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Identification and Characterization of a Gene Responsible for Cadmium-Induced Disease

Daniel W. NEBERT, Lei HE, Bin WANG, Scott SCHNEIDER, Jodie REED & Timothy P. DALTON

Department of Environmental Health, University of Cincinnati Medical Center

P.O. Box 670056-0056, Cincinnati, Ohio 45267-0056, U.S.A.

Cadmium (Cd^{+2}, **Cd**) is a nonessential metal, toxic environmental contaminant, and a human carcinogen. Cd induces testicular necrosis in all species having testes. Of 45 inbred mouse strains tested, 31 are sensitive and 14 are resistant to Cd-induced testicular necrosis. Resistance to Cd damage to the testis is an autosomal recessive trait, and the gene responsible for this trait was named *Cdm* [*Proc Soc Exp Biol Med* 1973; **143:** 629]. DBA/2J (**D2**) and 129S6/SvEvTac (**129S6**) strains are sensitive and C57BL/6J (**B6**) and **A/J** strains are resistant, to this trait. Screening 26 BXD/Ty recombinant inbred lines and using B6 x D2 genetic crosses, we narrowed down a >24-cM region on Chr 3 ultimately to an 880-kb region, containing three genes**;** the *Slc39a8* gene, showing homology to a ZIP metal transporter in plants was the most likely candidate. Several lines of convergent evidence included the robust ZIP8 in situ hybridization signal in testicular vascular endothelial (**TVE**) cells of sensitive, but not resistant, mouse strains [*PNAS* 2005; **102:** 3401]. From a 129S6 (sensitive) BAC library, we then isolated a 173-kb BAC clone containing only the *Slc39a8* gene and generated transgenic mice on the B6 (resistant) background. In situ hybridization demonstrated that ZIP8 mRNA accumulates in TVE cells from the transgenic TgB6.129/SvJ(*Slc39a8*)3Neb (**BTZIP8**) mice, but not their littermates. Further, the *trans*-gene converted the phenotype from Cd-resistance to Cd-sensitivity (testicular necrosis) in BTZIP8 mice. These data confirm that *Slc39a8* is the *Cdm* gene, and that regulatory sequences conferring TVE-cell-specific expression of ZIP8 are contained within this 173-kb BAC.

We have now determined that ZIP8 probably functions normally as Mn/HCO_3^- co-transporter (symporter) but has a high affinity for nonessential metals such as the hitchhiker Cd and may be responsible for the pathogenesis of other heavy metals as well. The effect of one dose of Cd ($CdCl_2$; s.c.; 30 μmol/kg), although sufficient to cause testicular damage in Cd-sensitive strains, is only mildly toxic to other organ systems and does not cause lethality**;** 12 h after such treatment, urinary output in BTZIP8 mice was >10-fold less than controls. Dramatic increases in BTZIP8 kidney Cd levels, blood urea nitrogen, red blood cells in urine, and urinary *N*-acetyl-β-*D*-glucosaminidase, glucose and protein reflected renal failure. We conclude that ZIP8, also in humans, very likely mediates Cd-induced renal Fanconi syndrome (proximal tubular metabolic acidosis) and osteomalacia. ***---Supported in part by NIH grants R01 ES10416, R01 ES012463, and P30 ES06096.***

Profile: Dr. Nebert is board-qualified in pediatrics and human genetics, has spent more than 20 years at the National Institutes of Health (Bethesda MD) and 16 years at the University Cincinnati Medical Center, and is author/coauthor of >550 highly-cited peer-reviewed publications, invited reviews, and book chapters. Since 1995 he has been a member of the External Advisory Board of the Human Gene Nomenclature Committee.