2012

Advancing Our Understanding of the Inheritance and Transmission of Pectus Excavatum

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Original Article

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Running title: Inheritance of pectus excavatum

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Abstract. Pectus excavatum is the most common congenital chest wall abnormality expressed in children, yet its inheritance is poorly understood. Here we present the first comprehensive assessment of the inheritance of this disorder. After evaluating 48 pedigrees and 56 clinical traits of probands and family members, we find strong evidence of autosomal recessive, genetic control for this disorder. Additionally there is likely more than one disease-associated allele as well as a relatively large number of disease allele carriers in the human population. Some clinical traits appear important and may serve as reliable indicators for predicting the likelihood of PE in children before severe symptoms present. Quantifying sex-ratio bias in probands demonstrates a highly significant male bias associated with PE. When combined with pedigree data, sex-bias is indicative of sex-linked, sex-limited, and/or epigenetic control such as X-inactivation, reiterating a point made with pedigrees alone, which is that more than one mutation is likely responsible for this disorder.

Key words: disease, heritable, genetic, association study

1. Introduction

Pectus excavatum (PE) is the most common congenital chest wall malformation, occurring in ~1/400 infants and children, primarily male (1-3). The PE phenotype is expressed as an anterior chest cavity depression that results from rotation, displacement, and depression of the sternum. PE has previously been qualitatively described to some degree, yet despite a very high rate of occurrence, its heritability has been only cursorily evaluated (2). PE probands present with various clinical features also found with Marfan syndrome, another connective tissue disorder. Additionally, over one-quarter (28%) of Marfan syndrome probands express PE (4-5).

Multiple, independent mutations in the *FBN1* (15q21) gene are associated with a range of clinical manifestations of Marfan syndrome (5-6). While no single gene, or large-scale genomic studies have been conducted on the inheritance of PE, several genes, including *FBN2* (5q23-31), *COL2A1* (12q13), *ACAN* (15q26.1), *TGFβ* (19q13) and receptors (*TGFβr1* (9q22), *TGFβr2* (3q22), and *TGFβr3* (1p33)) are associated with connective tissue disorders and thus could be considered candidate loci for PE. Very recent work evaluating one family via genome-wide linkage analysis suggested partial genetic control of PE on chromosome 18, however no causative genes were identified (7).

Here we assess 48 pedigrees and a broad array of clinical traits (n=56) in more than 2,000 individuals over 100 of whom have PE. Visual inspection of probands provides evidence for multiple, conspicuous clinical features (*e.g.* tallness, thinness, light-eyes) that commonly manifest with PE. We have quantified data related to several clinical traits and added this to conventional pedigrees that we have constructed. We find that this trait-related information contributes to predicting the likelihood that an individual inherits PE. We present results from our analysis of a comprehensive data set to provide evidence that PE is an autosomal, recessive

trait or results from polygenic inheritance. We suggest chromosomal regions relevant for future genome wide association studies for PE-alleles. We show evidence for the Carter effect (8), a polygenic inheritance pattern with a susceptibility threshold that differs for the sexes and that results in a predictable sex-related transmission pattern (9, 10). We demonstrate evidence of strong male bias in probands. Finally, we estimate the frequency of PE alleles and of carriers in the human population. Results from our study may prove useful for determining the likelihood of development of this disorder in unborn children and for locating genes controlling PE.

2. Materials and methods

Pedigree construction, inheritance, and sex-ratio

We have constructed pedigrees (Figure 1) based upon detailed information obtained during medical examination of probands and from self-reported data of immediate family members of individuals with PE severe enough to warrant surgical correction. Eastern Virginia Medical School IRB approved questionnaires were used to obtain these data, which address filial history of PE and a broad array of clinical features (discussed below). The population assessed is of national origin, though is comprised primarily of Caucasian males from the mid-Atlantic. They are a subset of the national registry of individuals at the Children's Hospital of The King's Daughters seeking surgical intervention. Forty-eight family pedigrees are constructed and each is comprised of at least four, and sometimes five, generations (though little information was typically known for the eldest generation).

The likelihood that spontaneous mutation, or a particular inheritance pattern (here considered: X-linkage, Y-linkage, autosomal dominant, autosomal recessive or semi-dominant control, sex-linkage coupled with an autosomal modifier, or polygenic inheritance), best explains

each pedigree is evaluated. This conventional analysis excludes impossible scenarios but does not yield a single, inheritance pattern for each family.

Therefore we supplemented the pedigrees with data regarding genes that control PE-associated clinical traits onto our pedigrees and re-evaluated for most probable mode of inheritance. Persons with autosomal traits that might be linked to PE genes demonstrate potential heritability information not available in the first analysis (*e.g.* what previously appeared, perhaps erroneously, to be a spontaneous mutation may now present with parent carrier phenotypes) and a polygenic mode of inheritance is plausible. Hence, the Carter effect is examined by assessing the relative rate of transmission of PE from females with PE (compared to males) to progeny with the expectation that women transmit to their progeny at a higher rate if a polygenic threshold model fits these data. Six additional families are added for this analysis only, to increase the sample size of the smallest cells in the contingency tables. The two by two contingency table is presented for all children (with and without PE) that have one parent that expresses PE (Table 2). Data are then presented for this same group of children, partitioned by sex. A binary logistic regression was performed to assess the association between mothers or fathers that had PE and their progeny that did (versus did not) express the disorder. An odds ratio is presented with 95% confidence intervals and the p value for the associated Chi-square test.

Another table (Table 3) is presented to evaluate the association between the number of siblings expressing PE (versus not) when the sex of the affected individual is male versus female. The same analyses are presented. Analyses were conducted using SPSS 12.0 (IBM, NY, USA).

Finally, we evaluated sex ratio bias in individuals with PE. Pearson chi-square analysis was conducted on the male: female ratio observed in the pedigrees (11). Several cases of pectus carinatum (PC, a similar disorder to PE except here the chest wall is everted) were reported in

families with PE. Since the inheritance of PC is also not understood, we analyzed the sex ratio for PC with an exact binomial test for goodness of fit for deviation from 1:1 (12). Miscarriage data were also evaluated to determine whether a lethal allele might drive the sex ratio bias observed in PE (*e.g.* X_{PE} causes PE in males and $X_{PE}X_{PE}$ is lethal in females).

Quantification of clinical traits

Fifty-eight clinical traits were assessed for 56 families to identify the traits most frequently associated with PE. Traits generally fell into broad categories including cardiac function, musculo-skeletal system function, and behavior. Some specific traits included mitral valve prolapse, height, finger length, skin tone, myopia, ADHD and depression. Table 1 ranks the 10 most common traits for individuals with PE and compares this to the 10 most common traits for family members without PE.

Using NCBI human genome data (13) to identify chromosomal locations of genes controlling the common traits assayed, we created a trait-related genomic map (Figure 2), allowing us to collect data on specific chromosomes that might be more likely to carry PE-related genes if linkage exists between these clinical trait genes and PE genes.

'Tall' and 'thin' are clinical traits commonly associated with PE but only qualitative data was included in our database regarding these. Therefore, we obtained independent quantitative data on these traits in individuals with PE from the thread found at the website dedicated to PE where individuals communicate regarding their condition (14). This thread included 179 self-reported responses to the Oct 6, 2004 query 'How tall/thin are you PE people?' We performed chi-square tests to compare average height and weight between individuals with PE and the U.S. adult population.

Estimating the frequency of heterozygotes

If PE arises from autosomal recessive mutations like many other human diseases then the expected frequency of heterozygous carriers can be calculated, assuming Hardy-Weinberg equilibrium. We perform this calculation using the phenotypic expression range found in the literature (1/400-1/1,000 individuals) for PE. We address single- versus multiple-gene involvement in PE and the effect on this calculation.

3. Results

Pedigree construction, PE-inheritance, and sex-ratio

A total of 2,147 individuals were evaluated from 56 families, wherein 116 individuals present with PE. Cumulatively, inheritance patterns across families reveal the likelihood that more than one PE-associated allele, and possibly an epigenetic effect are important in the heritability of this disorder.

As predicted, the conventional method of pedigree analysis is not an especially powerful technique with these data because of the small amount of information available on the pedigrees (e.g. one or a few individuals with PE). Thus, multiple inheritance patterns are possible explanations for disease for most families. When we test whether spontaneous mutation fits as an explanation for PE in each family, we find that it cannot be excluded as a possibility for 17 families (and it is a poor fit for 26 families). For five additional cases where PC is also present in the family, spontaneous mutation would explain PE only if PC is genetically unrelated.

When we test each pedigree for autosomal control, we find that this could explain the inheritance of PE in all families. However a standard null model for autosomal disorders is that about one-half of cases will be male and one-half female. Our data prove inconsistent with the 50:50 sex ratio given the large male-bias observed with PE. Autosomal recessive transmission here also requires multiple marriages to heterozygous individuals in many families, or an

163 epigenetic effect, or sex-limited expression. This fact spurred our Hardy-Weinberg calculation so
164 that we could evaluate the expected PE carrier rate.

165 Alternatively, sex-linked expression combined with an autosomal modifier describes the
166 pedigrees fairly well: X-linkage (or sex limited expression) plus a dominant or recessive
167 autosomal modifier can explain most cases of PE. The limitation of this analysis is that X-
168 linkage plus a modifier is not always a conservative explanation. X-linkage alone is supported
169 for 19 pedigrees and refuted for 19. For five additional cases, X-linkage is very unlikely. For the
170 remaining five cases X-linkage would be contingent upon the relationship between the
171 inheritance of PC and PE.

172 Finally, when we assess Y-linkage across pedigrees, Y linkage (or sex limited expression)
173 plus a dominant or recessive modifier could not be excluded as explaining the inheritance of PE.
174 However, in no case would Y-linkage alone be supported.

175 A more powerful analysis than the above is revealed when incorporating the relevant clinical
176 traits (Table 1) on the pedigrees atop the data regarding PE, since heterozygous individuals will
177 express all linked dominant or semi-dominant (or homozygous recessive) clinical traits, which
178 proves useful for identification of putative PE allele carriers. With this analysis, only two cases
179 of PE are predicted to result from spontaneous mutation, which is more consistent with the very
180 low *de novo* mutation rate for humans ($\sim 10^{-5}$-10^{-6}) (15) than the result from the first analysis.
181 The phenotypic expression of PE across generations, though skipping generations in many
182 pedigrees, reinforces the concept that carriers are likely to be important in the inheritance of this
183 disorder. In fact, the inheritance pattern on nearly all pedigrees suggests that linkage with
184 specific regions of chromosome 5 (or, plus 15 and/or 17) is worthy of future genome wide
185 analysis. Here, 19 pedigrees are best fit by a simple autosomal inheritance pattern (13 recessive

and six either dominant or recessive). In ~84% of them (16 out of 19 pedigrees), chromosome 5 associated-traits appear prominently in family members for whom they might be expected if these traits are linked to PE genes. In three of the autosomal recessive cases, chromosome 15 appears similarly.

Twenty pedigrees appear best explained by a polygenic effect, where again clinical traits from one or two chromosomes (namely 5 or 15, and/or 17) are inherited in a manner that is consistent with transmission of PE through the family and thus linkage to PE genes warrants future genome wide studies focusing on these chromosomes: chromosomes 5 and 17 appear relevant for five pedigrees, 5 and 15 for six pedigrees, and 5 or 15 and 17 for six more pedigrees. A role for chromosomes 15 and 17 appears relevant for three pedigrees, for chromosome 5 and 1 or X important for two pedigrees, and for chromosome 15 and either 7 or X for two more pedigrees. A role for chromosome 7 appears for three pedigrees and one of these looks to have a spontaneous mutation. A final pedigree also appears to present with a spontaneous mutation and no chromosomal traits appear important.

Thus, a role for chromosome 5 appears in over half (62.5%, or 30/48) and perhaps as many as 75% (36/48) of the pedigrees. Chromosome 15 appears important for between one-third (29%, 14/48) and ~42% (20/48) of the pedigrees. Chromosome 17 appears important in nearly one-fifth (18.75%, 9/48) and up to 31% (15/48) of the pedigrees.

Results from the test for a polygenic mode of inheritance with a threshold that differs by sex are shown in Table 2. The rate of transmission from affected mothers (with PE) to children is 64% (16/24) and affected fathers to children is 33% (20/59). The Chi-square P value is 0.008 and the risk of transmission from mothers over fathers is 3.900, though the confidence interval surrounding the odds ratio is 1.427-10.659. Partitioning this data by the child's sex shows that

mothers transmit to their female children in 70% of the cases (7/10) whereas fathers transmit to female children in 17.39% of the cases (4/23). Mothers transmit to sons in 64.28% (9/14) of the cases whereas fathers transmit to sons in 44.46% of the cases (16/36). Odds ratios in both cases suggest that the risk of transmission is higher when the affected parent is female (Table 2).

Table 3 demonstrates the rate of PE in affected mother's siblings versus affected father's siblings, which addresses the relative genetic load in affected females versus males. Mothers have 33.33% affected siblings (9/27) whereas fathers have 7.2% (7/96). The odds ratio (6.357) is skewed toward a higher likelihood of the disorder in siblings of affected females.

The strong deviation from the 1:1 sex ratio suggests that sex-chromosomes, sex-limited expression, sex-related lethal alleles, or sex-related epigenetic control must be involved in some cases of PE. No useful traits were available to assess on the Y chromosome and X-chromosome traits were considered important in at least three pedigrees.

In cases where PC is also found in a family, the analysis is challenging, since the literature does not indicate a specific, known genetic association between these two disorders. Despite this, 25% (12/48) of families with PE also demonstrate PC in our pedigrees (two families have multiple cases of PC).

Evaluating sex ratio across all PE cases, we observe a strong male bias of nearly 4:1, where the exact ratio is 92:24, which reduces to 3.833:1 and deviates strongly from 1:1 ($X^2_{0.05,[1]}$= 39.863, $p<0.0001$), or the conventional expectation for autosomal control whether inheritance is controlled by one gene or is polygenic. A more extreme, 8-fold male bias is observed in cases where the proband is an only child. Here, the exact sex ratio is 17:2, which reduces to 8.5:1 and deviates from 1:1 ($X^2_{0.05,[1]}$= 11.842, $p<0.00006$), indicating that 89% of these probands are male. Thirty families present with an only-child that has PE. For these, a 9-fold male bias occurs

and the exact sex ratio is 27 males: 3 females, which reduces to 9:1 and deviates from 1:1, $X^2_{0.05,[1]} = 19.2$, $p<0.0001$). In contrast, in families with sibships where at least one sibling has PE, there is a 3-fold male bias and the exact sex ratio is 50:14, which reduces to 3.57:1 and deviates from 1:1 ($X^2_{0.05,[1]} = 20.250$, $p<0.0001$). Similarly, the respondents to the online database questions who indicate their sex (131 individuals) also demonstrate a 3-fold male bias, where the exact sex ratio is 101:30, which deviates from 1:1 ($X^2_{0.05,[1]} = 38.481$, $p<0.0001$).

Since extreme male bias is suggestive of lethal $X_{PE}X_{PE}$, the sex ratio for the number of living offspring in a sibship where miscarriage was reported for the mother was evaluated and determined to be ~1:1 (12:13). This does not suggest a disproportionate number of reported miscarriages that were female. Only two sibships were comprised of both PE and miscarriage, and in each there was one live male child with PE. One of these mothers reported one miscarriage and the other mother reported five. Under 1:1 sex ratio, this would suggest that four females and two males were miscarried.

A second possibility explaining male bias involves the masking of a recessive X_{PE} by a wild-type X in females. This would result in male biased disease expression (X_{PE} is not masked by Y), as would biased X-inactivation in females, but no expectation of a sex ratio bias in living children was found in sibships with miscarriage. For the recessive X there is a predicted inheritance pattern (heterozygous-mother to expressing son), which is sometimes, but not always, evidenced in our pedigrees. Our pedigrees also include 14 cases of PC (79% in males; 11 male: 3 female), which demonstrate a reduced sex ratio of 3.66:1. This is similar to the bias observed for PE.

Quantification of clinical traits

The average number of clinical traits that individuals with PE have is 5.73 ± 3.48. For family members without the disorder the average is 0.14 ± 0.085. After excluding individuals with zero traits, the non-PE family members' mean is still lower, at 2.80 ± 0.87, than for those with PE.

The 10 most common clinical traits identified in individuals with PE and their families in this study are reported in Table 1. Ranked from 1-10 for individuals with PE these are: thinness (47% of individuals with PE), braces (41%), myopia (40%), tallness (33%), light eyes (29%), long fingers (25%), creativity (25%), crowded teeth (25%), fair skin (22%), and asthma (20%). The ranking of these traits in family members without the disorder changes and the frequency of the top 10 PE-related traits is always substantially less for family members than for individuals with PE.

The chromosomal locations for genes that are known to be associated with the traits are pictured in Figure 2. Some of these are: light eyes (5p13.2, 9p23, 15q11.2, 15q13.1), fair skin (5p13.2, 15q21.1 and 16q24.3), asthma (1q32.1, 5q31-33, 7p14.3, 17q12-21.1 and 20p13) and myopia (1p36, 2q37.1, 3q26, 4q22-q27, 4q12, 5p15.33-p15.2, 7q36, 7p15, 8p23, 11p13, 12q21-q23, 17q21-q22, Xq28, Xq23-q25).

Figure 3 lists the 10 most common PE-related traits and identifies which members of the immediate family (mother, father, proband) have each trait. Traits shared between the proband and both parents arise 21 times (yellow cells), between the proband and the mother, 29 times (pink cells), and between the proband and the father, 44 times (light blue cells). This figure also displays the traits that are found only in the proband, which arise 89 times (gray cells), only in the mother, which arise 33 times (dark red cells), only in the father, which arise 39 times (black cells), and between the mother and father, which arise 9 times (green cells). The proband has

276 over twice as many traits as either of his parents (2.69 times more than his mother and 2.28 times
277 more than his father).

278 Regarding height, boys and girls with PE are taller and thinner than the average male and
279 female in the U.S. The average height of adult men (20+ yrs) in the U.S. is 5'9.4" and the
280 average weight is 194.7 lb. (16). Analysis of the PE web site height/weight data indicates that
281 males with this disorder are taller than this, despite being younger: 47 of the 54 male individuals
282 whose data we could analyze (complete information supplied) exceeded 5' 9" and seven were
283 shorter. An appropriate null expectation for height (a normally distributed trait) is that about half
284 of the population (or here the PE subpopulation) will be taller than average (and for PE this
285 would be 27 males), and half will be shorter. Our data deviate substantially from this expectation
286 since 47 of 54 men with PE are taller than average than average ($X^2_{[0.05],1} = 29.629$, $p < 0.0001$).
287 Further, of the 7 males shorter than 5'9", three are less than18 yrs and four are less than 20 yrs
288 old. In addition, of the 47 males above 5'9", nine are not yet 18 yrs old and are expected to
289 continue to grow. The average age for males evaluated from the web site data was 22.63 yrs.
290 Individuals < 20 yrs were included in the comparison because we had complete data on them
291 (making inclusion possible, which seemed reasonable since this is a conservative action given
292 that they can only grow taller with increasing age). Removing the heights of the boys under 20
293 yrs from the analysis, the result remains highly significant. Similarly, of the 34 men ≥ 20 yrs for
294 which we could calculate weight, their average weight was 174.14 lbs. This is ~20 lbs less than
295 the average reported for U.S. men (16).

296 The average height of adult women in the United States is 5'3.8" and average weight 164.4
297 lbs. (16). Females with this disorder are also taller and thinner than the average woman ($X^2_{[0.05],1}$
298 $= 9.9$ and the $0.001 < p < 0.01$). Of the females' query responses we were able to analyze, five

299 females were < 5'4" and 20 were taller. The average age for the women evaluated from the web
300 site data was 26.1 yrs. Of the five women < 5'4", one was under 18 yrs and potentially still
301 growing. Of the 20 over 5'4", five were ≤18yrs and 2 were ≤ 20yrs. Similarly, of the 14 women
302 ≥ 20 yrs for which we could calculate weight, the average weight was 119.07 lbs which is ~45
303 lbs less than the average reported for U.S. women (16).

304 **Estimating the frequency of heterozygotes**

305 Our pedigree assessment indicates that autosomes are likely involved in this disorder but we
306 find that a relatively large number of marriages between heterozygous individuals must occur if
307 this is true. If PE is a result of a homozygous recessive genotype, then individuals that are
308 heterozygous for alleles causing PE are predicted to be relatively common. If the disease
309 phenotype is represented by the autosomal recessive genotype, '*rr'*, then the frequency *'rr'* in the
310 population is 0.0025 (from the reported 1 in 400 have the disorder). In Hardy Weinberg, p^2=
311 0.0025, so p = 0.05, q = 0.95 and the frequency of heterozygotes (2pq) equals 0.095, implying
312 that 9.5 in 100 individuals will carry '*r'*. This number could increase if PE is additive and
313 polygenic. If two independent mutations cause PE, then 19/100 carriers would be expected and if
314 three independent mutations cause PE then 28.5/100 carriers would be expected.

315 Similarly, if the frequency of '*rr'* = 0.001 (from the reported 1/1,000) then q=0.969 and 2pq
316 = 0.060. Thus, between 6/100 and 9.5/100 individuals would be carriers of an autosomal
317 recessive allele if one recessive mutation causes PE, and as many as 29 in 100 individuals if say,
318 three independent mutations cause PE. Thus, a large number of heterozygotes would exist in the
319 human population.

Many cases of PE fit the autosomal, recessive inheritance pattern well and those that do not generally appear to be sex-linked and under autsomal modifier control. The above calculation would also apply for an autosomal modifier.

4. Discussion

Little is known regarding the genetics and inheritance pattern of pectus excavatum. This work advances our knowledge on many fronts. Spontaneous mutations causing human disease occur on the order of ~10^{-5} to 10^{-6}, yet as many as 1 in 400 people express PE, indicating that the majority of cases must not result simply from *de novo* mutations. Knowledge regarding clinical traits commonly associated with PE (like thinness, myopia, crooked teeth and tallness) may be useful during genetic counseling for predicting the probability of transmission of PE alleles. The Carter effect addresses the likelihood of the lesser-affected sex carrying a higher genetic load and expressing a disorder less frequently (than the opposite sex) while being more likely to transmit to progeny and to also have siblings that are affected. Data from our assessment of the Carter effect may also be useful in genetic counseling since it points to a higher probability of mother's with PE (versus father's with PE) transmitting the disorder to offspring, as well as these women's siblings having a higher likelihood of being affected with PE. However, caution must be exercised in drawing full conclusions here since the confidence intervals surrounding these odds ratios tend to be large with our present sample sizes.

Since the average height and weight of individuals with PE deviates from the norm and demonstrates the unconventional pattern of a negative association (e.g. tall and thin), the predictive power of this trait combination is enhanced.

The relatively high frequency of PE in the human population makes it plausible that a substantial number of heterozygous individuals are involved transmission. The presence of

clinical traits in a disproportionate number of probands' parents and siblings may reflect an abundance of heterozygous carriers, predicted here to be at least 38-60 fold more common than diseased individuals. This indicates that marriages to heterozygous carriers could occur frequently.

The sex-ratio associated with PE deviates substantially from the conventional expectation for pure autosomal control. This is indicative of sex-linked or sex-limited (epigenetic) expression. Male bias may result if more than one independent mutation causes PE and at least one of these is sex-linked, or if females must have a higher number of specific alleles to express PE because of a sex-related susceptibility difference. Or it may result if gene interactions or sex-related gene silencing occur. Thus, the role for genes like *SOX5* (12p12.1) and *SOX9* (17q24.3-q25.1) that interact during chondrogenesis, activate transcription of *COL2A1* (12q13.11), are associated with sex determination and related to disease (17), should be further explored.

Alternatively the biased sex-ratio (which demonstrates even greater bias for sibships with an only child with PE) could result from lethal $X_{PE}X_{PE}$. The sex ratio produced by the mothers that reported miscarriages did not suggest a female-bias in miscarried individuals. However, the sibships comprised of individuals with PE and miscarriages could, except our sample is too small to evaluate objectively so further study is warranted. Another possibility involves the masking of a recessive X_{PE} by a wild-type X, which would result in male biased PE expression (X_{PE} is not masked by Y), as would biased X-inactivation in females. Here, there is no expectation of a sex ratio bias in living children found in sibships with miscarriage, but for the recessive X there is a predicted inheritance pattern (heterozygous-mother to PE-expressing son), which is sometimes, but not always, evidenced in our pedigrees. Noteworthy is that in sibships of more than one child and inclusive of a proband, sex ratio is less biased, potentially indicative of multifactorial

366 inheritance or more than one inheritance pattern and/or epigenetic effects for PE. Biased sex
367 expression, such as we see, is expected when there is a sex-dependent threshold for a trait, as
368 holds for the Carter effect.

369 Other human diseases, such as Prader-Walli syndrome, demonstrate *de novo* deletions on
370 chromosome 15 (at 15q11-13) exclusively of paternal origin, and in a few cases maternal
371 heterodisomy (where two different copies of chromosome 15 are inherited maternally) (18) is
372 indicative of epigenetic control. Unlike most human diseases, some (including neurofibromatosis
373 and Duchenne muscular dystrophy) are associated with a high frequency of *de novo* germ-line
374 mutations (19) which result from older sires (in many taxa, especially mammals) that express a
375 higher germ-line mutation rate (spermatogenic cells from old mice have higher mutation rates
376 than young- or middle-aged mice (19). While we cannot definitively state whether our probands
377 have novel mutations, we are in the process of evaluating whether sire age plays a role in PE
378 (85% of probands' sire are over 30 years old), as it does in Marfan syndrome (17) (which affects
379 ~0.0001-0.0005 of the population) (21-23).

380 There is definitive overlap in traits associated with PE and Marfan syndrome (9) including
381 myopia, dental crowding, scoliosis, and long-fingers (23-25). PE and PC are also identified in
382 about half of the individuals with Marfan syndrome, potentially suggestive of similar causation
383 (24-27) and given the abundance of clinical traits involved, leading us to recommend that PE
384 also be referred to as a syndrome.

385 However, Marfan syndrome demonstrates a 1:1 sex ratio (24) indicative of pure autosomal
386 control, unlike the Pectus disorders. The *FBN1* gene (15q21.1) has been implicated as the
387 predominant cause of Marfan syndrome (28-29) with additional mutations found in *TGFßR2*
388 (*e.g.* 3p24.1) (30). While chromosome 15 appears important in our analysis for PE, *TGFßR1* and

389 *TGFβR2* are typically associated with Marfan syndrome II, which is less similar to PE than
390 Marfan syndrome.
391 Our current knowledge suggests the potential for greater than one mutation to be associated
392 with PE and the likelihood of sex-biased, polygenic control. Our data points to a clear need for
393 genome-wide analysis of control of this disorder and follow through on establishing the
394 importance of the links identified in this paper in a quantitative analysis. Regions of
395 chromosomes 5, 15, and 17 are relevant for linkage mapping and candidate gene searches since
396 relevant PE-associated clinical traits are controlled by genes on these chromosomes. Genes
397 affecting cartilage are also found on these chromosomes and mutations in some of these genes
398 control other syndromes demonstrating symptoms similar to PE. Aggrecan (*ACAN*, 15q26.1, a
399 major proteoglycan of cartilage (31) accounts for 35% dry weight of cartilage (32) and two
400 fibrillin genes (e.g. *FBN1*, 15q15-21.3 and *FBN2*, 5q23-31) affect connective tissue, causing
401 Marfan and Marfan-like syndrome (33). While this work advances our knowledge regarding PE
402 substantially, candidate gene searches for PE-related mutations are a necessary next step to
403 identifying the causative agent of PE. Equally important are microarrays to look for differences
404 in gene expression between individuals with PE, without PE, and those predicted to be
405 heterozygous for this disorder.
406

Acknowledgement: Thanks to two anonymous reviewers for very constructive comments. This research was supported by a grant from the Norfolk Foundation to M. Stacey & L. Horth.

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647 **Figure Legends**

648

Figure 1. Pedigree with traits added for the mother, father and proband (additional family member traits not included here for clarity). Father and proband have pectus excavatum (darkened symbol). Proband is labeled with an arrow.

Figure 2. Genomic map of chromosomal gene locations for traits evaluated in this study of pectus excavatum The genes for asthma include CHI3L1 (1q32.1) (34), IL12B (5q31.1-q33.1) (35), NPSR1 (7p14.3) (36), ORMDL3 (17q12-q21.1) (37), and ADAM33 (20p13) (38). The genes used for arthritis include PTPN22 (1p13.3-p13.1) (39), FCRL3 (1q21-q22) (40), AFF3 (2q11.2-q12) (41), STAT4 (2q32.2-q32.3) (42), IL2 (4q26- q27) (43), HLA-DRB1 (6p21.3) (40), TRAF1 (9q33-q34) (42), and CCL5 (17q11.2-q12) (44). The genes used for ADHD include ADHD5 (2q21.1) (45), DRD5 (4p16.1) (46), SLC6A3 (5p15.3) (46), ADHD4 (5p13) (47), ADHD3 (6q12) (47), HTR1B (6q13) (46), DRD4 (11p15.5) (46), TPH2 (12q21.1) (46), ADHD6 (13q12.11) (45), ADHD1 (16p13) (47), ADHD2 (17p11) (47), and SNAP-25 (20p12-p11.2) (46). The genes used for depression include HTR1A (5q11.2-q13) (48), TPH1 (11p15.3-p14) (49), BDNF (11p13) (50), TPH2 (12q21.1) (51), SLC6A4 (17q11.1-q12) (52), and MAOA (Xp11.3) (53). The genes used for fair skin include SLC45A2 (5p13.2) (54), SLC24A5 (15q21.1) (55), HERC2 (15q13) (56), and MC1R (16q24.3) (57). The genes used for hearing loss include KCNQ4 (1p34) (58), OTOF (2p23.1) (59), GJB2 (13q11-q12) (60), MYO1C (17p13) (61), and MYO1F (19p13.3-p13.2) (61). The genes used for light eyes include SLC45A2 (5p13.2) (56), SHEP11 (9p23) (62), OCA2 (15q11.2-12) (63), and HERC2 (15q13) (64). The genes used for light hair include SHEP11 (9p23) (62), OCA2 (15q11.2-12) (65), HERC2 (15q13) (65), MC1R (16q24.3) (57), and ASIP (20q11.2-q12) (66). The genes used for migraines include MGR

672 (1q31) (67), MA (4q24) (68), MGR8 (5q21) (69), MGR3 (6p21.1-12.2) (70), MGR7 (15q11.2-
673 q12) (71), MGR5 (19p13) (72), CACNA1A (19p13.2-13.1) (73), and MGR2 (Xq24-q28) (74).
674 The genes used for mitral valve prolapse include AGTR1 (3q21-25) (75), MMVP2 (11p15.4)
675 (76), MMVP3 (13q31.3-q32.1) (77), and MMVP1 (16p12.1-p11.2) (78). The genes used for
676 myopia include MYP14 (1p36) (79), MYP12 (2q37.1) (80), MYP8 (3q26) (81), MYP11 (4q22-
677 q27) (82), MYP9 (4q12) (78), MYP16 (5p15.33-p15.2) (83), MYP4 (7q36) (84), MYP17 (7p15)
678 (85), MYP10 (8p23) (81), MYP7 (11p13) (81), MYP3 (12q21-q23) (86), MYP5 (17q21-q22)
679 (87), MYP1 (Xq28) (88), and MYP13 (Xq23-q25) (89). The genes used for seizures include
680 SCN2A (2q23-24) (90) and CDKL5 (Xp22) (91). The genes used for scoliosis include CHD7
681 (8q12.1-12.2) (92), IS4 (9q31.2-q34.2) (93), IS2 (17p11.2) (94), IS5 (17q25.3) (93), and AIS
682 (19p13.3) (95). The gene used for tallness is GDF5 (20q11.2) (96). The gene used for Congenital
683 Contractural Arachnodactyly (CCA, or Beals-Hecht syndrome), which can include symptoms
684 such as long fingers, tall, thin, scoliosis, and mitral valve prolapse (97-99), is FBN2 (5q23-q31)
685 (33). The gene used for Marfan Syndrome, which can include symptoms such as tall, thin, long
686 fingers, scoliosis, and mitral valve prolapse (8, 99, 100), is FBN1 (15q21.1) (7).
687
688 **Figure 3. Traits present in parents and/or proband.** Traits assayed here include the 10 traits
689 we find most frequently associated with pectus excavatum. Traits shared between the proband
690 and both parents are represented by yellow cells, between the proband and the mother by pink
691 cells, between the proband and father, by light blue cells, in the proband only, by gray cells, in
692 the mother only by dark red cells, in the father only by black cells, and between the mother and
693 father by green cells.
694

Table 1

The ten clinical traits found to be most frequently associated with pectus excavatum (PE), ranked by prevalence, for individuals with PE and their relatives

	PE		Non-PE	
Trait	%	Rank	%	Rank
Thinness	47.41	1	3.55	7
Braces	41.38	2	5.17	5
Myopia	39.66	3	10.93	1
Tallness	32.76	4	7.13	2
Light Eyes	29.31	5	7.09	3
Long Fingers	25.00	6	1.72	19
Creativity	25.00	7	3.35	8
Crowded Teeth	25.00	8	2.46	13
Fair Skin	21.55	9	5.81	4
Asthma	19.83	10	1.67	21
Light Hair	14.66	13	3.10	9
Arthritis	12.07	16	4.92	6
Depression	8.63	22	3.00	10

Table 2

Transmission of pectus excavatum (PE) from affected fathers versus affected mothers to their children.

Individuals	# children in sibship with PE	# children in sibship without PE	Chi-square P value	Odds Ratio (female: male)	95% Confidence Interval for Odds Ratio
Affected fathers (for all children)	20	39			
Affected mothers	16	8	0.008	3.900	1.42-10.65
Affected fathers	4	19			

Affected fathers (for female children)	4	19			
Affected mothers	7	3	0.006	11.083	1.966-
Affected fathers (for male children)	16	20			
Affected mothers	9	5	0.245	2.137	0.595-7.685

Table 3

Differential prevalence of pectus excavatum in siblings of affected mothers versus affected fathers.

Individuals	# siblings with PE	# siblings without PE	Chi-square P value	Odds Ratio (female: male)	95% Confidence Interval for Odds Ratio
Affected Fathers	7	89			
Affected Mothers	9	18	0.001	6.357	2.095-19.291