

Osteoarthritis and Cartilage



Defective WNT signaling may protect from articular cartilage deterioration – a quantitative MRI study on subjects with a heterozygous *WNT1* mutation



S. Lehtovirta ^{†‡a}, R.E. Mäkitie ^{§*a}, V. Casula ^{†‡}, M. Haapea ^{‡||}, J. Niinimäki ^{†‡||},
T. Niinimäki [¶], A. Peuna ^{‡||}, E. Lammintausta ^{‡||}, O. Mäkitie ^{§#††b}, M.T. Nieminen ^{†‡||b}

[†] Research Unit of Medical Imaging, Physics and Technology, University of Oulu, Oulu, FI-90014, Finland

[‡] Medical Research Center, University of Oulu and Oulu University Hospital, Oulu, FI-90220, Finland

[§] Folkhälsan Institute of Genetics and Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, FI-00290, Finland

^{||} Department of Diagnostic Radiology, Oulu University Hospital, Oulu, FI-90220, Finland

[¶] Department of Orthopedics, Oulu University Hospital, Oulu, FI-90220, Finland

[#] Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, FI-00290, Finland

^{††} Center for Molecular Medicine, Karolinska Institutet, and Clinical Genetics, Karolinska University Hospital, SE-171 76, Stockholm, Sweden

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SUMMARY

Objective: WNT signaling is of key importance in chondrogenesis and defective WNT signaling may contribute to the pathogenesis of osteoarthritis and other cartilage diseases. Biochemical composition of articular cartilage in patients with aberrant WNT signaling has not been studied. Our objective was to assess the knee articular cartilage in *WNT1* mutation-positive individuals using a 3.0T MRI unit to measure cartilage thickness, relaxation times, and texture features.

Design: Cohort comprised mutation-positive ($N = 13$; age 17–76 years) and mutation-negative ($N = 13$; 16–77 years) subjects from two Finnish families with autosomal dominant *WNT1* osteoporosis due to a heterozygous missense mutation c.652T>G (p.C218G) in *WNT1*. All subjects were imaged with a 3.0T MRI unit and assessed for cartilage thickness, T2 and T1ρ relaxation times, and T2 texture features *contrast*, *dissimilarity* and *homogeneity* of T2 relaxation time maps in six regions of interest (ROIs) in the tibio-femoral cartilage.

Results: All three texture features showed opposing trends with age between the groups in the medial tibiofemoral cartilage ($P = 0.020$ – 0.085 for the difference of the regression coefficients), the mutation-positive individuals showing signs of cartilage preservation. No significant differences were observed in the lateral tibiofemoral cartilage. Cartilage thickness and means of T2 relaxation time did not differ between groups. Means of T1ρ relaxation time were significantly different in one ROI but the regression analysis displayed no differences.

Conclusions: Our results show less age-related cartilage deterioration in the *WNT1* mutation-positive than the mutation-negative subjects. This suggests, that the *WNT1* mutation may alter cartilage turnover and even have a potential cartilage-preserving effect.

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Introduction

WNT signaling is important in chondrogenesis and its regulatory effect spans throughout the life from early embryonic development to maintenance of articular cartilage in adulthood¹. Previous studies indicate that altered WNT signaling may contribute to the pathogenesis of osteoarthritis (OA)—either due to inadequately constructed articular surface that is more susceptible

* Address correspondence and reprint requests to: R.E. Mäkitie, Folkhälsan Institute of Genetics, PO Box 63, FIN-00014, University of Helsinki, Helsinki, Finland. Tel.: 358-2941911.

E-mail address: riikka.makitie@helsinki.fi (R.E. Mäkitie).

^a The first and second authors contributed equally to this manuscript.

^b The final and penultimate authors contributed equally to this manuscript.

to deterioration or due to impaired healing of minor erosions. Several human and experimental mouse studies report that activated WNT signaling or overexpression of WNTs are present in early-onset of OA^{2–4}. Furthermore, genome-wide association studies (GWAS) have confirmed that genetic factors play an important role in OA and that polymorphisms in genes encoding WNT pathway components are associated with severe large-joint OA^{5,6}. However, despite the high worldwide prevalence of OA, most of its molecular and genetic bases and the strategies for effective treatment remain unknown^{7,8}.

WNT/ β -catenin signaling pathway is also recognized as a pivotal pathway in bone formation and its defective activation leads to various skeletal pathologies, such as osteoporosis-pseudoglioma syndrome, van Buchem disease and sclerosteosis^{9–11}. In 2013, we identified WNT1 as a key ligand to WNT signaling in bone, as *WNT1* mutations were shown to cause autosomal recessive and dominant osteoporosis¹². In a large Finnish family, 10 affected members with a novel heterozygous loss-of-function *WNT1* mutation c.652T>G (p.C218G) presented with severe, early-onset osteoporosis characterized by multiple peripheral and vertebral compression fractures and subsequent adult height loss. Transiliac bone biopsies showed low turnover osteoporosis¹². Since then, similar findings have been described in pediatric patients¹³ and other patients with other *WNT1* mutations^{14–17}.

To further explore the significance of WNT signaling for OA, we studied the effect of the heterozygous *WNT1* mutation on knee articular cartilage using several quantitative magnetic resonance imaging (qMRI) parameters. Cartilage thickness has been proposed as a morphological marker, but its correlation with the joint status is not clear^{18–23}. T2 relaxation time is an established qMRI parameter in the evaluation of cartilage structure^{24–27} and mechanical properties²⁸. Alterations in T2 relaxation reflect changes in collagen content, fibril network orientation and integrity, as well as hydration^{29–31}. T2 relaxation time increases in degenerated cartilage^{32,33}. T1 ρ relaxation time, another qMRI parameter, strongly associates with glycosaminoglycan loss in articular cartilage^{34–38}. Texture analysis methods are image processing tools that can be used to detect OA-related changes in articular cartilage^{33,39–42}. Our texture tool is based on gray level co-occurrence matrices (GLCM)⁴³.

Materials and methods

Subjects

We have previously identified two large Finnish families with autosomal dominant *WNT1* osteoporosis due to heterozygous missense mutation c.652T>G (p.C218G) in *WNT1*¹². These two families, both of ethnic Finnish descent, comprise altogether 25 *WNT1* mutation-positive members. In addition, the families consist of 41 individuals who have been determined mutation-negative based on genetic screening or mode of inheritance of the *WNT1* mutation.

For the current study, we offered all mutation-positive subjects over the age of 16 years from both families (Family A $n = 18$, Family B $n = 4$) the opportunity to participate in a study examining the skeletal and extra-skeletal consequences of the *WNT1* mutation, including MRI evaluation of knees. To form a suitable control group, reflective of normative data in a similar age- and gender-distributed population, we also offered participation to all over 16-year-old *WNT1* mutation-negative family members in the two families (Family A $n = 16$, Family B $n = 2$). Altogether 15 mutation-positive (hereafter: MP) and 13 mutation-negative (hereafter: MN) individuals consented to participate in the study. However, two MP subjects (female aged 74, male aged 52) were left out due to excess

metal implants and an imaging error, resulting in the final study group of 13 MP and 13 MN subjects. The research protocol was approved by the Research Ethics Board of Helsinki University Hospital.

Genetic evaluations

We performed *WNT1* mutation analysis on DNA extracted from peripheral blood, as previously described¹². Briefly, DNA was amplified with standard PCR, purified with ExoSAP (USB) and sequence reactions performed with BigDye Terminator v3.1 Cycle Sequencing—labeling (Applied Biosystems). Sequencing was done with an ABI730 Sequencer (Applied Biosystems) and chromatograms visualized and analyzed using Sequencer v.5.0 (Gene Codes Corporation). We screened all samples for the families' known heterozygous missense mutation c.652T>G (p.C218G) in exon 4 of the *WNT1* gene (NCBI Reference Sequence NM_005430.3). Primer sequences and PCR conditions are available from the authors upon request.

Clinical cohort characteristics

We clinically assessed the mutation-positive subjects at Helsinki University Hospital for skeletal characteristics, including possible lower limb or joint deformities and joint inflammation. We collected medical histories, previous radiographs and subject interviews to review previous complaints or medical and surgical treatments on knees or other joints.

Magnetic resonance imaging

All subjects underwent MRI examination on a 3.0 T MRI unit (MAGNETOM Skyra, Siemens Healthcare, Erlangen, Germany) used in combination with a 15-channel transmit-receive knee coil (QED, Mayfield Village, OH, USA). We systematically imaged the left knee joint of each subject; however, if the left knee had an arthroplasty or other metal implant, the right knee was chosen for imaging. The MRI protocol included a fat-suppressed PD-weighted and a T1-weighted turbo-spin-echo sequence, a Dual Echo Steady State (DESS) sequence, a T2 relaxation time mapping sequence and a T1 ρ relaxation time mapping sequence (Table 1)^{44,45}. The validity and reproducibility of these methods and the discriminatory and prediction power of T2 and T1 ρ have been previously reported by us and others. The used, in-house software also allows for correction of motion artefacts^{45–47}. The total acquisition time was approximately 20 min. The acquisition parameters are reported in Table 1.

We obtained mean T2 and T1 ρ relaxation time values from sagittal T2 and T1 ρ maps, respectively, by manually segmenting the weight-bearing area of the knee's articular cartilage. Segmentation was conducted by one of the authors (S.L., 2 years of experience) and verified by a more experienced reader (V.C., 5 years of experience). Segmentation resulted in total of six regions of interest (ROIs): anterior (acF) and posterior central femur (pcF) on lateral condyle (acFL, pcFL respectively), acF and pcF on medial condyle (acFM, pcFM respectively), medial central tibia (cTM) and lateral central tibia (cTL) (Fig. 1). More details on cartilage segmentation are reported in the Online Supplemental File.

For analysis of T2 relaxation time, we chose the three middlemost sagittal slices from each femoral condyle. For each ROI, we calculated the average of the three slices, weighted by the number of pixels. Due to the long acquisition time of T1 ρ , only one slice per condyle was imaged, in the same positions as the centermost slices of the T2 mapping sequence. These slices were chosen in areas that represent the highest weight-related stress on cartilage. Implemented cartilage thickness calculation tool was applied to measure

Table 1
MRI parameters used for Park-grading and quantitative MRI study protocol

Parameters	PARK			Quantitative MRI	
	FS PD-weighted	DESS	T1-weighted	T2-mapping	AdT1 ρ -mapping
Pulse sequence	2D Turbo spin echo	3D DESS	2D Turbo spin echo	2D Multi Echo Spin-Echo	trains of 0, 4, 8, 12 and 16 AFP, T _{SL} = 0, 24, 48, 72, 96 ms; followed by 2D spoiled gradient echo
TR (ms)	500	14.1	688	1300	4000
TE (ms)	8.6	5.0	18	13.8, 27.6, 41.4, 55.2, 69	3.36
Flip Angle (deg)	150	25	150	180	15
ETL	8	2	2	5	23 segments per acquisition
Bandwidth (Hz/pixel)	247	250	240	228	260
FOV (mm ²)	140 × 140	150 × 150	130 × 130	129 × 129	180 × 180
Matrix (px ²)	256 × 256	256 × 256	320 × 240	384 × 384	256 × 256
Plane	Sagittal	Sagittal	Coronal	Sagittal	Sagittal
Slices (n)	2	160	25	30	2
Slice Thickness (mm)	3	0.6	3	2.0	3.0
Scan Time (m:ss)	0:40	3:18	1:59	8:57	4:44

FS = Fat Suppressed; PD = Proton Density; DESS = Dual Echo Steady State.

AFP = adiabatic full passage hyperbolic secant pulses of the HS family ($n = 4$, spin lock frequency = 600 Hz).

T_{SL} = spin-lock time.

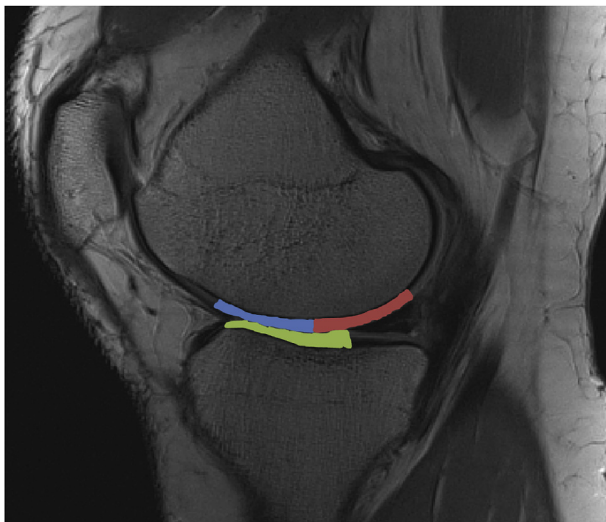


Fig. 1. Sagittal T2-weighted image of tibiofemoral joint from a representative subject (mutation-negative, female, 37 years) presenting the regions of interest selected for quantitative analysis: anterior central femur (blue), posterior central femur (red) and central tibia (green).

mean cartilage thickness of each ROI according to the segmentation of T2 maps. The thickness was calculated by solving Laplace's equation within the ROI⁴⁸.

We conducted the texture analysis for segmented T2 maps using an in-house developed software described earlier⁴⁹. For each ROI, the application computed the gray level co-occurrence matrix (GLCM) texture features in the direction parallel to bone-cartilage interface (BCI), i.e., every pixel within the ROI was assumed with an angle that presents offset direction that is parallel to BCI. Extrapolative soft-contour analysis for the offset endpoint was performed to correct for the tendency of the orientation vectors to point to pixel interfaces due to limited T2 map resolution. We chose to analyze three texture features; *contrast*, *dissimilarity* and *homogeneity*. Due to limited image resolution T1 ρ maps were not analyzed with texture analysis.

Park classification

We used the Park-scoring system⁵⁰ to review all MR images for their visual cartilage injury, osteophytes, bone marrow edema

(BME), and subchondral cysts. This scoring system is reported to correlate with the Kellgren–Lawrence (KL) grading for plain radiographs⁵⁰. All images were independently reviewed by an orthopedic surgeon (T.N.) and an experienced radiologist (J.N.), who were blinded to subjects' genotype and phenotype. A detailed description of the Park-scoring system is reported in the [Online Supplemental File](#).

Statistics

The MRI parameters (T2 and T1 ρ relaxation times and T2 texture features *contrast*, *dissimilarity* and *homogeneity*), and the thickness of articular cartilage, are presented as means with standard deviations. Differences between the two cohorts (MP and MN) were assessed using independent samples *t*-test. A *p*-value <0.050 was considered statistically significant. Associations between age and the MRI parameters are presented as scatter plots. Effect of the mutation status was analyzed using linear regression analysis. First, the regression coefficients of age, adjusted for sex, were calculated stratified by mutation status. Next, the interaction term age × mutation status was analyzed using analysis of variance with mutation status, sex, age and age × mutation status included in the models. The statistical analyses were conducted using IBM SPSS for Windows, version 24.

Results

Cohort characteristics

Mutation-positive subjects

The MP cohort comprised 13 subjects (age range 17–76 years) ([Table II](#), [Fig. 2](#), [Online Supplemental Table I](#)). Seven subjects had had some pain, swelling or stiffness in their finger joints and knee joints. Two subjects had undergone surgical treatment: a 72-year-old female (AII-2) a prosthetic surgery on her right hip due to OA and on her left knee due to secondary OA after severe patellar and distal femur fracture, and a 76-year-old male (AII-4) a hip prosthetic surgery due to OA. Ten of them had received osteoporosis medication prior to the study and for four subjects the medication was ongoing throughout the study.

Mutation-negative subjects

The MN cohort comprised 13 subjects (age range 16–77 years) ([Table II](#), [Fig. 2](#), [Online Supplemental Table I](#)). Five had had some

Table II

Demographic characteristics of 13 mutation-positive (MP) and 13 mutation-negative (MN) subjects with a heterozygous *WNT1* mutation p.C218G. Values are presented as frequencies and medians with ranges

Demographic characteristic	MP	MN	P-value
T2, Texture, Thickness			
Total	13	13	
Female, n (%)	10 (76.9%)	7 (53.8%)	0.216*
Male, n (%)	3 (23.1%)	6 (46.2%)	0.216*
Age, median (range) [years]	51.0 (17–76)	43.0 (16–77)	0.520†
Park grade, n (%)			
0	8 (61.5%)	10 (76.9%)	
1	2 (15.4%)	2 (15.4%)	
2	2 (15.4%)	0 (0.0%)	
3	1 (7.7%)	1 (7.7%)	
T1ρ			
Total	12	12	
Female, n (%)	10 (83.3%)	7 (58.3%)	0.178*
Male, n (%)	2 (16.7%)	5 (41.7%)	0.178*
Age, median (range) [years]	51.5 (17–76)	42.5 (16–59)	0.125†
Park grade, n (%)			
0	7 (58.3%)	10 (83.3%)	
1	2 (16.7%)	2 (16.7%)	
2	2 (16.7%)	0 (0.0%)	
3	1 (8.3%)	0 (0.0%)	

MP = Mutation-positive, MN = Mutation-negative.

* Chi-square test (exact sign.).

† Mann–Whitney test (exact sign.).

pain in their joints and two had undergone surgical treatment. None had received any previous osteoporosis medications.

MRI findings

Two individuals (MP subject aged 19 and MN subject aged 77) had incomplete T1ρ maps due to an error during the MR imaging, and these subjects were left out of the T1ρ analysis (Table II). Based on Park scores, visual inspection of the MR images displayed no clinical difference between the two groups (Table II). Park grade 0 was observed in eight of the thirteen MP subjects (61.5%) and in ten of the thirteen MN subjects (76.9%), indicating that the majority

of subjects in each group had normal knee cartilage on visual inspection.

Cartilage thickness and T2 and T1ρ relaxation times

No differences were observed between the MP and MN groups in cartilage thickness or in T2 values (Online Supplemental Table II). This suggests that the groups did not differ in signs of cartilage deterioration, such as hydration, collagen content, or fibril network orientation.

T1ρ values, reflective of cartilage quality and sensitive to glycosaminoglycan content, were significantly higher in the MP group compared with the MN group in pcFM and cTM region (Online Supplemental Table II). No association of age with the qMRI parameters or with cartilage thickness was found (Online Supplemental Figs 1 and 2 and Online Supplemental Table III).

Texture analysis

All three T2 texture features, *contrast*, *dissimilarity* and *homogeneity*, reflective of OA-related changes, displayed opposing trends with age between the MP and MN groups in the medial tibiofemoral (MTF) cartilage (Figs. 3–5); statistical analysis for the significance of the interaction term age × mutation status provided *p*-values of 0.020–0.085 (Table III). The MN group seemed to be affected by age in all texture features (*p*-values of 0.001–0.153) (Table III) in the MTF cartilage, indicating a physiological age-related cartilage degeneration. On the contrary, the MP group showed no association with age (Table III) in the MTF cartilage, suggesting that the articular cartilage is not as prone to age-related cartilage deterioration as in the MN, healthy subjects. In the lateral tibiofemoral (LTF) cartilage, the two groups behaved in a similar manner (Figs. 3–5) and showed no statistically significant differences (Table III). Comparison of means of the texture features did not reveal statistically significant differences in any of the three texture features (Online Supplemental Table II).

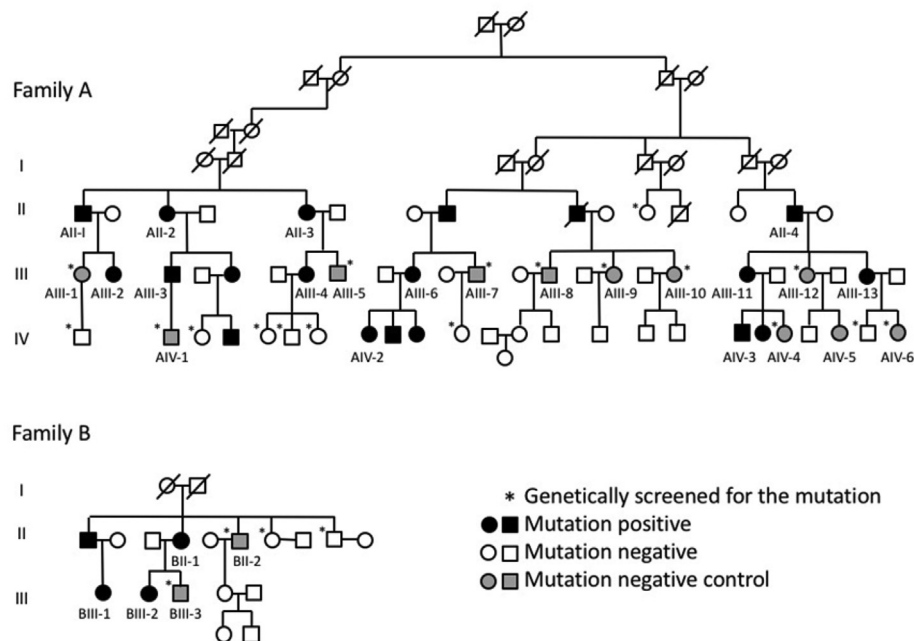


Fig. 2. Pedigrees of the two families with a heterozygous *WNT1* mutation p.C218G. Squares represent males, circles females, black symbols affected family members, grey symbols negative family members included in the study, and slashes deceased family members. All genetically tested, unaffected family members are indicated with an asterisk. Generations are numbered with roman numerals.

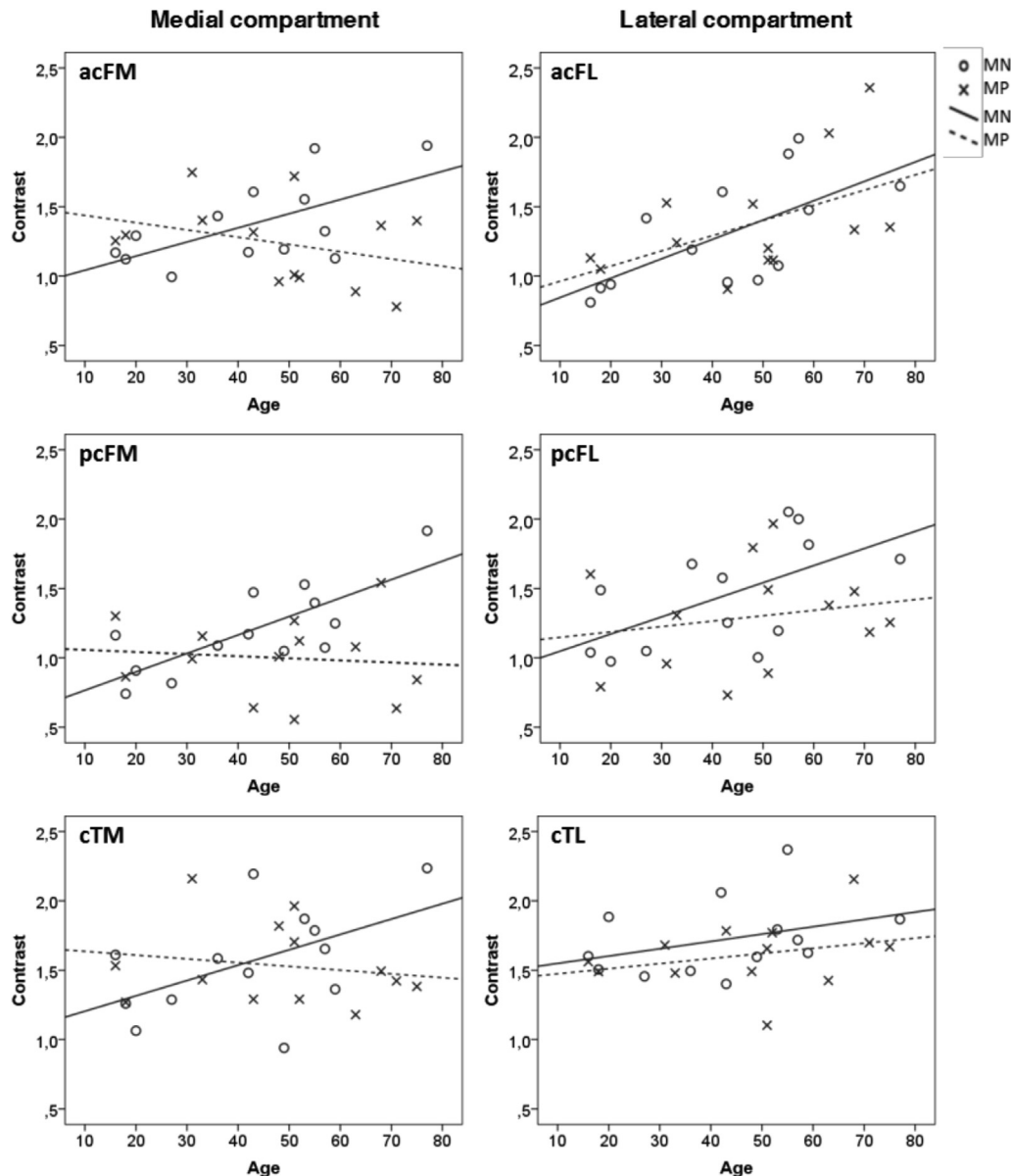


Fig. 3. Contrast dependency on subject age for 13 mutation-positive (MP) and 13 mutation-negative (MN) subjects with a heterozygous *WNT1* mutation p.C218G. Regression curves for both groups are displayed. Results are presented separately for different regions of interest: acF = anterior central femur, pcF = posterior central femur, cT = central tibia, in medial (M) and lateral (L) condyles.

Discussion

This study describes previously unreported findings of changes in knee articular cartilage quality in *WNT1* mutation positive children and adults. We assessed knee joints in 13 *WNT1* mutation-positive subjects from two large Finnish families using MR imaging, systematically reviewed T2 and T1 ρ relaxation times, and cartilage thickness for possible cartilage damage and early OA-related changes, and compared their results with a similar cohort of 13 mutation-negative, healthy subjects from the same two families. We have previously shown that the *WNT1* missense mutation p.C218G impairs WNT signaling and leads to progressive spinal pathology in the vertebral bodies, cartilaginous vertebral endplates and in intervertebral discs, among other tissues^{12,13,51}. The present study, our results show that the MP and MN groups did not differ in cartilage thickness: neither means nor association of

age with thickness displayed statistically significant differences. Similar results were observed in T2 relaxation time; means and association of age with T2 relaxation time were not different between the groups. We observed a significant difference in the means of T1 ρ : cTM and pcFM regions showed considerably higher T1 ρ value in the MP group compared with the MN group. Higher values may indicate compromised cartilage quality^{34–38}. However, having to leave two subjects (MP subject aged 19 Park score 0, MN subject aged 77 Park score 3) out of the T1 ρ analysis due to the imaging error alters the age distribution and Park scores of the groups. Although the age difference between the groups in T1 ρ analysis is not significant (Table II), we speculate that the changes in the groups' age distribution and Park scores might explain the significance in the results of T1 ρ analysis. Furthermore, we did not observe difference in association of age with T1 ρ relaxation time between the groups.

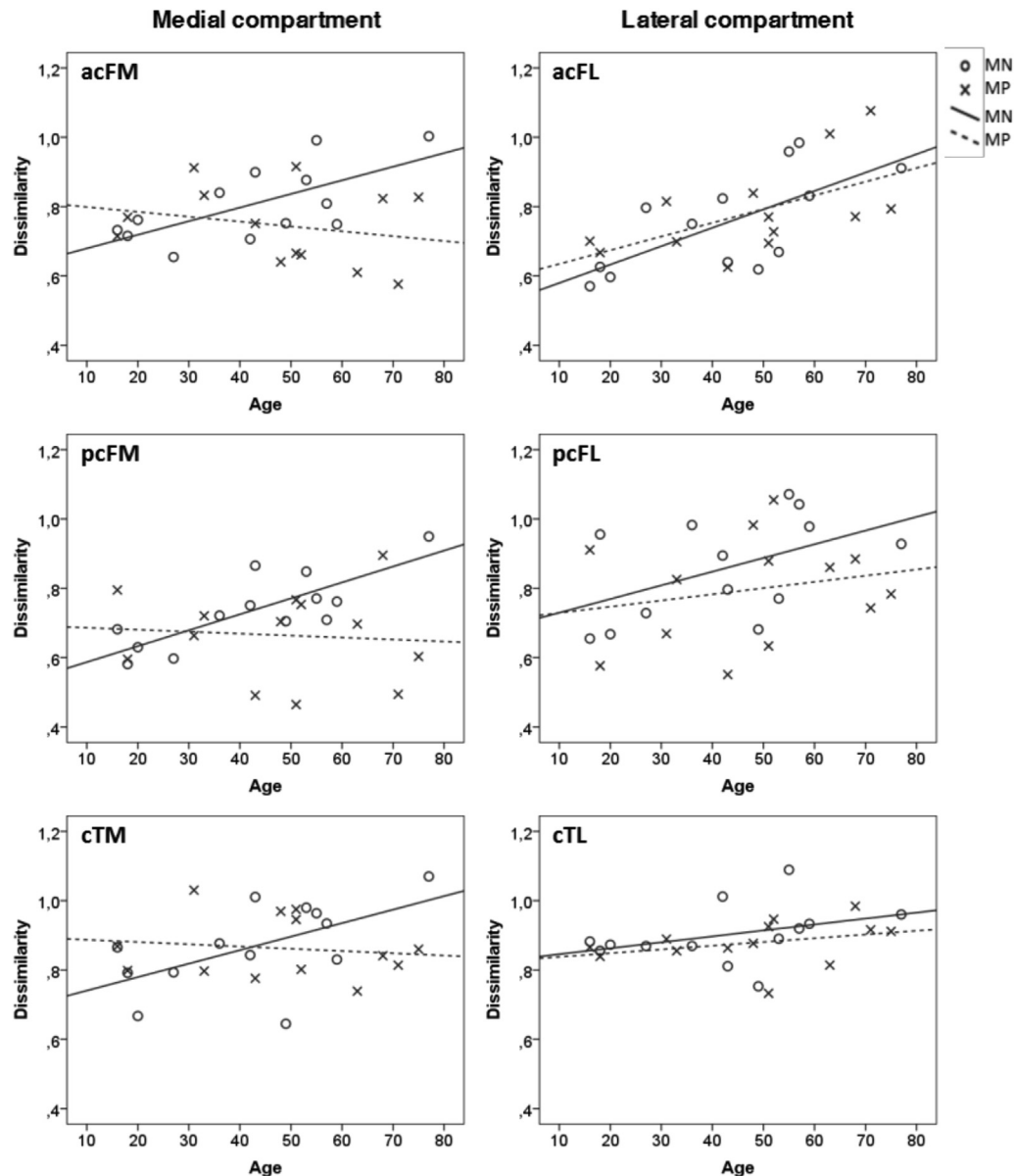


Fig. 4. Dissimilarity dependency on subject age for 13 mutation-positive (MP) and 13 mutation-negative (MN) subjects with a heterozygous *WNT1* mutation p.C218G. Regression curves for both groups are displayed. Results are presented separately for different regions of interest: acF = anterior central femur, pcF = posterior central femur, cT = central tibia, in medial (M) and lateral (L) condyles.

Texture analysis of T2 maps revealed opposing trends with age in MTF cartilage in all three texture features. The results for all three features differed significantly between the groups in five out of nine ROIs; statistical significance was not detected in all the ROIs possibly due to limited group sizes. On the contrary, the two groups had very similar trends on the LTF cartilage. It seems that the MP group has a different tendency in MTF and LTF compartments whereas the MN group sets similarly in both MTF and LTF compartments. The changes in *contrast* and *homogeneity* features in MN subjects most likely result from cartilage degeneration with age. This is in line with findings reported in previous studies, which have shown an increase in *contrast* feature^{40,41} and a decrease in *homogeneity* feature⁴⁰ in OA patients as compared with healthy controls. Moreover, a previous longitudinal study by Baum *et al.*³⁹ showed an increase over time in *contrast* feature for healthy subjects with and without OA risk factors in medial femoral cartilage.

However, our MP subjects show significantly opposing behavior in the MTF cartilage and we therefore speculate that the cartilage quality in the MP subjects may be superior to that of the MN subjects. *Dissimilarity* feature supports these findings by revealing opposing setting of the MP and MN group in the MTF cartilage. We believe that these differences were only observed in texture features because this analysis is more sensitive to local alterations in cartilage structure, whereas ROI-based T2 relaxation time measurements consider the ROI as a whole and underlying local alterations in relaxation times may be averaged out.

We speculate that our findings can be explained with altered cartilage turnover and altered WNT signaling in the MP subjects. Various studies have shown WNT signaling to be crucial in development and homeostasis of articular cartilage. In developmental stages WNT signaling stimulates chondrogenesis, chondrocyte differentiation and hypertrophy⁵². However, the effects of WNT

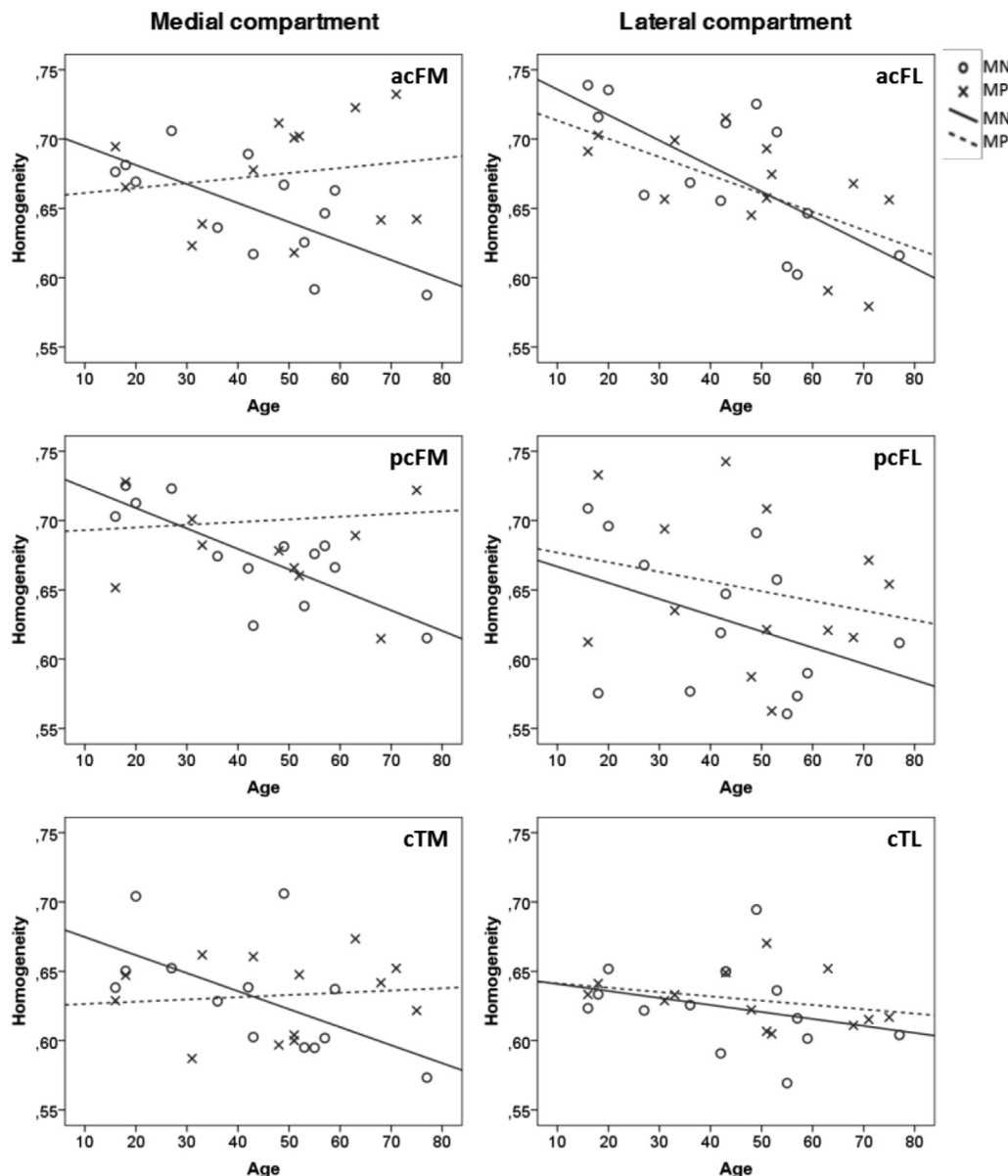


Fig. 5. Homogeneity dependency on subject age for 13 mutation-positive (MP) and 13 mutation-negative (MN) subjects with a heterozygous *WNT1* mutation p.C218G. Regression curves for both groups are displayed. Results are presented separately for different regions of interest: acF = anterior central femur, pcF = posterior central femur, cT = central tibia, in medial (M) and lateral (L) condyles.

signaling on adult cartilage are less pronounced and even opposite, and several prior studies have demonstrated how overactivation of WNT signaling may induce cartilage damage and contribute to OA pathology, and that WNT inhibitors may have therapeutic implications in OA^{53,54}. Experimental overexpression of Wnt8 and Wnt16 lead to increased protease activity and early OA-like cartilage deterioration², while loss of the WNT inhibitor Sclerostin promotes OA in mice³. Further, WNT co-receptor LRP5 is up-regulated in human and experimental OA tissue samples⁴.

These findings are resonated in our current findings in that reduced WNT stimulus in mature adult articular cartilage in MP subjects seems to slow down the cartilage's natural deterioration. Cartilage deterioration in OA patients is more prevalent in the medial femoral cartilage, suggesting that the MTF compartment may be under a higher stress compared with the LTF compartment in the knee joint^{55–58}. Chondrocytes are responsible for cartilage

homeostasis and they can react to modify cartilage extracellular matrix to altered mechanical demands⁵⁹. The partial suppression of the WNT signaling in the MP subjects could lead to increased differentiation of chondrocytes and enable the cartilage to better maintain its integrity against mechanical stress. Whether these findings are similar in other smaller and non-weight-bearing joints requires further investigation in future studies.

Another explanation for the differences in articular cartilage between the two groups could be the quality of underlying bone. MP individuals are known to have reduced bone turnover leading to a reduced bone mineral density (BMD)^{12,13}. We speculate that this could lead to a reduced cartilage stress, therefore enabling the cartilage to maintain its integrity even at older age. We have previously reported an inverse relation between cartilage and bone quality, as measured by quantitative MRI parameters⁶⁰ and similar findings of inverse relation between OA and osteoporosis have been

Table III

Regression coefficients of age, adjusted for sex, on T2 texture features (*contrast*, *dissimilarity* and *homogeneity*) for 13 mutation-positive (MP) and 13 mutation-negative (MN) subjects with a heterozygous *WNT1* mutation p.C218G

	Medial compartment			Lateral compartment		
	Regression coefficient (95% CI)	<i>P</i> [*]	<i>P</i> [†]	Regression coefficient (95% CI)	<i>P</i> [*]	<i>P</i> [†]
Contrast						
acF						
MP	−0.0051 (−0.0157, 0.0054)	0.305		0.0107 (−0.0023, 0.0238)	0.096	
MN	0.0103 (0.0007, 0.0198)	0.038		0.0161 (0.0049, 0.0272)	0.009	
Difference	−0.0155 (−0.0283, −0.0027)		0.020	−0.0036 (−0.0194, 0.0122)		0.640
pcF						
MP	−0.0013 (−0.0119, 0.0094)	0.796		0.0046 (−0.0088, 0.0180)	0.467	
MN	0.0108 (0.0048, 0.0168)	0.002		0.0139 (0.0019, 0.0260)	0.028	
Difference	−0.0141 (−0.0260, −0.0022)		0.022	−0.0096 (−0.0257, 0.0066)		0.230
cT						
MP	−0.0018 (−0.0112, 0.0077)	0.688		0.0041 (−0.0043, 0.0125)	0.306	
MN	0.0082 (−0.0036, 0.0199)	0.153		0.0058 (−0.0044, 0.0160)	0.237	
Difference	−0.0135 (−0.0288, 0.0018)		0.080	−0.0021 (−0.0139, 0.0096)		0.711
Dissimilarity						
acF						
MP	−0.0014 (−0.0054, 0.0026)	0.462		0.0039 (−0.0001, 0.0078)	0.054	
MN	0.0040 (0.0007, 0.0072)	0.022		0.0061 (0.0022, 0.0099)	0.005	
Difference	−0.0054 (−0.0100, −0.0007)		0.027	−0.0016 (−0.0066, 0.0035)		0.530
pcF						
MP	−0.0004 (−0.0052, 0.0043)	0.839		0.0020 (−0.0034, 0.0075)	0.424	
MN	0.0038 (0.0020, 0.0056)	0.001		0.0047 (−0.0001, 0.0096)	0.055	
Difference	−0.0050 (−0.0099, 0.0000)		0.048	−0.0027 (−0.0092, 0.0039)		0.407
cT						
MP	−0.0004 (−0.0035, 0.0026)	0.769		0.0012 (−0.0010, 0.0033)	0.248	
MN	0.0031 (−0.0006, 0.0069)	0.095		0.0017 (−0.0015, 0.0048)	0.276	
Difference	−0.0045 (−0.0092, 0.0002)		0.061	−0.0007 (−0.0041, 0.0027)		0.672
Homogeneity						
acF						
MP	0.0004 (−0.0011, 0.0018)	0.589		−0.0013 (−0.0025, −0.0001)	0.037	
MN	−0.0014 (−0.0025, −0.0003)	0.017		−0.0021 (−0.0034, −0.0008)	0.005	
Difference	0.0017 (0.0001, 0.0034)		0.036	0.0006 (−0.0010, 0.0022)		0.463
pcF						
MP	0.0001 (−0.0017, 0.0020)	0.859		−0.0008 (−0.0027, 0.0012)	0.395	
MN	−0.0012 (−0.0018, −0.0006)	0.002		−0.0015 (−0.0032, 0.0003)	0.095	
Difference	0.0016 (−0.0002, 0.0035)		0.085	0.0007 (−0.0017, 0.0030)		0.572
cT						
MP	0.0001 (−0.0009, 0.0011)	0.818		−0.0003 (−0.0010, 0.0003)	0.280	
MN	−0.0011 (−0.0023, 0.0001)	0.072		−0.0004 (−0.0016, 0.0008)	0.433	
Difference	0.0014 (0.0000, 0.0029)		0.056	0.0002 (−0.0010, 0.0014)		0.740

95% CI = 95% confidence interval, MP = mutation-positive, MN = mutation-negative, acF = anterior central femur, pcF = posterior central femur, cT = central tibia.

Number of MP/MN 13/13.

^{*} Significance of the regression coefficient B of age, adjusted for sex, from the models stratified by mutation status.

[†] Significance of the interaction term age × mutation status.

observed by other groups as well⁶¹. However, not all the MP subjects had low BMD. Furthermore, if the differences were solely secondary to impaired bone quality in the MP individuals, differences between the groups would be anticipated also in the LTF cartilage, indicating that the entire joint rather than only the medial compartment would benefit from the reduced joint stress. Our findings therefore suggest that the observed changes are directly related to the local effects of altered WNT signaling.

Bisphosphonate treatment in some of the MP subjects could have altered the natural course of cartilage deterioration. The cartilage preservation potential of bisphosphonates has been surveyed by other groups^{62–64}, but the issue is still underexplored in humans. An earlier study by Lehmann *et al.* reported bisphosphonates to correlate with urinary concentrations of collagen degradation product CTX-II, reflecting possible chondroprotective effects⁶⁵. However, it was concluded that doses required for cartilage protection are higher than those in clinical use for osteoporosis. Based on these data it is unlikely that our findings are solely due to bisphosphonate exposure in the MP group.

Our study has several strengths. The study setting is unique, and we have controlled for several potential confounders, such as ethnic and genetic background, age and sex, by recruiting both MP and MN

individuals from the same two Finnish families aiming at matched age range and sex distribution. Our study presents two main limitations—the relatively small cohort size and the cross-sectional nature of the study. Due to the limited sample size, we were not able to test for differences among different age groups in relaxation times, which are affected by ageing in the articular cartilage⁶⁶. In addition, further functional or *in vitro* experiments using cell lines to study the molecular effects of mutated WNT1 on chondrogenesis and articular cartilage would be highly beneficial but were unfortunately outside the scope of this study. However, despite these limitations, and given the rarity of *WNT1* mutation-positive individuals, we consider our results to bring novel information about WNT1-related OA pathology in humans and detected several significant differences between the groups in the unique study setting. Furthermore, this is the first systematic human study in a cohort with a genetic defect in WNT signaling pathway indicating that impaired WNT signaling may have important protective effects on articular cartilage—in line with previous findings from experimental studies.

We conclude that contrary to our hypothesis, this qMRI study on MP and MN subjects showed that the articular cartilage of the knee joint in *WNT1* mutation-positive subjects was better protected from age-related deterioration compared with the mutation-

negative subjects. The mutation-positive individuals with impaired WNT signaling activity may have altered cartilage turnover suggesting even a potential cartilage-preserving role of the gene defect. Further studies in larger cohorts, in patients with other *WNT1* mutations and in longitudinal study settings are required to confirm these findings and to evaluate whether medical therapies targeting WNT signaling could be used in OA treatment. Additional functional experiments and *in vitro* analyses on the effects of mutated *WNT1* on chondrogenesis and articular cartilage would be highly enlightening.

Authors' roles

Study design: REM, JN, TN, OM, MTN. Study conduct: SL, REM, JN, TN, OM, MTN. Data collection: SL, REM, VC, MH, JN, TN. Data analysis: SL, REM, VC, MH, JN, TN, AP, EL, OM, MTN. Drafting manuscript: SL, REM, VC, MH, JN, TN, AP, EL, OM, MTN. Revising manuscript content: all authors. Approving final version of manuscript: all authors. SL, REM, OM, and MTN take responsibility for the integrity of the data.

Competing interest statement

All authors state that they have no competing interests.

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Supplementary data

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