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Heritability of Raynaud's Phenomenon and Vascular Responsiveness to Cold: A Study of Adult Female Twins

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Introduction

The episodic vasospasm that characterizes Raynaud's phenomenon (RP) occurs in up to 15% of the adult population (1). Environmental trigger factors, including exposure to cold and to vibrating tools, make a well-recognized contribution to the clinical presentation. However, the extent to which the environment explains an individual's underlying susceptibility to the condition is unclear. Climatic variation, for example, does not fully explain the variation in RP prevalence between different populations (1). Indeed, few specific environmental risk factors have been identified despite numerous population-based studies. A contribution from genetic factors has been suggested by the identification of pedigrees with multiple members affected by RP (2) and by reports of an increased risk among first-degree relatives of patients (3). However, these observations may equally be explained by a contribution from the shared family environment.

We report the results of a study examining the occurrence of RP among a sample of female twins enrolled in the national TwinsUK Registry. The twin study design allows the separation of the contribution of genetic factors from those of the shared family environment. In common with other epidemiologic studies of RP, our assessment was based on individual respondents' recall of their symptom history and the presence or absence of RP was classified using standard criteria (4). In addition to this questionnaire-based assessment, we extended our evaluation by conducting a cold challenge test in a sample of respondents. In the past, cold challenge testing in RP has been confined to small studies of patients and required equipment impractical for use in a population setting. In developing the present study we validated a simple cold challenge protocol using a portable radiometer and demonstrated that it could be deployed reliably in the large-scale assessment of patients with RP (5). These thermographic measurements provide an additional objective assessment of the genetic and environmental contribution to the physiologic basis of RP that is less prone to potential bias than assessment based on recall alone.

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Subjects and Methods

Design. The study followed a 2-stage protocol: 1) an initial screening questionnaire was sent to a sample of adult women enrolled in the St Thomas TwinsUK Adult Twin Registry and 2) a subset of respondents, weighted in favor of RP-affected pairs, were invited to undergo clinical assessment by interview and cold challenge test.

Questionnaire sample. The initial sample was drawn from subjects enrolled in the St Thomas UK Register. Further details are available at www.twinsuk.ac.uk. This cohort of monozygotic (MZ) and dizygotic (DZ) twins was assembled through successive media campaigns recruiting healthy twin volunteers who had agreed to take part in medical research. For historic reasons, most of the twins were female. Their zygosity was ascertained by a standard questionnaire and in cases of uncertainty was confirmed by multiplex DNA fingerprinting.

Questionnaires were sent to 3,652 female twins between ages 30 and 60 years comprising 911 MZ and 915 DZ twin pairs. Stratified sampling was used to ensure an MZ-to-DZ ratio of 1:1 and equal proportions of subjects in 10-year age groups. The questionnaire did not indicate the primary objective of the study. RP screening questions were included in a larger set of questions asking about lifestyle and other health issues.

The questions that related to RP were designed to allow classification using standard clinical criteria (4). Twins were asked 1) whether their fingers were unusually sensitive to the cold; 2) whether their fingers sometimes showed

unusual color changes; 3) whether, if their fingers showed unusual color changes, these colors included white, blue, purple, or red; and 4) whether they experienced associated pain or numbness. A history of ≥2 color changes including white was considered positive for RP. Those that experienced pain and numbness were classified as having severe RP. Those who reported cold sensitivity without color changes, or color changes that did not include white, were classified as being cold sensitive.

Clinical assessment and cold challenge test. A sample of respondents, comprising 288 twin pairs (129 MZ and 159 DZ), were invited to undergo clinical assessment and cold challenge testing. The subset was weighted to balance the proportions of MZs and DZs among 3 groups of twin pairs: those that were 1) concordant for the presence of RP, 2) discordant for RP, and 3) concordant for the absence of RP. Because twin pairs in which 1 member reported RP (groups 1 and 2) would be most informative for analyses, a greater proportion of these pairs were invited for clinical assessment.

A study nurse interviewed twin pairs attending for assessment. The assessment included a reevaluation of their symptoms using the original set of questions. Subjects reporting color changes were asked to confirm these using standard color charts (6). Rheumatic symptoms were recorded, and blood samples were obtained for rheumatoid factor and antinuclear antibody. All attendees underwent a 15°C, 60-second cold challenge test following a standardized protocol (7).

Statistical analysis. *Twin studies.* An individual's phenotype is the result of the effects of both genotype and environment. To study the source of individual differences (i.e., the variance) in a phenotype, genetically related subjects are required. MZ twins share the same genetic makeup and DZ twins share on average 50% of their segregating genes. It is assumed that both types of twins have been exposed to the same shared environments, so any greater similarity between MZ twins than DZ twins is due to genetic influences.

Concordance. Casewise concordance for RP was determined. This is the probability of the co-twin of an affected twin reporting RP. Under complete ascertainment, casewise concordance is calculated from the formula 2C/(2C + D), where C is the number of concordant pairs and D is the number of discordant pairs. Greater than expected concordance indicates familial occurrence, whereas an excess of MZ compared with DZ concordance indicates the familial occurrence is mediated by genetic factors (8).

Genetic modeling. Heritability is a measure of the proportion of variation in a trait that is attributable to genetic variation and was estimated through maximum likelihood structural equation modeling (9,10). This approach assumes that the presence of RP is determined by a normally distributed underlying liability that leads to expression of the trait when it exceeds a certain threshold value. For dichotomous traits, the correlation in liability among twins can be estimated from the frequencies of concordant and discordant pairs. Comparison of the observed correlation in liability between MZ and DZ twins allows separation of the variance into shared additive genetic factors (A; a correlation of 1 in MZ twins and 0.5 in DZ twins), dominance genetic factors (D; correlation of 1 in MZ twins and 0.25 in DZ twins), the shared environment (C; correlation of 1 in both MZ and DZ twins), and the unique environment (E; uncorrelated in MZ and DZ twins). By sequentially removing these variance components in a stepwise manner from the full model, the significance and size of their contribution to the variation can be assessed and the deterioration in the fit of each submodel can be tested by hierarchical chi-square tests. This process leads to a model that explains the variance with as few variables as possible (the best-fitting model). Preliminary analysis was carried out in Stata software (11) and modeling was performed using Mx software (12).

Analysis of the cold challenge data included 3 measurements: baseline skin temperature, temperature after cold immersion, and the rewarming rate (the difference in temperature immediately after cold immersion and at 10 minutes). The relative contribution of the variance components A, C, D, and E was estimated using DeFries-Fulker regression (13) weighted to take account of the sampling proportions of the pairs.

Results

Of the 3,652 individuals who received questionnaires, 3,043 individuals (83%) responded. These included 702 MZ pairs (77%) and 727 DZ pairs (79%). Three twins were

Table 1. Characteristics of the sample*

	MZ n = 1,400 (700 pairs)	DZ n = 1,452 (726 pairs)
Age, median (range) years	48 (30–60)	49 (30–60)
Body mass index, mean (range) kg/m^2	25.5 (16.4–48.3)	25.6 (15.1–49.8)
Alcohol consumption, mean (range) units/week	5.7 (0–93)	5.9 (0–80)
Ever smoked, %	45	49
Cold sensitive, no. (%)	477 (34)	512 (35)
Classified as RP positive, no. (%)	142 (10)	174 (12)
Classified as severe RP, no. (%)	132 (9)	160 (11)

* MZ = monozygotic (identical) twin pairs; DZ = dizygotic (nonidentical) twin pairs; RP = Raynaud's phenomenon.

Table 2. Concordance and heritability of cold sensitivity, RP, and severe RP*

	MZ pairs (n = 700)			DZ pairs (n = 726)			Best fitting model	h^2 (95% CI)
	C(+/+)	D(+/−)	Cc %	C(+/+)	D(+/−)	Cc %		
Cold sensitivity	140	197	59	105	302	41	AE	53 (42–64)
RP	26	90	37	17	140	20	AE	55 (40–70)
Severe RP	23	86	35	14	132	18	AE	53 (38–68)

* RP = Raynaud's phenomenon; MZ = monozygotic (identical) twin pairs; DZ = dizygotic (nonidentical) twin pairs; 95% CI = 95% confidence interval; h^2 = heritability estimate; C(+/+) = concordant RP positive pairs; D(+/−) = discordant RP pairs; Cc = casewise concordance calculated using the formula Cc = 2C/(2C + D); AE represents best fitting model where A = additive genetic and E = unique environment components of variation.

identified in the sample as having autoantibodies suggestive of rheumatic disease (rheumatoid factor in 2 subjects, antinuclear antibody in 1). These twins and their co-twins were excluded from subsequent analyses. The characteristics of the final sample of 700 MZ and 726 DZ pairs are shown in Table 1. The 2 zygosity groups were matched for age, reported body mass index, smoking history, and alcohol history. They also reported a similar prevalence of cold sensitivity, RP, and severe RP (Table 1). The majority of subjects classified as having RP reported symptoms suggestive of severe disease.

Cold sensitivity, RP, and severe RP all showed a greater concordance among MZ twins than DZ twins, indicating a genetic contribution (Table 2). Variance components analysis indicated a heritability of 53%, 55%, and 53% for cold sensitivity, RP, and severe RP, respectively. A potential contribution from the shared environment was rejected for all 3 traits.

A total of 288 pairs (129 MZ and 159 DZ) underwent clinical assessment (Table 3). All pairs were selected at random from the respondents and none showed significant differences compared with the original sample (based on age, body mass index, smoking, and alcohol use). When subjects underwent clinical assessment the agreement with original questionnaire responses was fair: kappa statistics for cold sensitivity, primary RP, and severe primary RP were 0.64, 0.46, and 0.47, respectively. When attending for the clinical assessment, twins were no longer blinded to the study's hypothesis. Subjective data from the clinical assessments, therefore, were not used to alter the original disease status classification.

The results of cold challenge testing demonstrated that MZ and DZ twins had similar mean values of baseline temperature, drop in temperature after immersion, and rate of rewarming (Table 3). For all 3 measures, a greater correlation in response was seen among MZ twins compared with DZ twins. Variance components analysis showed a significant contribution from additive genetic factors of 65% for baseline skin temperature, 35% for temperature drop after immersion, and 24% for rewarming rate.

Discussion

RP is common and may affect as many as 1 in 4 adults (1). There is a well-documented association with autoimmune rheumatic disease developing before or after the onset of RP; other recognized causes of secondary RP include thoracic outlet syndrome, paraproteinemias, drugs, and chemicals (14). RP is also associated with a range of vascular conditions that have significant morbidity including stroke, migraine, and coronary artery disease. The etiology of RP remains poorly understood, although an underlying vascular defect has been proposed (15). It may be that a functional imbalance in vasoconstrictors and vasodilators observed in RP (14) is widespread, influencing a number of vascular beds.

Increased susceptibility to RP among relatives of affected probands has been demonstrated in population studies, suggesting a role for genetic factors. However, these studies have relied on probands' recalled account of symptoms among their relatives and may be prone to bias. Only one study has evaluated RP directly among relatives (3). To date, no RP study has distinguished the influence of genetic factors from that of the shared family environment.

By demonstrating a higher concordance in MZ twins compared with DZ twins, our data show conclusively that RP has a genetic basis, with a doubling of risk of RP in first-degree relatives of affected individuals. This justifies the search for disease susceptibility genes. To date only 2

Table 3. Results of thermographic testing and heritability in twin subjects attending for clinical assessment (cold challenge)*

	MZ (129 pairs)	DZ (159 pairs)	Model	h^2 (95% CI)
Sample size, no./total no.				
C(+/+)	19/26	9/17		
D(+/−)	49/90	69/140		
C(−/−)	61/584	81/569		
Baseline, °C	29.4 ± 3.4 (20.3–35.3)	29.6 ± 3.2 (20.4–35.1)	AE	65 (52–79)
Drop, °C	6.7 ± 2.0 (0–11)	7.0 ± 1.8 (1.2–12.8)	AE	35 (13–57)
Rewarm, °C	4.9 ± 3.5 (−1–12)	5.2 ± 3.6 (−1.6–12.5)	AE	24 (5–43)

* Values are the mean ± SD (range) unless otherwise indicated. C(+/+) = concordant trait positive pairs; D(+/−) = discordant pairs; C(−/−) = concordant trait negative pairs; see Table 2 for additional definitions.

such studies have been published, providing limited insight. A genome scan of 6 multicase families showed suggestive linkage at 3 chromosome areas (2). The result of a candidate gene study of 4 vasoactive genes was negative, but the study lacked power (n = 95 cases) (16). Both studies used clinical disease definitions based on subject recall. As yet, no genetic study has included physical responsiveness to cold in characterizing the RP phenotype.

In assessing our findings, a number of methodologic issues need to be considered. Our study was based on women and the findings relate more to primary RP than the secondary form of the condition. The twin study design itself is often criticized because of a potential lack of representativeness of twin samples and potential bias arising from unequal sharing of the common environment in MZ twins compared with DZ twins. These potential biases are likely to have minimal impact in this study because subjects enrolled in the TwinsUK Registry are representative of the UK population with respect to the frequency of common traits and diseases, as well as lifestyle factors (17). None of the twins included in the present study lived together and the differences in environmental sharing have been shown to be minimal (18). We have not found evidence of differences in recall between MZ and DZ twins for past events that might bias results.

One particular difficulty in studying RP is the lack of a gold standard in disease definition. The majority (67%) of those initially classified with severe RP retained the classification after use of the color chart at the interview. While we found good agreement between color charts and clinical criteria at the interview (83% agreement), it was striking that RP symptoms were elicited more frequently at the interview than they were on initial questionnaire. We believe this resulted from the unblinding of twins at the clinical assessment and it highlights the susceptibility of survey data to biased recall. In the present study, responses to the initial questionnaire were used to determine RP prevalence and heritability because they were thought to be a more reliable indicator of disease status.

Unravelling the genetic basis of complex diseases such as RP presents well-recognized challenges. Our observation that the cold challenge response is under partial genetic control provides strong corroboration of the evidence from the subjective data of a heritable basis for RP. Although not designed to provoke an attack of RP, the cold challenge responses can be taken to reflect the physiologic process giving rise to symptoms. We have previously demonstrated that baseline skin temperature, fall in temperature, and rewarming rate are highly correlated and all are associated with the report of RP (7). Demonstrating that these variables are heritable provides further rationale for the use of objective measures to investigate the mechanisms underlying the disease.

RP is associated with other vasospastic conditions such as migraine and hypertension, and we have demonstrated in the same twin population that a shared genetically determined mechanism appears to account for some of this association (19). Understanding the molecular genetic basis of RP and the physiologic processes that determine the wide range of normal peripheral vascular responsiveness may provide useful insight into a range of important pathologic conditions.

AUTHOR CONTRIBUTIONS

Drs. Cherkas and MacGregor had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Cherkas, Carter, Howell, Black, Spector, MacGregor.

Acquisition of data. Cherkas, Carter, MacGregor.

Analysis and interpretation of data. Cherkas, Spector, MacGregor.

Manuscript preparation. Cherkas, Williams, Black, Spector, MacGregor.

Statistical analysis. Cherkas, Williams, MacGregor.

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