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Alkaptonuria

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Abbreviations: AKU, alkaptonuria, BQA, benzoquinoneacetate; HGA, homogentisic acid; HGD, homogentisate 1,2-dioxygenase; OA, osteoarthritis; ASC, ascorbic acid; NTBC, 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione; FDA, Food and Drug Administration; HT1, hereditary tyrosinaemia type 1; HT3, hereditary tyrosinaemia type 3; MAA, maleylacetoacetic acid; FAA, fumarylacetoacetic acid; VBD, van Buchem disease

Alkaptonuria (AKU) is a rare disorder of autosomal recessive inheritance. It is caused by a mutation in a gene that results in the accumulation of homogentisic acid (HGA). Characteristically, the excess HGA means sufferers pass dark urine, which upon standing turns black. This is a feature present from birth. Over time patients develop other manifestations of AKU, due to deposition of HGA in collagenous tissues, namely ochronosis and ochronotic osteoarthropathy.

Although this condition does not reduce life expectancy, it significantly affects quality of life. The natural history of this condition is becoming better understood, despite gaps in knowledge. Clinical assessment of the condition has also improved along with the development of a potentially disease-modifying therapy. Furthermore, recent developments in AKU research have led to new understanding of the disease, and further study of the AKU arthropathy has the potential to influence therapy in the management of osteoarthritis.

Introduction

AKU is an iconic disease in medicine, historically used by Archibald Garrod in his Croonian lectures of 1908, to demonstrate the theory behind "inborn errors of metabolism". It was one of the first disorders in humans found to conform with the principles of Mendelian recessive inheritance.[1] It is a hereditary disorder and results from absence of homogentisate 1,2 dioxygenase (HGD), the enzyme, predominantly produced by hepatocytes in the liver and within the kidney, is responsible for the breakdown of HGA; an intermediate in the tyrosine degradation pathway (**Fig. 1**).[2]

Deficient HGD activity within the liver causes HGA levels to rise systemically. Large (gram) quantities of HGA are removed by urinary excretion on a daily basis.[3] Other incidental observations of HGA in bodily fluids and tissues has been documented, based on the darkening of fluids or pathological deposition in tissues.[4,5] However, the removal of HGA by urinary excretion is not sufficient to completely remove it from bodily tissues and fluids.

Characteristic early clinical presentation is the observation that urine darkens on standing. This is because the HGA polymerizes but can also be observed upon the addition of alkali substances.[2] This is the only symptom seen in the pediatric age group.[6] Over time the HGA polymer is deposited within connective tissues, causing ochronosis (a darkening of collagenous tissues).[7] Long-term ochronosis results in the development of ochronotic osteoarthropathy, often misdiagnosed as early onset osteoarthritis (OA),[2] unless observation of darkened urine has been seen before joint manifestations. Previous work has summarized the human disease, the genetics, and its manifestations.[8]

There is currently no approved cure for AKU. However, research surrounding the mechanism of disease progression has highlighted that HGA pigment deposition occurs within structurally intact cartilage. The research identified that ochronotic osteoarthropathy and subsequent joint failure appear to arise following initial changes in the calcified cartilage and subchondral bone becoming susceptible to damage following focal change. This has led to suggestions that there could be a large overlap between the pathogenesis of OA and ochronotic osteoarthropathy, increasing the potential avenues of research.[9]

Epidemiology

The worldwide prevalence of AKU is 1 case in 250 000–1 000 000 births.[2] So far, 950 AKU sufferers have been identified in 40 countries (**Fig. 2**).[10,11] It is a condition that is reported to be more prevalent in Slovakia, the Dominican Republic, India, and Jordan. The highest prevalence is in Slovakia where up to 1 in 19 000 are affected.[10] Analysis of the affected families revealed they typically live within isolated hamlets, leading to conclusions that the usually high incidence was predominantly due to the founder effect (loss of genetic variation) as a result of genetic isolation.[12] Although difficult to perform with such a rare condition, there is also no genotype-phenotype correlation; all mutations lead to the development of ochronosis.[13]

Research is still ongoing to fully understand the mechanism of disease progression; literature suggests the reporting of new cases has increased due to a raised profile of features associated with the disease.[10] However, even with the increase in reporting

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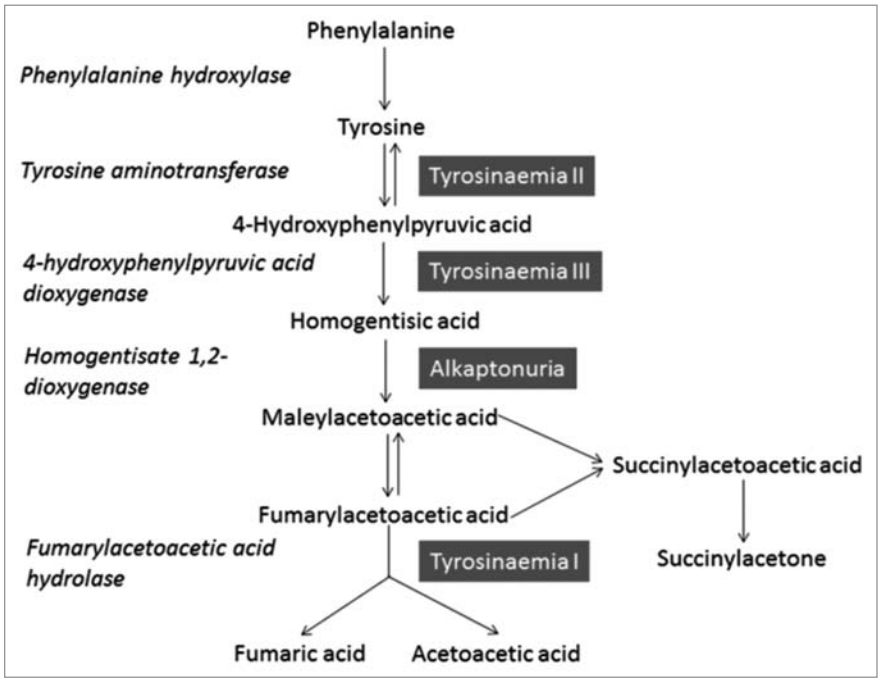

Figure 1. Adapted from reference 3. The diagram illustrates the normal phenylalanine and tyrosine degradation pathway, enzymes involved and defects that can occur (highlighted blue). The metabolism of HGA occurs in the liver, and HGD is an enzyme expressed in this organ.

of new cases, the number of individuals documented as suffering from the disease is well below what would be expected based on the incidence.

Clinical Features

AKU has three distinct clinical features; homogentisic aciduria, ochronosis, and ochronotic osteoarthropathy.[14-17] Each feature presents at various stages in life, the earliest being detection of HGA in urine.[18] The passing of black urine is the only manifestation of the condition known in pediatrics, leading to 21% of patients being diagnosed with AKU before 1 year of age.[2,6] Although newborn screening is not undertaken, incidental discoveries have been observed during other screening programs.[19] HGA plasma levels in AKU sufferers range between 0.018–0.165 mM in comparison to non-AKU sufferers plasma levels of 0.014–0.071 µM, a thousand fold difference.[2,20] The darkening of urine occurs because the HGA pigment oxidizes to Benzoquinoneacetate (BQA), which forms a melanin-like polymer that slowly turns urine black.[2]

Ochronosis develops as the BQA accumulates both intra- and extra-cellularly in connective tissue. This feature is commonly observed in the third through to fifth decades of life. Typically the pigment is seen clearly in the eyes and ears of patients but is also present in bodily fluids, including perspiration, which often results in skin discoloration.[21] Organs affected are: large joints, cardiovascular system, kidney, skin, and glands.[4,7,22-24] Other manifestations include: renal, prostate, gall bladder, and salivary gland stones, ruptures of tendons and ligaments, osteopenia, and fractures.[4,25-27]

Cardiac manifestations of the disease are not uncommon. Aortic valve stenosis is a frequent finding, often requiring surgical replacement.[23] It may be that mechanical forces play a key role in mediating ochronosis given that there appears to be little pigmentation in the venous component of the circulation compared with the arterial side.[17] Interestingly, while HGD expression and HGA metabolism occur in the liver, there are no reports to demonstrate that these tissues—along with those of the pancreas, gastrointestinal, lymphoreticular, or endocrine organs—develop ochronosis.[17]

The development of ochronotic arthropathy is the result of deposition of the HGA polymer within hyaline articular cartilage. Pigmentation is widespread, with all tissues of the joint organ being affected (**Table 1**). The affected tissues often become weak, brittle, and prone to chipping, fracturing, and cracking, causing rapid joint degeneration, which means that patients can be left profoundly disabled at a young age.[7] Although AKU does not affect longevity, it significantly affects quality of life due to these secondary pathologies.

Patients with ochronotic arthropathy usually present with lumbar pain as the initial joint manifestation. Larger weight bearing joints tend to be affected later in the progression of the condition.[28] Quite often this complaint is misdiagnosed as an early form of osteoarthritis (OA) or ankylosing spondylitis. The differences between the two conditions are summarized in **Tables 2 and 3**. It is interesting to note that the ossification of tissues in ochronosis is different from that observed in other forms of pathological ossification. This is because the ossification of tissues in ochronosis is seen with a variety of calcium crystals.[29,30] Furthermore, biomechanical studies have shown that ochronotic cartilage calcification is harder than normal cartilage calcification.[17,31,,32]

History of AKU

Garrod's use of AKU in the Croonian lectures brought the condition into the spotlight in 1908. Yet many descriptions of the triad of features associated with AKU date back long before this.[2] Documentation of the condition began in the 16th and 17th centuries.[24] The earliest clinical case of AKU was found in the Egyptian mummy *Harwa*, which is believed to date back as far as 1500 BC.[33] The name Alkaptonuria is derived from the Arabic word "alkali" (meaning alkali) and the Greek word meaning "to suck up oxygen greedily in alkali".[24] The name was created by Boedeker in 1859 after he discovered unusual reducing properties in the urine of a patient.[14,15]

Ochronosis was first described and named by Virchow in 1866, because under microscopy the HGA pigment appeared to be ochre (yellow/brown) in color.[34] In 1891 HGA was identified as the causative component and named so due to its close

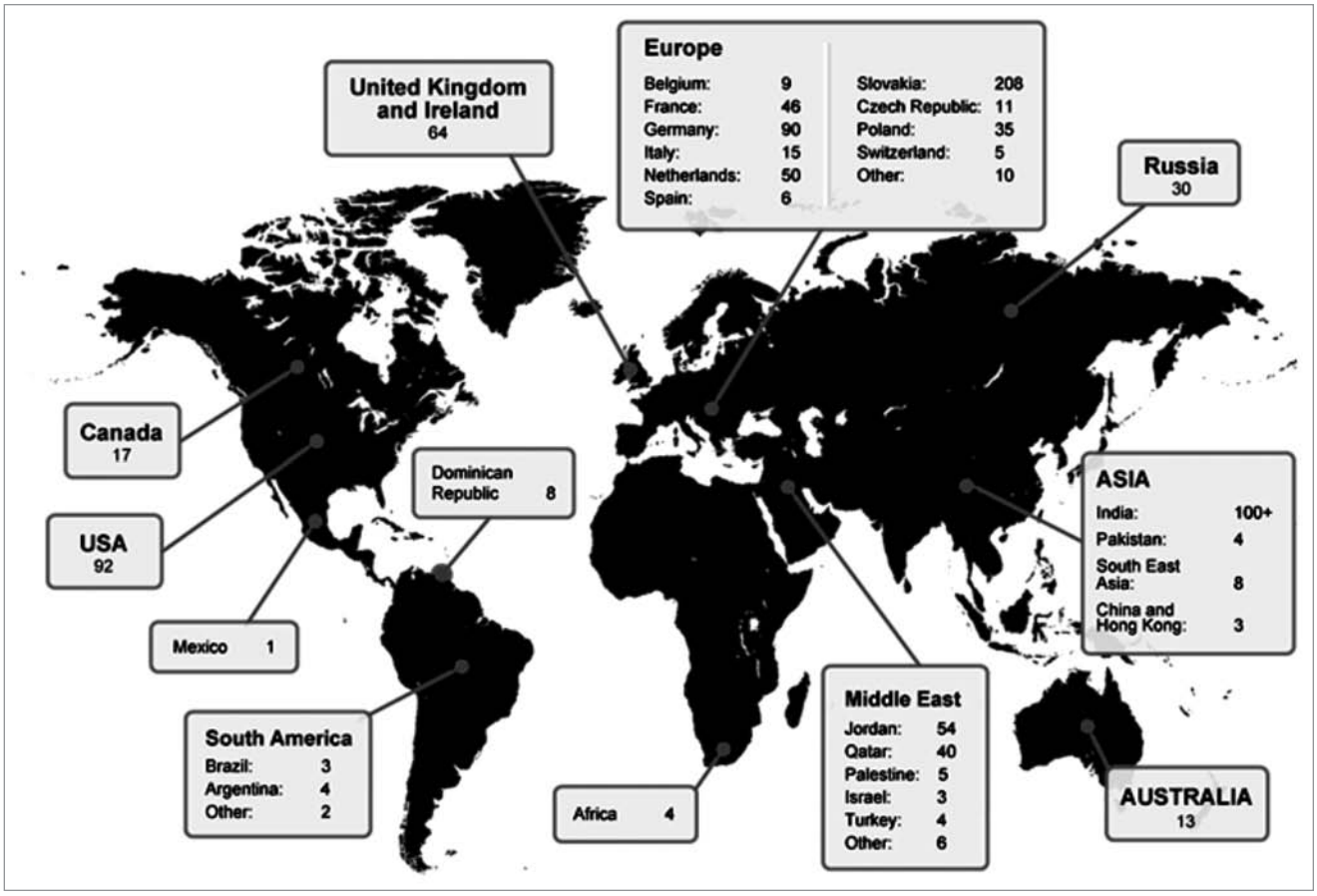

Figure 2. Taken from reference 11. The map illustrates the current number of AKU patients identified worldwide.

Table 1. Summarizes the location of HGA pigment deposition through all layers of an articulating joint.

Tissue	Areas of pigmentation
Bone	Intracellular
Cartilage	Intra- and extra-cellular
Synovium	Extracellular
Capsule	Intra- and extra-cellular
Ligament	Extra-cellular
Tendon	Intra- and extra-cellular

Bone has predominantly intracellular pigmentation, located in both osteocytes and osteoclasts. Mineralized bone matrix is resistant to pigmentation, it is thought that the mineral crystals located on bone collagen are protective against HGA and its subsequent binding and polymerization on the collagen fibers. The chondrocytes within cartilage show numerous intracellular deposits. Pigment deposition within the synovium appears to be caused by large fragments of ochronotic cartilage that have broken off from the articular surface and have embedded in the synovium.[28] Although ligaments show large extracellular deposits, little work has looked at the cellular components of ligaments. Investigation into tendons has demonstrated both intra- and extra-cellular pigmentation.[25]

structural relationship with gentisic acid, a derivative of benzoic acid.[1,21,35,36] By 1995 the genetic defect was discovered, cloned, and mapped to chromosome 3q21-q23.[22,37,38]

Therapies

Many therapies have been tried. However, currently as there is no effective therapy, the management of AKU remains palliative and involves physiotherapy, joint replacement surgery, and pain control. Ascorbic acid (ASC), more commonly known as vitamin C, is an antioxidant believed to reduce the conversion of HGA to BQA via oxidation. However, investigation revealed that although ASC reduced the HGA to BQA conversion, it did not affect HGA urinary excretion.[39] Furthermore, it was found to increase HGA production, contributing to the formation of renal oxalate stones. This is concerning, as AKU patients are already at high risk for developing renal calculi.[40,41] An additional study highlighted that vitamin C is a co-factor for 4-hydroxyphenylpyruvate

Table 2. Adapted from reference 21. The table shows the differences between peripheral ochronotic arthropathy and osteoarthritis.[31]

Peripheral Ochronotic Arthropathy vs. Osteoarthritis			
	Joint Involved	**Narrowing of Joint Space**	**Osteophytosis**
Ochronosis	Knees, shoulders +++ Hips, elbows and ankles ++ Sacroiliac Joints ±	Symmetric or asymmetric	Observed macroscopically not detectable radiographically
Osteoarthritis	Hips +++ Knees +++ Hands +++	Symmetric or asymmetric	Present

+++, significant involvement; ++, some involvement; ±, little or no involvement

Table 3. Adapted from reference 21. The table shows the radiographic differences between ankylosing spondylitis and ochronotic spondylarthropathy.

Ochronotic spondylarthropathy vs. ankylosing spondylitis (radiographic features)		
	Ochronosis	**Ankylosing spondylitis**
Calcification of intervertebral discs	++	±
Syndesmophytes	±	+++
Ossification of ligaments	+++	+
Erosion and fusion of sacroiliac joints	±	+++
Osteoporotic vertebral bodies	++	+

+++, significant involvement; ++, some involvement; +, minimal involvement; ±, little or no involvement

dioxygenase, which causes increased HGA production. In the cases of young infants there were profound increases in urinary levels of HGA, leading to conclusions that this is a highly unsuitable treatment.[42]

A low protein diet, although logical, is not sustainable in the long-term for many patients. Approximately 6% of dietary protein is degraded via the HGA pathway, and intensive supervision is required with younger patients, especially during growth periods.[43,44] Also, regardless of restrictions on dietary intake of tyrosine, tissue catabolism is likely to contribute to raised HGA plasma levels within individuals with AKU. There is also evidence to suggest that liver transplantation is a successful way to eradicate HGA from the body.[45] Many therapies have been trialed and are summarized in **Table 4**.

Other, more promising therapy includes a triketone herbicide, Nitisinone (NTBC), that inhibits 4-hydroxyphenylpyruvate, an enzyme involved in the conversion of hydroxyphenylpyruvate to HGA (**Fig. 1**). It significantly reduces urinary excretion of HGA in both murine models and humans.[47-49] Initially approved by the Food and Drug Administration (FDA) for the treatment of hereditary tyrosinaemia type I (HT1), there are, however, known side effects, including elevated plasma tyrosine levels causing corneal irritation. This can be ameliorated by reducing dietary intake of tyrosine. Other serious adverse events associated with elevated plasma tyrosine levels include thrombocytopenia, leukopenia, and porphyria.[49] However, complications of using this therapy are the development of hereditary tyrosinaemia type III (HT3) and rarely a deficiency of 4-hydroxyphenylpyruvate. These conditions cause neurological complications; tremor, ataxia, delayed development, and intellectual impairment.[2,50]

Enzyme replacement would be an ideal therapy for AKU, consisting of immediate replacement of HGD in the tyrosine degradation pathway. However, there are potentially fatal complications associated with this therapy. It is imperative that the HGD enzyme is delivered to the exact location of tyrosine metabolism within the hepatocytes of the liver. If not, spontaneous formation of succinylacetone (formed from the production of MAA and FAA in tyrosine metabolism) would occur, which is toxic and highly mutagenic. Build-up of this substance in the body and bloodstream would create complications more serious than the ochronosis of AKU.[51]

Models of AKU

Many spontaneous reports of AKU in animals have been documented: crab-eating macaque, chimpanzee, orangutan, Dalmatians, cattle, horses, dogs, and rabbits. All the reports identify the appearance of dark urine but rarely identify ochronosis or joint involvement.[52-56] Early attempts to study AKU in animal models were undertaken by intraperitoneal or intravenous and intra-articular injection of HGA into rabbits.[57] The study demonstrated the damaging effects of HGA. Animals that received multiple injections exhibited darkening of urine along with ochronosis of joint tissues, and those injected with HGA via intraperitoneal or intravenous routes did not exhibit ochronosis.[56] The first model study was made using rats fed on a diet of 8% L-tyrosine for at least a 9 month period. This appeared to induce ochronosis and osteoarthropathy.[58]

The first murine model of AKU was generated in 1994 and proved to be key in aiding to map the location of the HGD mutation to chromosome 16. The report stated that although the mice excreted high levels of urinary HGA, there was no evidence that they developed ochronosis or arthropathy. This was hypothesized to be the result of the endogenous production of ascorbic acid in the digestive tract of the mouse, thus inhibiting the polymerization of HGA.[59]

Other studies have developed murine models of AKU, also concluding that despite elevated levels of HGA, the mice did not

Table 4. Adapted from reference 44. Information on current and future therapies available from references 5 and 46.

Treatment	Summary
Ascorbic acid	Efficacy unproven, increases HGA production, may exacerbate condition
Low protein diet	Efficacy in adults unproven, compliance difficult
Lifestyle counselling	Underused, lack of evidence base
Physiotherapy	Underused
Pain control	Widely used, incompletely effective
Organ replacement	Unjustified for a condition with preserved lifespan
Palliative surgery	Effective but invasive
Liver transplant	Not frequently used, lack of evidence base
Reverse pigment binding	Not yet available
HGA lowering therapy:	
Nitisinone	Not shown to alter outcomes, increases tyrosine
Enzyme replacement	Not yet available
Gene replacement	Not yet available

exhibit the typical phenotypic ochronosis observed in the human presentation. Hypotheses as to why this occurred, aside from the production of ascorbic acid in mice, have stated that the mice do not live long enough for ochronosis to occur and that urinary excretion is efficient so that tissues are not exposed to the high concentration of HGA as seen in humans.[60,61] Other animal models have produced similar reports, although arguably not as reliable.[54]

Contrary to the above reports, Taylor et al. produced the first data of tissue ochronosis in a murine model. The data from this study demonstrated ochronosis in tissues and joints of mice with the AKU genotype, similar to the presentation seen in humans.[62] This was a novel finding and has subsequently enabled better understanding of the molecular pathology of the condition.

In more recent murine models, initiation and progression of pigmentation has been documented. At 15 weeks, pigmentation is visible, suggesting that ochronosis begins at an early age. Furthermore, ochronosis was seen in all mice, and pigmentation of chondrocytes also increased with mouse age.[48] These models, along with others, have been used to investigate the effects of NTBC. The trials have been successful and demonstrate the delayed progression of ochronosis after drug administration. The conclusions from these studies suggest beginning therapy at an early stage in the development of ochronosis results in a more beneficial outcome.[48,61]

Initial trials of low dose NTBC in two patients with AKU showed a 69% reduction in urinary HGA excretion. However, there was a significant elevation in plasma tyrosine levels, increasing the risk of corneal crystal formation, corneal epithelial damage, and photophobia.[2] A larger scale trial of nine patients was performed and also discovered a 95% reduction in urinary HGA excretion but an 11-fold increase in plasma tyrosine over a four month period. Although the tyrosinaemia did not cause eye complications in these patients, this is a concern when considering the efficacy of NTBC as a therapy for AKU.[3] A three year trial of NTBC performed on a cohort of 40 patients also demonstrated similar results.[47]

AKU as a Model of Osteoarthritis

The development of murine and in vitro models of AKU have enabled a better understanding of the pathophysiology involved in the progression of ochronosis and the related osteoarthropathy. So far, research has identified that HGA is present in healthy cartilage but only becomes susceptible to degeneration following focal change.[9] The potential overlap between the pathogenesis of OA and ochronotic arthropathy has stemmed from a better understanding of the importance of subchondral bone in the pathogenesis of OA[63,64] and determining factors that influence the integrity of articular cartilage: subchondral bone turnover, chondrocyte function, and biomechanical stresses, all of which are affected in the arthropathy of AKU.[63,65] Furthermore, AKU has already been documented as causing premature OA and mimicking the typical disease process; this extreme phenotype of OA could provide insights into OA.[66,67]

Summary

It has been over 100 years since Garrod first described AKU as an "inborn error of metabolism". Within this time, the causative molecule has been isolated and identified, the enzymatic defect located, and the genetic mutation mapped. Yet there is still no effective treatment for this iconic condition. Interest in AKU research has increased recently, generating hope that potential disease modifying therapy is within reach.

Furthermore, it appears that the arthropathy of AKU may have similarities to OA—not only in its presentation but also its pathogenesis—leading to speculation that it could prove a useful extreme phenotype in highlighting missing knowledge in understanding OA. This would not be the first time that a rare disease has brought more knowledge to a common condition. For

example in musculoskeletal conditions, the discovery of sclerostin and its importance in the Wnt signaling pathway, highlighted by the high bone mass phenotypes of those with van Buchem Disease (VBD), has led to therapies for osteoporosis.[68,69]

It is encouraging to consider that further research into this condition may contribute to the understanding and treatment of joint degeneration not only in AKU but also in OA.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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