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Association Between Polymorphisms in the *GSTA4* Gene and Risk of Lung Cancer: A Case–Control Study in a Southeastern Chinese Population

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GST Alpha 4 (GSTA4) has an important role in the protection against oxidative stress induced by carcinogens such as tobacco smoke. However, few studies investigated the association between *GSTA4* polymorphisms and lung cancer risk. We genotyped three selected *GSTA4* SNPs (rs182623 − 1718:T > A, rs3798804 + 5034:G > A and rs316141 + 13984:C > T) in a case–control study of 500 lung cancer patients and 517 cancer-free controls and evaluated the association between these SNPs and risk of lung cancer in this Han Chinese population. We found that there was a significant difference in genotype and allele frequency distributions of GSTA4 −1718 between the cases and the controls ($P = 0.006$ and $P = 0.003$, respectively). Compared with the *GSTA4* −1718TT genotype, individuals with the TA + AA genotypes had a significantly decreased risk of lung cancer (adjusted OR, 0.63; 95% CI, 0.47–0.84; $P = 0.006$). Although there were no such statistical differences between the cases and controls at the loci +5034 and +13984, nor for histological types, individuals carrying the genotypes of −1718TA, +5034GG and +13984CT had a significantly decreased lung cancer risk (OR, 0.37; 95% CI, 0.23–0.61; $P < 0.0001$), especially for those smokers who smoked ≤25 pack-years ($P < 0.000001$). These results need to be confirmed in larger studies with different ethnic groups.

Key words: lung cancer; glutathione *S* transferase A4 (*GSTA4*); genetic polymorphism; genetic susceptibility

INTRODUCTION

Lung cancer is the leading carcinoma in China, and the incidence rate has been still increasing to date. The etiology of lung cancer has been shown to be multifactorial, including genetic, epigenetic, and environmental factors such as tobacco smoking. Although smoking remains the most important etiological factor, accumulating evidence has showed that genetic factors may modify the risk of lung cancer [1,2]. Genetic polymorphisms of xenobiotic carcinogen-metabolizing enzymes, such as CYP1A1 (phase I), have been widely investigated, and their genetic variants are believed to play a role in modifying the effects of carcinogens on lung cancer risk.

Glutathione *S*-transferases (GSTs), the phase II metabolic enzymes, including five isoenzymes including GSTA, GSTM, GSTT, GSTP, and GSTZ, are involved in the biotransformation of carcinogens, such as polycyclic aromatic hydrocarbons, in the biological organisms. Genetic polymorphisms exist in the most of these enzymes, and mutations of the genes would change the enzyme activity and decrease its detoxification functions (e.g., reduced GST expression and enzyme activity) and thus may result in an increased susceptibility to cancer. Many studies have focused on the GST functions and their associations with cancer risk of various human malignancies [3–5], including lung cancer. Considering the results of association studies that are discrepant possibly because of the differences in

Abbreviations: *GSTA4*, glutathione *S* transferase A4; SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidential interval.

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environmental exposure and stratification of study populations, previous published investigations of lung cancer highlighted a complex nature of the effects metabolizing genes.

Tobacco smoking is one of the main causes of lung cancer, and there are several possible ways that cigarette smoke causes damage to DNA. One is oxidative stress that leads to mutations, contributing to the development of lung cancer. Among all GSTs, the isoenzyme GST Alpha 4 (*GSTA4*) has the highest efficiency in conjugating glutathione to 4-hydroxynonenal (4-HNE) [6], and GSTA4 over-expressing cells have lower steady-state levels of 4-HNE [7–9]. This compound is one of the major end products of lipid peroxidation and has been shown to induce apoptosis in a variety of cell lines [10]. *GSTA4* was found transiently induced during liver disruption and strongly correlated to oxidative stress [11,12]. However, few reports have addressed the association between *GSTA4* polymorphisms and lung cancer susceptibility.

Here we hypothesized that single nucleotide polymorphisms in the *GSTA4* gene may be associated with lung cancer risk. To test this hypothesis, we performed genotyping analyses for *GSTA4* polymorphisms at locus −1718 T > A (rs182623), +5034 G > A (rs3798804), +13984 C > T (rs316141) in a case–control study of lung cancer in an ethnic Han Chinese population.

MATERIALS AND METHODS

Study Populations

The detailed recruitment of study subjects have been described elsewhere [13]. Patients were consecutively recruited between July 2002 and December 2004 at the Cancer Hospital of Jiangsu province and the First Affiliated Hospital of Nanjing Medical University, Nanjing, China. Those with previous cancer history and radiotherapy or chemotherapy were excluded. Finally, total 500 incident patients with histopathologically confirmed lung cancer were enrolled with a response rate of 90.5% (500/552). Additional 517 cancer-free controls frequency-matched to the cases on age (±5 yr), sex and residential area (urban or rural areas) were randomly selected from 10 500 individuals participated in a community-based screening program for non-infectious diseases conducted in Jiangsu province during the same period with a response rate of 83.8% (517/617). All subjects were genetically unrelated ethnic Han Chinese and were from Nanjing City and surrounding regions in southeast China. Each subject was scheduled for an interview after a written informed consent was obtained, and a structured questionnaire was administrated by interviewers to collect information on demographic data and exposure information including tobacco smoking and family history of cancer. Pack-years smoked [(cigarettes per day/20) × (years smoked)] were calculated to determine the cumulative smoking dose. Smokers were dichotomized (light smokers: ≤25 pack-years and heavy smokes: >25 pack-years) according to the median cumulative smoking dose among controls. Non-smokers were defined as those smoked less than 1 cigarette per day and shorter than 1 yr in their lifetime. Family history of cancer was defined as any reported cancer in first-degree relatives (parents, siblings or children). After interview, approximately 5-mL venous blood sample was collected from each participant. The study was approved by the institutional review boards of Fudan University and Nanjing Medical University.

SNP Selection and Genotyping

Genomic DNA was extracted from the leukocyte pellet by phenol–chloroform and standardized to 100 ng/μL when working solution was diluted to 10 ng/μL [13]. The human *GSTA4* gene is mapped to chromosome 6p12, spanning 18 kb in length and containing 7 exons. Because no published genotyping study at the time we initiated the case–control study of *GSTA4* polymorphisms, we selected single nucleotide polymorphisms (SNPs) by location in the gene of approximately 1 SNP per 10kb with minor allele frequency (MAF) ≥0.05. As a result, only three SNPs were selected, −1718 T > A (rs182623), +5034 G > A (rs3798804), +13984 C > T (rs316141). These SNPs were genotyped at Chinese National Human Genome Center in Shanghai by using the Bead Lab Genotyping System made by Illumina Inc. (www.illumina.com), where the primers and probes used to test SNPs −1718, +5034, and +13984 were designed and synthesized (the assay information including the primers and probes was available upon request). Quality control was described elsewhere [14]. Briefly, one blank well and three repeated samples were set for each 96-well assay plate to prevent contamination. The assay products were hybridized to high-density, bead-based micro arrays and imaged on the Sherlock scanner and the ''GenCall'' software was used to run clustering and calling algorithms with the genotyping result output. Approximately 5% of the samples were randomly selected for genotype confirmation by sequencing, and the results were 100% concordant.

Statistical Analysis

Allele frequencies were calculated from the observed genotype frequencies. Bayesian algorithm in PHASE 2.1 was used to infer haplotypes based on the observed genotypes of the three SNPs and those haplotypes with frequency <5% were combined as ''others'' for further analyses. Differences in selected demographic variables, cumulative smoking, allele and genotype frequencies between the cases and the controls were evaluated by using χ^2-test; the Student's *t*-test was used for comparisons

of continuous data, such as age in years and pack-years, between cases and controls; and the associations between *GSTA4* SNPs and lung cancer risk were estimated by calculating odd ratios (ORs) and their 95% confident intervals (CIs) from multivariate logistic regression analyses. All the tests were two-sided tests. All the statistical analyses were performed with Statistical Analysis System software (v.8.0e; SAS Institute, Cary, NC).

RESULTS

The characteristics of the 500 lung cancers and 517 controls included in the analysis have been described previously [15]. There were no statistically significant differences between the cases and the controls in terms of the frequency of distribution of sex and age. However, the cumulative smoking dose was significantly higher in case group than that of control group ($P < 0.001$). The frequency of the reported family history of cancer in the first-degree relative was significantly greater in the cases than that in the controls ($P = 0.003$). Therefore, these variables were further adjusted in the multivariate logistic regression analysis.

Genotype frequency in the control group was consistent with those estimated from the Hardy–Weinberg equilibrium. There was a significant difference in genotype and allele frequency distributions of *GSTA4* −1718 between the cases and the controls ($\chi^2 = 10.36$, $P = 0.006$ and $\chi^2 = 8.62$, $P = 0.003$, respectively), which was remain significant after Bonferroni correction with P value as 0.017. However, there were no differences between the two groups in both genotype and allele frequency distributions for *GSTA4* +13984 ($\chi^2 = 0.39$ and 0.82 and $P = 0.09$ and 0.768, respectively) and for +13984 ($\chi^2 = 0.90$ and 0.64 and $P = 0.06$ and 0.803, respectively). After adjustment for age, sex, status of smoking, and family history, a significantly 37% decreased lung cancer risk was associated with the *GSTA4* − 1718 TA genotype (OR = 0.63; 95% CI = 0.47–0.84) but a non-significantly decreased risk with the homozygous AA genotype (OR = 0.64; 95% CI = 0.27–1.55) compared with the TT genotype. The combined TA + AA genotype was associated with a decreased risk by 37% (OR = 0.63; 95% CI = 0.47–0.84). However, for *GSTA4* +5034 and +13984, no statistical difference existed for the variant genotype of GA/CT and AA/TT when compared with their corresponding wild-type genotype GG and CC (Table 1).

As shown in Table 2, we performed stratification analyses according to the subjects' age, sex, cumulative tobacco use, and histological tumor types. For carriers of at least one variant −1718 A allele (TA and AA), the decreased risk was more pronounced in subgroups of cases with squamous cell carcinoma or small-cell lung cancer, compared with those carrying genotype TT (OR = 0.51, 95% CI = 0.32–0.80;

Table 1. Genotype and Allele Distributions of Selected *GSTA4* SNPs and Their Associations With Risk of Lung Cancer

				Cases ($n = 500$)		Controls ($n = 517$)				
SNPs	Location	Nucleotide change	Genotype/allele	n	%	n	%	OR (95% CI)	χ^2	P
rs182623	−1718	T > A	TT	385	77	352	68.1	1.00	10.36	0.006
			TA	105	21	153	29.6	0.63 (0.47–0.84)		
			AA	10	2	12	2.3	0.64 (0.27–1.55)		
			TA + AA	115	23	165	31.9	0.63 (0.47–0.84)		
			A	0.125		0.171			8.62	0.003
rs 3798804	+5034	G > A	GG	440	88.2	458	88.6	1.00	0.39	0.823
			GA	57	11.4	58	11.2	1.03 (0.69–1.54)		
			AA	2	0.4	1	0.2	1.54 (0.13–17.9)		
			GA + AA	59	11.8	59	11.4	1.04 (0.70–1.55)		
			A	0.061		0.058			0.09	0.768
rs 316141	+13984	C > T	CC	201	49	243	47	1.00	0.90	0.638
			CT	171	41.7	231	44.7	0.94 (0.71–1.25)		
			TT	38	9.3	43	8.3	1.09 (0.67–1.78)		
			CT + TT	209	51	274	53	1.97 (0.74–1.26)		
			T	0.301		0.307			0.062	0.803

ORs were adjusted by age, sex, status of smoking, family medical history. The *P*-value of OR was calculated by the Wald test using SPSS.

Table 2. Association Between Lung Cancer Risk and the *GSTA4* Genotypes by Lung Cancer Histological Types

Pathology type	*GSTA4* genotype		OR (95% CI)	*P*
GSTA4-1718	TT	TA + AA		
Control	352	165	1.00	
Lung adenocarcinoma	143	62	0.92 (0.651–1.314)	0.662
Lung squamous carcinoma	114	27	0.51 (0.319–0.799)	0.002
Small-cell lung carcinoma	29	5	0.37 (0.140–0.967)	0.025
Other	37	8	0.46 (0.210–1.013)	0.049
GSTA4 + 5034	GG	GA + AA		
Control	458	59	1.00	
Lung adenocarcinoma	170	34	1.55 (0.98–2.453)	0.063
Lung squamous carcinoma	131	9	0.53 (0.26–1.10)	0.071
Small-cell lung carcinoma	32	2	0.49 (0.11–2.08)	0.281
Other	40	5	0.97 (0.37–2.56)	0.951
GSTA4 + 13984	CC	CT + TT		
Control	243	274	1.00	
Lung adenocarcinoma	78	84	0.96 (0.671–1.359)	0.799
Lung squamous carcinoma	52	56	0.96 (0.631–1.447)	0.828
Small-cell lung carcinoma	16	11	0.61 (0.278–1.340)	0.213
Other	26	18	0.61 (0.278–1.339)	0.097

ORs were adjusted by age, sex, status of smoking, family medical history. The *P*-value of OR was calculated by the Wald test using SPSS.

OR = 0.37, 95% CI = 0.14–0.97, respectively) but not with adenocarcinoma. However, both *GSTA4* +5034 and +13984 polymorphisms were not associated with these histological types and genotypes. Since there was only a small number of other histological types, such as mixed adenosquamous lung cancer, undifferentiated carcinoma, they were excluded in this analysis (Table 2).

We then examined the joint effects of different genotypes of the three *GSTA4* polymorphisms as shown in Table 3. Compared with −1718TT/+5034GG/+13984CC genotypes, the percentage of −1718TA/+5034GG/+13984CT genotypes was lower in the cases than in the controls (5.0% vs. 15.9%, $P < 0.0001$), which was associated with a lower risk (OR = 0.37; 95% CI = 0.23–0.61), whereas carriers of −1718TT/+5034GG/+13984CT and −1718TA/+5034GA/+13984TT had a borderline higher risk (OR = 1.39; 95% CI = 1.00–1.92; $P = 0.052$ and OR = 3.06; 95% CI = 0.95–9.92; $P = 0.048$; respectively) (Table 3). Further stratified analysis by smoking status indicated that the joint effect among different genotypes was only significant for carriers with −1718 TA/+5034GG/+13984CT among both non-smokers (OR = 0.37; 95% CI = 0.15–0.87) and those who smoked for >25 pack-years (OR = 0.40; 95% CI = 0.20–0.82). Although the frequencies of *GSTA4* − 1718TA/+5034GG/+13984CT and −1718TT/+5034GG/+13984CT genotypes were significantly lower in the cases than in the controls (0.00% vs. 4.4%; $P = 1.37E{-}07$and 0.00% vs. 5.2%; $P = 1.61E{-}08$,

Table 3. Combination Effect of Different Genotypes of *GSTA4* −1718, +5034, +13984 and Risk of Lung Cancer

GSTA4 genotype			Cases		Controls			
−1718	+5034	+13984	*n*	%	*n*	%	OR (95% CI)	*P*
TT	GG	CC	195	39.0	239	46.2	1.00	
TT	GG	CT	113	22.6	100	19.3	1.39 (1.00–1.92)	0.052
TT	GG	TT	13	2.6	13	2.5	1.23 (0.56–2.71)	0.615
TT	GA	CC	4	0.8	12	2.3	0.41 (0.13–1.29)	0.105
TA	GG	CT	25	5.0	82	15.9	0.37 (0.23–0.61)	<0.001
TA	GG	TT	7	1.4	13	2.5	0.66 (0.26–1.69)	0.378
TA	GA	CT	35	7.0	51	9.9	0.84 (0.53–1.35)	0.469
TA	GA	TT	10	2.0	4	0.8	3.06 (0.95–9.92)	0.048
AA	GG	TT	2	0.4	8	1.5	0.31 (0.065–1.46)	0.102

The genotype combination with the number of subjects less than 10 for both controls and cases was excluded and ORs were adjusted by age, sex, status of smoking, family medical history. The *P*-value of OR was calculated by Wald test using SPSS.

respectively), the risk could not be estimated (Table 4).

Further haplotype analysis of the selected *GSTA4* SNPs showed that the frequency of TGT (haplotype 2) was higher in the cases than in the controls (30.9% vs. 20.4%; $P < 0.001$). Compared with carriers of TGT (haplotype 1), the risk of lung cancer increased by 1.55-fold for TGC (haplotype 2) carriers (OR = 1.55; 95% CI = 1.24–1.91). However, the frequency of AGT (haplotype 3) was lower in the cases than in the controls (9.4% vs. 18.1%; $P < 0.001$). Compared with carriers of TGC (haplotype 1), the risk of lung cancer for AGT (haplotype 3) carriers decreased by 47% (OR = 0.53; 95% CI = 0.40–0.70) (Table 5).

DISCUSSION

In this molecular epidemiologic study of lung cancer in a Chinese population, we investigated, for the first time, the role of three common *GSTA4* polymorphisms in the etiology of lung cancer. We found that genetic polymorphisms in *GSTA4* had modification effects on smoking-related lung cancer risk, particularly among patients with squamous cell carcinoma and small-cell-lung-cancer.

Environmentally, tobacco smoking is a well-established etiological factor for lung cancer. Pro-carcinogens, such as polycyclic aromatic hydrocarbons, nitrosamine, and arylamine in the tobacco smoke, can be activated by aryl hydrocarbon-hydroxylase, and the activated carcinogens react with DNA to form DNA adducts, which may lead to mutations or oncogene activation and thus to carcinogenesis [2,16]. However, having been catalyzed by glutathione *S* transferase (GST), glutathione combines with electropholic (carcinogenic agent, carcinogenic promoting agent, chemicals, environmental toxicant, etc.) and is transformed into hydrophilic molecular, which is prone to be excluded from bile and urine. Therefore, the normal cell is free from being attacked by carcinogenic factors and carcinogenic promoting factors [17]. It is likely that genetic polymorphisms of *GSTs* may change the molecular structure and functions of the enzyme. If the polymorphisms are located at the promoter and enhancer of control region and transcription regulator, the expression of the enzyme could be altered, and its deintoxication functions could be affected, and these changes may result in an altered susceptibility to cancers.

In 1998, Hubatsch et al. [6] reported that GSTA4 is highly effective in catalyzing 4-hydroxynonenal, an important product of peroxidative degradation of arachidonic acid and a commonly used biomarker for oxidative damage in humans. GSTA4 may regulate oxidative stress, a major pathway by which that cigarette smoke causes damage to DNA. Therefore, we genotyped three loci of *GSTA4* (one of five isoenzyme genes of *GSTs*), the T/A at locus −1718, G/C at locus +5034 and C/T at locus +13984, attempting to establish an association between *GSTA4* genetic variants and lung cancer risk. The results showed that the presence of A allele (genotype TA or AA) at locus −1718 of *GSTA4* was associated with a 37% significantly decreased risk of lung cancer, suggesting that this polymorphism may have some functional significance or in linkage with other disease-causing variants in the genome. The possible biological mechanism underlying this association is that the polymorphisms located in the control (regulation) region may enhance the transcription activity of the gene, increase the expression of GST, and elevate the deintoxication power of the enzyme.

Our results of different histological types of lung cancer also showed that individuals with genotypes of TA + AA at locus −1718 of *GSTA4* had a decreased risk of squamous cell carcinoma and small cell lung

Table 4. Stratified Analysis of the Combination Effect Between Different *GSTA4* Genotypes by Smoking Status

	GSTA4 genotype			Cases		Controls			
	−1718	+5034	+13984	*n*	%	*n*	%	OR (95% CI)	*P*
Non-smokers	TT	GG	CC	72	14.4	120	23.2	1.00	
	TT	GG	CT	39	7.8	44	8.7	1.48 (0.88–2.49)	0.142
	TA	GG	CT	7	1.4	32	6.2	0.37 (0.15–0.87)	0.023
	TA	GG	TT	4	0.8	9	1.7	0.74 (0.22–2.49)	0.628
	TA	GA	CT	14	2.8	25	4.8	0.93 (0.46–1.91)	0.850
No. of cigarettes ≤25	TT	GG	CC	53	10.6	53	10.3	1.00	
	TT	GG	CT	0	0.0	27	5.2		1.61E−08
	TA	GG	CT	0	0.0	23	4.4		1.37E−07
	TA	GA	CT	7	1.4	15	2.9	0.48 (0.18–1.24)	0.125
No. of cigarettes >25	TT	GG	CC	91	18.2	66	12.8	1.00	
	TT	GG	CT	50	10.0	28	5.4	1.30 (0.74–2.27)	0.366
	TA	GG	CT	15	3.0	27	5.2	0.40 (0.20–0.82)	0.012
	TA	GA	CT	7	1.4	10	1.9	0.51 (0.18–1.40)	0.191

The genotype combination with the number of subjects in the control and case less than 10 is deleted.

Table 5. Associations Between *GSTA4* Haplotypes and Risk of Lung Cancer

Haplotype	−1718 (T > A)	+5034 (G > A)	+13984 (C > T)	Cases		Controls		*P* value	OR (95% CI)
				n	%	*n*	%		
1	T	G	C	474	47.4	502	48.5	—	1.00
2	T	G	T	309	30.9	211	20.4	<0.001	1.55 (1.24–1.91)
3	A	G	T	94	9.4	187	18.1	<0.001	0.53 (0.40–0.70)
4	A	A	T	96	9.6	108	10.4	0.695	0.94 (0.70–1.27)
5		Others		27	2.7	26	2.5	0.779	1.10 (0.63–1.91)

Other haplotypes included haplotype TAC, AGC, and AAC and were combined because of frequency <5%.

cancer, although no biological mechanism can be inferred based on this observational data. There are several possible lines that support this finding. Firstly, the expression of *GST* is higher in the bronchial epithelium than in the terminal airway epithelium and moreover, the squamous cell carcinoma and small cell lung cancer mainly develop in the large bronchus [18]. Although no previously published study has focused on relation of GSTA4 and lung cancer risk, a positive association between the gene and hepatocellular carcinoma in males may suggest its role in cancer susceptibility [19]. In addition, a borderline significant protective effect was observed in other type lung cancer cases, which needs to be cautious to make conclusion because of the small sample size and heterogeneity of cancer type.

Interestingly, although the polymorphisms at the position +5034 and +13984 of *GSTA4* had no association with the risk of lung cancer, individuals with genotypes of TA at locus −1718, GG at +5034 and CT at +13984 of *GSTA4* had a significantly decreased risk for the development of lung cancer independent of smoking. These findings suggested a potential multiplicative joint effect among these three loci. Although the protective effect also existed in the individuals who smoked more than 25 pack-years and had the combined genotypes of *GSTA4* − 1718 TA, +5034 GG and +13984 CT, this effect apparently was no as strong as that seen in those who smoked ≤25 pack-years. The reason is likely that the main effect caused by heavy smoking, a high risk of lung cancer, counteracted this protective effect in the development of lung cancer, which suggested a possible gene-environment interaction between the *GSTA4* genetic polymorphisms and tobacco smoking in the etiology of lung cancer in the study population, which is consistent with the previous findings of the association studies in other ethnic populations or in other cancer types [20].

Recent studies of complex multifactorial common disease show that multi-locus haplotypes evaluated together can effectively identify genetic markers that correlated with disease and other related phenotypes [21]. Our study indicated that TGT haplotype carriers had a higher risk of lung cancer than those carrying the TGC haplotype, while AGT haplotype carriers had a lower risk of lung cancer. Although the sample size of current study was only moderate, our study on genetic susceptibility and haplotype analysis was more efficient in finding out the biological marker reflecting hereditary susceptibility to lung cancer.

Our study may have certain limitations to be addressed. First of all, this study was a hospital-based case–control study and cases were recruited from hospitals while controls were selected from community, thus selection bias may lead to spurious findings. However, potential confounding bias was minimized by frequency-matching controls to cases by age, sex and area and further adjusting potential confounding factors in analyses. Secondly, the sample size was relative small in this study, which may deduce false-positive or -negative results while the genotype frequency was low or stratified analyses were performed, though we had 80% statistic power to convince the significant association between GSTA4 −1718 and lung cancer risk according to present sample size and allele frequency of 0.17. Finally, three loci of GSTA4 were genotyped in this study, which did not cover all variants of GSTA4 and restricted the further interaction analysis with other gene in GST family.

In conclusion, our study provides evidence that the *GSTA4* polymorphisms may contribute to the etiology of primary lung cancer in this Chinese study population. However, ethnic variation in the *GSTA4* SNP distribution and its association with lung cancer risk warrant additional comparative studies to confirm our findings. Furthermore, more functional studies for this variant-cancer risk association are warranted.

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