Developing an Interdisciplinary Research Agenda for Genetics of Birth Defects: Summary of A 2014 NIH Workshop

Steering Committee
Gary M. Shaw
Andrew F. Olshan
Charlotte A. Hobbs
Lorette Javois

Speakers
Michael Bamshad
Leslie Biesecker
Stephen Chanock
Jan Friedman
Sonia Hernandez-Diaz
Benjamin Tycko
John B. Wallingford
Allen J. Wilcox

Discussants
Terri Beaty
Richard H. Finnell
Edward McCabe
Enrique Schisterman

Introduction

Human structural birth defects are often accompanied by physical or mental disability and are the leading cause of infant mortality in the U.S. The medical, economic, social, and family implications of birth defects are tremendous. Three of every 100 babies born in the U.S. will have a major birth defect.

For several decades the scientific community has stated that as many as 75% of birth defects have an unknown cause and only a small percentage of causes can be attributed to infections, environmental toxins, and a limited number of known genetic effects. It is commonly posited that many, if not most, structural birth defects are caused by the combined effects of a number of genetic factors that interact with environmental factors. Despite this prevailing idea, efforts to comprehensively examine the role of genetic variation and the interaction of these variants with environmental exposures and lifestyle factors in the etiology of birth defects have been insufficient.

Our nascent understanding of potential causes of birth defects is at a critical and especially timely juncture, particularly given the rapidly advancing genomic technologies and experience gained by scientific communities investigating the genomics of other human conditions. Our limited knowledge regarding genetic, epigenetic and non-genetic factors that impact occurrence of most human birth defects is partly rooted in what has historically been a lack of adequate data resources and limited technology. The necessary resources and technology are in place and an unprecedented opportunity to advance “omics” research of birth defects now exists. The next steps toward capitalizing on such opportunities are encountering remedial obstacles. Primary among these obstacles is a lack of a coordinated interdisciplinary national research agenda to study birth defect etiologies.

In January 2014 approximately 40 scientists attended a NIH-funded Workshop to consider future research needs associated with the substantive lack of information on etiologies of human birth defects as an initial effort toward overcoming such obstacles. There is substantial enthusiasm as well as intellectual capacity among scientists who have complementary expertise

in multiple basic and applied sciences to discover new causes of structural birth defects that will lead to effective primary prevention strategies. Integration of biologic concepts and human epidemiologic data, combined with thoughtful use of multiple new laboratory and analytical technologies undoubtedly will overcome barriers that for decades made the search for causes, especially genetic causes, elusive. The Workshop's goal was to assemble such scientists to identify approaches and formulate interdisciplinary strategies that are likely to have a substantial and sustained impact on discovering genomic and epigenomic risk factors for human birth defects.

Attendees included scientists from a wide range of disciplines and home institutions including NIH, CDC and Foundation officials and members. To optimize productive and engaging interactions between scientists, the Workshop was designed as multiple point/counter-point formal discussions. Opening remarks were made by NICHD Director, Dr. Alan Guttmacher, who announced the establishment of a NIH Trans-Institute Structural Birth Defects Working Group that will facilitate NIH efforts to coordinate efforts addressing birth defects. Dr. Guttmacher's remarks were followed by a one-day program consisting of four topical areas. These areas with their attendant point/counterpoint perspective, speakers, and discussants are shown in Figure 1. A brief summary of each area follows.

TOPIC #1: How informative are experimental model systems for understanding human birth defect etiologies?

Point: *Experimental model systems are critical to our understanding of human birth defects. (Dr. John Wallingford, University of Texas at Austin)*

Because of evolution, we can use animals to study human disease. The model organisms including the worm, *C. elegans*, the fly, *Drosophila melanogaster*, the chick, the frog, the fish, and the mouse that are used cluster together in one very small part of the tree of life. The reason these animals were chosen was because of their experimental tractability. However, these are not humans. They are not even primates.

Moving forward, there is really going to be a balance of use among these different organisms. I have several things I'll tell you that model organisms can do for us that make them really important. One is the basic biology of development. Almost all that we know about development comes from animal models, and birth defects are, without question, developmental defects. Whether they are genetic or environmental, they are a perturbation of the normal developmental process. In some model systems we can actually watch their development in real-time. We can actually watch all of the cells in the neural plate folding up and closing to learn some of the cell biology of how neural tube closure happens. We can go into one of these embryos a little closer and actually look at the individual movements of molecules within those cells. The entire field of developmental biology is centered on understanding how genes control cell biological processes which influence tissue movements that ultimately shape the animal. There are differences from humans, but the basic principles are the same.

As a second point, the ability to see these processes in model animals has provided most of what we know about the genes that control development. High-throughput screens have employed a variety of model organisms and have identified the functions of hundreds, probably thousands of genes involved in embryonic development. Because of evolutionary conservation,

the genes identified and characterized in the simpler, cheaper, and faster model organisms (invertebrate models) are generally found later to be involved in vertebrate development, usually in frogs, fish, or mice, and then finally in humans. With current gene sequencing power, we're actually beginning to discover human disease genes, birth defect genes, first from humans; we still need the model animals to determine what those genes do. There are numerous examples illustrating how model animals have allowed us to identify candidate genes such as planar cell polarity genes and neural tube defects (NTDs).

A third contribution of animal models to our understanding of birth defects is for candidate disease gene testing. We're making these critical discoveries. We believe that we're laying the foundation for clinicians to utilize the interesting things being found in animal models and guiding their research as we move forward. However, the incredible power of modern sequencing has really made the human population the greatest forward genetic screen there ever was. Now we're sequencing large human cohorts. We're finding a lot of disease genes *de novo* from the human population, but the problem is a lot of these disease genes turn out to be things that we know nothing about. For example, the ZNF452 or Chromosome-5 open reading frame 86, i.e., genes that have never been studied. We have absolutely no idea what they do. Many of these are not even annotated in the genome.

What do you do when the sequencing says this is the important gene and no one's ever studied it? The point that I would make here is that the model animals provide the context to test disease gene mutations. Many researchers when they have a human disease gene, will go to cell culture because it's very simple, it's inexpensive. The problem with that is developmental biologists have found time and again things that are true in cell culture are not always true *in vivo*. The reason is even the most fundamental cell-biological processes are cell-type specific. These cell culture models, while incredibly cheap, rapid and powerful, sometimes can lead you down the wrong road. With the modern advances and techniques, there's really nothing you can do in cell culture that you cannot do inside an organism now. The *in vivo* context of the organism is so important. I would argue *in vivo* in the mouse or even *in vivo* in the frog is actually more informative than a human cell on a plastic dish.

Moving forward to the future, who knows what's going to be possible. I have highlighted some things where it's well-established that model systems have been important.

I think we absolutely have to do a few things; we need to make sure that developmental biologists understand that they are birth defect researchers. I think a lot of developmental biologists don't feel connected with birth defects research. They feel like they are doing developmental biology. Often you will see the genes involved in development are reactivated in cancers. They feel like that is a better angle. There is no reason for them not to say, “We're birth defects researchers. This is what we're doing.” Every developmental biologist is a birth defects researcher.

We need to make sure epidemiologists and human geneticists understand what developmental biologists are actually doing and how it's relevant to what they're doing. I think there could be a lot more crosstalk between epidemiology, human genetics, and developmental biology.

We can engage the Society for Developmental Biology to try to make some birth defects initiatives. There are great journals. *Development's* the leading journal in developmental biology. *Developmental Biology* is another leading journal. We should reach out to them to run special issues on birth defects research. *Disease Models and Mechanisms* is another journal about “animal models of disease”; we can consider a special issue there.

The last thing that I would say is we need to establish some centers for birth defects research. Every medical school has a cancer center, and everyone wants to be the best cancer center, but you're not going to be because Sloan-Kettering will always be there, or MD Anderson, or whoever. However, if you just say we're a birth defects research center, you might be the best one in the world because I'm not sure anyone says they have a birth defects research center at their medical school. Simple things like this, as simple as that, I think really could be the game-changer for inter- and trans-disciplinary research.

Counter-Point: *Human translational research is critical to our understanding of human birth defects.*
(Dr. Leslie Biesecker, National Human Genome Research Institute, NIH)

I think that experimental model systems are critical to our understanding of human birth defects, *and* these systems cannot sufficiently inform the complex environmental, social and genetic underpinnings. I'm here to try, though, to represent the human genetics viewpoint and what we can do that animal model systems can't do. I found an issue of *Science* from 26 years ago that reviewed this question (Science June 10, 1988). The papers are about retroviruses, bacteria, yeast, *C. elegans,* the fly, plants, transgenic animals, primates, and then the last one is on the human. In thinking about how things have come along and which things are relevant today, I think overall, the higher, the non-human higher primates, are being lost as a model system in biology. They are hideously expensive and socially toxic, if you will.

Ray White and Tom Caskey wrote in 1988 with what I thought was little bit of a tongue and cheek, the title: “The Human as an Experimental System for Molecular Genetics.” I like that title because we commonly like to think that it's the human which is the end goal for understanding, and we use model systems to understand the human. The point they make is that, in fact, you should think about the human as the experimental system because the human has several biological and practical attributes that make it powerful. Their two arguments were the richness in the phenotyping and the genetic resources. I think both of these central arguments are still valid.

No other species is observed so closely for variation. The array of phenotypes that we encounter in a human is, in fact, astonishing, and I don't think in many other systems that you can find such phenotyping with such variation.

Long before biologists figured it out, we proposed that if disorders overlap phenotypically or exist in syndrome families, they are biologically connected to each other. Because these phenotypes overlap with Bardet-Biedl Syndrome, we proposed that the biology of GLI and Sonic Hedgehog was coupled with the ciliary transport, which was shown a few years later. Thus, our abilities to describe human phenotypes in detail can lead to biological insights.

There are also phenotypes that I would be so bold as to say you're just not going to find in an animal colony. For example, Proteus Syndrome. This phenotype is caused by a somatic post-zygotic mutational event and cannot be transmitted through the germline because it is 100 percent embryonic lethal. What's the first thing you do when a mutant animal arises in a colony? Observe the phenotype. If the animal's not in distress, you test it for transmission. If that trait does not transmit, it's not interesting and the animal is discarded. The fact that the animal model can't be used—makes it an intractable approach.

In the human, tractability is not our highest priority. We have to take care of patients irrespective of how tractable their problem is. This is a medical challenge that we have to deal with irrespective of whether it's a good scientific problem or not. We push the envelope in science because these problems have to be taken care of.

A second theme involves genetic resources for human disease. I would say that the human genomic and genetic resources are still superior to those in any other animal model. I think that's because the medical system drives interest in these technologies because they are clinically very useful to us. That pushes the technology faster and the side effect is that these resources are built at a furious pace, sometimes far outpacing our ability to use them. In science, we can exploit that and understand the biology of this model system, the human, to dissect the genetics and genomics in these disorders.

I think that John made a very good point. Mouse genetics are extremely powerful. Although, still somewhat behind the human, I would say in resources, the power of genetics to dissect traits, i.e. by crosses, we can't so much do it. Fly genetics are certainly fantastic, but humans, I would say, are still better. I would argue that both zebrafish and frog genetics are not so great for a number of reasons. I would predict that this medical utility driver will keep humans ahead of the model systems for the foreseeable future.

Most of us do hypothesis testing research. It's a very well-trod pathway to understanding biology. It works. It can and must continue, but it isn't the only way that you can do research. Hypothesis-generated research is really a spinoff of genomics in general. The notion is generate the data and ask questions based on the data. Don't start with questions; start with data. You can imagine just sequencing people. Then, what you do is you dig into those data and parse the data for patterns and perturbations, anomalies, outliers, etc. You use that perturbation of the data to generate an hypothesis. Then, you test that hypothesis with clinical research.

To give you an example of how we've done this, we've worked on methylmalonic acidemias, a subset of which is this disorder called combined malonic acidemia, a pediatric disorder of organic acidosis with childhood onset metabolic decompensation, infarcts, strokes, coma, and often death. A few years ago, we did the now standard drill of exome sequencing a single nuclear trio, filtered them for attributes of the gene that we thought would cause such a disorder, and identified a gene with a predicted mitochondrial functionality. We took it a step further, however, and screened an adult cohort for variants in this gene. The reasoning was that since this is a subset of a rare disease, wouldn't it be nice to know what the carrier frequency was in the population? From population genetics principles we could back calculate what the prevalence of the disease was. What we identified in our cohort was surprising. An adult in the sequencing cohort turned out to have biallelic mutations in this disorder. She was actually homozygous.

Adults walking around don't have this disease, obviously. That is the conclusion, right? Well, actually, it's not. We phenotyped this person in clinic. She's in her 60s, and it turns out she's been having neurological symptoms recently, memory problems, and incontinence. She also has wildly abnormal levels of malonic acid and methylmalonic acid in her blood. We had no idea what the phenotype actually was. Our preconceived notion that we thought we knew what the disease was turned out to be the problem and it took an hypothesis-generating approach to overthrow this incorrect assumption.

That is the power of hypothesis-generating research. The medical phenotyping that can be done and is out there to be had and just the amount of human sequencing that will be done will be

hugely informative. In the foreseeable future that I can see, unless NIH comes up with a lot more money, similar efforts such as routinely sequencing all the mice that are born in our colonies, or frogs, or fish are unlikely. We're just not going to do that, but we are going to be sequencing people. People are using the data now, making hypotheses, and figuring things out from those data because they're rich resources and the medical drivers.

Where are we? Supportive research by the public at large is essential for all of us, and the problem is so important and so compelling, in fact, that we're not just going to sacrifice animals for the results, but we're willing to actually risk harming children. Of course, this is not to say that harming human subjects is ever ok--it's the converse—that the research goals are so important that we, the ethicists, and the families accept that we have to tolerate risk of harm. That is the most compelling and powerful statement of the importance of the research enterprise that I can imagine.

It also gives us, I think a very powerful moral argument to say that we're not just harming animals for curiosity and scientists' interest in basic science. We have a really serious problem here, and we have to solve it, and we're going to do everything we can to solve these problems. We have to both do animal research and human research, keep the information flowing from the bench to the bedside and back again to solve this in the most multi-faceted, multi-focal way we possibly can. We know that basic science is absolutely critical to solving these problems. Curiosity-driven science of selective problems is the only way we will go forward in understanding the basic mechanisms of disease and normal physiology. But we also need to push these newer approaches of hypothesis-generating research in the human to maximize all of our options for progress. The purpose is to ameliorate disease and suffering and we should use all of the tools at our disposal to do so.

TOPIC #2: What is the preferred method(s) to discover genetic etiologies of human birth defects?

Point: *Genome-wide association studies continue to be legitimate, and in some circumstances, the preferred approach.*
(Dr. Stephen Chanock, National Cancer Institute, NIH)

In considering how to discover susceptibility alleles for pediatric disorders/diseases, I think it's really a question of numbers, cost, and most importantly the summation of possible questions one may want to ask with respect to the genetic disorders that present in the pediatric time period. At present, we have a number of different approaches to mapping different types of alleles. One of the things we have to take into account in the world of genetics is change in the trajectory of genetics, and we can see that we have gone from "candidate genetics," which is based on the hubris that we think we can predict SNP functions as well as the function of the SNPs in the gene, and think that that is a good test for its functional value and associate or map the genes.

And then, we began to look beyond one SNP, and thus, moved into the age of new technologies that enable us to look at many genetic markers simultaneously. First, we were able to look with microarrays in genome-wide association studies designed to identify markers of common variants. This followed the age of using linkage and Sanger sequencing to find the mutation of a highly penetrant variant. Now, we're into the world of whole genome and exome sequencing,

which has technical issues and also generates so much data. These are very exciting, but they're being applied at once—often with underpowered studies—so we are back to the age of sorting our way through the many false positives.

Only a few years ago, euphoria of genome-wide association studies (GWAS) excited the community. GWASs have been successful in adult cancers and recently, in select pediatric cancers, but large numbers are needed. We have over 400 loci in genome-wide association studies for more than 20 cancers. We have thousands of individuals that we've scanned to use for exploration of new hypotheses—biomarkers in an exhaustive approach.

The middle ground where most of the pediatric diseases are going to be falling - and we need a large number of individuals—the allele is low enough in frequency that the statistical capacity of finding it linked is limited. You're going to have to sort of combine analyses here. The laboratory analyses are going to have to be partnered with genetics. So our two extremes of approach—either large adequately powered studies or candidate gene analyses will only get us so far. That middle ground represents a fair amount of genetics and counseling disease models. We see they're going to have to be done by flagging through the variance and flagging specifically the biology to understand. Anyone who's done next-generation sequencing of whole genomes, you're always facing thousands and thousands of prospective variances and tests to consider for the disease model.

The epigenetic disorders are probably no different from cancer. A known fraction is complex in etiology. You'll have enough comparisons to be able to interrogate the data to model that like you started to do with cancer. You found five, six thousand Mendelian disorders with just really rare variance. We all know that in developmental clinics and pediatric clinics there are many more patients arriving who are not diagnosed with classical symptoms.

So, I'm one who has a hard time ascribing the majority of missing heritability to the singular, rare variant. That's a dangerous way to look at it. We know there's a spectrum of monogenic disorders for one mutation leading to phenotypic heterogeneity—again underscoring the importance of both genetic and environmental modifiers.

We can see that we're going to need to use some kind of hybrid approach. We simply can't effectively think that the current tools are sufficient to find new alleles. Instead, we will have to rely on putting together the numbers needed to discover variants that contribute to disease.

Next-generation sequencing (NGS) on the other side is genetics on steroids with direct testing of all variants but no real way to sort through the enormous field of false positives. It has very powerful elements, but it also has created newer and larger challenges. The technical problem for calling NGS data continues to be a major problem. The challenge is in the analytics, and particularly the existential crisis of "what is the coverage?". How well are we actually getting each base? Unlike the GWAS where you have very HIGH concordance and reproducibility, in NGS that's a work still very much in progress. It will get there, but it's not quite fixed. One worries an awful lot about the false-negatives, and there have been a number of recent papers published looking at target sequencing for exome sequencing, I think, that underscore this feeling that you need careful and lab-based corroborative data.

The steps ahead rely on the new biology of bioinformatics, which is both exciting but fraught with misunderstandings. As we look forward, we know there is a phenomenal cost in collecting samples in large numbers. We know the GWAS chips are inexpensive and take very little time to process and have stability.

NGS has minimal sample costs as we see improvements in the chemistry, but at the same time, we have created a crisis in cost for handling data and its complex analysis. You can do exomes and you can take months and months to go over, to really understand, which ones you want to take forward. The biggest issue in my mind is interpretation based on insufficient information. The challenge with respect to the ethical relationship is return of information to family and patients which is not in the scope of research. To those that spend time in the clinic it's always a challenge when you order a test—what are you going to do with this? And, will it do no harm? So, we have very opposite moral and technical challenges.

So, if we look at the cancer paradigm, we see the emergence of driver genes—both somatic (found in TCGA) and also in susceptibility with respect to biology, cancer risk assessment, and cancer prevention. The role of GWAS in cancer research has been very successful. There are 400 published; another 200 are going to be published very soon, which shows it's exponentially taking off. The biology uncovered in GWAS is quite surprising. This is where I think the GWAS has been far more useful than in clinical use, and that is in identifying new biologic processes. It has really underscored the importance of the silent, or the ignored part of the gene, where you have sort of this code maybe responsible for something. You have a very, very high fraction of disease including pediatric disorders. It's just much harder to go after something with less than very clear markers.

In the not too distant future I think we're going to look back at GWAS as sort of the golden age. The temptation to continue and do GWAS is fine, it shouldn't be banned, but we have to know our limitations, what part of the genetic architecture can be explained. We have to focus on that middle ground, so to speak, and the variances in sequencing, and in some circumstances the GWAS for variances.

We are not building large mammalian genome databases. This is already done in GWAS, but in sequencing we just don't have the comparisons to effectively evaluate NGS base by base. So, let me just finish by saying I think we want it to evolve. And, I think you have to continue to keep GWAS in mind for very particular things, but the advantage will be in sequencing.

Counter-Point: *Genome-wide sequencing or exome sequencing would be preferred.*
(Dr. Mike Bamshad, University of Washington)

I'm going to talk about genome sequencing as a tool for discovering genes and pathways for birth defects.

I agree with Steve Chanock in that I don't think whole genome sequencing is the only answer. I think that there are tradeoffs between whole genome sequencing and continuing to use GWAS by way of SNP typing. I do think genome sequencing has a place. Whole genome sequencing of even a modest number of cases or trios could lead to important discoveries of genes and pathways for common birth defects.

Several years ago, with the advances in next generation sequencing, we and others began to apply exome sequencing to the study of genes from Mendelian disorders. In a paper that we put together on identifying the gene for Miller syndrome using exome sequencing, we suggested that exome sequencing and eventually genome sequencing was likely to accelerate the discovery of genes underlying Mendelian disorders. Then ultimately, the goal should be identifying the gene for every known Mendelian disorder by way of sequencing.

Over the last couple of years the number of gene discoveries made using exome sequencing has accelerated very rapidly. To this end, two years ago, NHGRI supported a group of centers called the Centers for Mendelian Genomics. In the first two years that this program has been in existence, it's been highly successful. There are 691 phenotypes in the exome and genome sequencing pipelines across the three centers. I'd say half of those phenotypes are novel phenotypes, so they're not yet phenotypes that have an OMIM number. There's 11,000 exomes in the pipeline. Already, there are 232 new genes for Mendelian disorders that have been discovered. For more than 75 percent of the phenotypes for which genes have been discovered, they include a birth defect.

While I mentioned our successes to date, we've just started developing metrics which really measure those successes. For X-linked and for autosomal recessive disorders in populations, our success rate is very high. Where success has been lower involves phenotypes for which we didn't have mapping data. Arguably, this is where most birth defects lie. Most birth defects are likely to be complex traits. Thus, our challenge is to try and figure out ways in which we can use next generation sequencing to identify genes, a pathway, underlying this group of disorders for which we don't have a good model to use.

One of the things that we and others have been doing is to use trios and look for *de novo* variants. We got an estimated mutation of about 1.3×10^{-8} across all protein coding regions of the genome. That means there are about one to three genetic variants per exome.

It might be a challenge to identify extremes of phenotypes for birth defects. One could make an argument that you have to look at something like hypoplastic left heart disease and mitral valve prolapse. Perhaps those are two extremes. We think an alternate strategy in which you compare one extreme to a very large set of control data is something that is probably going to be much more useful to the community of researchers studying birth defects. This is taking the same extremes that I've talked about earlier, which is comparing each to a large set of controls.

In fact, for example, if you had only hypoplastic left heart or only several neural tube defects, you could take a small number of individuals, from either exome or genome data, and compare them to a much larger control dataset. These control datasets, whereas they didn't exist three and four years ago, they certainly exist now. In fact, there's more than a hundred thousand exomes that are now available and there are, or shortly will be, tens of thousands of genomes that will be available for comparison. Again, you could leverage that by looking at sequence data from a relatively small number of individuals with birth defects.

We have been working with samples from the National Birth Defects Prevention Study—one of the best curated set of cases of birth defects in the world including more than 15,000 trios. There are extensive data on environmental exposures. In fact, this really is a unique and highly valuable resource. How do we leverage the use of this resource? Well, the bad thing from a genetics and genomics perspective is that the DNA samples have been collected from buccal swabs. That is for many reasons. DNA quality is highly variable and relatively small amounts of DNA available.

We undertook, with NBDPS samples, a pilot to see whether we could do genome sequencing from these samples. We chose a phenotype—left-sided heart defects—partly because it's a long-standing interest as well as a phenotype that we think has high public health interest. We chose an extreme phenotype within the phenotype of left-sided heart defects, that of hypoplastic left heart, which is not common and occurs in about 1 in forty-four hundred births, or about a thousand cases each year in the U.S.

With respect to what we typically use for assessing our confidence in the quality of the calls, the number of variants was about on par with other samples, 3.9 million variants per genome, about 430 to 450,000 novel variants. In our pipeline, for example, with the standard Mendelian we put through the Center for Mendelian Genomics, a postdoc can do the analysis looking at four different models, with dominant, recessive, etc, in an hour.

As an example, in one trio was an isolated congenital heart defect, hypoplastic left heart syndrome, aortic atresia, mitral valve stenosis. In the *de novo* model, we identified two protein coding variants that were of interest. One was in a gene called CABLES2. The other in a gene called MVP. Both were non-conserved. This one's changes had high GERP scores and high CAD scores. Both of these genes are expressed in the heart. MVP encodes a protein called the major vault protein. The interesting thing about MVP is that it interacts directly with PTPN11, which is the gene that causes Noonan syndrome. Those individuals with Noonan syndrome have congenital heart disease.

I'm just going to summarize here, that whole genome sequencing for NBDPS samples is technically feasible, minimal special processing was required. The cost is comparable to the use of a standard sample in our pipeline. Does the cost compare to using a GWAS chip? Higher. Sharing it's a comprehensive set of genetic variants, enables the use of a full array of analytical models. Standard GWAS can still be performed or informed from those data.

Clearly, this is just a first step. This is just identifying the molecular basis. In many cases, it's just coming up with a set of candidates that would need to be validated by other strategies, whether it's the use of an in vitro model or an animal model. I'll go back to the point again, which is that I think whole genome sequencing of even a modest number of cases or trios could lead to important discoveries of genes or pathways for common birth defects.

TOPIC #3: How valuable will the investigation of epigenomics be toward understanding the etiologies of human birth defects?

Point: *Epigenomics have theoretical relevance but owing to logistical and scientific challenges such studies of human birth defects are currently impractical.*
(Dr. Jan Friedman, The University of British Columbia)

I will give you my view on the role of how epigenetics contributes to our understanding birth defects. Epigenetics, just to remind you, has been around since 1940 when Waddington described it as the interactions of genes with their environment that bring the phenotype into being. At that time, no one understood how this might happen, but we now know a lot more. We know that there are a variety of different mechanisms that can be involved in regulating the expression of genes. The one that people talk about most often is DNA methylation, but there are others like histone modification. Histones can be ubiquinated or phosphorylated or changed in other ways that affect expression.

The unique combination of stability and plasticity is what makes epigenetics attractive to people who want to study birth defects because we know that, although there are some birth defects that are basically caused by genetic factors and others that are basically caused by environmental factors, most seem to have a cause that lies between genetic and environmental factors.

We call this “multifactorial”, but that’s really mostly hand waving because we don’t know what the factors are. The unique combination of stability and plasticity makes epigenetics a good candidate for being involved. Epigenetic states can be transmitted from a cell to its daughter, so they are stable in that sense. They can also be altered by environmental exposures. We know that they can vary from tissue to tissue. In fact, they do. We know that they can vary during embryonic development of a tissue at different stages. We know that they can vary over time within a fully developed tissue, as well.

Epigenetic abnormalities can cause birth defects in some circumstances. There’s no question at all about that. They can do it in two different ways. One is that there are genomic alterations that can distort normal epigenetic control. The other is that there can be mutations in genes that control normal epigenetic mechanisms.

Those are both well established mechanisms. The classic examples of genomic alterations are Prader-Willi and Angelman syndrome. Prader-Willi syndrome is characterized by intellectual disability, compulsive overeating, consequent obesity, and a number of other features with a characteristic phenotype that includes an ability to solve jigsaw puzzles.

On the other hand, there’s Angelman syndrome, which is a completely different disorder associated with more severe intellectual disability, often with seizures, with unusual ataxic gate, and with laughing at completely the wrong time, like when you’re sticking the kid to draw blood. We know that Prader-Willi syndrome and Angelman syndrome both can be produced by abnormalities of the same region of the genome, or at least overlapping regions of the genome.

Within this region, there are genes that are normally expressed just from the maternal chromosome and other genes that are just expressed from the paternal chromosome. If you lose the expression of either the maternal chromosome or the paternal chromosome, you get one or the other of these syndromes. If you lose the paternal allele, you end up with Prader-Willi syndrome. If you lose the maternal allele, you end up with Angelman syndrome.

The other circumstance where you can have epigenetic influences on birth defects is when you knock out a gene that controls epigenetic factors. There are genes like *MECP2* and *ATRX* that control transcriptional regulation. There are genes that control chromatin remodeling, like *JARID1C*. Mutations in these genes can cause birth defects, as well.

The real question is not whether epigenetics can ever be involved in birth defects (it can), but whether abnormal epigenetic control is the mechanism by which teratogenic exposures interact with genes to cause common birth defects.

If we’re interested in common birth defects, we need to try to understand this, and I think this is where the idea of epigenetics gets confused because an epigenetic mechanism is not an alternative in the sense that some diseases are caused by mutations and some are caused by environmental factors and some are caused by epigenetics. That’s not right. Rather, it’s a mechanism by which either the genes or the environment or both could interact. It’s a way that a mutation or a teratogenic insult could screw up a signaling pathway or the ability of a structure to be made. It’s a developmental mechanism rather than a causal mechanism.

Conventional studies of common birth defects generally have assessed the epigenetic status of only one or a few loci at a time. Technology now permits genome-wide analysis of the entire epigenetic landscape. Then, you can use those data and compare tissues, one to the other; or developmental stages, one to the other; or the effect of a toxic exposure to non-exposure; or the

effect of a particular disease. You can develop catalogs of normal tissue states. There's lots of information you can get. You can also use epigenetics not as a separate study but as a way to increase the information provided by genetic studies—looking, for example, at identical twins and the differences that they have if one twin has a birth defect and the other doesn't.

Alternatively, epigenetic studies could be incorporated into epidemiological studies of longitudinal cohorts to study the changes that occur over time, for example, at different stages in pregnancy. One can develop these models or these ways to look at epigenetic factors in a much more powerful way than we ever could before. The problem is that there's a huge amount of variability in interpreting these data. Different tissues show different epigenetic patterns, and usually, in humans at least, we don't have access to the tissue that you're really interested in. We have to study tissue we can get, like white blood cells.

Epigenetic patterns change with time. We usually don't have access to the right tissue or often to any tissue at all at the time that's most important for the development of a birth defect.

Epigenetic patterns are complex and they're normally variable, so with the signal, there is lots and lots of noise. Epigenetic studies, at best, may be able to provide some evidence of gene by environment interactions, but they don't provide information about either the genetic or the environmental contribution. They don't tell you what the gene is, and they don't tell you what the environment is that caused the epigenetic change. They may tell you that there's been some interaction and that something has changed, but they don't tell you what's causing that change.

Defining the etiology in a study requires assessing genetic and environmental factors directly. If we want to understand the cause, we have to look at the possible causal factors. We can't do it by looking at the epigenetics. There's no free lunch. We have to study the genetic factors directly. We have to study the environmental factors directly. In this context, the epigenetic studies may be helpful as an addition, but they're not a substitute. They may assist us in understanding how the genetic and environmental factors are interacting, but they're not helpful in deciding whether or not they're there or what they are.

Counter-Point: *Epigenomics represent a critical, timely, and currently practical area for focus towards understanding human birth defects.*
(Dr. Benjamin Tycko, Columbia University)

Epigenetics has both broad and narrow definitions. Here we'll be thinking of this as cytosine methylation and histone modifications.

When methylation occurs in promoter regions it enforces gene silencing, but when it occurs at insulator elements, it actually can activate the expression of adjacent genes. Importantly, it can be manipulated by drugs, such as the hypomethylating agent decitabine (5aza-dC) and also by folic acid, betaine and vitamin B12 attained through methyl-donor-rich diets, which can help to maintain methylation levels in situations such as developmental stress and preneoplasia. But I want to concentrate here mostly on epigenomic mapping, since mapping CpG methylation can give very substantive insights into genetic-epigenetic interactions.

Genetic mutations in chromatin modifier genes can influence epigenetic patterning, and such mutations are now documented in a minority of birth defects, such as syndromic and possibly non-syndromic cardiac defects associated with *MLL2*, *KDM5B*, *CHD7*, and *RNF20* mutations or haploinsufficiency—which clearly links epigenetic pathways to some birth defects. Chromosome losses and deletions can influence epigenetic patterns throughout the genome, both in cis and

in trans, and I'll return to that. In addition, we know from studies of imprinting that pure epimutations can cause disease. Beckwith-Wiedemann syndrome and Silver-Russell syndrome are the classic examples. These are growth disorders (over-growth in BWS and intrauterine growth restriction in SRS). Most of those cases are not due to mutations. Rather, they are caused by epimutations early in post-zygotic development, where there's no change in a gene's sequence; only in the methylation status of its regulatory region. These epimutations cause the disorder in the proband, but since the DNA sequence is not altered and the epimutation is erased in germline cells, they are not transmitted to future generations. For Prader-Willi and Angelman syndromes, the other two imprinted disorders that are the most famous ones, some of the cases are due to epimutations, while most of the cases are not, instead being due to physical DNA deletions affecting the same regulatory sequences.

So, epimutation as a cause of developmental disorders is a very real phenomenon. Whether it occurs outside of imprinted regions, and whether it has a role in more classical birth defects such as cardiac defects, cleft palate and so on, is not yet clear, but I would bet that it does, albeit probably rarely. It simply needs more research at this point.

Getting back to the important topic of genetic/epigenetic interactions, we know so much about the genome now that we really should be asking this question, how do genomic changes affect the epigenome globally?

The most straightforward example would be a mutation in an epigenetic modifier gene. The example here that has struck me from the literature is the paper from Rick Lifton's lab on ostensibly sporadic congenital heart defects where they found about 10 percent of cases are explained by de novo mutations in histone modifier genes (***).

***Zaidi S, Choi M, et al. De novo mutations in histone-modifying genes in congenital heart disease.Nature. 2013 Jun 13;498(7453):220-3. doi: 10.1038/nature12141. Epub 2013 May 12. PMID: 23665959; PubMed Central PMCID: PMC3706629.

What about genetic/epigenetic interactions, since this is something that my lab has worked on since we published the phenomenon, originally in 2008, in a *Nature Genetics* paper that I think was very important in showing that such interactions occur in cis (**). We showed that haplotypes can affect DNA methylation locally. This is not due to deletion or creation of CpG dinucleotides by SNPs in the haplotype. Rather, the context of the haplotype can affect the probability that a (non-polymorphic) CpG dinucleotide is methylated in that haplotype. Another way of looking at that is that CpG methylation in the human genome is a quantitative trait that is dictated in cis by the local haplotypes.

**Kerkel K, Spadola A, et al. Genomic surveys by methylation-sensitive SNP analysis identify sequence-dependent allele-specific DNA methylation. Nat Genet. 2008 Jul;40(7):904-8. doi: 10.1038/ng.174. PMID: 18568024

We initially discovered this phenomenon, which can be abbreviated as "hap-ASM," using a simple adaptation of Affymetrix SNP arrays. Those were the old days. We now do this by Nextgen bisulfite sequencing, both targeted and genome-wide. One can identify pronounced allele asymmetry. As an example in the locus shown here, in person after person after person, one can predict that it's always going to be the A allele that's more methylated than the B allele. This is a clear example of genetics dictating epigenetics in cis, and it does affect gene expression. The effects can be as strong as for imprinted loci, and in fact haplotype-dependent DNA methylation is more common than imprinting. The end result looks a lot like imprinting, but

this is genetically-determined. It is in fact a Mendelian effect, in contrast to imprinting, which is a non-Mendelian phenomenon where the same allele can be methylated or unmethylated, simply depending on what type of parent (male or female) it has most recently passed through.

We know quite a bit about molecular mechanisms of imprinting. So, can we get at the mechanism of non-imprinted haplotype-dependent methylation? We used Fluidigm Access Array as a high sample throughput platform for bisulfite PCR, followed by long-read Nextgen methylation sequencing. We looked at a large number of normal human samples of different tissue types and mapped the epicenters of the allelic asymmetry for hap-ASM. The striking finding is in this heat map, not showing net methylation but rather showing allelic asymmetry in methylation in the red color. In heterozygotes, this small 1 kilobase segment is the only place within this 100 kb region where we found an epicenter of allelic symmetry. So, whatever the mechanism is, there's a very discrete sized differentially methylated region—a DMR. This is very reminiscent of imprinting, but here, this allele-specific methylation is non-imprinted; it is haplotype dependent.

This slide is showing an allelic asymmetry that's physically in the DNA. One allele is differently methylated than the other. If you have even a sub-threshold GWAS peak, and it overlaps with this physical signal, you can be pretty sure that there's a true regulatory element here that is polymorphic between individuals. That's the argument; it strongly bolsters the evidence that there's a disease association there. It also applies now in exome sequencing; not exome standard but "exome plus" and whole genome sequencing (WGS), where you're looking not only at coding changes, but also at the regulatory regions.

Now an equally interesting note about trans effects of genetics on epigenetics. We've studied the most elegant possible model for this, and it's also a clinically interesting topic: Down syndrome (DS). You have a tiny chromosome, the smallest human chromosome, duplicated to give a 1.5X gene dosage. What is the effect of that discrete genetic abnormality on the epigenome? It turns out to have very well-defined effects.

These effects need to be examined in a tissue-specific manner. Jan Friedman brought up the idea that you need to state what tissue you're examining as the epigenome of different tissues is very different; we care a lot about that, as well. We started with studying blood, and I wouldn't have looked at blood had there not been a blood phenotype in DS. It wouldn't make sense. But there is a DS blood phenotype—namely, increased autoimmunity, alopecia, celiac disease, perhaps the most common, but also thyroid disease, as well as recurrent bacterial and viral infections. So, there is an immune abnormality in DS and one can justify looking at the epigenome in blood cells, including whole blood and isolated lymphocytes.

We saw differences that are not as strong as the kinds of differences that you see for example in malignant tumors, but they're recurrent differences in DNA methylation. As you can see here, some of these differences are so different, in fact, between DS and controls that it's virtually diagnostic (*****).

*****Kerkel K, Schupf N, Hatta K, Pang D, Salas M, Kratz A, Minden M, Murty V, Zigman WB, Mayeux RP, Jenkins EC, Torkamani A, Schork NJ, Silverman W, Croy BA, Tycko B. Altered DNA methylation in leukocytes with trisomy 21. PLoS Genet. 2010 Nov 18;6(11):e1001212. doi: 10.1371/journal.pgen.1001212. PMID: 21124956; PubMed Central PMCID: PMC2987931.

For future projects, we have wondered if a methyl-donor-rich diet in this case might be useful for making the methylation patterns more normal and might improve immune function or decrease

autoimmunity in older children and adults with DS. I think that's an interesting clinical research topic.

I just want to finish with the other organ, the brain, which is very relevant. This is now submitted, but not yet published. These autopsy brains are like gold; we collaborated with Jerzy Wegiel at NYSIBR in Staten Island and Gudrun Moore at UCL in the U.K. Nearly all of the changes in the cerebrum are gains in methylation, just with some losses in the cerebellum, but still mostly gains. It's very different from the blood. The genes are different too. This comes back to what Jan Friedman was saying about tissues having different epigenomes. There's a systematic change, in the epigenome downstream of chromosome 21, but it's different in the brain than in the blood.

We even did further purification of cell types. We can purify glia and neurons by nuclear FACS. It's quite beautiful, actually. We didn't invent this. People do this with a Neu-N monoclonal antibody. Here's the neuronal nuclear prep and the glia nuclear prep. These cells (glia and neurons) have different genes affected with about a 30 percent overlap of different genes hypermethylated. It's about a 30 percent overlap between these cell types. Here's the neuroligin gene (NLGN1), which is important for synapse formation. It's a very good hit, but it only gains methylation in the neurons and in the whole brain, not in the glia. This is a neuron-specific epigenetic affect due to a chromosome aneuploidy in a gene that is relevant to brain development and probably intellectual disability in DS. It suggests a therapeutic target. I think it's very nice, and there are multiple other genes that we found to be recurrently affected that also participate in brain development.

I just showed you that downstream of chromosome 21 there are losses in blood cells but gains in the brain. You can think about timing of methyl donor supplementation—which perhaps should not be attempted until brain development is completed—that is—not started until late childhood. But, on the other hand, if these methylation changes in the brain are actually compensatory and normalized the gene networks, then you might want to supplement early in development because it might be that these methylation changes are beneficial to the intellectual function in brain development.

DS is an elegant example, but there are other syndromes with sub-chromosomal deletions, duplications, and so on, and cancer is the other large area where, clearly, aneuploidies are going to have systematic trans-acting effects on the epigenome.

TOPIC #4: Are epidemiological approaches suitable for answering pertinent questions involving risk/preventive factors for human birth defects?

Point: *Modern epidemiologic approaches are invaluable to furthering our understanding of risk factors for human birth defects.*
(Dr. Sonia Hernandez-Diaz, Harvard School of Public Health)

Less than 50% of defects can be predicted or prevented, which supports the need for further research to identify risk/preventive factors for human birth defects. What are the ***pertinent questions***? One could argue that these questions would aim to either: 1) identify modifiable causes of birth defects in order to intervene and prevent cases (e.g. avoiding non-genetic exposures or teratogens); or 2) identify predictors of birth defects in order to inform family planning (i.e. quantify absolute risk in specific human populations) and reduce morbidity through targeted screening.

If these are the pertinent questions, which *approaches* other than epidemiological studies would be suitable? To support that "*Epidemiologic approaches are valuable to identify risk factors for human birth defects*" we can review the historical evidence. For major teratogens such as thalidomide, which increases over 100-fold the risk of specific malformations, clinical observation may identify clusters. However, even for thalidomide, it took over 10,000 affected children for us react. To avoid another thalidomide conundrum, we have proactive surveillance systems such as pregnancy registries, pooling of health care databases, or case-control surveillance studies. These designs have successfully identified more modest teratogens (e.g. valproate). Another example is periconceptional folic acid supplementation; probably the flagship for birth defects prevention research. We learned from the folic acid studies that well-conducted epidemiological studies can be as valid as randomized clinical trials. Nowadays, with the new surveillance systems established, epidemiological studies may be valid and much more efficient than clinical trials to identify risk or preventive factors for human birth defects. Therefore, epidemiological studies have proven to be valuable.

What would be the alternatives to epidemiological studies for pharmaceuticals, and for other factors? Based on its pharmacologic class, one can often predict a drug's adverse effects in adults. However, for teratogenic effects we rarely know the mechanism, and therefore, toxicologic studies provide limited information. Moreover, animal studies are seriously limited in their ability to predict human teratogenesis because of considerable variations in teratogenic effects (even among various non-human mammalian species) and the usual absence of concordance between effects in animals and humans. In humans, clinical trials usually exclude women who might become pregnant, particularly if there is any suspicion from animal studies. Regarding exposures other than prescription medications, most risk factors would be non-randomizable for logistic (e.g. genes) or ethical (e.g. smoking) reasons. Therefore, we are left with well-designed epidemiological studies.

Some epidemiological studies are specifically designed to study birth defects. An example would be the U.S. Collaborative Perinatal Project, a *large cohort* of 52,000 pregnancies. However, despite the large sample size, this study was not able to evaluate most drugs, given the relatively small number of women exposed and the relatively rare outcomes under consideration. Nowadays, we most commonly use cohorts of pregnancies specifically exposed to the factor (drug) of interest: *pregnancy registries*. Yet, it still takes time to find and enroll exposed women soon after conception; and the registries have limited sample sizes for looking at specific malformations. For specific malformations we use *case-control studies*, if the

exposure is not too infrequent. Finally, in the last decade, epidemiologists are increasingly using information from *health care administrative databases*, i.e., claims and electronic medical records. These data sources were not specifically designed to study birth defects. Yet, even with some limitations, health care databases studies can be less costly, include large numbers of pregnancies, and have no recall bias.

Strong risk factors can be identified with *crude simple epidemiologic methods*. To rule out strong teratogenic effects (e.g., over 20% risk of malformations after prenatal exposure to thalidomide), enrollment of 100 exposed pregnancies in a simple uncontrolled cohort might suffice. The effect of major teratogens is so large as to overwhelm the potential impact of common methodological biases on relative risks. However, weaker effects are more difficult to identify, require larger samples and accurate estimates, i.e., carefully designed studies. Validity is crucial for modest effects. For these modest risks it is important to stick to *First Principles*: I) consider specific birth defects; II) consider specific factors (e.g., individual drugs); III) focus on the etiologically relevant gestational period; IV) obtain accurate measures of exposure and outcome; V) enroll enough to attain sufficient statistical power, VI) avoid preferential publication in the context of multiple comparisons; VII) replicate studies to confirm or refute initial findings; VIII) write a detailed protocol (well-designed study, well-specified comparisons, appropriate analytic plan, etc.) that could be registered; IX) carefully publish (expose and discuss limitations, sensitivity analysis that quantify uncertainty, rational reporting and interpretation of scientific evidence); and, X) transparently communicate to stakeholders.

In addition to first principles, sometimes we may need *modern epidemiologic approaches to* avoid selection bias (e.g. by not adjusting for intermediate variables), evaluate indirect effects (e.g., mediation analyses), or reduce confounding (e.g., propensity scores). Modern causal approaches such as the use of direct acyclic graphs (DAGs) may be instrumental in our causal thinking for birth deects. These causal networks can help us identify confounders and colliders.

To quantify absolute risk in humans and inform couples based on their genetic and non-genetic characteristics we need data from humans and large sample sizes (i.e., epidemiological studies). To identify causes (particularly if preventable) of birth defects in humans we need data from humans, large sample sizes and causal thinking (i.e., epidemiological studies). Epidemiologic approaches can be valuable to both identify new teratogens (e.g. drugs, environmental exposures) and “furthering our understanding of (known) risk factors.” To identify potential new teratogens we learned from thalidomide that we need active surveillance in place. To “furthering our understanding of risk factors”, epidemiologic studies have been crucial to understand the relevance of folic acid pathways for birth defects. We learned from folic acid that epidemiological plausibility can support biological findings as much as biological plausibility can support epidemiological ones. However, we must acknowledge that models from basic sciences and animal studies can be used to guide hypotheses. Indeed, although modern epidemiologic approaches are invaluable to furthering our understanding of risk factors for human birth defects, interdisciplinary approaches and collaborations may be essential to further our knowledge.

Counter-Point: *Current epidemiologic approaches are unlikely to offer additional salient information on causes of human birth defects.*
(Dr. Allen Wilcox, National Institute of Environmental Health Sciences, NIH)

As an attempt at defending this counterpoint, I am going to discuss three issues. First, Sonia Hernandez-Diaz talked about folic acid as the flagship example, and I think in some ways, it’s

an example that's hard to replicate. Sonia spoke a lot about the things that make epidemiology a very good context for reproductive studies, but in fact, there are some problems with studying the origins of birth defects by epidemiology, and I'll touch on those. Finally, I'll get into a question I'm even less informed about, whether a certain level of birth defects is really inevitable and not preventable. Let me start with the first. Is folic acid and neural tube defects (NTDs), this wonderful public health advance, is this really an exception to the rule of birth defects in human populations?

We certainly know it was a great accomplishment, but in some ways, neural tube defects may be, if not unique, quite unusual among human birth defects. I think one way it's unusual is that even before we knew about folic acid, we had a lot of reasons to think that there was something preventable here because of the epidemiology of neural tube defects. The risk of NTDs is highly variable among human populations across geography and over time. It's just that kind of variability that points out that there's something going on that we can intervene in. We also see that migrant populations acquire the risk of the country that they move to in terms of neural tube defects, another cardinal sign of a preventable cause. In 1991, we had the definitive randomized clinical trial by the Medical Research Council in the U.K. showing that folic acid reduced the risk of neural tube defects 70 percent. In the 1990s, there was a widespread public health push to encourage food fortification and supplements during early pregnancy, and then victory. There's no other birth defect that has the variability of NTDs, nor, I would suggest the same potential for prevention.

As a second issue, I will discuss roadblocks to the current epidemiologic approaches. With respect to birth defects we can only observe the prevalence as it exists at some point in time after the birth defect was formed, usually before gestational week 10. This wouldn't be so important except there's a big rate of miscarriage after the birth defect has been created that might distort the prevalence. The total risk of miscarriage is 12 to 15 percent in women at their healthiest years of reproduction (age 25 to 35). What we observe at delivery, which is about three percent of births with severe defects, actually is not a lot of information about what percent of embryos actually had defects at the time the embryo was formed. Such inability to measure incidence can actually make problems for studying the etiologies of birth defects. Measuring incidence or the inability to measure incidence of birth defects is a problem that not even randomized clinical trials can solve.

As a third issue to discuss, I'm going to enter into an area which I know least of all. Let me propose that maybe birth defects—the complete eradication of birth defects—is not something that you can even practically attempt.

The literature that I found on this is not huge, and the way this is talked about is something called developmental noise. The idea here is that variability in biology is fundamental, and in fact, error in biology is also intrinsic to the processes. The machinery of embryonic construction is complicated, and it has to be imperfect. Would we ever think otherwise? Just to do the thought experiment. Can we imagine that it might be possible to provide an environment for a population in which no woman was exposed to any teratogen, and we manage to remove all the allelic variants that might increase the risk of a birth defect? If we could provide that perfect environment for a population, can we imagine that we would ever get zero percent birth defects? I would suggest to you the answer is no.

I think birth defects are not simply the product of environment and genes, and my example for this more concretely is monozygous twins. What is the concordance of birth defects in

monozygous twins? They have nearly identical genes, nearly identical exposures, and the proportion of concordance with birth defects is 40 percent at the very highest. It is probably more like 20 or 30 percent. Why is that? I would suggest there's an element of randomness here as well.

Does this mean we can stop worrying about finding the causes of birth defects? Absolutely not. Surveillance for new teratogens is absolutely essential, and here I can't help but step away from my talk title and say, "I am really alarmed at the decline of political will to support birth defects registries." If we're not going to keep track of—good track of—the prevalence of birth defects, we're going to miss new teratogens. We should always be looking for new preventable causes. We've had so many interesting examples even in the recent past, but I do think that there are some serious obstacles facing epidemiology. The fraction of birth defects with preventable causes may be limited, and we can't expect future successes to be as major as the prevention of neural tube defects. However, I'm hoping that recognizing these limitations may even improve our chances of success.

Discussion

This one-day workshop, along with another workshop held four years earlier (Olshan, Hobbs & Shaw, 2011), highlighted current challenges and new opportunities for studying the role of genetic and interacting nongenetic factors in the causes of human birth defects. Both workshops provided salient discussions pertaining to the use of animal models; genetic technologies; key elements of population-based study designs; the need for national collaborative projects, biorepositories, and consortia; investigation of new types of structural genetic variants; examination of gene-exposure interactions; and, strategies for genetic variant discovery. Importantly, they have brought diverse, but complementary disciplines together to share ideas. We as scientists are far too comfortable working in disciplinary silos, and this has resulted in a lack of the necessary intellectual mixing. As NIEHS Director Birnbaum astutely pointed out at this workshop—let's not choose sides between genes and environment. Both, and much more, will need to be considered to find causes and preventives for birth defects.

Indeed, if our intention is to unravel the etiologies of any, let alone all, birth defects it's going to take the collective intellectual contributions of numerous fields: developmental biologists, geneticists, epidemiologists, clinicians, teratologists, among others. The motivation for the workshop was to increase action toward advancing discoveries about the causes, particularly genetic ones, of birth defects. This was a good first step. While the discussions pointed toward what is scientifically possible, next steps are not without obstacles. Primary among the obstacles is the lack of comprehensive and sustained funding devoted to interdisciplinary birth defects research in the U.S. and the lack of coordinated political or research agendas to prevent or reduce the occurrence of birth defects.

At this time, to overcome roadblocks to the discovery of birth defect causes, priorities will need to be set and resources to support these priorities will need to be made available. There is a critical need for more funding opportunities and mechanisms to support a national collaborative approach among scientists. A national agenda will be a powerful substrate to attract and train the next generation of birth defects researchers.

One of the topics discussed at the workshop was that birth defects research needs better branding. Indeed, Dr. Bamshad articulately noted, "*I don't think I've used the phrase "birth*

defect" with a family for 20 years. I do think there is this issue of branding. I'm not certain what is the correct term or phrase to use, but typically when I'm working with—when I talk about the differences that a child or children have compared to other children—to be honest, this phrase never even enters my mind in the discussion with a family of the newborn."

Birth defects are not one single outcome. Unlike cancer, there is not one Institute at NIH whose sole purpose is to fund scientific efforts targeting birth defects. Part of the difficulty of developing an integrated approach to all structural birth defects rests in the diversity of phenotypes that can be affected during embryogenesis. Across the vast majority of phenotypes a common feature is a disruption of embryogenesis that affects the structure of one or more developing organs. Etiologic investigations of structural birth defects have solid tenets in teratology and developmental biology. The NIH has 11 Institutes and Offices with an interest in structural birth defects. The NICHD is the logical leader and champion to spearhead an integrated national movement. The advent of the Trans-NIH Structural Birth Defects Working Group at NIH, announced at this workshop by Dr. Guttmacher, Director of NICHD, is an important first step.

Our nascent understanding of potential causes, including factors that may reduce the risk of birth defects, is at an important juncture given the rapidly advancing genomic technologies and experience gained by scientific communities investigating the genomics of other human conditions. Even if small budgetary fractions of NIH's 11 Institutes and Offices were to be redirected and coordinated, our research community would be poised to unravel the genetic mysteries of birth defects that plague our most vulnerable members of society.

Figure 1: Agenda of the 1-Day NIH Workshop January 2014

Topic 1: How informative are experimental model systems for understanding human birth defect etiologies?

- Point: Experimental model systems are critical to our understanding of human birth defects; Dr. John Wallingford
- Counter-Point: Experimental model systems can not sufficiently inform the complex environmental, social, and genetic underpinnings of human birth defects; Dr. Leslie Biesecker
- Discussant: Dr. Richard Finnell

Topic 2: What is the preferred method(s) to discover genetic etiologies of human birth defects?

- Point: Genome wide association studies continue to be legitimate, and in some circumstances, the preferred approach; Dr. Stephen Chanock
- Counter-Point: Genomewide sequencing or exome sequencing would be preferred; Dr. Mike Bamshad
- Discussant: Dr. Terri Beaty

Topic 3: How valuable will the investigation of epigenomics be toward understanding the etiologies of human birth defects?

- Point: Epigenomics have theoretical relevance but owing to logistical and scientific challenges such studies of human birth defects are currently impractical; Dr. Jan Friedman
- Counter-Point: Epigenomics represent a critical, timely, and currently practical area for focus towards understanding human birth defects; Dr. Benjamin Tycko
- Discussant: Dr. Edward McCabe

Topic 4: Are epidemiological approaches suitable for answering pertinent questions involving risk/preventive factors for human birth defects?

- Point: Modern epidemiologic approaches are invaluable to furthering our understanding of risk factors for human birth defects; Dr. Sonia Hernandez-Diaz
- Counter-Point: Current epidemiologic approaches are unlikely to offer additional salient information on causes of human birth defects; Dr. Allen Wilcox
- Discussant: Dr. Enrique Schisterman