Pedigree Analysis of Lumbar Developmental Spinal Stenosis: Determination of Potential Inheritance Patterns

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Key Words: Developmental spinal stenosis; lumbar; pedigree; family

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Running title: Developmental spinal stenosis family pedigrees

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ABSTRACT

Lumbar developmental spinal stenosis (DSS) refers to multilevel pre-existing narrowed spinal canals which predisposes to neural compromise. The objective of study is to identify any inheritance pattern of DSS by utilizing pedigree charts. This was a case series of 13 families with a total of 80 subjects having magnetic resonance imaging (MRI) from L1-S1. Cases (subjects with DSS) or controls (subjects without DSS) were identified by measuring their anteroposterior (AP) vertebral canal diameters. Multilevel model analyses were also performed to evaluate whether there is substantial clustering of observations within the families, and the effect of multilevel DSS. Intraclass correlation coefficient (ICC) and Akaike information criteria (AIC) were compared between models. Correlations between subject demographics and AP vertebral canal diameter were statistically insignificant at all levels. Only vertebral canal cross-sectional area and axial and sagittal vertebral canal diameter were found to be statistically different between cases and controls at all levels (all $p<0.05$). Both males and females were affected by DSS and there was no skipping of generation, which highly suggested DSS followed an autosomal dominant inheritance pattern. After accounting for multilevel DSS, there was a drop of more than 10 in AIC and some variances were also explained within families. This is the first study which suggests multilevel lumbar DSS to have an autosomal dominant inheritance pattern. Within families with a background of DSS, subjects had a smaller canal size, contributed by shortened axial and sagittal AP vertebral canal diameter, and smaller canal cross-sectional area.

Key Words: Developmental spinal stenosis; lumbar; pedigree; family

Level of evidence: Diagnostic level III

INTRODUCTION

Lumbar developmental spinal stenosis (DSS) is described as a pre-existing narrowed lumbar vertebral canal by Verbiest in 1954[1]. It is of great clinical importance as a minor degree of degeneration, such as disc protrusion and facet hypertrophy, may already lead to sufficient neural compression. It is important to differentiate it from lumbar spinal stenosis with dural sac compression which results from degenerative changes. DSS is a developmental narrowing of the neural tube which is independent from degenerative causes.[2] Throughout the years, multiple authors proposed different radiological cut-offs to diagnose DSS[1; 3-5]. It is well-recognized that the anteroposterior (AP) vertebral canal diameter and lamina are significantly shorter in patients with DSS than the general population[3; 4; 6-8]. Due to its developmental nature[9; 10], multilevel stenosis is expected and justified by multiple authors[6; 7; 11]. According to a large-scale multilevel DSS study[12], its prevalence is reported to be 7.3% in the southern Chinese. Clinically, Cheung *et al*[13] reported a 22% reoperation rate for patients who did not receive prophylactic decompression for levels of DSS during index operation. This poor prognostic factor is likely a result of multilevel stenosis as asymptomatic stenotic levels tend not to be operated.

Several genetic mutations are identified to be associated with lumbar spinal stenosis, such as Trp2 and Trp3[14; 15]. In addition, low-density lipoprotein receptor-related protein 5 (LRP5) which plays an important role in bone development is specifically associated with DSS[9]. Nonetheless, majority of the proposed genetic associations are not specific to DSS but to spinal stenosis in general which may be influenced by disc degeneration factors. Furthermore, it is proposed that DSS is a result of genetic disturbance during fetal and postnatal lumbar vertebrae development[16-18]. To understand the role of genetic factors in the pathogenesis of DSS, potential inheritance patterns should be elucidated. Hence, this study aims to study potential inheritance patterns using familial pedigrees of individuals with DSS.

METHODS

Study design and population

This was a pedigree analysis of 13 families with demographic and standardized magnetic resonance imaging (MRI). All probands were subjects of the Hong Kong Disc Degeneration Cohort Study, in which they were openly recruited by advertisement from the general population[4; 5; 19-21]. This is a population-based cohort of approximately 3500 subjects with axial and sagittal lumbosacral (L1-S1) MRI. Probands with extended family members (at least three generations alive and multiple siblings) were invited to participate and encourage their family members to undergo imaging. All subjects were of Chinese ethnicity, and had no previous spinal surgeries. Demographic data including sex, age, weight, height and body mass index (BMI) were obtained. Informed consents were also acquired, and ethics was approved by a local institutional review board.

MRI Measurements

All subjects used 1.5 or 3T HD MRI machines for imaging in supine position. T1- and T2-weighted MRI were utilized[22] in this study for measurement of bony parameters. For axial MRI, the field of view was 21cm×21cm, slice thickness was 4mm, slice spacing was 0.4mm, and imaging matrix was 218×256. For sagittal MRI, the field of view was 28cm×28cm, slice thickness was 5mm, slice spacing was 1mm, and imaging matrix was 448×336. The repetition time were 500ms-800ms and 3320ms, while the echo time were 9.5ms and 85ms for T1- and T2-weighted MRI respectively. 11 slices were available per vertebral level and parallel slices were made according to the disc and pedicle levels.

One investigator was blinded to all clinical information before and throughout the measuring process. The imaging files were provided to the investigator randomly to avoid bias.

The slice that showed the pedicle, lamina, spinous process and vertebral body clearly with the thickest pedicle diameter were identified as optimal for axial MRI measurements. The following measurements were obtained (Figure 1) for L1-S1 axial scans: midline AP vertebral body diameter, mid-vertebral body width, AP vertebral canal diameter, interpedicular distance, left and right pedicle width, vertebral canal cross-sectional area (Figure 2), and left and right facet joint angle (Figure 3). The AP vertebral canal diameter was measured by a line from the midpoint of the base of the vertebral body to the base of the spinous process. The vertebral canal cross-sectional area was measured by drawing the boundary of the vertebral canal. The angle made by the junction between a line joining the medial and lateral opening of the facet joint and the transverse plane was the facet joint angle. The midsagittal cut that showed the most prominent spinous process were identified as optimal for L1-S1 sagittal MRI measurements. The following measurements were obtained (Figure 4) for sagittal scans: midline AP vertebral body diameter, mid-vertebral body height and vertebral canal diameter. The vertebral canal cross-sectional area was measured by ImageJ (U.S. National Institutes of Health, USA). All other MRI measurements were obtained using Centricity Enterprise Web V3.0 (GE Medical Systems, St. Louis, MO).

Definition of DSS

A developmentally narrowed canal was defined if the AP vertebral canal diameter was at L1<19mm, L2<19mm, L3<18mm, L4<18mm, L5<18mm, S1<16mm[4]. Any subjects with AP vertebral canal diameters below these level-specific cut-offs in at least 3 levels were identified as cases of DSS. Subjects with 2 or less levels of narrowed canal were identified as controls.

Pedigree Chart Illustration

All pedigree charts in this study followed the standardized human pedigree nomenclature proposed by the Pedigree Standardization Task Force of the National Society of Genetic Counselors[23; 24]. Subject and family confidentiality were carefully considered, and only minimum amount of information was included in each pedigree chart. Question mark '?' indicated family members who disagreed to join the study and hence missing MRI data. All pedigree charts were drawn by using Genial Pedigree Draw (Genial Genetics Solutions Ltd, Chester, UK).

Statistical Analysis

Descriptive and frequency statistics were performed. The means and ranges of subject demographics were calculated. To account for any confounding factors between demographics and AP vertebral canal diameter, nonparametric correlation analyses using Kendall tau's b and Spearman's correlation were performed for binary and continuous variables respectively. In addition, Spearman's correlation analyses were conducted to evaluate the impact of other imaging phenotypes on the AP vertebral canal diameter. A correlation coefficient of -0.3 to 0.3 was noted to be poor and negligible, while a coefficient of 0.3 to 0.5 or -0.3 to -0.5 was noted to be low correlation, 0.50 to 0.7 or -0.50 to -0.70 was noted to be moderate, and 0.70 to 0.90 or -0.70 to -0.90 was noted to be high[25]. Mann-Whitney U test was also performed to detect measurement differences between cases and controls.

Multilevel modelling analyses were then performed for each spinal level. Basic model was first established without any factors. Significant models with p-value <0.05 indicated variations exist. Intraclass correlation coefficient (ICC) ≥ 0.05 was an indication of substantial clustering observations within families[26]. Multilevel DSS (Yes/No) was then introduced into the models to evaluate its effects within these families. Akaike information criteria (AIC) were used to compare the in-sample fit between the basic model and the multilevel model. An AIC

difference between models of less than 2 was considered as no difference, a difference of 4-7 had little difference, while a difference of more than 10 was considered as substantial difference[27]. A P-value of less than 0.05 was considered as statistically significant. All statistical analysis was performed using SPSS Statistics 26 (IBM SPSS Inc., Chicago, Illinois).

RESULTS

There were 13 families and a total of 233 family members. There were 80 subjects available for analysis while 153 individuals did not undergo MRI and were labelled as "?" in the pedigree charts. Up to 71 cases with multilevel DSS (27 males and 44 females) and 9 controls (5 males and 4 females) were identified. Demographics of all cases and controls are presented in Table 1. The mean values and ranges of their imaging parameters are listed in Table 2. The measurements were statistically significantly different between cases and controls for vertebral canal cross-sectional area and axial and sagittal AP vertebral canal diameter. The results of nonparametric correlation analyses are presented in Table 3. Only axial vertebral canal cross-sectional area and sagittal vertebral canal diameter were correlated to axial AP vertebral canal diameter at all levels (all $p<0.05$).

Pedigrees of interest

All families showed DSS involvement of 2 or more successive generations and all first-degree relatives. All pedigree charts demonstrated involvement of males and females, and there was no skipping of generations. A basic model and multilevel model were then constructed, and the AIC and ICC values are presented in Table 4. The models were statistically significant at L1-S1 ($p<0.001$).

For detailed illustration of the inheritance pattern of DSS, we selected families with sample size larger than 5 and at least 1 control. 4 out of 13 families were chosen. The other 9

families were included in the appendix. Of those 9 families, there was a total of 44 cases, 1 control, and 111 subjects without MRI.

Family 1 – 10 cases (4 males and 6 females) and 2 controls (1 male and 1 female) were identified across two generations (Figure 5). There were 14 individuals without MRI. The mean age of cases was 33.8 (range=13.1-46.8) and mean BMI was 23.7 kg/m^2 (range=19.6-30.9), while controls had mean age of 31.5 (range=14.3-48.6) and mean BMI of 29.4 kg/m^2 (range=26.0-32.8). The average AP vertebral canal diameter for cases was as follows: L1=15.8mm, L2=15.7mm, L3=15.1mm, L4=15.0mm, L5=15.4mm, S1=15.3mm. For controls, their mean measurement was: L1=17.9mm, L2=17.6mm, L3=19.3mm, L4=19.6mm, L5=22.8mm, S1=19.6mm. The proband (1-21) was a member of the third generation, and his sister (1-22) was also a case. Their father (1-7) was affected by multilevel DSS. Throughout the second generation, 4 siblings (1-7, 1-9, 1-13 and 1-14) were identified as cases, and one sibling (1-5) was a control. Furthermore, in the subfamily where both parents (1-9 and 1-10) were affected, both of their offspring (1-23 and 1-24) were also identified as cases. It was noted that the subfamily on the left had an unaffected offspring (1-15) even though the mother (1-4) was a case. All cases had canal narrowing at L1-S1, except individual 1-4, 1-7 and 1-13 who had L1-L5 narrowing.

Family 2 – 5 cases (2 males and 3 females) and 4 controls (2 males and 2 females) were identified across three generations (Figure 6). There were 12 individuals without MRI. The mean age of cases was 39.5 (range=23.0-50.5) and mean BMI was 26.9 kg/m^2 (range=22.1-33.5), while controls had mean age of 43.9 (range=18.4-68.1) and mean BMI of 26.7 kg/m^2 (range=21.4-31.4). The average AP vertebral canal diameter for cases was as follows: L1=17.4mm, L2=17.3mm, L3=16.3mm, L4=18.2mm, L5=18.8mm, S1=18.5mmm. For controls, their mean measurement was: L1=17.9mm, L2=17.5mm, L3=18.9mm, L4=20.9mm, L5=20.9mm, S1=18.9mm. The proband (2-8) was a member of the second generation with

narrowed vertebral canal at L1-L5. Throughout the second generation, at least 3 siblings (2-5, 2-8 and 2-13) were cases, with narrowed L1 and L3-L4, L1-L5 and L1-L5 respectively. However, two additional siblings (2-3, 2-10) of the proband were controls. Moreover, their mother (2-2) was also not affected by multilevel DSS. 2 affected parents (2-5 and 2-6) in the second generation had an affected daughter (2-16) with narrowed L1-L3, but also an unaffected son (2-17).

Family 3 – 5 cases (3 males and 2 females) and 1 control (1 female) were identified across three generations (Figure 7). There were 8 individuals without MRI. The mean age of the cases was 43.8 (range=30.3-58.6) and mean BMI was 26.7 kg/m^2 (range=23.5-29.8). The only control was 43 years of age with a BMI of 17.7 kg/m^2. The mean AP vertebral canal diameter of the cases was as follows: L1=18.2mm, L2=16.8, L3=15.7, L4=16.8, L5=17.3, S1=16.8, while the control had L1=21.3mm, L2=20.0, L3=20.2, L4=17.9, L5=19.8, S1=N/A. The proband (3-4) was a member of the second generation with narrowed canal at L1-S1, who also had at least 2 siblings (3-5 and 3-9) being affected, with narrowed L1-L3 with L5-S1 and L1-L5 respectively. Their mother (3-2) also had multilevel DSS at L1-L4. However, it was noted that two affected parents (3-3 and 3-4) in the second generation had a daughter (3-11) without multilevel DSS.

Family 4 – 7 cases (3 males and 4 females) and 1 control (1 male) were identified across three generations (Figure 8). There were 8 individuals without MRI. The mean age of the cases was 43.2 (range=18.3-69.6) and mean BMI was 23.9 kg/m^2 (range=19.5-33.5). The only control was 18.3 years of age with a BMI of 31.4 kg/m^2. The mean AP vertebral canal diameter of the cases was as follows: L1=17.1mm, L2=17.2mm, L3=17.2mm, L4=18.3mm, L5=20.1mm, S1=18.9mm, while the control had L1=19.0mm, L2=16.4mm, L3=20.4mm, L4=20.2mm, L5=22.6mm, S1=N/A. The proband (4-4) was a member of the second generation with narrowed vertebral canal at L1-L3, and at least 1 of her siblings (4-7) were affected at L1-

L4. Their mother (4-2) also had multilevel DSS at L1-L4. In addition, both of their husbands (4-3 and 4-6) had narrowed vertebral canal at L1 and L3-L4 and L1-L5 respectively. Both subfamilies had affected parents and offspring (4-11 and 4-14), except individual 4-12 who was a control.

DISCUSSION

Multilevel DSS impacts the outcome of patients with lumbar spinal stenosis as it contributes to a low threshold of compressive symptoms and high risk of reoperations[13] due to shorter laminae and AP vertebral canal diameter as compared to normal individuals. Current literature identified several genetic components that associated with spinal stenosis, such as Trp2, Trp3 and LRP5[9; 14; 15]. In addition, it is recognized that the AP vertebral canal diameter ceases to change beyond pubertal growth and skeletal maturity[28]. In patients with DSS, this evidence points towards a genetic abnormality as the cause. However, the distribution of DSS and its inheritance pattern within a family is unknown. Family studies provide us with knowledge of its mode of inheritance and to estimate the probability of having the disease phenotype among offspring. Also, we can determine the familial risks and stratify these risks by family history. By utilizing pedigree analysis, it is also possible to separate environmental causes from genetic causes[29; 30]. Our pedigree charts showed involvement of both males and females with no skipping of generation, which suggests multilevel DSS to be an autosomal dominant disease.

All families showed involvement of multilevel DSS in first-degree relatives and in at least 2 generations, which suggests the entity as highly integrated between close relatives. Due to its relatively high prevalence of 7.3% in the population[12], it is possible and reasonable that both parents being cases of multilevel DSS could marry each other as shown in Family 1 to Family 4. It is also important to note that the unaffected mother (2-2) in Family 2 gave birth to

at least 3 cases (50%) and 2 controls (33%), which may imply the father (2-1) inheriting the disease gene to his offspring. This is consistent with our proposed inheritance pattern as 50% of the offspring was affected. Therefore, it is likely that either of the parents or both parents could give the disease gene to their offspring, which suggests multilevel DSS to have an autosomal dominant inheritance pattern. In addition, some families had unaffected individuals. This is coherent with the autosomal dominant inheritance pattern as uncommonly, the individual receives two recessive genes from both parents that spare the individual from having the pathology. When investigating into the levels of canal narrowing, the parental and offspring patterns were similar in most cases. In Family 1, all paternal and offspring had at least 5 levels of canal narrowing. Likewise, mother with L1-L4 narrowing had offspring with at least 4 levels of canal narrowing in Family 2, with L1-L3 being their common levels. Family 4 also reiterated our observations in which parents with L1-L4 narrowing had offspring with L1-L3 and L1-L4 narrowing as shown in Family 4. This suggests that the inheritance is level-specific to a certain extent. It also allows us to predict the affected levels and the probability of having DSS in the offspring of individuals with DSS. However, our results are still preliminary, and a larger sample size should be utilized to justify the relationship in the future.

The vertebral canal size is the imaging phenotype that is consistent in the pedigrees and inherited within families. Parameters that are regarded as measurements of vertebral canal size included axial and sagittal AP vertebral canal diameter, interpedicular distance and vertebral canal cross-sectional area. Except for the interpedicular distance, the other three parameters were well correlated. In contrast, the remaining MRI measurements were poorly or negligibly[25] correlated, which highlights their limited association by inheritance in our model. It is coherent with the current evidence[28] that subjects' demographics were not correlated with the AP vertebral canal diameter. Our results provide additional confirmation that the canal size is an independent structure and it does not vary with age, sex, and body size. Comparing with the

vertebral canal cross-sectional area that requires precise outlining of the canal circumference, the axial AP vertebral canal diameter plays a more important role in representing the canal size as it is the simplest and most convenient measurement with minimal variations and inconsistency. It is less likely to be affected by the curvature of the vertebrae and disease of the disc or endplate[4], which may lead to variations in the sagittal AP vertebral canal diameter. Furthermore, a multilevel model was used to indicate the role of multilevel DSS and canal diameter within a family. By introducing the multilevel DSS factors, variations are lessen and resemblance of these families increases as indicated by a fall in ICC. There are also substantial differences[27] between models after accounting for multilevel DSS as suggested by a drop in AIC.

Although our study provides additional insights to the genetic background of DSS, there are several limitations. Firstly, there were missing data in families as some subjects refused to participate. However the autosomal dominance inheritance pattern is supported based on the expected high prevalence rate of 7.3% in the population which suggests a high chance of both parents to be cases. Hence we only focused on large families with three generations for appreciation of the inheritance pattern of DSS. Nevertheless, it is important to note that the conclusions could be further strengthened with more subjects analysed. We are not sure if the other individuals had DSS or not. Secondly, further analyses of the family cohort with gene sequencing is required to better understand the causative genetic polymorphism responsible. Lastly, the generalizability of the study is limited as we only investigated the inheritance pattern of DSS in Chinese. Nevertheless, concentrating in only one ethnicity allows us to minimize variations. Further investigations should expand to different ethnicities and focus on identifying the genetic sequence of DSS. The role of epigenetics could also be a direction for future studies. It is also important to note that the spinal canal should be studied

in a three dimensional manner. Future study with a three dimensional measurement is warranted.

CONCLUSION

Utilizing pedigree charts, this is the first study that identifies the inheritance pattern of DSS in the Chinese. There is evidence that points towards an autosomal dominant inheritance pattern. Only the vertebral canal size is affected within families, which highlights its likely independent inheritable role in bony maldevelopment. Although preliminary, our study provides additional understanding to the potential genetic background of DSS. Future studies should investigate the role of DSS in other ethnicities, and identify its genetic origin.

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FIGURE LEGENDS

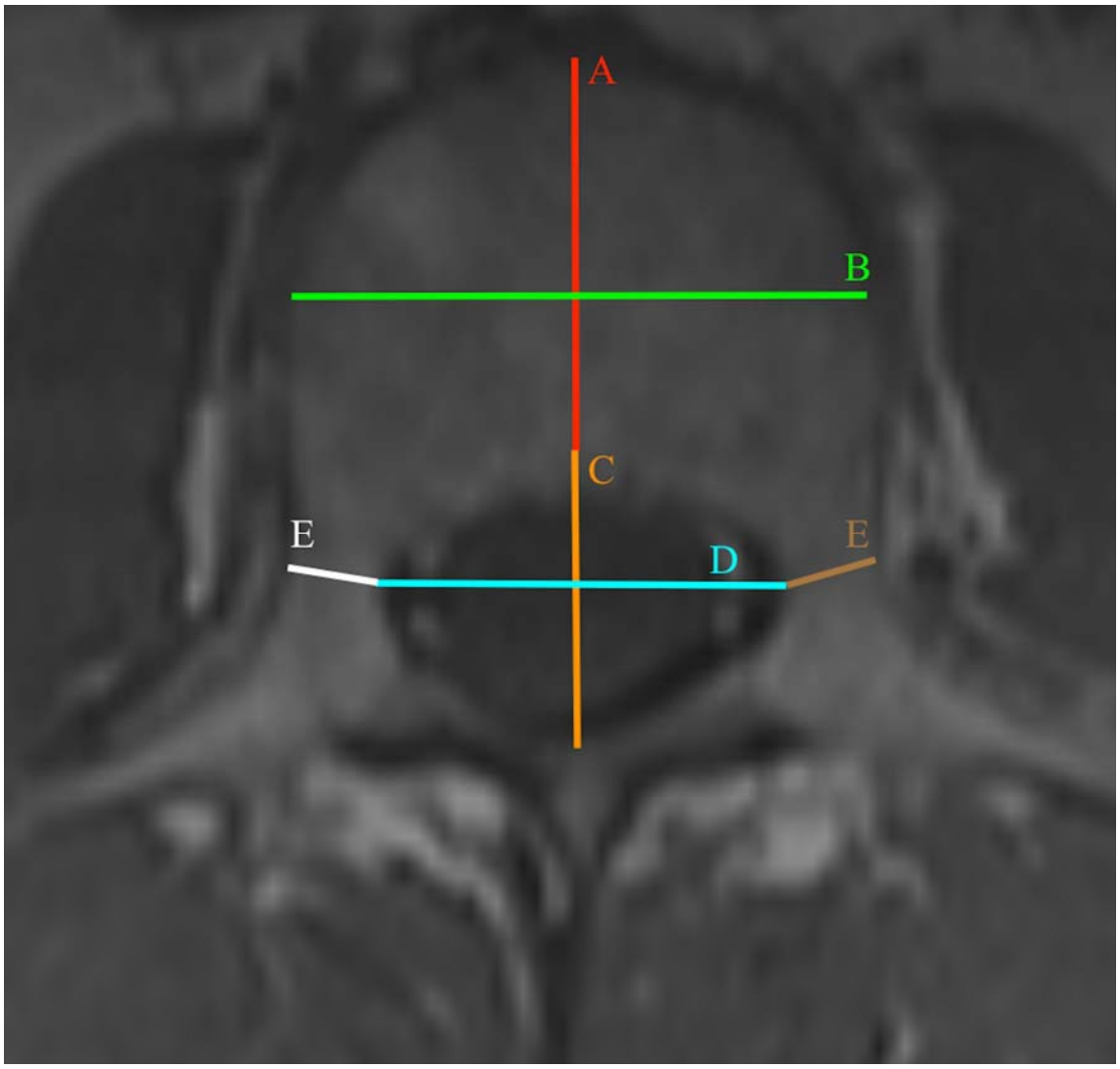


Figure 1. Axial magnetic resonance imaging measurements: (A) midline AP vertebral body diameter; (B) mid-vertebral body width; (C) AP vertebral canal diameter; (D) interpedicular distance; (E) left and right pedicle width.

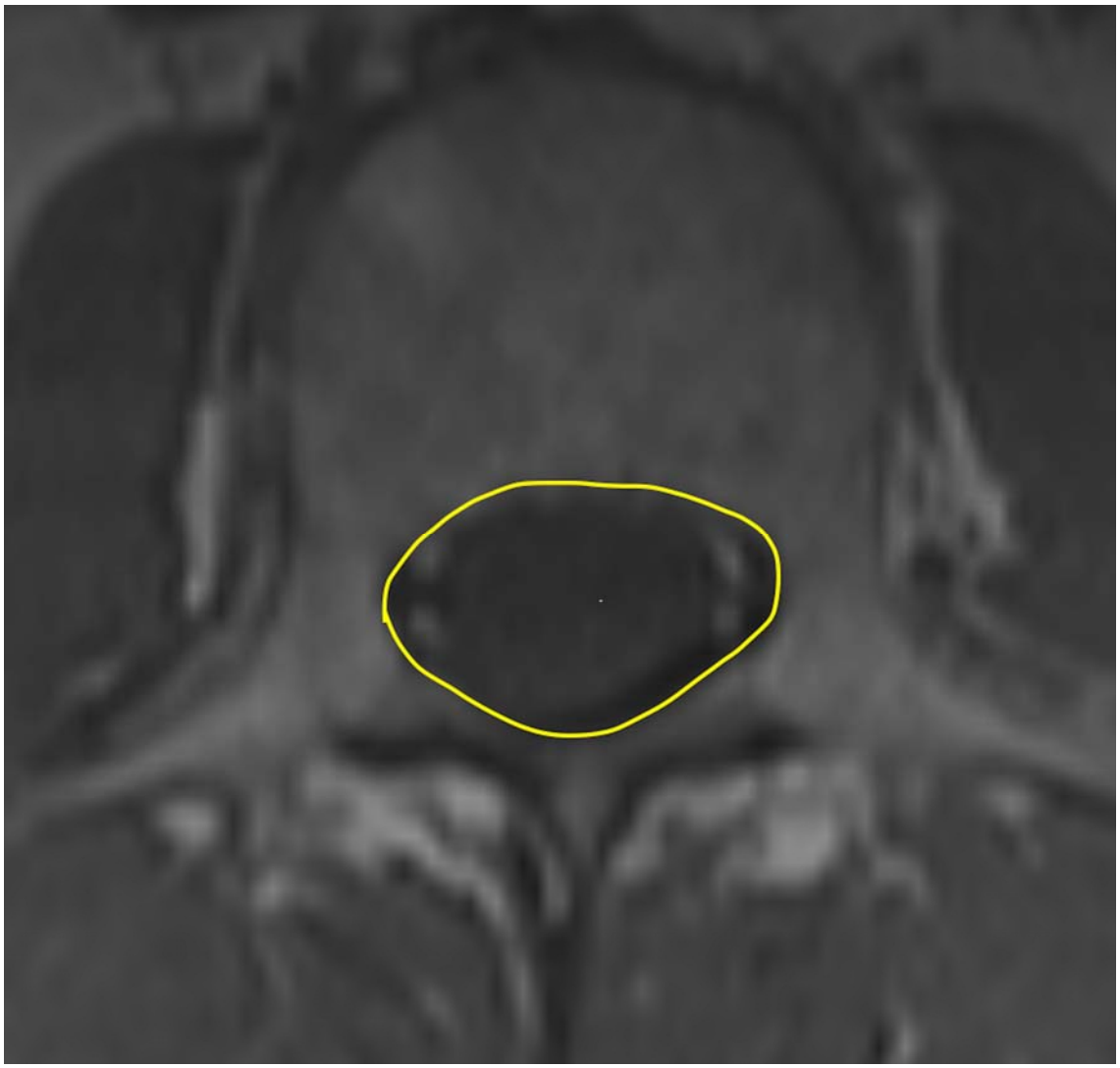


Figure 2: Axial magnetic resonance imaging measurement of the vertebral canal cross-sectional area (measured by drawing the boundary of the vertebral canal).

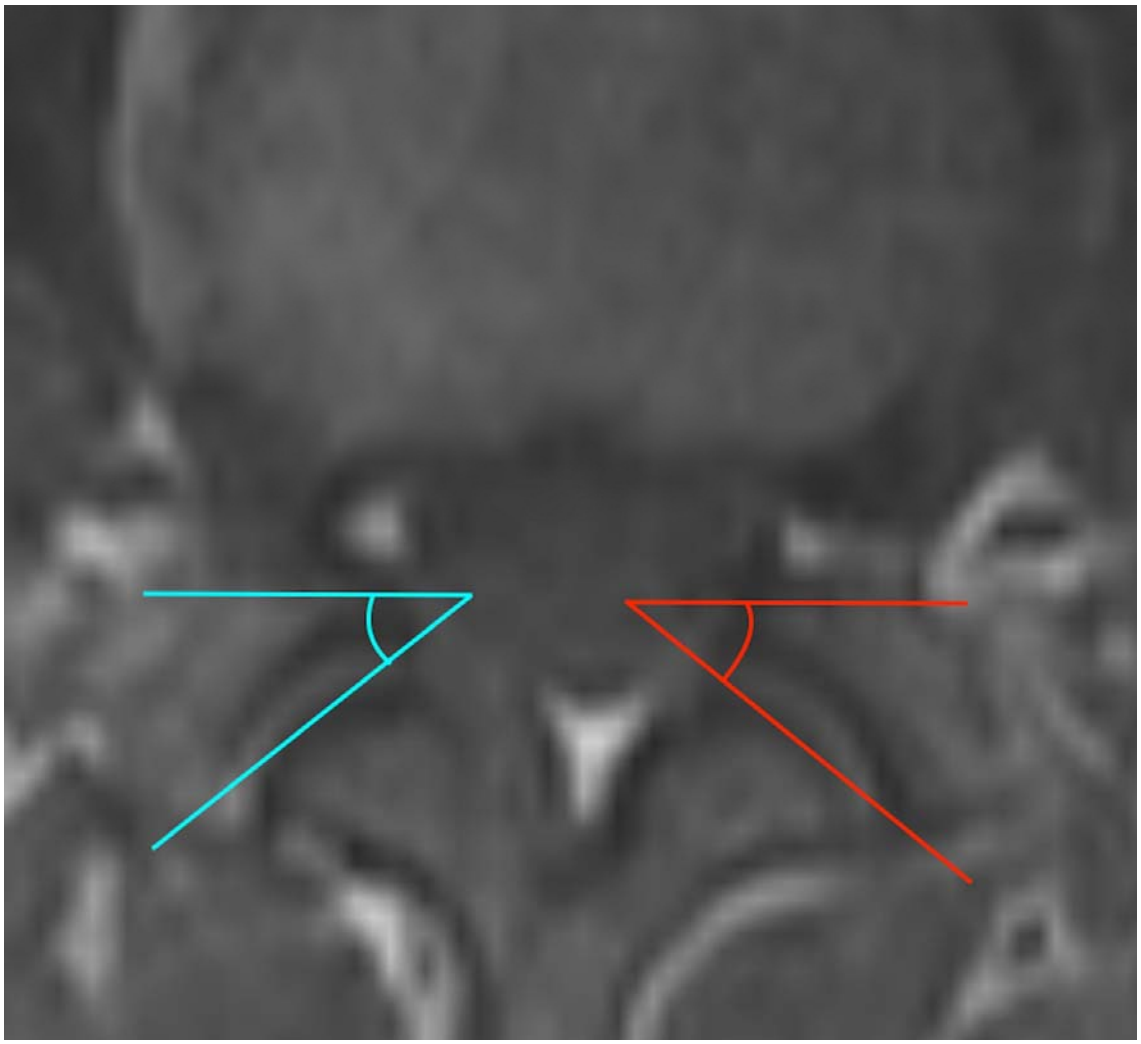


Figure 3. Axial magnetic resonance imaging measurements of the left and right facet joint (angle made by the junction between a line joining the corners of the facet joint and the transverse plane was the facet joint angle).

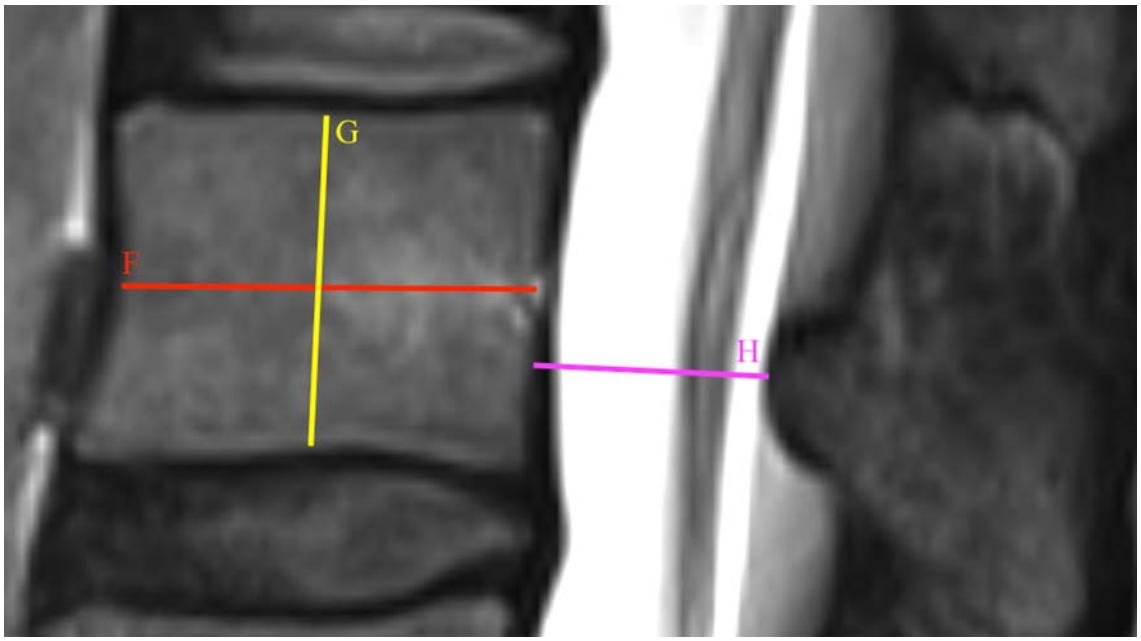


Figure 4. Sagittal magnetic resonance imaging measurements: (F) midline AP vertebral body diameter; (G) mid-vertebral body height; and (H) vertebral canal diameter.

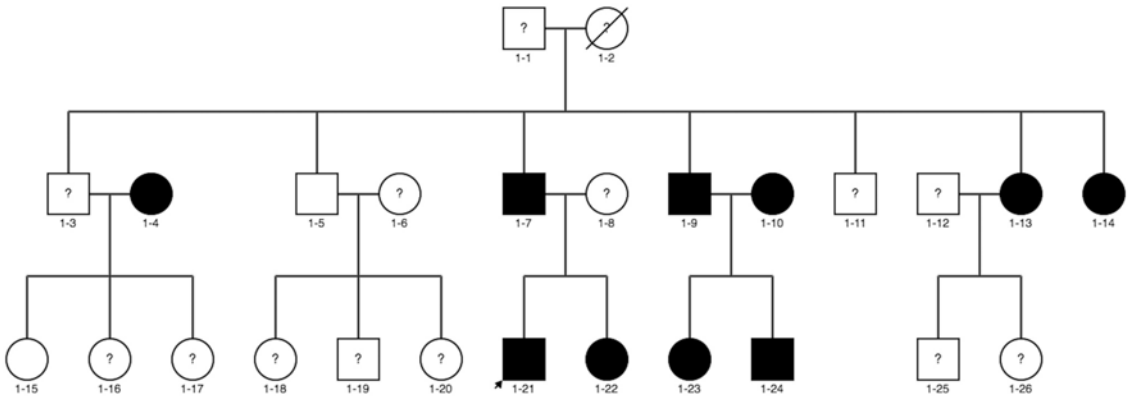


Figure 5. *Family 1* – 10 cases (4 males and 6 females), 2 controls (1 male and 1 female) and 14 individuals without MRI were identified across two generations. The proband (1-21) was a member of the third generation.

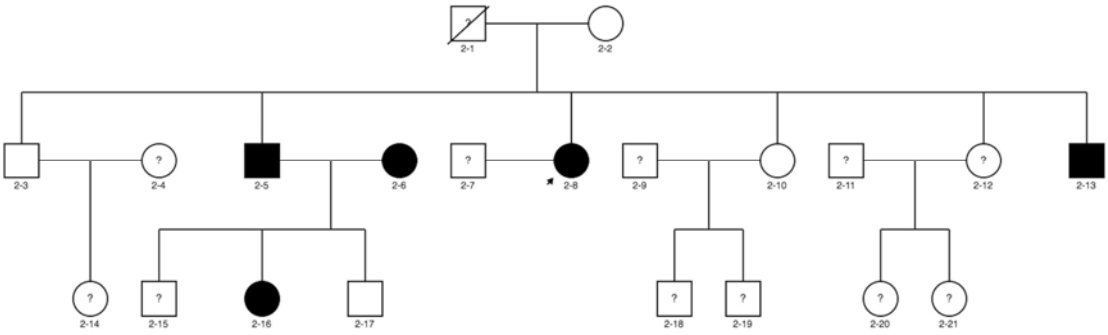


Figure 6. *Family 2* – 5 cases (2 males and 3 females), 4 controls (2 males and 2 females) and 12 individuals without MRI were identified across three generations. The proband (2-8) was a member of the second generation.

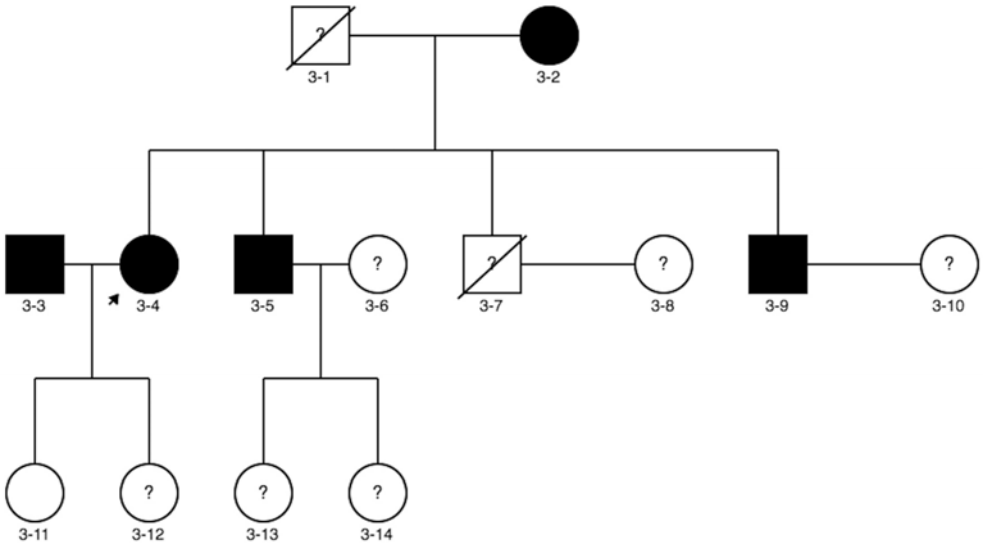


Figure 7. *Family 3* – 5 cases (3 males and 2 females), 1 control (1 female) and 8 individuals without MRI were identified across three generations. The proband (3-4) was a member of the second generation.

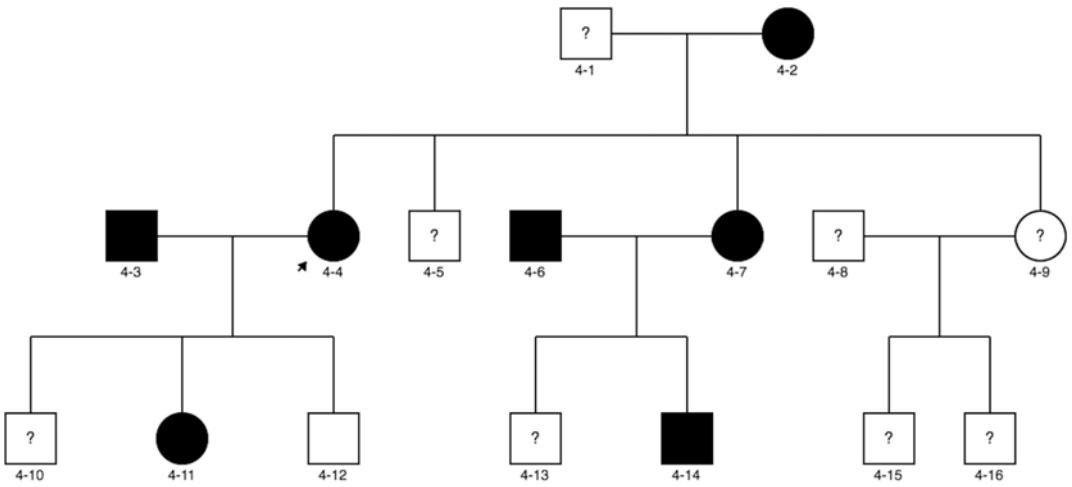


Figure 8. *Family 4* – 7 cases (3 males and 4 females), 1 control (1 male) and 8 individuals without MRI were identified across three generations. The proband (4-4) was a member of the second generation.

Table 1: Demographics of Cases and Controls

	Cases Mean (range)	Controls Mean (range)
Number of subjects	71	9
Age (years)	40 (13-70)	33 (14-68)
Weight (kg)	62.5 (45.0-98.0)	72.0 (43.0-94.0)
Height (m)	1.62 (1.46-1.80)	1.65 (1.56-1.73)
Body Mass Index (kg/m^2)	26.0 (17.7-32.8)	23.9 (18.3-33.5)

Table 2. Mean Measurements of All Imaging Phenotypes

	Cases Mean *mm*, Except for Cross-sectional Area (mm^2) and Facet Joint Angle (^{o}) (range)	Controls Mean *mm*, Except for Cross-sectional Area (mm^2) and Facet Joint Angle (^{o}) (range)	P-Value
Axial Midline Anteroposterior Vertebral Body Diameter			
L1	26.3 (21.9-32.5)	28.1 (23.1-33.3)	<0.001
L2	27.5 (21.5-33.1)	28.7 (24.2-33.8)	0.002
L3	29.3 (22.6-35.8)	29.9 (25.7-34.6)	<0.001
L4	29.6 (25.4-38.4)	30.0 (28.0-32.2)	<0.001
L5	30.6 (24.7-37.3)	31.2 (27.8-36.2)	<0.001
S1	29.8 (17.9-37.5)	31.7 (29.2-35.1)	0.138
Axial Mid-Vertebral Body Width			
L1	35.1 (26.8-43.3)	36.8 (31.3-41.1)	0.095
L2	36.2 (29.0-46.2)	38.0 (33.9-42.6)	0.232
L3	37.1 (30.3-45.1)	38.2 (33.5-41.4)	0.571
L4	39.1 (29.3-47.1)	40.2 (36.4-44.0)	0.577
L5	43.7 (32.1-54.8)	46.7 (38.6-54.2)	0.762
S1	50.0 (29.1-63.1)	48.9 (45.2-52.0)	0.347
Axial Anteroposterior Vertebral Canal Diameter			
L1	16.7 (13.4-19.4)	18.7 (17.3-21.3)	0.235
L2	16.2 (12.6-19.4)	17.9 (16.4-20.0)	0.151
L3	15.6 (11.6-20.0)	19.4 (18.3-20.4)	0.326
L4	15.9 (11.6-20.7)	19.8 (17.7-26.6)	0.469
L5	16.6 (12.3-25.1)	21.2 (18.7-24.2)	0.121
S1	16.1 (10.1-23.8)	18.0 (14.6-20.3)	0.582
Interpedicular Distance			
L1	22.5 (19.0-27.8)	23.2 (21.2-25.6)	0.503
L2	23.1 (19.1-27.9)	23.9 (18.5-29.9)	0.235
L3	23.9 (19.1-30.4)	25.9 (21.7-30.9)	0.051
L4	25.7 (21.7-30.3)	26.3 (20.7-29.2)	0.271
L5	30.1 (24.3-35.0)	29.2 (22.5-32.7)	0.579
S1	32.6 (26.5-37.4)	34.3 (31.1-36.9)	0.263
Vertebral Canal Cross-sectional Area			
L1	302.7 (223.4-405.6)	370.8 (328.1-422.5)	<0.001
L2	299.7 (199.8-426.9)	367.4 (300.3-445.9)	<0.001
L3	298.4 (201.7-468.6)	374.4 (346.6-444.8)	<0.001
L4	313.3 (217.5-499.8)	418.4 (347.2-516.3)	<0.001
L5	380.6 (249.4-643.0)	505.9 (383.0-587.4)	<0.001
S1	420.3 (254.4-612.2)	495.5 (383.2-636.2)	0.129
Right Pedicle Width			

L1	5.2 (2.3-8.6)	4.9 (3.6-6.5)	0.523
L2	5.3 (3.2-9.2)	6.1 (3.8-8.4)	0.120
L3	7.0 (3.6-10.0)	6.8 (5.2-9.2)	0.608
L4	9.1 (4.1-13.2)	8.3 (2.4-10.6)	0.922
L5	13.0 (7.6-18.0)	13.8 (6.8-18.4)	0.470
S1	16.6 (9.3-22.6)	15.6 (11.6-19.9)	0.683
Left Pedicle Width			
L1	5.3 (2.0-9.3)	5.0 (4.1-6.2)	0.615
L2	5.4 (2.7-8.5)	5.8 (3.2-7.9)	0.458
L3	7.1 (4.4-10.6)	7.3 (4.9-10.0)	0.832
L4	9.1 (6.2-12.7)	8.8 (6.3-10.3)	0.623
L5	12.5 (6.3-16.7)	13.1 (8.8-16.9)	0.565
S1	17.3 (10.4-24.7)	16.5 (13.0-20.9)	0.653
Right Facet Joint Angle			
L1	54.0 (42.1-69.2)	51.9 (46.7-60.8)	0.250
L2	53.1 (36.8-67.8)	51.6 (41.8-57.2)	0.597
L3	47.3 (32.6-69.3)	42.5 (28.8-59.0)	0.136
L4	40.5 (18.6-63.7)	34.1 (24.2-48.7)	0.035
L5	35.7 (18.1-55.8)	34.7 (23.0-46.6)	0.875
Left Facet Joint Angle			
L1	59.5 (40.3-77.8)	55.1 (48.8-65.2)	0.063
L2	57.4 (42.9-76.4)	48.9 (40.2-55.1)	0.004
L3	49.9 (33.0-67.3)	42.2 (35.2-52.6)	0.016
L4	40.1 (22.1-61.4)	36.3 (19.8-51.5)	0.424
L5	35.0 (16.1-61.2)	29.7 (23.2-37.2)	0.260
Sagittal Midline Anteroposterior Vertebral Body Diameter			
L1	25.7 (21.1-31.8)	27.3 (20.9-33.6)	0.147
L2	26.6 (21.3-32.8)	27.8 (23.9-32.2)	0.214
L3	28.2 (22.0-39.2)	27.9 (24.0-32.6)	0.943
L4	29.1 (23.3-37.8)	29.1 (26.0-32.5)	0.890
L5	28.2 (23.3-36.4)	29.7 (27.0-34.0)	0.244
S1	20.4 (14.8-27.3)	20.8 (17.7-23.6)	0.512
Sagittal Mid-vertebral Body Height			
L1	22.7 (18.4-27.3)	22.9 (17.2-27.4)	0.678
L2	23.5 (19.5-27.4)	23.4 (17.2-27.4)	0.694
L3	23.7 (20.4-27.7)	24.1 (18.7-27.4)	0.492
L4	23.0 (19.8-27.3)	24.1 (21.2-26.3)	0.136
L5	22.5 (18.7-27.5)	23.1 (20.7-25.9)	0.349
S1	24.6 (19.8-30.8)	25.5 (20.7-27.8)	0.198
Sagittal Vertebral Canal Diameter			
L1	15.9 (12.6-19.4)	16.6 (14.0-18.1)	0.195
L2	15.3 (10.0-18.2)	17.1 (16.3-18.7)	<0.001

L3	14.9 (10.7-18.7)	17.7 (16.2-19.3)	<0.001
L4	14.8 (10.5-19.8)	17.3 (15.2-20.1)	<0.001
L5	15.3 (10.2-22.5)	17.9 (14.5-19.8)	<0.001
S1	12.1 (8.6-15.9)	14.1 (12.4-18.6)	0.012

Table 3. Correlation of AP Vertebral Canal Diameter with Demographics and Different Imaging Phenotypes

	L1	L2	L3	L4	L5	S1
Age	-0.053	0.046	-0.105	-0.067	-0.077	0.123
Sex	-0.022	-0.098	-0.036	0.086	0.069	0.195
BMI	0.056	-0.136	-0.087	0.100	0.192	0.196
AP Vertebral Body Diameter	-0.007	-0.231*	-0.228*	-0.126	-0.042	-0.165
Mid-vertebral body width	0.073	-0.040	-0.152	-0.065	0.087	0.207
Interpedicular distance	0.126	0.075	0.254*	0.213	0.018	-0.089
Vertebral Canal Cross-sectional Area	0.591*	0.578*	0.672*	0.677*	0.756*	0.689*
Left pedicle width	-0.256*	-0.123	-0.039	-0.163	0.130	0.254
Right pedicle width	-0.287*	-0.206	-0.245*	-0.194	0.135	0.186
Right Facet Joint Angle	-0.112	-0.302*	-0.278*	-0.336*	-0.284*	N/A
Left Facet Joint Angle	-0.308*	-0.380*	-0.442*	-0.318*	-0.211	N/A
Sagittal vertebral body diameter	0.002	-0.100	-0.156	-0.019	-0.036	0.031
Sagittal mid-vertebral body height	0.040	-0.092	0.167	0.274*	0.153	-0.100
Sagittal vertebral canal diameter	0.419*	0.480*	0.663*	0.719*	0.656*	0.558*

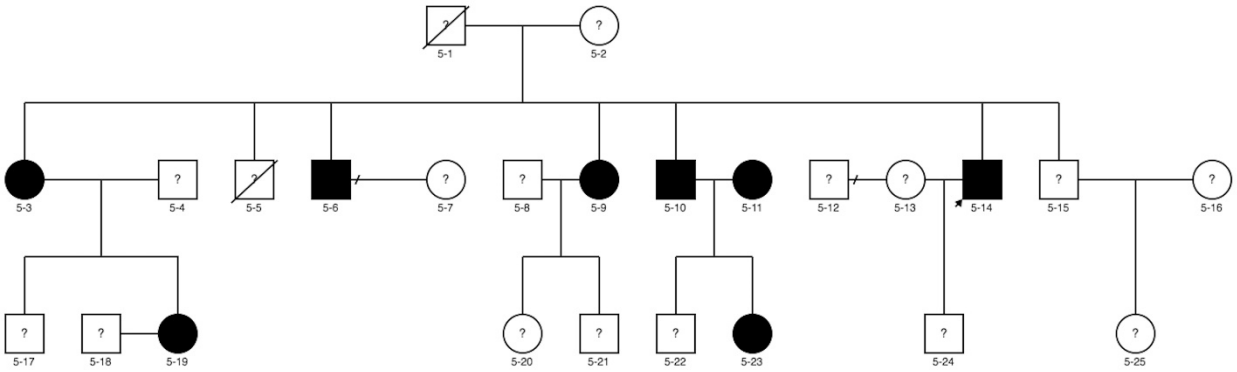
**Statistically significant at 0.05 level.*
AP, anteroposterior; BMI, body mass index; N/A, not applicable.

Table 4. AIC and ICC Before and After Introducing Multilevel DSS

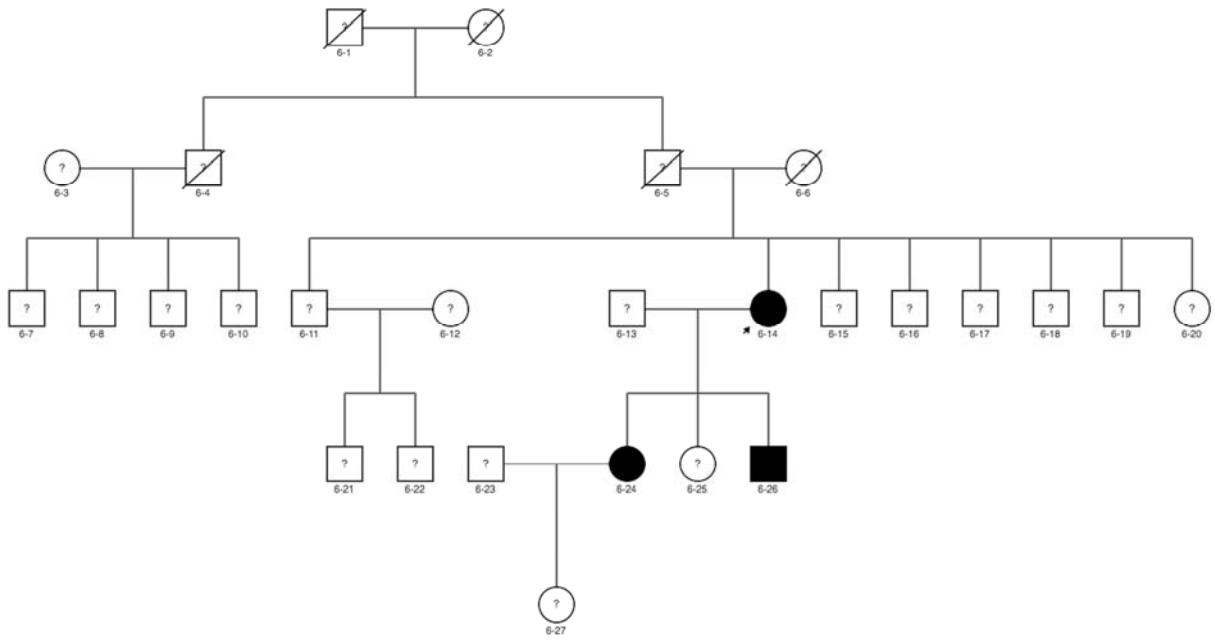
	Before Introducing Multilevel DSS			After Introducing Multilevel DSS		
	Overall Intercepts of Models	AIC	ICC	Overall Intercepts of Models	AIC	ICC
L1	16.96*	267.538	0.302	18.63*	247.525	0.313
L2	16.34*	297.974	0.156	17.79*	288.682	0.085
L3	15.96*	331.595	0.239	19.17*	295.175	0.222
L4	16.21*	351.260	0.378	18.95*	331.872	0.329
L5	16.96*	370.872	0.406	20.39*	345.554	0.395
S1	16.44*	284.530	0.205	17.82*	280.843	0.191
	**Statistically significant at the 0.05 level.* DSS, developmental spinal stenosis; AIC, Akaike information criteria; ICC, intraclass correlation coefficient.					

APPENDIX

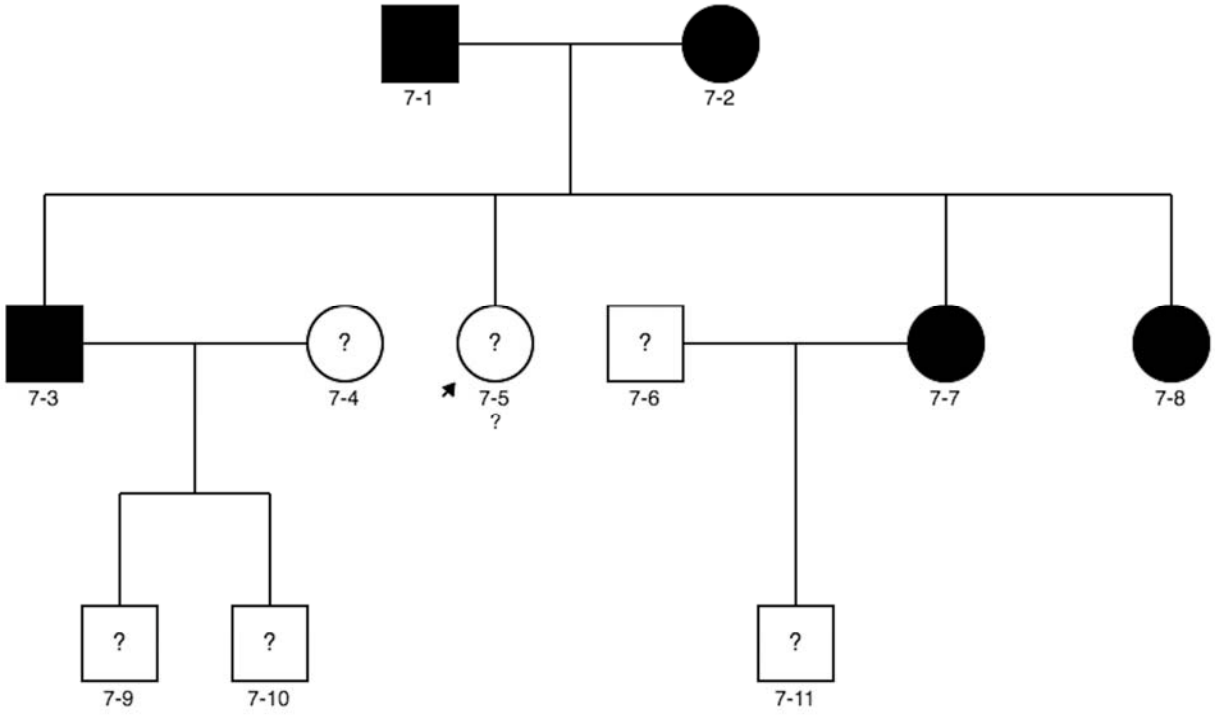
Appendix 1. Family 5 had 8 cases (3 males and 5 females) and no controls across two generations. The proband (5-14) was a member of the second generation. Both parental (5-3, 5-10 and 5-11) and their offspring (5-19 and 5-23) had multilevel DSS.



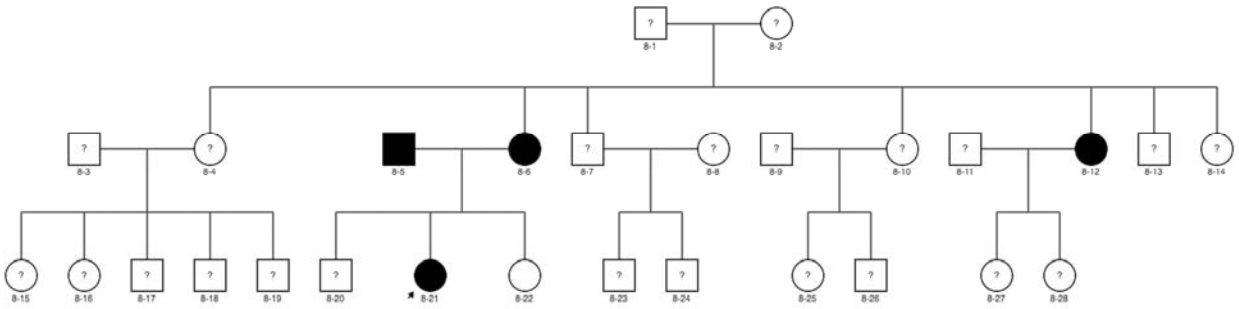
Appendix 2. Family 6 had 3 cases (1 male and 2 females) and no controls across two generations. The proband (6-14) was a member of the third generation. Both parental (6-14) and offspring (6-24 and 6-26) had multilevel DSS.



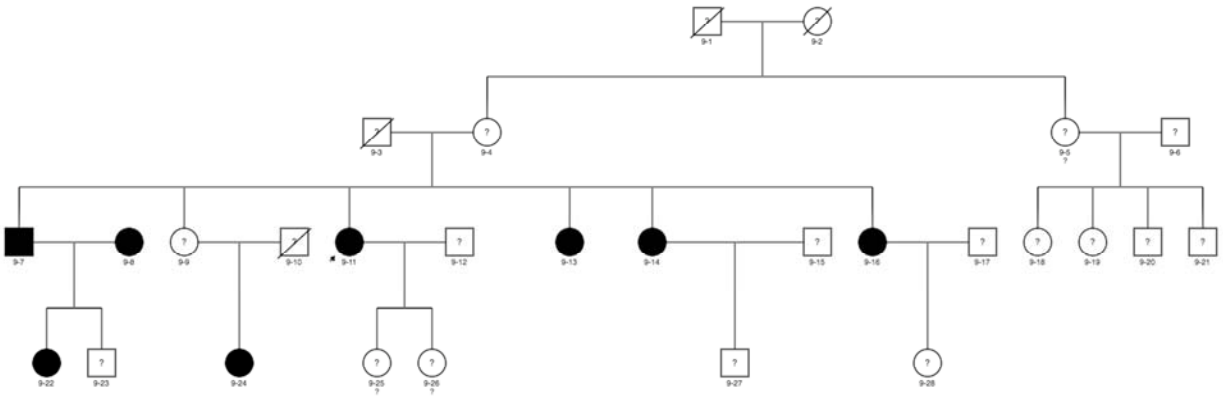
Appendix 3. Family 7 had 5 cases (2 males and 3 females) and no controls across two generations. The proband (7-5) was a member of the second generation. Both parental (7-1 and 7-2) and their offspring (7-3, 7-7 and 7-8) had multilevel DSS.



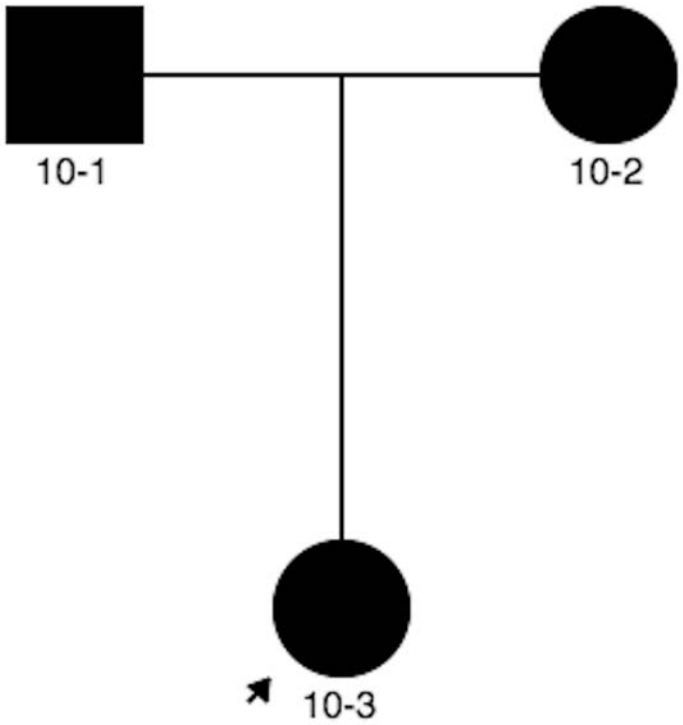
Appendix 4. Family 8 had 4 cases (1 male and 3 females) and 1 control (1 female) across two generations. The proband (8-21) was a member of the third generation. Both parents (8-5 and 8-6) had multilevel DSS, while one of their offspring (8-21) had multilevel DSS, and another was a control (8-22).



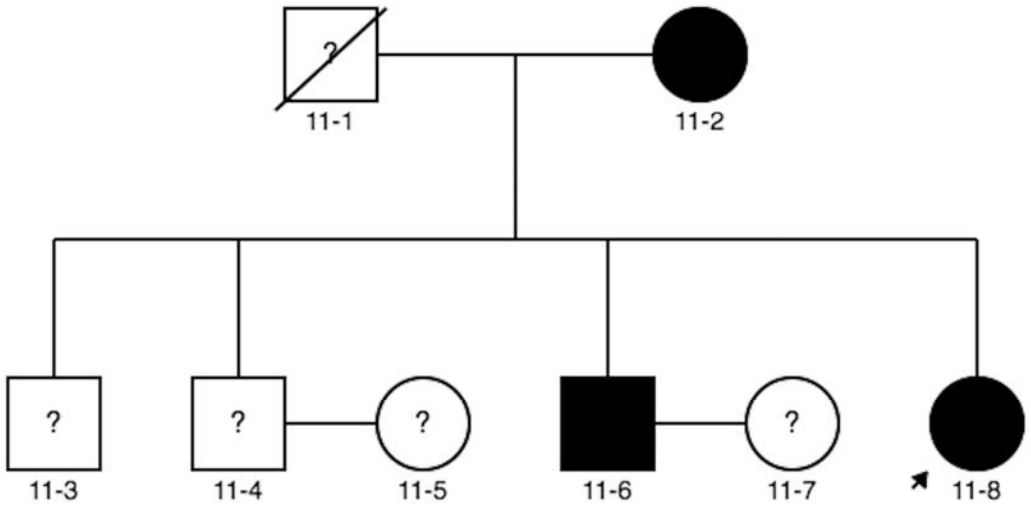
Appendix 5. Family 9 had 8 cases (1 male and 7 females) and no controls across two generations. The proband (9-11) was a member of the third generation. Both parental (9-7 and 9-8) and their offspring (9-22) had multilevel DSS.



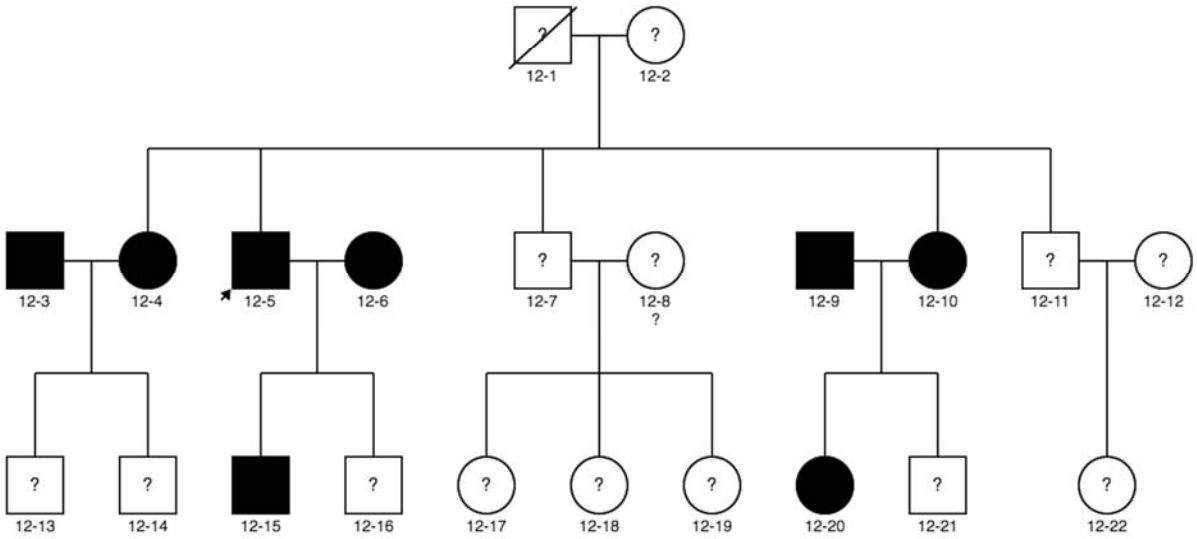
Appendix 6. Family 10 had 3 cases (1 male and 2 females) and no controls across two generations. The proband (10-3) was a member of the second generation. Both parental (10-1 and 10-2) and their offspring (10-3) had multilevel DSS.



Appendix 7. Family 11 had 3 cases (1 male and 2 females) and no controls across two generations. The proband (11-8) was a member of the second generation. Both parental (11-2) and offspring (11-6 and 11-8) had multilevel DSS.



Appendix 8. Family 12 had 8 cases (4 males and 4 females) and no controls across two generations. The proband (12-5) was a member of the second generation. Both parental (12-5, 12-6, 12-9 and 12-10) and their offspring (12-15 and 12-20) had multilevel DSS.



Appendix 9. Family 13 had 2 cases (1 male and 1 female) and no controls across two generations. The proband (13-3) was a member of the second generation. Both parental (13-2) and offspring (13-3) had multilevel DSS.