

1 **Age related deviation of gait from normality in alkaptonuria**

2

3 Gabor J. Barton, Stephanie L. King, Mark A. Robinson, Malcolm B. Hawken, Lakshminarayan R.

4 Ranganath

5

6

7 Corresponding author:

8 G J Barton

9 Research Institute for Sport and Exercise Sciences,

10 Liverpool John Moores University,

11 Byrom Street,

12 Liverpool L3 3AF, UK

13 Tel: +44 (0)151 9046263, Fax: +44 (0)151 9046284, E-mail: g.j.barton@ljmu.ac.uk

14

15

16 S L King, M A Robinson, M B Hawken

17 Research Institute for Sport and Exercise Sciences,

18 Liverpool John Moores University,

19 Byrom Street,

20 Liverpool L3 3AF, UK

21

22 L R Ranganath

23 National Alkaptonuria Centre,

24 Royal Liverpool University Hospital,

25 Prescott Street,

26 Liverpool L7 8XP, UK

27

28

29 **Word count:**

30 2661 Text (excluding summary, acknowledgments, references and figure legends)

31 250 Summary/Abstract

32 1 Table

33 3 Figures

1 **Summary**

2

3 Alkaptonuria is a rare metabolic disease leading to systemic changes including early and severe
4 arthropathy which affects mobility. For unknown reasons, the onset of degenerative changes is
5 delayed to around 30 years of age when both objective and subjective symptoms develop. In order to
6 complement description of the structural changes in alkaptonuria with measures of movement
7 function, clinical gait analysis was added to the list of assessments in 2013. The aim of this study was
8 to describe the deviation of gait from normality as a function of age in patients with alkaptonuria.
9 Three-dimensional movement of reflective markers attached to joints were captured during walking
10 in 39 patients and 10 controls. Subsequent to processing the data to emphasise the shape of marker
11 trajectories, the mean Movement Deviation Profile was generated for all participants. This single
12 number measure gives the deviation of a patient's gait from a distributed definition of gait normality.
13 Results showed that gait deviation roughly follows a sigmoid profile with minimal increase of gait
14 deviations in a younger patient group and an abrupt large increase around the second half of the 4th
15 decade of life. Larger variations of gait deviations were found in the older group than in the younger
16 group suggesting a complex interaction of multiple factors which determine gait function after
17 symptoms manifest. Continued gait analysis of adults with AKU, extended to younger adults and
18 children with AKU, is expected to complete understanding of both the natural history of alkaptonuria
19 and how interventions can affect movement function.

20

21

22 **Take-home message**

23

24 Deviation of gait from normality shows an abrupt increase in the second half of the 4th decade of life
25 in alkaptonuria.

1 **Compliance with Ethics Guidelines**

2

3 **Conflict of interests**

4 Gabor Barton received a grant from the National Alkaptonuria Centre.

5 Stephanie King is employed from the same grant income.

6 Mark Robinson and Malcolm Hawken are named co-investigators in the same grant.

7 Lakshminarayan Ranganath is Director of the National Alkaptonuria Centre.

8

9 **Informed Consent**

10 All procedures followed were approved by the local NHS Ethics Committee (07/H1002/111
11 amendment 6, and 07/Q1505/29 amendment 9) and were in accordance with the ethical standards
12 of the responsible committee on human experimentation (institutional and national) and with the
13 Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients
14 for being included in the study.

15

16 **Contributions of individual authors**

17 GJB conceived the study, performed the initial analyses and was lead author of the manuscript. SLK
18 performed some of the data collection, parts of the data analysis and collaborated in writing the
19 manuscript. MAR was involved in defining the test protocol and contributed to revising the manuscript.
20 MBH was involved in the advanced analysis of results and in writing the manuscript. LRR contributed
21 to revising the manuscript.

1 Introduction

2

3 Alkaptonuria (OMIM #203500) is an ultra-rare autosomal recessive metabolic disease with an
4 estimated incidence of 1:250,000-1:1,000,000 in the US (Introne and Gahl, 2013). A defect of
5 homogentisate 1,2-dioxygenase (EC 1.13.11.5) blocks the catabolism of phenylalanine and tyrosine
6 resulting in elevated levels of homogentisic acid. After its oxidation, a melanin-like polymer is
7 produced which binds to virtually all fibrous connective tissues including cartilage, leading to
8 ochronosis (Ranganath et al. 2013). While alkaptonuria begins at conception, there is only anecdotal
9 evidence of joint pain in younger patients followed by a rapid increase of symptoms around 30 years
10 of age (Introne and Gahl, 2013; Ranganath and Cox, 2011). Among various degenerative changes
11 affecting the cardiovascular and renal systems, a characteristic of the disease is early and severe
12 arthropathy (Abimbola et al. 2011; Aquaron, 2013). Despite mounting information about structural
13 changes in alkaptonuria, little is known about the functional effects of arthritic changes on movement.
14 A prospective randomised clinical trial showed that nitisinone reduced homogentisic acid levels by
15 95%, but the total hip range of motion, spinal flexion, functional reach, timed get up and go, and 6-
16 minute walk test did not improve (Introne et al. 2011). In order to better understand how movement
17 degenerates in alkaptonuria, in 2013 we started performing gait analysis on adults who visit the
18 National Alkaptonuria Centre in Liverpool.

19

20 Gait analysis is a routine non-invasive procedure which allows the collection of objective and
21 quantitative information in order to identify abnormalities, postulate their causes and propose
22 treatments for those with walking problems (Davis et al. 2004). Body segment motion is captured by
23 infrared cameras which track reflective markers attached to the legs and pelvis, and ground reaction
24 forces are recorded by force platforms embedded in a walkway over which the participant walks.
25 Dynamic joint angles, moments and powers are calculated in all anatomical planes over several strides.
26 Interpretation of the results by gait analysts can uncover reasons for gait changes, allowing specific
27 anatomical structures to be targeted for interventions.

28

29 The traditional interpretation of gait results has recently been complemented by the successful
30 derivation of simplified measures of gait deviation, often represented by a single number. Notable
31 and widely used examples are the Gait Deviation Index (GDI, Schwartz and Rozumalski, 2008) and the
32 Gait Profile Score (GPS, Baker et al. 2009). The Movement Deviation Profile (MDP, Barton et al. 2012;
33 Barton et al. 2015) is a single curve generated by an artificial neural network model which shows
34 quantitatively how much a patient's gait deviates from normal gait. A summary measure of the MDP,

1 the MDP_{mean} , has demonstrated advantages over the GDI and GPS while showing good agreement
 2 with the GDI (Barton et al. 2012). The MDP_{mean} offers a simple and effective means of quantitatively
 3 describing disease progression in alkaptonuria. The aim of this study was to examine the development
 4 of gait deviations in a cross-sectional sample of adults with alkaptonuria with a view to employing our
 5 method of objective assessment of gait function to monitor disease progression and responses to
 6 treatment.

9 **Participants and Methods**

11 A group of 40 patients with alkaptonuria (AKU) and 10 unimpaired controls underwent clinical gait
 12 analysis at Liverpool John Moores University between May 2013 and October 2014. One patient
 13 refused consent for the use of their data in the following analysis. Thirty-two of the patients were
 14 receiving 2 mg/day nitisinone treatment (13 of them started treatment within 3 days of testing) which
 15 is expected to slow progression of the disease, but not to reverse the effects. Eighteen of the patients
 16 had had one or more joints replaced. Table 1 shows descriptive statistics together with duration of
 17 nitisinone treatment and any joint replacements.

20 *Table 1: Characteristics of patients with alkaptonuria (AKU) and unimpaired controls.*

	AKU	Controls
N	39	10
Male/female	23/16	4/6
Age* (years)	24.5 (12.7)	34.2 (13.1)
Height* (m)	1.66 (0.10)	1.66 (0.08)
Mass* (kg)	74.1 (18.2)	69.5 (11.6)
Body Mass Index* (kg/m ²)	26.8 (5.4)	24.3 (4.7)
Nitisinone use		
On nitisinone for 1 year	19	n/a
On nitisinone for 1-3 days [†]	13	n/a
No nitisinone treatment	7	n/a
Joint Replacement		
Knee or hip	14	n/a
Knee only	9	n/a
Knee and hip	5	n/a
Other	6	n/a

21 *mean (standard deviation); [†]not included in "Nitisinone" group; n/a: not applicable

1 The gait analysis procedure followed the guidelines of the Clinical Movement Analysis Society of UK
2 and Ireland. Fifteen reflective markers were attached to the skin or tight fitting clothes over the feet,
3 lower legs, thighs and the pelvis according to the Helen-Hayes model (Davis et al. 1991). Several walks
4 were performed at self-selected speed on a 10 m long walkway while the 3D coordinates of markers
5 were captured by a 10 camera Qualisys Oqus or 16 camera Vicon T10/T160 motion capture system.
6 The first three clean walks (without marker dropouts or measurement artefacts) of each patient and
7 the first two walks of each control were selected for analysis.

8

9 The MDP (together with the GDI and GPS) was validated using three-dimensional joint angles but there
10 is no universally accepted way to describe spatial joint angles given the mathematically equivalent but
11 effectively very different 16 Euler rotation sequences (Baker 2006; Lees et al. 2010). To circumvent
12 the uncertainties attached to selecting one particular Euler rotation sequence over others without
13 clear justification, we used marker positions directly to describe movement as suggested by Federolf
14 et al. (2013). Marker coordinates attached to body segments are used to calculate joint angles and so
15 the information contained in 3D angles is also contained in marker coordinates.

16

17 Processing of the X, Y and Z coordinates of the 15 markers involved subtraction of each marker from
18 a calculated straight line fitted onto the progression of the centre of the pelvis during one gait cycle
19 (means of the X, Y and Z coordinates of the two ASIS markers and the sacrum marker), followed by
20 subtracting its mean from each of the X, Y and Z coordinates of the 15 markers and division by their
21 respective standard deviation. The mean correction and normalisation to unit standard deviation
22 equalised the differences between the different amplitudes of markers attached to proximal and distal
23 segments, thereby placing the emphasis on the shape of the marker trajectories as opposed to their
24 amplitudes.

25

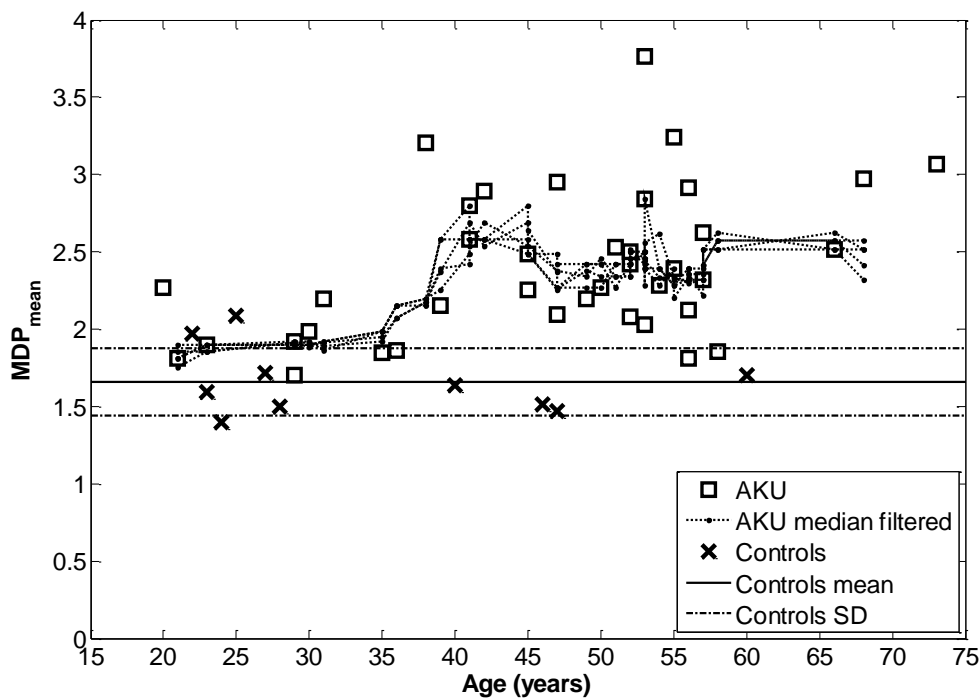
26 The processed marker coordinate data (X, Y and Z of 15 markers) was resampled to 101 values over
27 one gait cycle and then concatenated into a single column with $3 \times 15 \times 101 = 4545$ rows. Repeated gait
28 cycles of each participant (2 for controls and 3 for patients) were then added as further columns, giving
29 two sets of numerical data, one for controls and one for patients. A model of normal gait was created
30 by passing the data of controls to the self-organising neural network for training in the MDP freeware
31 program (Barton et al. 2012). MDP_{mean} values for the patients were then derived by the program using
32 the trained neural network model, and processed further in MATLAB.

33

1 To summarise, the mean deviation from normal for each patient and control was calculated by
 2 averaging the MDP_{mean} values from the three walks of patients and two walks of controls, and plotted
 3 against age. As there was considerable scatter in the data, explorative median filtering was applied in
 4 order to investigate the possibility of a transitional increase in deviation from normal gait over a small
 5 age range. Median filters, under the right circumstances, preserve transitions better than more
 6 common linear filters (Huang and Lee, 2006).

7
 8
 9 **Results**

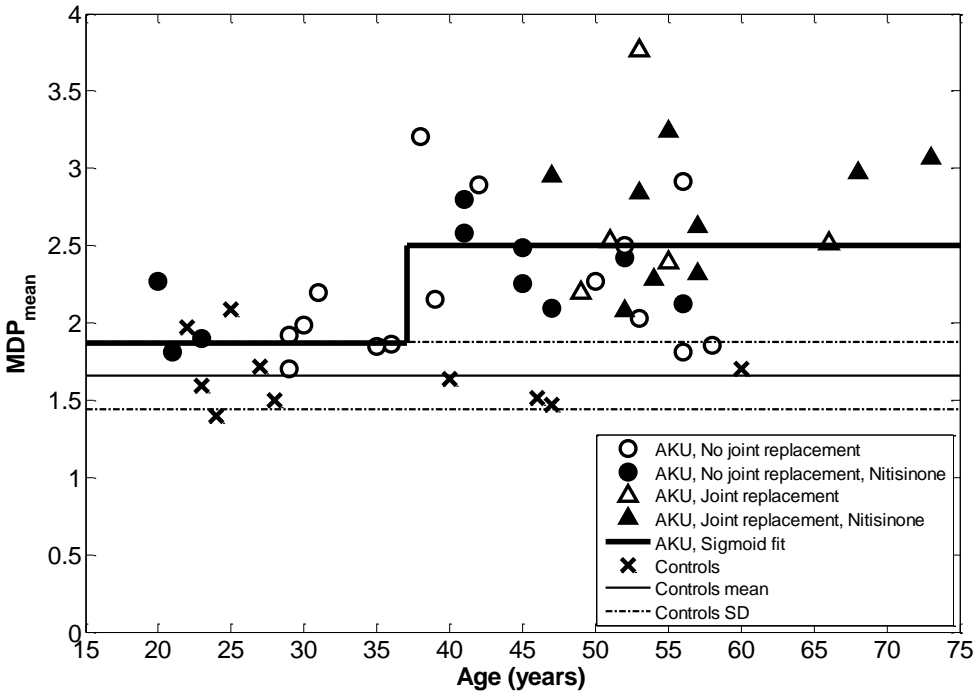
10
 11 Preliminary examination of the values of MDP_{mean} plotted against age (Figure 1) showed that they
 12 increase significantly with age, however, the linear association is not particularly strong ($p = 0.005$, R^2
 13 $= 0.193$). Further examination suggested that there might be an abrupt change around ages 35-40.
 14 This pattern becomes more obvious when overlaying the series of median filtered data (with filter
 15 points ranging between 5-9) which suggests a sigmoid profile. The upper half of the relationship
 16 (above 35-40 years) showed more variability than the lower half (under 35-40 years).



18
 19 *Figure 1: Deviation of gait from normality (MDP_{mean}) as a function of age in patients with*
 20 *alkaptonuria (AKU) compared to unimpaired controls (individuals and their mean \pm standard*
 21 *deviation). A series of median filtered curves of the AKU group indicates an abrupt increase around*
 22 *35-40 years of age.*

1 Based on the effect of median filtering, individual regressions over (a) age<35 and (b) age>40 were
 2 fitted to the patient data and showed no significant variation with age. An appropriate sigmoid
 3 was fitted to the data ("R P", 2014) using starting and ending values from the regressions at the end
 4 of (a) and at the start of (b) respectively. The fitted curve showed a discontinuity with its 50% point
 5 around age 37, providing support for the conjecture suggested by the median filtering. Adjusting the
 6 start and end of the respective regressions to 37 years and making minor adjustments to the start and
 7 end values of the sigmoid curve from the revised regressions produced an almost identical sigmoid
 8 curve (Figure 2) which was used to model the change of gait deviation as a function of age in our AKU
 9 sample.

10



11

12 *Figure 2: Subsets of AKU patients with and without joint replacement and with and without nitisinone*
 13 *treatment shown against controls. A sigmoid curve fitted on the AKU group confirmed the discontinuity*
 14 *of gait deviation around 37 years of age.*

15

16

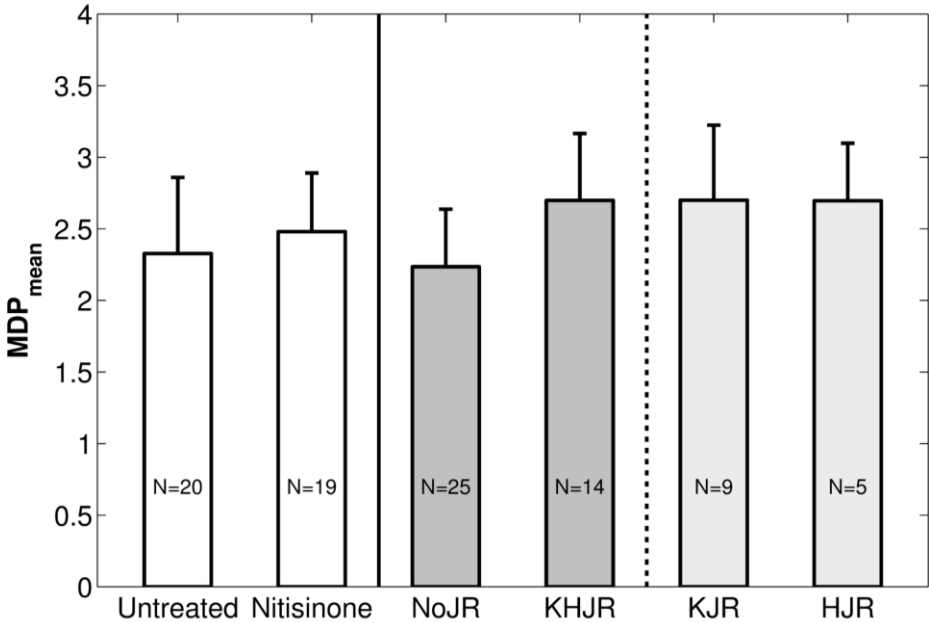
17 All AKU patients under 37 years show some level of gait deviation as their MDP_{mean} values are greater
 18 than the mean of controls. The majority of AKU patients over 37 years (28 out of 30) demonstrated
 19 more gait deviations than the mean+1SD of controls (1.88), but only 23 out of 30 were above the 95%
 20 confidence interval of the control group (2.159).

21

1 Only patients above 47 years had joint replacements (red empty and filled triangles in Figure 1) and
 2 their gait deviation was greater (2.70 ± 0.47 , $N=14$) than that of patients without joint replacements
 3 (2.24 ± 0.4 , $N=25$, red empty and filled circles in Figure 1). There are 8 AKU patients in the age range
 4 37-47 years without joint replacements but considerable deviations from normal gait.

5
 6 In an attempt to clarify the association of gait deviations with nitisinone treatment and joint
 7 replacements, the MDP_{mean} of sub-groups of AKU patients were plotted in Figure 3. While clear cause-
 8 effect mechanisms cannot be established without longitudinal data of individual patients,
 9 nevertheless the comparisons of sub-groups revealed some interesting findings. The 19 patients under
 10 nitisinone treatment showed higher deviations of gait from normality than the 20 patients without
 11 nitisinone treatment. Similarly, the gait of 14 patients with knee or hip joint replacements is further
 12 away from normality than those 25 without any joint replacements. Comparing the effects of knee
 13 and/or hip replacement on gait normality showed negligible differences. Nine AKU patients had a
 14 single knee replacement and an additional five had hip and knee replacements.

15



16
 17 *Figure 3: A comparison of subsets of AKU patients (group means and SD with the size of group indicated*
 18 *on each bar). **Untreated** (no nitisinone, no joint replacement), **Nitisinone** (on nitisinone with or without*
 19 *joint replacement), **NoJR** (no joint replacement, with or without nitisinone), **KHJR** (knee or hip joint*
 20 *replacement, with or without nitisinone), **KJR** (knee joint replacement only, with or without nitisinone)*
 21 *and **HJR** (hip joint replacement, all 5 with knee replacement, with and without nitisinone).*

22
 23

1 Discussion

2

3 An objective measure of gait function, the MDP_{mean} , showed a pattern of deterioration in alkaptonuria
4 similar to other indices of disease severity (Ranganath and Cox, 2011). Our data suggests a non-linear
5 deterioration of gait function in the second half of the 4th decade which differs somewhat from the
6 findings of Ranganath and Cox (2011) who described a gradual change in the 3rd decade followed by
7 an accelerated progression of the disease in the 5th and 6th decades of life using clinical measures and
8 patient questionnaires. Considering that AKU is an ultra-rare disease, gait related measures of 39
9 patients can be regarded as a large pool of data but certainly this particular sample of patients might
10 have influenced the fit therefore caution is advisable with setting a firm age threshold. Although the
11 regression line in the AKU group over 37 years has a low slope (0.0026 units of $MDP_{mean}/year$)
12 indicating minimal change as a function of age, there are considerable inter-individual differences in
13 their gait deviations. An interaction of several factors which may influence gait function (causing gait
14 variability) justifies individualised clinical assessment for patients in this group in order to identify their
15 specific problems which can then be targeted with appropriate treatment.

16

17 Adults with AKU under 37 years of age show some movement deviations although their MDP_{mean} is
18 only one standard deviation away from normality. Our youngest patient was 20 years of age and so
19 we have no information about the gait function of even younger patients and children with AKU. Early
20 detection of movement problems may trigger focused management in order to prevent cumulative
21 deterioration. Especially in case of children, treatment with nitisinone may have to be delayed to
22 prevent any potential side effects (Bendadi et al. 2014). Evidence of gait deviations in the young would
23 support earlier intervention (either nitisinone or alternatives e.g. physiotherapy), and conversely a
24 lack of movement problems would support delaying nitisinone treatment to a later time when the
25 chance of side effects is minimal.

26

27 Adults older than 37 years of age with AKU show more deviation from normal gait than the younger
28 group. Nevertheless if our method based on the MDP_{mean} was used to decide if a patient moved away
29 from normality, then the sensitivity/specificity would likely be low with false positives given that 23%
30 of AKU patients fall within the 95% confidence interval of the control group. Further evaluation of the
31 MDP_{mean} as a method to separate a patient with AKU from controls is necessary using a larger sample.

32

33 Patients with AKU and joint replacements were all above 47 years and their gait deviations were higher
34 than those of the group without joint replacements. The higher age of this group likely reflects clinical

1 decisions of when a joint replacement is indicated. Gait does not easily return to normality; in
2 osteoarthritis, gait was shown not to return to normal until about 11 months after total hip
3 arthroplasty (Beaulieu et al. 2010). Potential mechanisms underlying slow recovery are: unnecessary
4 but continued pain avoidance strategies and persistent muscle weakness due to disuse, both of which
5 may hinder not only the affected but the contralateral side too. Persisting gait abnormality following
6 joint replacement in AKU is likely to be due to the systemic nature of the disease which affects
7 anatomical structures beyond the joint itself. AKU potentially affects all joints including their related
8 ligaments, tendons and muscles and this might explain why repairing a single joint may not lead to a
9 rapid recovery.

10

11 Deviation of controls from normality, as measured by the MDP_{mean} , shows minimal increase as a
12 function of age. This might be explained by the self-organising neural network's method of operation
13 underlying the MDP method. Subsequent to defining an internal model of gait normality based on the
14 gait of controls, the deviation from the closest matching variant of normal gait is calculated (Barton et
15 al. 2012). As the control group included older individuals (40, 46, 47 and 60 years of age) the MDP_{mean}
16 of AKU patients could reflect the deviation from this subgroup of controls with altered gait due to their
17 age. In order to determine if the gait of the older control sub-group affected the calculation of the
18 MDP_{mean} , a separate neural network model was generated by training with the younger group of
19 controls and then calculating the MDP_{mean} of the older control group. The similar MDP for the older
20 control group ($MDP_{mean} = 1.82$) compared to younger controls (22-28 years, $MDP_{mean} = 1.67$) indicates
21 that only minimal gait deviations occur as a result of ageing. As such the increased MDP_{mean} of the
22 older individuals with AKU is referenced to a rather coherent definition of gait normality regardless of
23 age. Increased gait deviations therefore were due to genuine deterioration of gait related to AKU
24 together with other factors like joint replacements and nitisinone treatment. More in-depth sub-
25 group analyses will be required to differentiate between these factors.

26

27 A limitation of our method is that on its own the single number MDP_{mean} can only be used to flag an
28 increased deviation of gait from normality. This should then be followed up by establishing the specific
29 gait problems which eventually is expected to lead to improved clinical decision making and treatment.
30 Movement of body-attached markers was used to quantify deviation of gait but this approach can be
31 complemented by including the forces acting on the body at each joint. Such an extension of the
32 method may offer further advantages due to its focus on joint loading which is the ultimate cause of
33 pain in alkaptonuria. An inevitable limitation of our study was the low number of participants and the
34 cross-sectional nature of gait data.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34

Conclusions

This was the first attempt to evaluate gait function in patients with alkaptonuria using a summary measure of gait deviation (the MDP). Patients with AKU showed minimally increased gait deviations between 20-37 years, followed by an accelerated increase around the second half of the 4th decade of life. The older group of patients was characterised by elevated and varied levels of gait deviation. Together with continued gait analysis of adults with AKU, evaluation of gait deviations in younger adults and children with AKU is necessary to complete and refine our understanding of disease progression moderated by the influence of interventions including joint replacements and nitisinone treatment.

Acknowledgements

We gratefully acknowledge the support of participants in our study and the continued help and assistance by the AKU Society. We also thank Kimberley Lewin for performing gait analysis on the very first group of patients at Liverpool John Moores University.

References

1. Abimbola O, Hall G, Zuckerman JD (2011) Degenerative arthritis of the knee secondary to ochronosis. Bull NYU Hosp Jt Dis 69:331-334
2. Baker R (2006) Gait analysis methods in rehabilitation. J Neuroeng Rehabil 2006 3:4.
3. Baker R, McGinley JL, Schwartz MH, et al (2009) The gait profile score and movement analysis profile. Gait Posture, 30:265-269
4. Barton GJ, Hawken MB, Holmes G et al (2015) A gait index may underestimate changes of gait: a comparison of the Movement Deviation Profile and the Gait Deviation Index. Comput Methods Biomech Biomed Engin 18:57-63
5. Barton GJ, Hawken MB, Scott M et al (2012) Movement Deviation Profile: a measure of distance from normality using a self-organizing neural network. Hum Mov Sci 31:284-294
6. Beaulieu ML, Lamontagne M, Beaulé PE (2010) Lower limb biomechanics during gait do not return to normal following total hip arthroplasty. Gait Posture 32:269-273

- 1 7. Bendadi F, de Koning TJ, Visser G et al (2014) Impaired cognitive functioning in patients with
2 tyrosinemia type I receiving nitisinone. *J Pediatr* 164:398-401
- 3 8. Davis RB (2004) The motion analysis laboratory. In Gage J ed. *The treatment of gait problems*
4 *in cerebral palsy*. London: Mac Keith Press. 90-98.
- 5 9. Davis RB, Ounpuu S, Tyburski D et al (1991) A gait analysis data collection and reduction
6 technique. *Hum Mov Sci* 10:575-587
- 7 10. Federolf PA, Boyer KA, Andriacchi TP (2013) Application of principal component analysis in
8 clinical gait research: identification of systematic differences between healthy and medial
9 knee-osteoarthritic gait. *J Biomech* 46:2173-2178
- 10 11. Huang HC, Lee TCM (2006) Data adaptive median filters for signal and image denoising using
11 a generalized SURE criterion. *IEEE Signal Proc Let* 13:561-564
- 12 12. Introne WJ, Gahl WA (2003) Alkaptonuria. In *NORD guide to rare disorders*. Lippincott Williams
13 & Wilkins. Philadelphia: PA. 431
- 14 13. Introne WJ, Perry MB, Troendle J et al (2011) A 3-year randomized therapeutic trial of
15 nitisinone in alkaptonuria. *Mol Genet Metab*, 103:307-314
- 16 14. Lees A, Barton G, Robinson M (2010) The influence of Cardan rotation sequence on angular
17 orientation data for the lower limb in the soccer kick. *J Sports Sci* 28:445-450
- 18 15. "R P" (2014) Sigmoid / logistic curve fit. Matlab Central, Retrieved from
19 <http://uk.mathworks.com/matlabcentral/fileexchange/42641-sigmoid---logistic-curve-fit> on
20 27/11/2014
- 21 16. Ranganath LR, Cox TF (2013) Natural history of alkaptonuria revisited: analyses based on
22 scoring systems. *J Inherit Metab Dis* 34:1141-1151
- 23 17. Ranganath LR, Jarvis JC, Gallagher JA (2013) Recent advances in management of alkaptonuria
24 (invited review; best practice article). *J Clin Pathol* 66:367-373
- 25 18. Schwartz MH, Rozumalski A (2008) The gait deviation index: a new comprehensive index of
26 gait pathology. *Gait Posture* 28:351-357