PNAS Plus Significance Statements

Multifunctional, inexpensive, and reusable nanoparticle-printed biochip for cell manipulation and diagnosis

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Point-of-care diagnostics in the developing world and resource-limited areas require numerous special design considerations to provide effective early detection of disease. Of particular need for these contexts are diagnostic technologies featuring low costs, ease of use, and broad applicability. Here we present a nanoparticle-inkjet-printable microfluidics-based platform that fulfills these criteria and that we expect to significantly reduce the footprint, complexity, and cost of clinical diagnostics. This reusable $0.01 platform is miniaturized to handle small sample volumes and can perform numerous analyses. It can perform complex, minimally invasive analyses of single cells without specialized equipment and personnel. This inexpensive, accessible platform has broad applications in precision diagnostics and is a step toward the democratization of medical technologies. (See pp. E1306–E1315.)

Estrogen receptor α wields treatment-specific enhancers between morphologically similar endometrial tumors

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This study shows that the hormonal environment in which a tumor originates may affect a hormone receptor's enhancer usage. We further show that enhancer function is less tissue specific than previously thought. By implementing ChIP sequencing in a unique patient cohort, we compared estrogen receptor α (ERα) profiles in endometrial tumors that developed in different hormonal environments and integrated these comparisons with transcriptomic data. Our data show that tumors associated with therapeutic intervention have a distinct ERα DNA-binding signature with regulatory potentials that resemble ERα-binding patterns in breast cancer. These results highlight the value of cistromic analyses in clinical specimens, which enabled us to distinguish subtypes of tumors on the level of transcriptional regulation. (See pp. E1316–E1325.)

Kinetics, subcellular localization, and contribution to parasite virulence of a *Trypanosoma cruzi* hybrid type A heme peroxidase (*Tc*APx-CcP)

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Trypanosoma cruzi, the causative agent of Chagas disease, affects 8–10 million people in Latin America. Parasite antioxidant systems are essential for parasite survival and infectivity in the vertebrate host. Herein, we characterized the enzymic properties, subcellular localization, and contribution to parasite virulence of a *T. cruzi* hybrid type A member of class I heme peroxidases. The enzyme reacts fast with hydrogen peroxide and utilizes both ferrocytochrome *c* and ascorbate as reducing substrates [*T. cruzi* ascorbate peroxidase (*Tc*APx)-cytochrome *c* peroxidase (CcP)]. A unique subcellular distribution of *Tc*APx-CcP in the infective stages suggests a role during parasite–host interactions. Infection of macrophages and cardiomyocytes, as well as in mice, confirmed the involvement of *Tc*APx-CcP in pathogen virulence as part of the parasite antioxidant armamentarium. (See pp. E1326–E1335.)

Reaction dynamics analysis of a reconstituted *Escherichia coli* protein translation system by computational modeling

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Biological systems are driven by multiple components and interactions that form a complex reaction network. We developed a method to analyze their dynamics by focusing on the component in temporal plateaus, or a quasi-stationary state (QSS). The analyses, using a computational model of a minimal in vitro protein synthesis system, showed that components in a QSS form clusters that grow over time. However, the growth is not in a linear fashion: The process involved collapse and regrowth of the formed clusters, where the collapse was closely related to the phase transition in the reaction network. These observations suggest that the studies focusing on the QSS might be useful for understanding of complex reaction dynamics. (See pp. E1336–E1344.)

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Cdon deficiency causes cardiac remodeling through hyperactivation of WNT/β-catenin signaling

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Upon injury, reactivated Wnt/β-catenin signaling is implicated in cardiac remodeling and cardiomyopathy, thus it is an attractive target for intervention of cardiac diseases. Here, we demonstrate a role of a cell surface receptor Cdon in preventing cardiac remodeling through suppression of Wnt signaling. *Cdon*$^{-/-}$ mice develop cardiac dysfunction and fibrosis with altered expression of remodeling genes. Cdon deficiency causes aberrant localization and function of gap junction protein connexin 43, correlating with hyperactivated Wnt signaling. Blocking of Wnt signaling in Cdon-depleted cardiomyocytes attenuates aberrant intercellular coupling. Conversely, Wnt activator causes aberrant activation of gap junction with decreased Cdon levels, suggestive of a feedback mechanism. These data suggest that Cdon is required for the control of Wnt signaling to prevent cardiac remodeling. (See pp. E1345–E1354.)

Phosphorylation of cardiac myosin binding protein C releases myosin heads from the surface of cardiac thick filaments

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Cardiac myosin binding protein C (cMyBP-C) is an important regulator of myocardial contraction, but its mechanism of action is unclear. In this study, we examined the structure of thick filaments from the hearts of mice in which the three serine residues that are phosphorylated by protein kinase A in the m-domain of cMyBP-C were replaced by either alanine or aspartic acid to mimic either the nonphosphorylated or phosphorylated state of cMyBP-C. In contrast to earlier work on rat cardiac filaments, the results support a model in which nonphosphorylated cMyBP-C stabilizes the relaxed/superrelaxed ordered "off-state" conformation of the heads while phosphorylation weakens the binding of the heads to the thick filament surface, increasing the probability of interaction with actin. (See pp. E1355–E1364.)

Molecular basis of fatty acid selectivity in the zDHHC family of S-acyltransferases revealed by click chemistry

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S-acylation, the attachment of different fatty acids onto cysteine residues, regulates the activity of a diverse array of cellular proteins. This reversible posttranslational modification is essential for normal physiology, and defects are linked to human disease. S-acylation is catalyzed by a large family of zDHHC S-acyltransferases that use a cellular pool of diverse fatty acyl-CoAs as substrates. Using chemically synthesized probes, we show that individual zDHHC enzymes have marked differences in fatty acid selectivity and identify the underlying molecular basis for this property. This study identifies how acyl chain heterogeneity of S-acylated proteins is generated and is significant because the chemical nature of the attached S-acyl chain can fundamentally impact protein behavior. (See pp. E1365–E1374.)

Myofibril breakdown during atrophy is a delayed response requiring the transcription factor PAX4 and desmin depolymerization

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Muscle wasting as occurs with disuse, spinal injuries, aging, and many diseases (including cancer, sepsis, and renal failure) results primarily from the accelerated destruction of the myofibrillar apparatus, although the molecular mechanisms for this effect are largely unclear. To gain insight into the sequence of events leading to myofibril destruction, we studied the atrophy induced by denervation. We found that phosphorylation and ubiquitination of the desmin cytoskeleton precede its depolymerization, which eventually causes myofibril destruction. We further uncovered a delayed phase in the atrophy process, which involves the induction of genes that facilitate myofibril breakdown, including the AAA-ATPase p97/VCP, by the transcription factor paired box 4 (PAX4). Consequently, desmin phosphorylation, p97/VCP, and PAX4 may represent new therapeutic targets to reduce myofibril breakdown during atrophy. (See pp. E1375–E1384.)

Myosin-driven transport network in plants

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A key feature of plant cells is rapid cytoplasmic streaming that is driven by myosin motors. However, specific mechanisms of myosin-dependent streaming are poorly understood. Here, we characterize a dense network of plant myosins and their receptors and adaptors that, jointly with myosins, appear to mediate cytoplasmic streaming through distinct endomembrane compartments. We additionally present data suggestive of a myosin-dependent nucleocytoplasmic trafficking pathway. The myosin network is an ancient functional module that was already present in the common ancestor of green algae and land plants but underwent a major expansion in the latter, probably contributing to land colonization by plants. (See pp. E1385–E1394.)

Targeting IRE1 with small molecules counteracts progression of atherosclerosis

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Endoplasmic reticulum (ER) stress is linked to the development of complex metabolic diseases, including diabetes, obesity, and atherosclerosis. Irremediable ER stress can push the unfolded protein response (UPR) toward proinflammatory and proapoptotic signaling. The need to dissociate the adaptive UPR responses from its destructive outputs has become a major challenge for therapeutic strategies aimed at mitigating ER stress that is often observed in chronic diseases. Our findings show that inositol-requiring enzyme 1 (IRE1) plays a critical role in metaflammation and that administering IRE1-specific inhibitors to hyperlipidemic mice counteracts atherosclerosis progression. (See pp. E1395–E1404.)

An amidase is required for proper intercellular communication in the filamentous cyanobacterium *Anabaena* sp. PCC 7120

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The filamentous cyanobacterium *Anabaena* has become a widely studied model to determine the molecular mechanisms involved in establishing and maintaining the pattern of heterocyst differentiation in response to the removal of fixed nitrogen from the environment. Heterocysts develop from vegetative cells, usually spaced about 10 cells apart, converting an oxic cell capable of division into an anoxic factory for nitrogen fixation that does not divide. Genetic analysis to elucidate the mechanisms of intercellular material exchange between heterocysts and vegetative cells is in an early phase. Here we show that an amidase is involved in the function of channels that penetrate the rigid peptidoglycan walls that separate cells in the filaments. (See pp. E1405–E1412.)

RhoA knockout fibroblasts lose tumor-inhibitory capacity in vitro and promote tumor growth in vivo

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In order for cancer to develop, normal tumor-inhibitory fibroblasts need to change into tumor-promoting, cancer-associated fibroblasts. We created Ras homolog family member A (*RhoA*) gene knockout fibroblasts and found that even though these cells lacked common markers of classic cancer-associated fibroblasts, they had lost their normal tumor-inhibitory capacity and induced tumor-cell migration and proliferation in vitro and tumor growth in vivo. *RhoA* knock-out cells also showed an altered cytoskeleton, reduced contractile force, and induced stiffness of the fibroblasts. *RhoA* knockout also induced a loss of α-smooth muscle actin and an activated proinflammatory state, which was reflected by interference with a number of Rho signaling cascades. Our data indicate that RhoA is a key regulator of the switch from tumor-inhibitory to tumor-promoting fibroblasts. (See pp. E1413–E1421.)

Gata4 potentiates second heart field proliferation and Hedgehog signaling for cardiac septation

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Dominant GATA4 mutations cause congenital heart defects in humans, but the mechanistic basis whereby Gata4 haploinsufficiency causes disease is unknown. We demonstrate that Gata4 is required in a subset of cardiac progenitor cells called the "second heart field" for cardiac septation. Furthermore, we identified two distinct pathways downstream of *Gata4*, phosphatase and tensin homolog (*Pten*)-modulated cell-cycle transition and Hedgehog signaling, which appear to act independently. Restoration of either *Pten*-mediated cell-cycle transition or Hedgehog signaling restored cardiac septation in *Gata4*-mutant mice, suggesting that defects in both pathways are required for disease causation. This work generates a working model for understanding the molecular basis of human congenital heart disease caused by dominant transcription factor mutations. (See pp. E1422–E1431.)

Intestinal NCoR1, a regulator of epithelial cell maturation, controls neonatal hyperbilirubinemia

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In many parts of the world, especially in low- and middle-income countries, severe neonatal hyperbilirubinemia (SNH) is associated with substantial mortality and long-term morbidities. Although the immediate and rapid rise in total serum bilirubin (TSB) originating from lysis of red blood cells has been linked to genetic predisposition, preterm births, and blood type incompatibilities, the inability to efficiently metabolize bilirubin results from delayed expression of UDP-glucuronosyltransferase 1A1 (UGT1A1). In this study, the mechanism associated with delayed expression of the human *UGT1A1* gene in neonatal mice that are humanized for the *UGT1* locus is described. Neonatal humanized *UGT1* (*hUGT1*) mice develop SNH and control TSB levels by nuclear receptor corepressor 1 (NCoR1)-directed repression of intestinal epithelial cell maturation, an event linked to expression of the *UGT1A1* gene. (See pp. E1432–E1440.)

Reconciling fisheries catch and ocean productivity

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Phytoplankton provide the energy that sustains marine fish populations. The relationship between phytoplankton productivity and fisheries catch, however, is complicated by uncertainty in catch estimates, fishing effort, and marine food web dynamics. We enlist global data sources and a high-resolution earth system model to address these uncertainties. Results show that cross-ecosystem fisheries catch differences far exceeding differences in phytoplankton production can be reconciled with fishing effort and variations in marine food web structure and energy transfer efficiency. Food web variations explaining contemporary fisheries catch act to amplify projected catch trends under climate change, suggesting catch changes that may exceed a factor of 2 for some regions. Failing to account for this would hinder adaptation to climate change. (See pp. E1441–E1449.)

Selection maintains signaling function of a highly diverged intrinsically disordered region

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Intrinsically disordered regions (IDRs) are widespread, have diverse functions, and are involved in human disease. Because standard sequence analysis methods identify little sequence homology in IDRs, it is not currently understood whether (or how) the functions of these protein regions are preserved over evolution. Here we show that orthologous IDRs can preserve regulatory functions despite near-complete sequence divergence. This suggests that natural selection maintains aggregate molecular properties in IDRs, which we propose to be quantitative traits. Consistent with this, we find signatures of stabilizing selection on the electrostatic properties of IDRs. Thus, in analogy to the rapid evolution of noncoding DNA in eukaryotic enhancers, divergence in primary amino acid sequence does not imply functional divergence in IDRs. (See pp. E1450–E1459.)

Dynamics of genome size evolution in birds and mammals

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Deciphering the forces and mechanisms modulating genome size is central to our understanding of molecular evolution, but the subject has been understudied in mammals and birds. We took advantage of the recent availability of genome sequences for a wide range of species to investigate the mechanism underlying genome size equilibrium over the past 100 million years. Our data provide evidence for an "accordion" model of genome size evolution in birds and mammals, whereby the amount of DNA gained by transposable element expansion, which greatly varies across lineages, was counteracted by DNA loss through large segmental deletions. Paradoxically, birds and bats have more compact genomes relative to their flightless relatives but exhibit more dynamic gain and loss of DNA. (See pp. E1460–E1469.)

Low escape-rate genome safeguards with minimal molecular perturbation of *Saccharomyces cerevisiae*

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As the use of synthetic biology grows, there is an increasing need to ensure biocontainment both to protect engineered proprietary strains and to mitigate potential inadvertent or advertent release to the environment. Here, we screen for the best-performing construct for essential gene-dependent transcriptional safeguards (SGs) in yeast. We have engineered strains that have near-WT fitness and a low escape rate, and grow in the presence of a nanomolar concentration of essential supplement. We also improved our "safeguard" construct by promoter engineering, as well as by analyzing a series of potential "decoy molecules" that could be used to mask the existence of critical chemical ligands required to grow the SG strain. (See pp. E1470–E1479.)

TGF-β inhibitor Smad7 regulates dendritic cell-induced autoimmunity

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Smad7 is a negative regulator of TGF-β signaling, a cytokine with anti-inflammatory properties. Although TGF-β was implicated in the development and function of dendritic cells (DCs), the in vivo role of Smad7 in DCs remains elusive. Here, we demonstrate that DC-specific Smad7 deletion affects the development of splenic $CD8^+CD103^+$ DCs by regulating expression of the transcription factors Batf3 and IRF8. In addition, Smad7 directs DC function by regulating the expression of indoleamine 2,3-dioxygenase in response to IFN-γ signaling. Hence, absent Smad7 in DCs mediates resistance of mice to the development of autoimmunity via protective regulatory T-cell induction. These findings demonstrate that Smad7 expression governs splenic DC subset differentiation and affects tolerogenic DC function in vivo. (See pp. E1480–E1489.)

Dnmt3a restrains mast cell inflammatory responses

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Methylation of genomic DNA is an epigenetic modification at the interface between genetic information and environmental stimuli underlying many phenotypic variations in the human population as well as the pathogenesis of complex diseases. Accordingly, mutations in the de novo DNA methyltransferase enzyme *DNMT3A* have been identified in a number of diseases, including mast cell-related disorders. However, the role of DNA methylation and DNMT3A in regulating mast cell physiology still needs to be elucidated. Here, we found that *Dnmt3a* plays a critical role in modulating mast cell responsiveness to acute and chronic stimulation, potentially implicating DNA methylation-mediated processes in all types of mast cell-related diseases. (See pp. E1490–E1499.)

SGK3 sustains ERα signaling and drives acquired aromatase inhibitor resistance through maintaining endoplasmic reticulum homeostasis

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Acquired aromatase inhibitor (AI) resistance is a major clinical issue in the treatment of estrogen receptor alpha (ERα)-positive breast cancer. Most AI-resistant breast tumors still express ERα and rely on its signaling for growth. The current study shows that serum- and glucocorticoid-inducible kinase 3 (SGK3), a kinase transcriptionally regulated by ERα in breast cancer, sustains ERα signaling and drives the acquired AI resistance by protecting against endoplasmic reticulum (EnR) stress-induced ERα down-regulation and cell death through preserving sarcoplasmic/EnR calcium ATPase 2b (SERCA2b) function. Our study reveals regulation of ERα expression mediated by the EnR stress response and highlights SGK3 inhibition as a potential effective treatment of acquired AI-resistant breast cancer. (See pp. E1500–E1508.)

Gene activation of SMN by selective disruption of lncRNA-mediated recruitment of PRC2 for the treatment of spinal muscular atrophy

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Autosomal recessive mutations or deletions of the gene *Survival Motor Neuron 1* (*SMN1*) cause spinal muscular atrophy, a neurodegenerative disorder. Transcriptional up-regulation of a nearly identical gene, *SMN2*, can functionally compensate for the loss of *SMN1*, resulting in increased SMN protein to ameliorate the disease severity. Here we demonstrate that the repressed state of *SMN2* is reversible by interrupting the recruitment of a repressive epigenetic complex in disease-relevant cell types. Using chemically modified oligonucleotides to bind at a site of interaction on a long noncoding RNA that recruits the repressive complex, *SMN2* is epigenetically altered to create a transcriptionally permissive state. (See pp. E1509–E1518.)

Bordetella PlrSR regulatory system controls BvgAS activity and virulence in the lower respiratory tract

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Bordetella spp. includes *Bordetella pertussis*, the causal agent of whooping cough. The *Bordetella* virulence gene (BvgAS) two-component regulatory system (TCS) is considered the "master virulence regulator" in *Bordetella*, as it controls expression of all

known virulence factor-encoding genes. We show here that another TCS, PlrSR, is required for BvgAS activity in the lower respiratory tract (LRT) and for virulence even when BvgAS is rendered constitutively active, suggesting that it controls critical functions for bacterial survival in the LRT independently of BvgAS. Our data introduce a new layer of complexity to a paradigm of *Bordetella* virulence control that has held for more than 30 y, and they indicate the existence of previously unknown bacterial factors that may serve as vaccine components and therapeutic targets. (See pp. E1519–E1527.)

ACSS2-mediated acetyl-CoA synthesis from acetate is necessary for human cytomegalovirus infection

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Viruses rely completely on host cell metabolism to provide the building blocks and energy required for producing progeny virions. Infection by human cytomegalovirus (HCMV) induces significant alterations in glucose metabolism by increasing glucose uptake and glycolysis as well as redirecting glucose carbon to support the synthesis of biomolecules such as lipids. The significance of acetate as a nutrient has been ignored for a long period. Our studies show that glucose carbon can be converted to acetate and used to make cytosolic acetyl-CoA by acetyl-CoA synthetase short-chain family member 2 (ACSS2) for lipid synthesis, which is important for HCMV-induced lipogenesis and the viral growth. The study provides greater understanding of HCMV pathogenesis and suggests strategies to develop antiviral therapies. (See pp. E1528–E1535.)

Proteomic analysis reveals O-GlcNAc modification on proteins with key regulatory functions in *Arabidopsis*

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Studies in mammalian systems have shown important functions of O-linked *N*-acetylglucosamine (O-GlcNAc) modification of proteins (O-GlcNAcylation) in a wide range of cellular, physiological, and disease processes. Genetic evidence indicates that O-GlcNAcylation is essential for plant growth and development. However, very few O-GlcNAc–modified proteins have been identified in plants. Here, we report identification of 262 O-GlcNAc–modified proteins in *Arabidopsis*, revealing both conserved and distinct functions of O-GlcNAc modification in plants. This study uncovers potentially important functions of O-GlcNAcylation in many cellular and developmental pathways and also provides a large number of modification sites for further genetic and molecular dissection of these specific functions. Our study provides the framework of an O-GlcNAc modification network underlying plant growth and development. (See pp. E1536–E1543.)

Loss of GET pathway orthologs in *Arabidopsis thaliana* causes root hair growth defects and affects SNARE abundance

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Root hairs are unicellular extensions of the rhizodermis, providing anchorage and an increase in surface area for nutrient and water uptake. Their fast, tip-focused growth showcases root hairs as an excellent genetic model to study physiological and developmental processes on the cellular level. We uncovered a root hair phenotype that is dependent on putative *Arabidopsis* orthologs of the Guided Entry of Tail-anchored (TA) proteins (GET) pathway, which facilitates membrane insertion of TA proteins in yeast and mammals. We found that plants have evolved multiple paralogs of specific GET pathway components, albeit in a compartment-specific manner. In addition, we show that differential expression of pathway components causes pleiotropic growth defects, suggesting alternative pathways for TA insertion and additional functions of GET in plants. (See pp. E1544–E1553.)

PPR-SMR protein SOT1 has RNA endonuclease activity

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Our results demonstrate that SUPPRESSOR OF THYLAKOID FORMATION 1 (SOT1), an *Arabidopsis* pentatricopeptide repeat (PPR) protein with a small MutS-related (SMR) domain, has endonuclease activity. The SMR moiety of SOT1 performs the endonucleolytic maturation of 23S and 4.5S rRNA through the PPR domain specifically recognizing a 13-nucleotide RNA sequence in the 5′ end of the chloroplast 23S–4.5S rRNA precursor. Our results also show that SOT1 can be engineered to recognize and cleave a predicted RNA substrate. Our findings suggest that SOT1 could be used as a tool for RNA manipulation in the future. (See pp. E1554–E1563.)

Human and rat gut microbiome composition is maintained following sleep restriction

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It is widely presumed that there is a relationship between sleep and the gut microbiome because both sleep restriction and dysbiosis of the gut microbiome are associated with metabolic diseases such as obesity and diabetes. Here, we report sleep restriction over several consecutive days does not overtly influence the composition of the microbiome of either rats or humans, despite both species showing other changes associated with sleep loss. These analyses suggest that sleep loss and microbial dysbiosis have independent effects on the development of metabolic diseases. (See pp. E1564–E1571.)