

## Impaired Flush Response to Niacin Skin Patch Among Schizophrenia Patients and Their Nonpsychotic Relatives: The Effect of Genetic Loading

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We previously reported familial aggregation in flush response to niacin skin patch among schizophrenia patients and their nonpsychotic relatives. However, little is known about whether this abnormal skin response is associated with genetic loading for schizophrenia. This study compared the niacin flush response in subjects from families with only one member affected with schizophrenia (simplex families) with those from families having a sib-pair with schizophrenia (multiplex families). Subjects were patients with schizophrenia and their nonpsychotic first-degree relatives from simplex families (176 probands, 260 parents, and 80 siblings) and multiplex families (311 probands, 180 parents, and 52 siblings) as well as 94 healthy controls. Niacin patches of 3 concentrations (0.001M, 0.01M, and 0.1M) were applied to forearm skin, and the flush response was rated at 5, 10, and 15 minutes, respectively, with a 4-point scale. More attenuated flush response to topical niacin was shown in schizophrenia probands and their relatives from multiplex families than in their counterparts from simplex families, and the differentiation was better revealed using 0.1M concentration of niacin than 0.01M or 0.001M. For the highest concentration of 0.1M and the longest time lag of 15 minutes, a subgroup of probands (23%), parents (27%), and siblings (19%) still exhibited nonflush response. Flush response to niacin skin patch is more impaired in schizophrenia patients and their relatives from families with higher genetic loading for schizophrenia, and this finding has implications for future genetic dissection of schizophrenia.

*Key words:* nicotinic acid/niacin skin test/prostaglandin/vulnerability indicator

### Introduction

Niacin (nicotinic acid), both a vitamin and a drug for hyperlipidemia,<sup>1</sup> can induce a visible skin flush response that is caused by prostaglandin-mediated cutaneous vasodilatation.<sup>2,3</sup> The observation of reduced flush response in patients with schizophrenia in early research on high-dose niacin treatment<sup>4</sup> had led to the proposal of niacin challenge as a simple biochemical test for this illness.<sup>5</sup> Studies utilizing oral niacin<sup>6–8</sup> or topical skin patch of methyl nicotinate (a skin-permeable ester of nicotinic acid)<sup>9–15</sup> consistently found attenuated flush response in patients with schizophrenia. Depending on the criteria to define niacin nonresponse, the prevalence rates of attenuated or absent response to niacin skin patch in patients with schizophrenia ranged from 49% to 90%, compared with 8%–23% in healthy controls.<sup>9,11,16</sup>

The attenuated flush response to locally applied niacin in schizophrenia patients, however, was not observed in patients with depression,<sup>17</sup> bipolar disorder,<sup>8,15,16</sup> or autism.<sup>18</sup> The reduced niacin flush response in patients with schizophrenia was not affected by medication status,<sup>10,19</sup> antipsychotic drug doses,<sup>8,14,15</sup> or substance use such as cigarette smoking,<sup>10,12,15,16,20</sup> coffee drinking,<sup>15</sup> or alcohol consumption.<sup>10,15</sup> Moreover, 2 previous studies reported abnormal flush response to niacin skin patch in first-degree relatives of schizophrenia patients,<sup>21,22</sup> and our recent study showed familial aggregation in niacin flush response among schizophrenia patients and their nonpsychotic relatives, with the greatest estimated heritability ranging from 47% to 54%.<sup>23</sup> Thus, niacin flush response appeared to be a potential marker of genetic susceptibility to schizophrenia.

Schizophrenia is postulated to occur through a multifactorial process involving several genes with environmental factors.<sup>24</sup> To identify subgroups of patients with greater etiological homogeneity, a familial-sporadic approach was initially proposed by discriminating between patients with and without a family history.<sup>25</sup> Because those lacking family history may still have

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genetic vulnerability, a continuum of more genetic to less genetic variation in the etiology of schizophrenia was suggested.<sup>26</sup> This led to a similar approach but with more stringent definition on familial/genetic cases by discriminating between multiplex families (families having at least 2 schizophrenia patients) and simplex families (families with only one member with schizophrenia). The utility of this strategy has been demonstrated in detecting the association of higher genetic loading with more severe neuropsychological deficits, which are potential markers for genetic susceptibility to schizophrenia, in nonpsychotic relatives of schizophrenia patients.<sup>27–29</sup>

In this study, we aimed to compare the niacin flush response of schizophrenia patients and their relatives with that of healthy controls and to examine the effect of genetic loading, assuming that subjects from families having a sib-pair with schizophrenia (multiplex families) have higher genetic loading for schizophrenia than those from families without positive history in first-degree relatives other than the proband (simplex families). We predicted that the skin response to niacin would be abnormal in patients with schizophrenia and their relatives and that the abnormalities would be more prominent in subjects from multiplex families than in their counterparts from simplex families.

## Methods

### *Subjects*

The present study recruited probands of simplex and multiplex families as well as their nonpsychotic relatives from 2 studies on schizophrenia. The first study recruited specifically probands of simplex families from inpatient or outpatient units at the Department of Psychiatry, National Taiwan University Hospital, Taipei, and Ju Shan Hospital, a private psychiatric hospital in Taoyuan County, if they met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for schizophrenia or schizoaffective disorder, depressive type. Any family having 2 coaffected siblings was not included for the study. Part of the recruited subjects (153 probands, 217 nonpsychotic parents, and 70 nonpsychotic siblings) had participated in our previous study.<sup>23</sup> However, among them 2 families were found to have one parent affected with schizophrenia and were thus excluded from the subsequent analyses. Further, any subject with mental retardation, dementia, or prominent substance use problem was excluded, too. For the final analysis, subjects from simplex families included 176 schizophrenia probands, 260 unaffected parents, and 80 unaffected siblings. Whether probands or their first-degree relatives had schizophrenia was determined by means of face-to-face interview or information provided by directly interviewed relatives on those who were not interviewed personally, and, among a total

of 849 alive first-degree relatives from simplex families, 420 (49.5%) were directly interviewed.

Subjects from multiplex families constituted a subgroup of participants in a second study, the Taiwan Schizophrenia Linkage Study (TSLs), which has been described in detail elsewhere.<sup>30</sup> In brief, the TSLs was aimed at collecting a large nation-wide sample of schizophrenia families with at least 2 siblings fulfilling DSM-IV criteria for schizophrenia or schizoaffective disorder, depressive type, and successfully recruited 607 families, comprising 2490 subjects (1258 probands, 922 parents, and 310 unaffected siblings). The initial research protocol of the TSLs did not include niacin skin patch test until the last year of the project, and thus only 633 out of 2490 (25.4%) subjects (350 probands, 223 parents, and 60 unaffected siblings) were assessed with the skin test. The subjects who undertook niacin skin test and those who did not, regardless of probands, parents, or siblings, were comparable in terms of age, gender ratio, and years of education (all  $P > .05$ ). For probands, there was no difference in age at onset between those who were assessed with niacin skin test and those who were not ( $P > .05$ ). After excluding those with mental retardation, dementia, or prominent substance use problem, the final study subjects from multiplex families consisted of 311 schizophrenia probands, 180 unaffected parents, and 52 unaffected siblings.

Healthy volunteers were recruited from members of the hospital staff of National Taiwan University Hospital who reported a negative history of any psychiatric disorder and constituted the control group. All participants in this study, regardless of the multiplex, simplex, or control group, were Taiwanese Han Chinese in ethnic origin and had to meet the following criteria before undertaking niacin skin patch test: no history of alcohol and drug abuse, no pregnancy, no major systemic illness (especially heart disease, allergic skin illness, and asthma), and no usage of anti-inflammatory drugs (eg, aspirin, nonsteroidal anti-inflammatory drugs, and steroids) within 3 days before the niacin skin test. Subjects were included in the study after they had given written informed consent to participate, and the study was approved by the institutional review boards of the participating hospitals.

### *Diagnostic Procedures*

For both simplex and multiplex groups, all the schizophrenia probands and their first-degree relatives were interviewed with the Diagnostic Interview for Genetic Studies (DIGS).<sup>31</sup> Interrater reliability of the Chinese version of the DIGS for the diagnoses of schizophrenia, bipolar disorder, and major depression was reported to be good, with the kappa values ranging from 0.86 to 0.93.<sup>32</sup> The Chinese version of the Family Interview for Genetic Studies (FIGS)<sup>33</sup> was used by the interviewers to obtain relevant family history information on those relatives who were not directly interviewed for the study. Interviewers were research assistants who had received rigorous training on the Chinese version of the DIGS

and the FIGS.<sup>30</sup> Best-estimate final diagnosis according to the DSM-IV criteria was made independently by 2 board-certified psychiatrists using all available information, including the DIGS, the FIGS, the hospital records, and the interviewers' notes. When the 2 diagnosticians disagreed, a senior research psychiatrist (H-G.H.) would be sought and a consensus in diagnosis was reached after discussion.

All of the probands from both the multiplex and simplex groups received the final diagnosis of schizophrenia except 2 probands from multiplex families who were diagnosed as schizoaffective disorder, depressive type. These 2 subjects and their relatives were included in the following analyses because the study results remained unchanged after excluding them. In addition, the DIGS contains a section of the modified Structured Interview for Schizotypy (SIS),<sup>34</sup> which measures both schizotypal symptoms and signs. Information obtained by the SIS was used to diagnose DSM-IV schizotypal, paranoid, and schizoid personality disorders in nonpsychotic relatives.<sup>35</sup> Among siblings from simplex families, 0 (0%), 0 (0%), and 1 (1.3%) subject was diagnosed as having schizotypal, paranoid, and schizoid personality disorders, respectively, and the corresponding figures among parents from simplex families were 0 (0%), 4 (1.5%), and 1 (0.4%). The study results remained unchanged when we treated those with these personality disorders as affected cases and accordingly reassigned their families to the multiplex group (for an affected sibling) or excluded these families from the subsequent analyses (for an affected parent).

#### *Niacin Skin Test*

We used a modified protocol that was based on Ward et al.<sup>9</sup> In the form of aqueous methyl nicotinate (AMN), one drop of 0.001M, 0.01M, or 0.1M niacin solution was put onto 3 patches of absorbent paper, respectively. The concentration of 0.0001M evoked nearly no flush response even in the healthy controls and thus was not used in this study.<sup>16</sup> Using adhesive tapes, the 3 AMN patches, as well as a blank one for negative control use, were applied to each subject's forearm skin for 5 minutes and then removed. The following criteria with a 4-point scale was used to rate the skin flush reaction at 5, 10, and 15 minutes after application of the patches: 0 = no erythema, 1 = incomplete erythema, 2 = complete erythema within the definite area of the patch, and 3 = erythema plus edema beyond the definite area of the patch. Focusing on lack of flush, we also treated the scoring as binary using the cut-off point of 2 and categorized score 0 or 1 as nonflush response.<sup>9,19</sup>

In a preliminary study of 50 subjects (34 patients with schizophrenia, 4 patients with bipolar disorder, and 12 healthy controls), the interrater reliability for the flush scoring by 2 psychiatrists (S-S.C. and C-M.L.) was shown to be good with the intraclass correlation coefficients

ranging from 0.85 to 0.94 for different concentrations of AMN.<sup>16</sup> Five research assistants trained by the 2 psychiatrists then assessed flush response for all subjects in this study. In another preliminary evaluation of 50 subjects (25 patients with schizophrenia and 25 healthy controls), the interrater reliability of the 5 research assistants was good, with the intraclass correlation coefficients for the 3 different concentrations of AMN being 0.76 (95% CI: 0.67, 0.84), 0.74 (95% CI: 0.65, 0.83), and 0.69 (95% CI: 0.59, 0.79), respectively.

#### *Data Analysis*

Group comparisons in demographic features were conducted using Student's *t* test for continuous variables and  $\chi^2$  or Fisher's exact test (when the cell number was less than 5) for categorical variables. Because familial aggregation in niacin flush response had been demonstrated in our prior work,<sup>23</sup> a linear mixed-effects model that allows for adjustment for intrafamilial correlation was adopted.<sup>36</sup> This model could also properly account for correlation between repeated measurements on the same subject,<sup>37</sup> which, in the present study, included 9 flush scores rated at 3 time points (5, 10, and 15 minutes) for 3 concentrations of AMN (0.1M, 0.01M, and 0.001M). Therefore, family, time, and concentration were treated as random effects in the mixed-effects model if indicated, and SAS PROC MIXED (SAS Institute, Cary, NC) was used for the analyses.

For each of the comparisons between healthy controls and probands, parents, or siblings, a multivariate linear mixed-effects modeling analysis was conducted to examine the overall effects of group (multiplex, simplex, and control), concentration, time, interactions (among group, concentration, and time), and other covariates (age, gender, cigarette smoking, coffee drinking, and history of food or drug allergy). A *P* value of less than .05 was considered significant. If a 2-way interaction term involving group was significant, group differences with respect to individual levels of the other variable (ie, concentration or time) were examined using either generalized linear modeling or mixed-effects modeling analyses, as indicated, after adjustment for other covariates. The nominal value of significance was adjusted accordingly (*P* value of less than .006, ie, .05 divided by 9). For any significant group effect, post hoc pairwise comparisons among the 3 groups were conducted with Bonferroni correction. Effect sizes in niacin flush scores were calculated by subtracting the mean values between the groups of interest and then divided by the pooled SD, representing group differences in SDs.

## **Results**

### *Sample Characteristics*

Table 1 summarizes the demographic features of all study subjects. Probands from multiplex and simplex families were comparable in age of onset. The multiplex and

**Table 1.** Demographic Characteristics of Schizophrenia Probands, Nonpsychotic First-Degree Relatives, and Healthy Controls.

	Schizophrenia Probands				Parents				Siblings				Healthy Controls	
	Multiplex ( <i>N</i> = 311)		Simplex ( <i>N</i> = 176)		Multiplex ( <i>N</i> = 180)		Simplex ( <i>N</i> = 260)		Multiplex ( <i>N</i> = 52)		Simplex ( <i>N</i> = 80)		(N = 94)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age, years	37.7 <sup>ab</sup>	8.0	31.6 <sup>c</sup>	9.0	66.2 <sup>ab</sup>	9.1	58.9 <sup>c</sup>	9.5	39.8 <sup>ab</sup>	10.2	32.1	10.7	34.1	10.1
Age at onset, years	22.1	6.1	21.2	7.0										
	<i>N</i>	% <sup>d</sup>	<i>N</i>	% <sup>d</sup>	<i>N</i>	% <sup>d</sup>	<i>N</i>	% <sup>d</sup>	<i>N</i>	% <sup>d</sup>	<i>N</i>	% <sup>d</sup>	<i>N</i>	%
Male	202 <sup>b</sup>	65.0	103	58.5	80	44.4	114	43.8	32	61.5	42	52.5	45	47.9
Cigarette smoking	123 <sup>ab</sup>	39.9	48 <sup>c</sup>	27.6	41 <sup>ab</sup>	23.2	29	11.2	22 <sup>ab</sup>	42.3	15	19.2	10	10.6
Coffee drinking	21 <sup>b</sup>	6.8	17 <sup>c</sup>	9.7	7 <sup>b</sup>	4.0	19 <sup>c</sup>	7.4	3 <sup>b</sup>	5.8	12	15.4	21	22.3
History of allergy	7 <sup>a</sup>	2.3	12	6.8	12	6.7	27 <sup>c</sup>	10.4	2	3.8	5	6.3	2	2.1

<sup>a</sup>*P* < .05, multiplex versus simplex group by *t* test (for continuous variables) or  $\chi^2$  test (for categorical variables).

<sup>b</sup>*P* < .05, multiplex versus control group by *t* test or  $\chi^2$  test.

<sup>c</sup>*P* < .05, simplex versus control group by *t* test or  $\chi^2$  test.

<sup>d</sup>Data were missing for some subjects, so percents were based on varying numbers.

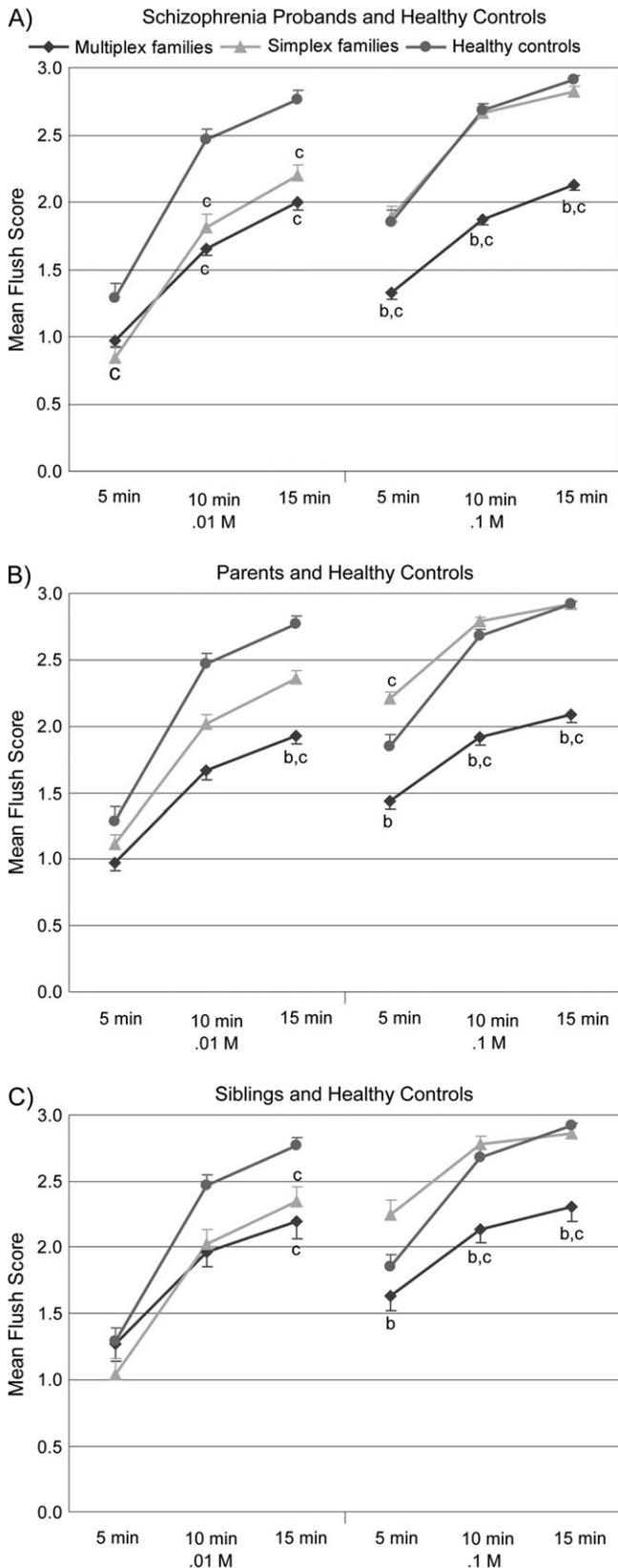
simplex groups, regardless of probands, parents, or siblings, did not differ in gender ratio, rate of coffee drinking, and rate of allergy history (except for a lower rate in probands from multiplex families), whereas subjects from multiplex families were older and had higher prevalence rate of cigarette smoking than their counterparts from simplex families. When the multiplex families were compared with healthy controls, there were more males for

probands, the age was older, the smoking prevalence was higher, whereas the prevalence of coffee drinking was lower for all family members. Meanwhile, in comparing the simplex families with healthy controls, the age was younger and the smoking prevalence was higher for probands, the age was older and the rate of allergy history was higher for parents, as well as the prevalence of coffee drinking was lower for both probands and parents.

**Table 2.** Mixed-Effects Model Results of Niacin Flush Response in a Variety of 3-Group Comparisons.

Overall Effects	Multiplex Probands ( <i>N</i> = 311), Simplex Probands ( <i>N</i> = 176), and Controls ( <i>N</i> = 94)			Multiplex Parents ( <i>N</i> = 180), Simplex Parents ( <i>N</i> = 260), and Controls ( <i>N</i> = 94)			Multiplex Siblings ( <i>N</i> = 52), Simplex Siblings ( <i>N</i> = 80), and Controls ( <i>N</i> = 94)		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Group	26.97	2 4705	<.0001	24.07	2 4340	<.0001	7.81	2 1776	.0004
Concentration	19.66	1 4705	<.0001	25.02	1 4340	<.0001	15.82	1 1776	<.0001
Time	7.13	1 4705	.008	8.98	1 4340	.003	5.30	1 1776	.02
Group-by-concentration	132.87	2 4705	<.0001	137.75	2 4340	<.0001	32.20	2 1776	<.0001
Group-by-time	13.69	2 4705	<.0001	14.35	2 4340	<.0001	8.24	2 1776	.0003
Concentration-by-time	88.82	1 4705	<.0001	45.41	1 4340	<.0001	25.68	1 1776	<.0001
Age	0.20	1 4705	<sup>a</sup>	2.56	1 4340	<sup>a</sup>	1.95	1 1776	<sup>a</sup>
Gender	2.29	1 4705	<sup>a</sup>	14.19	1 4340	.0002	0.83	1 1776	<sup>a</sup>
Cigarette smoking	2.76	1 4705	<sup>a</sup>	5.20	1 4340	.02	3.41	1 1776	<sup>a</sup>
Coffee drinking	2.10	1 4705	<sup>a</sup>	0.25	1 4340	<sup>a</sup>	0.16	1 1776	<sup>a</sup>
History of allergy	0.53	1 4705	<sup>a</sup>	0.23	1 4340	<sup>a</sup>	0.14	1 1776	<sup>a</sup>

<sup>a</sup>Not significant (*P* > .05).



**Fig. 1.** Mean Niacin Flush Scores at 3 Time Points for 0.01M and 0.1M Concentrations of Aqueous Methyl Nicotinate in Schizophrenia Probands, Nonpsychotic First-Degree Relatives, and Healthy Controls. (a) Significant results were of post hoc pairwise comparisons with Bonferroni correction following

### Profile of Niacin Flush Response

Table 2 summarizes the results of mixed-effects modeling analyses comparing the flush response of different groups across the concentrations and time lags of the niacin skin test. For each of the comparisons among healthy controls and the multiplex and simplex groups of probands, parents, or siblings, respectively, 3-way group-concentration-time interaction was insignificant and thus dropped from subsequent analyses. Significant overall effects were demonstrated for group, concentration, time, and their pairwise 2-way interactions, whereas the effects of other covariates were mostly not significant except for gender and cigarette smoking when comparing parents with healthy controls. Significant concentration-by-time interaction indicated that the increase of skin flushing over time depended on applied AMN concentration. Further, group-by-concentration and group-by-time interactions indicated that the effect of genetic loading on niacin flush response differed by applied AMN concentration and observation time. Hence, group differences for each of the concentration-time combinations were depicted in figure 1 for examination in more detail.

The profiles of niacin flush response in schizophrenia probands, their relatives, and healthy controls showed that the flush response tended to become more intense for both higher AMN concentration and longer time lag (figure 1, see online supplementary figure 1 for a color image). For the concentration of 0.001M, the flush scores were generally approaching zero, and group differences in flush response were not significant across time. Therefore, the results of 0.001M were not shown in figure 1 (but see in online supplementary figure 1). Indeed, most of the subjects in any group (83%–98%) showed nonflush response for this concentration even at 15 minutes (see online supplementary figure 2).

For the remaining 2 concentrations of 0.01M and 0.1M, the reductions in mean flush scores for multiplex families as compared with those for simplex ones were more profound for 0.1M, with significant differences in post hoc comparisons between the 2 groups (figure 1) and relatively large effect sizes across the 3 time points (0.6–1.0 for probands, 0.9–1.3 for parents, and 0.7–1.0 for siblings). In contrast, for 0.01M, flush response scores in multiplex and simplex families were comparable except for lower mean flush score in parents from multiplex families at 15 minutes than in their counterparts from simplex families. Compared with healthy controls, reduced flush response in subjects from simplex families was more profound for the concentration of 0.01M than that of 0.1M,

significant group effect in multivariate mixed-effects or generalized linear modeling analysis for each of the 9 concentration-time lag combinations. A  $P$  value of less than .006 (.05 divided by 9) was considered significant. (b)  $P < .006$  multiplex versus simplex group. (c)  $P < .006$  versus control group. A vertical bar indicates the standard error of the mean flush score.

with small to medium effect sizes across the 3 time points (0.4–0.6 for probands, 0.2–0.5 for parents, and 0.2–0.5 for siblings). When multiplex families were compared with healthy controls, subjects from multiplex families had less skin flushing for both 0.01M and 0.1M, particularly at 10 and 15 minutes, with relatively large effect sizes for these 2 time lags (1.0–1.3 for probands, 1.0–1.4 for parents, and 0.6–1.0 for siblings).

Of note, for the setting in which the maximal flush response was expected in the present study, ie, 15 minutes following the application of 0.1M AMN skin patch, a substantial proportion of probands (23%), parents (27%), and siblings (19%) from multiplex families still exhibited nonflush response, whereas complete erythematous response could be seen in all of the healthy controls and nearly all but 2%–5% of the subjects from simplex families. The distributions of original flush response scores, proportions of nonflush response, as well as the mean and SD of the scores at 3 time points for 3 AMN concentrations by groups of subjects were demonstrated in the online supplementary figure 2.

## Discussion

To our knowledge, this is the first study comparing flush response to niacin skin patch among families with different genetic loading for schizophrenia. The results revealed impaired niacin flush response in schizophrenia patients and their relatives and that the impairment was more pronounced in probands, parents, and siblings from families with higher genetic loading for schizophrenia (multiplex families) than in their counterparts from families with lower loading (simplex families). Furthermore, the optimal concentration of niacin for distinguishing between multiplex and simplex families was 0.1M, when a subgroup of subjects from multiplex families still displayed little response to the strongest niacin stimulation for this study.

The present study found significant differences by means of collecting a sample size with adequate power to discriminate between subjects with different genetic loading for schizophrenia<sup>38,39</sup> and adopting strategies to address methodological concerns such as personal interviews or obtaining family history information using validated tool for diagnosing relatives and controlling for potential confounders.<sup>40</sup> In contrast, one previous study compared flush response to oral niacin between schizophrenia patients with positive family history and those without,<sup>6</sup> and the insignificant result in that study could be attributed to insufficient power (33 schizophrenia patients in total) and other methodological problems related to subtyping schizophrenia using family history.<sup>40</sup>

An important feature of this study is that a wide range of AMN concentrations and time lags were examined. The finding that group differences varied with different concentration-time combinations is worthy of discussion.

First, schizophrenia probands and their relatives from multiplex families consistently showed less skin flushing than their counterparts from simplex families and healthy controls for 0.1M. Furthermore, a significant proportion of subjects from multiplex families still presented with little skin flushing in the setting the maximal response was expected. Second, subjects from simplex families tended to show less skin flushing than healthy controls for 0.01M, with significant difference across time found between healthy controls and probands but not their relatives. However, simplex families did not differ from healthy controls for 0.1M, and this could be due to the ceiling of skin flush response in both groups for this concentration. Therefore, simplex families differed from healthy controls in the distributions of flush response, instead of the maximal response, and this finding was consistent with a previous study.<sup>14</sup> Third, the AMN concentrations of 0.1M and 0.01M characterized the group differences in niacin flush response better than 0.001M, and this finding was consistent with our previous report that flush scores data obtained using 0.1M and 0.01M yielded the greatest estimated heritability and recurrence risk ratios.<sup>23</sup> Thus, stimulation using 0.1M and 0.01M of AMN would provide more variations in skin flush response, whereas 0.001M could not evoke meaningful flushing among subjects. These findings imply that future genetic studies in schizophrenia should use niacin stimulation with these 2 concentrations of 0.1M and 0.01M to better discriminate between subjects' flush response.

Another feature of this study is that many potential confounders were controlled for in the analyses. Unlike the reported influence of age and gender on the flush response to niacin skin patch in one previous study,<sup>41</sup> we found that the effects of age, coffee drinking, and history of allergy were not significant, a finding consistent with several studies on niacin skin test,<sup>14,15,17</sup> and that the effects of gender and cigarette smoking were significant only when comparing parents with healthy controls. Nevertheless, our finding of the genetic loading effect on niacin flush response was robust after accounting for these covariates.

Whether to include those disorders thought to be genetically linked to schizophrenia such as schizotypal, schizoid, or paranoid personality disorders in the definition of family type might influence the study results.<sup>29</sup> This influence was assessed in this study by regrouping or excluding those simplex families having a sibling or a parent diagnosed with these personality disorders, respectively, and the main results remained unchanged.

Our findings have important implications on the use of niacin skin patch test in genetic studies of schizophrenia. First, our results, together with our previous finding of family aggregation,<sup>23</sup> suggest that abnormal flush response to niacin may be an endophenotype for schizophrenia.<sup>42</sup> The finding that only a subgroup but not all

of the patients showed abnormal skin flushing implies that impaired niacin flush response might be an endophenotype rather than a diagnostic marker for schizophrenia. Schizophrenia patients and their relatives who presented with deficits in niacin flush response might share some specific pathophysiological characteristics or genotypes responsible for vulnerability to schizophrenia and thus could be an etiologically homogenous group for genetic linkage or association study. However, further studies are needed to examine whether the abnormal niacin flush response is independent of disease states and associated with certain candidate genes or gene regions of schizophrenia. In addition, the use of niacin flush response as an endophenotype may also be applied in imaging, cognition, or treatment studies on schizophrenia. For example, a recent study reported the association of peripheral niacin flush response with central metabolism by showing negative correlation between skin flushing and the levels of cerebral energy metabolism measured by 31-phosphorus magnetic resonance spectroscopy in male schizophrenia patients.<sup>43</sup>

Second, a further understanding of the biochemical processes underlying the abnormal niacin flush response and related genetic regulations may lead to new directions for etiological research of schizophrenia. Because the niacin flush response has been demonstrated to be mediated by prostaglandin, particularly prostaglandin D<sub>2</sub>,<sup>2,3</sup> the underlying mechanism of the abnormal skin response to niacin might involve any step in the process of prostaglandin signaling.<sup>44</sup> Prostaglandins are derived from metabolism of membrane lipids, and disturbed lipids metabolism in both central and peripheral tissues was repeatedly shown in schizophrenia.<sup>45,46</sup> One previous study had shown the usefulness of niacin skin test in identifying a gene polymorphism related to a key enzyme in lipids metabolism and further used this marker in genetic association study on schizophrenia.<sup>47</sup>

Finally, the niacin skin test may be used as a research tool to test new etiological hypotheses of schizophrenia with an emphasis on the inflammatory process. For example, a novel theory of schizophrenia has reviewed the evidence that inflammatory vascular disease of the brain can lead to psychosis and hypothesized schizophrenia as a genetically mediated central nervous system microvascular inflammation disease.<sup>48</sup> Because the impairment in flush response to niacin might be one peripheral marker of a centrally as well as systemically disturbed inflammatory process, the niacin skin test might be used as a screening tool for abnormal inflammatory reaction and related aberrant genetic regulations.

The study has several limitations. First, a mean age of 32 years in siblings from simplex families in our sample implied that some of them had not passed through the age of risk and this might lead to misclassification of family type. Second, we directly interviewed only half of alive first-degree relatives from simplex families, and, for those

who were not directly interviewed, a failure to diagnose schizophrenia in affected relatives might cause misclassification of family type, too. Nevertheless, we used a validated tool on collecting family history information to reduce false negatives. Furthermore, above misclassifications tended to categorize multiplex case as simplex one and thus would diminish rather than increase differences in niacin flush response between the 2 groups. Third, most of the patients with schizophrenia in this study were receiving medication treatment, and this might influence our results because some studies have reported alteration of flush response to niacin by antipsychotic treatment.<sup>19,49</sup> However, most of the previous studies found that medication status did not influence niacin flush response,<sup>10,14,15,17</sup> and our findings in nonpsychotic relatives in addition to probands further suggested niacin flush response as a genetic trait independent of medication or disease status.

In summary, impaired flush response to topical niacin was associated with genetic loading for schizophrenia, indicated by more blunted skin response in probands, parents, and siblings from multiplex families than their counterparts from simplex families. In 3-by-3 combinations of niacin concentration and time lag, the differentiation between subjects with different genetic loading was better revealed using 0.1M concentration of niacin skin patch than 0.01M or 0.001M. Heterogeneity is indicated by the finding that a subgroup of patients and their relatives from multiplex families still displayed blunted skin response in the setting when subjects from simplex families or healthy controls exhibited complete erythema. Our findings support that abnormal niacin-induced skin flush response is a vulnerability trait for schizophrenia and might be useful in future genetic dissection of schizophrenia.

### Supplementary Material

Supplementary figures 1 and 2 are available online at <http://schizophreniabulletin.oxfordjournals.org>.

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