Gait & Posture 91 (2022) 312-317

Identifying joint-specific gait mechanisms causing impaired gait in alkaptonuria patients

H.R.Shepherd[1], M.A.Robinson[1], Lakshminarayan R Ranganath[2], G.J.Barton[1]

[1]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool L3 3AF
[2]National Alkaptonuria Centre, Liverpool University Hospitals NHS Foundation Trust, Liverpool L7 8XP.

Keywords: *alkaptonuria, rare disease, statistical parametric mapping, gait, osteoarthritis*

Abstract

Background: Alkaptonuria is a rare genetic disease that leads to structural joint damage and impaired movement function. Previous research indicates that alkaptonuria affects gait, however the detailed mechanisms are unknown. **Research question:** What are the joint-specific gait mechanisms which contribute to impaired gait in alkaptonuria patients? **Methods:** The gait of 36 alkaptonuria patients were compared to those of 21 unimpaired controls. The AKU patients were split into three age groups (young 16-29 years, *n* = 9, middle 30-49 years, *n* = 16 and old 50+ years, *n* = 11), and the kinematic and kinetic gait profiles were compared to speed-matched controls using a *spm1d* two-sample t-test. **Results:** The young AKU group showed significant differences in the sagittal plane of the knee joint compared to speed-matched controls. The middle group showed deviations in the knee and hip joints. The old group showed significant differences in multiple joints and planes and exhibited gait mechanisms which may be compensation strategies. **Significance:** This study is the first to identify and describe joint-specific mechanisms during gait in alkaptonuria patients. Gait deviations were evident even in young AKU patients, including a 16-year-old, much earlier than previously thought. The knee joint is an important focus of future research and potential interventions as deviations were found across all three AKU age groups.

1. Introduction

Alkaptonuria (AKU), also known as the 'black bone disease', is one of 7000 known rare diseases [1] with an estimated incidence of 1:250,000-100,000 worldwide [2]. Alkaptonuria is due to inheriting two copies of the faulty homogentisate 1,2-dioxygenase (HGD) gene, one from each parent. As a result of the disrupted tyrosine catabolic pathway, a melanin-like polymer is produced which binds to connective tissues leading to ochronosis. The dark discoloured ochronotic tissues [3] accounts for the name 'Black bone disease'. The hyaline cartilage, like other connective tissues, becomes stiff and brittle leading to structural damage [4] and related pain. The ochronosis spreads across the articular

surface and is observed in regions that are subjected to the greatest loads during locomotion [5], suggesting that even normal tissue stresses during activities of daily living can trigger cartilage damage, and joint destruction. Ultimately, this decline in joint health leads to pain, disability and loss of movement function [6].

Despite AKU's structural damage and painful debilitating symptoms, there are only three studies that have investigated the effect of AKU on gait. Two studies investigated the age-related deviations of gait from normality in an AKU cohort, using the Movement Deviation Profile (MDP) [7, 8]. The MDP provides a summary measure (MDP_{mean}) which quantitatively shows how much an individual's gait deviates from normal gait, across the duration of the gait cycle. The MDP uses a self-organising map (SOM, [9]) which is presented with pre-processed marker trajectories captured during gait. When analysing the gait of AKU patients between the ages 20-74 years, Barton [7] found an abrupt increase in MDP_{mean} around ages 35-40 years and all AKU patients showed a MDP_{mean} greater than the mean of the controls. King [8] found a similar abrupt increase in MDP_{mean} at around ~55 years in AKU patients between 19-72 years of age. The most recent study investigated a European AKU cohort. The results showed all but one AKU patient had an MDP_{mean} greater than the mean of controls, an abrupt rise at ~50 years and most notably, high MDP_{mean} scores in AKU patients as young as 16 years [10]. Summary measures are effective tools for monitoring disease progression, however, to further understand the effects of AKU on gait, an in-depth analysis of joint level gait mechanisms is needed. Due to the changes in MDP_{mean} scores across the lifespan in all three studies it is important to separate the gait mechanisms over different periods of the lifespan. The analysis should consider the abrupt increase at ~50 years, the differences in gait mechanisms leading to the increase at 30-40 years and factors contributing to the high MDP scores seen in the younger group under 30 years.

To comprehensively understand the joint-specific mechanisms in AKU gait, all lower limb angles, moments and powers should be analysed and incorporate the magnitude of difference as well as the timing during the gait cycle. Statistical Parametric Mapping (SPM) is a method which can be used to

overcome errors associated with predetermined discrete parameters when comparing gait waveform data. The method allows analysis over the entire waveform instead of specific data points and indicates exactly where in the gait cycle there is a significant difference [11]. Using this tool on multiple gait variables is expected to identify any significant differences between AKU gait and unimpaired gait and from this granular information joint-specific gait mechanisms can be described.

It is clear from previous research that AKU patients exhibit some gait deviations from normality. However, the joint-specific differences and gait mechanisms associated with AKU are currently unknown. To further understand gait in AKU, to help inform clinical decision making and treatment plans and to describe AKU gait mechanisms for the first time, a nonbiased approach is required to evaluate all clinically relevant one-dimensional data which describe movement together with their causes across the gait cycle. Therefore, the aim of this study is to identify the underlying gait mechanisms which contribute to the gait deviations seen in AKU patients by comparing joint angles, moments and powers to the gait of controls using SPM.

2. Methods

2.1. Participants

All 36 AKU patients within this study were recruited from the National Alkaptonuria Centre based at the Royal Liverpool University Hospital. Patients at the time of testing were not receiving or had previously received nitisinone treatment. Any previous lower limb injuries or surgeries were recorded (Table 1). The AKU population included various European nationalities and were from the same AKU cohort as the Cox [10] study. NHS ethical approval was obtained by the sponsor (Royal Liverpool University Hospital) according to the site agreement and granted by NRES (07/Q1505/29), all participants provided written informed consent.

For comparison, 21 unimpaired controls, who reported no musculoskeletal disease or injuries and aged between 19-60 years were recruited based on convenience sampling. See Table 1 for participant characteristics.

Table 1: Characteristics of AKU patients and controls.

	AKU	Control
N	36	21
Male/female	22/14	8/13
Age (years, mean ± SD)	41 ± 16	33 ± 12
Height (m, mean ± SD)	1.67 ± 0.09	1.71 ± 0.09
Body Mass (kg, mean ± SD)	68.4 ± 13.58	74.81 ± 12.29
Joint Replacements		
Knee	8	
Hip	5	
Other		
Meniscus removals	2	
Ankle ligament ruptures	4	

2.2. Equipment

A ten-camera motion capture system (T10/T160, Vicon Motion Analysis Inc., Oxford, UK) was centred around two adjacent embedded 600 x 400 mm force plates (Kistler 9281B; Kistler Instruments Ltd., Winterthur, Switzerland). The 3-dimensional marker coordinates and ground reaction forces (GRF) for consecutive foot contacts were simultaneously recorded in Vicon Nexus (V1.85). Kinematic data were sampled at 120 Hz and force data at 1000 Hz.

2.3. Protocol

To track the lower limbs 15 reflective markers (1.4 cm diameter) were attached in accordance with the Helen-Hayes model [12]. All walking trials were performed barefoot to remove the effects of footwear or prescribed insoles. Alkaptonuria patients walked along a 10 m walkway at their self-selected walking speed (1.11 ± 0.29 m/s), three successful trials were collected for each patient. For a speed-matched comparison, the control group repeated the same protocol at two different

speeds: self-selected speed (mean ± SD = 1.28 ± 0.13 m/s) and slow speed (mean ± SD = 0.98 ± 0.14 m/s) to represent the typical walking speed range seen in AKU patients.

2.4. Data Analysis

The AKU patient data were split into three age groups (Young 16-29 years (*n* = 9), Middle 30-49 years (*n* = 16) and Old 50+ years (*n* = 11)) based on the three previous studies. Patients with more established disease severity are likely to walk slower, and speed may have a greater effect on kinematics than age or gender [13]. Therefore, each AKU age group were compared with the controls walking at the closest mean speed. AKU Young (1.26 ± 0.14 m/s) and Middle (1.17 ± 0.15 m/s) were compared with controls at self-selected speed (1.28 ± 0.13 m/s), and AKU Old (0.89 ± 0.40 m/s) with controls at slow speed (0.98 ± 0.14 m/s).

All movement data were processed in Vicon Nexus (V1.85, Vicon Motion Analysis Inc., Oxford, UK). Automatic gait events were calculated, stance phase was defined when the vertical GRF vector was greater than the 20 N threshold, gait events were then auto correlated. The data were exported to .c3d files, filtered using the 6 Hz low-pass Butterworth filter and time-normalised to percentage gait cycle in Visual 3D (V6, C-Motion, Inc., Germantown, MD, USA). The Helen-Hayes lower limb 7-segment model was applied to calculate angles, and inverse dynamics to calculate internal net moments and powers normalised to body mass.

2.5. Statistical Parametric Mapping analysis

A two-sample t-test using SPM compared the mean joint angles, moments and powers between AKU and speed-matched control gait data using the source *spm1d* package (v M.0.4.5, [11]) in Matlab (R2017b, Mathworks Inc., Natick, MA, USA). To reduce the likelihood of false positives caused by multiple comparisons, alpha was corrected using the *spm1d* Bonferroni correction. The 21 gait curves compared are shown in Table 2.

Table 2: The gait curves of both sides used in the comparison between AKU patients and controls.

Angles	**Moments**	**Powers**
Ankle plantar/dorsiflexion	Ankle plantar/dorsiflexion	Ankle
Ankle in/out-toeing	Knee flexion/extension	Knee
Knee flexion/extension	Knee ab/adduction	Hip
Knee ab/adduction	Knee rotation	
Knee rotation	Hip flexion/extension	
Hip flexion/extension	Hip ab/adduction	
Hip ab/adduction	Hip rotation	
Hip rotation		
Pelvic tilt		
Pelvic obliquity		
Pelvic rotation		

For each comparison, the univariate *t*-statistic (SPM {t}) was calculated at each data point of the waveform and a critical threshold was calculated. The two-group means were considered significantly different if the SPM {t} curve exceeded this threshold. Consecutive points where the SPM {t} curve exceeds the critical threshold are known as a cluster and show where in the gait cycle the two means significantly differed. Very high and/or broad clusters occur with a low probability according to the height-threshold and cluster-breadth procedure [14]. Cluster-specific *p*-values were also calculated.

3. Results

The number of significant differences between AKU and control gait curves (represented by the number of clusters) increased with age (Table 3). The young and middle groups differed from the control group in only the sagittal plane angles and moments. The AKU young group only showed significant differences at the knee joint. The middle group showed significant differences at the knee and hip, whereas the old group showed significant differences at all lower limb levels (ankle, knee, hip and pelvis). The old group showed significant differences in all three planes of motion within the angle curves and predominantly frontal plane differences within the moment curves. The knee joint

moment showed significant differences across all three age groups in both the sagittal and frontal planes.

Table 3: The significant differences derived from the time-based differences between AKU and control gait angles, moments and power curves within the Young, Middle and Old AKU age groups (the descriptions provided should be interpreted as observations in the patient group). The observed differences are ordered and ranked with respect to their *p*-value from smallest to largest within each group and variable category.

Angles						
Group	Rank	*P* Value	Joint	Plane	% Gait cycle	Description
Young	1	***p*=0.021**	Knee	Sagittal	35-42	Increased extension
Middle	1	***p*=0.008**	Hip	Sagittal	82-100	Reduced flexion
	2	***p*=0.045**	Hip	Sagittal	22-26	Increased extension
Old	1	***p*<0.001**	Foot	Transverse	0-68	Increased out-toeing
	2	***p*<0.001**	Knee	Frontal	7-55	Increased abduction
	3	***p*=0.002**	Pelvis	Frontal	55-76	Reduced depression
	4	***p*=0.002**	Pelvis	Frontal	5-25	Reduced elevation
	5	***p*=0.016**	Hip	Frontal	55-72	Increased adduction
	6	***p*=0.022**	Ankle	Sagittal	62-68	Reduced plantarflexion
	7	***p*=0.034**	Foot	Transverse	92-100	Increased out-toeing
	8	***p*=0.039**	Ankle	Sagittal	3-6	Increased dorsiflexion
Moments						
Group	Rank	*P* Value	Joint	Plane	% Gait cycle	Description
Young	1	***p*<0.001**	Knee	Sagittal	35-42	Increased flexion moment
Middle	1	***p*=0.014**	Hip	Sagittal	55-57	Reduced flexion moment
Old	1	***p*<0.001**	Knee	Frontal	38-55	Reduced abduction moment
	2	***p*<0.001**	Knee	Frontal	11-25	Reduced abduction moment
	3	***p*=0.032**	Hip	Transverse	58-59	Reduced external moment
	4	***p*=0.049**	Knee	Frontal	98-98	Increased abduction moment
Powers						
Group	Rank	*P* Value	Joint	Plane	% Gait cycle	Description

Young	1	***p*=0.002**	Knee	Sagittal	42-45	Increased generation
Middle	1	***p*=0.046**	Knee	Sagittal	56-56	Reduced absorption

3.1. Joint-specific gait deviations in the Young AKU patients

Figure 1 displays the results of the SPM analysis of the kinematic and kinetic waveforms in the Young AKU group. Patients in this group walked with increased knee extension at mid- to terminal stance (35-42% gait cycle) (p=0.021). At the same period of the gait cycle they had an increased knee flexion moment (p<0.001). Knee power absorption was also increased during this period of the gait cycle although this was not significant. This is followed by a significantly greater power absorption at 42-45% gait cycle (p=0.002) than speed-matched controls.

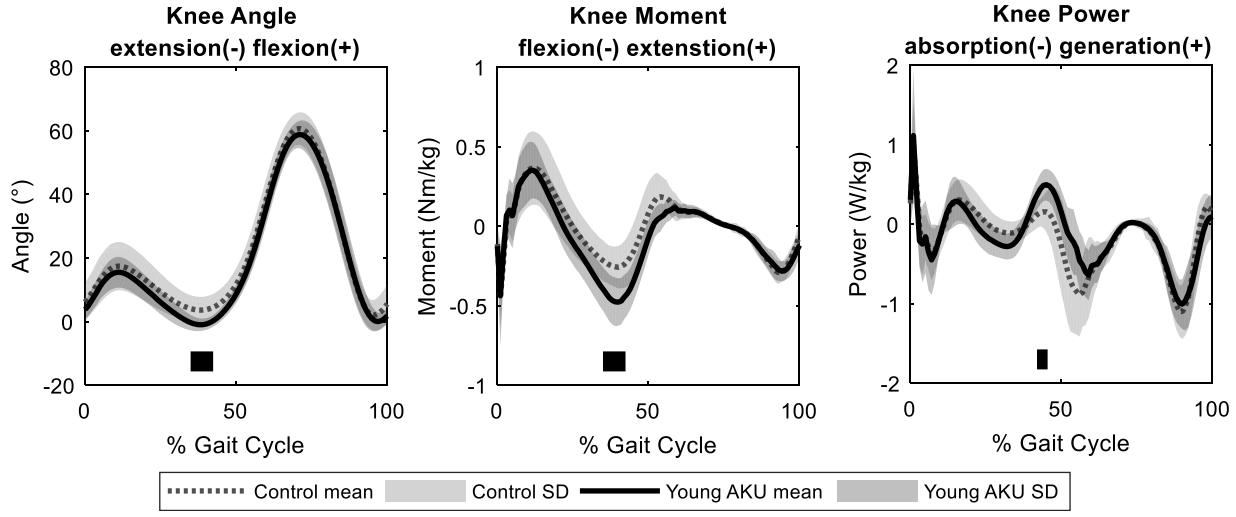

Figure 1: The knee angle, moment and power curves for the AKU Young (black) and speed-matched controls (grey dotted) throughout the gait cycle. The lower black bars represent where the differences were significant indicated by the SPM{t} statistic along the gait cycle.

3.2. Joint-specific gait deviations in the Middle AKU patients

The largest significant difference determined by the smallest p-value between AKU Middle group and control group was the reduced hip flexion at terminal swing (82-100% gait cycle) (p=0.008). The

Middle AKU group walked with a significant offset to hip extension during stance between 22-26% gait cycle (p=0.045). At 55-57% of the gait cycle at terminal stance there was a reduced hip flexion moment (p=0.014) with a simultaneous reduced knee power absorption at 56% gait cycle (p=0.046) (Figure 2).

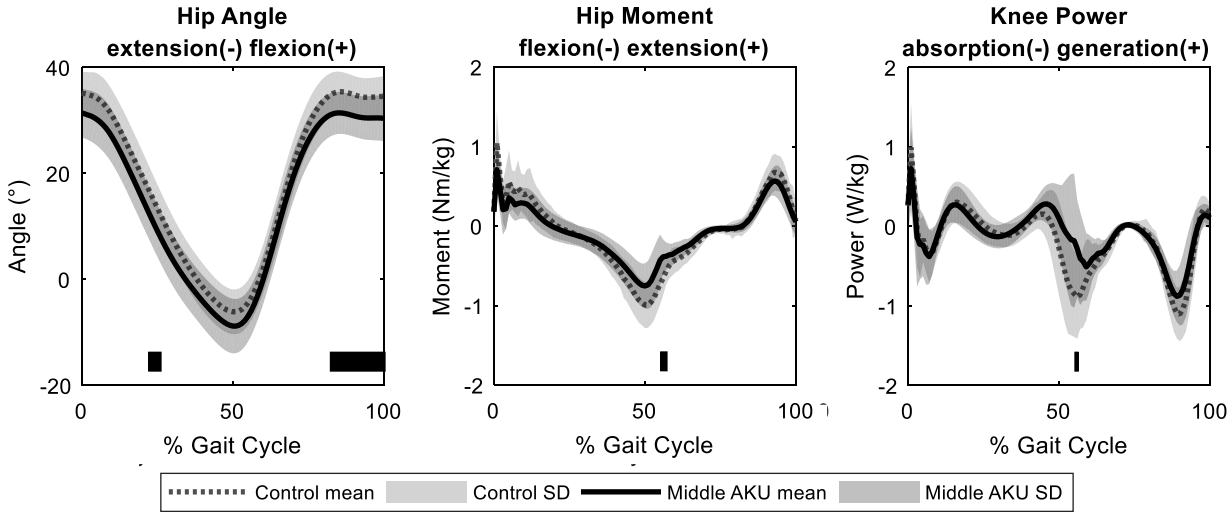

Figure 2: The hip angle, hip moment and knee power curves for the AKU Middle (black) and speed-matched controls (grey dotted) throughout the gait cycle. The lower black bars represent where the differences were significant indicated by the SPM{t} statistic along the gait cycle.

3.3. Joint-specific gait deviations in the Old AKU patients

Figure 3 shows the largest significant difference determined by the smallest p-value between the Old AKU group and the control group in the transverse plane foot progression angle between 0-68% gait cycle (p<0.001). Secondly, there was increased knee abduction between 7-55% gait cycle (p<0.001). There were significant differences shown in the pelvic obliquity; reduced depression 55-76% gait cycle and reduced elevation 5-25% gait cycle (p=0.002). There was also increased hip adduction during 55-72% gait cycle and reduced plantarflexion during 62-68% gait cycle during push-off.

The largest significant difference between the Old AKU group and the control group in the moment curves was the reduced knee abduction moment (p<0.001) between 38-55% of the gait cycle, i.e. the

second half of the stance phase. The second largest difference was during 11-25% of the gait cycle indicating reduced knee abduction moment during the first half of the stance phase (p<0.001).

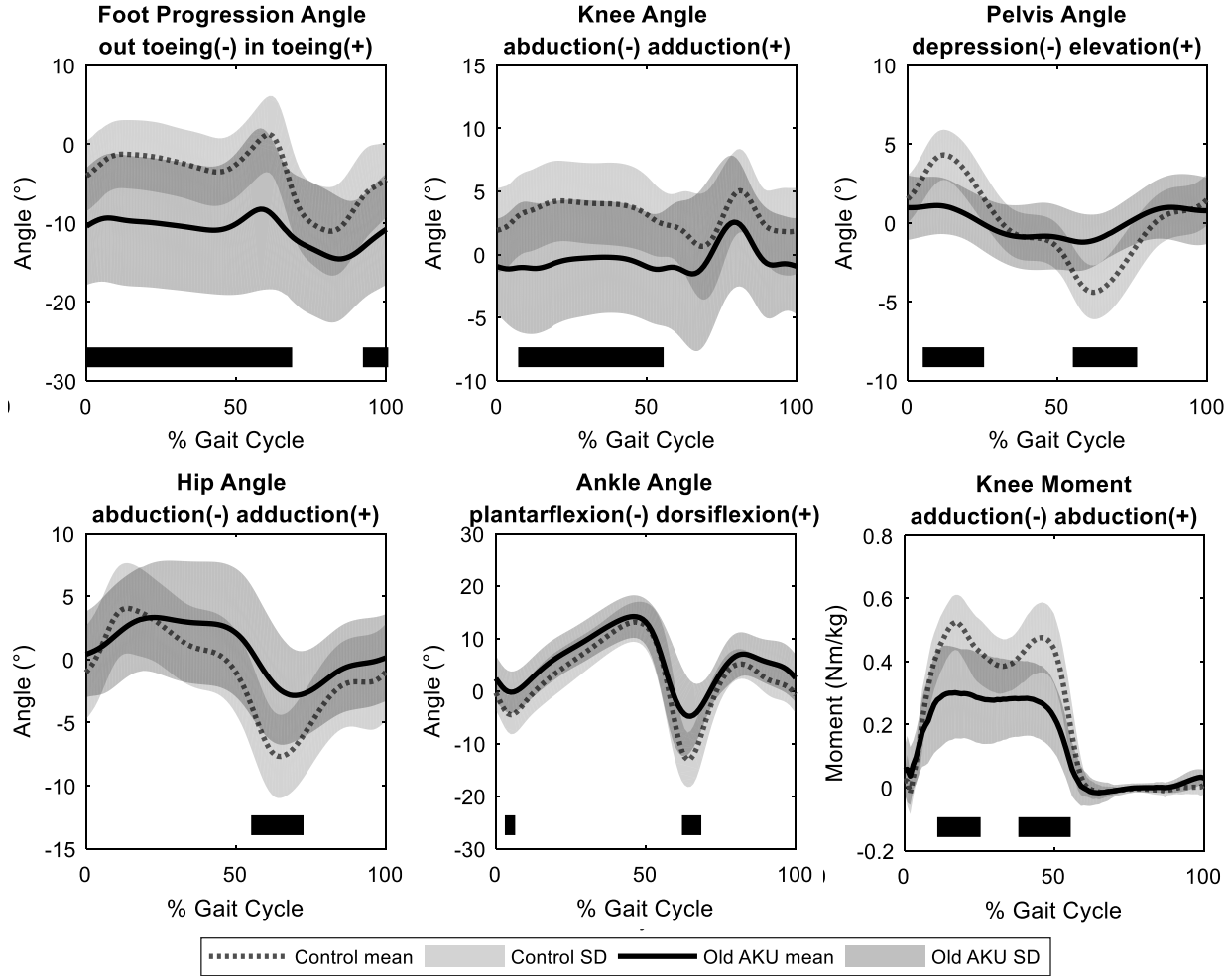

Figure 3: The kinematic and kinetic curves where significant differences occurred between the Old AKU (black) and speed-matched controls (grey dotted) throughout the gait cycle. The lower black bars represent where the differences were significant indicated by the SPM{t} statistic along the gait cycle.

4. Discussion

When observing all three AKU age groups the number of significant differences from controls increased with age, coinciding with the progression of the disease, and the increase in movement deviations (MDP_{mean}) previously reported [7, 8, 10]. Although the number of differences increased with age, the deviations changed across age groups. As age increased there was a shift from sagittal

plane joint deviations to frontal and transverse planes, demonstrating that AKU gait is affected in all three planes of motion, and the knee joint was affected in all three age groups.

Alkaptonuria symptoms typically manifest around 30 years of age due to the accumulation of harmful HGA-pigment [3, 15]. However, in this study the younger AKU group (<30 years) exhibited significant differences at the knee joint in the sagittal plane compared to controls. There was a reduced second rocker indicating an early heel rise leading to an excessive plantarflexion knee extension couple [16] evidenced by the increased knee extension angle and increased knee flexion moment in mid-stance. Typically, during mid- to terminal stance, the knee power curve is negligible as the GRF stabilises the knee in extension and muscular contribution is minimal [17] as seen in the control group. However, in AKU gait there is an increased power absorption followed by increased generation. As the knee is in increased extension, the increased flexion moment is likely to be a result of the passive resistance of the posterior knee soft tissue structures absorbing power whilst being elongated. As the knee joint flexes, the stretched tissues are released which may contribute to the increased generation of power occurring directly afterwards. These gait mechanisms are likely to put additional strain on the posterior supporting ligaments of the knee, which are also susceptible to AKU ochronosis-related damage [2, 18].

Sagittal plane moments are often overlooked in osteoarthritis (OA) research; however, Erhart-Hledik [19] found that the first peak extension moment had the greatest association with degeneration of the posterior tibial region in less severe OA patients. This, along with the current findings, suggests that sagittal plane moments have a substantial influence on the joint and the potential damage to the integrity of the AKU knee joint in the younger group. Our findings confirm that there are gait deviations in the younger AKU patients coinciding with the findings by Cox [10] and describe gait mechanisms with potential long-term consequences.

In the middle AKU age group the largest significant differences were the reduced hip flexion during terminal swing, and an offset to hip extension during early mid-stance. With a (non-significant)

offset to extension during terminal stance one would expect an increased hip flexion moment. However, there was a reduced hip flexion moment in terminal stance, at the same time as a reduced knee power absorption. One possible explanation for this is a forward trunk lean. Spinal disc degradations were seen at 40-50 years of age [10], which could result in structural changes and orientation of the trunk during gait, however, trunk position was not measured during this study. These findings also reflect the large rise in gait deviations (MDP_{mean}) at the ages 35-40 years reported in the Barton [7] study despite a different AKU cohort.

The older AKU group showed the most complex multi-planar differences. The results highlighted several mechanisms which contribute to a reduced internal knee abduction moment arm. Firstly, the increased out-toeing throughout stance translates the GRF vector laterally towards the knee centre. Additionally, the increased knee abduction and hip adduction angle suggest a valgus knee alignment, bringing the knee closer to the GRF vector. Both mechanisms reduce the frontal plane knee moment arm length, consequently reducing the knee abduction moment. High knee abduction moments and a varus alignment are often associated with severe OA as an indicator of medial compartment loading [20]. In contrast, our older and more severe AKU group had a reduced internal knee abduction moment, and a valgus alignment. Although AKU leads to premature OA, there are underlying physiological differences between the two diseases as well as between gait profiles which may require specific AKU treatment plans. It is difficult to determine whether the gait mechanisms, specifically out-toeing, are modifications to decrease the frontal plane knee moment in response to pain [6] and joint damage [10], or a natural change occurring during healthy ageing as the internal hip rotators weaken or to increase the base of support [21, 22]. The older group also showed a reduced range of pelvic obliquity motion in the frontal plane, this is likely due to spine stiffness and lower back pain which is reported by 88.9% AKU patients aged between 40-74 years [6].

Although there are observed gait differences across age groups in AKU, it is difficult to determine whether the changes are a cause or an effect. Alkaptonuria patients may adopt a walking pattern in

response to pain caused by the pathology, or their walking pattern may contribute to and cause structural damage and pain. Previous literature has suggested that mechanical loading in the large weight bearing joints contributes to the structural damage in AKU joints [23, 24]. Therefore, it may be possible to learn from load reducing movement patterns seen in the older AKU group to implement earlier gait modification interventions targeted at the knee within younger patients. This may have the potential to delay the progression of the disease within the knee and delay the need for invasive joint replacements.

There were a few limitations to this study. Only lower extremity variables were measured, description of the upper body would provide details of full body movement. Although it is known that pain and structural damage increases with age [6, 10], there are currently no direct associations between gait deviations and disease severity, which should be considered in future studies. There were large inter-patient variations in the older AKU group indicated by the large standard deviations across the waveform data. This indicates that AKU gait becomes heterogenous and cohort analysis may not identify all movement patterns and the deviations seen in the young and middle groups maybe hidden in the older group. Finally, the study had a sample size of 36 AKU patients, however it should be considered that AKU is an ultra-rare disease, and that the study included AKU patients who met the inclusion criteria and were not involved in nitisinone clinical trials.

4.5. Conclusions

This study described joint and age-specific mechanisms of gait for the first time in AKU and identified altered gait patterns when compared to unimpaired controls. In contrast to the common perception that AKU only affects older people, we found significant differences within all three age groups. The results identified potentially harmful gait deviations in the younger AKU group and multiple gait mechanisms in the older AKU group contributing to a reduction in the frontal plane knee moment. The knee joint was affected in all three age groups highlighting it as an important focus in clinical management. It remains unclear whether the mechanisms in each age group are driven by the

progression of the disease, or if they contribute to the progression of the disease. Answering this question would require longitudinal monitoring across all ages of AKU. The results of this study contribute to understanding the biomechanical mechanisms of AKU gait aiding researchers and clinicians to monitor the progression of the disease and plan for future treatments and interventions.

[1] S. Nguengang Wakap, D.M. Lambert, A. Olry, C. Rodwell, C. Gueydan, V. Lanneau, et al., Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database, European Journal of Human Genetics 28(2) (2020) 165-173. https://doi.org/10.1038/s41431-019-0508-0.
[2] C. Phornphutkul, W.J. Introne, M.B. Perry, I. Bernardini, M.D. Murphey, D.L. Fitzpatrick, et al., Natural history of alkaptonuria, N Engl J Med 347(26) (2002) 2111-21. https://www.ncbi.nlm.nih.gov/pubmed/12501223.
[3] W.J. Introne, W.A. Gahl, Alkaptonuria, in: M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, G. Mirzaa, et al. (Eds.), GeneReviews((R)), Seattle (WA), 1993.
[4] A.M. Taylor, T.J. Batchelor, V.L. Adams, T.R. Helliwell, J.A. Gallagher, L.R. Ranganath, Ochronosis and calcification in the mediastinal mass of a patient with alkaptonuria, Journal of Clinical Pathology 64(10) (2011) 935-6. https://www.ncbi.nlm.nih.gov/pubmed/21551467.
[5] T.P. Andriacchi, S. Koo, S.F. Scanlan, Gait mechanics influence healthy cartilage morphology and osteoarthritis of the knee, J Bone Joint Surg Am 91 Suppl 1 (2009) 95-101. https://www.ncbi.nlm.nih.gov/pubmed/19182033.
[6] M. Rudebeck, C. Scott, N. Sireau, L. Ranganath, A patient survey on the impact of alkaptonuria symptoms as perceived by the patients and their experiences of receiving diagnosis and care, JIMD Reports 53(1) (2020) 71-79. https://onlinelibrary.wiley.com/doi/abs/10.1002/jmd2.12101.
[7] G.J. Barton, S.L. King, M.A. Robinson, M.B. Hawken, L.R. Ranganath, Age-Related Deviation of Gait from Normality in Alkaptonuria, JIMD Rep 24 (2015) 39-44. https://www.ncbi.nlm.nih.gov/pubmed/25786642.
[8] S. King, M. Hawken, H. Shepherd, J. Gallagher, L. Ranganath, G. Barton, A protective effect in females with alkaptonuria: relationships between gait deviations and ochronosis, Gait & Posture 57 (2017) 149-150.
[9] T. Kohonen, The self-organizing map, Proceedings of the IEEE 78(9) (1990) 1464-1480.
[10] T. Cox, E.E. Psarelli, S. Taylor, H.R. Shepherd, M. Robinson, G. Barton, et al., Subclinical ochronosis features in alkaptonuria: a cross-sectional study, BMJ Innovations 5(2-3) (2019) 82-91. https://innovations.bmj.com/content/bmjinnov/5/2-3/82.full.pdf.
[11] T.C. Pataky, One-dimensional statistical parametric mapping in Python, Computer Methods in Biomechanics and Biomedical Engineering 15(3) (2012) 295-301. https://doi.org/10.1080/10255842.2010.527837.
[12] R.B. Davis, S. Ounpuu, D. Tyburski, J.R. Gage, A gait analysis data-collection and reduction technique, Hum Movement Sci 10(5) (1991) 575-587. <Go to ISI>://WOS:A1991GM10500008.
[13] J. Røislien, Ø. Skare, M. Gustavsen, N.L. Broch, L. Rennie, A. Opheim, Simultaneous estimation of effects of gender, age and walking speed on kinematic gait data, Gait and posture 30(4) (2009) 441-445.
[14] W.D. Penny, K.J. Friston, J.T. Ashburner, S.J. Kiebel, T.E. Nichols, Statistical parametric mapping: the analysis of functional brain images, Elsevier2011.

[15] L.R. Ranganath, T.F. Cox, Natural history of alkaptonuria revisited: analyses based on scoring systems, J Inherit Metab Dis 34(6) (2011) 1141-51. https://www.ncbi.nlm.nih.gov/pubmed/21748407.
[16] J.R. Gage, The treatment of gait problems in cerebral palsy, Mac Keith2004.
[17] D. Winter, The biomechanics and motor control of human gait. Waterloo, University of Waterloo Press, 1987.
[18] R.V. Manoj Kumar, S. Rajasekaran, Spontaneous tendon ruptures in alkaptonuria, J Bone Joint Surg Br 85(6) (2003) 883-6. https://www.ncbi.nlm.nih.gov/pubmed/12931812.
[19] J.C. Erhart-Hledik, J. Favre, T.P. Andriacchi, New insight in the relationship between regional patterns of knee cartilage thickness, osteoarthritis disease severity, and gait mechanics, Journal of biomechanics 48(14) (2015) 3868-3875.
[20] S. Meireles, F. De Groote, N. Reeves, S. Verschueren, C. Maganaris, F. Luyten, et al., Knee contact forces are not altered in early knee osteoarthritis, Gait & posture 45 (2016) 115-120.
[21] J.O. Judge, R.B. Davis, 3rd, S. Ounpuu, Step length reductions in advanced age: the role of ankle and hip kinetics, J Gerontol A Biol Sci Med Sci 51(6) (1996) M303-12. https://www.ncbi.nlm.nih.gov/pubmed/8914503.
[22] F. Prince, H. Corriveau, R. Hébert, D.A. Winter, Gait in the elderly, Gait & posture 5(2) (1997) 128-135.
[23] A. Taylor, A. Preston, N. Paulk, H. Sutherland, C. Keenan, P. Wilson, et al., Ochronosis in a murine model of alkaptonuria is synonymous to that in the human condition, Osteoarthritis and Cartilage 20(8) (2012) 880-886.
[24] A.M. Taylor, T.J. Batchelor, V.L. Adams, T.R. Helliwell, J.A. Gallagher, L.R. Ranganath, Ochronosis and calcification in the mediastinal mass of a patient with alkaptonuria, J Clin Pathol 64(10) (2011) 935-6. https://www.ncbi.nlm.nih.gov/pubmed/21551467.