The results of the study can be easily generalized and applied to general population. Each health examination data was measured independently during 18 years. By using all the longitudinal data, we believe the validity and reliability of the measurements can be appropriate for study purposes. The cotwin control study design adopted in the study has specific strength in detecting and interpreting the environmental effects, minimizing the noise from confounding factors, such as genomic background, other life styles, intrauterine environments, etc.

However, although this study applied the limitation of height difference to reduce dizygotic twins, the zygosity discrimination was not performed by standard methods using genotype information. This could have caused certain amount of uncertainty in the results. The smoking rate of women was very low among Korean population, which resulted in very small female twin pairs discordant for smoking habits. The small sample size made the confidence intervals of results wider for women. We only measured obesity in terms of BMI. There were several reports saying smoking habits are specifically associated with sub-types of obesity, such as central obesity or visceral obesity (Duffy, 2000; KIHASA, 2002b). We could not produce data on these specific obesity measures.

Conclusion

We concluded that the common belief that smoking may help reduce the body weight has no scientific base. Conversely, the smoking significantly increased the obesity in men. If we consider the other adverse effects on the riskfactors of metabolic syndrome, such as blood pressure and total serum cholesterol levels, the smoking effects on obesity should be amplified in terms of overall health risk. If smoking does not decrease the obesity itself or actually increase it, the smoking cessation campaign can utilize the evidence to increase the effect of public health intervention.

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