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Advancing Our Understanding of the Inheritance and Transmission of Pectus Excavatum

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Original Article

Advancing our understanding of the inheritance and transmission of pectus excavatum

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

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

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

A more powerful analysis than the above is revealed when incorporating the relevant clinical traits (Table 1) on the pedigrees atop the data regarding PE, since heterozygous individuals will express all linked dominant or semi-dominant (or homozygous recessive) clinical traits, which proves useful for identification of putative PE allele carriers. With this analysis, only two cases of PE are predicted to result from spontaneous mutation, which is more consistent with the very low *de novo* mutation rate for humans ($\sim10^{-5}$-10^{-6}) (15) than the result from the first analysis. The phenotypic expression of PE across generations, though skipping generations in many pedigrees, reinforces the concept that carriers are likely to be important in the inheritance of this disorder. In fact, the inheritance pattern on nearly all pedigrees suggests that linkage with specific regions of chromosome 5 (or, plus 15 and/or 17) is worthy of future genome wide 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 over twice as many traits as either of his parents (2.69 times more than his mother and 2.28 times more than his father).

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

The average height of adult women in the United States is 5’3.8” and average weight 164.4 lbs. (16). Females with this disorder are also taller and thinner than the average woman ($X^2_{[0.05],1} = 9.9$ and the $0.001 < p < 0.01$). Of the females’ query responses we were able to analyze, five females were < 5’4” and 20 were taller. The average age for the women evaluated from the web site data was 26.1 yrs. Of the five women < 5’4”, one was under 18 yrs and potentially still growing. Of the 20 over 5’4”, five were ≤18yrs and 2 were ≤ 20yrs. Similarly, of the 14 women ≥ 20 yrs for which we could calculate weight, the average weight was 119.07 lbs which is ~45 lbs less than the average reported for U.S. women (16).

Estimating the frequency of heterozygotes

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

Similarly, if the frequency of ‘*rr’* = 0.001 (from the reported 1/1,000) then $q=0.969$ and $2pq = 0.060$. Thus, between 6/100 and 9.5/100 individuals would be carriers of an autosomal recessive allele if one recessive mutation causes PE, and as many as 29 in 100 individuals if say, three independent mutations cause PE. Thus, a large number of heterozygotes would exist in the 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 inheritance or more than one inheritance pattern and/or epigenetic effects for PE. Biased sex expression, such as we see, is expected when there is a sex-dependent threshold for a trait, as holds for the Carter effect.

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

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

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

TGFßR2 are typically associated with Marfan syndrome II, which is less similar to PE than Marfan syndrome.

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

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References

1. Chung CS, Myrianthopoulos NC. Factors affecting risks of congenital malformations. I. Analysis of epidemiologic factors in congenital malformations. Report from the Collaborative Perinatal Project. Birth Defects Orig Artic Ser 1975; 11:1–22.
2. Creswick HA, Stacey MW, Kelly RE, et al. Family study of the inheritance of pectus excavatum. J Pediatr Surg 2006; 41:1699–1703.
3. Kelly RE, Shamberger RC, Mellins RB, et al. Prospective multicenter study of surgical correction of pectus excavatum: design, perioperative complications, pain, and baseline pulmonary function facilitated by internet-based data collection. J Am Coll Surg 2007; 205:205–216.
4. Dietz HC, Cutting CR, Pyeritz RE, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 1991; 352: 337–339.
5. Faivre L, Collod-Beroud G, Loeys BL, et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. Am J Hum Genet 2007; 81: 454–466.
6. Judge DP, Biery NJ, Dietz HC. Characterization of microsatellite markers flanking FBN1: utility in the diagnostic evaluation for Marfan syndrome. Am J Med Genet 2001; 99: 39–47.
7. Gurnett CA, Alaee F, Bowcock A, et al. Genetic linkage localizes an adolescent idiopathic scoliosis and pectus excavatum gene to chromosome 18 q. Spine 2009; 34: E94–E100.

8. Carter, CO. Inheritance of congenital pyloric stenosis. British Medical Bulletin 1961; 117: 251-253.
9. Kantarci, OH, Barcellos LF, Atkinson EJ, Ramsay PP, De Andrade M, Hauser SL, Weinshenker BG. Men transmit MS more often to their children vs women: the Carter effect. Neurology 2006; 67: 305-310.
10. Kruse LM, Dobbs MB, Gurnett, CA. Polygenic threshold model with sex dimorphism in clubfoot inheritance: the Carter effect. J Bone Joint Surg Am 2008; 90:268-2694.
11. Graph Pad Software [Chi Square Calculator]. La Jolla, CA: Graph Pad Software, Inc, 2005.
12. McDonald JH. Handbook of Biological Statistics. Baltimore: Sparky House Publishing, 2008: 21–28.
13. NCBI: National Center for Biotechnology Information [Human Genome Resources]. Available at: http://www.ncbi.nlm.nih.gov/projects/genome/guide/human Accessed March 2, 2010.
14. “How Tall/Thin are you PE people??” Pectus Support Group, 2004. Available at: www.pectusinfo.com/board/stats.php Accessed December 1, 2008.
15. Crow JF. The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet 2000; 1:40–47.
16. McDowell MA, Fryar CD, Ogden CL, Flegal KM. (2008) Anthopometric reference data for children and adults: United States, 2003-2006. National Health Statistics Report 10. US Dept of Health and Human Services, Center for Disease Control and Prevention, National Center for Health Statistics, 2008:1–48.

17. Lefebvre V, Li P, de Crombrugghe, B. A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. *Embo J* 1998; 17:5718–5733.

18. Nicholls RD, Knoll JH, Butler MG, Karam S, Lalande M. Genetic imprinting suggested by maternal heterodisomy in nondeletion Prader-Willi syndrome. Nature 1998; 342:281–285.

19. Walter CA, Intano GW, McCarrey JR, McMahan CA. Mutation frequency declines during spermatogenesis in young mice but increases in old mice. Proc Natl Acad Sci USA 1998; 95:10015–10019.

20. Risch N, Reigh EW, Wishnick MW, McCarthy JG. Spontaneous mutation and parental age in humans. A detailed review and analysis of the classical literature on the paternal age effect. Am J Hum Genet 1987; 41:218–248.

21. Dean JCS. Marfan syndrome: clinical diagnosis and management. Euro Jour Hum Genet 2007; 15:724–733.

22. Frydman M. The Marfan Syndrome. Isr Med Assoc J 2008; 10:175–178.

23. Judge DP, Dietz HC. Marfan’s Syndrome. Lancet 2005; 366:1965–1976.

24. DePaepe A, Dietz HC, Devereux RB, Hennekem R, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996; 62:417–426.

25. Lopez VM, Perez AB, Moisés VA, et al. Serial clinical and echocardiographic evaluation in children with Marfan syndrome. Arq Bras Cardiol 2005; 85:314–318.

26. De Backer JF, Devos D, Segers P, et al. Primary impairment of left ventricular function in Marfan syndrome. Int J Cardiol 2006; 112:353–358.

27. Taub CC, Stoler JM, Perez-Sanz T, et al. Mitral valve prolapse in Marfan syndrome: an old topic revisited. Echocardiography 2009; 26: 357–364.

28. Hollister DW, Godfrey M, Sakai LY, Pyeritz RE. Immunohistologic abnormalities of the microfibrillar-fiber system in the Marfan syndrome. N Engl J Med 1990; 323:152–159.

29. Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L. Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med 1990; 323:935–939.

30. Mizuguchi T, Collod-Beroud G, Akiyama T, et al. Heterozygous TGFBR2 mutations in Marfan syndrome. Nat Genet 2004; 36:855–860.

31. Doege KJ, Coulter SN, Meek LM, Maslen K, Wood JG. A human-specific polymorphism in the coding region of the aggrecan gene. Variable number of tandem repeats produce a range of core protein sizes in the general population. J Biol Chem 1997; 272:13974–13979.

32. Skandalis SS, Theocharis AD, Vynios DH, et al. Cartilage aggrecan undergoes significant compositional and structural alterations during laryngeal cancer. Biochim Biophys Acta 2006;1760:1046–1053.

33. Putnam EA, Zhang H, Ramirez F, Milewicz DM. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. Nat Genet 1995;11:456–458.

34. Ober C, Chupp GL. The chitinase and chitinase-like proteins: a review of genetic and functional studies in asthma and immune-mediated diseases. Curr Opin Allergy Clin Immunol 2009;9:401–408.

35. Randolph AG, Lange C, Silverman EK, et al. The IL12B gene is associated with asthma. Am J Hum Genet 2004;75:709–715.

36. Castro-Giner F, de Cid R, Gonzalez JR, et al. Positionally cloned genes and age-specific effects in asthma and atopy: an international population-based cohort study (ECRHS). Thorax 2010;65:124–131.

37. Wu H, Romieu I, Sienra-Monge JJ, Li H, del Rio-Navarro BE, London SJ. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. Allergy 2009;64:629–635.

38. Weiss ST, Raby BA, Rogers A. Asthma genetics and genomics 2009. Curr Opin Genet Dev 2009;19:279–282.

39. Pazár B, Gergely P, Nagy ZB, et al. Role of HLA-DRB1 and PTPN22 genes in susceptibility to juvenile idiopathic arthritis in Hungarian patients. Clin Exp Rheumatol 2008;26:1146–1152.

40. Kochi Y, Suzuki A, Yamada R, Yamamoto K. Ethnogenetic heterogeneity of rheumatoid arthritis-implications for pathogenesis. Nat Rev Rheumatol 2010;6:290-295.

41. Hinks A, Eyre S, Ke X, et al. Association of the AFF3 gene and IL2/IL21 gene region with juvenile idiopathic arthritis. Genes Immun 2010;11:194–198.

42. Hinks A, Eyre S, Ke X, et al. Overlap of disease susceptibility loci for rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). Ann Rheum Dis 2009; doi: 10.1136/ard.2009. 110650.

43. Albers HM, Kurreeman FA, Stoeken-Rijsbergen G, et al. Association of the autoimmunity locus 4q27 with juvenile idiopathic arthritis. Arthritis Rheum 2009;60:901–904.

44. Yao TC, Tsai YC, Huang JL. Association of RANTES promoter polymorphism with juvenile rheumatoid arthritis. Arthritis Rheum 2009;60:1173–1178.

45. Rommelse NNJ, Arias-Vasquez A, Altink ME, et al. Neuropsychological endophenotype approach to genome-wide linkage analysis identifies susceptibility loci for ADHD on 2q21.1 and 13q12.11. Am J Hum Genet 2008;83:294.

46. Banaschewski T, Becker K, Scherag S, Franke B, Coghill D. Molecular genetics of attention-deficit/hyperactivity disorder: an overview. Eur Child Adolesc Psychiatry 2010;19:237–257.

47. Ogdie MN, Fisher SE, Yang M, et al. Attention deficit hyperactivity disorder: fine mapping supports linkage to 5p13, 6q12, 16p13, and 17p11. Am J Hum Genet 2004;75:661–668.

48. Villafuerte SM, Vallabhaneni K, Sliwerska E, McMahon FJ, Young EA, Burmeister M. SSRI response in depression may be influenced by SNPs in HTR1B and HTR1A. Psychiatr Genet 2009;19:281–291.

49. Viikki M, Kampman O, Illi A, et al. TPH1 218A/C polymorphism is associated with major depressive disorder and its treatment response. Neurosci Lett 2010;468:80–84.

50. Lavebratt C, Aberg E, Sjöholm LK, Forsell Y. Variations in FKBP5 and BDNF genes are suggestively associated with depression in a Swedish population-based cohort. J Affect Disord 2010; doi:10.1016/j.jad.2010.02.113.

51. Utge S, Soronen P, Partonen T, et al. A population-based association study of candidate genes for depression and sleep disturbance. Am J Med Genet B Neuropsychiatr Genet 2010;153B:468–476.

52. Frodl T, Reinhold E, Koutsouleris N, et al. Childhood Stress, Serotonin Transporter Gene and Brain Structures in Major Depression. Neuropsychopharmacology 2010;35:1383–1390.

53. Rivera M, Gutiérrez B, Molina E, et al. High-activity variants of the MAOA polymorphism increase the risk for depression in a large primary care sample. Am J Med Genet B Neuropsychiatr Genet 2009;150B:395–402.

54. Lucotte G, Mercier G, Diéterlen F, Yuasa I. A decreasing gradient of 374F allele frequencies in the skin pigmentation gene SLC45A2, from the north of West Europe to North Africa. Biochem Genet 2010;48:26–33.

55. Sturm RA. Molecular genetics of human pigmentation diversity. Hum Mol Genet 2009;18:R9–R17.

56. Bouakaze C, Keyser C, Crubézy E, Montagnon D, Ludes B. Pigment phenotype and biogeographical ancestry from ancient skeletal remains: inferences from multiplexed autosomal SNP analysis. Int J Legal Med 2009;123:315–325.

57. Latreille J, Ezzedine K, Elfakir A, et al. MC1R gene polymorphism affects skin color and phenotypic features related to sun sensitivity in a population of French adult women. Photochem Photobiol 2009;85:1451–1458.

58. Hildebrand MS, Tack D, McMordie SJ, et al. Audioprofile-directed screening identifies novel mutations in KCNQ4 causing hearing loss at the DFNA2 locus. Genet Med 2008;10:797–804.

59. Rodríguez-Ballesteros M, Reynoso R, Olarte M, et al. A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy. Hum Mutat 2008;29:823–831.

60. Bartsch O, Vatter A, Zechner U, et al. GJB2 Mutations and Genotype-Phenotype Correlation in 335 Patients from Germany with Nonsyndromic Sensorineural Hearing Loss: Evidence for Additional Recessive Mutations Not Detected by Current Methods. Audiol Neurootol 2010;15:375–382.

61. Zadro C, Alemanno MS, Bellacchio E, et al. Are MYO1C and MYO1F associated with hearing loss? Biochim Biophys Acta 2009;1792:27–32.

62. Sulem P, Gudbjartsson DF, Stacey SN, et al. Two newly identified genetic determinants of pigmentation in Europeans. Nat Genet 2008;40:835–837.

63. Sturm RA, Larsson M. Genetics of human iris colour and patterns. Pigment Cell Melanoma Res 2009;22:544–562.

64. Sturm RA, Duffy DL, Zhao ZZ, et al. A single SNP in an evolutionary conserved region within intron 86 of the HERC2 gene determines human blue-brown eye color. Am J Hum Genet 2008;82:424–431.

65. Shekar SN, Duffy DL, Frudakis T, et al. Linkage and association analysis of spectrophotometrically quantified hair color in Australian adolescents: the effect of OCA2 and HERC2. J Invest Dermatol 2008;128:2807–2814.

66. Tully G. Genotype versus phenotype: human pigmentation. Forensic Sci Int Genet 2007;1:105–110.

67. Lea RA, Shepherd AG, Curtain RP, et al. A typical migraine susceptibility region localizes to chromosome 1q31. Neurogenetics 2002;4:17–22.

68. Bjornsson A, Gudmundsson G, Gudfinnsson E, et al. Localization of a gene for migraine without aura to chromosome 4q21. Am J Hum Genet 2005;76: 715.

69. Nyholt DR, Morley KI, Ferreira MAR, et al. Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet 2005;77:500–512.

70. Carlsson A, Forsgren L, Nylander PO, et al. Identification of a susceptibility locus for migraine with and without aura on 6p12.2-p21.1. Neurology 2002;59:1804–1807.

71. Russo L, Mariotti P, Sangiorgi E, et al. A new susceptibility locus for migraine with aura in the 15q11-q13 genomic region containing three GABA-A receptor genes. Am J Hum Genet 2005;76:327–333.

72. Nyholt DR, Lea RA, Goadsby PJ, Brimage PJ, Griffiths LR. Familial typical migraine: linkage to chromosome 19p13 and evidence for genetic heterogeneity. Neurology 1998;50:1428–1432.

73. Lea RA, Hilton DA, MacMillian JC, Griffiths LR. An analysis of clinical characteristics in genetically linked migraine-affected pedigrees. Cephalalgia 2003;23:808–813.

74. Nyholt DR, Curtain RP, Griffiths LR. Familial typical migraine: significant linkage and localization of a gene to Xq24-28. Hum Genet 2000;107:18–23.

75. Szombathy T, Jánoskúti L, Szalai C, et al. Angiotensin II type 1 receptor gene polymorphism and mitral valve prolapse syndrome. Am Heart J 2000;139:101–105.

76. Freed LA, Acierno JS, Dai D, et al. A locus for autosomal dominant mitral valve prolapse on chromosome 11p15.4. Am J Hum Genet 2003;72:1551–1559.

77. Nesta F, Leyne M, Yosefy C, et al. New locus for autosomal dominant mitral valve prolapse on chromosome 13: clinical insights from genetic studies. Circulation 2005;112:2022–2030.

78. Diss S, Abergel E, Berrebi A, et al. Mapping of a first locus for autosomal dominant myxomatous mitral-valve prolapse to chromosome 16p11.2-p12.1. Am J Hum Genet 1999;65:1242–1251.

79. Wojciechowski R, Moy C, Ciner E, et al. Genomewide scan in Ashkenazi Jewish families demonstrates evidence of linkage of ocular refraction to a QTL on chromosome 1p36. Hum Genet 2006;119:389–399.

80. Chen CY, Stankovich J, Scurrah KJ, et al. Linkage replication of the MYP12 locus in common myopia. Invest Ophthalmol Vis Sci 2007;48:4433–4439.

81. Hammond CJ, Andrew T, Mak YT, Spector TD. A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of dizygotic twins. Am J Hum Genet 2004;75:294–304.

82. Zhang, Q, Guo, X, Xiao, X, Jia, X, Li, S, Hejtmancik, JF. A new locus for autosomal dominant high myopia maps to 4q22-q27 between D4S1578 and D4S1612. Molec Vis 2005;11:554–560.

83. Lam CY, Tam POS, Fan DSP, et al. A genome-wide scan maps a novel high myopia locus to 5p15. Invest Ophthal Vis Sci 2008;49:3768–2778.

84. Naiglin L, Gazagne C, Dallongeville F, et al. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. J Med Genet 2002;39:118–124.

85. Paget S, Julia S, Vitezica ZG, Soler V, Malecaze F, Calvas P. Linkage analysis of high myopia susceptibility locus in 26 families. Molec Vis 2008;14:2566–2574.

86. Young TL, Ronan SM, Alvear AB, et al. A second locus for familial myopia maps to chromosome 12q. Am J Hum Genet 1998;63:1419–1424.

87. Paluru P, Ronan SM, Heon E, et al. New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. Invest Ophthal Vis Sci 2003;44:1830–1836.

88. Bartsocas CS, Kastrantas AD. X-linked form of myopia. Hum Hered 1981;31:199–200.

89. Zhang Q, Li S, Xiao X, Jia X, Guo X. Confirmation of a genetic locus for X-linked recessive high myopia outside MYP1. J Med Genet 2007;52:469–472.

90. Bergren SK, Chen S, Galecki A, Kearney JA. Genetic modifiers affecting severity of epilepsy caused by mutation of sodium channel Scn2a. Mamm Genome 2005;16:683–690.

91. Erez A, Patel AJ, Wang X, et al. Alu-specific microhomology-mediated deletions in CDKL5 in females with early-onset seizure disorder. Neurogenetics 2009;10:363–369.

92. Gao X, Gordon D, Zhang D, et al. CHD7 gene polymorphisms are associated with susceptibility to idiopathic scoliosis. Am J Hum Genet 2007;80:957–965.

93. Ocaka L, Zhao C, Reed JA, et al. Assignment of two loci for autosomal dominant adolescent idiopathic scoliosis to chromosomes 9q31.2-q34.2 and 17q25.3-qtel. J Med Genet 2008;45:87–92.

94. Salehi LB, Mangino M, De Serio S, et al. Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11. Hum Genet 111:401–404.

95. Chan V, Fong GCY, Luk KDK, et al. A genetic locus for adolescent idiopathic scoliosis linked to chromosome 19p13.3. Am J Hum Genet 2002;71:401–406.

96. Sanna S, Jackson AU, Nagaraja R, et al. Common variants in the GDF5-UQCC region are associated with variation in human height. Nat Genet 2008;40:198–203.

97. Tunçbilek E, Alanay Y. Congenital contractural arachnodactyly (Beals syndrome). Orphanet J Rare Dis 2006;1:20.

98. Callewaert BL, Loeys BL, Ficcadenti A, et al. Comprehensive clinical and molecular assessment of 32 probands with congenital contractural arachnodactyly: report of 14 novel mutations and review of the literature. Hum Mutat 2009;30:334–341.

99. Gao LG, Luo F, Hui RT, Zhou XL. Recent molecular biological progress in Marfan syndrome and Marfan-associated disorders. Ageing Res Rev 2009; doi:10.1016/j.arr.2009.09.001.

100. Mamada M, Yorifuji T, Yorifuji J, et al. Fibrillin I gene polymorphism is associated with tall stature of normal individuals. Hum Genet 2007;120:733–735.

Figure Legends

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

(1q31) (67), MA (4q24) (68), MGR8 (5q21) (69), MGR3 (6p21.1-12.2) (70), MGR7 (15q11.2-q12) (71), MGR5 (19p13) (72), CACNA1A (19p13.2-13.1) (73), and MGR2 (Xq24-q28) (74). The genes used for mitral valve prolapse include AGTR1 (3q21-25) (75), MMVP2 (11p15.4) (76), MMVP3 (13q31.3-q32.1) (77), and MMVP1 (16p12.1-p11.2) (78). The genes used for myopia include MYP14 (1p36) (79), MYP12 (2q37.1) (80), MYP8 (3q26) (81), MYP11 (4q22-q27) (82), MYP9 (4q12) (78), MYP16 (5p15.33-p15.2) (83), MYP4 (7q36) (84), MYP17 (7p15) (85), MYP10 (8p23) (81), MYP7 (11p13) (81), MYP3 (12q21-q23) (86), MYP5 (17q21-q22) (87), MYP1 (Xq28) (88), and MYP13 (Xq23-q25) (89). The genes used for seizures include SCN2A (2q23-24) (90) and CDKL5 (Xp22) (91). The genes used for scoliosis include CHD7 (8q12.1-12.2) (92), IS4 (9q31.2-q34.2) (93), IS2 (17p11.2) (94), IS5 (17q25.3) (93), and AIS (19p13.3) (95). The gene used for tallness is GDF5 (20q11.2) (96). The gene used for Congenital Contractural Arachnodactyly (CCA, or Beals-Hecht syndrome), which can include symptoms such as long fingers, tall, thin, scoliosis, and mitral valve prolapse (97-99), is FBN2 (5q23-q31) (33). The gene used for Marfan Syndrome, which can include symptoms such as tall, thin, long fingers, scoliosis, and mitral valve prolapse (8, 99, 100), is FBN1 (15q21.1) (7).

Figure 3. Traits present in parents and/or proband. Traits assayed here include the 10 traits we find most frequently associated with pectus excavatum. Traits shared between the proband and both parents are represented by yellow cells, between the proband and the mother by pink cells, between the proband and father, by light blue cells, in the proband only, by gray cells, in the mother only by dark red cells, in the father only by black cells, and between the mother and father by green cells.

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