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ZINC DEFICIENCY AND ITS INHERITED DISORDERS – A REVIEW

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ABSTRACT: ***Zinc is an essential trace element required by all living organisms because of its critical roles both as a structural component of proteins and as a cofactor in enzyme catalysis. The importance of zinc in human metabolism is illustrated by the effects of zinc deficiency, which include a diminished immune response, reduced healing and neurological disorders. Furthermore, nutritional zinc deficiency can be fatal in newborn or growing animals. While zinc deficiency is commonly caused by dietary factors, several inherited defects of zinc deficiency have been identified. Acrodermatitis enteropathica is the most commonly described inherited condition found in humans. In several of the few cases that have been reported, this disorder is associated with mutations in the hZIP4 gene, a member of the SLC39 family, whose members encode membrane-bound putative zinc transporters. Mutations in other members of this family or in different genes may account for other cases of acrodermatitis in which defects in hZIP4 have not been detected. Another inherited form of zinc deficiency occurs in the lethal milk mouse, where a mutation in ZnT4 gene, a member of the SLC30 family of transmembrane proteins results in impaired secretion of zinc into milk from the mammary gland. A similar disorder to the lethal milk mouse occurs in humans. In the few cases studied, no changes in ZnT4 orthologue, hZnT4, were detected. This, and the presence of several minor phenotypic differences between the zinc deficiency in humans and mice, suggests that the human condition is caused by defects in genes that are yet to be identified. Taking into account the fact that there are no definitive tests for zinc deficiency and that this disorder can go undiagnosed, plus the recent identification of multiple members of the SCL30 and SLC39, it is likely that mutations in other genes may underlie additional inherited disorders of zinc deficiency.***

KEY WORDS: Acrodermatitis, Zinc and immune response, Zinc secretion, zinc transporters, Zinc and wound healing

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Zinc deficiency

Zinc has a unique and extensive role in biological processes. Since the discovery of this element as an essential nutrient for living organisms (Raulin, 1869, Maze, 1914, Todd *et al.*, 1934), many diverse biochemical roles for it have been identified. These include roles in enzyme function (Vallee and Auld, 1990), nucleic acid metabolism (Miller *et al.*, 1967, Brown *et al.*, 1985), cell signalling (McNulty and Taylor, 1999) and apoptosis (Zalewski *et al.*, 1993). Zinc is essential for physiological processes including growth and development (Prasad, 1985), lipid metabolism (Cunnane, 1988), brain and immune function (Prasad, 1985, Endre *et al.*, 1975).

The importance of zinc for plant and animal metabolism has been recognised for many years. In 1969, zinc was shown to be essential for growth of *Aspergillus niger*, the common bread mould (Raulin, 1869). Subsequently zinc was found to be essential for plants (Maze, 1914) and for normal growth of rats and mice (Todd *et al.*, 1934). It is only more recently that zinc deficiency in humans was identified. In the first reported study, published in 1961, symptoms of severe anemia, growth retardation, hypogonadism, skin abnormalities, geophagia and mental lethargy described in men from Iran, were attributed to nutritional zinc deficiency (Prasad *et al.*, 1961). Subsequently, there were many other reports (Prasad *et al.*, 1963a, Prasad *et al.*, 1963b) and the recognition that nutritional zinc deficiency is a potentially widespread problem, not only in developing countries, but also in highly industrialised ones (Sandstead, 1991).

While dietary factors that reduce the availability of zinc are the most common cause of zinc deficiency, inherited defects can also result in zinc deficiency. Both nutritional and inherited zinc deficiency produce similar symptoms. An outstanding feature of zinc deficiency is the broad range of pathologies produced. This is not surprising considering the number of physiological processes for which zinc is required and that over 300 mammalian enzymes are zinc-dependant (Vallee and Auld, 1990). The initial effects of zinc deficiency include dermatitis, diarrhoea, alopecia and loss of appetite (Aggett, 1983, Danks, 1990). More prolonged deficiency results in growth impairment and neuropsychological changes such as emotional instability, irritability and depression (Halsted *et al.*, 1972, Prasad, 1991, Vallee and Falchuk, 1993). Immune

deficiency syndromes have also been recorded, leading to increased susceptibility to infections and that may lead to the death of patients (Rodin and Goldman, 1969, Julius *et al.*, 1973, Beach *et al.*, 1980).

Data on the magnitude of zinc deficiency, whether nutritional or inherited, are difficult to obtain due to the lack of a simple and reliable method to determine body zinc status (Aggett, 1991; Hambidge, 2000; Ramakrishnan, 2002). Plasma zinc concentrations are frequently used to estimate zinc status, however, this parameter may not be affected in mild zinc deficiency (Mack *et al.*, 1989) and furthermore, plasma zinc levels are altered by infections or stress (King, 2000). Even in severe zinc deficiency, plasma zinc concentrations can remain unchanged (Aggett and Comerford, 1995; Krebs and Hambidge, 2001).

Acrodermatitis enteropathica

Of the inherited forms of zinc deficiency, acrodermatitis enteropathica is the most commonly described condition. This rare, recessively inherited disorder was first reported by Wende in 1902 (Wende, 1902) but received its current name in 1942, when Danbold and Closs described it in more detail (Danbolt and Closs, 1942). The symptoms of this condition included skin lesions, alopecia, diarrhoea, neuropsychological disturbances and reduce immune function and led to death of the patient in the absence of treatment (Aggett, 1983). Acrodermatitis enteropathica was first identified as a zinc deficiency disease when it was discovered that the symptoms could be abolished by oral zinc supplementation (Moynahan, 1974). Prior this finding, the antibiotic amphotericin B, which increases membrane permeability to divalent cations, was used effectively in the treatment of symptoms, presumably because the defective zinc transport system was bypassed (Aggett *et al.*, 1981). The defect in this disorder was shown to result in an impairment of zinc absorption in the gut, where patients with acrodermatitis enteropathica showed decreased intestinal absorption of ^{65}Zn (Weismann *et al.*, 1979) and nett intestinal secretion of ^{65}Zn was reduced (Aggett *et al.*, 1978). Jejunal mucosal biopsies also showed reduced ^{65}Zn accumulation (Atherton *et al.*, 1979). Assays in cultured fibroblasts from patients with acrodermatitis enteropathica demonstrated a decreased rate of cellular zinc accumulation (Grider *et al.*, 1998; Grider and Young, 1996) in some instances but not in others (Ackland *et al.*, 1989).

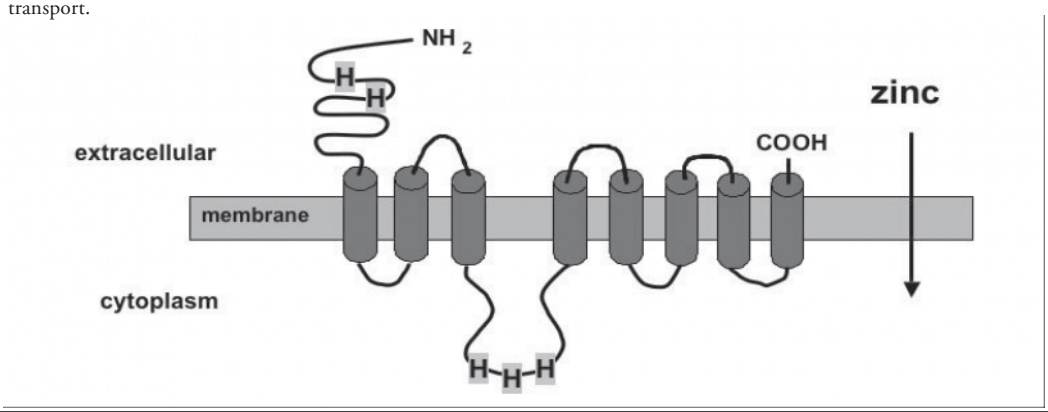
Histological studies on gut tissue from patients have shown that acrodermatitis enteropathica is associated with cellular abnormalities. Filamentous inclusion bodies in the cytoplasm (Mack *et al.*, 1989; Bohane *et al.*, 1977) and abnormal lysosomal inclusions (Jones *et al.*, 1983) occur in the intestinal Paneth cells of patients. This pathology is considered to be a secondary effect of the disease because it disappears after zinc treatment. The inclusion bodies are not specific to acrodermatitis enteropathica, but are found in other diseases such as coeliac disease which may also be associated with zinc deficiency.

In acrodermatitis enteropathica the symptoms of zinc deficiency often first become manifest with the change from breast milk to cows milk (Neldner and Hambidge, 1975), indicating the protective role of human milk, possibly due to the presence of low molecular binding agents, which increase zinc bioavailability (Arnaud and Favier, 1992). Disturbances in lipid metabolism including reduced enteral absorption of unsaturated fatty acids also occur (Mack *et al.*, 1989). The plasma zinc levels in untreated patients with acrodermatitis enteropathica, are generally reduced (ranging from 0.33mmol/L to 8.1μmol/L) (Chandra, 1980; Ozkan et al., 1999; Anttila et al. 1984; Neldner and Hambidge, 1975; Weismann et al., 1983; Weismann et al., 1980; Aggett et al., 1981; Graves et al., 1980; Oleske et al., 1979; Walravens et al., 1978; Bohane et al., 1977; Kelly et al., 1976; Bronson et al., 1983), although normal (11.5-22.5μmol/L) (Mack et al., 1989, Chandra, 1980) and higher (23.2μg/g dry weight relative to normal 10.4-11.9μg/g dry weight) (Garretts and Molokhia, 1977) serum zinc levels have been reported. The variations in serum zinc in both nutritional and inherited forms of zinc deficiency suggest that serum zinc levels do not reflect overall body zinc status and that the symptoms of zinc deficiency may be due to a depletion of zinc from specific intracellular pools, for example membrane-bound zinc fractions (Jackson et al., 1984; Bettger and O'Dell, 1981).

The gene responsible for acrodermatitis enteropathica was recently mapped to chromosome region 8q24.3 (Wang and Walsh, 2001). Intensive search of this region identified a novel gene encoding a putative zinc-transporting molecule, ZIP4, which harboured a range of point mutations, splice-site modifications and deletions in patients with the disorder (Nakano *et al.*, 2002; Wang *et al.*, 2002; Kury *et al.*, 2002). The human *ZIP4* gene shows high homology to other members of the ZIP (SLC39) family of metal uptake transporters. ZIP proteins have eight conserved transmembrane domains, often contain a histidine-rich intracellular loop between transmembrane domains III and IV and are also rich in histidines at the extracellular amino terminus (Fig.1) (Gaither and Eide, 2001). The similarity of the ZIP4 protein with other members of the ZIP family is consistent with a proposed role for it in zinc transport into cells of the small intestine (Wang *et al.*, 2002). Further studies have demonstrated that expression of *hZIP4* gene was restricted to small intestine, stomach, colon and kidney (Wang *et al.*, 2002). The mouse homologue of human ZIP4 protein was localised to apical surface of mature enterocytes, consistent with its function in uptake of dietary zinc in small intestine (Wang *et al.*, 2002; Dufner-Beattie *et al.*, 2003a). Human cells over-expressing *mZIP4* gene showed increased accumulation of ^{65}Zn which was concentration-dependant and saturable, indicating a carrier-mediated uptake process (Dufner-Beattie *et al.*, 2003b). Dietary zinc deficiency resulted in up-regulation of *ZIP4* mRNA and protein in mouse small intestine and conversely, zinc supplementation produced the opposite effect (Dufner-Beattie et al., 2003a, Liuzzi et al., 2004). *In vitro* studies on cells over-expressing mouse or human *ZIP4* gene (Kim *et al.*, 2004) and also *in vivo* studies on mouse (Dufner-Beattie *et al.*, 2003b) have shown that ZIP4 undergoes posttranscriptional regulation in response to zinc levels. In conditions of zinc deficiency, ZIP4 protein was concentrated on the plasma membrane of the cells whereas in zinc-replete cells ZIP4 was endocytosed and was mainly found in intracellular compartments.

Figure 1. Predicted structure of ZIP4 protein.

Transmembrane domains are represented by dark grey barrels, the conserved histidine residues (H) are boxed in light grey. Arrow indicates the direction of zinc transport.



In addition to the gross mutations of the ZIP4 gene detected in acrodermatitis enteropathica such as frame-shifts, premature termination of protein and large deletions, some single amino acid missense mutations were identified mainly within conserved transmembrane domains. The effect of six such mutations on zinc transport was investigated by Wang et al. (Wang *et al.*, 2004). Cultured cells containing these mutations showed a decrease in ^{65}Zn uptake. CHO cells stably expressing these different mutations showed variations in the amount of N-glycosylation of the ZIP4 protein. In addition, the ZIP4 protein was mislocalised and detected in the nuclear envelope and endoplasmic reticulum, in contrast to the plasma membrane where it was located in cells transfected with the wild-type allele. The mislocalisation of ZIP4 was attributed to misfolding of the protein, thus preventing its proper glycolysation and localisation. Two other mutants showed increased accumulation of ZIP4 at the plasma membrane relative to the control and a failure to response to changing zinc concentrations, suggesting a defect in a zinc sensing mechanism which controls mZIP4 trafficking (Wang *et al.*, 2004, Kim *et al.*, 2004).

Despite an intensive search, no modifications in coding, intronic or promoting sequence of *ZIP4* gene could be found in some patients affected by acrodermatitis enteropathica (Kury *et al.*, 2003). This indicates a possible presence of yet unidentified regulatory region of *ZIP4* gene, harbouring mutations in these individuals. Alternatively, another zinc transporter may be affected in some cases of acrodermatitis enteropathica (Kury *et al.*, 2003).

Table 1. Genotypic and phenotypic differences between 3 zinc deficiency disorders.

Table 1 summarises the phenotype and genotype of acrodermatitis enteropathica in relation to two other disorders of zinc deficiency described in the subsequent paragraphs.

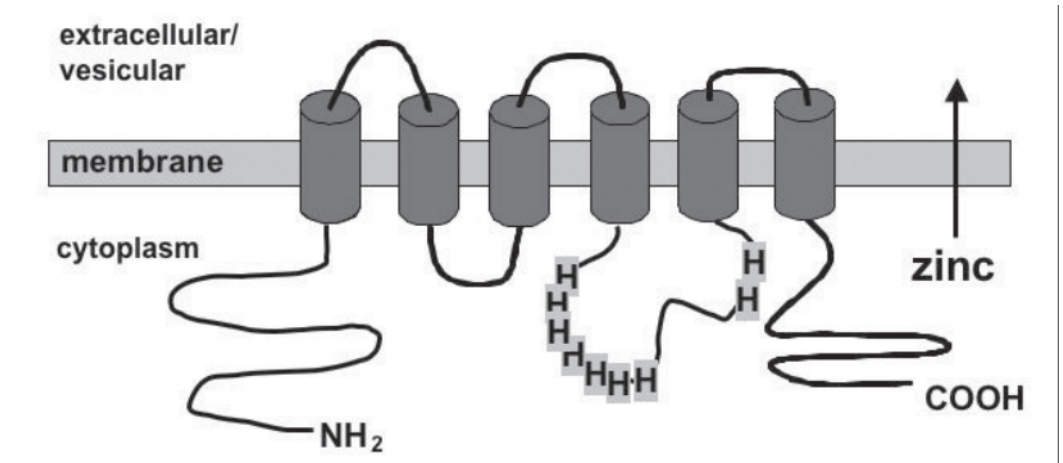
Zinc deficiency disorder	Genotype	Phenotype
Acrodermatitis enteropathica	Mutations in *ZIP4* gene	Normal zinc levels in breast milk Symptoms develop after weaning – protective role of breast milk Life long zinc supplementation required for affected individuals Defect of zinc absorption in gut
'Lethal milk' mouse	Mutation in *ZnT4* gene	Low zinc levels in breast milk Symptoms develop during breast feeding Zinc supplementation not required for offspring after weaning Maternal zinc supplementation effective Defect of zinc secretion in breast Zinc deficiency symptoms re-appear in old age
Zinc deficiency in breast fed infants	Unknown	Low zinc levels in breast milk Symptoms develop during breast feeding Zinc supplementation not required for offspring after weaning Maternal zinc supplementation not effective Defect of zinc secretion in breast Re-occurrence of zinc deficiency not recorded

The lethal milk mouse

Lethal milk is an inherited disorder of zinc deficiency occurring in mice. New born mice suckling dams with the "lethal milk" (*lm*) mutation develop zinc deficiency and die within a week. The *lm* mutation is an inherited disorder of zinc metabolism in mice which has provided an opportunity to investigate a specific lesion in zinc metabolism. Lethal milk is a recessive phenotype in mice caused by a mutation on chromosome 2 (Green, 1973). The most prominent defect is found in the lactating dams. When suckled to a homozygous mutant dam, both normal (+/+) and *lm*/*lm* pups develop symptoms characteristic of nutritional zinc deficiency. This leads to death of the pups before weaning (Piletz and Ganschow, 1978). Mutant pups survive if fostered to a normal dam. They also survive if zinc supplementation is given either to the pups (Piletz and Ganschow, 1978) or to the mothers (Erway and Grider, 1984). This suggested a defect in the production of *lm*/*lm* milk, which causes a reduction in the amount or the availability of zinc to the pups.

Figure 2. Predicted structure of ZnT4 protein.

Transmembrane domains are represented by dark grey barrels, the conserved histidine residues (H) are boxed in light grey. Arrow indicates the direction of zinc transport.



In the lethal milk mouse, a defect in the secretion of zinc from the mammary gland was demonstrated where the zinc concentration in the milk was reduced by 34% relative to the normal (Piletz and Ganschow, 1978, Ackland and Mercer, 1992, Lee *et al.*, 1992). Zinc supplementation of pups or fostering them on normal dams reduced their mortality (Ackland and Mercer, 1992). A nonsense mutation at arginine codon 297 in the ZnT4 zinc efflux transporter, resulting in premature protein termination, was reported to be responsible for this disorder (Huang and Gitschier, 1997). ZnT4 belongs to ZnT (SLC30) family of metal transporters, which have 6 conserved transmembrane domains and histidine-rich zinc binding region between transmembrane domains IV and V (Fig.2). These findings, together with the capacity of ZnT4 to confer zinc resistance when expressed in zinc-sensitive Δ*zrc1* yeast strain (Huang and Gitschier, 1997) and its ability to bind zinc (Murgia *et al.*, 1999), suggested that mouse ZnT4 plays a role in the transport of zinc from the breast into milk.

The zinc deficiency in the mutant milk is the most obvious feature of the *lm/lm* mutation, but it is not the only abnormality seen in these animals. In early adult life they are phenotypically normal, having survived the neonatal period by being fostered to a normal dam. However, in late adult life, mutant animals usually develop less dramatic symptoms of zinc deficiency (Erway and Grider, 1984).

Zinc deficiency in premature breast fed infants

An inherited form of zinc deficiency similar to that of the lethal milk mouse is found in humans. The disorder manifests itself in premature breast fed infants, who demonstrate symptoms characteristic to nutritional zinc deficiency including dermatitis, diarrhoea, alopecia, loss of appetite, impaired immune function and neuropsychiatric changes (Aggett et al., 1980, Prasad, 1985). This condition has been reported in pre-term babies (27 to 33 weeks gestation) (Aggett et al., 1980; Zimmerman et al., 1982; Weymouth et al., 1982; Connors et al., 1983; Parker et al., 1982; Heinen et al., 1995) and less commonly in term babies (Stevens and Lubitz, 1998; Khoshoo et al., 1992; Bye et al., 1985; Glover and Atherton, 1988).

This zinc deficiency disorder found in premature babies is a consequence of reduced levels of zinc in the maternal milk (Sharma *et al.*, 1988). Analysis of maternal milk indicated zinc levels were less than 40% that of normal milk at matched weeks of lactation (Weymouth *et al.*, 1982, Zimmerman *et al.*, 1982). Maternal zinc deficiency was not responsible for the low zinc levels in the milk (Weymouth *et al.*, 1982, Zimmerman *et al.*, 1982). Pedigree analysis has indicated that the condition, which predisposes mothers to produce zinc-deficient breast milk, is inherited.

Oral zinc supplementation induced a remission of zinc deficiency in these babies. Maternal zinc supplementation, in most cases, did not increase zinc levels in milk (Weymouth *et al.*, 1982, Zimmerman *et al.*, 1982, Parker *et al.*, 1982, Connors *et al.*, 1983) or maternal plasma zinc levels (Weymouth *et al.*, 1982; Parker *et al.*, 1982; Dorea, 2000). Prematurity does not account for the zinc deficiency, despite the higher requirements of pre-term babies for zinc due to rapid growth, however prematurity may lead to a predisposition to zinc deficiency. Premature babies are in negative zinc balance at birth because of the lower than in

term babies capacity for gut absorption, however they regain positive zinc balance in few week after birth when fed on normal breast milk (Dauncey *et al.*, 1977; Vileisis *et al.*, 1981; Widdowson *et al.*, 1974). On zinc deficient milk, on other hand, their body zinc levels remain low (Aggett *et al.*, 1980, Zimmerman *et al.*, 1982, Weymouth *et al.*, 1982, Connors *et al.*, 1983, Parker *et al.*, 1982, Heinen *et al.*, 1995).

The aetiology of the zinc deficiency of neonates fed on breast milk is distinct from acrodermatitis enteropathica, in several ways. While zinc deficiency in the breast fed babies is caused by the low levels of zinc in the maternal milk, in acrodermatitis enteropathica the maternal milk is protective and the symptoms of zinc deficiency develop after weaning (Aggett, 1983). No impairment in zinc uptake in the gut has been found in the breast fed zinc deficient babies (Aggett *et al.*, 1980). This is in contrast to acrodermatitis enteropathica, where mucosal zinc uptake in the small intestine of patients was lower than normal (Atherton *et al.*, 1979).

The clinical picture of the zinc deficiency found in premature babies is similar to that seen in the "lethal milk" mouse, previously described. The murine disorder is associated with a mutation in the *ZnT4* gene, a member of the SLC30 family. The possibility that a defect in maternal *hZnt4* was responsible for the production of zinc deficient milk in the mothers of the pre-term babies has been investigated. Sequence analysis of the reading frames of *hZnT4* cDNA from lymphoblasts, fibroblasts and mouthwash buccal cells showed no differences between control individuals and mothers of the infants with zinc deficiency. Furthermore, no differences between *hZnT4* mRNA levels in affected mothers and controls were found (Michalczyk *et al.*, 2003). Protein levels of hZnT4 in extracts of lymphoblasts, fibroblasts and buccal cells and the intracellular localisation of hZnT4 protein was similar in lymphoblast, fibroblast and buccal cells from mothers of the infants with zinc deficiency compared to controls. Interestingly, in all 3 cell types, the hZnT4 protein did not co-localise with intracellular pools of zinc detected with Zinquin, which may be in the vesicular secretory pathway. This suggests that the hZnT4 transporter may not be pumping zinc into zinc-containing vesicles that are destined for secretion (Michalczyk *et al.*, 2003). These results indicate that the 'lethal milk' mouse is unlikely to be the corresponding model for the human mammary zinc secretion disorder.

There are some differences between the mouse and human disorders, which support the conclusion that the human and murine disorders are different. In old age, the mouse shows symptoms of zinc deficiency (Piletz and Ganschow, 1978), while zinc deficiency in women with defective zinc mammary secretion has not been reported. Maternal zinc supplementation in the "lethal milk" mouse is effective in alleviating pup zinc deficiency (Piletz and Ganschow, 1978) but maternal zinc supplementation in humans does not increase milk zinc levels (Weymouth *et al.*, 1982, Zimmerman *et al.*, 1982, Parker *et al.*, 1982, Connors *et al.*, 1983). Finally, utricular otoconia are absent in the "lethal milk" mouse (Erway and Grider, 1984) but abnormalities in balance, which might be a consequence of defective utricular otoconia have not been reported in humans.

Previous studies on the 'lethal milk' mouse (Ackland and Mercer, 1992) provide evidence for alternative zinc transporters apart from ZnT4. The milk produced by the 'lethal milk' mouse has a approximately one third reduction in zinc concentrations relative to the control, thus the rest gets through presumably by other transporters. Zinc in milk is bound to a number of different components including casein 14%, albumin 28%, low-molecular-weight ligands 29% and fat 29% (Lonnerdal *et al.*, 1982). It is possible that different zinc transporters are involved in incorporation zinc into different types of vesicles, which deliver zinc into various milk components.

Several other members of the SLC30 have been screened to test the hypothesis that one or more of them may be responsible for impaired zinc secretion into the breast milk. Significantly reduced levels of *hZnT5* and *hZnT6* mRNA were detected in fibroblasts and lymphoblasts from two patients in comparison to corresponding controls (Michalczyk unpublished data). These findings suggest that defects in *hZnT5* and/or possibly *hZnT6* may underlie the disorder of reduced zinc secretion into the milk.

In conclusion, the genetic basis of two inherited disorders of zinc deficiency, acrodermatitis enteropathica and the lethal milk mouse, is known. In another disorder of zinc deficiency found in humans and which results in the production of zinc-deficient milk, no mutations have been detected. As there are no definitive tests for zinc deficiency, it is considered that other zinc disorders may go undiagnosed. It is therefore likely that defects in other genes, in addition to *ZnT4* and *ZIP4*, may contribute to the aetiology of zinc deficiency.

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