Crystalline Cataract and Uncombable Hair

Ultrastructural and Biochemical Findings

PAUL T. V. M. de JONG, MD, PhD, ELIZABETH M. BLEEKER-WAGEMAKERS, MD, PhD, GIJS F. J. M. VRENSEN, PhD, RENÉ M. BROEKHUYSE, PhD, JOHANNA D. R. PEEREBOOM-WYNIA, MD, PhD, J. WILLEM DELLEMAN, MD, PhD

Abstract: A 7-year-old girl was found to have a progressive axial crystalline cataract located in the embryonal, fetal, and infantile nucleus. She also had the unknown association of crystalline cataract with uncombable hair. Samples of the aspirate after extracapsular cataract extraction (ECCE) showed elongated, trigonal crystals on scanning electron microscopy. On transmission electron microscopy, the crystals were surrounded by a membrane sometimes consisting of up to 30 concentric layers. The crystals were found to contain carbon, oxygen, nitrogen, sulfur, and disulfide bonds. The findings suggest that a major constituent of the crystals was a sulfur-containing aminoacid, probably cystine. Protein analysis of the remaining lens material showed elevated $\alpha_1$, $\beta_2$, and $\gamma_2$ crystallin levels. Analysis of the hair root status showed hair loss in the resting phase of the hair cycle with abnormal sheathing in most hairs that were in the growth phase. Ophthalmology 1990; 97:1181–1187

There are more than 70 different morphologic descriptions of cataract, of which crystalline cataract is one. Duke-Elder subdivides this uncommon cataract in a coralliform and a rarer needle-shaped one. The coralliform cataract has round or elongated processes, often with ampullary endings, radiating out axially from the center of the lens, never actually reaching the capsule. Synonyms for the needle-shaped cataract are spear-shaped (German: Spies), aculeiform, fasciculiform, and frosted cataract. In the needle-shaped cataract, crystals are arranged in parallel bundles like a witches' broom. These bundles are scattered at irregular angles within the axial region of the lens both in the outer layers of the embryonic and in the inner layers of the infantile nucleus.

Uncombable hair syndrome is an autosomal dominant disorder characterized by irregular bending and varying calibre of the scalp hair shaft. On scanning electron microscopy (SEM), grooves rotating around the longitudinal axis of the hair shaft are visible, and the hair may be triangular or kidney-shaped on cross-section. This anomaly results in shiny hair and these characteristics have led to the synonyms pili trianguli canaliculi and spin glass hair. Some authors think that wooly hair belongs to the same group of hair disorders. Since the first descriptions in 1973, approximately 50 patients with uncombable hair have been described in the literature.

In sporadic cases, other abnormalities were present such as Wilson's disease, ichthyosis vulgaris, atop dermatitis, and dental abnormalities as well as hemangiomas. Recently, a pedigree has been described with uncombable hair, dystrophy of the retinal pigment epithelium, juvenile
central pulverulent cataract, myopia, and short metacarpals. We report the presence of a new form of crystalline cataract in combination with uncombable hair. New ultrastructural and biochemical findings in lens and hair are given.

**CASE REPORT**

A 5-year-old girl was found, at school, to have a subnormal visual acuity. Her mother had been in contact with rubella during the third month of pregnancy. There was no history of drug or alcohol use and she did not smoke during pregnancy. The pregnancy and delivery were uneventful. The girl had always been healthy and had been on no prolonged medication. Intellectually, she was normal. She had shiny, very blond, wiry hair that was difficult to handle. It broke easily and only had to be cut once in 2 to 3 years (Fig 1). Her eyebrows were blond and looked normal and she had dark regular lashes. She was the second child and had a healthy older sister. Her father's family had a history of deafness. Otherwise, the family history was negative.

Result of general examination by a pediatrician showed no abnormality apart from her hair, cataracts, and reduced but symmetric tendon reflexes. Specifically, her nails and teeth were normal.

On ophthalmic examination, visual acuity was 6/36 in both eyes with a symbol test chart and sphere of −2.00. After mydriasis, stenopeic visual acuity was 6/18 in the right eye and 6/30 in the left. She had blue irides with no diaphany and no nystagmus. The embryonic and infantile nuclei of both lenses were filled with golden-colored, transparent, nonpolychromatic crystals radiating out from the center of the nucleus toward the capsule (Fig 2). The crystals did not reach as far as the lens capsule, and the lens substance around the crystals was completely transparent. A slightly hypopigmented fundus, normal-pigmented macula, and no clear foveal reflex were discernible.

Results of dermatologic examination showed blond hair with a metallic sheen. She had normal down hair with a normal implantation pattern for her age. On her right upper leg, she had a light-brown pigmented nevus of 10 × 4 cm with irregular borders. Otherwise, her skin was normal.

Results of her ear, nose, and throat examination were normal, but her audiogram showed 10-dB perception loss.

She had a normal female karyogram. Results of genealogic examination showed no consanguinity for six generations and no linkage with known families with juvenile cataract. Both parents had full vision. Her mother had fine crystals in the anterior lens nucleus of the lens in both eyes. Her father had tiny non-specific lenticular opacities. There were no other ocular abnormalities. Results of ocular and dermatologic examination of the elder sister were normal and she, as well as her parents, had normal hair. Results of electron microscopic (EM) examination also were normal.

Over the next 3 years, the number of crystals gradually increased while the surrounding lens substance remained clear. Visual acuity dropped to 6/60 and glare became so bad that she did not dare to leave the house on bright days. An extracapsular cataract extraction (ECCE) of the right lens was performed when she was 8½ years of age. The lens was washed out through two small keratomes with an irrigation and suction cannula using Ringer's solution. A small piece of the lens aspirate was put into fixation solution; the remainder was frozen at −23°C. Her visual acuity improved to 6/12 on the Snellen chart with a +12.0 spherical contact lens.

At the last follow-up visit, at 10 years of age, 22 months after the operation, the right posterior lens capsule was completely clear without a trace of postcataract. The visual acuity of the right eye again was 6/12 with a +11.5-diopter contact lens. Visual
acuity of the left eye remained 6/60. We could not determine if
the subnormal visual acuity of the right eye was due to a slight
foveal hypoplasia or to slight deprivation amblyopia. The left
lens which has an identical cataract also showed an increase in
crystals but has not yet been removed. At the last visit, the crystals
in the left eye did not reach the lens equator or the anterior lens
cortex.

LABORATORY METHODS

ELECTRON MICROSCOPY OF THE LENS

The small piece of lens material was fixed in a solution
of 1.0% glutaraldehyde and 1.25% paraformaldehyde in
0.08 M of cacodylate (pH 7.3). Subsequently, the material
was rinsed in cacodylate buffer, dehydrated, critical point-
dried with carbon dioxide, fractured, and then gold coated.
The specimen was examined in a Philips SEM-505 (Philips
Industries, Eindhoven, The Netherlands), using a secondary
emission detector.

The elemental composition of the lens crystals was an-
ylyzed in the following way. A few crystals were isolated
from the SEM specimen and fractured to obtain nongold-
coated surfaces. Subsequently, energy-dispersive x-ray
analysis was carried out using an EDAX PV9900 system
with a windowless ECON (Mawah, USA) detector con-
ected to a Philips SEM-525M.

For transmission electron microscopy (TEM), the pellet
obtained after centrifugation (see Biochemical Lens
Analysis section, below) was fixed in the glutaraldehyde/
paraformaldehyde solution described above, rinsed, de-
hydrated, and embedded in Epon 812. Ultrathin sections,
stained with lead citrate and uranyl acetate were inspected
in a Philips EM-400.

BIOCHEMICAL LENS ANALYSIS

After thawing, the main bulk of lens material was ex-
amined by crossed immunoelectrophoresis, isoelectric fo-
cusing, and SDS-polyacrylamide gel electrophoresis.
Crossed immunoelectrophoresis and tentative identifi-
ation of the obtained precipitation patterns were carried
out as described earlier.14 The agarose layer contained
10% rabbit antiserum against human lens crystallin.15,16
This antiserum was raised against the total soluble lens
fraction of clear human lenses and contained high titer
antibodies against α-, β-, and γ-crystallins. The standard
technique for isoelectric focusing and identification of
crystallin bands was used.17 Human lens crystallins were
isolated as the soluble fraction by centrifugation of the
lens homogenate (10^3 X g for 30 minutes) and were com-
pared with a similar lens fraction from an 8-year-old, nor-
mal human lens that had been intracapsularly removed and
stored for several years in physiologic saline solution
at −80°C.

HAIR EXAMINATION

The trichogram, or description of the hair root status,
was carried out in a standardized method.18 For SEM,
the hair was mounted without further treatment on SEM

stubs with conducting carbon cement. The specimens
were gold coated and were examined in a Philips SEM-
505 operating at 15 kV.

RESULTS

Results of hematologic and biochemical examinations,
including liver function tests, were normal apart from a
plasma zinc level of 79.2 μmol/l (94–112 μmol/l). Plasma
copper level was 14.3 μmol/l (13–25 μmol/l), and plasma
eruloplasmin level was 36 mg/100 ml (20–40 mg/100
ml). Specific tests for rubella, cytomegalic inclusion dis-
ease, homocystinuria, mucopolysaccharidosis, and phe-
nylketonuria were negative. Sorbitol dehydrogenase levels
in blood cells were normal. Quantitative column chro-
matography for plasma amino acid analysis showed, in
1985 and 1989, normal concentrations for her age. In
particular, the cystine level was normal with 0.01 to 0.03
mmol/l. Urine amino acid analysis showed a normal total
excretion and fractioning pattern with no indication of
cystinosis. The urate/creatinine ratio was normal as well
as the mucopolysaccharide excretion. The urine organic
cacid fraction pattern was normal.

ELECTRON MICROSCOPY OF THE LENS

The main finding of the SEM inspection was the pres-
ence of elongated, trigonal crystals (asterisks) between
disorganized lens fibers (Fig 3). Energy dispersive x-ray
analysis showed (Fig 4) that the crystals contained carbon,
nitrogen, oxygen, and sulfur, indicating that they were
organic in nature and most likely had a proteinaceous charac-
ter with a large number of sulfur-containing mol-
cules.

The lens proved on TEM to contain numerous crystals
in an electron-lucent region, which were surrounded by
membranes and vacular elements with an electron-
opaque content. The crystals were surrounded by nu-
merous membranes (Fig 5). At high magnification, the
crystals proved to have a fine linear spacing.

BIOCHEMICAL LENS ANALYSIS

The protein patterns in the crossed immunoelectro-
phoresis showed similar immunoprecipitin lines for the
patient’s and the normal lens (e.g., 1 α- and 3 β-crystall-
ils), whereas the γ-crystallins were not detected in the
precipitin patterns (Fig 6). The isoelectric focusing plate
(Fig 7) showed similar patterns. In the patient’s sample,
however, the densitometric reading for the β2-crystallin
was 3.7 times higher and for the γ2-crystallin 2.5 times
higher than obtained in the normal lens. SDS-polyacryl-
amide gel electrophoresis showed no difference in α- and
β-crystallins between the lens from our case and the con-
trol lens.

Preliminary Raman-spectroscopic analysis indicated
that the lens crystals contained numerous disulfide bonds.
HAIR EXAMINATION

The trichograms, descriptions of hair root status, showed telogen effluvium, or abnormal hair loss, resulting from mass precipitation of hair in the resting phase of the hair cycle. The hair sheaths were detached at their distal ends in 23 of 26 anagen hairs (hairs in the synthetic phase of the cycle) from the left temporal and cranial scalp region. Hair amino acid analysis showed high lysine and valine and low serine and half-cystine levels.

Light microscopy and SEM showed longitudinal grooves on the hair shaft and a triangular shape on cross-section of the shaft as in uncombable hair (Fig 8). Some hairs had a severely disturbed cuticular cell pattern.
Fig 5. Transmission electron micrograph of a crystalline body with the actual crystals (asterisks) in the center surrounded by a clear zone with numerous opaque vacuoles (arrowheads) 30-fold surrounded by membranes (original magnification, ×30,000).

Fig 6. Crossed immuneelectrophoresis of crystallins in age-matched normal (N) human lens and in patient’s (P) lens shows an increase of the α-crystallin fraction.

DISCUSSION

Crystalline inclusions in lenses can be composed of lipids, phospholipids, cholesterol, polysaccharides, and proteins or can be of inorganic nature (e.g., calcium). Because no elements such as calcium, zinc, etc., were found, the crystals must be organic in nature. The presence of a substantial amount of nitrogen in addition to carbon and oxygen on x-ray analysis also ruled out lipid, phospholipid, cholesterol, or polysaccharide as the origin of the crystals. Amino acids, either in the form of proteins or as polyaminoacids, are the most likely candidates. The evident sulfur peak suggests a substantial amount of sulfur-containing amino acids pointing to methionine, cysteine, or cystine. The observation, using Raman spectroscopy, of the presence of disulfide bonds in the crystals indicates that cystine is present in these structures. Earlier observations on coralliform cataracts also pointed to cystine crystals. Another argument in favor of the cystine nature of the crystals is that cystine is the least-soluble, naturally occurring amino acid, whereas cysteine is highly soluble in water.

Crystals of equal size have been found inside many cell types in patients with cystinosis. These cystine crystals are
Fig 7. Isoelectric focusing of crystallins of age-matched normal (N) human lens and proband’s (P) lens. Notice increased protein in the \( \beta_2 \) and in the \( \gamma_2 \) fraction.

Fig 8. A, scanning electron magnification of proband’s hair. Longitudinal grooves on and irregular kinking of the hair shaft (original magnification, \( \times920 \)). B, scanning electron magnification of normal hair of proband’s 2-years-elder sister (original magnification, \( \times800 \)).

limited to cell membranes and proved to be of lysosomal origin.\(^{20}\) Whether the cystine crystals and the coralliform crystals are in some way comparable remains to be established. The main difference is that in cystinosis the crystals are cytoplasmic, whereas in cataract they are located outside the cytoplasm of the fibers.\(^{20}\)

To our knowledge, the multiple membranous coating of the lens crystals has not been described before. In this respect, one should bear in mind that differentiated normal lens cells do not have cellular organelles including lysosomes. The most likely origins of these membranes are the limiting membranes of the lens fibers and tentatively it may be suggested that they serve to separate the crystals from the neighboring lens fibers. Recently, membranes around crystals in a conjunctival mesenchymal cell of a patient on clofazimine therapy have been described.\(^{21}\) These membranes were only one or two layers thick and no explanation as to their origin was given.

The water-soluble \( \beta_2 \) - and \( \gamma_2 \)-crystallins are higher concentrated in the patient’s than in the normal sample (Fig 7). This could very well be due to the cysteinization of the free sulphydryl groups of these crystallins (protein-SH + cystein-SH \( \rightarrow \) protein-S-S-cystein). This prevents protein-S-S-cystein from becoming engaged in the formation of disulfide bonds with other proteins and from disappearing from the water-soluble moiety. The lens of the normal case showed lower water-soluble \( \beta_2 \) - and \( \gamma_2 \)-crystalline values due to interprotein disulfide bonds (protein-S-S-protein) leading to insolubilization.\(^{22}\) The higher densitometric readings for \( \beta_2 \) - and \( \gamma_2 \)-crystallins in the patient’s lens thus can be explained by shielding of these crystallins by cysteinization. Normally, the \( \beta \)- and \( \gamma \)-crystallins decrease progressively with increasing age. In this case, the patient’s lens seems to be younger than its age because these crystallins are cysteinized and are thus prevented to disappear from the pattern by insolubilization. The \( \gamma \)-crystallin precipitin line in Figure 6 was lacking both in the patient and the control pattern due to the use of a polyvalent antiserum.

Uncombable hair has been connected once before with cataract\(^ {12} \) but this cataract had a pulverulent form that is quite different and less rare than the crystal one. Crystalline cataract is considered to be congenital and nonprogressive;\(^ {6} \) our case showed definite progression but because she only showed signs of diminished vision at 5 years of age, we do not know when the cataract started. The presence of crystals in the whole nucleus, from the fetal to the infantile, could point to an early onset. In that case, it could not have been a very dense cataract because there was no nystagmus. Crystals in this type of cataract spread through the whole lens without following the lens fiber pattern and without confining themselves to a special part of the nucleus. Thus, one cannot deduce from the distribution of the crystals the exact timing of onset of the cataract because the crystals also might grow inward toward the center of the nucleus.

Both crystalline cataract and uncombable hair are considered to be rare autosomal dominant disorders,\(^ {12} \) although a recessive inheritance of the cataract has been described as well.\(^ {4} \) The pathogenesis of crystalline cataract
is unknown and there is no known association with other
malformations or general diseases. The tiny crystals in
the mother’s eyes could indicate that the cataract was in-
herited from her, in which case dominant inheritance with
incomplete expression would be the likely transmission.
If it were a recessive disorder, then this could point to
heterozygosity in the mother.

Also, the pathogenesis of uncombable hair is unknown.
Our patient had normal serum copper levels in contrast
to other patients but her plasma zinc level was too low.
Zinc deficiency may cause alopecia but we could not find
an association between uncombable hair and low plasma
zinc levels in the dermatologic literature. Regarding
her uncombable hair, one would have to assume that
the proband would be a new mutation because both par-
ents and her sister had no trichologic abnormalities.

Trichograms in uncombable hair syndrome have not
yet been studied. Telogen effluvium was obvious in two
locations. This could be explained by slow hair growth.
The aberrations in the hair shaft could be the result of the
abnormal sheathing of most developing hair roots. It
remains uncertain why these sheaths were open in contrast
to the normally closed distal ends. High levels of lysine
and valine and low levels of serine and half-cystine have
dev not yet been reported in uncombable hair syndrome.

Despite a careful search, we could not find any common
cause for crystalline cataract and uncombable hair. From
this, it may be clear that we cannot determine if the com-
bination of the lens and hair anomalies in our proband is
a coincidence or not.

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