Role of copper in Indian childhood cirrhosis\textsuperscript{1,2}

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\textbf{ABSTRACT} Of the cirrhoses that affect Indian children, Indian childhood cirrhosis (ICC) is a discrete clinical and histologic entity in which large amounts of copper are deposited in the liver. The evidence linking copper deposition to increased dietary copper intake in infancy was reviewed. Prevention of this feeding pattern prevents ICC, and the disease has now largely disappeared from many parts of India. Penicillamine, if given before the terminal clinical stage of ICC, reduces mortality from 92\% to 53\%. Long-term survivors show a sequence of histologic resolution, resulting either in inactive micronodular cirrhosis or in virtually normal histologic appearance. Twenty-nine treated ICC patients reexamined at 8.8 y of age (range: 6.3–13 y), 5–12 y after diagnosis, were well and had normal results from liver function tests. Clinical and epidemiologic evidence show that there must be excessive copper ingestion for ICC to develop, but the lack of an animal model, the inconstant relation between liver copper concentrations and liver damage, and the rarity of liver disease in adults suggests that other etiologic factors contribute. Two mechanisms are discussed: 1) that copper may be acting in synergy with a hepatotoxin, or 2) that there may be a genetic predisposition to copper-associated liver damage, as suggested recently for Tyrolean childhood cirrhosis. Although ICC is now rare, sporadic cases of an ICC-like disorder in infants continue to occur. There should be a greater awareness among pediatricians of this disease to enable early diagnosis. Penicillamine should be used early and adverse prognostic factors recognized as indications for early transplantation and unregulated water supplies should not be used to prepare infant feeds. \textit{Am J Clin Nutr} 1998(suppl);67:1074S–81S.

\textbf{KEY WORDS} Indian childhood cirrhosis, ICC, copper, pyrrolizidine alkaloids, Wilson disease, children, genetic determinants, toxicity

\textbf{INTRODUCTION}

On finding out that I was a pediatrician, the young man sitting next to me on the airplane announced, “I had Reye’s syndrome when I was seven.” “Had you had aspirin?” I asked. “Yeah, I had had the flu and mom gave me aspirin. At the time we didn’t know it was a poison.” Is aspirin a poison? Large numbers of middle-aged men taking it as prophylactic against myocardial infarction hope not. Yet the epidemiologic evidence linking it to Reye syndrome was sufficiently strong to generate a ban of its use in children in the United States and the United Kingdom with influenza. Since then, Reye syndrome in older children has virtually disappeared, suggesting that, indeed, it was a bad thing to give aspirin to children with influenza. The young man went on to say, “But lots of kids in my class who got flu were given aspirin, and they were okay. Only [me] got Reye’s.” Why was that? I did not have an answer to that question except to point out that the Reye-like illnesses we now see in younger children are frequently attributable to an inborn error in metabolism. We parted, he worrying about his genes and me pondering the relation between environmental and genetic causes of liver disease in childhood. Perhaps this is as good an introduction as any to a review of the role of copper in Indian childhood cirrhosis (ICC) and ICC-like disorders.

\textbf{DEFINITION OF ICC}

There are many causes of cirrhosis in Indian children. The early studies offer various definitions of ICC, reflected by the different views as to its epidemiology, clinical features, and response to therapies such as steroids, \(\gamma\)-globulin, and levamisole. Nayak and Ramalingaswami (1) proposed a histologic definition incorporating necrosis of hepatocytes with ballooning and Mallory’s hyaline, pericellular intralobular fibrosis, and inflammatory infiltration. Poor regenerative activity and no fatty changes were also characteristic of ICC, with cholestasis occurring only at an advanced stage. This distinctive histologic entity, ICC, was not described in the Western literature at this time but was peculiar to India.

This histologic definition of ICC was adopted by our group. In doing so we recognized that we were excluding two important categories of children: 1) those whose biopsies had some features of ICC but were not typical, and 2) those with other histologically identifiable cirrhoses (eg, biliary cirrhosis, chronic aggressive hepatitis with cirrhosis, cryptogenic inactive macro- and micronodular cirrhosis, and venous outflow obstruction) and those with marked fatty changes. Other authors (2) found that there were causes of cirrhosis in Indian children other than ICC, but they used different terminology. This tightly defined histo-

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logic group proved to have a homogeneous clinical picture and a high mortality (3–5).

It was in this rigidly defined group that orcein staining was used as a diagnostic tool. By 1982, at a workshop on ICC attended by several eminent pathologists in India (6), orcein staining had become accepted as a diagnostic criterion and was being used to help classify borderline cases. Two probable forms of ICC then became apparent. First, there was a group of older children with inactive micronodular cirrhosis (IMC) (7). The relation between ICC and IMC was shown by an observed evolution of ICC to IMC, by ICC and IMC in siblings, and by some IMC patients having a family history suggestive of ICC. Subsequently, penicillamine treatment allowed numerous examples of evolution of ICC to IMC to be studied. Second, some younger siblings of patients with ICC had hepatomegaly, abnormal results from liver function tests, or both. Evolution to ICC had been reported previously (8). Orcein positivity was reported in younger siblings (9), but in the Pune series it did not progress to ICC. This we now attribute to the fact that the population in Pune had been advised to not use brass utensils to prepare milk feeds for their infants (10).

DISCOVERY OF COPPER INVOLVEMENT IN ICC

The credit for this serendipitous finding, ie, the value of orcein staining, goes to Bernard Portman, who in 1978 used orcein to stain a series of biopsies from Pune children with ICC and other liver disorders, seeking evidence of hepatitis B infection. The granular orcein staining that he detected (11) suggested copper loading, a finding corroborated 1 y later by copper assay in Pune. The granular orcein staining had become accepted as a diagnostic criterion and was used as a diagnostic tool. By 1982, at a workshop on ICC in Pune had been advised to not use brass utensils for milk. The use of copper utensils for water was found as frequently in control subjects as in patients. The epidemiologic difference between patients and control subjects lie in the use of brass utensils for milk. For this reason, attention was focused on copper contamination of milk rather than water, but it is possible that the water-carrying copper utensils were also relevant. This may explain reports of ICC occurring even when brass utensils were not used in the preparation of milk feeds (21).

Second, in contrast with the findings in Tyrol, Austria, where Tyrolean childhood cirrhosis occurred (22), copper vessels were never used for milk. The brass vessels were traditionally tinned, but as their use declined the tinning became more expensive and infrequent.

SOURCE OF HEPATIC COPPER IN ICC

A retrospective review of patients with ICC in Pune showed that none were wholly breast-fed, that all had received animal milk, and that the age of introduction of animal milk was earlier than in other disease groups (19). A subsequent detailed comparison was made between the feeding history of 100 children with ICC and 100 age-, sex-, and caste-matched control subjects from the same village (20). This comparison generated the hypothesis that ICC results from the early introduction of cow or buffalo milk feeds contaminated with copper from brass utensils.

Two points from these studies deserve emphasis. First, most of the patients with ICC lived in small rural communities. The families obtained water from a well or village tap and carried it to their houses in copper containers. The use of copper utensils for water was used because of its cupriuretic effect, its effectiveness was not necessarily a result of its removing excess copper or even of detoxifying copper (28). It is an antiinflammatory drug that may act by inducing metallothionein, replacing glutathione, or inhibiting collagen cross-linking (29).

USE OF D-PENICILLAMINE

Untreated, ICC had a mortality of 45% within 4 wk, 74% within 8 wk, and 86% within 6 mo of the patient coming to the hospital. Most of these patients were in a very late stage of the disease and it was common clinical experience that the appearance of jaundice, ascites, and chest infection heralded a rapid terminal decline. A trial of penicillamine in such cases had no benefit. In a second randomized, controlled trial, only patients who had not yet progressed to the stage of developing ascites or jaundice were included. Penicillamine was given at a dose of 20 mg·kg⁻¹·d⁻¹, with or without prednisolone. Mortality was 92% in the placebo group and 53% in the treatment group, with no additional benefit from prednisolone (23). Similar results were reported from other groups (24, 25).

More recently, the cases of 29 treated ICC patients who had survived for > 5 y were reviewed (26). Treatment had begun at 1.5 y of age (range: 0.5–4 y) and the patients were reexamined at a mean of 8.8 y (range: 6.3–13 y), 5–12 y after diagnosis. All patients were well and thriving and had normal results from liver function tests. Four patients who still had hepatosplenoemegaly underwent liver biopsies, which in each case showed inactive micronodular cirrhosis. The most recent biopsies in the other 25 patients were reviewed. Ten of these biopsies were essentially normal, showing minimal portal fibrosis; 12 (performed < 3 y from diagnosis) showed inactive micronodular cirrhosis but were not repeated because the children were well; and 3 (performed during the first year after diagnosis) showed continuing features of ICC. The mean duration of penicillamine therapy was 3.5 y (range: 1–7 y). The four patients with hepatosplenoemegaly are still receiving treatment, the remaining patients have received no treatment for = 6–16 y. There were no major side effects from penicillamine.

The sequence of histologic changes in children treated with penicillamine, from ICC to nodular change to inactive micronodular cirrhosis and to residual fibrosis and even to an almost normal histologic appearance, was described in detail by Pradhan et al (27). With treatment, liver copper concentrations fell to near normal concentrations, in contrast with persistently high liver copper concentrations in patients with Wilson disease receiving penicillamine treatment. Although penicillamine was used because of its cupriuretic effect, its effectiveness was not necessarily a result of its removing excess copper or even of detoxifying copper (28). It is an antiinflammatory drug that may act by inducing metallothionein, replacing glutathione, or inhibiting collagen cross-linking (29).

PREVENTION OF ICC

The feeding data clearly suggested that ICC was preventable...
by a change in infant feeding practice. To evaluate this, the incidence of ICC in the older and younger siblings of 100 children with ICC was compared. Parents were told of the likely association between ICC and contamination of infant milk feeds with copper from brass utensils and were advised to use aluminum or stainless steel vessels if a subsequent child was to receive animal milk. Of 125 older siblings, 30 had had ICC, whereas of 86 subsequent siblings only 1 (who was known to have received copper-contaminated milk) developed ICC.

To evaluate this hypothesis further, a large intervention study in the Pune District of Maharashtra was performed (30). ICC prevalence and the use of brass utensils were documented in two population blocks, one of which then received an intensive program of health education advising them against the use of brass utensils for preparation of infant feeds. In the study population of the Pune District (population 4.1 million), the use of brass utensils fell from 13% to 4% and the incidence of ICC declined to near zero. However, the use of brass utensils and the incidence of ICC in the control area, Ahmednagar (population 2.7 million), also rapidly declined, probably because the advice to the experimental population to not use brass utensils had “leaked” into the control population. However, the incidence of ICC in a more remote area, Chandigarh, did not change. Second, when the study population was revisited, only 35% of the families interviewed who had ceased to use brass utensils had done so because they were told of the dangers to their infants. The authors concluded that the dramatic decrease in the incidence of ICC was associated with a decline in the use of brass utensils for preparation of infant feeds, but that this change had occurred spontaneously with increasing popularity of aluminum and stainless steel vessels. The health education program had accelerated an existing sociologic trend. When brass utensils became more widely used in Indian households, it was usual for a visiting “Kallai wallah” to regularly coat the inside of the brass utensil with tin. Tinning completely prevents the uptake of copper from brass by milk (31). As brass became used less often, the visits by the Kallai wallah became less frequent, and his fees rose. Thus, paradoxically, a decline in the use of brass possibly led to an increasing number of households in which untinned or inadequately tinned brass vessels were used to prepare infant milk feeds and thus an increase in the incidence of ICC before its decline as brass was replaced.

Despite the lack of proof of a cause-effect relation between a change in infant feeding practices and ICC eradication, the evidence for this association is sufficiently strong that it is considered negligent to not advise parents to avoid using brass utensils to prepare infant milk feeds for subsequent children after a previously born child has been diagnosed with ICC, and negligent to not give this same advice to rural, brass-using communities.

COPPER AND HEPATOCYTE DAMAGE

Copper-laden hepatocytes in ICC patients show evidence of severe damage. The hepatocytes are enlarged and the cytoplasm is vacuolated and contains hyaline inclusions. Prominent endstage copper-rich and sulfur-rich lysosomes are evident, as are severe morphologic abnormalities of the mitochondria. The cell is surrounded by collagen and shows little evidence of regeneration. Prasad et al (32) suggest that the nucleus is the principal site of copper cytotoxicity. In livers from ICC patients with a copper content ≈43-fold greater than that of control subjects, 73% of the copper was within the nuclear fraction, and there was evidence of significantly increased DNA fragmentation.

Exposure of DNA to hydrogen peroxide in the presence of Cu(II) and a reducing agent is known to result in the induction of a variety of oxidative lesions, including DNA strand breaks and base modifications. Cu(II)-dependent DNA damage was induced by ascorbate, reduced glutathione, NADH, and NADPH in that order of potency, the predominant cleavage sites being thymine residues located at positions 5’ or 3’ to guanine (33). Copper-containing metallothionein caused DNA cleavage with a similar site specificity (34). By contrast, Milne et al (35) found that glutathione, which occurs in cell nuclei at relatively high concentrations, was inefficient at promoting damage to DNA. They suggest that the reduced glutathione in cell nuclei serves to prevent rather than to promote copper-dependent damage to DNA.

Putatively copper-induced bulky DNA lesions were shown in the liver of patients with Wilson disease and primary hemochromatosis (36), and copper-dependent formation of miscoding etheno-DNA adducts was shown in the liver of Long-Evans cinnamon rats (37). The probability that these lesions are related to hepatoma formation in primary hemochromatosis and in Long-Evans cinnamon rats leaves unanswered the question of why hepatoma is rare in patients with Wilson disease, but raises the possibility that children who have recovered from ICC may later develop hepatoma.

The mitochondrion, rather than the nucleus, is suggested to be the site of oxidant damage in copper-loaded rats (38), in dogs (39), in humans with Wilson disease, and in primary hepatocyte cultures (40). In addition, there is evidence that α-tocopherol may ameliorate the lesion (41).

IS EXCESSIVE COPPER INGESTION A SUFFICIENT EXPLANATION FOR ICC?

The etiologic role of copper seems clear on the basis of the following findings: 1) ICC is associated with gross hepatic copper overload; 2) gross hepatic copper overload is attributable to dietary copper excess; 3) ICC is preventable by preventing excess copper ingestion; 4) the existence of conditions with pathologies similar to ICC, such as Tyrolean childhood cirrhosis (22) and infantile well-water associated cirrhosis (42–47); 5) ICC is treatable with penicillamine and survivors have no apparent evidence of liver disease; and 6) copper is known to be cytotoxic. However, there are several flaws in this hypothesis.

1) Although acute liver injury may be produced experimentally by copper, there is no animal model in which copper ingestion alone causes cirrhosis of the liver. In sheep, copper supplementation of the diet rapidly leads to hepatic copper accumulation, initially without evidence of liver cell damage (48). A hemolytic crisis then occurs that is associated with acute hepatic necrosis from which the animal either recovers or dies. In rats, copper ingestion produces focal hepatic necrosis, but continued administration of copper appears to induce tolerance (49). A suggested animal model in which rats were given copper nitritolriacetate (50) could not be reproduced.

2) There is an inconsistent relation between liver copper concentrations and liver damage. For example, a very high liver copper concentration is found in some species, such as white perch (51), and in presymptomatic patients with Wilson disease, yet there is minimal liver damage.

3) The liver of a 1-y-old child with ICC might weigh ≈900 g and have a copper concentration of ≈1500 μg/g dry wt (500 μg/g wet wt), and hence a total hepatic copper concentration...
of 450 mg. If this child had received 1 L milk each day (a generous estimate) containing 6 mg Cu/L (the highest concentration seen in in vitro experiments), the child would have ingested 2190 mg Cu in 1 y and would therefore have needed to retain ≈20% of his ingested copper in the liver. With normal biliary excretion of copper, this high retention seems unlikely.

4) Cases of liver disease in adults attributable to excess copper ingestion are rare. A 58-y-old psychiatric patient who ingested 275 American coins, which were largely made of copper, died from profound hemolysis. However, postmortem examination revealed no cirrhosis (52). A 26-y-old man who had ingested 10 times the maximum dose of dietary copper for 2 y, and who had acute liver failure requiring emergency liver transplant, had a successful outcome (53). The rarity of liver disease resulting from excess copper ingestion in adults has led to the view that infants are more susceptible to the adverse effects of excess copper ingestion, perhaps because infants have higher physiologic concentrations of copper and because of the physiological cholestasis of newborns. However, there is no direct evidence in humans or in other mammals that newborns are more susceptible than are adults to hepatic damage from copper ingestion.

Two other mechanisms have therefore been suggested for the etiologic role of copper in the development of ICC: 1) copper may act in synergy with a hepatotoxin, or 2) patients who develop ICC may have a genetic predisposition to copper-associated liver damage.

SYNERGISTIC TOXICITY

ICC might result from the combined effect of copper loading and another insult. For example, a hepatotropic virus might “detonate” copper-laden hepatocytes, although no serologic evidence has been found to support this suggestion (54). Contrary to this hypothesis, galactosamine (55) and carbon tetrachloride (56), two well-studied hepatotoxins, produced significantly less liver damage in copper-laden rats than in controls, even when there was a deficiency of vitamin E (57).

Pyrrolizidine alkaloids (PAs) have long been known to be hepatotoxic in livestock (58–62), particularly sheep and cattle. The toxicity of PAs in humans was first recognized as veno-occlusive disease in the West Indies (63). Sporadic examples of veno-occlusive disease have resulted from the ingestion of herbal remedies containing PAs (64–68), particularly comfrey (69, 70). Fatal hepatic veno-occlusive disease occurred in newborn infants whose mothers had consumed an herbal medicine containing PAs daily during pregnancy (71). This finding showed in humans the occurrence of a phenomenon seen in livestock—that the ingestion of a toxic substance by the mother may have no adverse effect to her but may severely damage her infant. Outbreaks of PA-induced veno-occlusive disease have been seen in India (72–75) and elsewhere (76).

The synergy of toxicity between PAs and copper was first shown in sheep. Ingestion of Ecchium plantagineum L. (salvation Jane) by sheep grazing on copper-contaminated pastures produced severe hepatotoxicity with copper accumulation (77). This was confirmed in an experimental study of copper and heliotrope intoxication in sheep. Heliotrope alone produced severe liver damage in 1 of 10 sheep. Copper alone produced hemolysis in 3 of 11 sheep, with liver copper concentrations being two times greater than those in controls. Copper and heliotrope given together produced severe liver damage in 13 of 14 sheep, with liver copper concentrations being three times greater than in controls. Sheep who were given heliotrope and who later received copper also had a severe toxic reaction to the two substances and an excessive accumulation of copper in the liver (78).

Rats given copper (2 g CuSO 4 /kg diet) and the PA retrorsine (25 mg · kg body wt -1 · wk -1 by gavage) developed severe liver damage manifested by rising plasma bilirubin, falling plasma albumin, histologic changes (nuclear dysplasia, megalocytosis, bile duct proliferation, and hemosiderin deposition), and massive liver copper accumulation (79). Lactating rats given retrorsine showed no adverse effects, whereas suckling newborns showed hepatic copper accumulation, and, if subsequently given copper and retrorsine, showed severe liver damage and copper retention (80). It remains to be determined whether copper accentuates PA toxicity by causing thiol depletion.

It is therefore hypothesized (81) that PAs excreted in the milk of lactating cows or buffalo may, if this milk is subsequently contaminated with copper, produce severe liver injury in infants given the milk. There are a small number of children in the Pune series in whom venous outflow obstruction was seen and in whom PA ingestion was suspected as the cause. This hypothesis may also be applied to Tyrolean childhood cirrhosis (22) because infants in remote Alpine villages would have been given milk from cows who would have been fed silage during the winter that may have been contaminated with ragwort.

PAs may act similarly to other toxins, such as aflatoxin, in that they may act synergistically with copper. Acute aflatoxin B 1 hepatotoxicity, well known in animals, was responsible for an outbreak of hepatitis in Western India in 1975 caused by contamination of maize heavily contaminated with Aspergillus flavus (82). A group of malnourished children given peanut meal contaminated with aflatoxin developed liver disease with progression to cirrhosis (83).

GENETIC ASPECTS OF ICC

In families with ICC, consanguinity is common and more than one sibling is often affected. Family data from the Pune series (1982) showed that of 136 children with ICC, 36 (26%) were from consanguineous parents. Of 120 children with other liver disorders (31 with chronic hepatitis, 26 with cholestasis of infancy, 17 with cryptogenic cirrhosis, and 46 other), 16 (13%) were from consanguineous parents. However, consanguinity is common among the rural India population as a whole. Of 168 older siblings of children with ICC, 22 (14%) had had ICC, whereas of 156 older siblings of children with other liver disorders, 12 (7%) had a history of a similar disorder. These findings suggest a genetic etiology in ICC. The calculated segregation ratio for this data, assuming incomplete ascertainment, is 0.155 ± 0.03 (3), less than the 0.25 value expected for autosomal recessive inheritance.

There was a marked predominance of ICC in males: 105 of the 136 patients were boys. This finding is not compatible with simple autosomal recessive inheritance, but may have been attributable to a superimposed cultural influence. For example, the family’s cow or buffalo milk supply, a precious resource in rural Indian families, may have been preferentially given to boys rather than to girls. In addition, male infants might likely have been brought to the hospital for treatment when ill, whereas
female infants may not have been. Both of these possibilities, however, were vehemently denied in discussions with affected families. That there was also a male predominance in the 120 children with other hepatic disorders, 87 of whom were boys, is evidence that such factors might be operative. Whatever the explanation for the sex difference in ICC, there was no sex difference seen for Tyrollean childhood cirrhosis (22); boys and girls were equally affected.

Several families were seen in whom there had been a child who developed ICC, the father of whom subsequently produced with a second wife another child that developed ICC. This pseudodominant inheritance was interpreted as being strongly suggestive of an environmental rather than a genetic etiology (3). However, it is possible that both wives may have had a responsible recessive gene if it were highly prevalent in the community, or if they were distantly related. Thus, the possibility of a genetic component to the development of ICC was possible.

Possible candidate genes

Wilson disease and its animal models

ICC and Wilson disease are quite dissimilar clinically. Whereas plasma ceruloplasmin is characteristically low in Wilson disease, it is normal or high in ICC. There are also marked histologic differences. For example, fatty infiltration is frequently seen in early Wilson disease but is never encountered in ICC. The dense pericellular fibrosis characteristic of ICC is not seen in Wilson disease. The dense orcein staining deposits characteristic of ICC are not apparent in early Wilson disease but is never encountered in ICC. The dense pericellular fibrosis characteristic of ICC is not seen in Wilson disease. The dense orcein staining deposits characteristic of ICC are not apparent in early Wilson disease. In the liver clinic in Pune, where many children with Wilson disease are seen, no family affected by Wilson disease had a family history of ICC.

Since the Wilson disease gene was identified in 1994, we and others have studied patients by haplotype and mutational analysis. Close to the Wilson disease gene locus are several closely linked microsatellite markers, areas in which there are CA (cytosine and adenine) repeats of variable length that enable a haplotype to be described. We found that most of the Wilson disease patients in the United Kingdom whom we studied were double heterozygotes rather than homozygotes. Homozygosity is found when the parents are consanguineous, as was the case in a high proportion of our Wilson disease patients in India. It is, therefore, logical to determine whether ICC patients from consanguineous parents are homozygous at the Wilson disease gene locus.

We recently studied a child with ICC whose parents were first cousins. This child indeed proved to be homozygous by haplotype at the Wilson disease locus. Such a result might be expected by chance 1 in 64 times. However, in DNA from three other ICC families, of whom one was consanguineous, there was heterozygosity at the Wilson disease locus (M Durkie, unpublished observations, 1996). On balance, therefore, it seems highly unlikely that a gene at or near the Wilson disease locus is involved in ICC but clearly this should be clarified in a larger study.

Metallothionein

In one case of an ICC-like disorder occurring in an American child (84), a defect in metallothionein synthesis was shown in cultured skin fibroblasts (85). However, this defect was not found in three ICC patients from Pune, nor in an Irish child with well-water–associated infantile cirrhosis, nor in an older child with non-Wilsonian copper-associated cirrhosis (86). Whether the metallothionein defect was artifactual or whether it represents only one of several disorders that might predispose to copper-associated cirrhosis remains to be determined. It is worth noting that David Danks in Melbourne succeeded in creating “knockout” mice lacking the metallothionein gene, which apparently show no defect in copper handling (J Mercer, personal communication, 1996).

Bedlington terrier disease

The putative gene defect in the Bedlington terrier and other animal models, such as the ferret, are possible candidate genes. In the search for a putative gene contributing to the causation of ICC, there is no reason to limit the search to known aspects of copper metabolism. It is equally likely to be in an unrelated area of metabolism, such as glutathione, P-450, cytokine, or collagen synthesis or degradation. The logical approach to this problem is to use homozygosity mapping (87–91), a powerful technical used to locate the gene causing a rare recessive disorder by looking for regions of homozygosity for polymorphic DNA markers in inbred affected children. This approach has been successful in several autosomal recessive disorders (92–105) and we hope to apply this technique to ICC. Identifying a responsible genomic site would make possible a search for the same mutation in the parents of children who died from Tyrollean childhood cirrhosis (22).

CONCLUSIONS AND RECOMMENDATIONS

It is clear that copper ingestion is associated with ICC. Just as Reye syndrome has largely disappeared since aspirin ceased to be used in children with influenza, ICC has largely disappeared since brass utensils ceased to be used in the preparation of milk feeds for infant. However, it is less clear whether an excessive intake of copper alone is sufficient to cause ICC or whether aspirin use alone in children with influenza is sufficient to cause Reye syndrome. Two competing theories suggesting a second factor are viable. If copper acts in synergy with a plant, fungal, or other biocidal toxin, then our failure to identify that toxin may have long-term effects, such as cirrhosis or malignancy in later life. If ICC resembles glucose-6-phosphate dehydrogenase in requiring both a genetic vulnerability and an environmental challenge, then the putative gene must be identified.

Sporadic cases of an infantile ICC-like disorder will likely continue to occur. The lessons learned from the study of ICC must be applied to these children. There should be a greater awareness among pediatricians of the disease to enable earlier diagnosis. Penicillamine should be used early in treatment and adverse prognostic factors, particularly jaundice, should be recognized as indications for early transplantation. Given the fact that many of the patients with infantile ICC-like disorders from countries other than India were from rural families with a private well, unregulated water supplies should not be used to make up infant feeds.

Finally, given that infantile copper toxicosis may have a genetic component to its causation, and older children with non-Wilsonian copper-associated cirrhosis may have an as yet uncharacterized genetic disorder, DNA from these patients and their families should be collected and stored for possible future analysis.

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