The Journal of Nutrition
Symposium: Systems Genetics in Nutrition and Obesity Research

Systems Genetics of Mineral Metabolism[1–3]

James C. Fleet,[4,5]* Rebecca Replogle,[4] and David E. Salt[5,6]

[4]Department of Foods and Nutrition, and [5]Center for Gene-Environment Interactions, and Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47906

Abstract

Minerals are essential and toxic elements that have an impact on human health. Although we have learned a tremendous amount about the metabolism, biological roles, and health effects of minerals with the tools of biochemistry, cell biology, and molecular genetics, there are gaps in our knowledge of mineral biology that will benefit from new approaches. Forward genetics, whereby variations in phenotypes are mapped to natural genetic variation in the genome, has been successfully used to increase our understanding of many biologically important traits but has not yet been used extensively for mineral metabolism. In addition, the well-appreciated existence of interactions between minerals justifies a broader, systems approach to the study of mineral metabolism, i.e., ionomics. This short review will explain the value of forward genetics and ionomics as tools for exploring mammalian mineral metabolism. J. Nutr. doi: 10.3945/jn.110.128736.

Introduction

Inorganic elements (metals, metalloids, and nonmetals commonly called "minerals" in nutrition) are essential for human health. They are critical to life as enzyme cofactors (e.g. Zn, Cu, Fe), stabilizers of organic molecules (e.g. Zn, Cu for proteins; Mg for DNA; Co in vitamin B-12), structural components of bone (e.g. Ca, P, Mg), second messengers (e.g. Ca), regulators of acid-base balance (e.g. Na, K), and participants in redox reactions (e.g. Mn, Fe, Cu, Se), and for the maintenance of cellular pH and electrical gradients (e.g. Na, K). Traditional reductionist approaches have revealed many aspects of mineral metabolism and function, but knowledge gaps still exist. To fill these knowledge gaps, we need new approaches that complement the traditional methods. For example, in the field of Ca metabolism, researchers spent 40 y using traditional methods in biochemistry and cell biology to build the facilitated diffusion model describing intestinal Ca absorption and its regulation by factors like vitamin D (1). However, when knockout mice were developed that permitted the direct testing of this model (i.e. calbindin D_{9k} and transient receptor potential cation channel, subfamily V, member 6 knockout mice), researchers were surprised to learn that intestinal Ca absorption was not affected, nor was regulation by vitamin D eliminated (2,3). There are several ways to interpret the lack of an expected phenotype in a gene knockout mouse. On one hand, it may be a reflection of compensation by functionally related proteins. On the other hand, it could indicate a refutation of the original model. Regardless, it is clear that reapplying the traditional approaches used to generate the original biological model is unlikely to yield a new outcome, emphasizing the need for new approaches to make progress and overcome our uncertainty related to biological areas like mineral metabolism.

Like others who presented at this symposium, we propose that forward genetics is an important new approach that has been virtually untapped for the evaluation of mammalian mineral metabolism. We think that this will allow researchers to identify new macromolecular players involved in metal ion homeostasis and trafficking, thereby giving us insight into roles of metals in cell biology and physiology, especially for those essential elements where their metabolic functions are not yet clearly established.

The basic concept underlying forward genetics is that there is natural variation with the genome and that this variation will influence nutritionally relevant phenotypes, e.g. tissue mineral levels. By examining controlled crosses between genetically well-characterized inbred mouse lines, one can correlate the variation in phenotype to sequence variations in the genome, identify the genes that contain this variation, and learn new biological roles for genes and their protein products (4). This approach has been remarkably successful in identifying the genetic loci controlling a wide variety of factors affecting health including fat mass (5), various behaviors (6,7), response to infection (8), and neurology (9). Yet even though the genetic differences between inbred mice can result in a wide range in tissue mineral levels [e.g. for spleen and liver Fe (10,11) and for brain Cu, Fe, and Zn levels (12)], forward genetics has not been used extensively for mineral metabolism. Still, the promise of this approach was recently demonstrated for Fe metabolism. Wang et al. (10) identified

[1] Published as a supplement to *The Journal of Nutrition*. Presented as part of the symposium entitled "Systems Genetics in Nutrition Research" given at the Experimental Biology 2010 meeting, April 27, 2010, in Anaheim, CA. The symposium was supported by an educational grant from USDA-NIFA, AFRI Award, and sponsored by the Nutrient Gene Interaction RIS and the University of Tennessee Obesity Research Center. The symposium was chaired by Naima Mousstaid-Moussa and cochaired by Brynn Voy. Guest Editor for this symposium publication was Kevin Schalinske. Guest Editor disclosure: Kevin Schalinske had no relationships to disclose.
[2] Supported by NIH awards DK054111 (J.C.F.), ES019103 (J.C.F.), and GM078536 (D.E.S.) and funds from Purdue University.
[3] Author disclosures: J. C. Fleet, R. Replogle, and D. E. Salt, no conflicts of interest.
* To whom correspondence should be addressed. E-mail: fleet@purdue.edu.

doi: 10.3945/jn.110.128736.

significant variation in spleen Fe levels between inbred mouse lines. By conducting genetic crosses between C57BL/10J and SWR/J mice, they were drawn to a locus on chromosome 9 that accounted for 30% of the variation in spleen Fe levels. Within this locus they identified variation in the Mon1a vacuolar fusion protein MON1 homolog A gene and used this information to determine that Mon1a is a critical component of spleen Fe uptake and recycling of RBC Fe within macrophages. Thus, even though we have learned a tremendous amount about Fe metabolism over the last 15 y from traditional approaches and rare genetic mutations (13), forward genetics permitted researchers to add another piece to this already complex picture.

Forward genetics for defining gene-diet interactions

It is likely that forward genetics will reveal a great deal about mineral metabolism. However, it also has the potential to inform us about the interaction between mineral metabolism and dietary mineral intake. This issue is at the heart of current suggestions that personalized nutrition, based on individual genetic variation, is possible.

To illustrate the potential of forward genetics for defining gene-diet interactions related to mineral metabolism, we will describe several scenarios. First, there are data available that suggest the response to changes in dietary Fe is dependent upon genetic factors. We know that although the C282Y mutation in the HFE gene is present in 85% of adult cases of the Fe overload disease hemochromatosis (14), only one-third or fewer of the individuals with the HFE mutation experience the clinical consequences of the disease (15). This suggests that other genetic or lifestyle factors may modify the penetrance of hemochromatosis. Another example of a potential gene-diet interaction affecting Fe metabolism comes from unpublished data from Dr. Byron Jones and the late Dr. John Beard, both from Penn State University, that has been deposited on a genetic resource called The GeneNetwork (16). They fed either a 3 or 270 mg/kg Fe diet from weaning until 120 d of age to 20 lines from a panel of recombinant inbred (RI) mice developed from a cross of C57BL/6J and DBA/2J mice [i.e. the BXD[6] RI panel (17)] and they examined the Fe levels of various tissues. **Figure 1***A* shows that there is a large degree of variation in liver Fe of male mice from each BXD line that is dependent upon the dietary Fe level. Using genetic mapping tools on the GeneNetwork, we found that no genetic loci were significantly associated with liver Fe on the low-Fe diet, regions of the genome on chromosome 4 (peak at 144 Mb) and 7 (peak at 92 Mb) were identified as controlling liver Fe when consuming the high-Fe diet, and regions on chromosome 1 (peak at 25 Mb) were identified as controlling the difference in Fe accumulation between high- and low-Fe consumption. Genes known to control Fe metabolism are on chromosome 1 (ferroportin, at 46 Mb), 4 (IRP at 40.1 Mb, ferritin light chain at 30.6 Mb), and 7 (hemoglobin β at 111 Mb, hepcidin at 31.7 Mb). However, none of these genes fall within genetic intervals that were identified by the mapping. This suggests that natural genetic variation in the mouse genome controls liver Fe accumulation on high- but not low-Fe diets, that the response of liver Fe accumulation to changing dietary Fe is controlled by different genetic variation than the maximal accumulation of liver Fe, and that we could conduct additional experiments to refine these regions and find new genes whose protein products control these traits. In addition, it highlights a unique feature of forward genetics that databases are available that can be used to conduct preliminary studies and generate new hypotheses related to mammalian mineral metabolism.

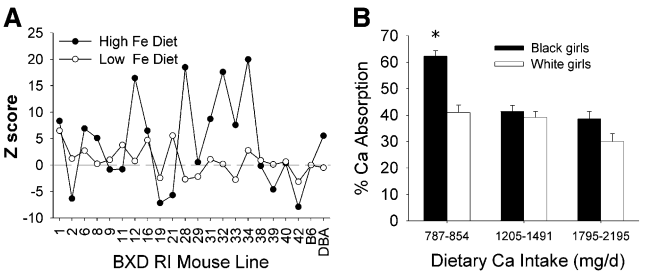

FIGURE 1 Evidence for genetic differences in the response to changes in dietary mineral levels. (*A*) Mice in the BXD recombinant inbred panel were fed diets containing high or low Fe leading to differences among the lines in liver Fe. Data were obtained as means ± SE of the mean liver iron from the Web QTL database (16) and are expressed as Z-scores normalized to the mean value from C57BL/6J mice (B6) within each diet group. (*B*) After feeding adolescent girls high-, medium-, or low-Ca diets, black girls had a robust adaptive upregulation of intestinal Ca absorption (determined from Ca balance experiments), whereas white girls did not [adapted from Weaver et al. (20) with permission].

Another example of where forward genetics could be used to identify the genetic response to diet is for Ca metabolism. We have known for some time that there are differences between racial groups in bone density (18) and Ca metabolism (19). For example, Weaver et al. (20) recently reported that there are racial differences in how young girls adapt to dietary Ca restriction. The traditional thinking is that when a person consumes inadequate dietary Ca, their bodies will adapt by increasing the production of the active hormonal form of vitamin D (1,25 dihydroxyvitamin D) and the 1,25 dihydroxyvitamin D will act upon the intestine to increase the efficiency of intestinal Ca absorption (1). However, when black or white girls aged 11–15 y were provided controlled diets containing high, medium, or low levels of Ca, adaptation to low dietary Ca intake was strong in black girls and only modestly affected by diet in the white girls (Fig. 1*B*). The potential role for genetic controls on Ca absorption is also supported by research demonstrating differences in basal and 1,25 dihydroxyvitamin D-induced Ca absorption between inbred mouse lines (21,22). Phenotypic differences between racial groups or inbred lines of model organisms like mice are a first indicator that a phenotype might be controlled by natural genetic variation. Given the fact that recent studies have raised doubt regarding the validity of the facilitated diffusion model traditionally used to describe Ca absorption (e.g. the lack of phenotypes in calbindin D_{9k} and transient receptor potential cation channel, subfamily V, member 6 knockout mice), mapping the source of the variation influencing Ca absorption has the potential to expand our understanding of intestinal Ca absorption and its regulation by vitamin D. One possible mechanism for this incongruity has already been partially evaluated: polymorphisms in the vitamin D receptor (VDR) gene. Studies have shown that the F allele of the *Fok*I gene polymorphism at the start site of the VDR gene shortens VDR protein by 3 amino acids and makes the VDR more transcriptionally active (23). Two studies have reported that individuals homozygous for the shorter, more transcriptionally active F allele have greater Ca absorption efficiency compared with individuals with the f allele (24,25). However, these investigators did not evaluate whether the *Fok*I genotype influenced adaptation of Ca absorption to low-Ca diets. In

[6] Abbreviations used: BXD, C57BL/6J by DBA/2J inbred mouse cross; ICP-MS, inductively coupled plasma MS; RI, recombinant inbred.

JN THE JOURNAL OF NUTRITION

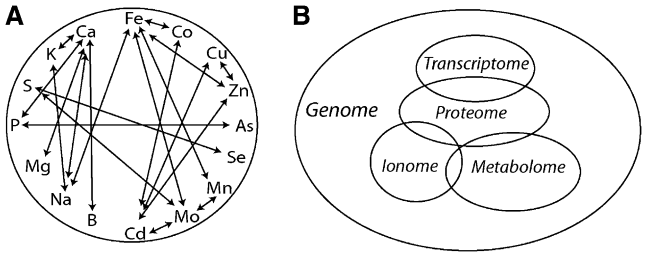

FIGURE 2 Mineral biology can be viewed as a system or ionome. (*A*) Interactions between individual elements of the ionome. (*B*) The ionome is integrated into the biology of the cell.

addition, although others have shown that the more active FF genotype is more common in African Americans (65 vs. 37% in whites) (26), there are many other DNA-level differences between racial groups, so it is unlikely that the response of Ca absorption to dietary Ca restriction is controlled by polymorphisms in a single gene.

The ionome: a new concept in mineral biology

Since 1970, when Hill and Matrone (27) published their seminal paper explaining the critical importance of chemical similarity among elements as the basis for biologically relevant mineral-mineral interactions, we have understood that single element research may have limitations. This concept has been expanded to recognize that some mineral-mineral interactions are indirect. For example, copper-dependent proteins (e.g. ceruloplasmin, ferroportin) are critical for various aspects of Fe metabolism (13), changes in electrolyte intake/metabolism can alter cellular pH or ion gradients necessary for mineral and other nutrient transport, and disruption of metabolism by the loss of mineral-dependent enzymes could indirectly alter physiology in ways that compromise mineral transport and other aspects of metabolism. To account for such direct and indirect interactions, it has been argued that mineral biology should be examined as a system, or the ionome (**Fig. 2***A*).

The ionome is defined as the mineral nutrient and trace element composition of an organism; it represents the inorganic component of cellular and organismal systems. Ionomics is the study of the ionome. It involves the simultaneous measurement of the elemental composition of an organism as well as the changes in this composition in response to environmental, physiological, or genetic modifications. Ionomics requires the application of high-throughput elemental analysis technologies [e.g. inductively coupled plasma atomic emission spectrometry or inductively coupled plasma-MS (ICP-MS)] and their integration with both bioinformatic and genetic tools. It has the ability to capture information about the functional state of an organism under different conditions. In addition, because the ionome is integrated into the overall biology of a cell, i.e. with links to the metabolome, proteome, transcriptome, and ultimately the genome (Fig. 2*B*), alterations within the ionome can be mapped back to sequence variation in the genome and may also reflect broader biologically relevant disturbances. Interested readers are encouraged to read one of several interesting reviews describing the use of ionomics in biology (28,29).

How can ionomics be used for discovery?

Although ionomics is at a nascent stage compared with other systems biology approaches, there are already several important studies that demonstrate its utility. An early example of this was reported by Eide et al. (30), who examined how gene deletions in budding yeast were linked to changes in the ionome. They conducted a 13 element profile for each of 4385 yeast gene deletion mutants and found that 212 mutants had disturbances in at least 1 member of the ionome of at least 2.5 SD from the wild-type yeast mean. By using bioinformatic tools to identify clusters of functionally related genes within the list of the 212 mutants, they found that shifts in the ionome were a reflection of specific biological functions. For example, 27 of the 212 gene deletion mutants influenced mitochondrial function and these mutants were characterized by lower Se and Ni accumulation (**Fig. 3***A*). This pattern of mineral accumulation was different from a profile of low Co, P, Se, Mg, and Ni, combined with high Mn, Ca, S, and Cu, that was associated with genes controlling vacular biogenesis and/or function. This highlights the point that alterations in the ionome reflect changes in critical biological functions.

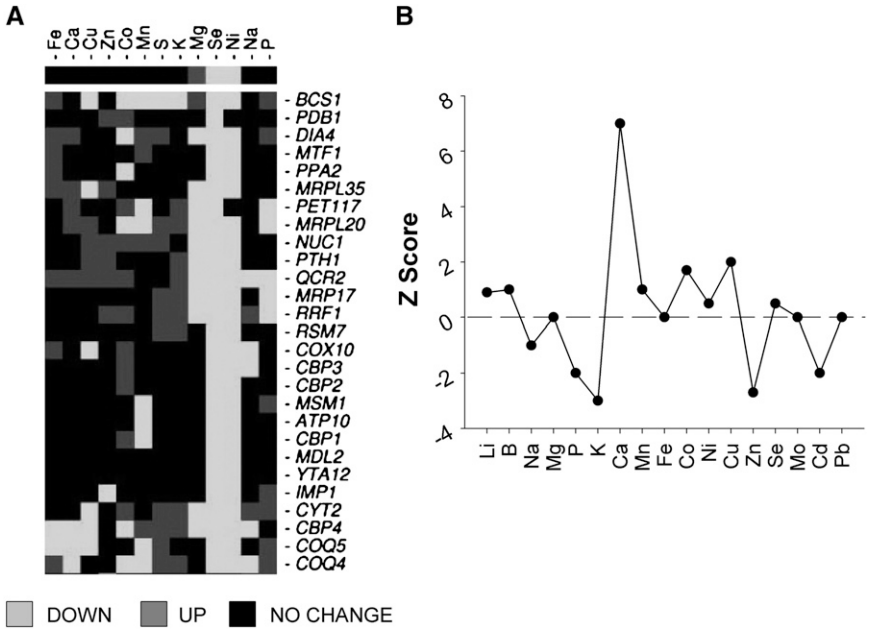

FIGURE 3 Changes in the ionome accompany gene deletions in model organisms. (*A*) Ionomic analysis was conducted on yeast gene deletion mutants and profiles from mutant lines were examined based on gene functions. Twenty-seven gene deletion lines with functional defects in mitochondrial function were characterized by low selenium and nickel levels. [Adapted from Eide et al. (30) with permission]. (*B*) Embryos from ZIP2 knockout mice fed Zn deficient diets and then whole embryos were analyzed by ICP-MS. Data from ZIP2 knockout embryos are expressed as Z-scores normalized to values seen in wild-type embryos fed Zn-deficient diets. This analysis shows that Zip2 knockout embryos have low Zn, high Ca, and low K levels. [Adapted from data in Peters et al. (31) with permission].

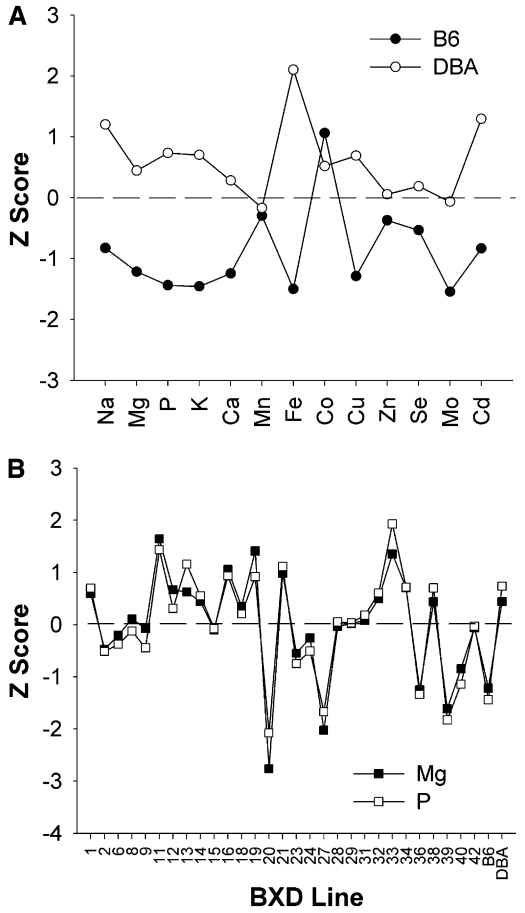

FIGURE 4 Ionomic differences within the liver of the BXD panel of RI mice. Livers of mice from BXD parental and RI lines (n = 6–10/line) were analyzed for mineral content by ICP-MS. Data for each line were normalized to the average mineral level for the entire BXD RI and parental line population and are expressed as Z-scores. (*A*) Differences in the liver ionome between the parental lines of the BXD panel [i.e. C57BL/6J (B6), DBA/2J (DBA)]. (*B*) The liver levels of Mg and P have coordinate changes across 29 BXD lines, suggesting they are regulated by similar genetic variation in the mouse genome.

Another concept that has been demonstrated is that targeted disruption of the metabolism of a single element leads to disturbances in multiple members of the ionome. Peters et al. (31) examined the impact of dietary Zn deprivation on embryos of mice lacking the Zn transporter ZIP2 (Zrt and Irt-like protein 2). As expected, they found that embryo Zn was lower in Zn-deficient ZIP2 null embryos compared with wild-type embryos. However, by examining the ionome, they revealed that ZIP2 deletion also led to an increase in embryo Ca and a reduction in embryo K (Fig. 3*B*). Although the molecular basis for this change is not clear, these findings show that even in complex systems, the ionome can be a biological indicator. The use of an ionomic profile may even be useful as biomarker for specific mineral inadequacies. Using the model plant *Arabidopsis thaliana*, Baxter et al. (32), identified multi-element predictors of plant Fe or phosphorus deficiency. They noted that the shoot Fe level was so tightly regulated that it is a poor indicator of a plant's Fe status. By examining the shoot ionome under a range of Fe nutritional conditions, Baxter et al. found that the combination of shoot Mn, Co, Zn, Mo, and Cd levels could be used to discriminate plants with different Fe nutriture. Similarly, shoot B, P, Co, Cu, Zn, and As performed better than P level alone as a model for detecting plants that are P deficient. In mammalian biology we have been faced with the difficulty of assessing the status of several mineral elements, e.g. serum Zn levels change only slightly over a wide range of Zn intakes (33). It will be interesting to determine whether this concept of ionomic profiling can also be useful to identify specific mineral deficiencies in mammals.

Application of ionomic profiling to genetic screens

As we suggested above, differences between inbred mice are an early indicator that there is genetic variation controlling a trait. With this in mind, we have compared the mouse liver ionome between C57BL/6J and DBA/2J mice, the parent lines of the BXD recombinant inbred mouse panel. **Figure 4***A* shows the normalized data for this analysis. Overall, the DBA line accumulates more of many elements compared with B6 mice, suggesting that a full ionomic screen of the BXD RI panel could reveal a huge number of loci controlling the liver accumulation of individual elements (i.e. making it more cost effective that repeating mapping studies for each individual element). However, the ultimate value of ionomic analysis is that the interaction of elements is incorporated into the analysis. As a result, while most elements have their own unique pattern of mineral accumulation across the BXD panel (e.g. see liver Fe levels in BXD mice in Fig. 1), some elements may change in unison across the lines. For example, we found that the liver levels of Mg and P had the same pattern of variation across the 29 BXD lines we examined (Fig. 4*B*). This suggests that there is a common genetic cause for the variation and that the metabolism of Mg and P is biologically linked. This has not been previously reported and thus provides us with the opportunity to discover something unique about mineral metabolism in mammals.

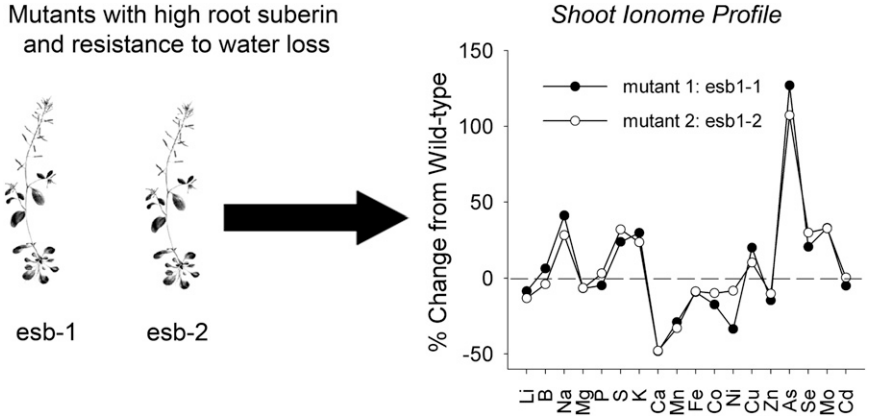

FIGURE 5 Mutant lines of *A. thaliana* with high root suberin levels have distinctive shoot ionome profiles. Shoots from wild-type *A. thaliana* and 2 mutant lines with polymorphisms in the suberin gene (esb1–1 and esb 1–2) were analyzed for mineral content using ICP-MS. Data are expressed in mutant lines as a percent change from the wild-type plant value. Although suberin is a chemical in roots that influences water transpiration rates, it leads to increased Se, Na, S, and K, but low Ca, in the shoot. [Adapted from data in Baxter et al. (35) with permission].

A final example of how ionomics can be linked to genetics for expanding our understanding of biology comes from a study in Arabidopsis. Previously, Lahner et al. (34) identified many ionomic mutants in a screen of 6000 M2 plants produced from fast neutron-mutagenized *A. thaliana*. Baxter et al. (35) subsequently cloned and characterized one of these mutants and found that it had high levels of root suberin, a compound that acts as an extracellular transport barrier limiting apoplastic radial transport of water and solutes. This mutant (*esb1–1*) and a related mutant (*esb1–2*) both had significant increases in their shoot concentrations of Na, S, K, As, Se, and Mo with concomitant reductions in Ca, Mn, Zn, and Fe (**Fig. 5**). In addition, this increase in root suberin levels leads to the reduced transpiration rates that accounts for the ability of this genetic mutant to use water efficiently and resist wilting. Thus, the shoot ionome profile could be used as a screening tool for identification of plants with drought resistance. Federal and international funding agencies are currently expending a huge amount of resources making genetically mutant mice using either homologous recombination [Knockout Mouse Project and International Gene Trap Consortium (36)] or N-ethyl-N-nitrosourea mutagenesis (37); it will be interesting to determine whether a serum or tissue ionomic profile can be used to identify physiologic disruption and thus aid our understanding of the functional roles of the genes that have been mutated.

Forward genetics coupled to ionomics is a novel approach to the study of mineral metabolism. In its purest sense, it is a discovery tool to identify the genes whose protein products regulate mammalian mineral metabolism. In this light, it is a complement to the tools of biochemistry, cell biology, and molecular biology that traditionally have been used to explore mineral metabolism. In addition, by looking at mineral metabolism as a system, we can take advantage of the well-recognized existence of interactions between mineral elements and the fact that the disruption within the ionome may reflect broader disturbances in physiology that are relevant to mammalian biology, human health, and nutrition.

Acknowledgments

J.C.F wrote the first draft of the manuscript and had primary responsibility for the final content; R.R. contributed ideas to the manuscript, edited the manuscript; D.E.S. contributed ideas to the manuscript, provided several sections for the first draft, and edited the manuscript. All authors read and approved the final manuscript.

Literature Cited

1. Fleet JC. Molecular regulation of calcium metabolism. In: Weaver CM, Heaney RP, editors. Calcium in human health. Totowa (NJ): Humana Press; 2006. p. 163–90.
2. Kutuzova GD, Sundersingh F, Vaughan J, Tadi BP, Ansay SE, Christakos S, DeLuca HF. TRPV6 is not required for 1alpha,25-dihydroxyvitamin D3-induced intestinal calcium absorption in vivo. Proc Natl Acad Sci USA. 2008;105:19655–9.
3. Benn BS, Ajibade D, Porta A, Dhawan P, Hediger M, Peng JB, Jiang Y, Oh GT, Jeung EB, et al. Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. Endocrinology. 2008;149:3196–205.
4. Flint J, Valdar W, Shifman S, Mott R. Strategies for mapping and cloning quantitative trait genes in rodents. Nat Rev Genet. 2005;6:271–86.
5. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Perusse L, Bouchard C. The human obesity gene map: the 2005 update. Obesity (Silver Spring). 2006;14:529–644.
6. Willis-Owen SA, Flint J. The genetic basis of emotional behaviour in mice. Eur J Hum Genet. 2006;14:721–8.
7. Tarantino LM, Bucan M. Dissection of behavior and psychiatric disorders using the mouse as a model. Hum Mol Genet. 2000;9:953–65.
8. Vidal SM, Malo D, Marquis JF, Gros P. Forward genetic dissection of immunity to infection in the mouse. Annu Rev Immunol. 2008;26:81–132.
9. Douglas DS, Popko B. Mouse forward genetics in the study of the peripheral nervous system and human peripheral neuropathy. Neurochem Res. 2009;34:124–37.
10. Wang F, Paradkar PN, Custodio AO, McVey WD, Fleming MD, Campagna D, Roberts KA, Boyartchuk V, Dietrich WF, et al. Genetic variation in Mon1a affects protein trafficking and modifies macrophage iron loading in mice. Nat Genet. 2007;39:1025–32.
11. Jones BC, Beard JL, Gibson JN, Unger EL, Allen RP, McCarthy KA, Earley CJ. Systems genetic analysis of peripheral iron parameters in the mouse. Am J Physiol Regul Integr Comp Physiol. 2007;293:R116–24.
12. Jones LC, Beard JL, Jones BC. Genetic analysis reveals polygenic influences on iron, copper, and zinc in mouse hippocampus with neurobiological implications. Hippocampus. 2008;18:398–410.
13. Andrews NC. Forging a field: the golden age of iron biology. Blood. 2008;112:219–30.
14. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Ellis MC, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13:399–408.
15. Ajioka RS, Kushner JP. Clinical consequences of iron overload in hemochromatosis homozygotes. Blood. 2003;101:3351–3.
16. Williams RW. The GeneNetwork [cited 2011]. Available from: www.genenetwork.org.
17. Peirce JL, Lu L, Gu J, Silver LM, Williams RW. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. BMC Genet. 2004;5:7.
18. Wood RJ, Fleet JC. The genetics of osteoporosis: vitamin D receptor polymorphisms. Annu Rev Nutr. 1998;18:233–58.
19. Walker MD, Novotny R, Bilezikian JP, Weaver CM. Race and diet interactions in the acquisition, maintenance, and loss of bone. J Nutr. 2008;138:S1256–60.
20. Weaver CM, McCabe LD, McCabe GP, Braun M, Martin BR, Dimeglio LA, Peacock M. Vitamin D status and calcium metabolism in adolescent black and white girls on a range of controlled calcium intakes. J Clin Endocrinol Metab. 2008;93:3907–14.
21. Chen C, Kalu DN. Strain differences in bone density and calcium metabolism between C3H/HeJ and C57BL/6J mice. Bone. 1999;25:413–20.
22. Armbrecht HJ, Boltz MA, Hodam TL. Differences in intestinal calcium and phosphate transport between low and high bone density mice. Am J Physiol Gastrointest Liver Physiol. 2002;282:G130–6.
23. Jurutka PW, Remus LS, Whitfield GK, Thompson PD, Hsieh JC, Zitzer H, Tavakkoli P, Galligan MA, Dang HT, et al. The polymorphic N terminus in human vitamin D receptor isoforms influences trascriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol. 2000;14:401–20.
24. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene Fok1 polymorphisms predicts calcium absorption and bone mineral density in children. J Bone Miner Res. 1999;14:740–6.
25. Huang ZW, Dong J, Piao JH, Li WD, Tian Y, Xu J, Yang XG. [Relationship between the absorption of dietary calcium and the Fok I polymorphism of VDR gene in young women]. Zhonghua Yu Fang Yi Xue Za Zhi. 2006;40:75–8.
26. Harris SS, Eccleshall TR, Gross C, Dawson-Hughes B, Feldman D. The vitamin D receptor start codon polymorphism (FokI) and bone mineral density in premenopausal American black and white women. J Bone Miner Res. 1997;12:1043–8.
27. Hill CH, Matrone G. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. Fed Proc. 1970;29:1474–81.
28. Salt DE, Baxter I, Lahner B. Ionomics and the study of the plant ionome. Annu Rev Plant Biol. 2008;59:709–33.
29. Baxter I. Ionomics: studying the social network of mineral nutrients. Curr Opin Plant Biol. 2009;12:381–6.
30. Eide DJ, Clark S, Nair TM, Gehl M, Gribskov M, Guerinot ML, Harper JF. Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in Saccharomyces cerevisiae. Genome Biol. 2005;6:R77.

31. Peters JL, Dufner-Beattie J, Xu W, Geiser J, Lahner B, Salt DE, Andrews GK. Targeting of the mouse Slc39a2 (Zip2) gene reveals highly cell-specific patterns of expression, and unique functions in zinc, iron, and calcium homeostasis. Genesis. 2007;45:339–52.
32. Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot ML, Salt DE. The leaf ionome as a multivariable system to detect a plant's physiological status. Proc Natl Acad Sci USA. 2008;105:12081–6.
33. de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. Food Nutr Bull. 2007;28:S480–4.
34. Lahner B, Gong J, Mahmoudian M, Smith EL, Abid KB, Rogers EE, Guerinot ML, Harper JF, Ward JM, et al. Genomic scale profiling of nutrient and trace elements in Arabidopsis thaliana. Nat Biotechnol. 2003;21:1215–21.
35. Baxter I, Hosmani PS, Rus A, Lahner B, Borevitz JO, Muthukumar B, Mickelbart MV, Schreiber L, Franke RB, et al. Root suberin forms an extracellular barrier that affects water relations and mineral nutrition in Arabidopsis. PLoS Genet. 2009;5:e1000492.
36. Guan C, Ye C, Yang X, Gao J. A review of current large-scale mouse knockout efforts. Genesis. 2010;48:73–85.
37. Hrabe de Angelis MH, Flaswinkel H, Fuchs H, Rathkolb B, Soewarto D, Marschall S, Heffner S, Pargent W, Wuensch K, et al. Genome-wide, large-scale production of mutant mice by ENU mutagenesis. Nat Genet. 2000;25:444–7.