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Genetic Basis of Susceptibility to Teratogen Induced Birth Defects

BOGDAN J. WLODARCZYK,* ANA M. PALACIOS, CLAUDIA J. CHAPA, HUIPING ZHU, TIMOTHY M. GEORGE, AND RICHARD H. FINNELL

Birth defects remain the leading cause of infant death in US. The field of teratology has been focused on the causes and underlying mechanisms of birth defects for decades, yet our understanding of these critical issues remain unacceptably vague. Conclusions from years of animal and human studies made it clear that the vast majority of birth defects have multifactorial origins, with contributions from environmental and genetic factors. The environment comprises not only of the physical, biological, and chemical external environment surrounding the pregnant woman, but it also includes the internal environment of the woman's body that interact with the developing embryo in a complex fashion. The importance of maternal and embryonic genetic factors consisting of countless genetic variants/mutations that exist within every individual contribute to birth defect susceptibility is only now being more fully appreciated. This great complexity of the genome and its diversity within individuals and populations seems to be the principal reason why the same teratogenic exposure can induce severe malformation in one embryo, while fail to do so to other exposed embryos. As the interaction between genetic and environmental factors has long been recognized as the first ''Principle of Teratology'' by Wilson and Warkany [1965. Teratology: Principles and techniques. Chicago: University of Chicago Press], it is only recently that the appropriate investigative tools have been developed with which to fully investigate this fundamental principle. The introduction of high throughput technologies like whole genome sequencing or genome-wide association studies are promising to deliver an enormous amount of new data that will shed light on the genomic factors that contribute susceptibility to environmental teratogens. In this review, we attempt to summarize the epidemiological and experimental literature concerning birth defects whose phenotypic expression can be clearly related to the interactions between several select environmental factors and those genetic pathways in which they are most likely to have significant modifying effects.

KEY WORDS: birth defects; teratogen; genome; mutations; gene–environment interaction

How to cite this article: Wlodarczyk B, Palacios A, Chapa C, Zhu H, George TM, Finnell RH. 2011. Genetic basis of susceptibility to teratogen induced birth defects. Am J Med Genet Part C Semin Med Genet 157:215–226.

INTRODUCTION

Birth defects are abnormalities present at or before birth, and may involve multiple organs including: the brain, heart, lungs, liver, bones, intestinal tract, limbs and other structures. These defects can occur for a variety of reasons including inherited (genetic) conditions, toxic exposure of the embryo and fetus, and as is most often the case, for as yet unknown reasons. In the

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DOI 10.1002/ajmg.c.30314
Published online 15 July 2011 in Wiley Online Library (wileyonlinelibrary.com).

United States, 1 out of every 33 babies is born with a major birth defect (http://www.nbdpn.org/docs/US_2010_C.pdf). The most common types of birth defects include congenital heart defects (CHDs) and neural tube defects (NTDs), and these defects can be very serious, often life-threatening. Another group of common birth defects, craniofacial malformations, include cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO), while less commonly life-threatening, they require comprehensive medical and surgical attention.

Currently, about 70% of the causes of birth defects are unknown. The etiology of the majority of birth defects whose causes are unclear is believed to involve both environmental and genetic factors. The list of environmental factors that have been associated with birth defects is long and remarkably varied [Blom et al., 2006]. Some examples include: maternal smoking [Shi et al., 2007; Shea and Steiner, 2008; Suarez et al., 2008, 2011], organic solvents [Brender and Suarez, 1990; Brender et al., 2002], environmental toxicants like pesticides, nitrates, and heavy metals [White et al., 1988; Sever, 1995; Shaw et al., 1999b], inadequate intake of nutrients [Shaw et al., 1999a; Carmichael et al., 2003], maternal fever/influenza [Shaw et al., 1998a; Lundberg et al., 2003], maternal obesity and diabetes [Watkins and Botto, 2001; Watkins et al., 2003; Waller et al., 2007]. On the other hand, there is an abundance of supportive evidence that complex birth defects have a genetic component [Campbell et al., 1986; Shaw et al., 1994; Blom et al., 2006]. One important source of scientific evidence supporting a genetic contribution to the etiology of birth defects comes from studies involving animal models. For example, more than 240 spontaneous and genetically modified mouse models of NTDs exist, with 205 of them representing specific genes [Harris and Juriloff, 2010]. These models provided abundant candidate genes for human studies; however, very few mutations have been found in these genes providing evidence as having a major role for human NTDs. Instead, variants from multiple genes have been linked to NTDs risk individually with rather small effect [van der Put et al., 1995; Ou et al., 1996; Volcik et al., 2003a,b; Zhu et al., 2003, 2005, 2006, 2007; van der Linden et al., 2006, 2007; Parle-McDermott et al., 2007; Davidson et al., 2008], leading us to believe that these small effects interact with select environmental factors to create a more significant developmental disequilibrium beyond which these genetic factors could have produced individually, disrupting normal development, and resulting in the abnormal phenotype [Prescott et al., 2001; Murray, 2002; Finnell et al., 2004; Graham and Shaw, 2005; Shi et al., 2008]. One commonly cited birth defect-oriented example of a GxE interaction is the effect of maternal smoking and an important detoxification gene, *CYP1A1*, on risk for oral clefts in the exposed embryos [van Rooij et al., 2001; Shi et al., 2007]. This is consistent with the extreme complexity of the developmental processes during early embryogenesis.

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Biological interactions and their role in etiological mechanisms require further functional studies [Ottman, 1990]. Herein we will review both human and animal studies on the genetic basis of environmentally induced birth defects. Inherited diseases caused by chromosomal abnormalities, as well as defects caused by single genes are not within the scope of this review.

FOLATE PATHWAY GENES AND NTDs

It has been demonstrated that folic acid supplementation prevents NTDs [Smithells et al., 1980; Czeizel, 2000; Au et al., 2010; Blencowe et al., 2010; Obican et al., 2010; Collins et al., 2011] and other birth defects such as conotruncal heart defects [Botto et al., 2003b; Bailey and Berry, 2005]. Folate metabolism is a complex process involving a large number of enzymatic reactions, producing a variety of intermediates that function in vital physiological processes such as nucleotide synthesis, cell division, and tissue growth. Perturbation of this complex process may result in frank folate deficiency and/or accumulation of homocysteine. Animal studies have generated several hypotheses regarding the mechanisms underlying the preventive effect of folic acid. Inactivation of folate pathway genes such as *Folr1*, *Folr2*, or *Mthfr* renders the developing embryo susceptible to an increased risk of NTDs following in utero exposure to specific teratogens. Human studies exploring the interaction between folate pathway genes and teratogens on NTDs risk have been limited by the insufficiency of statistical power due to small sample size. There have been reports of possible interactions between the MTHFR

C677T polymorphism and maternal smoking [Hobbs et al., 2006], BHMT and obesity, as well as transcobalamin II (TCII) and maternal alcohol use [Hobbs et al., 2010], on CHD risk. Blanton et al. [2011] recently explored a number of folate pathway genes (including folate transport genes) as risk factors for non-syndromic CLP, and found possible interactions between polymorphisms in *FOLR1* and *FOLR2* and maternal smoking.

Data from animal studies concerning the possible involvement of folate pathway genes in teratogen-induced birth defects remain equivocal. In our laboratory, we have treated four different knockout mouse strains (*Rfc1*, *Folr1*, *Folr2*, and *Mthfr*) that are involved in folate transport or metabolism with sodium arsenate, a known mouse neural tube teratogen [Wlodarczyk et al., 2001, 2006a; Spiegelstein et al., 2005]. The *Rfc1* knockout mice treated with 40 mg/kg of sodium arsenate, failed to show any genotype dependent differences in teratogenic outcomes. The rates of early embryonic loss (resorptions) and exencephaly (NTDs) among fetuses from treated wildtype and heterozygous *Rfc1* dams did not differ significantly. However, there was a substantial difference seen in arsenic embryotoxicity between heterozygous $Rfc1^{+/-}$ and heterozygous $Folr1^{+/-}$ dams, another folate transport mutant mouse line described in this study [Spiegelstein et al., 2005]. In $Rfc1^{+/-}$ mice treated with arsenic, 12–22% of the embryos were resorbed, whereas in $Folr1^{+/-}$ mice embryolethality reached 82% of the conceptuses. Despite the fact that these two mutant mouse strains were not compared directly in that study, this is still a compelling example that genetic variants can deeply modulate the outcome to a teratogen exposure. It should be also noted, that only wildtype and heterozygous animals were used in this study, since *Rfc1* and *Folr1* homozygous mutations are embryolethal. Having one active copy of these genes is apparently sufficient for viability and it appears that the heterozygous mice can compensate for their genetic deficit in folate metabolism [Spiegelstein et al., 2005].

When the *Folr2* knockout mice, which do not present with any pathological phenotype even in the nullizygous state, were administered sodium arsenate, the treatment was significantly more teratogenic to $Folr2^{-/-}$ mice than to wildtype ($Folr2^{+/+}$) mice. To further investigate how additional environmental factors (potentially teratogenic) can modulate this gene–teratogen interaction, the *Folr2* knockout mice were fed a folate deficient diet during the periconceptional period and throughout the pregnancy and the arsenate was administered during the period of neural tube closure. In this experiment, the teratogenic effect observed in $Folr2^{-/-}$ was considerably exacerbated by the low folate diet (exencephaly rate increased from 40 to 64%), whereas in $Folr2^{+/+}$ mice, the rate of NTDs did not change [Wlodarczyk et al., 2001]. These results illustrate that the gene–teratogen interactions can be further complicated when more than one teratogen is considered. Some teratogens can act in a synergistic manner in individuals with "sensitive" genotype, as exemplified by arsenic and folate deficiency in $Folr2^{-/-}$ nullizygous mice.

The results following the treatment of *Mthfr* knockout mice in utero with arsenic were somewhat similar to those observed in the *Rfc1* mice. There was no difference in teratogenic response to arsenic when the pregnancy outcomes in dams of different *Mthfr* genotypes (+/+, +/−, and −/−) were compared. However the *Mthfr* mutant mice were significantly more sensitive to arsenic teratogenicity than were the $Rfc1^{+/-}$ or $Folr2^{-/-}$ knockout mice [Wlodarczyk and Finnell, unpublished data].

MATERNAL EXPOSURE TO CIGARETTE SMOKE

Embryonic exposure to cigarette smoke (first-hand and/or passive) is a known risk factor for structural defects such as orofacial clefts [Lie et al., 2008; Shi et al., 2008] and gastroschisis [Feldkamp et al., 2008]. Although there are a few studies suggesting that maternal exposure to cigarette smoking is "protective" against NTDs and some CHDs [Kallen, 1998; Grewal et al., 2008], the vast majority of studies have reported an increased risk of adverse pregnancy outcomes including: birth defects, preterm delivery, spontaneous abortion, growth restriction, or negative neurodevelopmental outcomes in the exposed offspring of women who smoked [Shea and Steiner, 2008; Suarez et al., 2008, 2011; Shaw et al., 2009], or were breathing in second hand smoke [Venners et al., 2004; Meeker et al., 2007; Li et al., 2008; Suarez et al., 2008, 2011]. Association between maternal cigarette smoking and non-syndromic orofacial clefts (NSOFC) has been observed in a number of epidemiological studies [Lorente et al., 2000; Little et al., 2004; Shaw et al., 2009].

Hwang et al. [1995] was the first to suggest a possible interaction between the transforming growth factor alpha (*TGFα*) Taq1 variant and maternal smoking on NSOFC [Hwang et al., 1995]. TGFα is a secretory protein that binds to the epidermal growth factor receptor. It plays important roles in cell migration and differentiation, and is also a potent promoter of angiogenesis [Kumar et al., 1995]. Hwang reported that *TGFα* Taq1 variant was associated with a higher risk of non-syndromic cleft lip with or without cleft palate in mothers who smoked 20 or more cigarettes/day. Infants carrying the less common C2 allele who were exposed to maternal smoking of 10 or less cigarettes per day showed a 6.16-fold increase in the risk for CPO [95% confidence interval (CI): 1.09–34.7], while the risk was 8.69-fold higher (95%CI: 1.57–47.8) if the mothers smoked more than 10 cigarettes/day.

More recently in a population-based case control study in California, Shaw et al. [1998b] observed an elevated risk associated with the same *TGFα* Taq1 C2 allele in smoking mothers, resulting in an OR of 6.1 (95%CI: 1.1–36.1) for cleft lip with or without cleft palate (CL/P), and an OR of 9 (95%CI: 1.4–61.9) for CPO. It is noteworthy that when analyzing interactions, the ORs, although elevated, often have wide CIs, indicating the rather imprecise risk estimations.

A meta-analysis examining five case–control studies found a significant overall smoking effect for CL/P (OR = 1.64, 95%CI = 1.33–2.01) and CPO (OR = 1.42, 95%CI = 1.06–1.90). There was no evidence of increased risk for CPO if the infant carried the C2 allele among non-smoking mothers. However, if the mother reported smoking and the infant carried the C2 allele, there was an overall increased risk for CP, with an OR of 1.95 (95%CI: 1.22–3.10). Based on this meta-analysis, the *TGFα* genotype failed to demonstrate an increased risk for CL/P, regardless of maternal smoking status. The authors concluded that gene–environment interactions between the infant's *TGFα* Taq1 polymorphism and maternal smoking were limited to CPO [Zeiger et al., 2005].

Folic acid is an essential B vitamin required for both amino and nucleic acid biosynthesis. It also participates in the one-carbon unit transfers, a key step in biomethylation reactions. Quickly proliferating cells of a rapidly growing embryo/fetus have a high demand for folate; therefore adequate supply of folic acid is especially important for women during pregnancy. Cigarette smoking has been documented to lower folate and increase homocysteine blood levels [Ozerol et al., 2004; Jauniaux and Burton, 2007]. High level of homocysteine during pregnancy might affect important developmental processes such as neural crest cell motility and migration [Brauer and Tierney, 2004]. Additionally, high level of homocysteine and its conversion to homocysteine–thiolactone, have been associated with post-translational modification of the folate receptors. As a result, creation of neoantigens might be inducing a pathological maternal immune response that ultimately will impair folic acid uptake [Jakubowski et al., 2009]. In a recently published study, Blanton et al. [2011] analyzed 89 single nucleotide polymorphisms (SNPs) in fourteen folate metabolism-related genes in non-Hispanic white and Hispanic NSCLP families. Evidence was documented for interactions with smoking, and SNPs in the *FOLR1* and *FOLR2* genes.

Maternal smoking has been identified as a risk factor for defects other than craniofacial, including CHD [Malik et al., 2008; Kuciene and Dulskiene, 2010] and gastroschisis [Lammer et al., 2008; Chabra et al., 2011]. In a population-based case–control study, women who smoked and carried a TCII—776 CG or GG genotype—were 1.8 times more likely to have a CHD-affected fetus than were women who smoked and carried a CC genotype. The CHDs were: non-syndromic septal, conotruncal, right or left-sided obstructive heart defects [Hobbs et al., 2010]. TCII is an important enzymatic transporter of cobalamin from the intestinal lumen to the bloodstream, and eventually to target tissues, suggesting a role for vitamin B12 in the etiology of this group of defects [Quadros et al., 1999]. Torfs et al. [2006] assessed the interaction between maternal smoking and selected candidate genes in a case–control study of 57 cases of gastroschisis and 506 controls. Thirty-two genes involved in angiogenesis, blood vessel integrity, inflammation, wound repair, and dermal/epidermal strength were interrogated for variants. Of these genes, *NOS3* glu298asp, *ICAM1* gly241arg, and *NPPA* T2238C showed a strong interaction with maternal smoking. Women who smoked and carried one or two of the aforementioned variants were at a significantly higher risk of having an infant with gastroschisis, when compared to women with wildtype alleles who did not smoke [*NOS3* OR = 5.2 (95%CI: 2.4–11.4); *ICAM1* OR = 5.2 (95%CI: 2.1–12.7); and *NPPA* OR = 6.4 (95%CI: 2.8–14.6)] [Torfs et al., 2006].

MATERNAL ALCOHOL (ETHANOL) EXPOSURE

Fetal alcohol syndrome (FAS) was initially described in the French medical literature by Lemoine et al. [1968]. Subsequently, Jones and collaborators were the first to systematically define the association between maternal alcohol abuse and the pattern of birth defects associated with the FAS [Jones et al., 1973]. Maternal alcohol use has also been associated with birth defects that are not typically considered to be a part of the FAS. For example, one study found that women who consumed alcohol at least once a week had a 2.1-fold increased risk (95%CI: 1.1, 4.0) of having a child affected with an NTD [Grewal et al., 2008].

Studies on attempting to understand the genetic basis of susceptibility to the etiology of this condition have not been successful. Although a recent population-based case–control study reported that variants in the alcohol dehydrogenase 1C (*ADH1C*) gene may modify the association between alcohol and oral clefts, it did not extend to the whole pattern of FAS dysmorphic features [Boyles et al., 2010]. Alcohol dehydrogenase (ADH) is an enzyme that oxidizes ethanol to form acetaldehyde during its metabolism. Functional SNPs in *ADH1C* gene are associated with a reduced ratio of alcohol oxidation. In this study, mothers who consumed five or more alcoholic drinks per sitting during the first trimester of pregnancy had an elevated risk for a malformed infant with an oral cleft (OR of 2.6, 95%CI: 1.4–4.7). This increased risk was evident only in mothers or children who carried *ADH1C* haplotype that was associated with slow alcohol metabolism (OR of 3.0, 95%CI: 1.4–6.8) [Boyles et al., 2010]. There was no evidence of alcohol-related risk when both mother and infant carried only the rapid metabolism *ADH1C* variant (OR of 0.9, 95%CI: 0.2–4.1). Hobbs et al. [2010] reported a possible gene–environment interaction in infants with CHDs exposed in utero to alcohol. Women who consumed alcohol while pregnant and were carriers of the *TCII*—776 CG or GG genotype—were 1.7 times more likely to have a CHD-affected fetus, than were women who drank and carried a CC genotype.

MATERNAL HYPERGLYCEMIA/ GLUCOSE METABOLISM

An extensive epidemiological literature indicates that maternal diabetes is a

strong risk factor for birth defects and perinatal mortality [Mills et al., 1979; Myrianthopoulos and Melnick, 1987; Reece and Homko, 1993; Waller et al., 1994; Shaw et al., 1996, 2000; Werler et al., 1996; Hendricks et al., 2001; Eriksson et al., 2003; Honein et al., 2003; Watkins et al., 2003; Anderson et al., 2005]. Maternal metabolic syndrome, obesity and pre-pregnant weight are also associated with risk of central nervous system birth defects [Waller et al., 1994; Shaw et al., 1996; Watkins et al., 1996; Werler et al., 1996; Kallen, 1998; Hendricks et al., 2001; Ray et al., 2007; Correa et al., 2008] as well as other birth defects such as CHDs, omphalocele and instances where the child presents with multiple anomalies [Watkins and Botto, 2001; Watkins et al., 2003]. Gestational diabetes mellitus (GDM) may also be associated with increased risk of central nervous system birth defects [Anderson et al., 2005]; however, the increased risk may in fact be due to undiagnosed type 2 diabetes. Therefore, GDM has not been recognized as a risk factor due to inconsistent findings [Janssen et al., 1996; Savona-Ventura and Gatt, 2004; Mitanchez, 2010]. In vivo and in vitro studies using animal models suggested that hyperglycemia is a major teratogen capable of inducing malformations in diabetic animal models, while other associated factors such as hyperinsulinemia [Hendricks et al., 2001], ketone bodies, branched amino acids, and triglycerides may also exert some adverse effects on the developing embryos [Styrud and Eriksson, 1992; Suzuki et al., 1996; Reece et al., 1996a; Eriksson et al., 2000; Zhao and Reece, 2005]. There is sufficient evidence to suggest that compromised maternal glucose control predisposes embryos to developmental anomalies, even among non-diabetic women [Groenen et al., 2003; Shaw et al., 2003]. Maternal diabetes, obesity, and metabolic syndrome create a compromised in utero environment for the developing embryo. The genetic basis underlying the susceptibility to abnormal glucose metabolism induced birth defects involves both maternal and embryonic gene variants.

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Early human embryos do not have pancreatic function until the development of β-cells, usually after gestational week 7. Maternal hyperglycemia forces an increased glucose flux into embryonic cells through glucose transporters during early organogenesis; therefore, the embryonic cells suffer from an increased metabolic overload which is directed at their mitochondria, leading to increased formation of reactive oxygen and oxidative stress [Eriksson et al., 2000; Wentzel et al., 2001; Beemster et al., 2002]. Hyperglycemia causes a myo-inositol deficiency with a competitive inhibition of ambient glucose, which might have been associated with a diminished phosphoinositide signal transduction. Dietary supplementation of deficient substrates for example, arachidonic acid or myo-inositol [Goldman et al., 1985; Reece et al., 1988, 1996b; Baker et al., 1990; Khandelwal et al., 1998], either in vitro or in vivo, has been shown to reduce the incidence of diabetes-related malformations in the offspring of diabetic pregnant animals. Gene expression profiling studies showed that maternal diabetes alters transcriptional programming in developing embryos by down-regulating a large group of transcriptional factors [Pavlinkova et al., 2009; Salbaum and Kappen, 2010].

Studies have shown that the increased level of oxygen free radicals in embryos of diabetic rats are contributing to the observed malformations, and that the removal of these reactive oxygen species reduces the likelihood of fetuses expressing the diabetic embryopathy [Eriksson and Borg, 1991; Hagay et al., 1995; Eriksson and Siman, 1996; Sivan et al., 1996; Wentzel et al., 1997; Fine et al., 1999]. Increased glucose delivery to embryos, or activation of pathways that are stimulated by high glucose such as the hexosamine biosynthetic pathway or hypoxia, increase oxidative stress in embryos. Blocking these pathways, or providing antioxidants such as reduced glutathione or vitamin E, suppress the adverse effects of excess glucose [Loeken, 2005, 2006]. Impaired embryonic gene expression resulting from oxidative stress and consequent apoptosis or disturbed organogenesis, may be a general mechanism to explain the features observed in the diabetic embryopathy [Kambe et al., 1996; Wu et al., 1996; Phelan et al., 1997; Fine et al., 1999; Pani et al., 2002a,b; King and Loeken, 2004; Loeken, 2006; Dheen et al., 2009]. For example, oxidative stress induced by excess embryonic glucose metabolism inhibits the expression of *Pax3*, a critical developmental gene, leading to the appearance of birth defects [Morgan et al., 2008; Zabihi and Loeken, 2010].

PAX3 gene encodes a transcription factor required for normal neural tube closure. Murine embryos with a non-functional *Pax3* gene exhibit open NTDs, exencephaly and spina bifida, with 100% penetrance [Pani et al., 2002a; Wlodarczyk et al., 2006b; Burren et al., 2008]. PAX3 protein is required during neural tube development to suppress p53-dependent cell death and consequent failure to close the neural tube. PAX3 decreases steady-state levels of the p53 tumor-suppressor protein, such that when PAX3 is deficient, p53 protein increases, leading to increased

neuroepithelial apoptosis which may occur prior to the completion of neural tube closure. Supplementation with folic acid or 5-methyltetrahydrofolate rescues the normal phenotype in spontaneous *Pax3* mutant (*Sp/Sp*) embryos [Wlodarczyk et al., 2006b]. A recent mouse study suggested the presence of a gene–environment interaction between embryonic folate levels and the *Pax3* genotype. Folate deficiency does not cause NTDs in wildtype mice, but causes a significant increase in cranial NTDs among mutant embryos that carry an intragenic deletion in the *Pax3* gene (Sp^{2H}). Control treatments, in which intermediate levels of folate are supplied, failed to induce NTDs, suggesting that NTD risk is related to embryonic folate concentration, not maternal blood folate concentration. This study suggested that folate deficiency increases the risk of NTDs in genetically predisposed Splotch embryos, probably via embryonic growth retardation [Burren et al., 2008]. Variants in human *PAX3* gene potentially associated with risk of spina bifida have been suggested [Lu et al., 2007]; therefore, interaction between these variants and environmental factors including folic acid use and maternal hyperglycemia merit evaluation.

Glucose is transported across the lipid bi-layers of cell membranes by a family of structurally related membrane spanning glycoproteins called glucose transporters [Joost and Thorens, 2001; Joost et al., 2002]. Erythrocyte-type glucose transporter (*GLUT1*) is the major type of glucose transporter expressed during early organogenesis [Maeda et al., 1993; Matsumoto et al., 1995]. Consistent with the high demand for glucose, GLUT1 protein is expressed at a high level in the embryonic tissues undergoing differentiation, mainly in the neural tube, while expression declined as gestational age progressed [Shepard et al., 1970; Tanimura and Shepard, 1970; Akazawa et al., 1989; Matsumoto et al., 1995]. Homozygous *Glut1* knockout mice die in utero [Ohtsuki et al., 2006]. *Glut1*-deficient mice generated by antisense-Glut1 exhibit developmental malformations including: intrauterine growth retardation (IUGR), caudal regression, anencephaly with absence of the head, microphthalmia, and micrognathia, which are similar to those defects observed in the diabetic embryopathy [Heilig et al., 2003]. A recent study reported that one silent variant, Pro196, may be associated with risk of spina bifida by affecting the transcription and spicing of the *SLC2A1* (alias: *GLUT1*) gene [Davidson et al., 2008].

A recent report suggested that *GLUT2* may be a risk factor involved in NTD occurrence in diabetic pregnancies [Li et al., 2007]. In animal models, maternal diabetes and hyperglycemia do not down-regulate *Glut1* and *Glut2* expression in the embryos and visceral yolk sac during the critical periods of organogenesis, as they do in pre-implantation embryos; therefore, embryonic glucose transport continues and results in increased glucose flux into the cells [Maeda et al., 1993; Takao et al., 1993; Trocino et al., 1994; Li et al., 2007]. Inactivation of *Glut2* gene in a mouse strain protects embryos from maternal diabetes-induced NTDs [Li et al., 2007]. The interactions between human *SLC2A2* (alias: *GLUT2*) and maternal glucose metabolism; however, have not yet been evaluated.

EXPOSURE TO ANTIEPILEPTIC DRUGS

Epilepsy is the most common neurologic disorder requiring medical treatment in pregnant women [Hvas et al., 2000]. Antiepileptic drugs (AEDs) have long been recognized as having a teratogenic potential capable of disrupting normal development in exposed infants. Several epidemiological studies indicated that infants of mothers treated with AEDs during pregnancy have a two- to threefold higher risk of having birth defects when compared to the general population [Holmes et al., 2001; Perucca, 2005]. In the USA annually almost 25,000 children are born to women with epilepsy and most of them require AEDs such as valproic acid, carbamazepine, phenytoin, phenobarbital, or lamotrigine [Meador et al., 2008]. About 3–10% of these infants present with birth defects, and approximately 11–20% of them exhibit neurodevelopmental impairment, with or without associated structural abnormalities [Finnell, 1991; Kaneko et al., 1999; Dean et al., 2002; Mortensen et al., 2003; Adab et al., 2004; Gaily et al., 2004; Vinten et al., 2005]. Intrauterine exposure to valproate or to multiple AEDs seems to represent the highest malformation risk [Breen and Davenport, 2006; Pennell, 2006; Veiby et al., 2009]. Irrespective of which AED was studied, the results consistently showed that not all exposed fetuses were born with birth defects [German et al., 1970; Jones et al., 1989; Dansky and Finnell, 1991]. These observations indicate that in most instances, AEDs alone are insufficient to induce teratogenic effects in exposed fetuses. The observed birth defects are multifactorial in their origin and beside exposure to AEDs, some other interacting components, most likely genetic, must be involved in order to predispose the developing embryo to express an abnormal phenotype following the in utero AED exposure. It seems

Intrauterine exposure to valproate or to multiple AEDs seems to represent the highest malformation risk. Irrespective of which AED was studied, the results consistently showed that not all exposed fetuses were born with birth defects. These observations indicate that in most instances, AEDs alone are insufficient to induce teratogenic effects in exposed fetuses. The observed birth defects are multifactorial in their origin and beside exposure to AEDs, some other interacting components, most

likely genetic, must be involved in order to predispose the developing embryo to express an abnormal phenotype following the in utero AED exposure.

that either the mother or the embryo carry genetic factors determining susceptibility to AED induced adverse effects. Epidemiological study showed that the recurrence risk of NTDs among pregnant women who were exposed to VPA was higher than the occurrence risk of NTD in women exposed to VPA, suggesting possible genetic contributions to VPA sensitivity [Dansky and Finnell, 1991]. Other studies concerning NTDs demonstrated that these defects are more common if there was an existing family history of NTDs [Delgado-Escueta and Janz, 1992; Malm et al., 2002].

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that plays a key role in folate metabolism. It catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), which is the main methyl donor in the homocysteine to methionine remethylation cycle. Studies of *MTHFR* in human revealed a common polymorphism (677C→T substitution) in this gene that is associated with hyperhomocysteinemia and a slightly increased risk for birth defects [Botto and Yang, 2000]. Epileptic mothers taking AEDs who were *MTHFR* 677T homozygotes were found to have a three- to fourfold higher risk for having a child with the fetal anticonvulsant syndrome, compared with 677CC homozygous mothers receiving AED treatment [Deen et al., 1999; Kini et al., 2007; Dean et al., 2008]. This syndrome consists of a characteristic pattern of neurodevelopmental impairment, facial features, and major birth defects including NTDs, orofacial clefts, heart, and renal defects. It appears that there are significantly lower plasma folate concentrations in children taking VPA and carbamazepine, comparing to a control group of healthy children [Vurucu et al., 2008]. Based on this finding, one can speculate that VPA lowers an already limited supply of folate in pregnant *MTHFR* 677TT homozygous women, pushing the developing embryo beyond the threshold of normal development and ultimately leading to NTDs. Insight from the animal studies demonstrating that the teratogenicity of VPA can be prevented/diminished by folic acid supplementation tend to provide biological plausibility to this hypothesis [Padmanabhan and Shafiullah, 2003; Dawson et al., 2006]. Unfortunately, there is no concrete evidence that supplemental folic acid protects against NTDs in humans [Ornoy, 2009]. On the other hand, rather surprising results were reported from studies on *Mthfr* knockout mice treated with VPA. $Mthfr^{+/+}$ wild-type mice turned out to be more sensitive than $Mthfr^{+/-}$ heterozygotes when treated with VPA. There were no cases of exencephaly in the fetuses from $Mthfr^{+/-}$ dams, whereas 21% of fetuses from $Mthfr^{+/+}$ had NTDs. Further analysis revealed that VPA lowered homocysteine plasma level and increased the *Mthfr* expression which resulted in lower teratogenicity in *Mthfr* deficient mice [Roy et al., 2008]. Much of our understanding of how genetic factors modulate teratogenic action of drugs or other chemicals comes from studies on animal models. Results of VPA teratogenicity studies in mice clearly demonstrated a strong genetic component involved in determining sensitivity to the induction of NTDs. Different strains of mice reacted very differently to VPA treatment during pregnancy as evidenced by the presence of fetuses with exencephaly, a form of NTD equivalent of human anencephaly [Finnell, 1991; Hall et al., 1997; Malm et al., 2002]. More specifically SWV/Fnn, AKR/J, C3H/HeJ, and P/J mice turned out to be the most sensitive; LM/Bc had an intermediate sensitivity, while DBA and C57BL/6J were highly resistant [Azarbayjani and Danielsson, 1998; Faiella et al., 2000; Spiegelstein et al., 2003; Wiltse, 2005]. These differences in sensitivity cannot be explained by the slight strain dependent variation in timing of neural tube closure, as evidenced by the study which failed to produce at least moderately high level of NTDs in C57 mice treated at various timepoints during pregnancy [Beck, 1999]. The observed strain specific sensitivity to VPA was also confirmed in an in vitro study where 6–8 somite stage mouse embryos of two different strains; SWV and C57BL/6J, were cultured in the serum supplemented with VPA for 48 hr. As in the in vivo studies, the SWV embryos were more likely to have open NTDs than were the C57BL/6J embryos. Additionally, the minimum lethal dose of VPA was also significantly lower for SWV cultured embryos [Naruse et al., 1988]. Results of this study point out that besides variation in the maternal genes that interact with environmental factors, the embryonic genetic component alone can modulate the teratogenic outcome.

Complex birth defects are known to have multifactorial etiologies and the combination of genetic susceptibility as well as the dose and the time of exposure to certain environmental compounds (e.g., drugs and other toxic xenobiotics) appear to account for significant variation in the human response. Both maternal and fetal genotypes can affect placental transport, absorption, metabolism, distribution, and receptor binding of the drug, influencing its teratogenicity [Hall et al., 1997; Spiegelstein et al., 2003]. Polymorphisms in maternal and fetal genes involved in xenobiotic detoxification may modify risks of adverse pregnancy outcome in response to maternal use of AED. Many drugs and endogenous compounds need to be metabolized in order to be activated or inactivated and ultimately excreted. The enzymes responsible for these reactions belong to the extensive cytochrome P450 (CYPs) gene family. The human genome contains 115 cytochrome P450 genes, of which at least 57 are functional. Among CYP families 1–3 (responsible for drug metabolism), there are 22 different P450 isoforms and a high degree of polymorphism has been identified [Ingelman-Sundberg and Sim, 2010;

Johansson and Ingelman-Sundberg, 2010]. Almost all of the P450 metabolizing genes are polymorphic, and the known allelic CYP variants are too extensive to describe here (see web page http://www.cypalleles.ki.se/). This polymorphism translates into phenotypes that differ in the ability and/or speed of drug metabolization, from "low metabolizers" having defective alleles and inactive enzymes, to "ultrarapid metabolizers" carrying two or more active gene copies. Pregnant women who are so called "slow metabolizers" may be at increased risk of having an affected baby due to the drug/teratogen accumulation and slower excretion rate that extends the time when the developing embryo is exposed to that toxicant. Conversely, pregnant women who are "fast metabolizers" can more quickly activate some drugs, including proteratogens, resulting in higher plasma concentration of reactive intermediates or active teratogens which ultimately can also adversely affect the embryo.

Two cases of intoxication with an AED drug—phenytoin, showing women with significant neurological symptoms, have been published. In both cases, a very slow clearance (fourteen times slower than average) from blood, caused by mutations in *CYP2C9* (the main enzyme responsible for phenytoin metabolism) was responsible [Brandolese et al., 2001; Kidd et al., 2001]. A very elegant study by Buehler et al. [1990] involved monitoring 19 pregnancies exposed to phenytoin monotherapy. In the amniocytes collected from these pregnancies at midgestation, they measured the activity of epoxide hydrolase, an enzyme involved in phenytoin metabolism. If the enzyme activity was low (less than 30% of the standard), they predicted that the embryo would be at higher risk of having the fetal hydantoin syndrome. In four cases, significantly lower activity of epoxide hydrolase was found, and after delivery, all four infants presented with clinical symptoms of fetal hydantoin syndrome (FSH). The remaining 15 fetuses with higher enzyme activity were not considered at risk, and all of them were born without characteristic FSH features. This study indicated that an adverse pregnancy outcome can be predicted on the basis of an enzymatic biomarker. More studies are needed to decipher all the genes engaged in drugs metabolism and their genetic variants. This knowledge would be very helpful for new drug development and for clinicians in order to prescribe the right medicine, compatible with the specific patient genotype.

CONCLUSIONS

Experimental developmental toxicology studies repeatedly demonstrate the unquestioned importance of genetic variation in determining risks to teratogen-induced developmental abnormalities. As described earlier, inbred strains of mice, representing different mouse genomes, show significant variation in sensitivity to teratogenic pharmaceutical or xenobiotic agents. Clinical research in this area; however, remains quite limited. Although we are still far away from individualized preventive measures for birth defects, understanding the genetic basis of the susceptibility to specific environmental factor-induced birth defects may yield critical clues that will ultimately lead to novel approaches that can be implemented to prevent, preventable birth defects. Pharmacogenomics is an emerging science aiming to identify genetic polymorphisms that affect drug efficacy and safety. Genome-wide association studies will screen numerous genetic markers without a prior knowledge. Whole genome sequencing is the ultimate way to identify novel functional mutations, especially the rare ones that may modify individual's susceptibility to teratogens. Together with pharmaco- and toxicogenomics research, these genome-wide high throughput/high information content assays will provide essential information allowing physicians to choose the "right" drug at the "right" dose for each patient in order to avoid any adverse drug reaction and ultimately to make the personalized medication possible.

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