Impaired WNT Signaling and the Spine – Heterozygous *WNT1* Mutation Causes Severe Age-Related Spinal Pathology

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ABSTRACT

Background

WNT signaling plays a major role in bone and cartilage metabolism. Impaired WNT/β-catenin signaling leads to early-onset osteoporosis, but specific features in bone and other tissues remain inadequately characterized. We have identified two large Finnish families with early-onset osteoporosis due to a heterozygous *WNT1* mutation c.652T>G p.C218G. This study evaluated the impact of impaired WNT/β-catenin signaling on spinal structures.

Methods

Altogether 18 *WNT1* mutation-positive (age range 11–76 years, median 49 years) and 14 mutation-negative subjects (10–77 years, median 43 years) underwent magnetic resonance imaging (MRI) of the spine. The images were reviewed for spinal alignment, vertebral compression fractures, intervertebral disc changes and possible endplate deterioration. The findings were correlated with clinical data.

Results

Vertebral compression fractures were present in 78% (7/9) of those aged over 50 years but were not seen in younger mutation-positive subjects. All those with fractures had several severely compressed vertebrae. Altogether spinal compression fractures were present in 39% of those with a *WNT1* mutation. Only 14% (2/14) mutation-negative subjects had one mild compressed vertebra each. The mutation-positive subjects had a higher mean spinal deformity index (4.0 ± 7.3 vs 0.0 ± 0.4) and more often increased thoracic kyphosis (Z-score $>+2.0$ in 33% vs 0%). Further, they had more often Schmorl

nodes (61% vs 36%), already in adolescence, and their intervertebral discs were enlarged.

Conclusion

Compromised WNT signaling introduces severe and progressive changes to the spinal structures. Schmorl nodes are prevalent even at an early age and increased thoracic kyphosis and compression fractures become evident after the age of 50 years. Therapies targeting the WNT pathway may be an effective way to prevent spinal pathology not only in those harboring a mutation but also in the general population with similar pathology.

KEY WORDS: WNT signaling; magnetic resonance imaging; vertebral compression fracture; thoracic kyphosis; Schmorl node; intervertebral disc

1. INTRODUCTION

The importance of WNT/β-catenin signaling for bone mass development was first discovered when mutations in the co-receptor, low-density lipoprotein receptor-related protein 5 (LRP5) were identified as the cause for the osteoporosis-pseudoglioma syndrome [1,2]. Since then, other skeletal disorders, including early-onset osteoporosis and various high bone mass disorders such as van Buchem disease and sclerosteosis due to either decreased or increased WNT signaling, respectively, have further underlined this pathway's significance in bone homeostasis. [3,4,5,6] Furthermore, several genome-wide association studies (GWAS) have identified *LRP5* and other components of the WNT signaling pathway as major determinants of bone mass and fracture risk. [7,8]

WNT signaling plays a major role in bone metabolism throughout life –in skeletal development during embryonic phase, in bone mass accrual during childhood and adolescence, and in maintenance of bone homeostasis and renewal in adulthood [9]. In adult bone, the WNT pathway stimulates bone formation by committing common mesenchymal osteochondroprogenitor cells towards the osteoblastic lineage, by promoting their proliferation and differentiation and by preventing the apoptosis of uncommitted progenitor cells and mature osteoblasts. [10,11] WNT signaling also indirectly both stimulates and represses osteoclastogenesis through osteoblast-derived receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoprotegerin (OPG), respectively. [12,13] These mechanisms have been elucidated in several genetically modified mouse models, in which impaired WNT signaling leads to defects in bone formation and an osteoporotic phenotype [14,15]. Concurrently, WNT

signaling also mediates chondrogenesis, sustains mature cartilage homeostasis in adults and, when improperly activated, leads to cartilaginous tissue pathology [16,17].

WNT1 has been identified as a key ligand for the WNT/β-catenin pathway in bone, as several loss-of-function mutations in *WNT1* were found to cause variable degrees of early-onset osteoporosis. [15,18,19] Apart from VCFs of the thoracic spine seen in plain radiographs [15], little is known about the effects of defective WNT signaling in the axial skeleton. Access to a unique research cohort with a large number of individuals with WNT1 osteoporosis prompted us to examine in detail the consequences of impaired WNT signaling in the axial skeleton with magnetic resonance imaging (MRI) –specifically, the prevalence and characteristics of VCFs, the possible changes in vertebral endplates and intervertebral discs, and the relationship of spinal changes with age. Our findings on 18 mutation-positive subjects indicate that impaired WNT signaling due to mutated *WNT1* causes significant and progressive changes in the spine.

2. SUBJECTS AND METHODS

2.1 Subjects

During the course of our research program on early-onset osteoporosis we identified two large Finnish families with autosomal dominant WNT1 osteoporosis. Initially, the first family included ten mutation-positive family members (Family A) [15]. All first-degree relatives at risk were subsequently offered genetic screening for the family's *WNT1* mutation c.652T>G (p.C218G). Through genetic screening of an additional 21

family members, 10 other subjects were found to harbor the mutation. The whole *WNT1* gene was Sanger sequenced also for altogether >250 other Finnish index subjects with early-onset osteoporosis and another, seemingly unrelated family (Family B) was then found where four out of eight subjects were positive for the same *WNT1* mutation as Family A. Thus altogether 24 *WNT1* mutation-positive subjects were identified in these two families, both of ethnic Finnish background. In addition, 21 family members in these two families were confirmed by genetic screening to be negative for the mutation.

We offered all mutation-positive subjects from Families A (n=20) and B (n=4) an opportunity to participate in studies exploring the skeletal and extra-skeletal consequences of the heterozygous *WNT1* mutation, including MRI evaluation of the spine. In order to be able to compare the findings with normal data, a control group with similar age and sex distribution was formed by offering participation to *WNT1* mutation-negative individuals in families A (n=17) and B (n=2), aiming at similar age and sex distribution in the mutation-positive and control groups. Altogether 18 mutation-positive and 14 mutation-negative individuals consented. All subjects gave a written informed consent upon participation in the study. The research protocol was approved by the Research Ethics Board of Helsinki University Hospital.

2.2 Genetic evaluations

We performed genetic validations on DNA extracted from peripheral blood, as described previously. [20] We screened all samples for the families' known *WNT1* heterozygous missense mutation c.652T>G (p.C218G) in exon 4 of the *WNT1* gene (NCBI Reference Sequence NM_005430.3).

2.3 Clinical cohort characteristics

Mutation-positive subjects were clinically evaluated at Helsinki University Hospital for clinical features, including anthropometry, alignment and abnormalities of spine. For all subjects, data on sustained fractures and previous medical and surgical treatments were collected by interview and from medical records. History of back pain was recorded by interview; pain that required long-term medication, absence from work or professional help (e.g. physiotherapy) was considered disabling. All secondary causes of skeletal fragility (e.g. long-term illness or glucocorticoid treatment, low vitamin D status, or other biochemical or endocrine abnormality) had been ruled out previously [15,20].

2.4 Magnetic resonance imaging

Magnetic resonance imaging (MRI) of the thoracic and lumbar spine were performed in 2015-2016 at the Oulu University Hospital with a 1.5-T scanner (MAGNETOM Aera; Siemens Healthcare, Erlangen, Germany) using a 32-channel spine coil. The imaging sequences included T1-weighted turbo inversion recovery magnitude (TIRM) sagittal images (TR/TE/TI 1900/10/860 ms, FOV 280x280 mm, matrix 320x240 pixels, slice thickness 4 mm, and 0.8 mm interslice gap) and T2-weighted turbo spin echo (TSE) sagittal images (TR/TE 4000/99 ms, FOV 280x280 mm, matrix 448x358 pixels, slice thickness 4mm, and 0.8 mm interslice gap) of lumbar spine and T2-weighted TSE sagittal images (TR/TE 3500/97 ms, FOV 280x280 mm, matrix 384x269 pixels, slice

thickness 4 mm, and 0.8 mm interslice gap) of thoracic spine. All images were obtained in supine position with the subject lying down, legs extended horizontally.

The MRIs were first independently assessed for spinal changes by an orthopedic surgeon and an experienced radiologist who were blinded to the subjects' genotype and phenotype. The final conclusions were evaluated for discrepancy by calculating Intraclass Correlation Coefficient (ICC) for 10 randomly selected cases; for all the cases the ICC score was greater than 0.80 and therefore the analyses were considered highly consistent and reproducible. The MRI data were assessed as described later.

2.4.1 Vertebrae and intervertebral discs

Changes were evaluated separately for each vertebral body. Thoracic and lumbar vertebrae were first classified according to the Genant et al. semi-quantitative method by grading each vertebral body and VCF from T4 to L4 as 0) normal; 1) mild (decrease in height 20–25%); 2) moderate (decrease in height 25–40%); or 3) severe (decrease in height >40%). [21] The extent of vertebral changes was further evaluated using the spinal deformity index (SDI; scale 0–39) described by Minne et al. [22]–The SDI classification integrates number and severity of VCFs by summing all compression fracture scores. This classification has been proven accurate in assessing VCFs in osteoporotic patients. [23]

2.4.2 Endplates

The number, location and extent of Schmorl nodes (SN) were recorded by using modified classification introduced by Samartzis et al. [24] The recorded domains were: vertebrae level (T4–L4), endplate involvement (rostral, caudal, or both) and size (<1/3,

<2/3, or >2/3 endplate). The vertebrae having moderate or severe VCF (SDI >1) were not included in assessment.

2.4.3 Spinal alignment

Changes in spinal curvature were evaluated by calculating degrees for thoracic kyphosis and lumbar lordosis. Values were measured by selecting upper and lower vertebral bodies: T1 and T12 for thoracic spine, and L1 and L5 for lumbar spine. The angle was calculated using a modified Cobb's method by drawing tangent lines to these vertebral bodies; median values were used to compare differences between the two groups. [25] Measured values were also transformed into Z-scores based on age and sex-specific normative data according to Fon et al. [26]

2.4.5 Statistical analysis

Descriptive data are reported as median and range or as mean ± SD. Normality of the data was assessed using Kolmogorov-Smirnov and Shapiro-Wilk and visually using histograms. Unpaired 2-tailed Student t and Mann-Whitney U test were used as appropriate (SPSS Statistics 21; IBM Corporation, Armonk, NY, USA). A P-value <0.050 was considered statistically significant.

3. RESULTS

3.1 Cohort characteristics

3.1.1 Mutation-positive subjects

The 18 *WNT1* mutation-positive subjects (13 females, 5 males) ranged in age from 11 to 76 years (median 49 years) (Figure 1, Table 1). Altogether 16 (89%) of them had sustained at least one previous fracture. Twelve subjects (67%) reported back pain; six of them considered the pain disabling. Five adults reported height loss of ≥4 cm. None of the subjects reported high-impact trauma that would have caused spinal fractures. Nine subjects had received osteoporosis medication before the diagnosis of WNT1 osteoporosis and prior to the study and for three subjects, the treatment was still ongoing (Table 1).

Table 1. History of fractures and previous osteoporosis medication for 18 subjects with a heterozygous p.C218G *WNT1* mutation and 14 mutation-negative subjects. ALE, alendronate; ZOL, zoledronic acid; BP, bisphosphonate; O, denosumab; N/A, data not available; VCF, vertebral compression fracture.

SUBJECT	AGE (years)	SEX (M/F)	OSTEOPOROSIS MEDICATION	PERIPHERAL FRACTURES		HISTORY OF VCF	BACK PAIN	HEIGHT LOSS (cm)
				Number	Age at 1st fracture (years)			
Mutation-positive subjects								
AIV-6	11	F	None	1	0	-	-	0
AIV-5	13	M	None	4	N/A	-	-	0
AIV-8	13	F	None	1	8	-	-	0
AIV-4	17	F	None	8	0	-	-	0
AIV-7	19	M	None	1	12	-	-	0
BIII-2	31	F	ALE 2008-2013	11	0.6	-	+	0
AIII-2	34	F	None	2	N/A	-	-	0
AIII-12	44	F	None	0	-	-	+	0
AIII-7	48	F	ZOL 2010-	4	N/A	-	+	0
BII-1	51	F	BP (name N/A) 2002-2007	2	35	-	+	N/A
AIII-14	52	F	None	0	-	-	+	N/A
AIII-3	52	M	N/A	2	10	+	+	0
AIII-5	53	F	RIS 2001-2003 PTH 2012-2013	0	-	+	+	4
AII-1	63	M	ZOL 2007-2010 PTH 2011-2013	3	18	+	+	7
BII-2	68	F	ALE 2007-2014	5	N/A	-	+	2
AII-2	72	F	Estrogen (name N/A) RIS 2006-	9	7	+	+	13
AII-3	74	F	ALE 2001-2006 PTH 2006-2008 ZOL 2008-2011 DENO 2011-	6	42	+	+	17
AII-4	76	M	ZOL 2008 PTH 2011-2012	1	N/A	+	+	4

Table 1. (continued) History of fractures prior to study assessment and previous osteoporosis medication for 18 subjects with a heterozygous p.C218G *WNT1*

mutation and 14 mutation-negative subjects. ALE, alendronate; ZOL, zoledronic acid; BP, bisphosphonate; O, denosumab; N/A, data not available; VCF,

vertebral compression fracture.

Mutation-negative subjects								
AIV-1	10	M	None	0	-	-	-	0
AIV-2	16	M	None	0	-	-	-	0
AIV-10	18	F	None	0	-	-	-	0
AIV-8	21	F	None	0	-	-	-	0
AIV-9	27	F	None	0	-	-	-	0
AIII-1	37	F	None	0	-	-	-	0
AIII-5	42	M	None	0	-	-	-	0
BIII-3	43	M	None	0	-	-	-	0
AIII-12	49	F	None	0	-	-	-	0
AIII-7	53	M	None	0	-	-	-	0
AIII-10	56	F	None	5	19	-	+	4
AIII-9	57	F	None	0	-	-	-	0
AIII-8	59	M	None	5	23	-	+/-	0
BII-2	77	M	None	1	48	-	+	0

3.1.2 Mutation-negative subjects

The 14 *WNT1* mutation-negative subjects (7 females and males) ranged in age from 10 to 77 years (median 43 years) (Figure 1, Table 1). Altogether 3 (21%) of them had sustained one or more previous peripheral fractures, none had been diagnosed with compression fractures, three had mild back pain and one adult reported a 4 cm height loss. None had received any osteoporosis medications.

3.2 MRI findings

3.2.1 Vertebrae

VCFs were frequent and increasingly prevalent with age in the mutation-positive subjects; none of the subjects younger than 50 years had VCFs but seven out of nine (78%) of those over 50 years had multiple VFCs ranging from mild to severe (Figure 2a). In comparison, two out of the five mutation-negative subjects over the age of 50 years had a VCF, only one Genant grade 1 fracture each. The mean SDI-score for the mutation-positive subjects was 4 ± 7.3 and for the mutation-negative subjects 0 ± 0.4 ($p = 0.07$) (Figures 2 and 3a-c). In the mutation-positive subjects, VCFs were more prevalent in the thoracic spine and either mild (Genant grade 1) or severe (Genant grade 3) while moderate changes (Genant grade 2) were present mainly in the lumbar spine.

3.2.2 Vertebral endplates

In two mutation-positive subjects, severe VCFs prevented the evaluation of SNs. Eleven (69%) of the remaining 16 mutation-positive subjects had SN. In all of them SNs were present in the thoracic vertebrae and in six subjects, also in the lumbar spine (Figures 3a and 4). Median number of SN in the mutation-positive subjects was 3 (range 0-16). In all these 11 subjects,

the SNs were multilevel (i.e. seen in more than one endplate), present in all vertebral endplates, predominantly situated in T7 to L1 and most common in T9 (15%). Altogether 57% of the SNs were rostral, 63% involved one third of an endplate, and none involved more than two thirds of an endplate. In mutation-negative subjects the SNs were less prevalent (p = 0.07) as 5 (36%) of the 14 subjects had SN; four in the thoracic spine, one in the lumbar spine and one subject in both. Median number of SN was 0 (range 0–3). They were mostly rostral (64%), most common in T11 (36%) and mostly (64%) one third of an endplate in size.

3.2.3 Intervertebral discs

Intervertebral discs in the lumbar spine appeared enlarged in the mutation-positive group (Figure 3d). Median surface area of the intervertebral discs from L3 to L5 tended to be greater in the mutation-positive than in the mutation-negative subjects but this difference was not statistically significant (962 vs 844 mm^2, p = 0.24). Enlarged discs were biconcave in shape ("fish" or "hourglass vertebrae"). No enlarged discs were observed in the thoracic spine.

3.2.4 Spinal alignment

VCFs caused changes in the WNT1 subjects' spinal alignment. Thoracic kyphosis was significantly increased in the mutation-positive subjects and the median value for kyphosis was 39° (vs 34°, p = 0.01). The median values of kyphosis for the mutation-positive and mutation-negative subjects under the age of 50 years were 36° in both (p = 0.49), and for subjects over the age of 50 years 57° and 31° (p = 0.01), respectively. When the degree of kyphosis was compared with published age- and sex-specific normative data, there was a significant difference in kyphosis Z-scores between the mutation-positive and -negative subjects (Figure 2b), especially after age 50 years. Thoracic kyphosis was significantly (p = 0.02) increased in the mutation-positive subjects; 6 out of 18 mutation-positive subjects had a

kyphosis Z-score >+2.0 while all mutation-negative subjects had a Z-score within the -1.5 to +1.5 range. There was also an increase in kyphosis with increasing age (Figure 3a-c). The median values for lumbar lordosis were 41° and 44° in the mutation-positive and -negative subjects, respectively (p = 0.47); for subjects under the age of 50 years the values were 35° and 40° (p = 0.40) and for those over the age of 50 years, 48° and 54° (p = 0.32), respectively.

4. DISCUSSION

This study reports for the first time a systematic evaluation of the spine by MRI in a large cohort of patients with an inherited defect in WNT signaling. We assessed the spine in 18 mutation-positive subjects in two families with autosomal dominant WNT1 osteoporosis and in 14 mutation-negative relatives from the same two families. We previously showed that this *WNT1* missense mutation p.C218G impairs WNT signaling [15] and that affected individuals have increased peripheral fractures and compromised bone mass accrual in childhood and early-onset osteoporosis in adulthood [20]. Findings of the present study, involving pediatric and adult subjects, suggest that the negative effects of impaired WNT signaling on the spine accumulate with age and lead to significant spinal pathology by mid adulthood. Comprehensive evaluation of the spine, including vertebrae, cartilaginous tissue, and intervertebral discs, by MRI showed that all these spinal structures are affected.

VCFs were common and caused significantly increased kyphosis in mutation-positive adults. All subjects with fractures were older than 50 years, fracture prevalence being 78% in that age group. Fractures were also more severe and involved more vertebrae than in the mutation-negative subjects (mean SDI 4 ± 7.3 vs 0 ± 0.4). Further, the *WNT1* mutation-positive subjects

had more often increased thoracic kyphosis (33% vs 0%) and SNs (61% vs 36%) than the mutation-negative subjects.

The wide age distribution in our cohort enabled us to make some observations about the vertebral changes' relationship to age. We have previously shown that some skeletal changes are already seen in early childhood and that adults present with severe early-onset osteoporosis. [4,18] The present study showed phenotypic variability among subjects with same age. Most of the spinal changes occurred only past the age of 50 years, when a notable increase in pathological findings was seen in both males and females. Similarly, functionally significant *LRP5* polymorphisms modulate bone mass accrual during childhood and lead to subnormal lumbar spine BMD by adulthood. [3,27,28] In 10 individuals with defective WNT signaling due to a heterozygous *LRP5* mutation we showed that the magnitude of BMD deterioration and presence of VCFs associated with the patient's age. It seems likely that the changes in the axial spine accumulate during childhood and young adulthood and when a certain threshold BMD is reached, spinal changes (i.e. compression fractures and kyphosis) occur.

WNT signaling is also of key importance in cartilaginous tissues. WNTs function as a molecular switch in determining mesenchymal stem cells' commitment to either osteoblastic or chondrogenic lineage, and orchestrate their sequential proliferation and differentiation. [29] In the present study, spinal cartilaginous tissue was studied by evaluating defects in spinal endplates and intervertebral discs. SNs were twice as common in the mutation-positive subjects (69% vs 36%), were present at all ages and were atypically more frequent in the thoracic spine. They were also more common at an earlier age (one subject had 10 SNs at 17 years). SNs are intravertebral disc herniations often associated with disc degeneration and

weakened vertebral bone, and usually seen near the thoracolumbar junction. [30] While our results suggest low BMD to be a risk factor for SNs, the *WNT1* mutation may also hamper normal formation of cartilage, making the end plates more prone to herniation. As the SNs were more frequent and had earlier onset in the mutation-positive subjects, the presence of SN may be predictive of future VCFs. This however needs to be evaluated in a longitudinal study. Further, the overall role of WNT signaling in cartilage needs to be addressed in future studies.

Our study has some limitations, mainly concerning the relatively small cohort size and the cross-sectional nature of the study. Also, some of the mutation-positive subjects had received osteoporosis treatment prior to the study, influencing the natural course and improving the skeletal pathology. Importantly however, the presence of significant spinal pathology despite several years' medical treatment suggests that the available treatment modalities are not optimal. We did not use validated methods to evaluate the degree and actual cause of back pain, and physical and quality-of-life consequences of spinal changes; these would be important to gain more understanding of the disease burden. The MRI images were taken in supine position and compared against data taken in standing position. Several studies have indicated that kyphosis is decreased in supine versus standing position [31] and hence our study may have underestimated the degree of kyphosis. Lastly, as our study focuses solely on the spine, no other parts of the skeleton (e.g. knee or hip joints) were included. Evaluation of the appendicular skeleton in future studies will provide more comprehensive understanding of the skeletal and cartilaginous effects of the *WNT1* mutation. Despite these limitations, our study provides new and important information regarding the clinical implications of impaired WNT signaling on the spine. This is the largest reported genetically homogeneous cohort of subjects with a mutation in the WNT signaling pathway. The presence of an identical mutation in all affected subjects of all ages, and inclusion of mutation-negative subjects from only two

families allowed us to make several observations regarding the spinal changes' relationship to aging.

We conclude that defective WNT signaling due to the *WNT1* mutation p.C218G causes significant changes in the spine that are present both in bony vertebrae and in cartilaginous tissues, are evident in almost all subjects over the age of 50 years and become increasingly severe with age. VCFs and exaggerated kyphosis are a clinically significant and common problem, causing pain, physical limitations, impaired quality of life and increased morbidity. [32] Whether these changes are apparent in other tissues of the appendicular skeleton is left unexplored and will be subject to future studies. Therapies targeting the WNT pathway may be an effective way to prevent spinal pathology not only in those harboring a mutation but also in the general population with similar pathology.

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FIGURE LEGENDS

Figure 1. Pedigrees of the two Finnish families with a heterozygous p.C218G *WNT1*

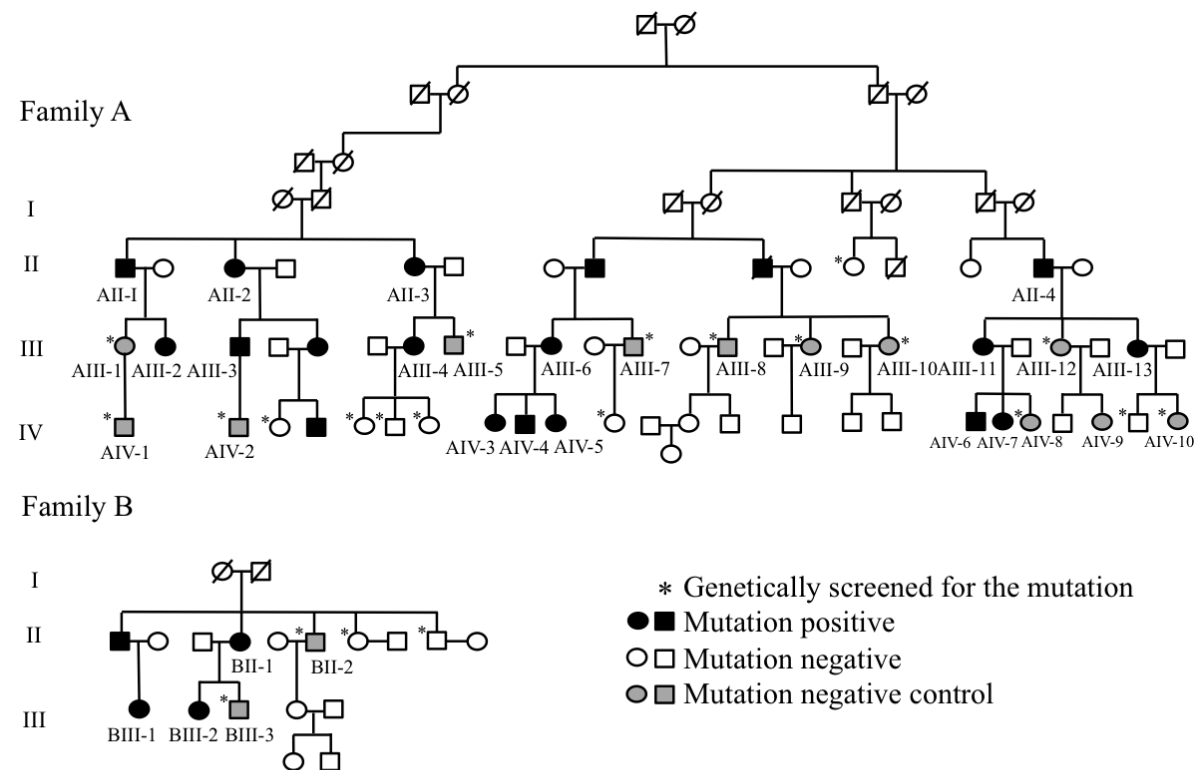mutation. The pedigrees have been modified to ensure anonymity.

Figure 2. A) Spinal deformity index (SDI) and B) kyphosis *Z*-scores in 18 *WNT1* p.C218G

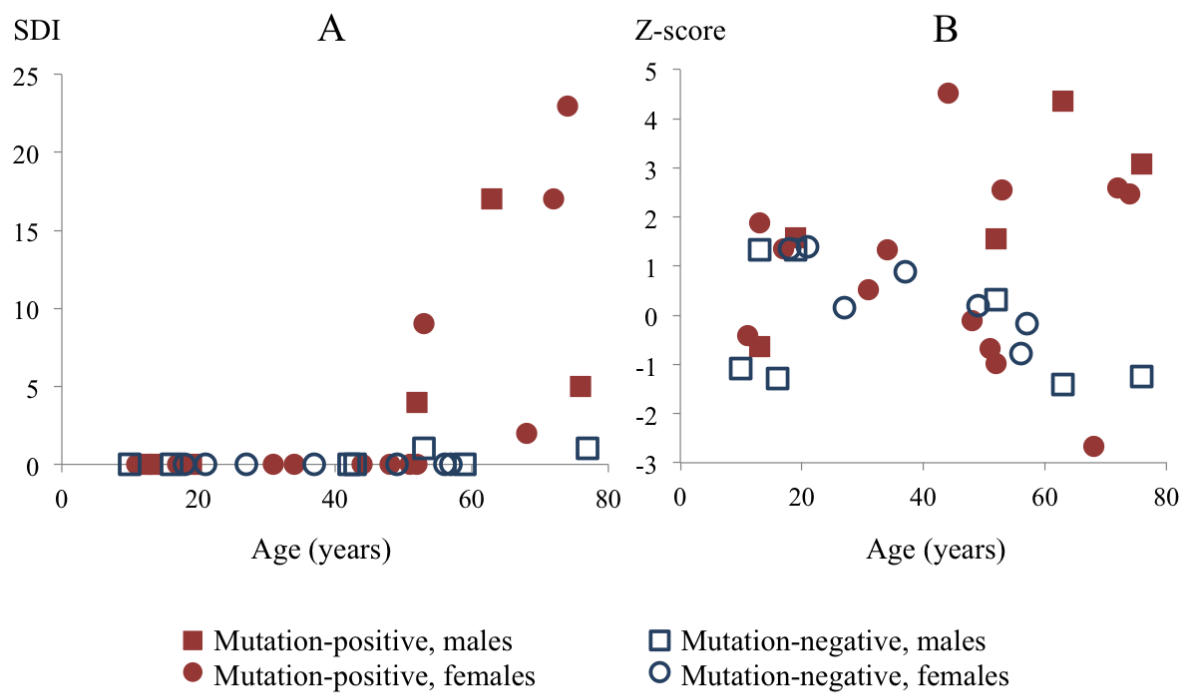mutation-positive and 14 mutation-negative subjects (to be printed in color).

Figure 3. Spinal magnetic resonance images of four *WNT1* p.C218G mutation-positive subjects. A) Thoracic spine of a 17-year-old female showing multiple Schmorl nodes (arrow). B) Thoracic spine of a 44-year-old female showing exaggerated thoracic kyphosis. C) Thoracic spine of a 76-year-old male showing several compressed vertebrae, kyphotic stature and Schmorl hernia (arrow). D) Lumbar spine of a 74-year-old female showing several compressed vertebrae and enlarged intervertebral discs (arrows).
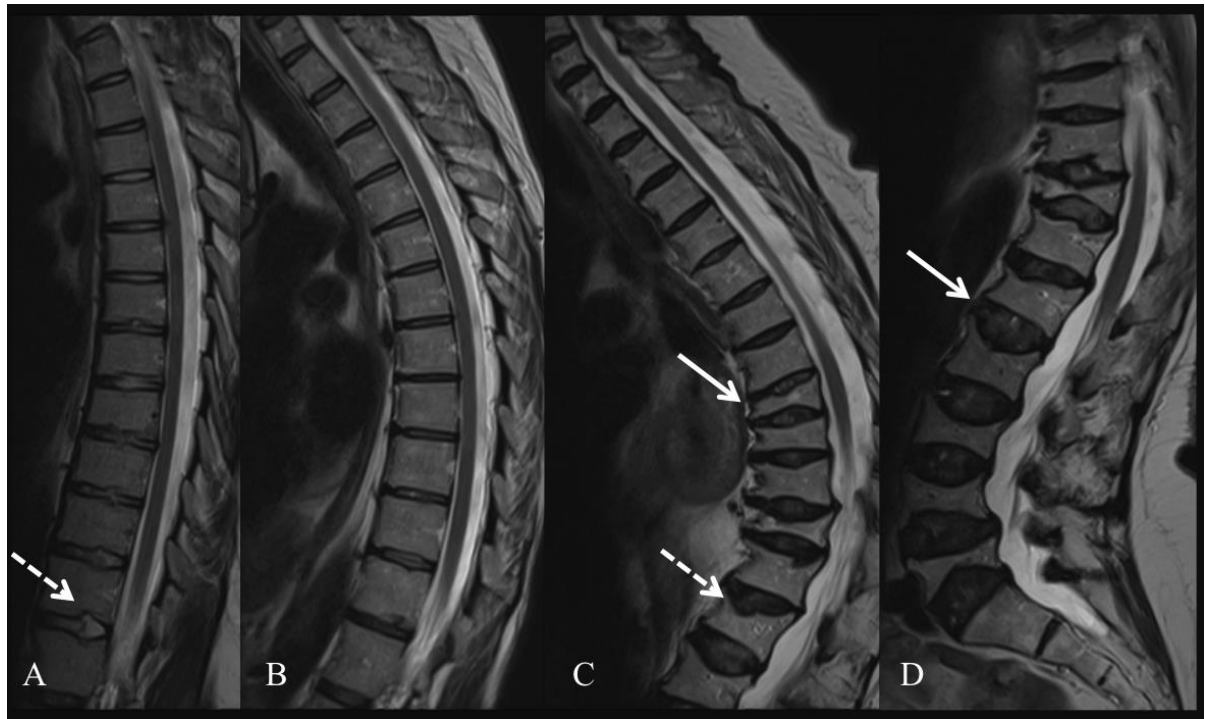
Figure 4. Number of Schmorl nodes and unassessable vertebrae (SDI > 1) in 18 *WNT1*

p.C218G mutation-positive and 14 mutation-negative subjects (to be printed in color).