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Newborn Screening: After the Thrill Is Gone The Donough O'Brien Presidential Lecture Presented at the 2007 Annual Meeting of the Society for Inherited Metabolic Disorders Nashville, TN

Jerry Vockley

Department of Pediatrics, School of Medicine, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Children's Hospital of Pittsburgh, Pittsburgh, PA, Phone: (412) 692-5070, Fax: (412) 692-7816, e-mail: gerard.vockley@chp.edu

Abstract

Expanded newborn screening for inborn errors of metabolism by tandem mass spectrometry has raised the stakes for specialists in metabolic medicine. New disorders and a broader clinical spectrum of disease call for new paradigms in approaching inborn errors of metabolism. The Society for Inherited Disorders has been at the forefront of advances in newborn screening for many years and faces new challenges in meeting new needs.

Introduction

Dr. Vladutiu, fellow SIMD members, distinguished guests, it is an honor to deliver the Donough O'Brien presidential lecture at this year's annual Society for Inherited Metabolic Disorders (SIMD) meeting. I must admit that it hardly seems almost 20 years ago that I attended my first Society meeting and it seems even less possible that my two-year term as president concludes with this one. The poet and philosopher George Santayana wrote, "Those who cannot remember the past are condemned to repeat it." So before starting with the scientific portion of my talk, I will take the luxury of spending a few minutes sharing my journey through inborn errors to date and detailing the forces that have shaped my view of this field.

I arrived as a freshman in biology at Carnegie Mellon University in Pittsburgh during the relatively tumultuous early 70's. That of course was the pre-recombinant DNA technology era when genetics was a much different discipline. There, I was lucky enough to stumble upon both my love of genetics and the love of my life, my wife Cate. 32 years later I continue to marvel at my great fortune over both.

As a first year MD, PhD student at the University of Pennsylvania in 1978, I sat captivated as a rather "hobbit-ish" appearing professor named Harry Harris lectured on variation in a human genetics course. This led to a thesis project in his laboratory where I not only learned to think as an independent scientist but was regaled with tales of the early days of genetic research in the Galton labs at Oxford with the likes of Penrose, Haldane, and Childs.

My interest in inborn errors of metabolism was sealed during my pediatric residency at The Denver Children's Hospital where I had the luxury of learning clinical management of these

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challenging patients from two founding members of the SIMD, Drs. Ed McCabe and Steve Goodman. Both have remained life long mentors and friends. There, I even had the opportunity to serve under Dr. O'Brien, the namesake of this lecture, as my attending physician on the pediatric ward. The final stages in my early development were guided by Dr. Kay Tanaka at Yale University where I met Dr. Piero Rinaldo who had arrived as a fellow in the lab several months earlier. Our paths have seemed inexorably entwined ever since.

I attended my first SIMD meeting in 1988 in Orlando, FL, and as near as I can remember, haven't missed one since. I started my independent career at the Mayo Clinic in 1991 with Dr. Virginia Michels, a long time SIMD member, as my department Chair. My most recent move to the Children's Hospital of Pittsburgh has returned me to the city where I began my academic journey with the incumbent challenges of starting anew.

The title of the scientific portion of my talk this evening is "Newborn Screening: After the Thrill Is Gone." The history of IEMs is fraught with fits and starts. New technology brings us new abilities to identify new biochemical phenotypes. That, however, is just the beginning. Only more extensive clinical and laboratory studies allow us to distinguish variation in human biology from pathologic conditions, a chore often less glamorous than that of discovery. Nowhere has this been more dramatic than with the application of tandem mass spectrometry to newborn screening. This audience needs no reminder of the impact of newborn screening on the clinical outcome of potentially devastating diseases. In this context I have generally fallen into the camp of believing "the more, the merrier," not to mention "the sooner, the better." However, this approach is not without its risks and its detractors. The "cause" of mass screening, especially of newborns, leads to the necessary "effect" of the dedication of appropriate resources to treat the disorders so identified. Otherwise, we cannot meet the goal of screening to maintain the health of our children: allowing them to reach their maximum potential. It also implies an inherent understanding of the natural history of a disease in all of its manifestations. Of particular import to newborn screening is the recognition that mild variants of otherwise serious conditions may exist. And finally, it is imperative to differentiate disease markers from benign variation. The tremendous expansion in our knowledge over the past three decades has been matched only by the tremendous need to know even more as we move forward.

Acyl-CoA Dehydrogenases

I have been fortunate to be involved in identifying five new enzyme deficiencies in the new millennium and each has presented unique challenges in understanding their impact on metabolism and disease [1–5]. I would like to focus on my laboratory's work on the acyl-CoA dehydrogenase (ACD) family of enzymes over the past nearly two decades as it reflects many of these issues. The ACDs are an evolutionarily conserved family of enzymes involved in the metabolism of fatty acids and branched chain amino acids [6]. They perform a characteristic enzymatic reaction that begins by abstraction of an α -proton from substrate by a catalytic base with resolution of a stable charge-transfer complex intermediate by transfer of electrons from the reduced ACD to electron transfer flavoprotein. Each enzyme possesses a characteristic, often overlapping, pattern of substrate utilization. The ACDs are nuclear encoded proteins that are active in the mitochondria and as such they undergo a characteristic biogenesis consisting of import into mitochondria after translation in the cytoplasm, followed by processing to a final mature homotetrameric or dimeric form.

Deficiencies of most of the ACDs in humans have been identified and can result in clinical disease. Isovaleric acidemia (IVA) is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD), active in the leucine catabolic pathway. It holds an important place in the history of inborn errors of metabolism as it was the first organic acidemia to be described by Dr. Tanaka

through his novel application of the technique of gas chromatography for analysis of organic acids in urine [7]. Early literature on IVA, an autosomal recessive disorder, emphasized two apparent phenotypes [8]. The first was an acute, neonatal presentation with patients becoming symptomatic within the first two weeks of life. Patients appeared initially well, then developed vomiting and lethargy, progressing to coma. The second group presented with relatively non-specific failure to thrive and/or developmental delay (chronic intermittent presentation). Patients who survived an early acute presentation subsequently were indistinguishable from those with the chronic phenotype, and both groups of patients were prone to intermittent acute episodes of decompensation with minor illnesses. In reality it is now apparent that patients can fall anywhere on the spectrum of acute to chronic presentation and that there is probably little predictive value to the initial presentation.

Molecular cloning of the *IVDH* gene showed it to be located on chromosome 15q14-15, consisting of 12 exons that span ~15 kb of genomic DNA [9]. My laboratory has identified numerous point mutations in the protein coding region in patients that lead to the production of an inactive or unstable protein [10–15]. In addition, approximately half of the mutant alleles lead to abnormal splicing of the IVD RNA and subsequent complete lack of IVD protein. Studies of the bioprocessing of IVD protein in fibroblasts from patients with IVA have reflected the effects of these mutations but have not provided insight into the clinical variability seen in the disorder. In general, genotype and phenotype have not been well correlated.

With the advent of the use of tandem mass spectrometry to screen newborn blood spots for acylcarnitine concentrations, most if not all patients with IVA should be identified as newborns prior to the development of symptoms. However, one unexpected finding to arise from newborn screening studies was the identification of individuals with only mild elevations of isovaleryl-CoA related metabolites in plasma, urine, and fibroblasts, many fold lower than in the classic forms of IVA [10]. Nearly half of the mutant IVD alleles sequenced from these infants have a common recurring missense mutation, 932C>T; A282V. Family studies have shown the same genotype in their asymptomatic siblings (ages 3 to 11 years at the time of diagnosis) with a similarly mild biochemical phenotype. All of these individuals have remained asymptomatic with mild or no dietary protein restriction with or without carnitine supplementation. Prior to newborn screening, we had identified this mutation in only a single patient with mild IVD deficiency. He was evaluated for a tic disorder and slight developmental delay. The mutant A282V protein is stable *in vitro* but is kinetically impaired, exhibiting an increased K_m and a reduced catalytic efficiency [11]. It also has diminished thermal stability [16].

These findings lead to an interesting question. Because of our long pre-screening history with isovaleric acidemia, there can be no question of the pathologic nature of inactivation of this gene. However, had it first been identified through newborn screening, how would our view of this disorder be different? Would we still perceive it as a major health risk, a disorder with occasional catastrophic outcome, or would we be arguing about physiologic relevance as is currently occurring for deficiencies of other members of this gene family. Let's examine this question further.

Two other ACDs catalyze the equivalent step to IVD in isoleucine and valine metabolism [17]. Short/branched chain acyl-CoA dehydrogenase (SBCAD; also known as 2-methylbutyryl-CoA dehydrogenase) was differentiated in early experiments in Dr. Tanaka's lab and later in collaboration with Dr. Rima Rozen [18,19]. The precise role of SBCAD in intermediary metabolism wasn't clear until we subsequently cloned and characterized the enzymatic properties of isobutyryl-CoA dehydrogenase (IBD), showing it to be specific to the valine intermediate isobutyryl-CoA. The first patient with IBD deficiency was identified by another of our past presidents Dr. Steve Cederbaum with confirmatory molecular studies by my lab [3,20]. We subsequently characterized the first SBCAD deficient patient in

collaboration with our colleague Mike Gibson [2]. Given our then understanding of isovaleric acidemia it was logical to assume that the severe medical problems exhibited by both patients could be attributed to their enzymatic blocks. However, soon babies with both deficiencies began to be flagged through newborn screening and all was not as straightforward as one might hope [21,22].

Early on we noticed that many of our SBCAD deficient patients had a common surname and then recognized that they were all members of a rather unique new U.S. population, the Hmong [23]. The Hmong are an ancestral Chinese people that migrated to Southeast Asia many generations ago, settling largely in what is now Laos. Political unrest in the region led them to become a target for persecution in their country and many chose to migrate to the U.S. Nearly half of these now live in California and half in Minnesota and Wisconsin. Not surprisingly, we documented a common mutation in these patients that leads to an amino acid substitution of methionine for valine at position 389 in the mature protein. Subsequent studies showed that this mutation does in fact inactivate the enzyme when expressed in an *E. coli* system. It also causes mis-splicing of the SBCAD RNA. The outcome of these combined effects is to make this effectively a null allele. The first several Hmong babies we identified with SBCAD deficiency were treated with a low protein diet with or without isoleucine restriction. However, most patients ultimately discontinued diet because of cultural issues without apparent ill effect in spite of the complete biochemical block. Moreover, additional biochemically affected, but clinically well patients and sibs were identified through newborn screening and family studies. Clinical studies are underway to better characterize the long term outcome in these patients [21]. Only a handful of non-Hmong patients with SBCAD deficiency have been identified thus far through newborn screening. Each has had unique mutations in the SBCAD gene and all are well. We have thus come to view this as a biochemical phenotype of little or no clinical relevance, a phenomenon that we speculate is due to overlap in substrate utilization by other ACDs; however the potential for unanticipated long term effects cannot be discounted.

The story of IBD deficiency is less clear. We were able to show that Dr. Cederbaum's original patient was homozygous for inactivating mutations on each allele and thus was completely deficient of IBD activity [3,20]. The patient's major presenting sign, however, was a cardiomyopathy that resolved completely when a presumed secondary carnitine deficiency was corrected. She has remained well since. Of the additional patients whom we have had the opportunity to study (all identified through newborn screening), all but one have remained asymptomatic [22]. The symptomatic patient had recurrent episodes of emesis and dehydration with mild intercurrent illness accompanied by dramatic increase in the production of isobutyryl-CoA intermediates and their carnitine derivatives. Moreover, she dropped her blood carnitine level from normal to near zero during these times then returned to normal again with or without carnitine supplementation. Mild hypotonia and developmental delay that was present during the first two years of life became less evident as she got older. She currently is felt to be normal. Thus it appears that the intermittent organic acidemia in this disorder may cause sufficient urinary carnitine loss or the production of enough alternative metabolites to lead to symptoms. Clearly, however, most children are well. Does this apparently small risk of developing clinical sequela justify the potential negative impact of notifying parents of an abnormal newborn screen?

Short chain acyl-CoA dehydrogenase deficiency offers yet a different set of clinical challenges. It was first identified by Dr. Bill Rhead, another SIMD member, in a patient with a classic picture of an organic acidemia: overwhelming neonatal acidosis [24]. Enzyme and later DNA studies substantiated the defect and set the stage for characterization of additional patients. Additional experience soon proved the situation to be more complicated than was initially appreciated. Ethylmalonic aciduria (EMA), one of the biochemical hallmarks of this disorder, was found to be relatively frequent in the general population and molecular studies identified

two polymorphisms in the SCAD gene, a 625 G>A and 511 C>T [25]. These polymorphisms were clearly over-represented in individuals with increased EMA excretion, but their combined allele frequency in the general population was too high to attribute a major clinical affect to them [26]. Reports of a multitude of symptoms continue to surface in patients with a polymorphism on each SCAD allele. It is difficult to accept (and there is certainly no proof) that these genotypes directly lead to clinical disease, though it is clear that they cause mild biochemical and cellular derangements. My lab has shown that both substitutions lead to mild impairment of catalytic function when the variant proteins are produced in *E. coli* and purified, and Gregersen and his colleagues in collaboration with us have reported an increased tendency for the polymorphic variant proteins to misfold following import into mitochondria, especially with elevated temperature [27–30]. It is conceivable that these mild derangements in function may have some physiologic relevance. However, if so, it must be in conjunction with other as yet undefined genetic or environmental factors. Such a polygenic or multifactorial model remains conjecture until more traditional quantitative genetic approaches are applied to examine its validity.

Numerous patients have been identified with an inactivating mutation on one SCAD allele and a polymorphism on the other, as well as patients with inactivating mutations on both alleles. These individuals would seem to be better candidates to have true disease but no consistent clinical pattern has emerged. We have characterized an SCAD mutation frequent in the Ashkenazi Jewish population (a 319 C>T substitution with a 1:15 carrier rate) that completely inactivates the enzyme [31]. Individuals homozygous for this mutation have been described primarily with myopathic symptoms. Thus the spectrum of clinical findings appears to be somewhat less broad than in those individuals with just the common polymorphisms. Nevertheless, family and newborn screening studies show that individuals with this genotype can be asymptomatic. In addition, numerous asymptomatic patients with two novel inactivating mutations have been identified in this fashion. Wanders' group in Amsterdam recently published their experience with over 100 individuals with various combinations of SCAD mutations and polymorphisms and it mirrors ours [32]. They failed to find significant differences in phenotype among the genotypes and concluded that SCAD deficiency is not clinically relevant. I am not yet quite ready to accede this. Rather, I think it is reasonable to continue to be cautious with individuals with complete SCAD deficiency as there may be a small risk for episodes of metabolic decompensation, much akin to our IBD deficient patient. There could also be a synergistic effect with other genetic or environmental factors. At the risk of being repetitive, additional study will be required before the real answer will emerge.

New Paradigms in Treatment

Let me switch gears for a few minutes now and talk about treatment of inborn errors. The traditional paradigm has involved waiting for the development of symptoms to identify patients, then instituting therapy, often under extremis, and finally dealing with acute and chronic clinical problems. Too often our therapeutic interventions have been limited to life style manipulation, especially dietary measures, that are difficult to implement and still leave a patient at risk for additional complications. We need better options for treatment if we are to realize the ultimate goal of newborn screening, promoting the health and well being of babies. To this end, we have begun exploiting our extensive knowledge of ACD structure and function to investigate the development of designer drugs to rescue defective enzymes in deficient patients. Let me turn to MCAD deficiency as an example. As the most common inborn error of metabolism identified through newborn screening in the US, it exacts a huge personal and financial toll on affected patients and their families even though it can effectively be treated with diet. As a result of a founder effect, we now know that as many as 90% of alleles in patients with MCAD deficiency harbor a single 985A>G mutation leading to a Lys304Glu substitution and the production of unstable protein [33,34]. Co-expression of the mutant enzyme with the

molecular chaperonin GroEL/ES can rescue this unstable protein and lead to an active enzyme [35]. This has led us to consider the alternative of developing chemical chaperonins that can accomplish this same feat to use as drug to treat patients with at least one copy of the common mutation. Chemical chaperonins are small molecules that promote the correct folding of mutant proteins. Visualization of the molecular structure of MCAD shows that the residue affected in the common mutation orients to the center of the homotetramer [36–39]. Reversal of this charge likely plays a role in the destabilization of the enzyme. Using computer aided design, we have created a series of chemicals that can interact with the abnormally charged residue, bridging the tetramer core, and are now testing them for the ability to stabilize the mutant protein in patient cells. While the road ahead is still long, we hope to soon have compounds available to move through the next steps of drug development.

New Challenges in Inborn Errors

So what has all of this taught us? Perhaps the main message is that metabolism is complicated, as are patients with defects in it. I sing a consistent song to my lab members: if a question has a simple answer, we would probably already know it. Therefore we need to start anticipating the complexity that we see rather than being surprised by it. Transitioning to the post-Human Genome Project era will require that we examine our diseases and enzymes in ever more sophisticated fashion. Enzymes do not function in isolation nor do metabolic pathways. Rather, we must embrace systems biology and ask how deficiencies in the enzymes we study affect the organism as a whole. The lack of good genotype/phenotype correlations in most diseases may simply mean that we are not looking at the right combination of genetic or environmental factors [40]. Quantitative geneticists and bioinformaticists should become our allies in identifying modifier and interacting genes. The phenomenon of “moonlighting functions”, alternative, often non-enzymatic functions for enzymes or partial gene products requires further attention [41].

Our discipline faces major hurdles in the immediate future but we cannot let these deter us. Winston Churchill once said, “Every day you may make progress. Every step may be fruitful. Yet there will stretch out before you an ever-lengthening, ever-ascending, ever-improving path. You know you will never get to the end of the journey. But this, so far from discouraging, only adds to the joy and glory of the climb.”

It is not hard to identify those hurdles. Adequate compensation for our intellectual based practice is crucial if we are to be drawn back from the brink of financial oblivion. There will also be continued financial challenges in funding our research as competition for support from the NIH and other agencies increases. In part, our corporate partners can help fill the gap. Elias Zerhouni, director of the NIH, recently wrote in *Science*, “The greatest risk for science is to stop taking risks.” [42]. We can’t afford this risk. While we face significant obstacles in providing care for our patients, we must advocate for them. We must continue to remind insurance companies of the uniqueness of our patients and our clinical practice in order to provide them with vital dietary and cofactor supplements. We need a rational and consistent national policy that ensures a healthy beginning for every child and appropriate resources for optimum growth and development. Allying with patient support groups may offer us not only the best hope of success in lobbying lawmakers for changes in reimbursement practices, but also in a wide ranging political action agenda aimed at guaranteeing us the ability to treat our patients. Additionally, we must convince our own institutions that clinical genetics is a vital component of the academic medical puzzle and worthy of continued support. Finally, we need to cement our role in our health care system as the experts in newborn screening. The Secretaries Advisory Committee on Heritable Disorders and Genetic Diseases (<http://mchb.hrsa.gov/programs/genetics/committee/>) and the Newborn Screening Regional Collaborative Groups (<http://www.nccrcg.org/>), both prominently featuring SIMD members,

and the American College of Medical Genetics Newborn Screening ACT Sheets and Confirmatory Algorithms (<http://www.acmg.net/resources/policies/ACT/condition-analyte-links.htm/>) are good starts. But we must continue to be at the forefront in selecting new targets for screening and then developing treatments for them. Thomas Edison once said, “Many of life’s failures are people who did not realize how close they were to success when they gave up.” We must be persistent in the pursuit of our goals.

In order to ensure that patients receive the best care, it is vital to transition to an evidence based approach to our clinical practice. Remember that the plural of “anecdote” is not “data”! We must develop and embrace a collaborative, national clinical trials infrastructure. As individual practitioners we are nearly powerless to advance clinical practice in a rigorous way. Rather, I look to the day when every patient with a new diagnosis of an inborn error of metabolism is entered into a clinical trial that will guarantee that everyone receives at least the current standard of care but will also allow accumulation of the data needed to unequivocally evaluate the efficacy of new therapies. Our oncology colleagues have been enormously successful with this approach (<http://www.childrensoncologygroup.org/>). Their experience can serve as a basis for our efforts and on behalf of the SIMD I have been working with the American College of Medical Genetics in starting this dialogue at multiple levels.

H.G. Wells wrote, “Human history becomes more and more a race between education and catastrophe.” In the coming years, I hope that the Society will place as much effort in fostering current and future trainees as it did me. The Society’s annual meetings were an early catalyst in shaping my thinking about inborn errors and they have provided ample substrate to fuel the reaction ever since. I was afforded the opportunity to attend my first SIMD meeting and to present my research as the recipient of the very first SIMD young investigator travel award. I have come full circle and now serve as the principal investigator of the NIH grant that funds the program that has brought a half dozen of my own trainees to this meeting over the years. In addition, development by the SIMD of a national training curriculum in biochemical genetics, a national metabolic academy, is well underway with a curriculum outline in place and a faculty that has assembled for the first time at this meeting. These efforts will allow us to transfer our knowledge to the next generation of inborn error specialists and researchers. This will ensure the legacy of the Society and the continued vitality of our discipline, and all else will follow.

I would like to conclude by acknowledging how enormously grateful I am to the SIMD and its members. It has been an honor to serve as your president. Over the past two years as I have wrestled with the future role of the Society in academic and clinical medicine, it has become clear to me that we face significant challenges and the need for much change. I hope to continue to be able to serve the Society to help meet these challenges. Thank you.

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