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Longitudinal stability of molecular endotypes of knee osteoarthritis patients

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SUMMARY

Objective: To assess the longitudinal stability of biomarker-based molecular endotypes of knee osteoarthritis (KOA) participants from APPROACH and to evaluate the consistency of findings in an independent KOA population.
Methods: Nineteen biomarkers were measured longitudinally in 295 KOA participants from the APPROACH cohort. *K*-means clustering was used to identify the structural damage, inflammation, and low tissue turnover endotypes at the six-, 12-, and 24-month follow-ups. Endotype stability was defined as having the same independent endotype assignment longitudinally for patients with complete data (n = 226). Clinical and biochemical characteristics were compared between participants with longitudinally stable and unstable endotypes. The presence and longitudinal stability of the endotypes were evaluated in a different KOA population from the placebo arm of the oral salmon calcitonin trials.
Results: An average overall longitudinal endotype stability of 55% (Fleiss' Kappa of 0.53; 95% confidence interval [CI]: 0.46, 0.60) was demonstrated. An average stability of 59% (range: 54–59%) was observed for

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the structural damage endotype (Fleiss' Kappa 0.52; 95% CI: 0.45, 0.60), 54% (52–56%) for the inflammatory (Fleiss' Kappa 0.61; 95% CI: 0.53, 0.68), and 50% (49–52%) for the low tissue turnover endotype (Fleiss' Kappa 0.46; 95% CI: 0.39, 0.54). Participants with longitudinally unstable endotypes exhibited molecular properties of more than one endotype, which were detectable already at the first visit.

Conclusions: Our study showed for the first time that more than half of KOA participants exhibited a longitudinally stable endotype, highlighting the applicability of biomarker-based endotyping in a clinical trial setting.

Introduction

More than 500 million people worldwide affected by osteoarthritis (OA) are left without effective treatment options. Despite significantly different etiologies, clinical trial designs for evaluation of novel treatments still do not typically involve patient selection based on pheno- or endotypic traits. This has likely contributed to the lack of approved disease-modifying OA drugs (DMOADs) and the high risk of unsuccessful intervention trials in the field.[1] In recent years, various factors indicating diverse patient subpopulations of OA have been described, including phenotypes driven by cartilage, metabolic syndrome, subchondral bone, inflammation, and trauma injury, but their underlying pathobiological mechanisms have not been fully elucidated nor have they been validated by differential treatment response.[2,3]

An endotype is a subtype of a disease population that is mechanistically defined through a distinct pathobiological pathway.[4] There has recently been an emerging interest in studying endotypes of OA as they can provide a more precise definition of patient subpopulations than phenotyping alone when applied to strategic clinical development and precision medicine.[2] Several consortia have aimed to discover OA endotypes, including the Osteoarthritis initiative from the Foundation for the National Institutes of Health (OAI-FNIH)[5] and the **A**pplied **P**ublic-**P**rivate **R**esearch enabling **O**s-teo**A**rthritis **C**linical **H**eadway (APPROACH).[6] Recently, APPROACH described three endotypes driven by: i) structural damage to cartilage and bone, ii) connective tissue inflammation, and iii) low tissue turnover in a two-year, European, multi-center, observational cohort of 295 tibiofemoral knee OA (KOA) participants.[7] The endotypes were discovered at baseline while the study was ongoing using a panel of serum and urine biomarkers that reflect cartilage and bone turnover as well as inflammation.

Identifying endotypes in an OA population, specifically clinically actionable endotypes, may enable successful interventional drug trials by matching the right patients with their appropriate treatment options, thereby shifting to patient-centric personalized medicine. To facilitate the enrichment of a specific molecular endotype at the screening visit for a clinical trial, it is imperative to understand the longitudinal stability of the endotypes within the length of the trial. To our knowledge, this has yet to be assessed and remains an open question. Understanding the longitudinal stability of such endotypes is a crucial step to ensure the clinical applicability of endotyping for the enrichment of the right patient subgroups in clinical intervention trials.

The aim of this study was to evaluate the longitudinal stability of the biomarker-based endotypes discovered at baseline in APPROACH and to elucidate the molecular differences between participants exhibiting endotypic stability over time and those with unstable endotypes. Both the cross-sectional presence and longitudinal stability of the endotypes were assessed in an independent KOA population from the two trials evaluating the efficacy of oral salmon calcitonin (ClinicalTrials.gov IDs: NCT00486434 and NCT00704847).

Method

Participants

297 participants fulfilling the American College of Rheumatology (ACR) criteria for tibiofemoral KOA from APPROACH (ClinicalTrials.gov ID: NCT03883568) were considered for this study. The observational cohort included measurements of 14 serum and two urine biochemical markers collected at baseline and after six, 12, and 24 months.[6] Month 24 had a time window of −2/+6 months due to limitations arisen from the COVID-19 pandemic.[8] Two participants had 10 or more missing biochemical measurements and were excluded, resulting in 295 participants (77% female) at baseline. 226 participants (77%) had measurements available for all visits (Supplementary Table 1). At baseline, serum and urine samples were collected in a fasting state, when possible, and 73% of the participants had fasted (Supplementary Table 2). Due to logistical limitations arisen during sampling of follow-up data, this was no longer required for the follow-up visits. This resulted in a shift to mostly non-fasting sampling conditions from baseline to the follow-up visits. As fasting status is known to affect some biochemical measurements, especially those related to bone turnover,[9,10] data from month six to 24 were considered.

Biochemical marker data

In order to assess the longitudinal stability of the biomarker-based endotypes discovered at baseline in APPROACH, the 16 biomarkers originally measured at baseline were included in this study and measured at all visits as described in Angelini *et al*., 2022.[7] For an in-depth review of the biochemical markers used for endotyping in APPROACH, we refer to Hannani *et al.*, 2024.[11] In addition, PRO-C1,[12] PRO-C4,[13] and VICM[14] were measured. Thus, a total of 19 biochemical markers primarily reflecting cartilage turnover (ARGS-aggrecan, C2M, C10C, cartilage oligomeric matrix protein [COMP], Coll2–1, Coll2–1NO_2, hyaluronic acid [HA], PRO-C2, and uCTX-II), bone turnover (sCTX-I, N-MID, PRO-C1, and u-αCTX-I), and inflammation (C1M, C3M, CRPM, high-sensitivity C-reactive protein [hsCRP], PRO-C4, and VICM) were quantified (Supplementary Methods are referred to for in-depth methods descriptions).

Data preprocessing

Urinary biomarkers (u-αCTX-I and uCTX-II) were corrected for creatinine levels, and all biochemical measurements were log-transformed (natural logarithm). Extreme outliers on the log-scale were winsorized with Tukey's rule.[7] For each marker, extreme outliers were defined as points outside the 1.5 x interquartile range and measurements outside the lower and upper bounds were replaced by the 5th and 95th quantiles, respectively (Supplementary Table 3).

Clustering of longitudinal biochemical marker data

Clustering of the longitudinal biomarker data was adapted from Angelini *et al.*, 2022.[7] Biomarker data used for clustering was imputed for missing data (0.69–1.12%) with a random forest model for each visit separately (Supplementary Fig. 1). Differently from Angelini *et al.*, 2022,[7] the biochemical marker data was scaled (z-score transformation) separately for men and women for each visit to mitigate the sex-specific differences in the biomarkers observed at baseline (Supplementary Fig. 2).[7] To reduce the effect of correlations between markers (Supplementary Fig. 3),[15] a principal component (PC) analysis was performed on the scaled biochemical data for each visit separately. The PCs explaining 95% of the variance were used for *k*-means clustering for every visit using $k = 3$, representing the three discovered endotype clusters at baseline.[7] To obtain reliable endotype clusters, the clustering was repeated 100 times. The final endotype was assigned to participants per visit based on their majority endotype allocation.

Longitudinal endotype stability

Longitudinal endotype stability was defined as having the same independent endotype cluster assignment for all visits. Participants were divided into longitudinal endotypic profiles: i) stable inflammatory, ii) stable structural damage, iii) stable low tissue turnover, and iv) unstable. Longitudinal stability was assessed for participants with measurements available for all visits ($n = 226$) across all 100 clustering repetitions. As some participants may be assigned to the same endotype across all visits by chance, the reliability of the longitudinal stability of the endotypes was assessed with Fleiss' Kappa.[16]

Longitudinally stable and unstable endotypes

As the purpose of this work was exploratory in nature, statistical tests of significance were not appropriate.[17] Results should be interpreted in light of this and will be reported as estimates with ranges, standard deviations or confidence intervals (CIs). Clinical characteristics at month six (Supplementary Table 4) and changes from month six to 24 were estimated for the longitudinal endotype profiles. Mean differences in log-transformed marker levels (non-scaled and non-imputed) between the longitudinal endotype profiles were assessed with linear mixed-effects models (LMMs). For each biomarker, an LMM was run with interaction between visit and the longitudinal endotype profiles, adjusting for participant-specific random effects. A model was run with and without adjusting for known confounders (age, sex, and body mass index [BMI]).

Endotype membership degrees

A "fuzzy" clustering was run on the retained PCs to obtain endotype membership grades. The fuzzy *k*-means clustering algorithm *FKM* was used with $k = 3$. Participants were assigned to an endotype based on the highest membership degree to an endotype cluster. Longitudinal endotype stability was assessed as previously described.

Consistency of findings in external clinical trial data

The clustering process was replicated on biomarker data from the placebo groups of two phase III, randomized, placebo-controlled trials evaluating the efficacy of oral salmon calcitonin, CSMC021C2301 ($n = 1176$) (ClinicalTrials.gov ID: NCT00486434) and CSMC021C2302 ($n = 1030$) (ClinicalTrials.gov ID: NCT00704847) (collectively referred to as SMC)[18] to evaluate the consistency of the findings from APPROACH in an independent KOA population. Both the cross-sectional presence and longitudinal stability of the endotypes originally discovered in APPROACH were assessed.

Participants fulfilling the ACR criteria for KOA aged 51–80 with target knee Kellgren-Lawrence (KL) grade 2–3, medial joint-space width (JSW) ≥2 mm, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain > 150/500 and/or WOMAC function 510/1700 mm (high WOMAC indicating worse symptoms) were recruited in SMC. Biomarker data were available at baseline and month 24, and 524 participants with data available for more than five biomarkers at both visits were included (Supplementary Table 5). Overlapping biomarkers between APPROACH and SMC (C1M, C2M, C3M, CRPM, sCTX-I, uCTX-II, N-MID, PRO-C2, and VICM) were considered. Missing biomarker data (~4%) were imputed as previously described, as well as preprocessing and clustering. Clustering of the APPROACH participants was repeated using the nine overlapping biomarkers, considering data from month six and 24 ($n = 232$). Clinical characteristics at baseline (Supplementary Table 6) and clinical changes over time of the longitudinal endotype groups (Supplementary Table 7) were estimated.

Results

Longitudinal presence of molecular endotypes

Clustering analysis of longitudinal data of 19 biomarkers (month six to 24) from KOA participants from APPROACH[6] confirmed the presence of the three molecular endotypes driven by: i) inflammation, ii) structural damage, and iii) low tissue turnover (Fig. 1A) described in Angelini *et al.*, 2022.[7] To ensure the robustness of the endotype clusters, the clustering was repeated 100 times. Across the repetitions, an extremely high endotype agreement was observed for month six (Fleiss' Kappa of 0.998; 95% CI: 0.996, 0.999), 12 (Fleiss' Kappa of 1; 95% CI: 1, 1), and 24 (Fleiss' Kappa of 0.995; 95% CI: 0.989, 0.999). The final endotype was assigned to each participant based on the most frequent endotype assignment across repetitions for each visit independently.

The highest levels of markers of inflammation such as hsCRP, C1M, C3M, and PRO-C4 were observed in the inflammatory endotype, while bone and cartilage turnover markers such as N-MID, u-αCTX-I, and sCTX-I were highest in the structural damage endotype (Supplementary Figs. 4–7 & Supplementary Table 7). The low tissue turnover endotype was defined by overall low levels of most biomarkers. At baseline, 77% of the participants were female, and the same distribution was observed on average for the endotype clusters across all visits (74–79% female), indicating that the clustering was not driven by sex differences (Table I & Supplementary Fig. 8).

Longitudinal stability of molecular endotypes

Longitudinal endotype stability was defined as being independently assigned the same endotype for all visits (month six, 12, and 24) and was assessed for participants with data available at all visits ($n = 226$). Across 100 clustering repetitions, an average overall longitudinal endotype stability of 55% (range: 52–56%) was observed (Table II & Fig. 1B) with a chance-corrected agreement coefficient (Fleiss' Kappa of 0.53; 95% CI: 0.46, 0.60). Relative to the number of participants assigned to the endotype at the first visit (month six), the highest average longitudinal stability was observed for the structural damage endotype of 59% (range: 54–59%) followed by 54% (range: 52–56%) for the inflammatory, and 50% (range: 49–52%) for the low tissue turnover endotype. However, the sizes of the endotype groups at month six differed, ranging from average sizes of 65 – 86. Of the participants with longitudinally unstable endotypes, the most frequent bidirectional transition of 47% (range: 46–49%)

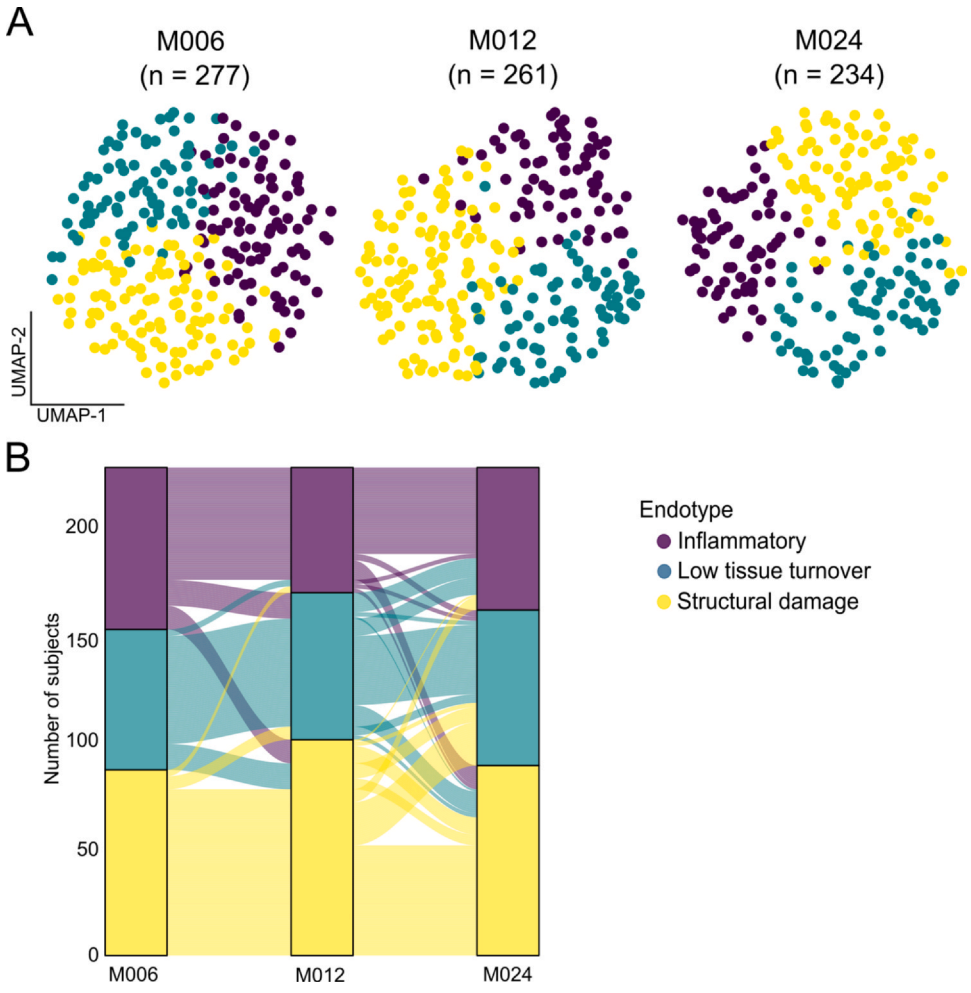

Fig. 1

Osteoarthritis and Cartilage

A) Uniform Manifold Approximation and Projections (UMAPs) of the six- (M006), 12- (M012), and 24-month (M024) follow-up visits of tibiofemoral OA participants from APPROACH.[6] UMAPs are based on the PCs from a PC analysis that explained 95% of the variance of scaled biochemical marker data for each visit separately. B) Sankey diagram of the longitudinal stability of endotype assignments for each participant with biochemical measurements available for all visits ($n = 226$).

occurred between the structural damage and low tissue turnover endotypes. 24% (range: 22–25%) of the longitudinally unstable endotypes transitioned between inflammation and low tissue turnover, and 25% (range: 23–26%) between inflammation and structural damage (Supplementary Table 9). Only 4% (range: 4–5%) were assigned to all endotypes longitudinally (interpreted as a "random" endotypic profile). Considering longitudinal stability of the endotypes between the first and last visit, an average overall stability of 64% (range: 62–66%) was achieved (Supplementary Table 10).

Clinical and molecular characteristics of longitudinally stable and unstable endotypes

Longitudinal changes in KL grades, JSW, and WOMAC scores of the longitudinal endotype profiles were estimated (Table III). No apparent differences were observed between the longitudinal endotype profiles for the traditional clinical parameters of OA. However, numerically higher WOMAC scores were observed for the longitudinally stable inflammatory endotype at month six (Supplementary Table 4). Percent changes in geometric mean biomarker levels between the longitudinal endotype profiles were estimated and adjusted for age, sex, and BMI (Supplementary Table 11). At month six, changes in inflammatory markers such as hsCRP (49.5%; 95% CI: 24.9%, 79.0%) and VICM (27.9%; 95% CI: 17.1%, 39.7%) were observed in the longitudinally stable inflammatory endotype relative to the unstable endotype (Fig. 2 & Supplementary Figs. 9–10). Changes in markers of bone turnover such as N-MID (24.0%; 95% CI: 13.2%, 35.7%) and PRO-C1 (11.3%; 95% CI: 4.2%, 18.9%) were observed for the longitudinally stable structural damage endotype compared to the unstable endotype. Negative changes in the longitudinally stable low tissue turnover endotype relative to the unstable endotype were found for markers of bone and cartilage turnover, and inflammation such as N-MID (−25.7%; 95% CI: −33.4%, −17.2%), C2M (−12.4%; 95% CI: −17.2%, −7.2%), and VICM (−12.6%; 95% CI: −20.6%, −3.9%).

Membership degrees of all endotypes

Rather than assigning KOA participants to one endotype with *k*-means clustering, a "fuzzy" *k*-means clustering approach was

Months	Endotype	No. of participants	Percentage female
6	Inflammatory	92 (90 – 92)	79% (79–79%)
	Structural damage	104 (104 – 109)	79% (78–79%)
	Low tissue turnover	81 (78 – 81)	74% (74–76%)
12	Inflammatory	68 (67 – 68)	78% (78–78%)
	Structural damage	116 (112 – 116)	78% (78–79%)
	Low tissue turnover	77 (77 – 82)	74% (73–74%)
24	Inflammatory	66 (66 – 67)	76% (76–76%)
	Structural damage	92 (87 – 93)	76% (76–77%)
	Low tissue turnover	76 (75 – 81)	77% (77–78%)

Shown are average (min – max range) results based on 100 repetitions of clustering and endotype assignment of participants.

Table I Osteoarthritis and Cartilage

Distribution of women in endotype clusters of tibiofemoral OA participants from APPROACH,[6] considering participants at all visits at month six ($n = 277$), 12 ($n = 261$), and 24 ($n = 234$), and participants with data available at all visits ($n = 226$).

applied that computes the degree of belonging to each endotype. There was an overall endotype assignment agreement between the *k*-means and fuzzy *k*-means clustering algorithms of 94% (Cohen's Kappa of 0.91; 95% CI: 0.89, 0.94) and a longitudinal endotype stability agreement of 92% (Cohen's Kappa of 0.84; 95% CI: 0.77, 0.91).

Participants located on the extreme ends of the UMAPs had endotype membership degrees dominated by mostly one endotype, while participants located at the borders between the endotype clusters had higher degrees of belonging to more than one endotype (Fig. 3A & Supplementary Figs. 11A-12A). Participants with longitudinally unstable endotypes were mostly located around the borders between endotype clusters (Fig. 3B & Supplementary Figs. 11B-12B).

Considering the distributions of the degrees of belonging to all endotypes for the longitudinally stable (Fig. 4A) and unstable (Fig. 4B) endotypes, participants with stable endotypes had membership degree distributions dominated by that particular endotype already at the first visit. Participants who transitioned between two endotypes exhibited higher membership degrees of belonging to both endotypes rather than having one dominant membership distribution, suggesting that the KOA participants displayed longitudinal molecular properties of more than one endotype that could be captured already at the first visit.

Consistency of endotypic findings in external study

Both the cross-sectional presence and longitudinal stability of the three molecular endotypes were assessed in an external clinical intervention study of 524 KOA participants from the placebo arm of the oral salmon calcitonin trials (SMC).[18] SMC included a larger Asian population compared to APPROACH, only recruited KL grades 2–3, had numerically lower medial JSW, and higher WOMAC pain, function, and stiffness scores (higher WOMAC scores indicated worse symptoms) (Supplementary Table 5). However, large variations were associated with the WOMAC scores. As such, the SMC participants represented a different population from a clinical trial setting with pronounced structural and pain severity of KOA.

As biomarker data was available at baseline and month 24 for SMC, data from month six and 24 was considered for APPROACH. Clustering of the SMC and APPROACH participants based on overlapping biomarkers (C1M, C2M, C3M, CRPM, sCTX-I, uCTX-II, N-MID, PRO-C2, and VICM) (Supplementary Table 12) revealed the cross-sectional presence of endotypes driven by inflammation, structural damage, and low tissue turnover with very similar biomarker profiles (Fig. 5 & Supplementary Fig. 13). When considering two visits and clustering of common biomarkers, APPROACH exhibited an average overall longitudinal endotype stability of 68% (range: 68–70%) and likewise SMC showed overall average stability of 68% (range: 67–69%) (Supplementary Tables 13–14 & Supplementary Fig. 14). For participants with longitudinally unstable endotypes, the most prevalent transitions for both cohorts occurred between structural damage and low tissue turnover at an average of 44% (range: 41–45%) for APPROACH and 36% (range: 33–38%) for SMC (Supplementary Tables 15–16).

Discussion

Molecular endotyping represents a promising tool for the enrichment of the right OA patient population in clinical trials of DMOADs.[19] In doing so, it is imperative to understand the longitudinal stability of the endotype assigned to the individual patients at the point of enrolment.[11] Markers of tissue remodeling have been utilized to endotype OA patients, and three biomarker-based endotypes have recently been discovered at baseline in 295 KOA participants from APPROACH.[7] As tissue remodeling may change over time in OA patients, this may also be reflected by their molecular endotype within the length of a clinical trial. Whether OA patients keep the same endotype longitudinally has yet to be assessed and remains an open question.[11]

Endotype	No. of participants at month six	No. of participants with stable endotype	Longitudinal endotype stability	Fleiss' Kappa (95% CI)	Stability measure
Inflammatory	75 (73 – 75)	40 (39 – 41)	54% (52–56%)	0.61 (0.53, 0.68)	Per endotype
Structural damage	86 (86 – 90)	51 (46 – 51)	59% (54–59%)	0.52 (0.45, 0.60)	
Low tissue turnover	65 (63 – 65)	32 (31 – 34)	50% (49–52%)	0.46 (0.39, 0.54)	
Total	226	123 (118 – 126)	55% (52–56%)	0.53 (0.46, 0.60)	Overall

CI; Confidence interval.
Overall longitudinal stability was defined as the total number of participants independently assigned to the same endotype at all visits (month six, 12, and 24) relative to the total number of participants. Longitudinal stability per endotype was defined as the number of participants stably assigned to that endotype relative to the number of participants assigned the endotype at the first visit (month six). Shown are average (min – max range) results based on 100 repetitions of clustering and endotype assignment of participants. Unweighted chance-corrected agreement coefficient across visits was assessed with Fleiss' Kappa.

Table II Osteoarthritis and Cartilage

Longitudinal endotype stability of tibiofemoral OA participants from APPROACH[6] with biochemical measurements available for all visits ($n = 226$).

	Stable Inflammatory ($n = 40$)[a]	Stable structural damage ($n = 51$)[b]	Stable low tissue turnover ($n = 32$)[c]	Unstable ($n = 103$)[d]
ΔBMI (kg/m^2)				
Mean (SD)	0.04 (1.55)	−0.07 (1.43)	0.04 (1.51)	0.06 (1.41)
ΔKL grade				
−1	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.1%)
−0	34 (91.9%)	47 (92.2%)	24 (77.4%)	83 (89.2%)
1	3 (8.1%)	4 (7.8%)	7 (22.6%)	9 (9.7%)
ΔJSW medial (mm)				
Mean (SD)	−0.09 (0.57)	−0.17 (0.53)	−0.09 (0.39)	−0.06 (0.49)
ΔJSW lateral (mm)				
Mean (SD)	−0.19 (1.27)	−0.10 (0.98)	0.27 (1.24)	−0.02 (1.12)
ΔJSW min (mm)				
Mean (SD)	−0.26 (0.77)	−0.18 (0.58)	−0.02 (0.82)	−0.03 (0.64)
ΔWOMAC Pain (%)				
Mean (SD)	−1.05 (15.73)	0.98 (15.49)	0.65 (19.09)	0.30 (17.10)
ΔWOMAC Function (%)				
Mean (SD)	−2.64 (14.43)	−0.31 (15.55)	−2.85 (14.43)	0.19 (15.32)
ΔWOMAC Stiffness (%)				
Mean (SD)	−6.88 (17.89)	−0.77 (18.29)	0.00 (25.41)	−0.87 (21.08)

Higher Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores indicate worse symptoms. Δ = Change from six- to 24-month follow-up; KL, Kellgren-Lawrence; JSW, joint-space width; SD, Standard deviation.
Data from target knee of participants with biomarker data available at all visits ($n = 226$) was considered.
[a] Missing data: KL grade ($n = 3$), JSW medial ($n = 5$), JSW lateral ($n = 5$), JSW min ($n = 5$), WOMAC Pain ($n = 2$), WOMAC Function ($n = 5$).
[b] Missing data: JSW medial ($n = 2$), JSW lateral ($n = 1$), JSW min ($n = 1$), WOMAC Function ($n = 4$), WOMAC Stiffness ($n = 2$).
[c] Missing data: KL grade ($n = 1$), JSW medial ($n = 2$), JSW lateral ($n = 2$), JSW min ($n = 2$), WOMAC Pain ($n = 1$), WOMAC Function ($n = 1$), WOMAC Stiffness ($n = 1$).
[d] Missing data: KL grade ($n = 10$), JSW medial ($n = 14$), JSW lateral ($n = 12$), JSW min ($n = 12$), WOMAC Pain ($n = 3$), WOMAC Function ($n = 16$), WOMAC Stiffness ($n = 2$).

Table III Osteoarthritis and Cartilage

Clinical changes from month six to 24 of longitudinal endotype groups of tibiofemoral OA participants from APPROACH.[6]

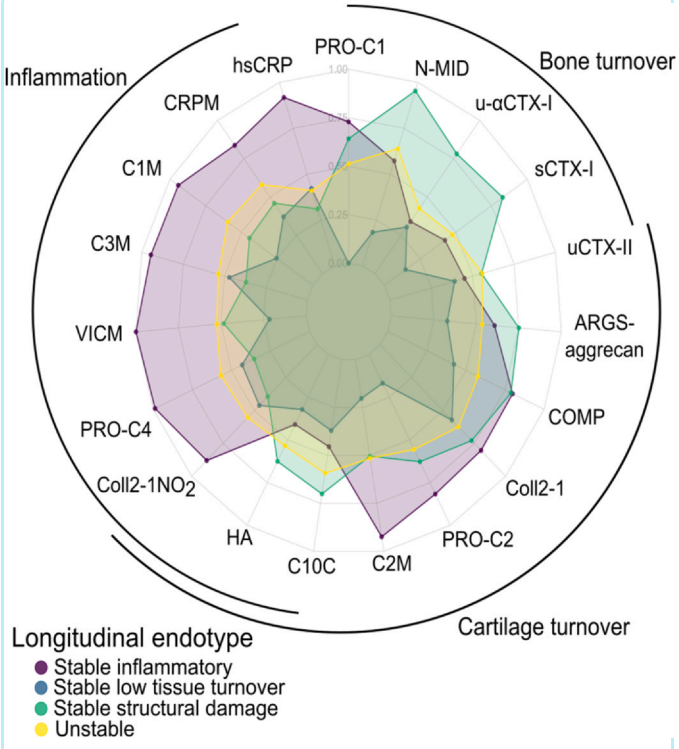

Fig. 2 Osteoarthritis and Cartilage

Biochemical marker profiles of 226 tibiofemoral OA participants with longitudinally stable and unstable endotypes from APPROACH.[6] Min-max normalized median biomarker measurements are shown for the six-month follow-up.

The longitudinal stability (> 6 months) of endotypes and phenotypes have been assessed in a limited number of diseases, most notably in respiratory diseases. Asthma phenotypes have been reported to exhibit longitudinal stability of 60% over 12 months.[20] Endotypes of chronic rhinosinusitis have shown longitudinal stability of 35% (median follow-up of 15.5 months),[21] while endotypes of sleep apnea have exhibited stability of 59–72% (mean follow-up of 6.5 years).[22] This study aimed to determine the longitudinal stability of endotypes of OA discovered in APPROACH.[7] Our study showed for the first time that, on average, 55% of the KOA participants exhibited longitudinal stability of their endotype across all follow-up visits over a period of 18 months. Considering the longitudinal stability between the first and last visit, as was done in previous studies evaluating the longitudinal stability of endotypes,[20–22] an average stability of 64% was demonstrated. We evaluated both the presence and, for the first time, the longitudinal stability of the endotypes in a larger, KOA population from the SMC clinical trials with pronounced structural and pain severity compared to the observational cohort from which they were originally described. An average of 68% of the SMC participants exhibited longitudinal endotype stability over 24 months. This indicated the consistency of biomarker-based endotyping even in a seemingly different KOA population and its potential generalizability and applicability in a clinical trial setting. However, future work is needed to establish the presence of the endotypes in other subpopulations such as trauma-induced OA.

Differences in baseline clinical characteristics and changes over time between the longitudinal endotype profiles of traditional clinical measures of OA appeared minimal. This may be attributable to large within-group variations and radiographic measurement uncertainty. This was reflected by cases of lowered KL grade and increased medial JSW over time.[23]

Another explanation for the lack of compelling clinical differences between the endotypes could simply be that they may not differ phenotypically. By definition, a phenotype comprises the observable presentation of a disease, while an endotype encompasses

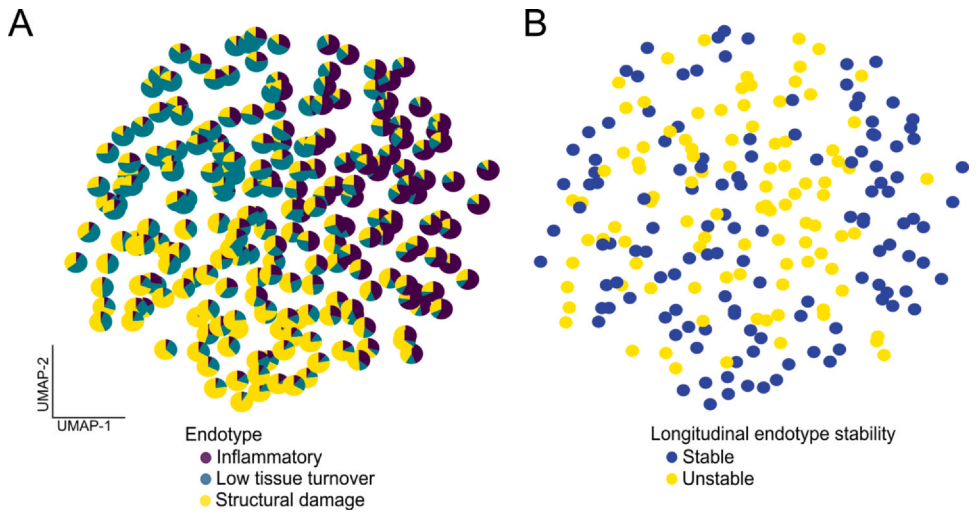

Fig. 3 Osteoarthritis and Cartilage

A) Pie charts of endotype membership degrees and B) longitudinal endotype stability status projected onto a Uniform Manifold Approximation and Projections (UMAP) of 226 tibiofemoral OA participants from APPROACH[6] at the six-month follow-up. UMAPs are based on the PCs from a PC analysis that explained 95% of the variance of scaled biochemical data.

the underlying pathobiology that drives the disease.[24] It is conceivable that heterogeneous OA populations may exhibit similar clinical manifestations as the disease progresses,[19] such as significant pain and ultimately needing total knee replacement. However, their underlying molecular path to the observable traits may differ depending on their endotypes and should be treated accordingly.[11] As such, the validity and clinical value of molecular endotypes perhaps need not rely on differences in traditional clinical

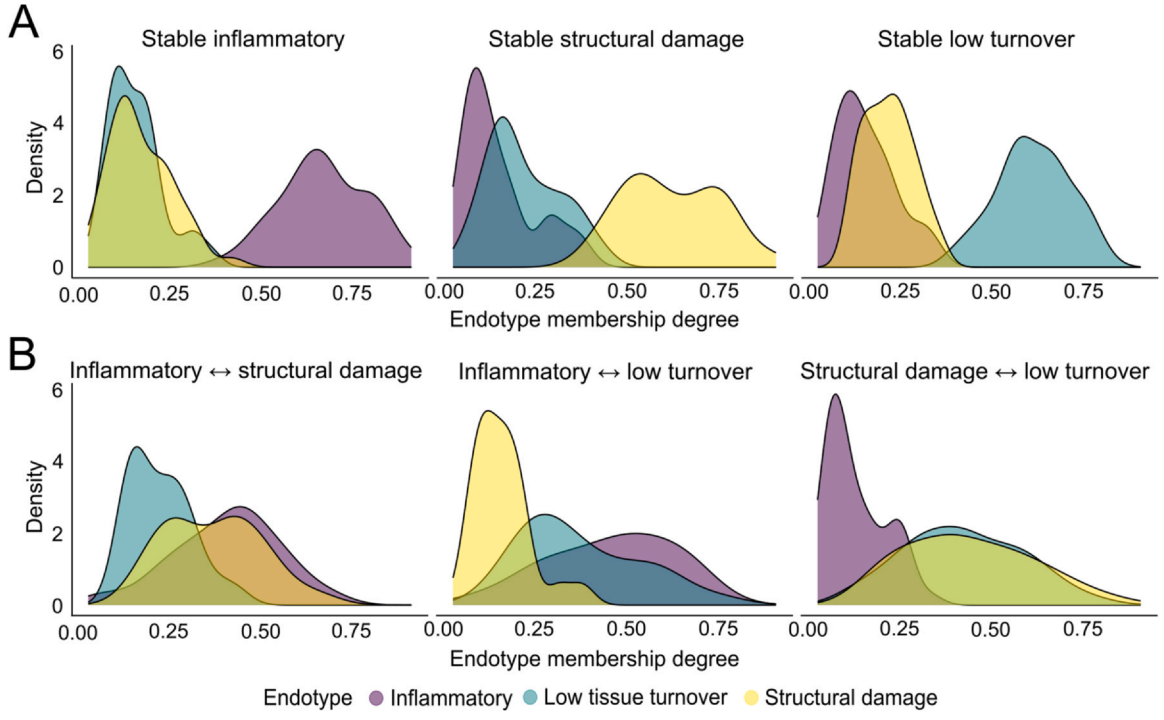

Fig. 4 Osteoarthritis and Cartilage

Distribution of endotype membership degrees of 226 tibiofemoral OA participants from APPROACH[6], grouped by longitudinally A) stable endotypes and B) unstable endotypes. Membership degrees are shown for the six-month follow-up.

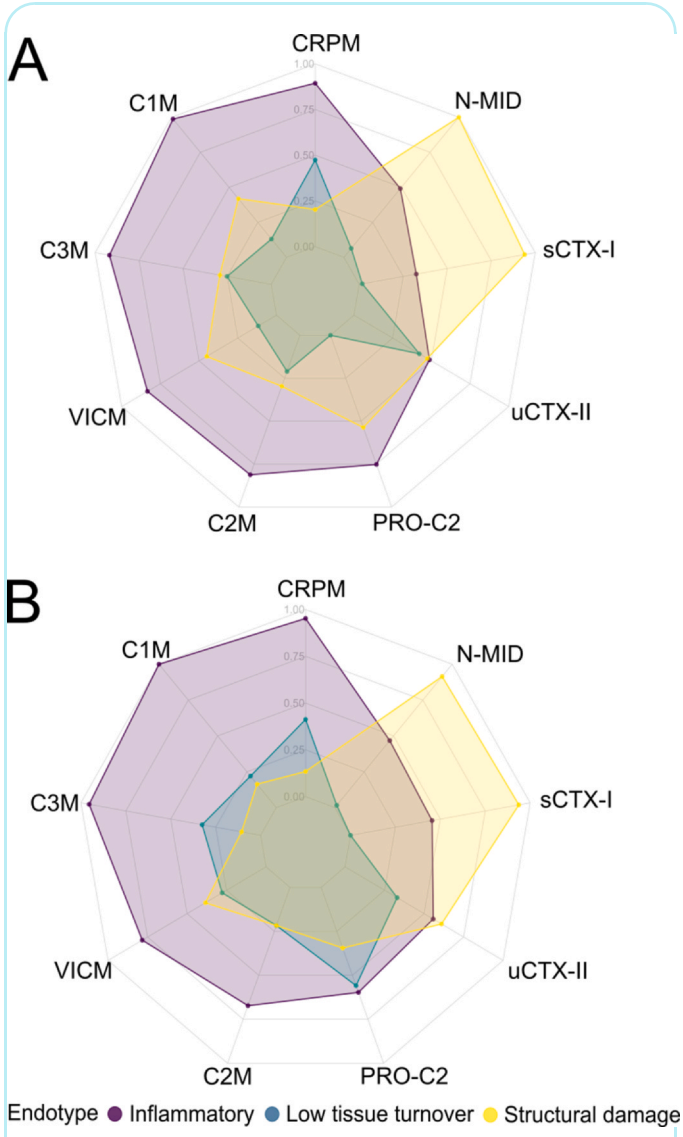

Fig. 5 Osteoarthritis and Cartilage

Biochemical marker profiles of A) 232 tibiofemoral OA participants from APPROACH[6] with biochemical measurements available for the six- and 24-month follow-up visits, and B) 524 tibiofemoral OA participants from the placebo-arm of the oral salmon calcitonin (SMC) clinical trials[20] with data available at baseline and 24-month follow-up. Min-max normalized median biomarker measurements are shown for the six-month follow-up for APPROACH and baseline for SMC.

measures of OA (such as KL grade and JSW) but rather their differential responses to DMOADs depending on their underlying pathobiological driver of the disease.

Three endotypes were identified at baseline with *k*-means clustering in KOA participants from APPROACH.[7] Driven by the same biomarker profiles, our study showed that the endotypes could be found at all visits with longitudinal stability. Participants with a longitudinally stable inflammatory endotype exhibited increased changes in inflammatory markers relative to the participants with longitudinally unstable endotypes, indicating that maintained higher levels of inflammation was associated with longitudinal stability. Increased changes in bone and cartilage turnover markers were found for the longitudinally stable structural damage endotype relative to the unstable endotype, indicating that maintained higher levels of structural damage was associated with longitudinal stability.

Participants with a longitudinally unstable endotype exhibited molecular features of several endotypes and we hypothesized that some OA participants may be driven by more than one endotype. Through "fuzzy" *k*-means clustering, endotypes were allowed to overlap and the degrees of belonging to every endotype were computed rather than assigning participants to only one.[25] As it has previously been suggested that molecular OA endotypes may overlap,[19] "fuzzy" clustering may allow for a more accurate depiction of the interwoven biology of tissue remodeling in OA. However, the concept of overlapping endotypes has yet to be established in OA and merits further investigation. An OA patient may be driven by structural damage as well as inflammation and may be dominated by structural damage at one point and inflammation at another. This was observed for an average of 12% (range: 11–12%) of the KOA participants. Assuming OA patients with an inflammatory endotype could benefit from anti-inflammatory treatment, such a subpopulation could benefit from anti-inflammatory treatment even if they did not have an endotype profile dominated only by inflammation. We believe these patients represent an interesting disease subpopulation and future investigation into why some OA patients transition between two endotypes is needed.

Limitations of this study included the low number of participants in APPROACH ($n = 295$) and SMC ($n = 524$), and the limited number of biomarkers measured in both cohorts (9/19). Thus, a true validation was not feasible, and the clustering procedure was rather replicated in both cohorts using common biomarkers, and the consistency of both the presence and longitudinal stability of the endotypes were assessed.[7] Another limitation was that it was not possible to include baseline data in APPROACH due to large fasting shifts from baseline to follow-up visits caused by changes in sampling requirements. Such shifts are known to affect biomarkers of bone turnover.[9,10] It was therefore decided to focus on the follow-up data to avoid undesirable biological variations between visits, resulting in longitudinal data of 18 months (−2/+6 months) rather than 24 for APPROACH. Biological variation of the biomarkers between men and women had previously been reported at baseline.[7] Efforts were made to limit these effects for clustering of the KOA participants, and differences in the biomarker levels between the longitudinal endotype profiles were adjusted for age, BMI, and sex. While not feasible in this study due to the low number of men in APPROACH (68/295), it would be informative to perform sex-specific endotyping in OA to elucidate the potential of gender-bias.[7,26] A larger and more diverse population is needed to investigate potential race-specific differences in endotypes of OA. Another limitation of this study was that *k*-means clustering was performed for each visit separately, which can potentially result in incorrect endotype allocations over time of a participant due to differing models. Therefore, some of the endotype instability may be modeling artefacts. Some participants may also be assigned to the same endotype across all visits by chance, and the longitudinal endotype stability was therefore reported alongside Kappa coefficients.

While the panel of biomarkers used to discover the molecular endotypes in OA[7] is based on over 20 years of research[11], the definition of the endotypes is limited to the biology of which they reflect. Other important biological aspects of the endotypes or entirely different endotypes could, therefore, be missed, and the use of a relatively restricted set of biomarkers is a limitation of this work. Synovial markers such as matrix metalloproteinase-3 and CD14 have been used to describe an inflammatory OA endotype[27] while serum metabolites such as butyrylcarnitine, arginine, and lysophosphatidylcholine have been associated with OA endotypes of muscle weakness, arginine deficit, and low inflammation.[28]

In conclusion, our study showed for the first time that more than half of KOA patients keep the same endotype longitudinally. We presented a novel way to depict endotype properties by computing their membership degrees of each endotype, indicating that some KOA patients exhibit molecular properties of more than one endotype. Both the presence and longitudinal stability of the endotypes driven by structural damage, inflammation, and low tissue turnover were shown for the first time in an independent KOA population from a clinical trial setting. These results support the applicability of biomarker-based endotyping for use in clinical trial settings to facilitate the successful development of DMOADs.

Ethics approval

This study involved human participants and complied with the protocol, Good Clinical Practice (GCP), the Declaration of Helsinki, and the ethical and legal regulatory requirements for all countries involved (Supplementary Table 17). All study participants provided written informed consent to participate in the study before taking part.

Role of the funding source

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Author contributions

All authors were involved in drafting the manuscript or revising it critically for important intellectual content. All authors provided approval of the version to be submitted. Study conception and design: Hannani, Thudium, Gellhorn, Larkin, Bacardit, Bay-Jensen. Acquisition of data: Struglics, Uebelhoer, Henrotin, Bihlet, Blanco, Haugen, Kloppenburg, Berenbaum, Bay-Jensen. Analysis and interpretation of data by Hannani, Thudium, Gellhorn, Larkin, Karsdal, Lisowska-Petersen, Frederiksen, Bager, Ladel, Mobasheri, Bacardit, Bay-Jensen.

Conflict of interest

Thudium, Karsdal, Frederiksen, Bager, and Bay-Jensen are full time employees and shareholders of Nordic Bioscience, a privately owned biotechnology company developing biomarkers for fibro-inflammatory diseases. Gellhorn is a fulltime employee of GlaxoSmithKline. Larkin is the founder of SynOA Therapeutics. Ladel is a consultant and provides consultancy to Regenosine, TrialSpark, Charité, Curnova, and RheumaNederland. Uebelhoer is a full-time employee of Artialis SA. Henrotin is a consultant and provides consultancy for Artialis, Kiomed Pharma, Grünenthal, Expanscience, Tilman, GeneQuine and Allegro. Bihlet is an employee of and shareholder in NBCD. Blanco reports funding from Gedeon Richter Plc., Bristol-Myers Squibb International Corporation (BMSIC), Sun Pharma Global FZE, Celgene Corporation, Janssen-Cilag International N.V, Janssen Research & Development, Viela Bio Inc., AstraZeneca AB, UCB BioSciences GmbH, UCB Biopharma SPRL, AbbVie Deutschland GmbH & Co.KG, Merck KGaA, Amgen Inc., Novartis Farmacéutica SA, Boehringer Ingelheim España SA, CSL Behring, LLC, GlaxoSmithKline Research & Development Limited, Pfizer Inc., Lilly SA, Corbus Pharmaceuticals Inc., Biohope Scientific Solutions for Human Health S.L., Centrexion Therapeutics Corp., Sanofi, Tedec-Meiji Farma SA, Kiniksa Pharmaceuticals Ltd., and Grünenthal. Haugen has provided consultancy for GlaxoSmithKline, Novartis, and Grünenthal, and received payment for lecture from AbbVie. Kloppenburg received grants from IMI-APPROACH, Dutch Arthritis Society, all paid to institution, and royalties from Wolters-Kluwer and Springer Verlag, all paid to institution. Kloppenburg has received fees for consultancy/advisory boards by AbbVie, Kiniksa, Galapagos, CHDR, Novartis, UCB, and GlaxoSmithKline, all paid to institution, and payments for lectures/educational events from Janssen and Galapagos, all paid to institution. Berenbaum reports personal fees from Aché Laboratories, AstraZeneca, Boehringer, CellProthera, Expanscience, Galapagos, Gilead, Grünenthal, GlaxoSmithKline, Eli Lilly, Heel, Merck Sereno, MSD, Nordic, Novartis, Pfizer, Roche, Sandoz, Sanofi, Servier, UCB, Peptinov, 4P Pharma, 4Moving Biotech, and grants from TRB Chemedica, outside the submitted work. Mobasheri is a senior advisor to the World Health Organization Collaborating Center for Public Health Aspects of Musculoskeletal Health and Aging and "Collaborateur Scientifique de l'Université de Liège" at the University of Liège, Belgium. Mobasheri has provided consultancy for Genacol, Sterifarma, Sanofi (Brazil), Janssen-Cilag (Brazil), Sanofi (France), Sanofi (USA), Pacira Biosciences, Grünenthal, Viatris, Novartis, Expanscience, Galapagos, Chiron, Dorian Therapeutics, Kolon TissueGene, Ampio Pharmaceuticals, Aptissen SA, Contura, HALEON, Nestlé, Nestlé Wobenzym, Synartro AB, Pfizer Consumer Healthcare, GSK Consumer Healthcare, and Aché (Aché Laboratórios Farmacêuticos). All authors have received grants from IMI-APPROACH (grant 115770).

Data availability

Data from APPROACH can be obtained upon reasonable request to the APPROACH Steering Committee.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.joca.2024.11.002.

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