



## Genes in their environment: how can we read the riddles?

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Once upon a time, as all the best children's stories begin, a lot of clever geneticists began a huge hunt for the "asthma gene." At first they thought they had found it, but then more and more of the hunters found different "asthma genes," and sometimes some gene hunters could not find the gene that previous hunters thought was the key one. Life became more and more confusing, and the root causes, which should have been thought of right from the start, have only recently begun to be identified. These include that there are many types of asthma, not likely all to have the same background, and thus careful clinical phenotyping is essential; that genes do not operate in isolation, but in their environment (which includes other genes around them), and this may modify their effects, and even completely reverse them. Also, statistical dangers lie in wait for those who merely collect DNA, feed it all into a good computer and turn the handle; and detailed attention to methodology is needed. The purpose of this editorial is to place the nice study of de Faria et al.<sup>1</sup> into the context of current issues for genetic association studies.

A recent review<sup>2</sup> identified more than 30 genes which have been shown to be associated with some aspect of asthma, and the association has been replicated in at least five studies. In broad terms, these fall into one of four groups: genes relating to innate immunity and immunoregulation; genes associated with TH2 cell differentiation and effector functions; genes associated with epithelial biology and mucosal immunity; and genes associated with lung function, airway remodeling and disease severity. These classes of genes find echoes in our

knowledge of the basic immunobiology of asthma. However, the bag is by no means empty; there are now three members of a class of unknown function, encoding transmembrane proteins located in the endoplasmic reticulum, which are implicated in asthma, the latest being ORM DL3.<sup>3</sup>

A number of important methodological issues were discussed which shed light on why so many studies have been conflicting. Linkage studies may implicate a particular locus, for example 5q31-33, but the locus may contain potentially a large number of

relevant genes,<sup>4</sup> and premature conclusions that a particular gene, and not one of its near neighbors, is important must be avoided. It is important to check the validity of the methods used for genotyping, preferably with validation by a second method; and also to check the association in at least one other population.<sup>5,6</sup> However, it should be noted that if the results do not appear to be important in a second population, this does not mean that the first study is invalid; it should instead provoke a careful look for differences between the studies, which may give important insights into novel gene-environment interactions.

Genes are relatively easy to study, but they exist not in a vacuum but in a complex environment, which the investigator ignores at his peril. Studies in mice have shown that gene-environment interactions may explain more phenotypic variance than either genetic or environmental effects considered separately.<sup>7</sup> There are many facets to these interactions. Unsurprisingly, the effect of environmental smoke

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No conflicts of interest declared concerning the publication of this editorial.

**Suggested citation:** Bush A. Genes in their environment: how can we read the riddles? *J Pediatr (Rio J)*. 2008;84(3):185-188.

doi:10.2223/JPED.1789

exposure is greater in children with null polymorphisms in glutathione S transferases, which are important antioxidant defense genes.<sup>8</sup> A large number of relatively simple interactions like this have been described. However, the phenomenon of phenotypic plasticity complicates the picture, whereby the effects of a gene may be opposite in different environments. CD14 is a molecule intimately bound up with the response to lipopolysaccharide, and is thus an obvious candidate as an "asthma gene."<sup>9</sup> However, a meta-analysis reported that the CD14 C-159T polymorphism was not associated with asthma or asthma severity.<sup>10</sup> When the relationships were studied in more detail, with focused hypotheses, the CD 14/-260 C/T polymorphism in the CD 14 promoter was associated with higher levels of both total and specific IgE to aeroallergens in children in regular contact with domestic pets, but the opposite relationship, not explained by endotoxin levels, in children in contact with stable animals.<sup>11</sup> Finally, the effects of poverty must not be forgotten; in cystic fibrosis, the most powerful modifying influence on mortality was not genetic, but the wealth of the family.<sup>12,13</sup> It is better to be rich and ill than poor and ill in many contexts.

There are important interactions within the cell nucleus as well. Polymorphisms in transcription factors may affect gene expression, in an organ specific manner.<sup>14,15</sup> Polymorphisms in a sequence of genes, each independently of little functional consequence, may synergize to magnify the effect on the risk of a particular phenotype.

Another prerequisite for genetic studies is careful phenotyping of the subjects, and careful selection of controls. Although doctor-diagnosed asthma, bronchial responsiveness and atopy overlap, they are not the same thing, and have different genetic associations. Asthma is a term that has in the past served a useful purpose in ensuring that children wheezing with viral colds were not given antibiotics. However, it may have outlived its usefulness therapeutically, because it may lead to the uncritical and unnecessary prescription of inhaled corticosteroids; and the term "asthma" certainly has in the context of genetic studies, unless it leads inexorably to the question "what sort of asthma." Preschool wheezing phenotypes offer a good example, and even here there is a need for more clarity. Preschool wheeze has been described as transient (present age 0-3 years, not age 3-6 years), persistent (present throughout the first 6 years of life) and late-onset (not present age 0-3 years, present age 3-6 years).<sup>16</sup> These phenotypes are only of any use retrospectively, since at age 2, although various indices have been found to predict remission of symptoms,<sup>17-19</sup> none is much use predicting persistence. They are also an oversimplification albeit one that has clarified our thinking. Recently, more sophisticated mathematical analysis suggests that a more detailed breakdown of wheeze patterns is required.<sup>20</sup>

These epidemiological phenotypes are sometimes uncritically confused with clinical ones; the patterns of intermittent

(viral) wheeze and multi-trigger wheeze can be distinguished with a good clinical history, and are sometimes thought to be the same as transient and persistent wheeze respectively, but this has never been shown. It would be naïve to believe that the nonatopic child who wheezes albeit severely three times a year with viral infection has the same underlying genetic makeup as a child with eczema, who wheezes every day.

Even in the school years, there are important international differences. In the western world, atopic asthma is the commonest phenotype, and, although nonatopic asthma exists in the school-age child, it is sufficiently unusual to prompt a careful review of the diagnosis. However, a recent paper from Porto Alegre has shown clearly that this phenotype is much commoner (around 50%), and associated with early severe bronchiolitis.<sup>21</sup> This valuable paper underscores the need to be critical about differences between populations apparently suffering from the same disease but in different countries.

There are further complicating factors. Genes may be operative only in a specific time window.<sup>22</sup> Epidemiological<sup>16,23-25</sup> and pathological<sup>26,27</sup> data have suggested that the first three years of life are crucial in determining long-term lung function, and that developmental processes may interact with genes at only specific time points. The inflammatory cellular phenotype of asthma may change over time.<sup>28</sup> The role of epigenetic modifications in the expression of crucial genes is little explored and may well be important.<sup>29</sup> The complexities of how genes and the environment (in the widest sense of both) interact to produce the multifaceted asthma phenotypes are far from being understood.

Where do the findings of de Faria et al.<sup>1</sup> fit into this complex picture? They have nicely generated the hypothesis that TGF- $\beta$ 1, CD14 and IL-4R, but not ADAM33 polymorphisms are implicated in asthma in Brazil. The literature is controversial about the effects of ADAM33, with some reporting important effects<sup>30-32</sup> and others finding none.<sup>33-35</sup> These discrepancies, which the present study<sup>1</sup> can not resolve, are strange; it may reflect the statistical power of the different studies, but it could also reflect population differences and the resolution of the discrepancies may lead to new discoveries. These studies now need taking forward in larger cohorts, to find more genes which apparently have different effects in Brazil than in the western world. Confirmatory studies are important, but the study that produces the unexpected, and is robust, is much more interesting. A prerequisite for success will be the generation of focused hypotheses, and rigorous methodology. This has to include the use of both a discovery and a second replication population. The rewards are potentially very great, because such differences can highlight novel mechanisms; remember that the observation of the protective effect against asthma of being born on a dairy farm, completely counter-intuitive to the received wisdom of

the time, leads to a whole stream of novel mechanistic papers. We will be watching this space!

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## Systemic infection and brain injury in the preterm infant

Richard A. Polin\*

In this issue of *Jornal de Pediatria*, Silveira et al.<sup>1</sup> investigated risk factors for the development of periventricular leukomalacia (PVL) in a cohort of very low birth weight (VLBW) infants. A significantly greater number of infants with PVL had documented sepsis and/or were ventilated for more than 24 hours. This well-designed study adds supportive evidence for the role of postnatal infection in the pathogenesis of PVL.<sup>2</sup> While the data are provocative, it is unclear from this article how the timing of the sepsis episodes is related to the development of PVL. If the study infants all had early-onset sepsis, it is somewhat surprising that coagulase negative staphylococcus was the most common pathogen recovered. In the USA that microorganism would be a rare cause of early-onset sepsis. If the study infants had late-onset infections, the question of timing of the infectious episode with the onset of PVL is particularly important. A major limitation in this study is the lack of data on the likelihood of antenatal infection. That question is particularly important given the high rate of bacteremia with *Mycoplasma* and *Ureaplasma* in infants born prematurely.<sup>3</sup> A third weakness in this study (as noted by the authors) is the lack of magnetic resonance imaging (MRI) information; MRI is a more sensitive way to diagnose PVL. Given these limitations, however, the authors should be congratulated for addressing this question.

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PVL is strongly associated with the development of cerebral palsy.<sup>4</sup> The pathogenesis of PVL has been linked with disorders resulting in hypoperfusion of the brain (e.g., hypoxia-ischemia, hypotension patent ductus arteriosus with reversed diastolic flow, etc.) and perinatal (antenatal and postnatal) infection. The final common pathway for both etiologies is likely to include microglial activation, cytokine and glutamate release and free radical production (Figure 1).

Late-onset infections are common among VLBW infants and up to 25% develop a systemic bacterial or fungal infection; 5-10% have documented meningitis.<sup>6</sup> Neurological abnormalities are common among survivors. In a recent prospective observational study by Stoll et al. (n = 6,093)<sup>7</sup> preterm infants with proven systemic infections, clinical infection

(negative blood culture), necrotizing enterocolitis (NEC) and meningitis were all more likely to exhibit neurological and growth abnormalities compared with an uninfected control group. Hearing impairment was more common in infants with NEC and in those infected with gram-negative microorganisms. In a case-control study, O'shea et al. also noted an association between clinical chorioamnionitis, sepsis and cerebral palsy.<sup>8</sup>

Over the past 10 years, there has been considerable interest in the relationship of antenatal infection and cerebral palsy.

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No conflicts of interest declared concerning the publication of this editorial.

**Suggested citation:** Polin RA. Systemic infection and brain injury in the preterm infant. *J Pediatr (Rio J)*. 2008;84(3):188-191.

doi:10.2223/JPED.1784