ferent J-codes forced providers to discriminate between these products and allowed better tracking of drug-specific adverse reactions. In January 2008, CMS re-merged the J-codes for HMW and LMW iron dextran. For the majority of U.S. physicians who are unaware that two iron dextrans even exist, there is high likelihood that the lower-priced HMW iron dextran will be selected by group purchasing organizations for distribution to ambulatory infusion centers and hospitals. As a result, physicians may unknowingly administer the HMW iron dextran, thereby increasing the likelihood of life-threatening, preventable adverse events. This substitution could accentuate the underutilization of intravenous iron that abounds already because of misinterpretation and misunderstanding of the incidence and nature of serious adverse events largely associated with HMW iron dextran. Given so much uncertainty surrounding the safety of ESAs in oncology as well as in nephrology practices, decreased utilization of intravenous iron would be counterproductive. There are now five publications in the oncology literature (three published and two in press),[9,12–15] demonstrating intravenous iron synergizes with ESAs in improving hemoglobin responses, decreasing the time to target hemoglobin and subsequently decreasing ESA exposure and cost of therapy. In all five of these studies, the benefits were independent of the pretreatment baseline iron parameters, including Fe/TIBC, ferritin, and bone marrow hemosiderin.

All countries in Western Europe have halted distribution of HMW iron dextran and removed the black box warning from the package insert or equivalent documents for the LMW formulation. The clinical community's larger perception of risk associated with the use of intravenous iron is antiquated and probably incorrect. While adverse event rates are driven higher by HMW iron dextran, both HMW and LMW iron dextrans, and to a lesser extent all intravenous iron formulations, suffer this stigma. HMW iron dextran is unsafe compared with other iron products and is medically unnecessary given the availability of three safer products in the United States. We urgently recommend avoiding use of HMW iron dextran in all clinical practice settings. We also recommend that the FDA withdraw this formulation of intravenous iron. Additional research into the optimal use of parenteral iron, particularly among persons treated with ESAs, is clearly warranted.

DISCLOSURES

Drs. Rodgers, Cella, Chertow, and Glaspy have nothing to disclose. Drs. Auerbach, Coyne, and Henry are consultants for Watson Pharmaceuticals, and Dr. Coyne has received research support from Watson Pharmaceuticals.

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Some Assembly Required: Renal Hypodysplasia and the Problem with Faulty Parts

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Renal hypodysplasia encompasses a broad spectrum of disorders that are all characterized by varying degrees of defective kidney formation. Dysplastic kidneys exhibit multiple types of pathology,[1,2] with defects evident as cystic tubule dilation, fibrosis, and dysregulated cell proliferation and cell death.[2] Renal hypodysplasia is characterized by a reduced number of nephrons, with compensatory changes in glomerular size driven by increased single-nephron GFR.[2] Overall, renal hypodysplasia is a leading cause of pediatric renal failure and can contribute to the development of hypertension in adults.[3]

In the search for genes that cause renal hypodysplasia, the link to renal development is compelling: What better candidate gene for a kidney that does not fully develop than a gene required for development? Kidneys form during embryogenesis by a tissue interaction between a ureteric bud epithelium and a loose population of stromal cells in the metanephric mesenchyme.[4] The information required to orchestrate kidney growth and development is passed between these two tissues in the form of secreted growth factors and growth factor antagonists. The dynamic interplay of secreted molecules that promote and inhibit epithelial outgrowth, along with the activity of transcription factors that regulate growth factor expression, shapes the tree-like architecture of the collecting system, drives initial nephron formation, defines overall kidney organ size, and determines the final number of nephrons in the mature kidney.[4] The RET/glial-derived neurotrophic factor (GDNF) signaling interaction is a central regulator of kidney morphogenesis. GDNF is a diffusible growth factor that is synthesized in the mesenchyme and binds to the its receptor Ret on the ureteric bud epithelium and drives growth and patterning of the collecting system.[5]

Previous studies linking mutations in well-studied kidney developmental regulators to renal hypodysplasia have encouraged the search for other regulatory genes that might be associated with this disease. The hereditary disorders renal coloboma syndrome (PAX2), renal cysts and diabetes syndrome (HNF-1β), and branchio-oto-renal syndromes (EYA1) all link mutations in developmental regulatory transcription factors with renal hypodysplasia.[2] Recent studies examining the GDNF and RET genes in cases of severe hypodysplasia have turned up multiple activating and inactivating mutations.[6] Still, mutations in *RET*, *GDNF*, *PAX2*, *HNF1*β, and *EYA1* do not account for all cases of renal hypodysplasia. The Fraser syndrome gene *FRAS1* (not to be confused with Frasier syndrome/WT1 mutations) and *glypican-3* (*GPC3*) both are mutated in renal hypodysplasia and encode proteins that might be expected to modulate or control the activity of other secreted kidney developmental regulators such as bone morphogenetic proteins (BMP) or fibroblast growth factors.[2] These findings beg the question of whether additional developmental signaling molecules mutate in patients with renal hypodysplasia.

In this issue of *JASN*, Weber *et al.*[7] identify new mutations in the gene encoding *BMP4* and the transcriptional regulator *SIX2* associated with renal hypodysplasia. BMP4 is a member of the BMP family, which comprises a subgroup of proteins within the TGF-β superfamily. BMP4 has complex functions in kidney development, including restricting the site of initial budding of the ureter to form a single ureter, support of smooth muscle development around the ureter, and promoting growth and survival of nephrogenic mesenchyme.[8,9] SIX2 is a transcription factor whose expression in the renal mesenchyme is required for synthesis of GDNF.[10] The association of missense mutations in these genes with renal hypodysplasia provide new potential links between development and disease; however, proving that the missense mutations are causal for the disease is another story.

Despite the wealth of data from genetic studies of kidney development for use in generating candidate genes for renal hypodysplasia, pinpointing disease-causing mutations in human syndromes often requires more than standard linkage analysis and sequencing. The main challenge is to assign biologic significance to what might be subtle missense mutations. For instance, how do you interpret a glycine to valine substitution? Is the addition of a single methyl group to a protein sufficient to disrupt its function? Or is this just a polymorphism? The short answer is, you don't know. Missense mutations, as opposed to more severe nonsense mutations, are the most common type of mutation associated with human disease. This trend is likely only to become more pronounced in current and future studies of complex, polygenic diseases for which disease severity is likely associated with a combination of missense mutations in several different genes; that is, the "mutational load" will determine many disease outcomes. When regulators of basic developmental processes are your best candidate genes, missense mutations that render a protein partially functional are likely to be the only types of mutations found, because anything more nasty would end things *in utero*. What is needed in this context is a reliable way to assess the function of a subtly altered protein.

Designing *in vitro* assay systems for mutant genes can be a trial-and-error process; developmental context is often important for molecular function, and mouse knock-ins to test multiple allelic variants can be prohibitively expensive. So why bother with assay development when Mother Nature has already done it for you? The fish embryo is an *in vivo*, self-contained, and sensitive assay system for any signaling pathway that is important in development.[11] Weber *et al.*[7] exploit this to show that missense alleles of human renal hypodysplasia genes show an altered function in the context of zebrafish embryogenesis, strengthening their argument that the missense mutations they identified are causative for disease. Comparative analysis using fish embryos to sort through candidate genes for human disease and to assay human mutant alleles has also been useful in other disease contexts[12–17]; Weber *et al.*[7] show that

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this approach can be particularly useful in studies of renal hypodysplasia.

Although these studies demonstrate a new use of the zebrafish embryo for assessing the severity of human hypodysplasia mutations, there is still room for improvement in this general approach. The method used by *Weber et al.*[7] relies on overexpression of proteins during developmental stages and in cells in which the normal endogenous protein would not be expressed. This forced expression is the reason that developmental defects are observed, even though the normal protein—and not mutant protein—is being expressed. Would these proteins act in the same way when expressed in their normal context? The assumption is yes, they would, but it will be important in future studies to demonstrate this directly. For instance, expression of mammalian genes in zebrafish embryos has been used to reverse or rescue zebrafish mutant phenotypes.[12] Would wild-type but not mutant mRNA encoding human SIX2 or BMP4 rescue zebrafish mutants in these genes? If so, then the experiments would be one step closer to assaying these genes in a normal developmental context. With either approach, it will also be important to confirm the expression of introduced mutant and wild-type proteins to avoid the potential pitfall that lack of observable effects could simply be due to reduced expression of mutant protein.

Zebrafish kidneys are not human kidneys, but the conservation of developmental mechanisms used to build them (and other organs) is remarkable.[18] On the basis of the work of *Weber et al.*[7] and other, similar studies, the fish embryo now occupies an experimental niche uniquely positioned between human hereditary disease pathology and cell culture assays of mutant genes. Genetic manipulation of the fish embryo coupled with careful, quantitative analysis of phenotypes is now an established way to assay gene function rapidly, *in vivo*, in a relevant developmental context.

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DISCLOSURES

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See related article, "*SIX2* and *BMP4* Mutations Associate With Anomalous Kidney Development," on pages 891–903.