Genetic Covariation of Pelvic Organ and Elbow Mobility in Twins and their Sisters

Narelle K. Hansell[1], Hans Peter Dietz[2], Susan A. Treloar[1], Barton Clarke[3], and Nicholas G. Martin[1]

[1] Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia
[2] Department of Urogynaecology, Royal Prince Alfred Hospital, Sydney, Australia
[3] Betty Byrne Henderson Centre, Royal Women's Hospital, Brisbane, Australia

A range of environmental risk factors, with childbirth the most notable, have been associated with the development of pelvic organ prolapse and urinary incontinence. However, indications of genetic influence (positive family histories, ethnic differences) have prompted research into the heritability of measures of pelvic organ descent and joint mobility, which have also been associated with prolapse and incontinence. Genes appear to influence about half of the variation in these measures and, furthermore, the pelvic organ measures are associated with elbow hyperextension at a phenotypic level ($r \approx .2$). We examined these measures in young, nulligravid women to determine if their association is due to a common genetic source. Data were collected from 178 Caucasian female co-twins and non-twin sisters, 50 of whom returned to be retested, which allowed reliability to be estimated and unreliable variance to be isolated in the multivariate analyses. Structural equation modeling was used to estimate genetic associations between latent elbow and bladder mobility factors for which heritabilities were estimated to be 0.80 and 0.64 respectively. The association between these factors appeared to be mediated by common genes (genetic $r = .48$, non-shared environmental $r = -.06$), with genes influencing latent elbow mobility accounting for 14% of the variation in latent bladder mobility. We speculate that genes influencing connective tissue structure may underlie this association.

Pelvic organ disorders, such as prolapse and urinary incontinence, are common among women. They have a substantial impact on women's health and have been associated with a lifetime risk of surgery of over 10% (Olsen et al., 1997). Previous studies have identified a range of risk factors relating to pregnancy and childbirth and to lifestyle factors such as obesity, constipation, smoking and heavy lifting (Swift & Theofrastous, 2001). In addition, positive family histories for genital prolapse (Rinne & Kirkinen, 1999) and ethnic differences in pelvic organ mobility (Dietz, 2003) point to genetic predeterminants. Analyses in our laboratory have found that approximately half of the individual variation in measures of pelvic organ descent (i.e., bladder neck and urethral mobility) was due to the influence of additive genes (Dietz et al., submitted for publication). Furthermore, we found that mobility in the elbow joint was associated, to a small degree (phenotypic $r = .19–.23$), with the pelvic organ measures of mobility. In order to gain some insight into the role of genes influencing pelvic organ descent and, possibly, pelvic organ disorders, we aimed in the present study to examine the association between measures of elbow and pelvic organ mobility to determine whether the relationship was due to a common genetic source.

Previous studies have found elbow hyperextension to be associated with an increased risk of postnatal urinary incontinence (Tincello et al., 2002) and a general measure of joint hypermobility to be associated with a higher prevalence of genital prolapse (Norton et al., 1995). These associations may reflect aspects of collagen metabolism, as metabolic changes have been associated with both prolapse (Jackson et al., 1996) and incontinence (Falconer et al., 1998). Furthermore, connective tissue strength may contribute to variability in measures of both joint and pelvic organ mobility, and genetic factors influencing connective tissue strength may be a source of covariation between these measures. We hypothesized that a common genetic source would be found to influence measures of both pelvic organ and joint mobility.

Method

Participants

Data were collected from 86 families, with 178 nulliparous, Caucasian females aged from 17.9 years to

24.9 years (M = 20.4, SD = 1.6) participating. Data from same sex twin pairs (46 monozygotic [MZ], 24 dizygotic [DZ]) were collected from 70 families and from the female co-twin in an opposite sex pair from a further 14 families in which there was an additional non-twin sister. In total, data were collected from 24 non-twin sisters (for 2 families, data were collected from a non-twin sister only). To allow the assessment of reliability, 50 individuals returned to be retested (17 MZ pairs, 7 DZ pairs, and a non-twin sister pair). The test–retest interval ranged from 32 to 122 days (M = 46.7, SD = 19.8). Participants had initially been recruited through mail-outs to secondary schools in the Brisbane region to participate in genetic studies of melanocytic naevi (moles; Zhu et al., 1999) and cognitive function (Wright et al., 2001). They received AUD$100 for their participation in the present study. Ethics approval from the Queensland Institute of Medical Research Human Research Ethics Committee and written, informed consent were obtained.

Zygosity was determined using the commercial kit (AmpFlSTR Profiler Plus Amplification Kit, ABI) which comprises 10 independent DNA markers (nine short tandem repeat [STR] loci D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820 and one homologous region on the X and Y chromosomes, amelogenin, which confirms gender). Same sex twin pairs were checked for concordance across the nine STR loci. These results were cross-checked with blood group results for the ABO, MNS, and Rh systems (Australian Red Cross Blood Service, Brisbane) and/or phenotypic data (hair, skin, and eye color), giving an overall probability of correct zygosity assignment of greater than 99.9%.

Measures of bladder neck mobility and urethral rotation were assessed by translabial ultrasound using a Toshiba Eccocee system with 5 Mhz curved array transducer. The examination was performed with participants supine and after bladder emptying. Examinations were performed by or under the direct supervision of the second author, or by staff trained by him for at least 100 separate assessments. The difference between measurements collected at rest and on the best of three Valsalva maneuvers were calculated as bladder neck descent in the craniocaudal plane, as proximal urethral rotation, and as total or oblique bladder neck descent in a cranioventral to dorsocaudal direction. For a more detailed description of the methodology used, see Dietz et al. (in press). Measures of bladder neck and urethral mobility were used as they are the most easily measurable indices of pelvic organ descent.

Joint mobility was assessed in the dominant limb and assessment was based on the Beighton method (Beighton et al., 1973). Joint angles were obtained using a goniometer for (a) passive dorsiflexion of the little finger at the metacarpophalangeal joint, (b) passive opposition of the thumb to the flexor aspect of the forearm, and (c) hyperextension of the elbow joint. Only elbow hyperextension was examined in the present article, as preliminary analyses found it to be the only joint mobility measure to correlate with our measures of pelvic organ descent.

Statistical Analyses

As estimates of test–retest reliability, intraclass correlations between test and retest data were computed in SPSS for Windows Version 11.5 (SPSS Inc., 1989–2002). All further analyses were run using the structural equation modeling software package Mx (Neale et al., 1999). As an initial exploration of the causes of variation, means and variances were examined for birth order, zygosity, and age effects using the method of maximum likelihood estimation from raw data observations (Eaves et al., 1978). An initial fully-saturated model, in which means and variances were free to vary and which included regression coefficients for age and age^2, was compared to successively more constrained models by likelihood ratio tests. Means, and then variances, were set equal for first- and second-born co-twins and then set equal for MZ and DZ pairs (a more detailed explanation of the procedure can be obtained elsewhere, McGregor et al., 1999). In addition, means and variances for non-twin sisters were compared to those for twins. To test for quadratic age effects on each variable, the regression coefficient for age^2 was dropped from the model. If this resulted in a loss of fit, the coefficient was retained. However, if there was no loss of fit, the regression coefficient for age was also dropped in order to test for linear age effects.

Twin correlations were computed for MZ pairs, DZ pairs, and twin/non-twin sister pairings. To test the validity of including sibling data, a model in which twin/non-twin sister correlations were set to equal the DZ twin correlations was compared to a model in which twin/non-twin sister correlations were free to vary from the DZ correlations. If no loss of fit was indicated by the likelihood ratio χ^2 test, DZ and twin/non-twin sister correlations were set equal in subsequent analyses.

An extended common pathway model (Neale & Cardon, 1992) was employed to examine sources of influence at a multivariate level (Figure 1). Retest data were included in analyses in order to differentiate reliable and unreliable variance, based on the assumption that variance common to test and retest data represents reliable variance and variance not common to test and retest data represents measurement error. To identify variance common to test and retest data, pathways from the latent factors were set equal for test and retest data for each variable, and based on the assumption that total variance for each variable was the same at test and retest, unreliable variance (U) was set equal at test and retest. Two latent factors were modeled: first, elbow mobility, which influenced test and retest data for elbow hyperextension and, second, bladder mobility, which influenced test and retest data for bladder neck descent, oblique
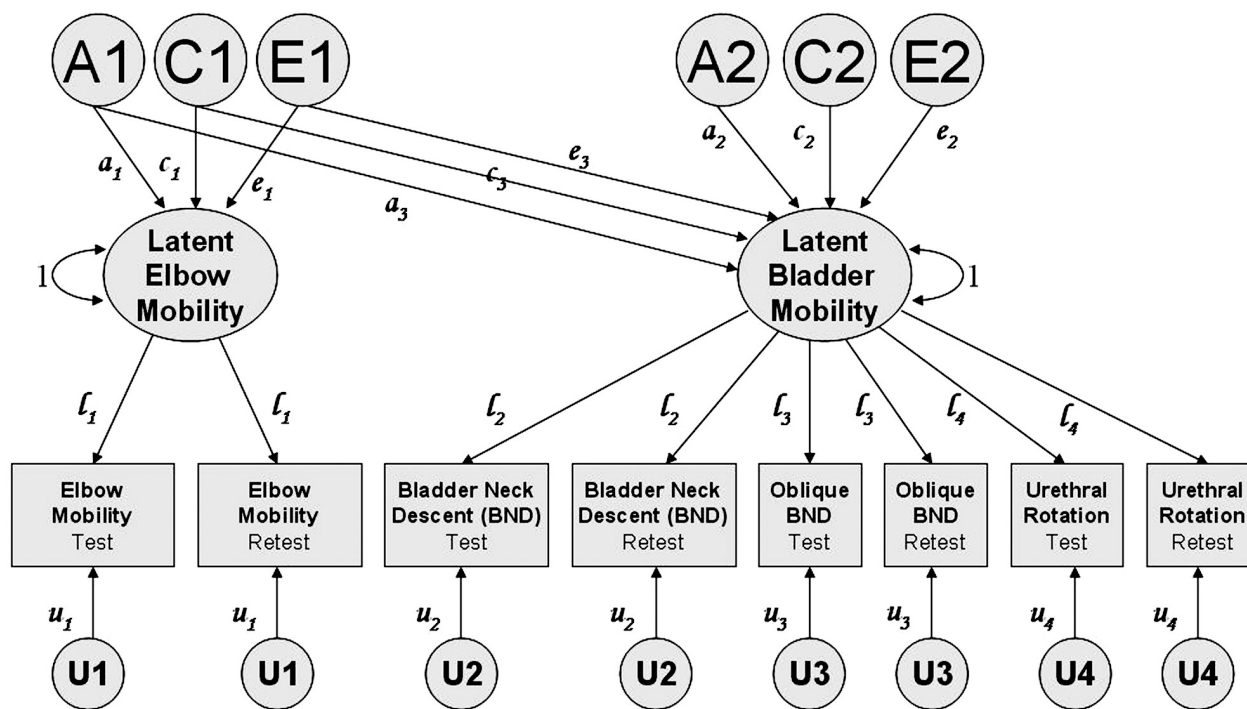
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Figure 1

Extended common pathway model of additive genetic (A), common environmental (C), and non-shared environmental (E) sources of influence, plus measurement error or unreliable variance (U), for measures of elbow and bladder mobility. Variance for the latent variables was constrained to 1. For each measure, reliable influences ($l_1 \ldots l_4$) and unreliable influences ($u_1 \ldots u_4$) were set equal for test and retest data. Co-twin correlations were set at 1 for MZ pairs and .5 for DZ pairs for A factors and 1 for both MZ and DZ pairs for C factors.

bladder neck descent, and urethral rotation. The variance for each latent factor was constrained to 1 and was decomposed into additive genetic (A), common environmental (C) and non-shared environmental (E) sources of influence by latent bivariate Cholesky decomposition. Nested models containing AE, CE, or only E sources of influence were compared to the fully-saturated ACE model by likelihood ratio χ^2 tests to determine the best-fitting model. Similar modeling based on split-half reliability has been reported by van Beijsterveldt and colleagues (2001), and in this laboratory, a similar approach based on independent pathway modeling has been used (Hansell et al., 2001).

Univariate and multivariate outlying families were identified using the %P option in Mx, which provided a likelihood statistic for each family conditional on the genetic model. Individual and family data were dropped if their z score value was greater than ±3 and the analyses were re-run. Data lost due to outliers accounted for less than 3% of the dataset. All data were standardized to mean of 0 and variance of 1 $N(0, 1)$ to facilitate comparisons of variance.

Results

Bladder neck descent and oblique bladder neck descent were near normally distributed and no transformations were required. In addition, elbow

hyperextension, which showed evidence of kurtosis but not skewness, was not transformed (kurtosis may be reduced through the use of a more stringent method of measurement, such as that suggested by Dijkstra and colleagues, 1994). A moderate positive skew was found for urethral rotation and this was corrected through square root transformation (Tabachnick & Fidell, 1989).

Test–Retest Reliability and Homogeneity of Means and Variances

Test–retest reliability was moderately high for the measures of pelvic organ mobility, with intraclass correlations ranging from .75 to .77 (Table 1), but was lower for elbow mobility ($r = .49$). Reliability of the elbow mobility measure may be improved by using a more rigidly standardized measurement protocol (for example, see Dijkstra et al., 1994).

Among the twin pairs, no significant differences in means and variances were identified for either birth order ($\Delta\chi^2_2$ ranged 0.5–4.5, critical value = 5.99) or zygosity ($\Delta\chi^2_1$ ranged 0.0–2.7, critical value = 3.84). Furthermore, all means and variances for siblings could be set equal to those of twins ($\Delta\chi^2_1$ ranged 0.0–3.2, critical value = 3.84). Quadratic age effects, as shown in Figure 2, were found to be associated with oblique bladder neck descent ($\Delta\chi^2_1 = 6.0$, $p = .01$) and urethral rotation ($\Delta\chi^2_1 = 6.7$, $p = .01$) and a linear age effect was found for bladder neck descent

Table 1

Intraclass Test–Retest Correlations and Maximum Likelihood Monozygotic (MZ), Dizygotic (DZ), Sibling/Co-twin, and Pooled Dizygotic and Sibling/Co-twin Correlations (95% Confidence Intervals) for Bladder Neck Descent (BND), Oblique Bladder Neck Descent (OBND), Urethral Rotation (URot), and Elbow Hyperextension (Elbow)

	Test–Retest Reliability*	MZr (44–46 pairs)	DZr (22–24 pairs)	Sib/Twin$r$ (21–28 pairings)	Pooled DZ & Sib/Twin$r$
BND	.77 (.63, .86)	.47 (.23, .65)	.48 (.07, .71)	.36 (−.08, .63)	.42 (.14, .62)
OBND	.76 (.61, .86)	.61 (.40, .75)	.15 (−.20, .46)	.25 (−.25, .55)	.18 (−.10, .44)
URot	.75 (.60, .85)	.45 (.21, .63)	.05 (−.34, .41)	.12 (−.35, .49)	.07 (−.22, .37)
Elbow	.49 (.24, .67)	.57 (.33, .72)	.17 (−.31, .53)	.29 (−.15, .60)	.23 (−.08, .48)

Note: *No allowance was made for non-independence of twins/siblings.
  Twin and twin/sibling correlations are based on a model with a single mean and a single variance.

($\Delta\chi^2_1$ = 4.3, p = .04). Consequently, means were adjusted for the effects of age in all Mx modeling, including the comparisons of means.

Twin Correlations

Correlations were examined for MZ co-twins, DZ co-twins, and for non-twin sister/twin pairings (Table 1). Non-twin sister/twin correlations could be set equal to those of DZ co-twins ($\Delta\chi^2_1$ ranged 0.0–0.2, critical value = 3.84), thus justifying the inclusion of sibling data in the analyses. There was a suggestion of genetic influence on all variables indicated by lower DZ and/or sister/twin correlations than MZ correlations. While the pattern of MZ and DZ correlations appeared to differ for bladder neck descent and oblique bladder neck descent (higher DZ compared to MZ correlations for bladder neck descent than oblique bladder neck descent), it should be noted that confidence intervals for the correlation estimates were broad, indicating a lack of power. In addition, these variables had a high phenotypic correlation (.87) suggesting that they were largely influenced by the same factors. A close examination of the data indicated that the high DZ correlation for bladder neck descent was largely due to the influence of just two DZ pairs.

Thus it appeared that sampling error may be masking genetic effects for this variable.

Genetic Modeling

All variables were examined in an extended common pathway model, as shown in Figure 1, that allowed for the influence of additive genetic (A), common environmental (C), and non-shared environmental factors (E) and measurement error (U). The fit of AE, CE, and E models was compared to that of the fully-saturated ACE model. Familial influence was significant, as a model allowing for only E influences offered a significantly worse fit to the data than the ACE model ($\Delta\chi^2_6$ = 37.5, p < .001). Neither the AE nor the CE model differed significantly in fit to the data compared to the ACE model ($\Delta\chi^2_3$ = 0.3 and 4.1 respectively). However, Akaike's Information Criterion (AIC), which combines the goodness-of-fit χ^2 of a model with its degrees of freedom to indicate parsimony, suggests that the AE model may be a better fit to the data than the CE model (AIC = −5.7 for the AE model and −2.0 for the CE model). On this basis, only the AE model was examined further.

Phenotypic correlation estimates indicated strong common influences among the measures of bladder mobility (r ranged .70–.82) and moderately low
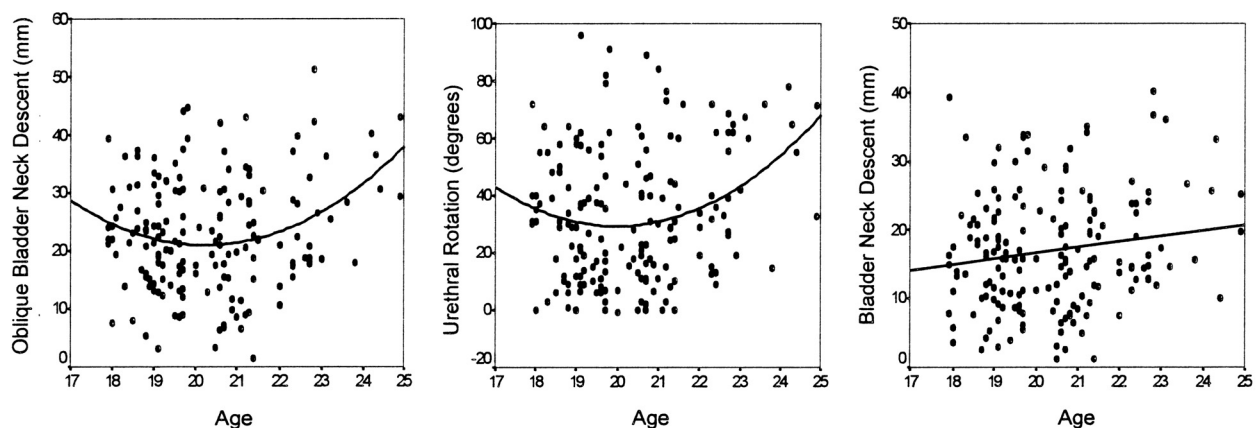
Figure 2

Scatterplots for Age × Oblique Bladder Neck Descent, Age × Urethral Rotation and Age × Bladder Neck Descent with quadratic regression curves fitted for Age × Oblique Bladder Neck Descent and Age × Urethral Rotation and a linear regression line fitted for Age × Bladder Neck Descent.

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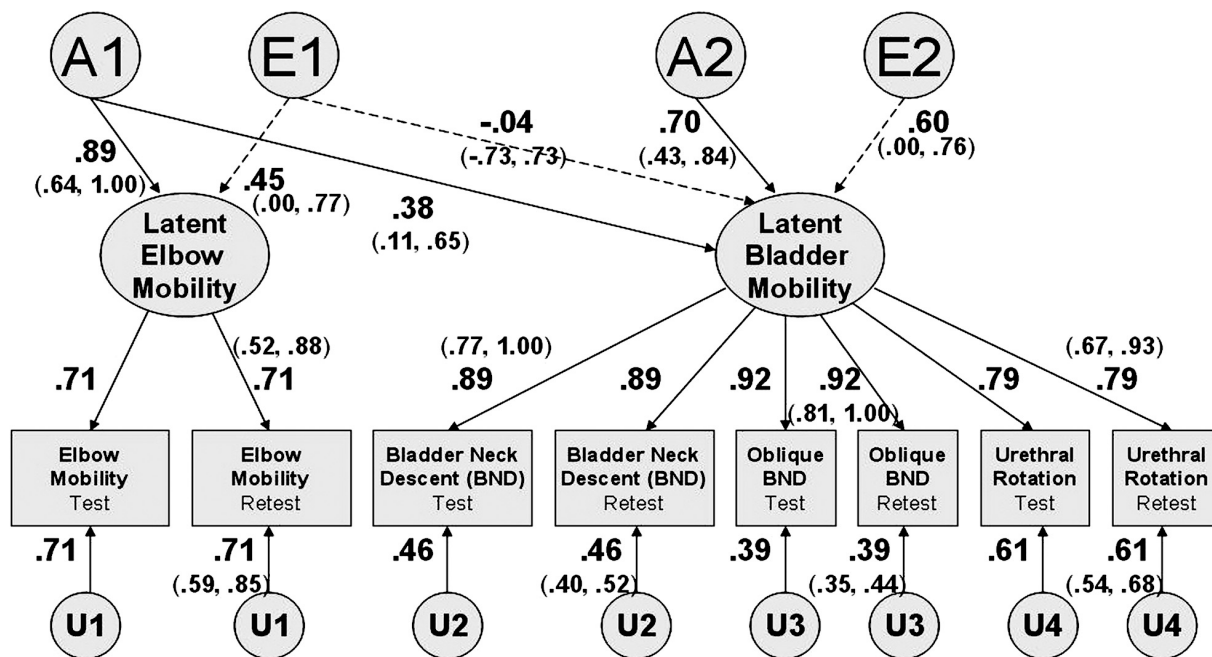
Figure 3

Common pathway modeling of additive genetic (A) and non-shared environmental (E) factors and measurement error (unreliable influence [U]) on measures of elbow mobility and bladder mobility (bladder neck descent, oblique bladder neck descent, urethral rotation) recorded at test and retest. Parameters are standardized and confidence intervals (95%) are shown in brackets. Nonsignificant pathways are shown as dashed lines.

correlations between elbow mobility and the bladder mobility measures (r ranged .18–.21). Genetic and environmental correlations show that an association between the latent factors is mediated by genes (r_g = .48, r_e = −.06). These associations are reflected in the path diagram (Figure 3) — the measures of bladder mobility load strongly onto a single latent factor and the two latent factors appear to be influenced by common genes.

Latent factors represent the reliable variance for elbow and bladder mobility. Heritability was estimated to be 0.80 (95% CIs = 0.41–1.00) for latent elbow mobility and 0.64 (CIs = 0.43–0.78) for latent bladder mobility. Genes influencing elbow mobility appeared to account for approximately 14% of the variance in bladder mobility, while non-shared environmental influences appeared to be specific to each latent factor (note that non-shared environmental influences are not significant based on confidence intervals). Latent factor loadings on the test and retest measures reflect measurement reliability, which was moderate for elbow mobility, but higher for the pelvic organ measures. Unreliability estimates indicate that 50% of the variance of elbow mobility was due to measurement error. Lower estimates were found for bladder neck descent (21%), oblique bladder neck descent (15%), and urethral rotation (37%).

Discussion

The central finding of this study is that the influence of common genes may underlie small phenotypic associations among measures of elbow mobility and pelvic organ descent in nulliparous young women. Specifically, genes influencing a latent elbow mobility factor were found to account for 14% of the variance in a latent bladder mobility factor. After removing variance due to measurement error, heritabilities of .80 and .64 were found for elbow and bladder mobility respectively. As measures of pelvic organ and joint mobility have previously been associated with disorders such as genital prolapse and incontinence (Dietz et al., 2002; Norton et al., 1995; Tincello et al., 2002), the present study supports the proposal that genetic influence may play a role in these disorders. Furthermore, the genetic association found between elbow and bladder mobility latent factors suggests the possibility of a genetically influenced mechanism, common to both pelvic organ and elbow mobility, which may play a small role in predisposing individuals to developing genital prolapse and incontinence.

The proposal that genes influencing the biochemical composition of collagen may be influencing pelvic organ mobility is biologically plausible. In biochemical studies, collagen characteristics have been found to differ in women afflicted with pelvic organ disorders compared with those not so afflicted (Falconer et al., 1998; Jackson et al., 1996). However, work in this field is of a very preliminary nature and it is difficult to be certain that altered biochemical characteristics of prolapsed tissues are the cause rather than the effect of increased pelvic organ descent. Another confounding factor is the influence of childbirth.

Increased pelvic organ mobility has been shown to be associated with easier childbirth (Dietz et al., 2003) and less pelvic floor trauma (Dietz & Steensma, 2003). This implies that the relationship between pelvic organ descent in young nulliparous women and the incidence of pelvic organ prolapse and stress urinary incontinence may be much more complex. Nevertheless, this study clearly provides a basis for future molecular genetic work in this field.

Another finding of the study was that pelvic organ mobility varied with age, which ranged from 18 to 25 years in the sample examined. The general trend was for the bladder neck measures and urethral rotation to increase with age, even within this restricted range, perhaps reflecting the impact of hormonal factors. Modeling suggested that common environmental factors, that is, those shared within the family, were not influential. However, a limitation of the study was the lack of power to significantly differentiate sources of influence due to additive genes and common environment. Nevertheless, the twin correlations for oblique bladder neck descent, urethral rotation, and elbow hyperextension showed no evidence of common environmental influence, with dizygotic correlations being less than half the monozygotic correlations. This limitation could be overcome with a substantially larger sample size.

Measurement error was considerable for elbow hyperextension, accounting for half of the total variance and indicating the need for a more rigidly standardized measurement protocol. By including retest data in the analyses, variance due to measurement error was isolated and elbow mobility emerged as a highly heritable trait. The measures of bladder mobility were less affected by measurement error, particularly bladder neck mobility where measurement error accounted for only 15% to 21% of the total variance.

The present study is the first to examine genetic associations between measures of pelvic organ and elbow mobility. The results indicate that multivariate molecular genetic studies, which aim to identify quantitative trait loci (QTLs — locations on the chromosomes at which specific genes contribute to a quantitative trait) may provide important insights into the etiology of pelvic organ mobility. Genes related to collagen, elastin and fibrillin structure or metabolism (i.e., genes implicated in clinical connective tissue disorders such as Marfan's and Ehlers Danlos syndrome, Hutchinson et al., 2003; Schwarze, et al., 2000) may be appropriate candidate genes for ensuing association studies, which will examine the relationship between allelic variation and degree of mobility in the measures of interest.

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