Association of Cytokine Gene Polymorphisms With Rate of Decline in Lung Function

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Learning Objectives

- Recall the association between earlier or accelerated pulmonary dysfunction, as reflected by declining 1-second forced expiratory volume (FEV_1), and the development of chronic obstructive pulmonary disease (COPD).
- Point out the implications of smokers whose lung function remains normal for genetic factors influencing susceptibility of COPD.
- Give examples of how variants of genes that regulate the expression of cytokines may be associated to some degree with the age-related decline in FEV_1.

Abstract

Objective: *To investigate whether genetic variants involved in cytokine expression are associated with the age-related rate of decline in forced expiratory volume in 1 second (FEV_1).* ***Methods:*** *Functional polymorphisms in the TNFα, TGFβ1, IL-1β, IL-1RN, IL-13, and IL-8 genes were investigated in 374 active firefighters with at least five pulmonary function tests.* ***Results:*** *A protective effect was found between the presence of the TGFβ1 −509 TT genotype and rate of decline in FEV_1 ($P = 0.043$). Carrying an A allele at TNFα −308 ($P = 0.010$) and GG genotype at TNFα −238 ($P = 0.028$) was associated with a more rapid rate of FEV_1 decline. The TNFα −308A/−238G haplotype was also associated with an increased rate of decline as compared with the other haplotypes.* ***Conclusions:*** *Interindividual variability in progressive decline in FEV_1 may be explained in part by genetic variations within genes involved in inflammatory responses.* (J Occup Environ Med. *2008;50:642–648*)

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Lung function, as measured by forced expiratory volume in 1 second (FEV_1), normally increases until reaching a plateau in early adulthood, and then declines in a relatively linear fashion as an individual ages.[1] An accelerated rate of decline or earlier age of onset of decline can exacerbate the development of chronic obstructive pulmonary disease (COPD).[2] In a recent study consisting of more than 15,000 adults in the United States, FEV_1 decline was found to be highly associated with COPD. Participants in a longitudinal cohort with Global Initiative for Chronic Obstructive Lung Disease stage 3 or 4 disease at baseline were at high risk for rapid decline in FEV_1.[3,4] Although cigarette smoking is a major risk factor for COPD, only 10% to 20% of smokers ever develop deteriorated lung function, suggesting that other factors, including genetics, determine the susceptibility in this process.[5] Homozygosity for the α1-antitrypsin Z allele has been associated with COPD and accelerated decline in FEV_1.[6] However, less than 1% of patients with COPD have this deficiency, indicating that other genetic factors may be involved. Gottlieb et al estimated a heritability component to decline in FEV_1 in the Framingham Heart Study population, accounting for 5% to 18% of the variance in rate of decline.[7] Evidence for familial correlation in decline in FEV_1, consistent with a recessive model of inheritance, was also reported among smoking-concordant siblings in the Tucson Epidemiological Study of Airway Obstructive Diseases.[8]

Inflammatory processes are of general importance in the pathology

of obstructive airway diseases. For example, inflammatory mediators are increased in the airways of patients with the progression of COPD, and are associated with a faster rate of decline in lung function.[9] It is also known that low-grade inflammation exists in the air spaces of clinically normal, older individuals as a part of the normal aging process and may contribute to decline in FEV_1 over time.[10] Previous studies of respiratory function in firefighters indicate that there may be differences in the rate of decline in FEV_1 in this occupational population as compared with the general population.[11,12] In addition, it has been shown that healthy nonsmoking firefighters show signs of inflammation, including increased proportion of lymphocytes and higher concentrations of fibronectin and hyaluronic acid in the lower airways, that can be attributed to smoke exposure.[13] On the basis of these observations, we hypothesized that genetic variants in inflammatory mediators, alone or in combination, influence the rate of FEV_1 decline over time, independent of the presence of disease. We examined selected polymorphisms known to regulate cytokine expression and that have been associated previously with pulmonary diseases in a group of firefighters with available longitudinal pulmonary function data.[14–16]

Materials and Methods

Study Population

The Phoenix Fire Department (Phoenix, Arizona) conducted medical monitoring from 1988 to 2003 that included compilation of demographic and pulmonary function test (PFT) data on 2492 active and retired firefighters. Following the study protocol approved by the University of Arizona Human subjects Institutional Review Board, we obtained informed consent from 451 of the active firefighters to collect blood or buccal cell samples for DNA analysis. Spirometry was performed as part of routine annual medical examinations in accordance with American Thoracic Society (ATS) recommendations[17] and corrected for temperature, pressure, and water saturation, but no information was available on the percentage of PFTs that met ATS standards. A total of 374 individuals for whom we had DNA samples and who had performed five or more annual PFTs over a period of five or more years were included in the analyses. Any change in FEV_1 values between surveys that was greater than 15% above or below preceding or subsequent PFTs was evaluated for validity and consistency with other PFT measures from the same individual. FEV_1 measures that met the above criteria for minimum number, duration and consistency were used to calculate the rate of decline in FEV_1 as a function of age using simple linear regression. Information on smoking history (ever vs never) was collected at the initial examination. Occupational exposure information was not available in the database.

DNA Preparation and Genotyping

Genomic DNA was prepared from whole blood and buccal cells using QIAamp DNA isolation kit (Qiagen, Valencia, CA). Genotype analysis was performed on genomic DNA, using a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique or a 5′-nuclease real-time PCR assay. Primers and probes for IL-1β −511 and +3953, IL-8 −251, IL-13 −1055, TGFβ1 −509, TNFα −308 and −238 were designed using Assay-by-Design service (Applied Biosystems, Foster City, CA) (Table 1). PCR amplification was performed in a volume of 25 μL containing 10 ng genomic DNA, 12.5 μL 2× Taqman Universal Master Mix (PE Applied Biosystems, Foster City, CA), 200 nM of probe, and 900 nM of primer. Cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 50 cycles at 92°C for 30 seconds and 60°C for 1 minute. Amplification was performed using an iCycler IQ (Biorad Laboratories, Hercules, CA) real-time thermal cycler. IL-1RN +2018 SNP was genotyped by PCR-RFLP as described previously.[16] Negative controls, without DNA template were included within each run of PCR amplification. Ten percent of the samples were randomly selected for repetition to ensure laboratory quality control.

Statistical Analyses

Hardy–Weinberg equilibrium was assessed individually for each SNP. Fisher exact test was used to assess gender and ethnic differences in SNP genotype frequencies. Mean pulmonary function measures were compared by gender, race or ethnic group, and smoking status, using one-way analysis of variance (ANOVA). For each firefighter, a simple linear regression of FEV_1 over age was used to calculate the slope of FEV_1.

For each SNP, differences in mean slope by genotype were assessed using one-way ANOVA and the Bonferroni correction to compare genotypes within SNPs. Stratified analyses were used to assess the relation between genotype and FEV_1 slope within each race or ethnic group, and to evaluate gender differences and effects of smoking status. Dominant, recessive, or codominant Mendelian genetic models were determined based on differences in mean FEV_1 slope between the genotypes in one-way ANOVA, and entered in multivariate linear regression models as predictors of slope. These models were adjusted for race or ethnic group, gender, smoking status, baseline FEV_1, age at last PFT, number of years of follow-up, and a root mean square error term (RMSE) that was included as a correction for the fit of the individual regressions used to calculate slope. In addition, these models were stratified by race or ethnic group to assess for effect modification.

Likelihood ratio tests were used to assess interaction effects and confounding. Linkage disequilibrium

TABLE 1
Primer and Probe Sequences for SNPs Studies

Cytokine SNP	Substitution	Probe and Primer Sequence
IL-1β −511	C→T	
rs16944*	Probe-C	VIC-AGAGCTCCCGAGGC-MGB
	Probe-T	FAM-AGCTCCTGAGGCAGA-MGB
	Primer-F	CAGAGGCTCCTGCAATTGACA
	Primer-R	GGTCTCTACCTTGGGTGCTGTTC
IL-1β +3953	C→T	
rs1143634	Probe-C	VIC-TGTGTCGAAGAAGAT-MGB
	Probe-T	FAM-TCCCATGTGTCAAAGA-MGB
	Primer-F	CCTAAACAACATGTGCTCCACATT
	Primer-R	ATCGTGCACATAAGCCTCGTTA
IL-8 −251	A→T	
rs4073	Probe-T	VIC- AGCATACATTTGATAATT-MGB
	Probe-A	FAM- AGCATACAATTGATAATT-MGB
	Primer-F	GTGTCACATGGTACTATGATAAAGTTATCTAGA
	Primer-R	TCAAATACGGAGTATGACGAAAGTT
IL-13 −1055	C→T	
rs1800925	Probe-C	VIC- TCCCTCGTTTTCC-MGB
	Probe-T	FAM- TCCCTCATTTTCC-MGB
	Primer-F	AACACCCAACAGGCAAATGC
	Primer-R	TGCAGCATGTCGCCTTTTC
TGFβ1 −509	C→T	
rs1800469	Probe-C	VIC-CCATCCCTCAGGTGT-MGB
	Probe-T	FAM-CATCCTTCAGGTGTC-MGB
	Primer-F	AAGGAGAGCAATTCTTACAGGTGTCT
	Primer-R	GCCTCCGGAGGGTGTCA
TNFα −238	A→G	
rs361525	Probe-A	VIC- CTCCCTGCTCTGATTC -MGB
	Probe-G	FAM- CCCTGCTCCGATTC -MGB
	Primer-F	CAGTCAGTGGCCCAGAAGAC
	Primer-R	AGCATCAAGGATACCCCTCACA
TNFα −308	G→A	
rs1800629	Probe-A	VIC- CCCGTCCTCATGCC -MGB
	Probe-G	FAM- CCCGTCCCCATGCC -MGB
	Primer-F	CCCCAAAAGAAATGGAGGC
	Primer-R	TCTTCTGGGCCACTGACTGAT

All assays were designed using Assay-by-Design service (Applied Biosystems).
*National Center for Biotechnology Information, dbSNP ID.

(LD) was tested between TNFα SNPs (−308 and −238) using LDA software to calculate D prime (D′) value. In addition, multivariate regression models were used to assess the predictive value of additive genetic effects on slope. Throughout, a critical value of $P < 0.05$ was considered statistically significant.

Results

Age, race or ethnic group, smoking status, and pulmonary function data of subjects are summarized in Table 2. The average rate of decline in FEV_1 was -0.034 ± 0.03 L/yr and was not significantly different by race or ethnic group or by gender. However, almost 18% of the firefighters (30% of women and 17% of men) had a mean annual rate of decline in excess of 60 mL. Ever-smokers had a significantly greater rate of decline as compared with never smokers ($P = 0.042$). The RMSE was positively related to rate of decline, but accounted for only 1% of the variance in the adjusted models. Nevertheless, likelihood ratio tests comparing models with and without RMSE were significant.

The population was in Hardy–Weinberg equilibrium for all of the SNPs analyzed and genotype frequencies were similar to those previously published.[14,15] There were significant differences in the distribution of genotype frequencies for IL-1β +3953, IL-1RN +2018, and TGFβ1 −509 by race or ethnic group (Fisher exact test, $P < 0.001$), but not for the other SNPs. The mean rates of decline in FEV_1 by SNP genotypes are shown in Table 3. Unadjusted mean slope values varied significantly by TNFα −308 ($P = 0.004$), TNFα −238 ($P = 0.043$) and TGFβ1 −509 ($P = 0.053$) genotypes, whereas IL-1RA +2018 ($P = 0.085$) approached significance. A slower rate of FEV_1 decline was associated with the TT genotype at TGFβ1 −509 in the whole population, but this effect was stronger among non-Hispanic Whites ($P = 0.0154$) and Hispanics showed no genotype effect. (There were no blacks with the rare TT genotype.)

In multivariate analyses, the rate of annual decline in FEV_1 was associated with the TGFβ1 −509, IL-1RN +2018, TNFα −238 and TNFα −308 variants adjusted for gender, race, smoking status, baseline FEV_1, age at last PFT, number of years of follow-up PFTs, and RMSE (Table 3). In the multivariate models, stratifying by ethnic or race group did not alter the results, and, in fact, confirmed them. Non-Hispanic Whites were the only group with sufficient sample size to detect the effects of genotypes on FEV_1 decline, and the coefficients and P values in the non-Hispanic Whites-only model were essentially identical to those in the whole population model. We also tested the whole population model with a genotype by race interaction term, and found no significant interaction between gene variants and race or ethnic group or gender for any of the SNPs. Thus, these interaction terms were not included in the final models.

There was also no significant interaction effect of baseline FEV_1 by race or ethnic group, nor was it an apparent confounder. A baseline FEV_1 by genotype interaction was also tested and was not significant in any of the models. In one-way ANOVA stratified by smoking status, ever-smokers with the

TABLE 2
Characteristics of the Study Participants

	Men	Women
Gender	364 (97.3)	10 (2.7)
Race or ethnic group		
non-Hispanic White	278 (76.4)	10 (100)
Hispanic White	71 (19.5)	0
African-American	15 (4.1)	0
Smokers		
Ever-smokers	72 (19.8)	3 (30.0)
Never smokers	292 (80.2)	7 (70.0)
Subjects with FEV_1/FVC <70%, any survey	58 (15.9)	0
Subjects with FEV_1 slope <−60 mL/yr	62 (17.0)	3 (30.0)
Mean initial age (yrs)	31.9 ± 6.4	29.7 ± 3.9
Mean age last PFT (yrs)	43.7 ± 7.4	41.2 ± 4.9
Mean yr follow-up	11.8 ± 2.5	11.6 ± 2.3
Mean number of PFTs	10.3 ± 2.1	10.5 ± 2.2
Mean initial FEV_1 (L)	4.39 ± 0.63	3.60 ± 0.43
Mean initial FVC (L)	5.46 ± 0.78	4.38 ± 0.45
Mean initial FEV_1/FVC	0.81 ± 0.07	0.82 ± 0.65
Mean FEV_1 slope (L/yr)	−0.034 ± 0.027	−0.038 ± 0.020

Values are given as *N* (%) or mean ± standard deviation.

TT genotype at IL-1RN +2018 had a significantly steeper rate of decline (−0.058 ± 0.014 L/yr) compared with ever-smokers carrying at least one C allele (−0.037 ± 0.025 L/yr, $P = 0.029$) (data not shown). Polymorphisms at IL-1β −511, +3953 and IL-8 −251 showed no relation to rate of FEV_1 decline in either the univariate or multivariate models.

Multivariate regression models were used to test for additive gene effects. When IL-1RN +2018, TGFβ1 −509, TNFα −308 and −238 SNP genotypes were included in the models, IL-1RN +2018 and both TNFα SNPs were significant predictors of the FEV_1 slope (Table 4). There were no significant interaction effects between TNF-α −238 and −308. This additive model accounted for 14.8% of the total variance in the slope.

Linkage disequilibrium (D′) between TNFα −308 and −238 was equal to 1.0, indicating that only three of four possible haplotypes were present in this population, and the third was uncommon ($n = 36$). In contrast, the r^2 value was less than 0.01, suggesting that LD between these SNPs is quite low. The TNFα −308A/−238G haplotype was associated with a significantly steeper rate of decline (−0.041 ± 0.031 L/yr) than −308G/−238A (−0.022 ± 0.029 L/yr) ($P = 0.004$) or −308G/−238G (−0.033 ± 0.028 L/yr) ($P = 0.031$) haplotypes in ANOVA (overall $P < 0.002$).

TABLE 3
Genotype Frequencies and Mean Rate of FEV_1 Decline by SNP Genotypes

SNP	Genotype	N (%)	Mean Slope	Unadjusted *P* Value*	Adjusted *P* Value†
IL-1β −511	CC	151 (40.4)	−0.036 ± 0.027		
	CT	165 (44.1)	−0.033 ± 0.029		
	TT	58 (15.5)	−0.030 ± 0.022	0.340	
IL-1β +3953	CC	254 (67.9)	−0.035 ± 0.027		
	CT	110 (29.4)	−0.032 ± 0.029		
	TT	10 (2.7)	−0.024 ± 0.022	0.351	
IL-1RN +2018	CC	192 (51.3)	−0.032 ± 0.028		
	CT	146 (39.0)	−0.034 ± 0.027		
	TT	36 (9.6)	−0.041 ± 0.027	0.085 (TT vs CC + CT)	0.052 (TT vs CC + CT)
IL-8 −251	TT	81 (21.7)	−0.035 ± 0.024		
	TA	169 (45.2)	−0.035 ± 0.025	0.383	
	AA	124 (33.1)	−0.031 ± 0.031		
IL-13 −1055	CC	241 (64.4)	−0.033 ± 0.028		
	CT	115 (30.7)	−0.034 ± 0.027		
	TT	13 (4.8)	−0.036 ± 0.023	0.952	
TGFβ1 −509	CC	171 (45.7)	−0.034 ± 0.027		
	CT	152 (40.6)	−0.035 ± 0.029		
	TT	51 (13.6)	−0.027 ± 0.022	0.053 (CC + CT vs TT)	0.043 (CC + CT vs TT)
TNFα −238	GG	339 (90.6)	−0.034 ± 0.027		
	GA	34 (9.1)	−0.025 ± 0.028		
	AA	1 (0.3)		0.043 (GG vs GA)	0.028 (GG vs GA)
TNFα −308	GG	271 (72.4)	−0.031 ± 0.028		
	GA	90 (24.1)	−0.040 ± 0.028		
	AA	13 (3.5)	−0.039 ± 0.019	0.004 (GG vs GA + AA)	0.010 (GG vs GA + AA)

*Oneway ANOVA.
†Multiple linear regression, adjusted for age at last PFT, gender, race or ethnic group, ever smoking, baseline FEV_1, yr of follow-up, RMSE.

TABLE 4
Multivariate Regression Model for the Slope of FEV_1 Including Additive Genetic Effects

Covariates	β	SE	$P > t$	(95% CI = for β)*	
Age at last PFT	−0.0009	0.0002	0.000	−0.0013	−0.0004
Men vs women	0.0151	0.0083	0.070	−0.0012	0.0313
Hispanic vs non-Hispanic White (ref)	0.0009	0.0035	0.800	−0.0060	0.0078
African-American vs non-Hispanic White (ref)	0.0030	0.0068	0.656	−0.0103	0.0164
Ever vs never smoker	−0.0047	0.0034	0.159	−0.0113	0.0019
Baseline FEV_1	−0.0121	0.0022	0.000	−0.0165	−0.0078
Total yrs of follow-up	0.0015	0.0006	0.018	0.0003	0.0028
Root MSE	0.0357	0.0150	0.018	0.0062	0.0653
IL-1RN 2018 TT vs CC + CT (ref)	−0.0107	0.0044	0.016	−0.0194	−0.0020
TGFβ1 −509 CC + CT vs TT (ref)	−0.0066	0.0039	0.095	−0.0143	0.0011
TNFα −238 GA vs GG (ref)	0.0090	0.0045	0.048	0.0001	0.0179
TNFα −308 GA + AA vs GG (ref)	0.0071	0.0029	0.015	0.0014	0.0129
Constant	0.0193	0.0157	0.220	−0.0116	0.0503

*Adjusted $R^2 = 0.1482$.
Ref indicates reference population.

Discussion

In the present study, we found that the TNFα −308, −238, and TGF β1 −509 SNPs and the TNFα −308A/−238G haplotype were associated with age-related decline in FEV_1 in a group of firefighters. This population provided a unique opportunity to test susceptibility genes in subjects who received routine annual spirometry for five or more years. Although the population was composed of firefighters, they may be representative of the general population as they had no medical history of occupationally related lung injury.

FEV_1 begins to decrease as a function of age and reflects various lung parameters such as lung volumes, pulmonary compliance, airflow rates, and muscle mass. After about age 20, the number of alveoli and lung capillaries gradually begin to decrease and the lungs become less elastic due to reduced elastic recoil. Some of these changes are physiologically similar to those associated with mild emphysema.[18] FEV_1 decrease is also an important outcome measure and a good predictor for COPD and cardiovascular diseases.[2,19] Smoking is the most widespread factor contributing to COPD in the general population, although airway hyperresponsiveness, atopy, childhood respiratory infections, air pollution, and occupational exposures also have been implicated, either alone or in combination, in the development of COPD.[20–22]

The −238 and −308 SNPs of the TNFα gene have been associated with numerous chronic pulmonary diseases including COPD.[23,24] In the present study, the −308A variant was associated with more rapid decline in FEV_1 compared with the −308G allele. Although recent studies have questioned whether the TNFα −238 SNP is associated with changes in protein levels,[25] we found an association between the TNFα −238 SNP and FEV_1 decline. The association may be due to a strong LD in the TNF region. As previously reported by other researchers,[26] we also found that TNFα −308 and −238 SNPs are in strong LD. Therefore, the association between the TNFα −308A/−238G haplotype and the steeper rate of decline in lung function might be explained by an allelic association with a functional marker in the TNFα locus or neighboring genes, possibly driven by the high producer −308A variant. Furthermore, when additive genetic effects were incorporated into the multivariate regression model, both TNFα SNPs were predictors of the FEV_1 slope.

A polymorphism at C−509T in the promoter region of TGFβ1 is associated with higher circulating concentrations of the protein.[27] An inverse association was found between −509 T allele and COPD in two independent studies.[28] More recently, the frequency of the −509T allele was found to be significantly higher in control subjects than in COPD patients.[29] We also found that the TT variant genotype is associated with a slower rate of decline in lung function. Our results are consistent with previous genetic studies and animal models of COPD where it was reported that increased production of TGFβ1 can protect against the development of COPD through inhibition of macrophage metalloelastase.[30,31] In contrast, two recent studies showed no significant association between individual SNPs of TGFβ1 gene (including C−509T) and rapid decline in lung function in the general population.[32,33] The lack of agreement may reflect differences in the study populations and design. Subjects in the study of van Diemen et al[32] were recruited from the general population and were followed for 25 years on average whereas the study population from Ogawa et al[33] was composed of smokers from NHLBI Lung Health Study.

In a pilot study, we found that the presence of the IL-1RN +2018 T allele was marginally associated with a more rapid decline in FEV_1 in 67 firefighters with at least four PFTs.[34] Similarly, in the current study, there was an increased lung function decline in carriers of the IL-1RN +2018 TT genotype, but this was not significant. The association was more evident when the data were stratified in the univariate model by

smoking status, however the number of smokers with the TT genotype was small ($n = 7$). The IL-RN (VNTR) A1/IL-1B −511T haplotype has previously been associated with rapid decline of lung function in smokers.[15] In the present study, there were no associations between IL-1β +3953 and −511 genotypes and lung function, either alone or in combination with IL-1RN +2018.

The major strengths of this study include the opportunity to examine the rate of change in lung function over time rather than a single cross-sectional measurement, a clearly defined phenotype, and examination of candidate genes based on their functional roles in lung disease. There were also a number of limitations to the current study. We lacked information on the quality of the spirometry. Longitudinal spirometry results are strongly affected by procedures, equipment and training, and poor quality could have increased variability. However, it seems unlikely that quality issues would have introduced any directional bias into the results. Although we found no evidence of confounding due to population stratification in our analyses, we cannot completely rule out this possibility. Another concern is the possibility of false positives due to multiple hypothesis testing. Although we show an effect of several genotypes on FEV_1 decline in this population, when we control for multiple comparisons,[35] we fail to reject the null hypothesis (at $P < 0.00625$). Conservatively, we would conclude that our rejection of three of eight hypotheses in multivariate regression analyses represent a false discovery rate and would be expected due to chance. However, this conclusion may be overly conservative, especially since most of our findings corroborate those in other studies. In addition, information on occupational exposures was not available. Firefighters may be a healthier than normal cohort which may reflect a "healthy worker effect" and may have had occupational exposures that were unaccounted for in this study.[36] Previous studies have shown that occupational lifetime exposure to gases, dust and fumes is associated with chronic bronchitis and airflow obstruction in the general population, independently of smoking status.[22] Also, certain products of combustion and organic compounds are known to cause significant adverse effects on respiratory function.[37] In addition, no quantitative information on years or quantity of cigarettes smoked was available.

In conclusion, interindividual variability in longitudinal lung function decline might be explained in part by variations in genes which regulate inflammatory processes in the airway response. Further longitudinal studies with adequate assessment of occupational exposure are warranted to assess the role of genetic as well as environmental and occupational factors in this process.

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