COMMENTARY

Why is the Impact of Genetic Polymorphisms on the Smoking Habit not Consistent? Possibly Diluted Association with the *Interleukin-1B* C-31T Polymorphism in Japanese Brazilians

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Abstract

The smoking habit is influenced by culture, psychological traits and physical factors. Recent studies on genetic polymorphisms have demonstrated that functional polymorphisms pertaining to neurotransmitters may affect smoking behavior, as well as psychological parameters and diseases. Our recent study demonstrated the *interleukin (IL)-1B* gene encoding IL-1β, a pro-inflammatory cytokine, to be associated with smoking; Japanese with the *IL-1B* C-31T *T/T* genotype, an inflammation-prone trait, were less likely to be smokers than those with *IL-1B* C-31T *C/C* (J Epidemiol 2001;11:120-125). This indicates that genetically determined biochemistry may also be an important factor for smoking behavior. We have investigated this association in another population, 963 Japanese Brazilians (399 males and 564 females) aged 33-69 years from Curitiba, Mogi das Cruzes, and Mirandopolis in Brazil. Current smokers were 15.3% among males and 11.6% among females. The sex-age-adjusted odds ratio (OR) for the polymorphism in males was around unity. In females, the adjusted OR of being current smokers vs. non-current smokers was 0.68 (95% confidence interval, 0.32-1.45) for the *T/T* genotype relative to the *C/C* genotype, and the adjusted OR of being ever smokers vs. never smokers was 0.85 (0.46-1.58). Significant reduction in the OR was not observed for either males or females with the *T/T* genotype. Although the inconsistent result could be caused by random variation, effect dilution caused by incorporation of a group with a smoking-free culture is a possible reason for the apparent anomaly. The effect of dilution may thus have to be taken into consideration, especially for studies on the smoking habit and genetic polymorphisms.

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Recent genetic polymorphism studies have provided new insights into the smoking habit. In addition to the culture of each society, genetic traits impacting on psychological and physiological parameters could influence whether individuals become confirmed users of tobacco. There have been many studies on the associations between smoking and polymorphisms relatied to neurotransmitters (serotonin and dopamine) and nicotine metabolism (Rossing et al., 1998) and although inconsistent results have been reported, some reproduced similar results for the same ethnic group, such as for the dopamine receptor D2 in Japanese (Hamajima et al., 2002). Since the psychological characteristics of smokers may differ among societies, heterogeneity of the effects of polymorphisms might be expected.

Since cigarette smoke induces inflammation to different extents depending on the individual, inflammation-proneness traits may also be related to smoking. IL-1β is a pro-inflammatory cytokine with multiple biological effects

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(Dianarello et al., 1996). The gene *Il-1B* encoding IL-1β has three C-to-T polymorphisms at -511, -31, and 3954 base pairs (bp) from the transcriptional start site (El- Omar et al., 2000) and an association between smoking and a low-expression allele (*C* allele) of *IL-1B* C-31T was found by our group for 241 non-cancer outpatients at Aichi Cancer Center Hospital and 462 examinees in a health checkup program provided by a local government (Hamajima et al., 2001). In this commentary we report on results obtained for Japanese Brazilians.

The subjects were adult Japanese Brazilians from four cities of Brazil, Sao Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis. Through Japanese Associations such as "Kenjin-kai" (Association named after the prefecture they immigrated from) in each city, a total of 969 participants were enrolled. After written informed consent was obtained, lifestyle data and blood samples were collected in the rooms of the associations on the occasions of festivals and sports competitions in March to May 2001 (Ito et al., 2001). Excluding six participants aged less than 30 years or more than 69 years, 963 subjects (399 males and 564 females) aged 33-69 years were selected. A 10 mL sample of peripheral blood was obtained from each and DNA was extracted by a simple salting out procedure (Miller at al., 1988). The *IL-1B* C-31T polymorphism was genotyped by a PCR-CTPP (polymerase chain reaction with confronting two-pair primers) method, as described previously (Hamajima et al., 2001).

An unconditional logistic model was applied for estimating sex-age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs). The Hardy-Weinberg equilibrium was examined by χ^2 test. All calculations were conducted with a computer program, STATA VersionV(STATA Corporation, College Station, TX). This study was approved by Ethical Committees at both Aichi Cancer Center in Japan (Approval No. 11-6) and the University of Sao Paulo in Brazil (Protocol No. 393/01).

Out of 963 samples, 947 could be successfully genotyped for the *IL-1B* C-31T. Table 1 shows the genotype distribution for 945 participants with their data on smoking habits. Never smokers were 73.0% of the 945 participants, former smokers 13.9% and current smokers 13.1%. Former smokers were defined as persons who quit smoking 1 year or before participation in the study. The current smokers who responded to questions on number of cigarettes, smoked an average of 13.9 cigarettes per day with a standard deviation (SD) of 8.5 in the 56 males and 12.7 cigarettes per day with a SD of 7.0 in the 49 females. The genotype frequency of *IL-1B* C-31T was 22.6% for the *C/C* genotype, 46.6% for the *C/T* genotype and 30.8% for the *T/T* genotype in the 393 males, and the corresponding values for the 552 females were 24.8 %, 45.1%, and 30.1%, respectively. The genotype distribution for all subjects was not in Hardy-Weinberg equilibrium (c^2=6.34, p=0.012), but the difference between the observed and expected was not substantial; 226 for 206.7, 432 for 470.5, and 287 for 267.7. There were no significant differences in the genotype frequency among current smokers, former smokers and never smokers. As shown in Table 1, the adjusted ORs in males were around unity. In females, the adjusted OR for being current smokers vs. non-

Table 1. Age-(sex-)adjusted Odds Ratios (ORs) of Smoking Status for *Interleukin 1B* (*IL-1B*) C-31T Genotype in Japanese Brazilians

Genotype	n	Never smokers	Former smokers[a)]	Current smokers	aOR 1[b)] (95%CI[d)])	aOR 2[c)](95%CI)
All participants						
C/C	226	167 (24.2)	30 (22.9)	29 (23.4)	1 (Reference)	1 (Reference)
C/T	432	311 (45.1)	59 (45.0)	62 (50.0)	1.16 (0.72-1.88)	1.08 (0.74-1.57)
T/T	287	212 (30.7)	42 (32.1)	33 (26.6)	0.83 (0.49-1.42)	0.95 (0.63-1.42)
Total	945	690 (100)	131 (100)	124 (100)		
Males						
C/C	89	54 (22.5)	22 (23.7)	13 (21.7)	1 (Reference)	1 (Reference)
C/T	183	114 (47.5)	41 (44.1)	28 (46.7)	1.11 (0.54-2.28)	0.92 (0.55-1.55)
T/T	121	72 (30.0)	30 (32.3)	19 (31.7)	1.01 (0.47-2.19)	1.06 (0.60-1.85)
Total	393	240 (100)	93 (100)	60 (100)		
Females						
C/C	137	113 (25.1)	8 (21.1)	16 (25.0)	1 (Reference)	1 (Reference)
C/T	249	197 (43.8)	18 (47.4)	34 (53.1)	1.21 (0.64-2.29)	1.25 (0.73-2.15)
T/T	166	140 (31.1)	12 (31.6)	14 (21.9)	0.68 (0.32-1.45)	0.85 (0.46-1.58)
Total	552	450 (100)	38 (100)	64 (100)		

% in parentheses.
[a)] Former smokers were defined as persons who had quit smoking at least 1 year before
[b)] Age-(sex-)adjusted OR of being current smokers vs. non current smokers
[c)] Age-(sex-)adjusted OR of being ever smokers vs. never smokers
[d)] 95% confidence interval

current smokers, was 0.68 (95%CI, 0.32-1.45) for the *T/T* relative to the *C/C* genotype and the adjusted OR of being ever smokers (former and current smokers) vs. never smokers was 0.85 (95%CI, 0.46-1.58). There was no statistical significance.

In our first study, smoking habit was associated with the *IL-1B* C-31T *C/C* genotype and therefore we conducted this second investigation in another population for confirmation. The genotyping method was exactly the same in both. The *T* allele makes a TATA box and the allele is thought to be responsible for a higher potential for *IL-1B* expression, implying that individuals harboring the *T* allele might be prone to inflammation. Cigarette smoke extract suppresses IL-1β production in human peripheral blood mononuclear cells (Ouyang et al., 2000), which is considered to be related to localized suppression of immune responses in the lung (Daniele et al., 1977). IL-1β release by cigarette smoke exposure was found to be greater from bronchial epithelial cell cultures of never smokers than from those of smokers (Rusznak et al., 2000). However, protein concentrations of IL1β in broncoalveolar lavage are higher among smokers than among non-smokers (Kuschner et al., 1996). These seemingly inconsistent biological findings should be re-examined taking into account the genotypes pertaining to the expression.

In our previous study, individuals with a *C/T* or *T/T* genotype had a reduced OR of being smokers relative to the *C/C* genotype (Hamajima et al., 2001). In the present study, no association between the smoking habit and the *IL1B* C-31T polymorphism was observed. The differences in the results could be caused by random variation, heterogeneous effects on Japanese populations from different sources by unknown modifiers, and/or effect dilution caused by the social environment or culture for smoking behavior. When the majority in the society live in a smoking-free culture, genetic traits relating to the smoking habit may be less influential. A smaller percentage of smokers among Japanese Brazilians than in Japanese in Japan may be one of the reasons for the apparent anomaly.

The inconsistent results thus may provide an example of effect dilution. Probably, other inconsistent findings for associations with polymorphisms could be explained by this phenomenon. In order to document marked associations, populations under relevant circumstances of risk should clearly be chosen for examination. This may be especially important for polymorphism studies on factors determining whether the smoking habit may become fixed.

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References

Daniele RP, Dauber JH, Altose MD et al. (1977). Lymphocyte studies in asymptomatic cigarette smokers. A comparison between lung and peripheral blood. *Am Rev Respir Dis* **116**, 997-1005.

Dianarello CA (1996). Biologic basis for interleukin-1 in disease. *Blood*, **87**, 2095-147.

El-Omar EM, Carrington M, Chow-W-H, et al (2000). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, 404, 398-402. Corrections (2001).442,99.

Hamajima N, Katsuda N, Matsuo K, et al (2001). Smoking habit and *interleukin 1B* C-31T polymorphism. *J Epidemiol*, **11**, 120-5.

Hamajima N, Ito H, Matsuo K, et al (2002). Positive association between smoking habits and dopamine receptor D2 *Taq*I A polymorphism in Japanese males. *J Epidemiol* (in press).

Ito LS, Oba SM, Hamajima N, et al (2001). *Helicobacter pylori* seropositivity among 963 Japanese Brazilians according to sex, age, generation, and lifestyle factors. *Jpn J Cancer Res*, **92**, 1150-6.

Kuschner WG, D'Alessandro A, Wong H, Blanc PD (1996). Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. *Eur Respir J*, **9**, 1989-94.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res* **16,** 1215.

Ouyang Y, Virasch N, Hao P, et al (2000). Suppression of human IL-1β, IL-2, IFN-γ, and TNF-α production by cigarette smoke extracts. *J Allergy Clin Immunol*, **106**, 280-7.

Rossing MA (1998). Genetic influences on smoking: candidate genes. *Environ Health Perspect*, **106**, 231-8.

Rusznak C, Mills PR, Devalia JL, et al (2000). Effect of cigarette smoke on the permeability and IL-1beta and sICAM-1 release from cultured human bronchial epithelial cells of never smokers, smokers, and patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* **23**, 530-6.