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Hereditary hyperferritinemia cataract syndrome: Ocular, genetic, and biochemical findings

A.R. ISMAIL[1], K.L. LACHLAN[2], A.D. MUMFORD[3], I.K. TEMPLE[2], P.R. HODGKINS[1]

[1]Southampton Eye Unit
[2]Wessex Clinical Genetics Service, Southampton University and Hospitals NHS Trust
[3]Department of Haematology, Bristol Royal Infirmary, Bristol - UK

PURPOSE. *To describe the cataract morphology and genetic and biochemical findings in a four-generation family with hereditary hyperferritinemia cataract syndrome (HHCS).*
METHODS. *Family members of the proband with HHCS were investigated. DNA sequencing was carried out to identify the iron responsive element (IRE) of the L-ferritin gene in affected and non-affected family members. Molecular modeling allowed prediction of the structure of the mutant IRE in affected cases. Serum ferritin and transferrin saturation were determined using standard methods. All family members underwent slit lamp examination by an ophthalmologist to document presence of cataract or lens status. Cataract morphology was documented where present.*
RESULTS. *This family with HHCS had the genetic heterozygous mutation G32C in the IRE of the L-ferritin mRNA. Lens opacities were detectable in young members of the family, and morphology of cataracts was consistent with previous reports. Biochemical testing demonstrated high serum ferritin levels in affected individuals.*
CONCLUSIONS. *The morphology of cataracts in HHCS seems to be similar in all cases. In the heterozygous G32C mutation, the age at onset of cataracts is very early. Greater awareness of this condition among ophthalmologists will lead to effective family counseling of those affected, by genetic testing or simple biochemical tests. Serum ferritin levels can be effectively used to screen for this condition in suspected families. (Eur J Ophthalmol 2006; 16: 153-60)*

KEY WORDS. *Cataract, Hyperferritinemia, Ferritin*

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INTRODUCTION

Hereditary cataracts may present in infancy or in later life. Hereditary hyperferritinemia cataract syndrome (HHCS) is an autosomal dominant disorder causing early onset cataracts. The syndrome is characterized by elevated serum ferritin, not related to iron overload, and early onset cataracts. To date, no other clinically relevant symptoms have been identified in patients with this disorder. Patients are often diagnosed with HHCS after investigation of unexplained hyperferritinemia or suspected hemochromatosis by hematologists. However, reports in the literature regarding the cataract morphology in patients with HHCS have shown a common phenotype consisting of multiple bread crumb-like opacities in the nucleus and cortex, with progression to a sunflower configuration (1, 2). Our findings presented in this study also agree with these clinical findings. Greater clinical awareness among ophthalmologists about the association of this relatively unusual form of lens opacity and HHCS may aid in the diagnosis of this disease in the ophthalmic setting. The age at onset of cataracts in HHCS appears to be variable upon both genetic and environmental factors (1). In all cases of HHCS, there is accumulation of L-ferritin within the crystalline lens, secondary to altered metabolic regulation of this protein, and it is believed that lens opac-

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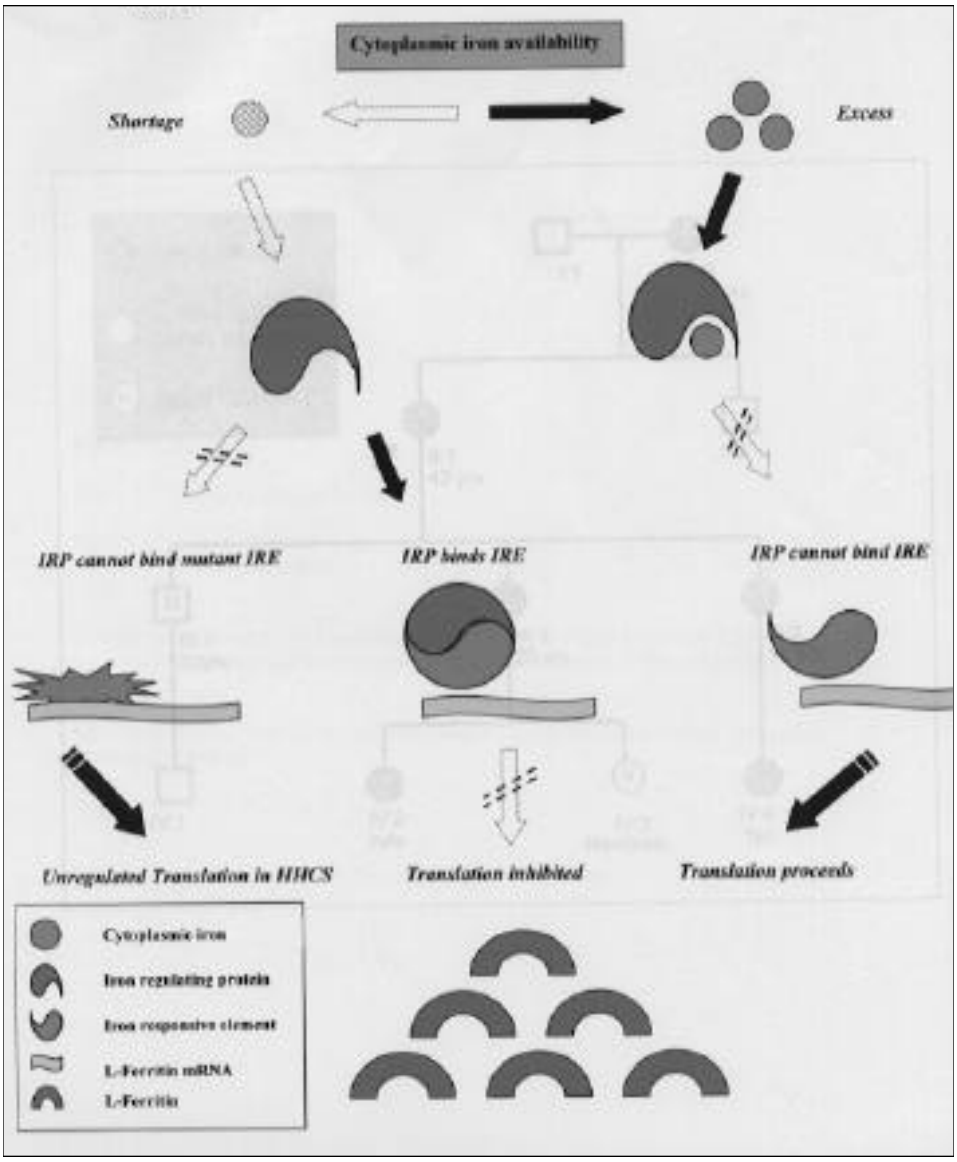

Fig. 1 - *Physiologic regulation of L-ferritin synthesis at translational level and unregulated L-ferritin synthesis due to mutant iron responsive element in hyperferritinemia cataract syndrome.*

ities are caused by abnormal interaction between L-ferritin and lens proteins, or by disturbed metabolism of L-ferritin within the lens (3). In patients without HHCS, ferritin synthesis is normally regulated precisely by iron availability (4). This physiologic regulation is shown diagrammatically in Figure 1. Translation of the L-ferritin gene, and thereby L-ferritin synthesis, is tightly controlled by interaction between iron regulatory proteins (IRPs) and a noncoding stem loop portion of the L-ferritin mRNA known as an iron responsive element (IRE). In the presence of excess cytoplasmic iron, the resulting structural change in the IRP prevents it from binding to the IRE, and therefore translation of the L-ferritin mRNA proceeds, leading to synthesis of L-ferritin. When there is a relative shortage of cytoplasmic iron, there is less interaction between iron and the IRPs, and therefore the IRP–IRE interaction is unaffected. This has the effect of inhibiting mRNA synthesis, and consequently reduces the production of L-ferritin. In HHCS, a variety of mutations in the mRNA IRE change the stem loop structure so that the IRP cannot bind, leading to unregulated translation of the L-ferritin gene, and high levels of circulating L-ferritin.

We present the ocular, genetic, and biochemical findings in a four-generation family with HHCS, revealing a genetic heterozygous mutation in the mRNA IRE associated with very early onset of cataracts.

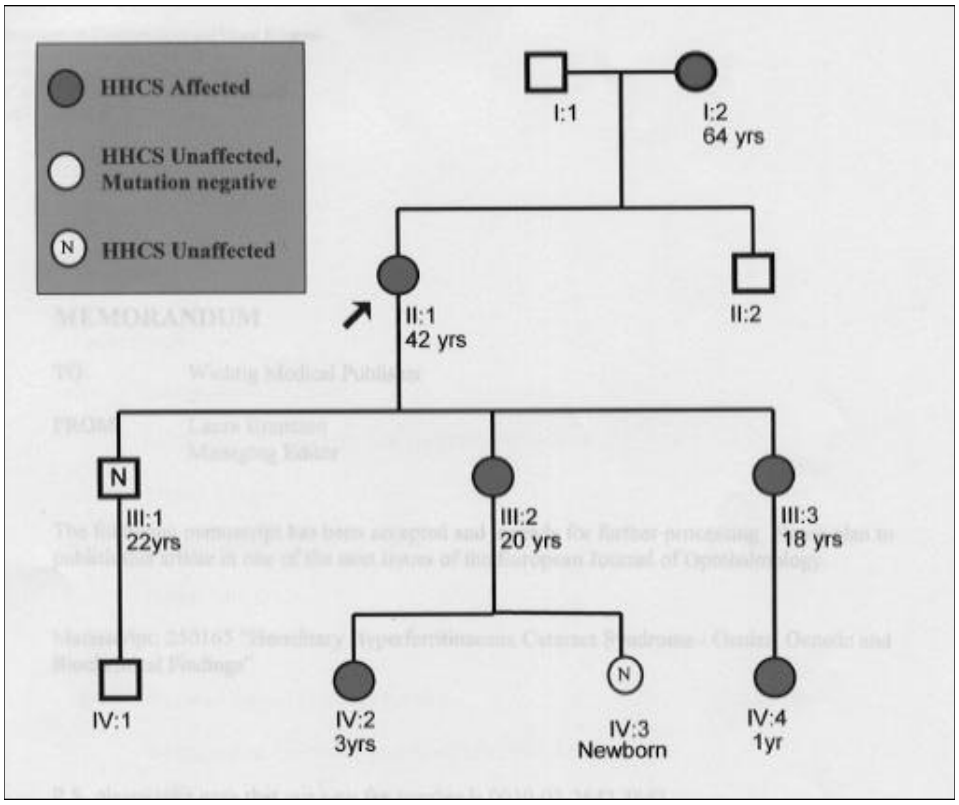

Fig. 2 - *Pedigree of four-generation family with hyperferritinemia cataract syndrome.*

METHODS

A 42-year-old woman was referred to a hematologist during the course of investigation of anemia.

She was found to have a very high serum ferritin level, and family history revealed that her mother had early onset cataracts. She herself had been diagnosed with cataracts in childhood and had them removed when she was 25 years old. HHCS was suspected, and the pedigree was traced (Fig. 2).

Anterior segment examination

Anterior segment examination was performed by an ophthalmologist to document the presence of cataract or lens status in all family members.

Genetic sequencing

Informed consent was obtained for genetic studies in all adult family members and Individual IV-4, on the basis of establishing the diagnosis at an early age for prognostic value.

The sequencing of the L-ferritin gene 5' untranslated region (IRE sequence) was performed as described previously by Arnold et al (5). The L-ferritin gene 5' untranslated region was PCR amplified from genomic DNA obtained from members of the kindred using the oligonucleotide primers AGAAGCCGCCCTAGCCACG (forward) and GAGCTAACCACAAAAACGGTGC (reverse). For the proband and Individual IV-4 the PCR amplicon was sequenced in both forward and reverse directions using the PCR primers.

The +32 G to C substitution observed in the proband creates an additional EarI recognition site within the PCR amplicon that is not present in the wild type allele.

Therefore, PCR amplicons generated from genomic DNA from the proband and from Individuals I-2, II-1, III-2, III-3, and IV-4 were also analyzed by restriction endonuclease digestion with EarI and agarose gel electrophoresis.

Molecular modeling

The predicted secondary mRNA structure of the wild type and mutant L-ferritin iron responsive elements was determined using mfold software obtained at the following Web site: www.bioinfo.rpi.edu/~zukerm/

Biochemical tests

Serum iron, ferritin, and transferrin saturation were determined using standard methods in relevant family members giving consent.

RESULTS

The pedigree is presented in Figure 2, and a summarized table showing clinical, genetic, and biochemical findings is presented in Table I.

Cataract morphology

The proband (arrow in Fig. 2), her mother, and two daughters were all pseudophakic at the time of diagnosis. Records of anterior segment photographs revealed her eldest daughter (III-2) to have had a sunflower-type morphology to her cataract prior to surgery (Fig. 4A). Examination of the two granddaughters (IV-2 and IV-4) revealed lens opacities that were bread crumb-like. The sparse opacities were mainly distributed in the posterior cortex (Fig. 4, B and C).

Genetic findings

DNA sequence analysis. Direct sequence analysis showed that the proband and Individual IV-4 were heterozygous for a G to C transversion at nucleotide +32 (numbering from +1 as the translational start site). Analysis of the PCR amplicon generated from other members of the kindred by EarI restriction endonuclease digestion confirmed the presence of this substitution in Individuals I-2, II-1, III-2, III-3, and IV-4 but not in Individual III-1.

Molecular modeling. The structure with the lowest free energy of association is a highly distorted motif in which the mutant C residue at position +32 (*) forms an energetically favorable Watson-Crick pairing with +48 G which in turn leads to structural disruption of the IRE apical loop (Fig. 3).

Biochemical findings

The serum ferritin results segregated with the results of the genetic analysis of the IRE (Tab. I). Family members with a high serum ferritin had cataracts. Serum ferritin levels in affected individuals varied from 767 µg/L to 1982 µg/L (normal range 12–200 µg/L) and was not age depen-

TABLE I - CLINICAL, GENETIC, AND BIOCHEMICAL FINDINGS

Family member	Age yr	Clinically affected	Lens status	Iron umol/L	Transferrin g/L	Transferrin saturation (%)	Ferritin ug/L	L-ferritin mutation (+32 G to C substitution)
Proband II-1	42	Yes	IOL	17	2.54	23	1960	Yes
I-2	64	Yes	IOL	16	2.63	27	1982	Yes
III-1	22	No	Clear	20	2.72	33	42	No
III-2	20	Yes	IOL	10	2.56	18	767	Yes
III-3	18	Yes	IOL	7	3.33	9	1063	Yes
IV-2	3	Yes	Sparse opacities	_	_	_	_	_
IV-4	1	Yes	Sparse opacities	_	_	_	1346	Yes

Normal values: Iron 11–28 µmol/L, transferrin 2.50–3.80 g/L, transferrin sat 16–45%, ferritin 12–200 µg/L
IOL = Intraocular lens

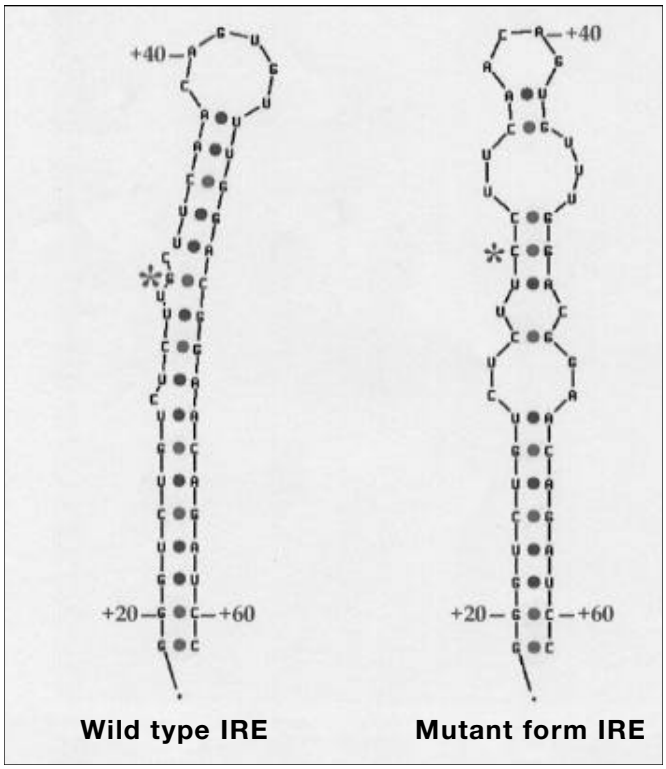

Fig. 3 - *Molecular model showing predicted secondary mRNA structure of the wild type and mutant L-ferritin iron responsive elements.*

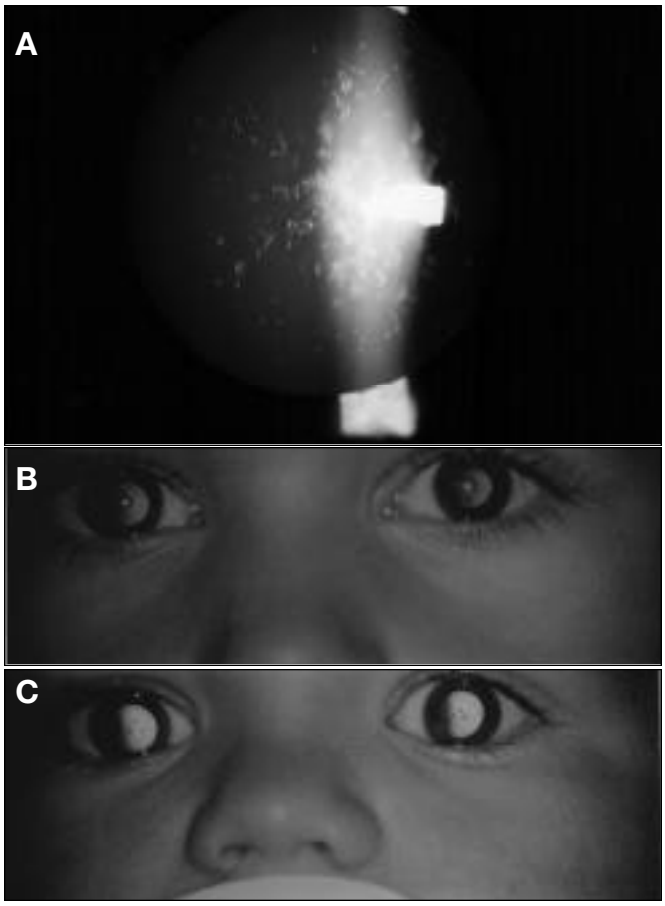

Fig. 4 - *(**A**) Sunflower configuration cataract morphology in Patient III-2. (**B**, **C**) Early sparse bread crumb-like opacities in Patients IV-2 and IV-4.*

dent. In the proband serum ferritin levels fluctuated over time between 1265 µg/L and 1960 µg/L. Serum iron and transferrin levels were found to be normal in both affected and unaffected members, confirming that iron overload was not present.

CONCLUSIONS

The linkage between hereditary hyperferritinemia and cataracts was first described by Girelli et al in 1995 (6). The initial patients studied were all found to have very high levels of circulating serum ferritin associated with bilateral cataracts, and the disease manifested in an autosomal dominant inheritance pattern. It was shortly afterwards discovered that HHCS provides an almost unique model of human translational pathology, since the genetic defect affects the IRE of L-ferritin mRNA, leading to breakdown of the normal translational regulation of L-ferritin synthesis (7).

We now know that there are a large number of different mutations in the IRE which can cause HHCS. A study of 62 affected patients in 14 families with HHCS found that there was a substantial variability in the age at onset of visual symptoms due to cataract among their patients, and that this variability could not be explained on the basis of the different mutations since it was also noted in patients sharing the same mutation (1). This finding led the authors to believe that additional environmental factors including age are likely to modulate lens involvement and rate of progression to severe cataract in patients with HHCS. It is likely that factors other than the IRE mutation play a role in cataract formation in HHCS, but it must be borne in mind that two individuals with identical lens opacities will not necessarily present with visual symptoms at the same age.

In our study, we have shown the association of very early onset cataracts with the heterozygous mutation G32C. This finding has been supported by the same observation in a second family with the same mutation (8).

TABLE II - POSSIBLE ETIOLOGY OF CATARACTS IN INFANCY AND CHILDHOOD

Intrauterine infection
- Rubella
- Rubeola
- Chickenpox
- Poliomyelitis
- Herpes simplex
- Cytomegalovirus
- Toxoplasmosis

Prematurity

Metabolic disorders
- Galactosaemia
- Hypoparathyroidism
- Diabetes mellitus
- Refsum syndrome
- Lowe syndrome
- Hypoglycaemia
- Alport syndrome
- Wilson disease
- Multiple sultatase deficiency
- Fabry syndrome
- Hyperferritinemia cataract syndrome

Ocular anomalies
- Microphthalmia
- Mesodermal dysgenesis
- Coloboma
- Aniridia
- Persistent pupillary membrane
- Posterior lenticonus
- Persistent hypoplastic vitreous

Trauma
- Laser
- Radiation
- Accidental

Musculoskeletal
- Chondrodysplasia punctata
- Myotonic dystrophy
- Albright osteodystrophy
- Congenital stippled epiphysis
- Potter syndrome
- Chondrodystrophic myotonica
- Smith-Lemli-Opitz syndrome
- Rhizomelic chondrodysplasia punctata

Central nervous system
- Marinesco-Sjogren syndrome
- Laurence-Moon-Bardet-Biedl syndrome
- Sjogren-Larsson syndrome
- Peroxisomal disorders
- Cerebral giantism
- Batten disease

Dermatologic
- Cockayne syndrome
- Poikiloderma atrophicans
- Incontinentia pigmenti
- Congenital ichthyosis
- Atopic dermatitis
- Ectodermal dysplasia
- Progeria

Craniofacial
- Hallermann-Streiff-Francois syndrome
- Rubenstein-Taybi syndrome
- Smith-Lemli-Opitz syndrome
- Cerebro-oculo-facial-skeletal syndrome
- Pierre Robin syndrome
- Oxycephaly
- Crouzon syndrome
- Apert syndrome

TABLE III - LABORATORY EVALUATION OF EARLY ONSET CATARACTS

Sample	Result	Possible diagnosis
Urine	Reducing substance	Galactokinase deficiency
	Aminoaciduria	Lowe syndrome
	Hematuria, proteinuria	Alport syndrome
	“Maltese cross” figures	Fabry disease
Blood		
	Erthyrocyte enzymes	Galactokinase deficiency
	Glucose	Hyper/hypoglycemia
	TORCH titres, VDRL test	Rubella, toxoplasmosis, CMV, herpes, syphilis
	Calcium, phophorus	Hypo or pseudohypoparathyroidism
	Ferritin	Hyperferritinemia cataract syndrome

VDRL = Venereal Disease Research Laboratory; CMV = Cytomegalovirus

This evidence rules out the possibility of age-related mechanisms playing a role in the development of lens opacities in these families and apportions a greater role for the IRE mutation in causing cataracts, in at least the subset of patients with HHCS with this particular mutation.

Analysis of the predictive molecular modeling of the L-ferritin mRNA IRE with the G32C mutation has revealed that this single nucleotide substitution disrupts Watson-Crick pairing in the stem of the structure, thereby greatly distorting the apical loop, which is responsible for IRP-IRE binding, and hence regulation of L-ferritin translation. This gross change in the structure of the critical apical loop on the IRE may explain why patients with the G32C mutation accumulate L-ferritin in the crystalline lens at a very early age, and therefore present with cataracts early in life. This would lead us to conclude that the severity of cataract in patients with HHCS is controlled to a significant extent by the binding interaction between mRNA IRE and IRP, depending on the degree to which the structure of the binding site is itself altered by genetic mutation. The fact that the newborn baby in our pedigree (IV-3) showed no signs of cataract indicates that simple transplacental transfer of L-ferritin from the affected mother is not sufficient to induce cataractogenesis, and that synthesis of L-ferritin in the lens epithelium is important.

Despite the apparent variability in severity of cataracts in HHCS, all reports in the literature tend to agree on the phenotypic morphology of the lens opacity regardless of the genetic mutation involved. The slit lamp appearances of cataracts in our study are a further example of the typically described sparse bread crumb-like or pulverulent type opacities which progress to a sunflower configuration later in life. The fact that the morphologic lens changes in HHCS are fairly constant and typical should alert the examining ophthalmologist to the possibility of this diagnosis, when this relatively unusual appearance of cataract is encountered. The fact that this diagnosis can easily be confirmed by the presence of high serum ferritin, and that an established diagnosis can allow accurate family counseling as to the presence of disease in individual members, has implications for the investigation of early onset cataracts in ophthalmic practice.

Bilateral infantile and early onset cataracts are a common finding in numerous multisystem disorders. Some of these disorders may have have developed due to intrauterine infection or prematurity, but many others have a genetic element that predisposes to abnormalities occuring throughout the body. When confronted with a case of bilateral infantile cataracts, the ophthalmologist should involve the help of a paediatrician to identify systemic features of known multisystemic disorders associated with cataract. Although there are a huge number of possible causes for infantile cataracts (Tab. II), in many cases, the diagnosis is clear from the clinical history (including family history) and examination. Exhaustive laboratory tests are not generally indicated, but usually, a simple screen from urinalysis and blood tests can aid diagnosis, especially in metabolic defects. A suggested protocol is shown in Table III, indicating pertinent tests used to diagnose cases of infantile cataract. The Table also shows the importance of taking an accurate family history to determine if a mode of inheritance is present. Hereditary hyperferritinaemia cataract syndrome is a further example of a diagnosis that can be easily confirmed by such means. At present, there have only been occasional reports of this disease in the ophthalmic literature, and it is suggested that a serum ferritin level is also performed in the investigation of infantile cataracts.

In summary, we report a large kindred with HHCS associated over four generations. We provide a further clinical description of the cataract, examine its relationship to serum ferritin, and finally, we determine the evolution of cataracts with increasing age. HHCS can be diagnosed simply by high serum ferritin detection in patients with ocular manifestations. In the G32C mutation type, the age at onset of cataracts is very early; however, other mutations causing less structurally inefficient IRE-IRP binding may present with cataracts later in life, and may require additional genetic or environmental factors to establish lens opacities. Finally, the morphology of cataracts in HHCS seems to be similar in all cases, and detection of the typical sparse bread crumb-like pulverulent opacities, progressing to the sunflower configuration later in life, should alert the ophthalmologist to this diagnosis, as accurate family counseling is easy to achieve.

The authors have no proprietary interest in any aspect of this study.

Reprint requests to:
A.R. Ismail, MD
Southampton Eye Unit
Tremona Road
Southampton SO16 6YD, UK
andreismail@btinternet.com

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