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Heritability of Respiratory Infection with *Pseudomonas aeruginosa* in Cystic Fibrosis

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Abstract

Objective—To quantify the relative contribution of factors other than cystic fibrosis transmembrane conductance regulator genotype and environment on the acquisition of *Pseudomonas aeruginosa* (*Pa*) by patients with cystic fibrosis.

Study design—Lung infection with *Pa* and mucoid *Pa* was assessed using a co-twin study design of 44 monozygous (MZ) and 17 dizygous (DZ) twin pairs. Two definitions were used to establish infection: first positive culture and persistent positive culture. Genetic contribution to infection (ie, heritability) was estimated based on concordance analysis, logistic regression, and age at onset of infection through comparison of intraclass correlation coefficients.

Results—Concordance for persistent *Pa* infection was higher in MZ (0.83; 25 of 30 pairs) than DZ twins (0.45; 5 of 11 pairs), generating a heritability of 0.76. Logistic regression adjusted for age corroborated genetic control of persistent *Pa* infection. The correlation for age at persistent *Pa* infection was higher in MZ twins (0.589; 95% CI, 0.222–0.704) than in DZ twins (0.162; 95% CI, –0.352 to 0.607), generating a heritability of 0.85.

Conclusion—Genetic modifiers play a significant role in the establishment and timing of persistent *Pa* infection in individuals with cystic fibrosis.

Cystic fibrosis (CF), the most common life-shortening autosomal recessive disorder in the Caucasian population, is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Progressive obstructive lung disease is the primary cause of mortality in CF.^{1,2} Respiratory infection by particular organisms, such as *Pseudomonas aeruginosa* (*Pa*), is associated with more rapid lung function decline³ and reduced survival.² Age at acquisition of infection varies considerably, and not all patients with CF acquire *Pa*,

indicating the presence of modifying factors. Several demographic and environmental factors have been shown to modify the risk of pulmonary infection with *Pa*.³⁻⁶

Multiple studies of risk factors for the acquisition of *Pa* have shown increased rates of *Pa* infection in patients with *CFTR* mutations associated with minimal chloride channel function (ie, severe mutations).⁷⁻⁹ Other genes also appear to modify infection status, including the mannose-binding lectin 2 (*MBL2*),¹⁰ transforming growth factor β 1,¹¹ and tumor necrosis factor α genes.¹² Some of these modifier genes have been associated with an earlier age of *Pa* acquisition (eg, *MBL2*);¹⁰ however, the majority of studies found no effect on *Pa* infection status.^{13,14} Although candidate modifier gene studies have provided evidence of potential genetic control of infection status in CF, the total contribution of genetic factors including both DNA variation and other heritable factors, such as epigenetic signatures to *Pa* infection, has not yet been elucidated. Estimating the degree to which variation in the host influences infection risk can inform the search for genetic modifiers of this important trait in CF. Deriving this estimate can be difficult, however, given the possibility that host factors affect if and when a patient will acquire *Pa*, whether a patient will enter a transient infectious state, and whether he or she will develop chronic infection. Therefore, a study of genetic risk factors should account for these different stages when assessing infection in patients with CF.

One approach to addressing the question of whether genetic effects contribute to the acquisition of *Pa* infection is to determine the heritability (ie, total genetic effect) of *Pa* infection. Heritability can be estimated using twins living concurrently in similar social and economic conditions, which reduces confounding effects related to the environment. Furthermore, limiting a study to twins reduces differences in exposures that might be attributable to age differences between siblings. A higher rate of concordance for disease among identical (monozygotic [MZ]) twins as opposed to nonidentical (dizygous [DZ]) twins provides evidence of the contribution of genetic factors to the acquisition of disease. Consequently, we undertook a twin study to estimate the heritability of *Pa* infection using respiratory infection data from the CF Twin and Sibling Study.

Methods

All subjects were recruited on the basis of having CF and a twin affected with CF,¹⁵ with 99% of subjects drawn from the United States. The zygosity status of twins was determined using an AmpFISTR Profiler (Applied Biosystems, Foster City, California). Only subjects with 2 *CFTR* class I, II, or III mutations were included, given the known relationship between the *CFTR* genotype and *Pa* acquisition.^{7,8} *CFTR* genotype was confirmed from the patient's medical record or by *CFTR* typing and sequencing.^{16,17} Written informed consent/assent was obtained from all subjects and/or their guardians. The study design was approved by Johns Hopkins University's Institutional Review Board.

Subjects were defined as having a positive culture if they had a respiratory culture (throat, sputum, or bronchoalveolar lavage culture) positive for *Pa* or mucoid *Pa* (*MPa*), regardless of the presence of other organisms in the culture. To ensure accurate assignment of the first positive culture, only those subjects with a previous negative culture for *Pa* before the first positive culture were included in the analysis. All subjects were followed from birth until December 31, 2008, using culture data provided from their care center and the CF Foundation Patient Registry. Subjects with no respiratory cultures performed before December 31, 2008, were excluded.

Two definitions were created to capture the extremes in stages of respiratory infection. First infection was defined as the first positive culture for *Pa* or *MPa*. Persistent infection was

defined as 3 consecutive years of culture data with at least one positive culture in at least 2 of the 3 years,¹⁸ and served as a proxy for chronic *Pa* infection. Subjects were defined as negative if neither *Pa* nor *MPa* had been cultured from the respiratory tract as of December 31, 2008.

Estimation of Genetic Effect and Statistical Analysis

Genetic contribution to infection was estimated first by subtracting the concordance in the DZ twins from the MZ twins and multiplying by 2, as described by Falconer and Mackay.¹⁹ Statistical significance for differences in concordance rate was calculated using the Fisher exact test (<http://www.unc.edu/preacher/fisher/fisher.htm>). Second, logistic regression as described by Ramakrishnan et al²⁰ was performed to calculate zygosity-specific ORs, which represent the risk of infection in one twin given the presence of infection in the co-twin. Whether a genetic model or an environmental model governs the data was determined using the equation $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2$, where Y is the probability that the index twin has infection; X_1 represents whether the co-twin has infection, with a value of 1 if the co-twin has disease and 0 if not; X_2 is an indicator with value of 1 for MZ twins and 0.5 for DZ twins; and $X_1 X_2$ is the product of X_1 and X_2 , representing the interaction between infection status and zygosity. When the coefficient for this interaction term is significantly different than 0 and the OR for the MZ twins is more than twice the OR for the DZ twins, then genetic factors are implicated. This model also allowed us to assess the contribution of potential confounding variables, such as age and lung function, to the genetic effect. The following covariates were tested using univariate logistic regression to determine whether they significantly altered this base model: sex (dichotomous variable: male or female); race (dichotomous: Caucasian or non-Caucasian); *F508del* (ordinal: 0, 1, or 2 mutations); age at the last clinic visit (continuous variable); forced expiratory volume in 1 second (FEV₁), measured as the maximum CF-specific percentile of FEV₁²¹ in the most recent year of pulmonary function test data (MaxFEV₁ CF percentile) (continuous variable with values of 0-1); and number of cultures per year for the subject (continuous). Any covariate with a P value <.10 was included in a multivariate model, and this model included current age, MaxFEV₁ CF percentile, and number of cultures. In this multivariate model, only age was found to be significant ($P < .05$) and thus was retained in the final model. Statistical calculations were performed using Intercooled Stata 10 (StataCorp, College Station, Texas).

Finally, age-of-onset methods involving residuals calculated from Cox regression analyses were used to determine whether age at first infection and persistent infection were affected by genetic modifiers. Time-to-onset methods were used, with time zero as birth of the subject and followed until a positive culture for *Pa* (ie, met the definition for first infection) was obtained. For age of persistent infection, subjects were determined to have 3 consecutive years of culture data with at least one positive culture per year in at least 2 of the years. The subject's age on the date of the first culture that met this criterion was entered as the age of persistent infection. Subjects were censored using the age at the last obtained culture if they never met the criteria for these definitions. Cox regression analysis was performed to identify variables that were significantly associated with age of onset of infection. Covariates to include were determined as described earlier for logistic regression. In this multivariate model, both MaxFEV₁ CF percentile and number of cultures were significant ($P < .05$) and were retained in the final model. Martingale residuals were calculated and power-transformed to create the deviance residual. The deviance residual for each individual was compared with his or her twin to determine the intraclass correlation coefficient for the MZ and DZ twins. Using a bootstrap technique, the intraclass correlation was resampled 100 000 times to formulate 95% CIs. Heritability was then calculated according to the method of Falconer and Mackay¹⁹ for linear traits by subtracting the

correlation coefficient of the DZ twins from that of the MZ twins and multiplying by 2. All calculations were performed with Intercooled Stata 10 (StataCorp).

Results

Eighty pairs of MZ twins, 25 pairs of DZ twins, 1 set of triplets, and 1 pair of twins with unknown zygosity were enrolled in the CF Twin and Sibling Study as of December 31, 2008. The set of triplets and twins of unknown zygosity were excluded, as were 7 sets of MZ twins and 1 set of DZ twins who had another sibling with CF. Seven sets of MZ twins with a class IV or V *CFTR* mutation, which is associated with “residual” CFTR function (which has previously been correlated with lower risk of *Pa* infection^{7,8}) were excluded. Five sets of MZ twins, in whom the effect of the mutation on CFTR function could not be determined, were excluded as well. Finally, 17 sets of MZ twins and 7 sets of DZ twins were excluded because the age at first positive culture could not be established due to lack of respiratory cultures before the first positive *Pa* culture. Table I presents clinical characteristics for the 44 pairs of MZ and 17 pairs of DZ twins with CF involved in this study. Seven sets of opposite sex DZ twins, 2 sets of female-only DZ twins, and 8 sets of male-only DZ twins were included in this analysis; there were no differences in outcome when opposite-sex twins were included. Also of note, the DZ twins were significantly younger than the MZ twins. The effect of this age difference was addressed using multivariate logistic regression techniques (see below). The MZ twins also had a larger proportion of subjects homozygous for the *F508del* *CFTR* mutation, which causes loss of CFTR function and pancreatic insufficiency. However, all of the MZ and DZ twin pairs had class I, II, or III mutations, which are also associated with loss of CFTR function and pancreatic insufficiency. The 122 subjects with CF in this study were classified according to *Pa* or *MPa* infection status (no infection, first infection, or persistent infection). For *Pa*, 23 subjects were negative for both first infection and persistent infection, 28 subjects were positive for first infection, and the majority (71 subjects) were positive for both first infection and persistent infection. The distribution was different for *MPa*, with 53 subjects negative, only 24 subjects positive for first infection, and 45 subjects positive for both first infection and persistent infection.

Genetic Modifiers Contribute to the Persistent Stage of *Pa* Infection

To determine the contribution of genetic and nongenetic (eg, environmental, stochastic) factors to airway infection, we compared concordance rates for each definition of infection with either *Pa* or *MPa* in the twin pairs with CF. Concordance rates for persistent infection with *Pa* were higher in MZ twins than in DZ twins (0.83 [25 of 30 pairs] vs 0.45 [5 of 11 pairs]; $P = .04$, Fisher exact test) (Table II). The estimated genetic contribution (ie, heritability) for persistent *Pa* infection was 0.76. On the other hand, the concordance rate for first infection with *Pa* did not differ between MZ twins and DZ twins (0.80 [32 of 40 pairs] vs 0.69 [11 of 16 pairs]; $P = .49$, Fisher exact test), indicating low genetic contribution (Table II). Furthermore, the concordance rates for absence of first infection and absence of persistent infection did not differ between the MZ twins and the DZ twins (Table II). There also were no differences in concordance rates for MZ and DZ twins for any definition of *MPa* infection or absence of infection with *MPa* (Table II).

To account for age differences between the MZ and DZ twins, we performed concordance analysis with age matching and a logistic regression that included age as a covariate. We matched 2 MZ twin sets to 1 DZ twin set for age at the last culture of the DZ set. After matching, the mean age was 11.41 ± 5.06 years for the MZ twins and 11.61 ± 5.16 years for DZ twins ($P = .86$, Student *t* test). The mean age at first infection was 5.67 ± 3.27 years for the MZ twins and 4.87 ± 3.64 years for the DZ twins ($P = .27$, Student *t* test). After

correcting for age differences, heritability for persistent infection assessed by concordance analysis remained robust at 0.73 ($P = .049$, Fisher exact test).

A regression model previously described by Ramakrishnan et al²⁰ was used to calculate zygosity-specific ORs, which represent the risk of infection in one twin given the presence of infection in the co-twin (see Methods for details on this modeling). Multivariate regression revealed subject's current age significantly altered the model and thus this variable was retained in the final model. The coefficient for interaction in the model for persistent *Pa* infection was 6.45 ($P = .003$) and the zygosity-specific OR for the MZ twins (3.69) was more than twice the DZOR (0.47), indicating that genetic factors play a substantial role (Table III). On the other hand, first *Pa* infection did not generate a significant coefficient of interaction ($P = .549$), nor did infection with *MPa* using either definition. We assessed the appropriateness of the application of the regression technique to our data by comparing the fit of 3 models (additive genetic, common environment, and common environment with additive genetic effect)²² to the parameters used in the regression. Wald χ^2 tests of these parameters revealed that the additive genetic effect model best fit our data.²⁰ Thus, both age-matched concordance analysis and logistic regression accounting for age were consistent with the conclusion that genetic modifiers play a substantial role in establishing persistent infection with *Pa*.

Genetic Modifiers Contribute to the Age at Which Subjects Develop Persistent Infection with *Pa*

To determine whether genetic modifiers affect the age at which patients with CF acquire *Pa*, we performed a time-to-event analysis of the age at which the subject met the definition for first infection and/or persistent infection. We used residuals calculated from the final model²³ to estimate the time difference between when a patient was predicted to be infected and when the patient was actually infected. In brief, positive residual values indicate that infection status was achieved before predicted, and negative values indicate that either infection status was achieved later than predicted or infection never occurred. To estimate the genetic effect on age at infection, we compared the intraclass correlation coefficients for MZ and DZ twins for these residuals. For age at persistent *Pa* infection, the correlation coefficient was higher in MZ twins than in DZ twins (0.59; 95% CI, 0.34-0.76 vs 0.16; 95% CI, -0.35 to 0.61) (Figure; available at www.jpeds.com). Heritability, calculated as described previously,¹⁹ was estimated to be 0.854 (95% CI, 0-1). For the age at first *Pa* infection, the correlation coefficient was minimally different between MZ twins and DZ twins (0.47; 95% CI, 0.19-0.68 vs 0.49; 95% CI, 0.01-0.79) (Table IV). Furthermore, the correlation coefficients for either age at first *MPa* or age at persistent *MPa* were not different between MZ and DZ twins.

Pulmonary disease in patients with CF is frequently complicated by infection with a variety of organisms, the hallmark being *Pa*. Identification of the factors predisposing to *Pa* infection has been an area of major importance in the treatment of patients with CF. Although some previous studies have assessed infection risk associated with shared environment^{4,5} or *CFTR* genotype,^{7,24} to the best of our knowledge, this is the first study to quantify the relative contribution of genetic modifiers to infection in CF. Our results indicate a highly heritable component for the development of a persistent state of *Pa* infection. Attaining chronic infection is age-dependent, given that progression from first infection to intermittent infection and finally to chronic infection can take many years. Our observations hold even after accounting for age differences that could affect establishment of persistent *Pa* infection. We also found that initial *MPa* infection and persistent *MPa* infection did not appear to be influenced by genetic modifiers, suggesting that environmental or stochastic factors mediate these traits. Although variation in the *CFTR* gene⁷⁻⁹ has a substantial role in initial infection with *Pa*, we found no evidence that genetic modifiers have an impact on

initial infection. Our results indicate that once a subject has had a *Pa* infection, genetic modifiers contribute to the establishment of persistent infection with *Pa*.

The present study has some limitations. First, there were fewer DZ twins than MZ twins in the study, and thus inferences based on these sample sizes are associated with wide CIs. Reanalysis of our data including an additional 17 MZ and 7 DZ twins pairs that were excluded due to lack of a negative culture before the first positive culture still showed evidence of heritability for persistent *Pa* infection, with a heritability estimate from concordance analysis of 0.37. Unfortunately, recruiting more DZ twins is not likely, given that we have ascertained >90% of the available twins with CF in the United States, and other countries have much lower numbers of individuals with CF and hence few twins with CF. Our small sample size also affects the power of this study. Determining the number of families in each of the 3 categories (concordant positive, discordant, and concordant negative) was not possible before the analysis; however, had all families been either concordant positive or discordant, the study would have had 80% power to detect a 50% difference in the proportion affected with *Pa* (equating to a heritability of 1.0). Given the actual numbers of twins classified as persistent *Pa*, our post hoc analysis revealed that the study had reasonable power (67%) to observe the results obtained. In contrast, the study had low power (27%) to detect genetic control of traits if heritability was 0.5. Thus, we cannot exclude the possibility that genetic factors beyond those associated with *CFTR* make a low to moderate contribution (ie, heritability <0.5) to the absence of *Pa* infection.

Another study limitation is the age difference between the MZ and DZ twins. Age, lung function, and infection are known to be interrelated.^{18,25} Although we attempted to control for the effect of age and lung function in this analysis using regression techniques, residual confounding may still remain. We were able to match the MZ and DZ twins for age; when we did so, heritability remained robust (0.73), suggesting the absence of significant residual confounding by age. However, due to the small number of DZ twins, we were unable to also match the twins according to lung function. Furthermore, there were too few twins to allow evaluation of other effects that have been associated with *Pa* acquisition, such as sex-dependent effects.^{6,18,26} Additionally, we used retrospective data collected by the CF Foundation Patient Registry. In this study, all subjects were required to have at least one negative culture for *Pa* before the first *Pa* positive culture to maximize confidence that the age at first infection was accurate. We extrapolated the absence of infection to the date of birth based on the assumption that genetic influence begins at birth, coincident with the initiation of exposure risk. Establishing the point of chronic infection is challenging, given that serial respiratory cultures are often intermittently positive for *Pa* before becoming chronically positive. The timing of transition from an intermittent state of infection to a persistent state can be imprecise in many patients^{27,28}; thus, defining subjects based on persistent infection might not accurately capture true chronic infection. However, strong association with lung disease severity has been noted by others using this definition,¹⁸ as opposed to first infection, and thus we feel confident in using this definition in our subjects.

Strengths of the present study include the use of both concordance and regression analysis to determine whether *Pa* infection in CF is under genetic control. Concordance analysis has been used to identify genetic differences in otherwise similarly exposed individuals. Age at onset of disease is a relatively new analysis technique used in genetic studies. Multiple methods have been used to address this question, but which method best estimates heritability remains unclear. Deviance residuals from Cox regression have been used to assess the age at onset of multiple complex diseases, including asthma²⁹ and diabetes,³⁰ and performing these analyses for a single gene disorder such as CF is a natural extension of this methodology. Furthermore, our work expands on current investigations into candidate modifier genes, such as *MBL2*,¹⁰ which have shown an association between *MBL2* and an

earlier age of first infection but have not evaluated for definitions of chronic infection. Our findings suggest that using a definition for chronic infection such as persistent infection may provide further evidence to either support or refute the role of these modifier genes. Even though the observed heritability of persistent infection is likely due to variation in genes other than *CFTR*, it is also possible that other inherited factors, such as epigenetic differences, could contribute to this estimate of genetic control. Further investigation into these genetic modifiers is warranted. Furthermore, the heritability for either presence of persistent *Pa* and age at onset of persistent *Pa* was <1.0, indicating that environmental and stochastic factors are also contributing to these traits. Thus, infection control policies and eradication procedures continue to be important factors for persistent infection with *Pa*.

In conclusion, our data suggest that chronic infection with *Pa* has high heritability, whereas initial infection with *Pa* is minimally influenced by genetic modifiers. The age at which a state of persistent infection is achieved also appears to be governed by genetic modifiers. These observations do not extend to mucoid forms of *Pa*, implying that genetic factors in the host have no significant influence on the conversion of this bacterium to a mucoid state. Thus, searches for genetic modifiers of *Pa* infection are most likely to be fruitful if patients are stratified according to definitions that characterize chronic infection status.

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Glossary

CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
DZ	Dizygotic twins
FEV₁	Forced expiratory volume in 1 second
MBL2	Mannose-binding lectin 2
MPa	Mucoid <i>Pseudomonas aeruginosa</i>
MZ	Monozygotic twins
Pa	<i>Pseudomonas aeruginosa</i>

Figure.

Correlation of deviance residuals of **A**, first infection with *Pa* and **B**, persistent infection with *Pa* for MZ and DZ twins. For each plot, the *x*-axis represents the residual for twin A, and the *y*-axis represents the residual for twin B. The points on each graph represent one twin pair. The best-fitting line is shown. In this representation, the correlation coefficient of MZ twins was 0.46 for first *Pa* and 0.41 for DZ twins, which equates to a heritability of 0.1. For persistent *Pa*, the correlation coefficient was 0.59 for MZ twins and 0.24 for DZ twins, equating to a heritability of 0.7.

Table I
Characteristics of study subjects

Characteristic	MZ twins (n = 88 individuals)	DZ (n = 34 individuals)	P value
Female sex, n (%)	42 (47.7)	11 (32.4)	.125 [*]
Caucasian, n (%)	76 (90.5)	30 (88.2)	.715 [*]
Clinical pancreatic insufficiency, n (%)	88 (100)	34 (100)	-
<i>F508del</i> mutations, n (%)			
0	2 (2.3)	0	
1	22 (25.0)	16 (47.1)	
2	64 (72.7)	18 (52.9)	.049[*]
Age at last visit, years, mean $\pm$ SD (range)	17.3 $\pm$ 9.0 (2.2-49.6)	10.8 $\pm$ 5.5 (0.80-21.9)	.0001[†]
Age at first <i>Pa</i> -positive culture, years, median (range) (n)	5.9 (0.2-44.8) (69)	2.9 (0.3-10.8) (26)	.005[†]
Age at PersInfx with <i>Pa</i> , years, median (range) (n)	6.6 (0.3-44.8) (55)	4.5 (0.3-12.9) (15)	.048[†]
Maximal FEV CF%, mean $\pm$ SD (n)	0.64 $\pm$ 0.29 (83)	0.77 $\pm$ 0.17 (31)	.065 [†]
Cultures per year per subject, mean SD, (n)	3.42 1.94 (82)	3.84 2.08 (34)	.363 [‡]
Prevalence of first <i>Pa</i> , n (%)	72 (81.8)	27 (79.4)	.505 [*]
Prevalence of first <i>MPa</i> , n (%)	54 (61.4)	15 (44.1)	.085 [*]

PersInfx, persistent infection (3 consecutive years of culture data with positive cultures in 2 of the 3 years).

Bold type indicates significance (ie, $P < .05$).

^{*} P reported from χ^2 , assumes equal variance in group means.

[†] P reported from Kruskal-Wallis test due to unequal variances in group means.

[‡] P reported from ANOVA due to equal variance in group means.

Table II
Concordance analysis of MZ and DZ twins for infection with *Pa* and *MPa*

	Total pairs	Pos/pos	Pos/neg	Neg/neg	P value for presence of infection	P value for absence of infection
<i>Pa</i>						
First Infx	MZ (n = 44)	32	8	4	.486	.615
	DZ (n = 17)	11	5	1		
Pers Infx	MZ (n = 44)	25	5	14	.041	.255
	DZ (n = 17)	5	6	6		
Mucoid <i>Pa</i>						
First Infx	MZ (n = 44)	23	8	13	.686	.703
	DZ (n = 17)	6	3	8		
Pers Infx	MZ (n = 44)	14	7	23	1.0	.399
	DZ (n = 17)	4	1	12		

Pos, positive; *Neg*, negative; *FirstInfx*, at least one positive culture for *Pa*; *Pers*, persistent. Bold type indicates significance (ie, *P* < .05).

Table III
Logistic regression for twins with 2 class I, II, or III CFTR mutations comparing zygosity status as a predictor of both twins developing infection with age as a covariate

	$\beta_{\text{co-twin}}$	β_{zygosity}	$\beta_{\text{interaction}}$	P value	$\beta_{\text{interaction}}$	β_{age}	β_{MZ}^*	$\beta_{\text{DZ}}^\dagger$
<i>Pa</i>								
FirstInfx	-0.53	-2.36	1.48	.549		0.18	0.95	0.21
PersInfx	-2.76	-3.92	6.45	.003		0.13	3.69	0.47
<i>MPa</i>								
FirstInfx	2.65	-0.05	-0.26	.906		0.10	2.39	2.52
PersInfx	6.62	1.82	-3.95	.215		0.07	2.67	4.64

$\beta_{\text{co-twin}}$: coefficient from regression for co-twin infection status; β_{zygosity} : coefficient from regression for zygosity status (MZ or DZ); $\beta_{\text{interaction}}$: coefficient from regression for interaction term of zygosity status and co-twin infection status; β_{age} : coefficient from regression for current age.

Coefficients in bold type are associated with $P < .05$.

* β_{MZ} twins calculated as $\beta_{\text{cotwin}} + \beta_{\text{interaction}}$.

† β_{DZ} twins calculated as $\beta_{\text{cotwin}} + 0.5 \beta_{\text{interaction}}$.

Table IV
Intrapair correlation coefficients estimated from residuals of Cox regression for age of onset of *Pa* infection

	MZ twins (38 pairs), <i>r</i> (95% CI)	DZ twins (15 pairs), <i>r</i> (95% CI)	Heritability (95% CI)
<i>Pa</i>			
FirstInfx	0.471 (0.185-0.684)	0.492 (0.007-0.793)	0 (0-1)
PersInfx	0.589 (0.338-0.762)	0.162 (-0.352-0.607)	0.854 (0-1)
<i>MPa</i>			
FirstInfx	0.501 (0.222-0.704)	0.733 (0.384-0.901)	0 (0-1)
PersInfx	0.394 (0.092-0.630)	0.795 (0.503-0.925)	0 (0-1)