

Natural history of alkaptonuria revisited: analyses based on scoring systems

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Abstract Increased circulating homogentisic acid in body fluids occurs in alkaptonuria (AKU) due to lack of enzyme homogentisate dioxygenase leading in turn to conversion of HGA to a pigmented melanin-like polymer, known as ochronosis. The tissue damage in AKU is due to ochronosis. A potential treatment, a drug called nitisinone, to decrease formation of HGA is available. However, deploying nitisinone effectively requires its administration at the most optimal time in the natural history. AKU has a long apparent latent period before overt ochronosis develops. The rate of change of ochronosis and its consequences over time following its recognition has not been fully described in any quantitative manner. Two potential tools are described that were used to quantitate disease burden in AKU. One tool describes scoring the clinical features that includes clinical assessments, investigations and questionnaires in 15 patients with AKU. The second tool describes a scoring system that only includes items obtained from questionnaires in 44 people with AKU. Analysis of the data reveals distinct phases of the disease, a pre-ochronotic phase and an ochronotic phase. The ochronotic phase appears to demonstrate an earlier slower progression followed by a rapidly progressive phase. The rate of change of the disease will have implications for monitoring the course of the disease as well as decide on the most appropriate time that

treatment should be started for it to be effective either in prevention or arrest of the disease.

Introduction

The natural history of alkaptonuria (AKU) is not fully understood (O'Brien et al. 1963; Phornphutkul et al. 2002). AKU affects virtually all connective tissues leading to a variety of clinical features and complications among which joint disease is a major feature (Helliwell et al. 2008). A new agent, called Nitisinone (NTBC, Orfadin), inhibits enzyme p-hydroxyphenyl pyruvate dioxygenase and decreases formation of homogentisic acid (HGA) (Suwannarat et al. 2005). It is a potential treatment for AKU. A major difficulty in utilising Nitisinone in AKU stems from the fact that there is a very long apparent disease free period followed by a progressive recognizable disease. It is possible that lack of quantifiable assessment methodology to describe disease also makes it difficult to develop suitable policies for clinical management as well as designing clinical studies with appropriate end points.

Alkaptonuria is a rare inborn error of metabolism characterized by high circulating homogentisic acid (HGA) due to a genetic deficiency in the enzyme homogentisate dioxygenase (HGD) (LaDu 2001). Conversion of HGA to a polymeric melanin-like pigment and binding to connective tissue, especially cartilage, called ochronosis, is the main pathophysiological event (Fernandez-Canon et al. 1996). The damaging effects of ochronosis include arthritis (especially spine and large weight bearing joints), stones (renal, prostatic, gall bladder and salivary), cardiac valve disease especially aortic (Goodfellow et al. 2005; Wilke and Steverding 2009), ruptures (muscle, tendons and ligaments), osteopenia and fractures (O'Brien et al. 1963). Virtually all connective tissue is affected.

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Alkaptonuria is a genetic disease that is present at birth, although overt ochronotic manifestations of the disease are delayed, typically beginning after 30 years of age. However, reason for the delayed appearance of ochronosis remains a mystery and the natural history incompletely understood.

We believe that the application of a scoring system, described elsewhere in this supplement (Cox and Ranganath 2011), along the lines described by others for allied disorders such as Gauchers and Fabry disease may allow better characterisation of patients and allow the natural history to be better understood (Beck 2006).

We performed a systematic clinical evaluation of a cohort of 15 patients with alkaptonuria by a combination of clinical assessments, imaging investigations, medical photography and detailed questionnaires with the objective of understanding and describing the features of AKU better. We also similarly undertook to describe and quantitate the features of AKU in a cohort of 44 people who only completed detailed questionnaires. More specifically the aims were to (1) score the clinical features of people with alkaptonuria for the clinical evaluation cohort and the questionnaire only cohort separately, and (2) to test the utility of these two scoring systems to inform on the evolution of the disease and thus clarify the natural history further.

Materials and methods

The study was approved by the local research ethics committee and all participants gave informed consent. We enrolled 15 individuals from 12 families for the clinical evaluation cohort. Patients with AKU travelled to Liverpool and were investigated over three days. The diagnosis of alkaptonuria was reconfirmed in all patients by typical pattern of external pigmentation and urinary homogentisic aciduria. All patients underwent clinical examinations, extensive imaging, medical photography and questionnaire analysis as described in another paper in this supplement (Cox and Ranganath 2011).

The Royal Liverpool University Hospital, in association with the alkaptonuria Society UK, undertook a programme to identify more people with AKU (JIMD in press) and this allowed us to establish contact with several patients both in the UK and outside the UK. 44 of these patients completed nine detailed questionnaires that included (1) demographic, (2) social, (3) diet/weight, (4) diagnosis, (5) family, (6) complications, (7) joint involvement, (8) quality of life, and (9) treatment aspects of AKU.

Data were extracted from both the clinical evaluation study (CLIN) and the questionnaire only study (QUEST) to populate scoring systems that included mostly similar items. These 2 scoring systems are described in Cox and Ranganath 2011.

Ocular ochronosis was scored for presence or absence of pigmentation in nasal and temporal aspects of sclera in each

eye in the CLIN group but only for presence or absence of pigment in right and left eye in the QUEST group. Auricular ochronosis was scored for no pigment, established pigment and marked pigmentation and scored 0, 2 and 4 respectively in each ear in the CLIN group. In the QUEST group, presence or absence of pigment was scored as 0 or 4 respectively.

Some features were due to excess circulating homogentisic acid (stones in prostate, kidney, salivary gland and gall bladder); some were due to ochronosis (ear/eye pigmentation, cysts in liver/kidney, central nervous system involvement such as stroke and Parkinsonism, bone disease (osteopenia), teeth and skin pigment, ENT features (such as middle ear and tympanic membrane pigmentation and hearing impairment), cardiac valve disease (aortic sclerosis/stenosis, congestive heart failure as well as arrhythmia); and others were due to damage to connective tissue leading to fractures, ruptures of tendons/ligaments/muscle, as well as spinal and non-spinal joint disease.

Later appearing features as well as those associated with breakdown of connective tissue or indicative of tissue/organ failure, such as fractures, ruptures (tendons, ligaments, muscle) and joint replacements were scored higher.

Spine was scored for pain in 4 major areas (cervical spine, thoracic spine, lumbar spine, sacroiliac). Scintigraphic scan employing Technetium labelled-methylene-bisphosphonate was used to identify spine involvement and scored to include 5 areas (cervical spine, thoracic spine, lumbar spine, sacroiliac, costochondral). Pubic symphysisitis was scored either by pain or on technetium scanning. Kyphosis and scoliosis were separately scored in the CLIN group and only as kyphosis in the QUEST group.

Non-spine joint involvement was scored for pain in major joint areas. These 14 major joint areas were defined as hips, knees, ankles, feet, shoulders, elbows, hands including wrists. The Technetium scan was also employed to score for these 14 major areas to serve as an objective measure of joint involvement. Interventions such as arthroscopies indicative of overt joint damage were also scored. Total joint failure requiring joint replacement was also scored.

Our aim was to arrive at a roughly 40/60 proportions for joint (spine and non-spine)/non-joint clinical features. This was to recognize the totality of clinical features rather than just joint disease.

The spine score, non-spine joint score, and non-joint clinical score were summed to derive an aggregate total AKU severity score index. This total aggregate score was called cAKUSSI for the CLIN group and qAKUSSI for the QUEST group.

Statistical methods

The scores of various features in the CLIN and the QUEST groups were assessed by CUSUM analysis

Table 1 Baseline characteristics of the clinical evaluation and the questionnaire only groups

	Clinical evaluation cohort	Questionnaire only cohort
Numbers of patients	15	44
Age (years) mean (all/men/women)	50.8 / 45.7 / 58.5	50.5 / 48 / 53.6
Age range	28 - 69	16-84
Male sex (%)	60	54.5
Hypertension (%)	33	31.8
Hyperlipidaemia(%)	60	36.4
Diabetes mellitus (%)	0	2.3
Current smoker (%)	20	16
Previous smoker (%)	13	18.2
Known coronary heart disease (%)	6	11.4

(cumulative sum, Grigg et al. 2003) and displayed as graphs to describe the rate of change of disease during the human lifecycle. The scores, individual components and well as aggregated, were analysed in terms of their relationships between the various features within CLIN and QUEST groups by Pearson’s correlation co-efficient with p values <0.05 being considered significant. Unpaired Students ‘t’ test was also used to assess relationship between groups with a value of P less than 0.05 considered significant.

Results

Baseline characteristics All baseline characteristics of both the clinical evaluation (CLIN) and the questionnaire only (QUEST) groups are detailed in Table 1. The prevalence of clinical features was higher in the clinical evaluation cohort compared with the questionnaire only cohort and is shown in Tables 2 and 3. The mean age (±SEM) was similar between the CLIN (50.8±3.1) and QUEST groups (Male+Female [48±3.3]; Male only [50.5±2.3]; Female only [53.6±3.1]). A range of cardiovascular risk factors was seen; prevalence of hypertension was similar but hyperlipidaemia was lower in the QUEST group. There was no diabetes in the CLIN group and only one person with new type 2 diabetes mellitus on diet treatment in the QUEST group. The self-reported prevalence of cardiac disease was low. No patient had a prior history of valvular heart disease or chronic heart failure. Only one patient had a prior history of coronary heart disease and had undergone percutaneous coronary angioplasty for chronic stable angina. No patient had a prior history of myocardial infarction.

The intention was to allow a proportion of 40/60 between articular (spine and non spine joints) and non-articular features. This has been shown in Table 4.

The rationale for the scoring system used and its validity for use in patients with AKU are described

elsewhere in this supplement (Cox and Ranganath 2011). The scores were used to derive cumulative scores that were then graphically represented and are shown in Figs. 1, 2 and 3.

CLIN group (Fig. 1, Table 5)

Two graphs are shown for each variable; the cumulative scores taken over the subjects ordered according to their age and the second which is a basic cusum chart based on the cumulative data. The cusum chart is started at the first subject to score more than zero, and as each point is added within the cusum,

Table 2 Prevalence of non-joint clinical features in the CLIN and QUEST groups

	Prevalence clinical evaluation cohort score	Prevalence questionnaire only cohort score
Prostate stones	3/9	4/44
Renal stones	6/15	4/44
Fracture	8/15	10/44
Osteopenia	7/15	-
Parkinsonism/Stroke	0	1/0
Ruptures (tendons, ligaments, muscle)	11/15	28/44
Hearing difficulties	7/15	-
Middle ear ochronosis	6/15	-
Known valvular heart disease (%)	3/15	7/44
Aortic sclerosis	3/15	
Aortic stenosis	3/15	
Arrhythmias (non-atrial fibrillation, atrial fibrillation, palpitations)	6	-
Congestive heart failure	0	1

Table 3 Analysis of joint replacements in CLIN (n=15) and QUEST (n=44) groups

	CLIN	QUEST
Age at first joint replacement	53±7.2	51.4±6
Number of people with joint replacements in each group	7	22
Total number of joint replacements in groups	17	76

the mean of the scores is subtracted (the mean being taken over all subjects, but starting with the first subject to score more than zero). This will have the effect that the final point in the plot will be back at zero. The usefulness of this type of plot is that it shows where the pattern of scores is changing with age. For example, if once the cusum starts, all subjects score the same, then the cusum will remain at zero for all points. If there is a region in the age range where scores are higher than average, then the cusum will have a positive slope in that range and conversely, if there is a range where scores are lower than average, then the cusum will have a negative slope. Note, a plot of cumulative scores against age would not be appropriate here since the actual profile of the ages in the sample would skew the plot and give false impressions of the behaviour of scores with age.

From the first two pairs of graphs in Fig. 1, eye ochronosis appears later than ear ochronosis (subject 2: 31 years of age, subject 6: 47 years of age). Then there is an initial increase in scores but then a marked increase in scores around age 58 years (subject 11). The same pattern is true for ear ochronosis but with the marked increase in scores at about 53 years of age (subject 8). Very similar patterns can be seen for all the other features (clinical without eye/ear, all clinical, spine, non-spine, all joint, cAKUSSI). Overall, there is a marked increase in scores within the 5th decade.

Most features except eye appear to become overt around age 30 years. Most parameters except spine showed a significant and sizeable correlation in the CLIN group. Older patients had more features of disease. Eye and ear ochronosis showed very good correlation suggesting similar underlying pathophysiological processes. Articular features show an excellent relationship with ear and eye ochronosis also confirming similar common mechanisms of pathogenesis.

Table 4 Ratios of eye and ear scores to all non-articular scores (EyeEar/AllClin), spine to all joint scores (spine/alljoint), and all non-articular scores to total AKUSSI (AllClin/AKUSSI) (Mean±SEM)

	EyeEar/AllClin	Spine/AllJoint	AllClin/AKUSSI
Clinical evaluation study (n=15)	0.35±0.08	0.48±0.04	0.43±0.03
Questionnaire only study male and Female (n=44)	0.41±0.05	0.45±0.04	0.38±0.03
Questionnaire only study male only (n=24)	0.34±0.06	0.49±0.05	0.38±0.04
Questionnaire only study female only (n=20)	0.49±0.08	0.40±0.05	0.37±0.04

AKUSSI scores showed excellent and strong relationship with most parameters except spine. (Note that careful interpretation of the correlations in Table 5 is required since the some features are comprised in part of other features also in the table; for example, an “All clin” score will include “Eye” and “Ear” scores.)

QUEST group (Fig. 2, Table 6)

Similar patterns of scores were found in the QUEST group as in the CLIN group. Eye ochronosis is generally later than ear ochronosis by approximately 10 years. For eye ochronosis and ear ochronosis, subjects tend to score 0 or 8, and once an 8 is scored for a subject, then the score remains at that level. Thus the cusum reflects the numbers of subjects that start to score 8 at particular age points rather than increases in scores within subjects. For eye ochronosis, by the middle of the 5th decade, all subjects are scoring 8. For ear/eye ochronosis, all subjects are scoring 8 by the middle of the 4th decade (the two sudden drops in the cusum chart are caused by some subjects scoring 0 while all their contemporaries are scoring 8). For “All Clinical” scores, scores start to increase at age 30 and then there is a marked increase at age about 60. For spine pain, the score start to increase at about age 40 and then there is a marked increase at age 54. For non-spine pain the initial increase is at age 51 and then the marked increase is at age 58. The same pattern applies for qAKUSSI. In summary, there appears to be an increase in score at age 50 and then an even larger increase at age 60.

All parameters including spine showed significant and sizeable correlations in the QUEST group. This suggests that older patients had more features of disease. Eye and ear ochronosis showed very good correlation suggesting similar underlying pathophysiological processes. Articular features excellent relationship with ear and eye ochronosis also confirming similar common mechanisms of pathogenesis. qAKUSSI scores showed excellent and strong relationship with all parameters including spine, but again noting the careful interpretation needed of the correlations.

Figure 3 shows cusum plots for qAKUSSI for males and females separately. It can be seen that the scores for both have three phases as seen in the previous analysis. For females, from the first phase to the second, the score

increases with age (age 40), but then the score markedly increases from phase two to phase three (age 60). The same pattern is true in males but with more profound effect. The two ages of increase for males are 32 and 52, putting males about 8 years ahead for qAKUSSI scores (see Cox and Ranganath 2011 for further details of this gender difference).

Further comparisons between males and females were made but the frequency and cusum charts are not shown here. In summary, eye ochronosis stated later in males than females, but by the middle of the fifth decade all subjects are scoring 8. Ear ochronosis starts earlier than eye ochronosis for both male and females, and by the start of the fourth decade, the majority of subjects score 8. For the

Fig. 1 Cusum (cumulative sum) charts for the clinical evaluation study group (n=15). For each feature, two cusum charts are shown. The left hand chart is a plot of the cumulative sum against subject number where subjects have been ordered by age. If all subjects score the same value for a feature, then the plot will be a perfectly straight line. Variations in the slope of the line show how scores vary with age – the steeper the slope in a particular age range, the higher the scores and correspondingly, the more shallow the slope, the lower the scores. The right hand chart is the same plot but as each individual score is added to the cumulative sum, the value of the overall mean is also subtracted. This chart helps to see the changes in score pattern with age. To interpret, if all subjects scored the same, then the plot would be a perfectly horizontal straight line; if there is an age range where scores are lower than average the chart will have a negative slope but will have a positive slope if the scores were higher than average. There are clear changes in slope for all features

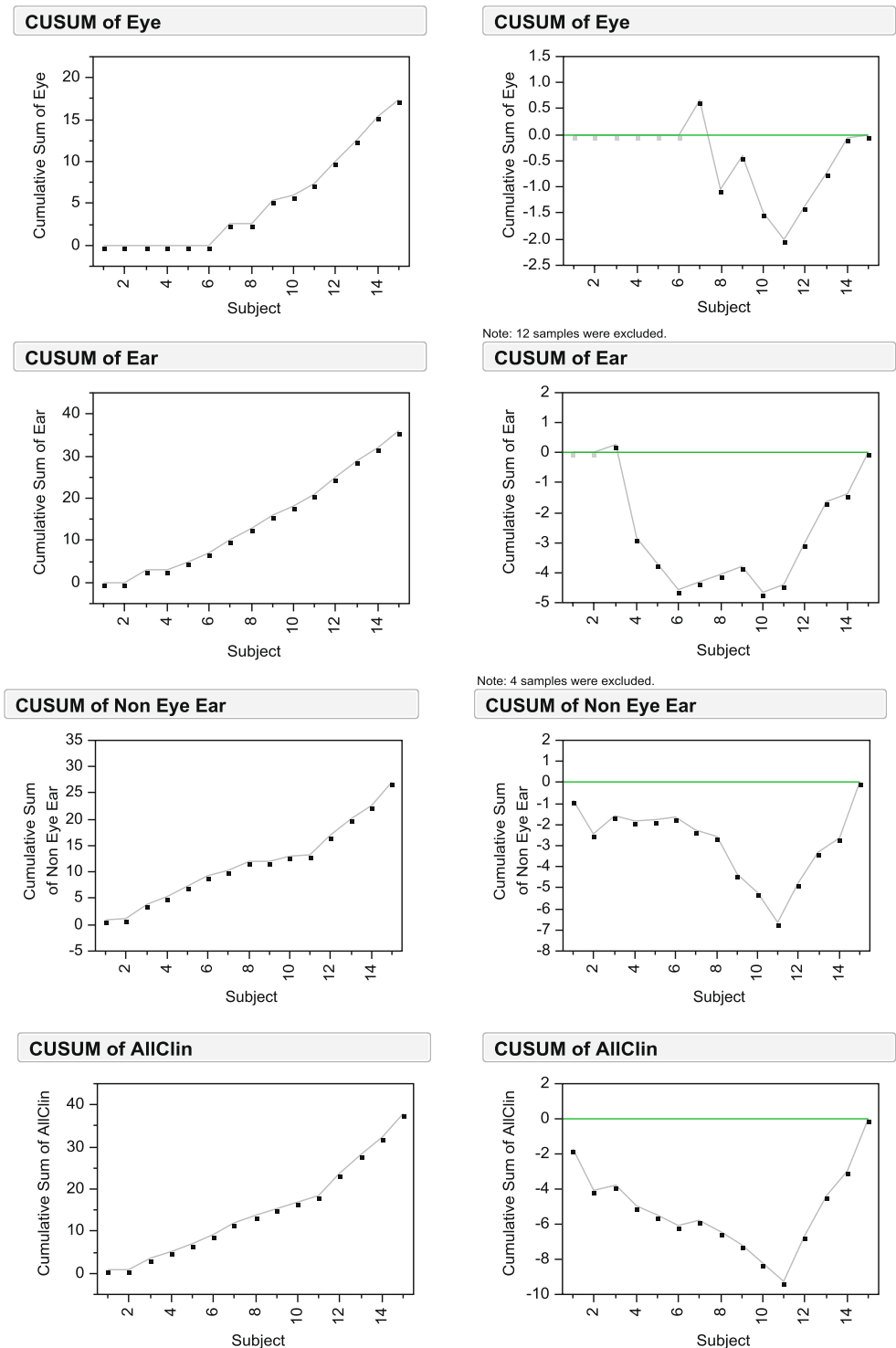
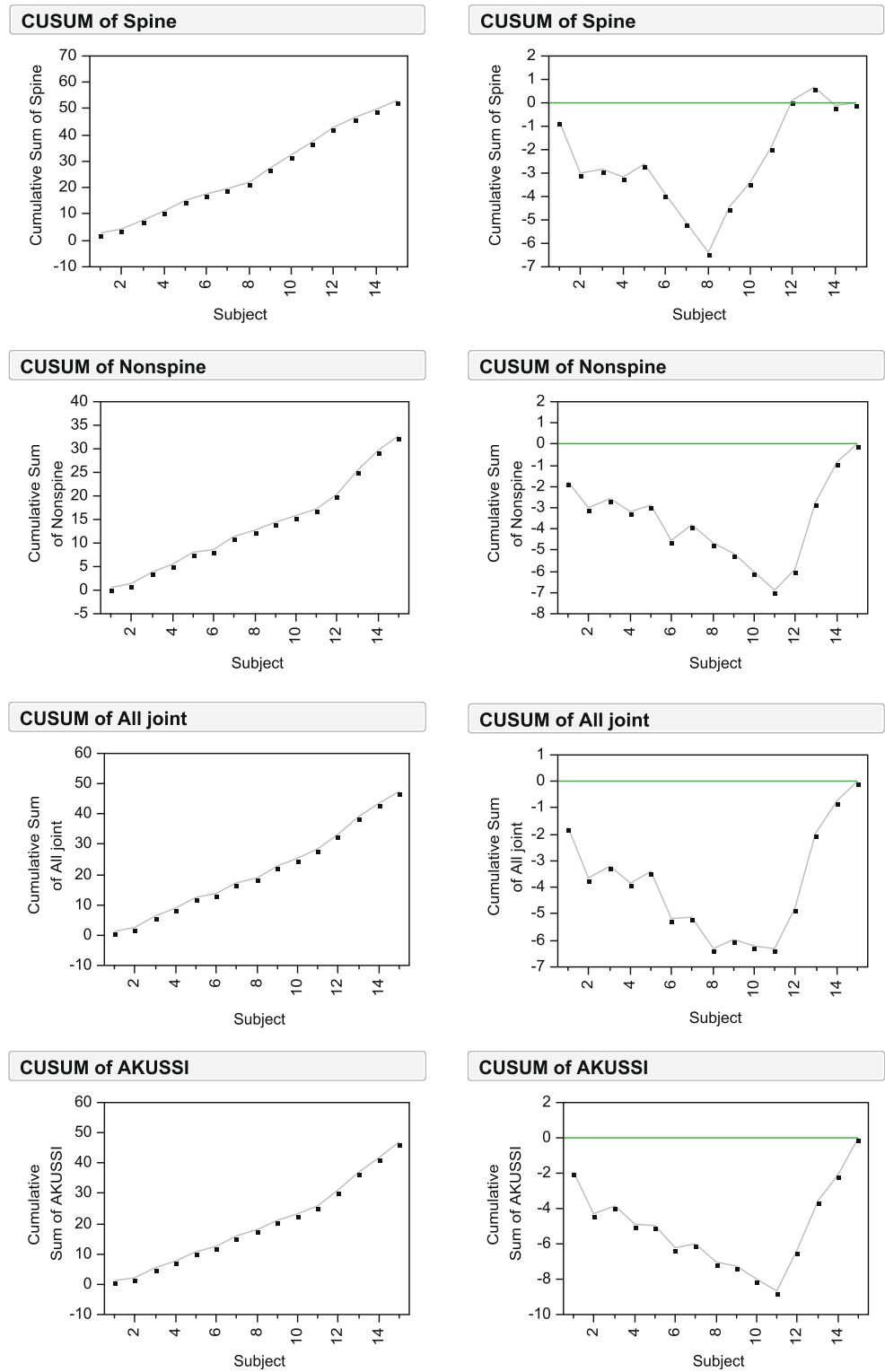


Fig. 1 (continued)



All clinical score, the marked increase in scores is at the age of about 50 for males and 58 for females. For spine pain, for non-spine pain and All joint pain, there is a marked increase at the age of 54 for males, but not so marked for females.

Discussion

This is the first time that an attempt has been made to describe the natural history by quantitating the features of the AKU. There have some good publications describing the natural

history (O’Brien et al. 1963; Phornphutkul et al. 2002) but in the main such descriptions remain qualitative and incomplete.

AKU is a genetic disease characterised by ochronosis due to high circulating HGA. Although the genetic defect is present at birth leading to increased urinary HGA, ochronosis is delayed for reasons that are not currently understood.

The present study investigated the natural history in AKU patients in different ways – one group had objective evaluation (CLIN) and the other group has subjective evaluation (QUEST). CLIN is more robust as it identified up more manifestations of AKU that were previously unrecognized.

Fig. 2 Cumsum charts for the questionnaire group (n=44). Clear changes in slope can be seen for all features

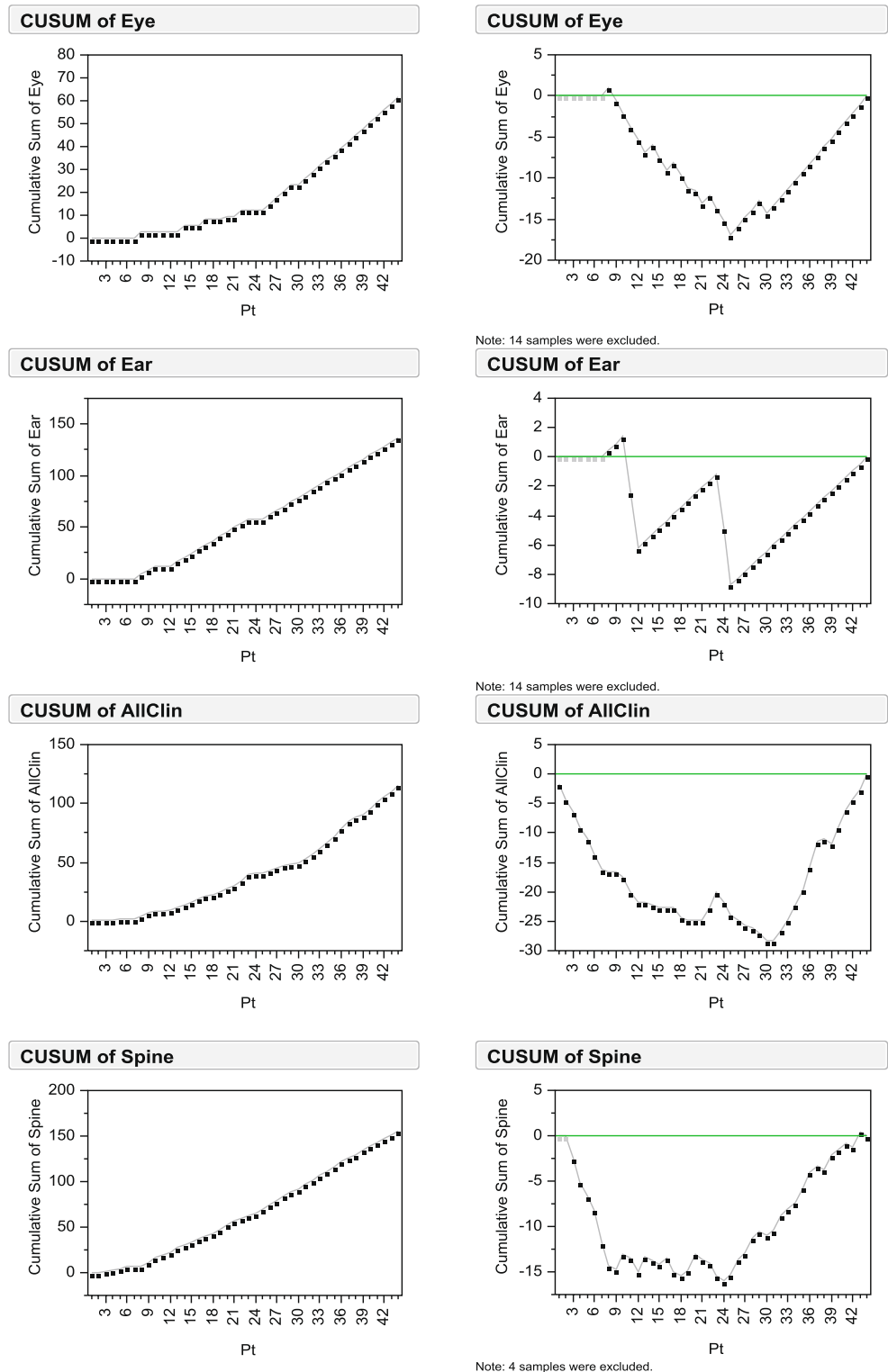
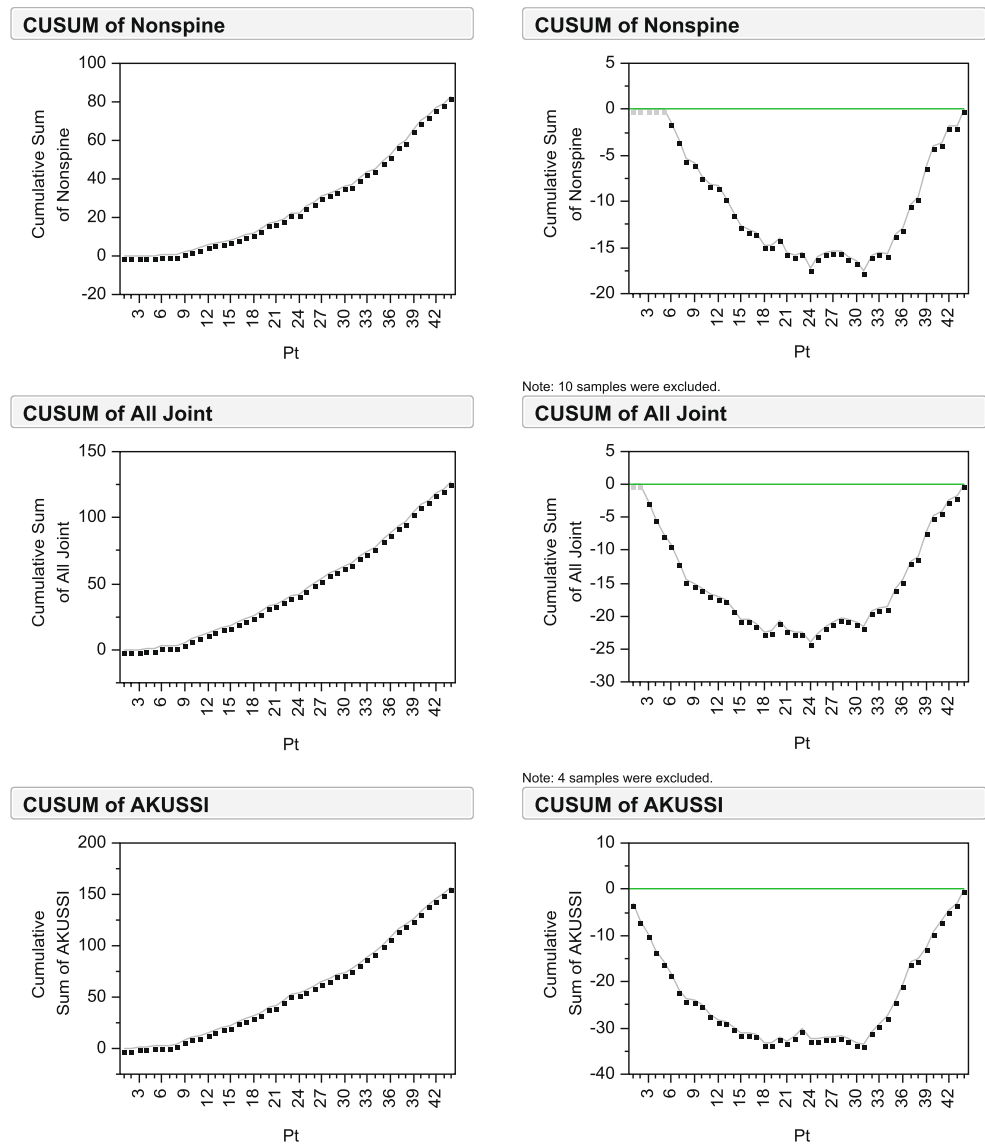


Fig. 2 (continued)



The information on the natural history is similar in both groups – very little happens in the first three decades of life in terms of objective ochronosis despite high urinary HGA at this time. Symptoms of joint pains in the first three decades are anecdotal and inconsistent.

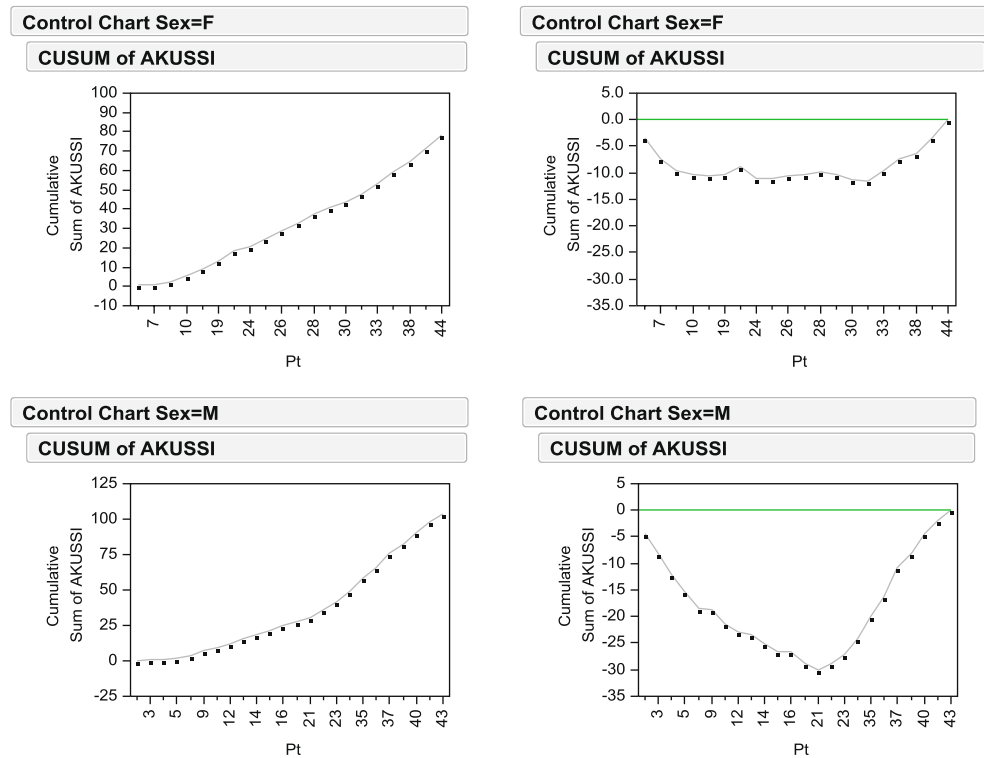
From the 3rd decade onwards all features progress at a similar rates in men and in women, joint and non joint features, spine and non-spine joint features. Isotope bone scan scores complemented the subjective scores and showed good correlation. This suggests that there is probably a similar systemic biological basis for this progression for these varied clinical features i.e. metabolic - high HGA causing ochronosis. There is currently little information of circulating HGA during the different phases of the disease.

We noted a large number of features in our assessment of the cohort of AKU patients. In order to avoid bias we

included all the features found including renal and liver cysts. Features such as liver and renal cysts may not necessarily be due to ochronosis but were included to prevent bias. Eventually we hope that the scoring system will evolve and features that do not add value will be removed from the scoring system – this is a first step and includes as many features as possible without bias. We plan to undertake a Delphi exercise to solicit input from leading practitioners and experts. However, it is worth remembering that the defective enzyme in AKU, homogentisate dioxygenase, is only expressed in health in kidneys and liver – therefore when we found these features in our patients on investigation, we felt it should be included.

The correlation between the non-joint clinical features and joint (spine and non-spine) was strong both in the CLIN and in the QUEST groups, which again suggests a similar biologic process. There was a very strong associa-

Fig. 3 Cusum charts for qAKUSSI for males and females



tion between age and the different manifestations in both the CLIN and QUEST groups. Total AKUSSI showed significant correlation with age.

In the QUEST group, men showed earlier onset of ochronosis and clinical features and more steady progression. Women on the other hand showed later onset; the reason for the difference between the genders is unclear.

The rate of progression was slower to begin with, but then with a marked increase in the 5th and 6th decades of

life. The reasons for this change in rate of progression are not clear. Random events such as tendon ruptures, muscle tears and fractures were scored in QUEST and CLIN groups and showed also good correlation with joint features and age. Predictable events such as ear, eye and joint features also showed excellent correlation between each other and with age. This may suggest that both the random and the predictable events have the same biological basis, i.e. ochronosis due to high HGA.

Table 5 Clinical evaluation cohort: correlation coefficient (n=15)

	Age	Eye	Ear	All clinical	Spine	Non spine	All joints	AKUSSI
Age		0.73 0.002	0.83 0.0001	0.73 0.002	0.5 0.06	0.59 0.02	0.68 0.005	0.74 0.002
Eye			0.7 0.004	0.69 0.005	0.43 <0.11	0.69 0.005	0.74 0.002	0.75 0.002
Ear				0.81 0.0002	0.49 0.06	0.67 0.007	0.75 0.002	0.82 0.0002
All clinical					0.33 <0.2	0.82 0.0002	0.81 0.0002	0.97 0.0001
Spine						0.24 <0.4	0.58 0.03	0.46 0.09
Non spine							0.93 0.0001	0.91 0.0001
All joints								0.94 0.0001
AKUSSI								

Data expressed as Pearson's correlation coefficient (r) (top row of each cell) with test of significance expressed as a p value (bottom row value of each cell)

Table 6 Questionnaire only cohort (Male+Female): correlation co-efficient grid (n=44)

	Age	Eye	Ear	All clinical	Spine	Non spine	All joints	AKUSSI
Age		0.67 0.0001	0.69 0.0001	0.68 0.0001	0.73 0.0001	0.47 0.0001	0.8 0.0001	0.82 0.0001
Eye			0.58 0.0001	0.63 0.0001	0.45 0.003	0.56 0.0001	0.57 0.0001	0.66 0.0001
Ear				0.69 0.0001	0.61 0.0001	0.48 0.002	0.56 0.0001	0.68 0.0001
All clinical					0.52 0.0003	0.62 0.0001	0.64 0.0001	0.89 0.0001
Spine						0.63 0.0001	0.8 0.0001	0.74 0.0001
Non spine							0.97 0.0001	0.89 0.0001
All joints								0.92 0.0001
AKUSSI								

Data expressed as Pearson’s correlation coefficient (r) (top row of each cell) with test of significance expressed as a p value (bottom row value of each cell)

The present understanding of the evolution of AKU is shown in Fig. 4. There is a recognizable ochronotic phase from the 3rd decade onwards with ear pigmentation and joint involvement with initial slower and later rapid progressive phases. In the pre-ochronotic phase there is an initial period when subjects are totally asymptomatic and this may be followed by a later intermittently symptomatic phase. It is not clear what pathophysiological events are occurring in the pre-ochronotic phases besides excessive urinary HGA excretion.

It is currently not clear whether potential effective treatment should be commenced at birth when subjects are

asymptomatic and non-ochronotic until around the third decade of life (Fig. 5). Potentially toxic treatments for AKU need to be justified on the basis of the natural history. Potential treatments with possible side effects could be justifiably administered if ochronosis is evolving from birth. It is currently unknown if ochronosis is reversible; if irreversible earlier treatments may be justified. With regard to influencing progression of the disease, there is concern whether the process can be arrested especially in the rapid phase of ochronotic disease. These considerations on ability to arrest or reverse the disease will be important aspects is designing, testing and employing potential treatments.

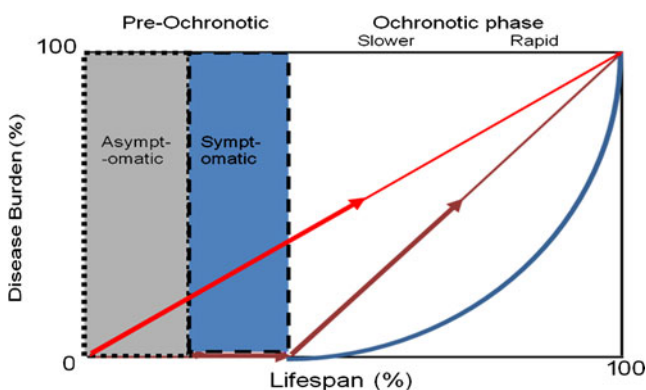


Fig. 4 A proposed model for evolution of ochronosis and other features in alkaptonuria. The ochronotic features of AKU become recognisable around the third decade of life. The lifespan can thus be divided into pre-ochronotic and ochronotic phases. One interpretation of overt ochronosis in the third decade is that it only starts to develop from this point onwards and is indicated by the purple line. An alternative possibility is that ochronosis is continuously evolving from birth reaching an overt threshold by the third decade of life. Slower and more rapid phases are recognisable in the ochronotic period. Symptoms have also been described in the pre-ochronotic phase

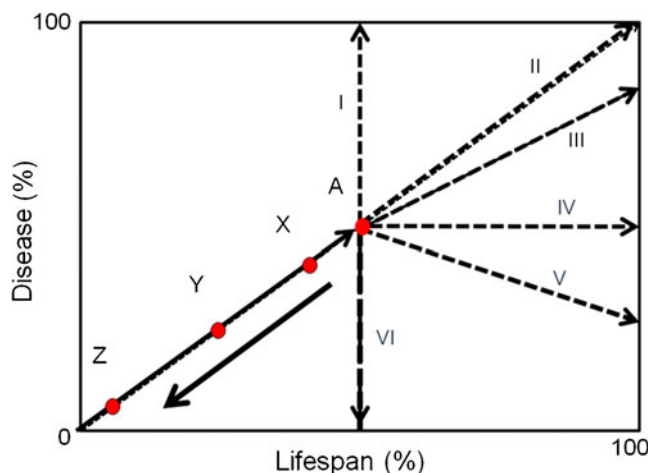


Fig. 5 Schema showing possible outcomes of intervention in AKU. The possible effect of intervention at point A in the natural history of AKU: I –potential worsening of disease; II – no change in evolution; III – attenuation of progression; IV – arrest of disease; V- decrease in burden of disease; VI – reversal of disease. Earlier intervention at points X, Y and Z will lead to less residual disease if decrease or reversal of disease is unlikely

In conclusion, we have described for the first time the evolution of AKU and ochronosis in two groups of patients, one group assessed by a variety of clinical testing and a second group only by questionnaire analysis. Validated scoring systems were used to quantify the disease thus allowing a better understanding of the natural history which is a pre-requisite to a fuller understanding of disease and optimal application of potential therapy.

References

- Beck M (2006) The Mainz Severity Score Index (MSSI): development and validation of a system for scoring the signs and symptoms of Fabry disease. *Acta Paediatr Suppl* 451:43–46
- Cox TF, Ranganath LR (2011) A quantitative assessment of alkaptonuria: testing the reliability of two disease severity scoring systems. *JIMD* (in press)
- Fernandez-Canon JM, Granadino B, Beltran-Valero de Bernabe D et al (1996) The molecular basis of alkaptonuria. *Nat Genet* 14:19–24
- Goodfellow RJ, Schwartz J, Leya F (2005) Black aorta: a rare finding at aortic valve replacement. *J Invasive Cardiol* 17:165–167
- Grigg OA, Farewell VT, Spiegelhalter DJ (2003) Use of risk-adjusted CUSUM and RSPRT charts for monitoring in medical contexts. *Stat Methods Med Res* 12:147–170
- Helliwell TR, Gallagher JA, Ranganath L (2008) Alkaptonuria - a review of surgical and autopsy pathology. *Histopathol* 53:503–512
- LaDu BN (2001) Alkaptonuria. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Vogelstein B (eds) *The metabolic and molecular basis of inherited disease*, vol. 2, 8th edn. McGraw-Hill, New York, pp 2109–2123
- O'Brien WM, La Du BN, Bunim JJ (1963) Biochemical, pathologic and clinical aspects of alcaptonuria, ochronosis and ochronotic arthropathy: review of world literature (1584–1962). *Am J Med* 34:813–838
- Phornphutkul C, Introne WJ, Perry MB et al (2002) Natural history of alkaptonuria. *N Engl J Med* 347:2111–2121
- Suwannarat P, O'Brien K, Perry MB, Sebring N, Bernardini I, Kaiser-Kupfer MI, Rubin BI, Tsilou E, Gerber LH, Gahl WA (2005) Use of nitisinone in patients with alkaptonuria. *Metab Clin Exp* 54:719–728
- Wilke A, Steverding D (2009) Ochronosis as an unusual cause of valvular defect: a case report. *J Med Case Rep* 3:9302