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To the Editor,

Acrodermatitis enteropathica (AE; MIM# 201100) is a rare autosomal recessive disorder characterised by zinc deficiency due to a defect of zinc absorption through the intestine¹. Acral dermatitis, diarrhoea and alopecia form the classical triad of AE although the clinical features may vary from one patient to another^{2,3}. The normal level of zinc in blood is around 160ug/dL but drops to <50ug/dL in patients with AE which can lead to an impairment of the immune system and increase the possibility of infections and eye problems. If not treated, AE can even progress to multiple organ failure and death. AE is caused by mutations in the SLC39A4 gene located in the 8q24.3 chromosomal region. *SLC39A4* encodes ZIP4, a 68kDa zinc transporter protein highly expressed in the duodenum and jejunum which are the main sites for zinc uptake⁴. Zip4 knockout mice develop systemic zinc deficiency and loss of intestinal integrity⁵. There are at least 40 mutations identified in the SLC39A4 gene so far that have been associated with AE. These mutations are spread along all 12 exons and include missense and nonsense mutations, small and large deletions or insertions causing frame-shifts, and splice-site substitutions⁶. A three generation family from the region of Tabuk in the north-western region of Saudi Arabia has two patients with AE (B03 and B06) Figure 1. In this family AE patients had a severe clinical phenotype. The first patient was a 30 year old Saudi male. He presented with painful oral ulceration and a pustular skin eruption affecting the face, scalp, chest, legs and feet. He had a very low plasma zinc level <26 ug/dL (normal: 60-120 ug/dL). He had been on oral zinc supplements since childhood and, on several occasions, the mouth ulceration and skin lesions had recurred during periods when he had stopped taking his regular dosage of zinc. The second patient was a female. She was five months old when she presented the first clinical features. These were similar to her

older brother (see above). Both patients respond perfectly to the treatment with symptoms gradually disappearing after starting take daily zinc supplements.

DNA samples from 11 individuals from this AE family were amplified by PCR after optimisation of PCR conditions. Primers and PCR conditions for each amplicon are as described in on-line supplemental materials. 15 kb of chromosome region 8q24.3, including the 12 exons of *SLC39A4* were sequenced in the 11 individuals. Note that there are two mRNA variants of *SLC39A* (variant 1 NM_130849.3 and variant 2 NM_017767.2) which differ in their 5' region. Therefore primers were designed to cover the regions encoding for both variants (Figure 1Sup; on-line supplemental materials). Sequence alignments using the generated sequence data of *SLC39A* in the 11 family members were performed (Figure 2Sup; on-line supplemental materials). The sequences were aligned by amplicon from all samples plus *SLC39A* reference sequence (accession number NM_130849.3). The *SLC39A* coding sequence was re-sequenced in another laboratory to confirm the results of the first round of sequencing.

Sequence alignments revealed several SNPs including synonymous and non-synonymous substitutions (Figure 2Sup; on-line supplemental materials). We focused on non-synonymous substitutions which gave rise to amino acid changes, of which there were five identified in these individuals (Table 1). None of these variations seems to segregate with disease phenotype except for one which gives a change from the neutral aliphatic amino acid (glycine) to the highly hydrophobic aromatic amino acid (tryptophan) at position p.Gly512Trp (c.1534G>T), with the genotype c.1534G>T showing perfect segregation with the AE phenotype in our family and appearing in homozygous state in the two affected members B03 and B06 (Table 1 and Figure 1). Our findings have been confirmed by

bioinformatics analysis using MutationTaster at www.mutationtaster.org. MutationTaster calculates probabilities for the alteration to be either a *disease mutation* or a harmless *polymorphism*. The prediction is based on the frequencies of all single features for known disease mutations/polymorphisms available in databases such as HGMD Professional and the 1000 Genomes Project. These were used as a root to calculate the probability for the alteration SLC39A4 G to T transversion at position 1534 (for sequence accession number NM_130849). The prediction gave a high score with probability of 0.99999 predicting that the SLC39A4 mutation G1534T is a disease causing mutation. In addition, the conservation of glycine at position 512 of human ZIP4 in different species including mammals, birds and fish indicate the importance of this amino acid in ZIP4 function (Figure 3Sup; on-line supplemental materials). Amino acids surrounding G512 are also conserved suggesting that this region of ZIP4 is under negative selection pressure, which allows this domain to preserve its function (Figure 3Sup; on-line supplemental materials).

Attempts to correlate genotype and phenotype of AE patients were possibly not successful because of the lack of a large data set including molecular and clinical data. However, Smith and colleagues also found no clinical difference between 35 AE patients carrying different SLC39A4 mutations⁶. Our AE patients present similar clinical features to those previously described in the literature but unlike AE patients heterozygous for certain SLC39A4 mutations (eg c1223_1227delCCGGG) the heterozygous members of our Saudi family were asymptomatic. One of the patients in the Saudi family has been admitted to hospital emergency services after omitting taking zinc supplement tablets for just a couple of days, suggesting that the SLC39A4 mutation G1640T might have caused a complete loss of ZIP4 function. The p.Gly512Trp (c.1534G>T) mutation is located within highly-conserved amino acids sequence ADG₅₁₂LA (Figure 2Sup) which corresponds to the fourth putative

transmembrane domain of ZIP4⁶. This mutation has also been reported in two AE families from North Africa⁶ but it has not so far been identified in families originating outside North Africa and the Middle East, suggesting the mutation originated in this part of the world. There is strong link between populations in North Africa and Middle East. In fact, during the seventh century the Arabs invaded North Africa and it could be hypothesised that p.Gly512Trp first appeared in the Middle East and has been spread in North Africa during Arab invasions⁸ but this hypothesis needs to be tested.

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