Epileptic Disord 2003; 5 (Suppl 2): S 35–S 43

Etiological classification of CNS malformations: integration of molecular genetic and morphological criteria

Harvey B. Sarnat, Laura Flores-Sarnat

Departments of Pediatrics (Neurology) and Pathology and Laboratory Medicine (Neuropathology), Cedars-Sinai Medical Center and University of California School of Medicine at Los Angeles (UCLA), Los Angeles, CA, U.S.A.

ABSTRACT – Classification is a creative activity that helps us understand relationships. The traditional classifications of central nervous system malformations was based exclusively upon descriptive morphology, but these criteria must now be integrated with molecular genetic data to enable an etiological classification that also remains useful to the clinician, radiologist and pathologist, who rely upon imaging and tissue examination for diagnosis. Many cerebral malformations previously thought to be a single disorder are now known to be common end-results of several independent genetic mutations. Examples are holoprosencephaly and lissencephaly. Gradients of genetic expression along the axes of the neural tube, established at the time of gastrulation, may explain many varieties or anatomical and clinical manifestations of cerebral malformations, including the involvement of non-neural tissues such as in midfacial hypoplasia, that may be attributed to abnormal neural crest migration. Genes of cellular lineage and of symmetry may explain some hamartomatous malformations, such as tuberous sclerosis and hemimegalencephaly. Modern classification should be applicable to the entire CNS as well as regions; schemes that attempt to artificially isolate the cerebral cortex for a "regional classification" may be erroneous even though the genetic defect primarily affects cortical structures because genetic gradients in the neuraxis are excluded and some involve a more subtle but still important expression in subcortical structures.

KEY WORDS: classification, genetic mutations, gradients of genetic expression, CNS malformations, axes of neural tube

Correspondence:
Dr. Harvey B. Sarnat MD, FRCPC, Cedars-Sinai Medical Center, Pediatrics 4221 North Tower, 4800 Beverly Blvd., Los Angeles, CA 90048, USA.
Tel. (310) 423-1299
Fax (310) 423-4131
E-mail: harveyb.sarnat@cshs.org

Why classify?

Classification is primordial to human thought processes. Everything we experience and understand since earliest infancy is subconsciously compared to stored memory engrams of previous experiences and it is these comparisons and relationships with all else in our fund of knowledge that gives everything in the world, from concrete objects to abstract thoughts, a place in our mind. This process of cerebral function transcends all cultures. It is as fundamental to human thought as are the instincts of less evolved species of animals.

Classification of simple concepts is almost reflexive but, at a high level of abstraction, classification requires the greatest creativity that the human mind can muster. Understanding relationships and impacts between physiological body functions and diseases is far from an automatic reflex, even after a prerequisite background of medical knowledge that only years of study can provide. In no category of disorders is classification more complex than in the field of diverse malformations of the nervous system. As with any scheme in which more factors are unknown than are known, periodic revision is essential to incorporate new data.

The discipline of classification thus may be performed at a basic level by filing clerks to encode health insurance forms, or at a loftier level to provide insight into etiologies, pathogenesis and mechanisms of diseases. We do not suggest discarding or rejecting traditional criteria used to classify nervous system malformations, based upon descriptive morphogenesis, but rather propose that these criteria be integrated with recent molecular genetic data to create new criteria. An etiological classification must be genetically accurate, have a practical application for the clinician who must rely upon traditional morphological criteria to even suspect a genetic defect, and must be flexible enough to incorporate continuous revision and change. The morphological recognition of CNS dysgeneses continues to advance in parallel with genetic revelations, through refinements in both imaging and neuropathological techniques and interpretations. The scheme presented here is an updated form of schemes we have recently published [1, 2].

Finally, we would like to emphasize the importance of considering the nervous system as a whole in any classification, regardless of a particular interest in one part of the brain. Schemes that attempt to isolate the disorders of cerebral cortical development, for example, for use by epileptologists, may be faulted in their basic premise. Firstly, many developmental processes such as defective genetic expression in the axes of the neural tube are not limited to the cortex; indeed they are often more readily understood in less complex structures of the brain, such as the spinal cord or brainstem, and then more easily extrapolated to understand cortical abnormalities. Secondly, many malformations that primarily involve the cerebral cortex are also associated with developmental abnormalities in subcortical structures and these may influence cortical function. An example is cerebellar dysplasias, sometimes associated with lissencephaly/pachygyria. The role of the cerebellum as an inhibitory organ influencing epilepsy, and its role in cognitive and language function are increasingly recognized, and malformations of the cerebellum may be important in the functional expression of some cerebral disorders of neuroblast migration. The importance of neural crest migration from the mesencephalic neuromere in some forebrain malformations is another example of why the entire nervous system must be considered in classification schemes, and why schemes limited to the cerebral cortex are incomplete and may be misleading by their exclusions.

Limitations of purely morphological schemes of classification

Traditional morphological classification schemes consist of categories of disturbances in normal developmental processes, such as neurulation, cell migration, axonal projection, synaptogenesis and myelination [3, 4]. While these categories retain a logic and practical validity, they were designed well before the advent of molecular genetic data on developmental programming in the final decade of the 20th century. Two examples are cited that represent major cerebral malformations for which the traditional schemes fail to provide the flexibility necessary to integrate the new data into an etiological classification. They both exemplify anatomical end-stages of defective developmental processes due to diverse genetic etiologies, perhaps best characterized as "syndromes", rather than the singular malformations that they were once regarded, and now as only a footnote in medical history.

Example 1: Holoprosencephaly

This malformation is traditional subdivided into *alobar*, *semilobar* and *lobar* forms, initially described by DeMyer *et al.* in 1964 [5], each defined by specific neuropathological and imaging criteria. A fourth form, known as the *median interhemispheric fusion variant*, was recently described [6]. However distinctive this classification and easy to apply from imaging or postmortem examinations, it cannot accommodate the recent identification of six distinct human genes responsible in various cases of holoprosencephaly (*SHH* at 7q36; *SIX3* at 2p21; *ZIC2* at 13q32; *TGIF* at 18p11.3; *PTCH* at 9q22.3 and *DKK* at 10q11.2) [7]. Some of these genes have a ventralizing effect in the vertical gradient (e.g. *SHH*) and others, a dorsalizing influence (e.g. *ZIC2*). In addition, at least seven other defective chromosomal loci are associated with holoprosencephaly, on chromosomes 3p26, 4, 5, 6, 14q21.1-q21.2, 20, 21q22.3, though the precise gene has not yet been isolated in patients with these abnormal karyotypes. The genes to be demonstrated defective in various cases of holoprosencephaly still only account for about 20% of the total cases examined by genetic analysis, indicating that many additional genetic defects are yet to be discovered.

It is increasingly evident that holoprosencephaly is a common end-result of several different genetic disturbances in cerebral development, rather than a single "midline defect" anomaly as had previously been concluded from anatomical studies. The anatomical classification defines the degree of severity of the result without addressing the issue of etiology or gradients of genetic expression in the

three major axes of the neural tube [8]. As final proof that these anatomical variants of holoprosencephaly represent degrees of severity or perhaps of residual genetic expression, each of the four variants have been demonstrated in patients with each of the six known genetic etiologies.

Example 2: Lissencephaly and pachygyria

The primary neuroblast migratory disorders represent a category of cerebral malformations more elucidated by molecular genetic research than many others. Abnormal cerebral gyration is an easy anatomical aberration to recognize by imaging and by macroscopic neuropathology. Heterotopia are another result of abnormal migration and also can be identified by imaging if the heterotopic neurons form nodules of grey matter within the white matter. A major limitation of imaging, however, is that the resolution is only as good as the naked eye, hence single, isolated heterotopic neurons can be recognized only by microscopic examination of tissue sections.

Morphological schemes cannot accommodate the many specific genetic defects now known to affect neuroblast migration and that could form a category of relatively complete etiological reclassification. Despite an expanded database of genetic information, the neuroradiologist and neuropathologist still have important roles to play in the initial diagnosis that only can be suspected from clinical phenotypes, the latter providing justification to proceed with neuroimaging and other investigations. As more genetic data are collected, the old, strictly anatomical schemes will become less and less relevant and eventually must be replaced by more flexible ones that incorporate, rather than exclude, genetic criteria. The genes and/or their transcription products that have been documented in human lissencephalies and related disorders of neuroblast migration are listed in *table 1*, category VI of the classification. Some of the key references are cited [9-18].

Pachygyria is a milder form of lissencephaly and both are evidence of disturbances of migration. Heterotopia, whether periventricular, within the subcortical white matter of the centrum semiovale, or even as focal areas of abnormal cortical lamination, also are evidence of a migratory disturbance, and leptomeningeal glioneuronal heterotopia result from overmigration rather than undermigration. None of these gross findings are specific and all may represent a primary disorder of migration or secondary disturbance, such as occurs in holoprosencephaly and in hemimegalencephaly, in which the primary pathogenesis is not one of migration. Nevertheless, the radiologist and the pathologist may identify some patterns so characteristic that they are nearly diagnostic of particular genetic defects. Examples include the Miller-Dieker type 1 or "classical" lissencephaly, type 2 lissencephaly, X-linked dominant periventricular nodular heterotopia, X-linked dominant subcortical laminar heterotopia and schizencephaly. Some of these disorders now can be definitively diagnosed by a molecular genetic marker from a blood sample, and undoubtedly more such diagnoses will be feasible in the future. Lissencephaly is, therefore, not a disease entity, but a convergence of several different genetic mechanisms to produce a common result. Neither the neuroradiologist nor the neuropathologist will become obsolete by the availability of genetic testing, but classifications of neuroblast migratory disorders based exclusively upon morphological criteria are already obsolete.

Limitations of functional clinical manifestations in the classification of CNS malformations

Clinical symptoms and signs, at times provide an important basis of classification for functional disorders that might have multiple etiologies. An excellent example is the epilepsies, particularly if the clinical seizure types are integrated with electrographic criteria. The classification of the epilepsies continues, and should continue, to undergo revision with the addition of new data and the evolution of new concepts, and has a very practical clinical usefulness for selection of treatments. However, clinical criteria have an extremely limited role in the classification of cerebral malformations because the clinical manfestations do not correlate in a predictable manner, often even in the most general terms. For example, again citing holoprosencephaly as a good prototype of a complex dysgenesis, epilepsy is mild or absent in many infants with this malformation, whereas others with nearly identical anatomical lesions by imaging and even after postmortem neuropathological examination, have severe, refractory seizure disorders. Degree of developmental delay and frank mental retardation are extremely variable as well. Some infants with holoprosencephaly have endocrine disorders of the hypothalamic-pituitary axis, especially diabetes insipidus, and others escape this complication.

We do not fault the value of clinical classifications for the clinical expression of defective nervous system function, as in the epilepsies and in neuroendocrine disturbances, but we suggest that clinical criteria do not have an important role to play in the classification of anatomical and genetic defects in CNS malformations.

Limitations of purely genetic schemes of classification

This type of scheme might be the most purely etiological and has great appeal to geneticists, but would be difficult to use for radiologists, pathologists and indeed for clinicians. Unfamiliarity and incomplete knowledge of many genes are initially problematic, but are temporary issues. Other, more serious limitations would not easily disappear

Table 1. Etiological classification of human nervous system malformations as patterns of genetic expression [1, 2]

I. Genetic mutations expressed in the primitive streak or node
A. Upregulation of organizer genes
1. Duplication of neural tube
B. Downregulation of organizer genes
1. Agenesis of neural tube
II. Disorders of ventralizing gradient in the neural tube
A. Overexpression of ventralizing genes
1. Duplication of spinal central canal
2. Duplication of ventral horns of spinal cord
3. Diplomyelia (and diastematomyelia?)
4. Duplication of neural tube
5. Ventralizing induction of somite
a. Segmental amyoplasia
B. Underexpression of ventralizing genes
1. Fusion of ventral horns of spinal cord
2. Sacral (thoraco-lumbo-sacral) agenesis
3. Arrinencephaly
4. Holoprosencephaly
III. Disorders of dorsalizing gradient of the neural tube
A. Overexpression of dorsalizing genes
1. Duplication of dorsal horns of spinal cord
2. Duplication of dorsal brainstem structures
B. Underexpression of dorsalizing genes
1. Fusion of dorsal horns of spinal cord
2. Septo-optic dysplasia (?)
IV. Disorders of the rostrocaudal gradient and/or segmentation
A. Increased homeobox domains and/or ectopic expression
1. Chiari II malformation
B. Decreased homeobox domains and/or neuromere deletion
1. Agenesis of mesencephalon and metencephalon (*EN2*)
2. Global cerebellar aplasia or hypoplasia
3. Agenesis of basal telencephalic nuclei (*EMX1*?)
V. Aberrations in cell lineages by genetic mutation
A. Non-neoplastic
1. Striated muscle in the central nervous system
2. Dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos)
3. Tuberous sclerosis
4. Hemimegalencephaly (also VIII. Disorders of symmetry)
B. Neoplastic
1. Myomedulloblastoma
2. Dysembryoplastic neuroepithelial tumours
3. Gangliogliomas and other mixed neural tumours
VI. Disorders of secretory molecules and genes that mediate migrations
A. Neuroblast migrations
1. Initial course of neuroblast migration
a. Filamin-1 (X-linked dominant periventricular nodular heterotopia)
2. Middle course of neuroblast migration
a. Doublecortin (DCX; X-linked dominant subcortical laminar heterotopia or band heterotopia)
b. LIS1 (type I lissencephaly or Miller-Dieker syndrome)
c. Fukutin (type II lissencephaly; Fukuyama muscular dystrophy)
d. Empty spiracles (EMX2; schizencephaly)
e. Astrotactin
3. Late course of neuroblast migration; architecture of cortical plate
a. Reelin (pachygyria and cerebellar hypoplasia)
b. Disabled-1 (DAB1; also VLDL/Apoe2R? App receptor defect; downstream of reelin, EMX2 and DCX)
c. L1-NCAM (X-linked hydrocephalus and pachygyria with aqueductal stenosis)
B. Glioblast migration
C. Focal migratory disturbances due to acquired lesions of the fetal brain
VII. Disorders of secretory molecules and genes that attract or repel axonal growth cones
A. Netrin downregulation
B. Keratan sulfate and other glycosaminoglycan downregulations
C. S-100 protein downregulation or upregulation (?)
VIII. Disorders of symmetry
A. Hemimegalencephaly (also see V. Aberrations of cellular lineages)
1. Isolated hemimegalencephaly
2. Syndromic hemimegalencephaly
a. Epidermal nevus
b. Proteus
c. Klippel-Trenaunay-Weber
d. Hypomelanosis of Ito
B. Hemicerebellar megalencephaly

despite experience and expanded knowledge. Cascades of sequential genes often occur, so that a mutation in the first gene in a series results in low expression of others that follow; it may be difficult to determine whether a detected underexpression of a later gene is primary or secondary. For example, defective *DAB1* causes underexpression of the downstream genes *LIS1*, *Reelin* and *EMX2*, all of which are important for various stages of neuroblast migration. Some genes enhance or suppress the expression of others, an extremely important relationship in normal development, but confusing when trying to identify the principal gene responsible for a malformation. Antagonistic expression also may be confusing: apparent overexpression of the ventralizing *SHH* gene may actually be due to underexpression of a dorsalizing gene of the *BMP*, *WNT* or *PAX* families. Even if the construction of a classification based entirely on specific genes were possible, it would be almost irrelevant to the clinical situation because of the total exclusion of morphological criteria. Finally, non-genetic malformations secondary to acquired lesions in fetal life (e.g. fetal cerebral white matter infarcts that interrupt radial glial fibers that guide migratory neuroblasts) could not be accommodated, by definition, in a purely genetic scheme.

Advantages of schemes defining *patterns of genetic expression*

The first and most important advantage of classifications based upon patterns of expression is that it is flexible enough to incorporate both morphological and genetic criteria. As well, multiple genetic deletions or mutations, secondary underexpression of downstream genes in a cascade and overexpression from loss of antagonistic balance can all be incorporated. Even malformations for which the exact gene is not yet identified can be included because the incorporation of traditional morphological criteria often allows the pattern of genetic expression to still be recognized. Impaired genetic programming or defective morphogenesis due to acquired fetal lesions, such as white matter infarcts in the cerebral hemispheres before neuroblast migration is complete, also find a place in this type of maximally accommodating scheme. This is the scheme that we previously proposed and continue to advocate.

Axes and gradients of genetic expression in the neural tube

It is not birth, marriage or death, but gastrulation which is truly the most important time in your life.

Lewis Wolpert, 1978

The 15th postconceptional day of human embryonic life is perhaps the most eventful of all of gestation because it is on that day that gastrulation occurs, associated with the appearance of the primitive streak and node and the birth of the nervous system with the earliest differentiation of the neuroepithelium.

The primitive streak establishes axes of growth that characterize all vertebrate and most invertebrate body plans: **1) bilateral symmetry**, by contrast with the primary radial symmetry of coelenterates, such as hydras, jellyfish and sea anemones, or the secondary radial symmetry of echinoderms such as starfishes and extinct crinoids; **2)** a **longitudinal axis** having rostral and caudal poles that establish head and tail ends; **3)** a **vertical axis** that establishes dorsal and ventral sides; and **4)** a **horizontal axis** that establishes medial and lateral structural growth.

The axes of the neural tube are associated with gradients of genetic expression during programming, with many genes often expressed more strongly in some regions and progressively decreasing expression in more distal regions. After the initiation of **segmentation** of the neural tube, the development of neuromeres or compartments that provide physical and chemical barriers to the unrestricted movement of cells, some genetic expression is restricted to certain neuromeres (e.g. *EGR2* in humans or *Krox-20* in animals, is expressed only in rhombomeres 3 and 5). Upregulation may allow these genes to become expressed in neuromeres that they do not normally influence, a phenomenon known as *ectopic expression* and a basis for some malformations. Failure of essential genes to become expressed in specific neuromeres, by contrast, may lead to an absence of those segments. Putative examples are the lack of mesencephalon and metencephalon with cerebellar hypoplasia in mice and humans due to defective *EN1*, *EN2* (*Engrailed1 and 2*) or *WNT1* (*Wingless1*) expression [19-24], or the failure of the basal telencephalic nuclei (i.e. *basal ganglia*) to develop with a putative *EMX1* or *MASH1* mutation [1, 2, 25].

Returning to holoprosencephaly, earlier presented as an example of why simple anatomical classifications are now inadequate, this malformation also provides a good prototype of the effects of gradients of genetic expression [8]. Whereas the genes known to be defective in some cases of holoprosencephaly have either a ventralizing or dorsalizing expression in the **vertical axis** of the forebrain, these same genes also affect the two other axes. In the case of the longitudinal axis, there is a **rostrocaudal gradient** that in some, but not all cases, causes noncleavage of the diencephalon as well as the telencephalon, with obliteration of the third ventricle and apparent "fusion" of the thalami and hypothalami of the two sides [3, 4, 26]. If the gradient of defective expression reaches the midbrain, the superior

colliculi may be noncleaved as a single midline colliculus, the cerebral aqueduct may be atretic and there may be continuity across the midline of the oculomotor nuclei [8]. The degree of severity of holoprosencephaly, as defined by the traditional alobar, semilobar and lobar forms, does not always correlate well with the associated midfacial hypoplasia, which may range from mild hypotelorism to extreme forms with cyclopia and a proboscis. It also fails to correlate with diabetes insipidus that is present in 67% of affected infants [27]. The correlations appear to relate to the rostrocaudal gradient of the genetic expression: if the gradient reaches the midbrain, it may interfere with neural crest formation or migration from the mesencephalic neuromere of the embryonic neural tube [8]. The most rostral neural crest tissue arises in the midbrain and forms not only neural structures such as the ciliary ganglion, but also membranous bones of the face, the orbits and much of the eyeball except the retina, lens and cornea [28, 29]. When mesencephalic neural crest comes in contact with an epithelium, cartilage is formed; when it contacts mesodermal cells, bone is formed; this difference explains why cartilage forms in the ears and nasal septum and bone forms the orbits. The cephalic neural crest extends back along the future cranium and scalp to the region of the future posterior fontanelle, hence the territory innervated by the sensory trigeminal nerve extends two thirds of the distance back on the scalp. The **mediolateral gradient** in holoprosencephaly is also seen in the most severely disorganized cerebral cortex in paramedian regions, less disordered cortex more laterally, and the cortex may be normally laminated with normal architecture in the most lateral regions. The extent of this mediolateral gradient may explain, in part, the variable degrees of mental retardation, cognitive deficits and epilepsy seen in children with this malformation [8].

A rostrocaudal gradient may also be seen in some malformations limited to the cerebral cortex. Lissencephaly type 1 is generally more severe in a posteroanterior gradient in the autosomal recessive form due to the microdeletion at 17q13.3 of the *LIS1* gene (Miller-Dieker disease), but follows an anterioposterior gradient in the X-linked recessive form of lissencephaly type 1 due to a defective *XLIS* gene. However, there is no apparent mediolateral gradient in the lissencephalic cortex in either form. Some disorders of pachygyria and of polymicrogyria also follow an anterioposterior gradient with more severe dysgenesis in the frontal, than the occipital lobes. The rostrocaudal gradient in the forebrain also may account in part for the difference between the alobar and semilobar forms of holoprosencephaly, in which semilobar holoprosencephalic brains show variable differentiation of two occipital horns and less severe architectural alterations in occipital than frontal cortex, whereas in the more severe alobar form a large dorsal cyst occupies the posterior half to two-thirds of the intracranial space and the monoventricle is total.

Defective patterns of genetic expression that induce abnormal development of the fetal nervous system

The genetic programming of the neural tube may be summarized as a series of 12 principles [1, 30]. The expression of the organizer genes in gradients along the three prinicipal axes of the neural tube is primordial. Amongst the most important concepts in its relevance to the induction of abnormal neural development are **1)** upregulation of genes acting in the vertical axis (dorsoventral or ventrodorsal) causing hyperplasia and/or duplication of structures, and suppressing the expression of antagonistic genes; **2)** downregulation of genes acting in the vertical axis and causing hypoplasia or noncleavage (apparent "fusion") of structures; **3)** upregulation of genes in the longitudinal axis causing ectopic expression in neuromeres where these genes are not normally expressed or altering the formation of structures in a more rostral or more caudal position than they normally occur; **4)** downregulation of genes in the longitudinal axis resulting in hypoplasia of midline structures or deletion of entire neuromeres; **5)** abnormal expression of genes of cellular lineage resulting in hamartomas with defective cellular growth and differentiation, in addition to defective tissue architecture or disorganized arrangement of essentially normal cells.

Table 1 is our proposal for a new classification of nervous system malformations that provides for the integration of both morphological and molecular genetic criteria. This scheme does not yet include all known cerebral dysgeneses. Some malformations may appear in more than one place in this scheme because more than one mechanism might produce the same result or two mechanisms might be operative. For example, duplication of the neural tube and certain other structures results from overexpression of the *Wnt-8c* gene as early as the time of gastrulation or primitive streak formation [31], and might explain some human cases of conjoined twinning or of diplomyelia. A similar duplication of the neural tube can occur from ectopic expression of s*onic hedgehog* (*SHH*) at the later time of neural induction by the notochord, with formation of a second floor plate, as shown in chick and mouse embryos [32].

A second example of different genetic influences producing a similar result is holoprosencephaly [1, 7, 8], already discussed in relation to the failure of purely morphological schemes to accommodate the new genetic data and cited under both overexpressed ventralizing influence (*SHH*) and overexpressed dorsalizing influence (*ZIC-2*).

Hemimegalencephaly is a third example of a malformation requiring, at least for the present, two positions in the classification scheme [2, 33-35]. This hamartoma of one hemisphere does not correspond to arrest in maturation at any stage of normal ontogenesis. It may occur as an isolated dysgenesis or as part of a syndrome, including

epidermal nevus, Proteus, Klippel-Trenaunay-Weber syndromes and in some cases of hypomelanosis of Ito [33]. Histopathologically, hemimegalencephaly, whether isolated or syndromic, is a disturbance of cellular lineage and cellular growth [34-36], hence is listed in *table 1* in this category, grouped with other non-neoplastic disorders of lineage such as tuberous sclerosis and dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos disease). However, a new category is also established for disturbances of symmetry of the brain, and hemimegalencephaly is listed a second time in this category. Several genes are now known to be involved in the establishment of bilateral symmetry in the vertebrate body and central nervous system [37-39], and a mutation of one of these might cause an asymmetrical dysgenesis.

This listing of some malformations in more than one place in a classification scheme is not, therefore, an ambiguity, but rather a recognition that there are sometimes multiple mechanisms of pathogenesis, involving different genes and different patterns of genetic expression, that converge to produce a similar anatomical end-result. This recognition is one of the most important features of a true etiological classification.

Finally, this table also accommodates secondary disturbances of neuroblast migration not due to primary genetic mutations, but rather as focal dysgeneses due to lesions acquired in fetal life. A frequent example is focal infarction of the subcortical white matter that destroys radial glial fibers that guide migrating neuroblasts and glioblasts to the cortical plate. Disruption of their monorail transport system leaves neuroblasts arrested in the middle course of migration, short of their destination, where they mature as heterotopic cells but are unable to establish their intended synaptic relations. The destruction of cells by such infarcts also destroys intrinsic genetic programs within these cells.

Other recent schemes of classification

Some authors continue to propose new schemes of classification based upon the 20th century criteria of morphogenesis. For example, Ikenouchi *et al.* recently proposed a reclassification of the lumbosacral neural tube defects that includes hydromyelia and abortive forms of frank meningomyelocele [40]. In all fairness, this type of scheme indeed has a practical usefulness, particularly since the precise genetic basis of this category of malformation is still unknown, but eventually it will require revision when the specific genes and their patterns of expression become known.

Barkovich and colleagues propose a classification "for malformations of cortical development" that is very similar to our scheme, attempting to integrate genetic with morphological criteria, with some reshuffling of the order in which categories are presented [41, 42]. Our principal objection to this scheme is that the central nervous system must be considered as a whole rather than artificially isolating the cerebral cortex, for reasons discussed in the first section of this paper. These authors also appear to have misunderstood our proposal because they state that it is purely based upon genetics alone.

Importance of semantic precision

Terminology must be precise in scientific communication, or the precision of the data or concepts that words convey becomes ambiguous and imprecise. The degradation of specificity to generality is a disservice to science, and both authors and journal editors share a responsibility to protect and preserve semantic precision in scientific communication. For example, *ectopia* and *heterotopia* are not synonymous and interchangeable terms [43, 44]. Ectopia are cells or tissues displaced *outside* their organ of origin, exemplified by isolated islands of neural tissue within the leptomeninges. Heterotopia, by contrast, are cells displaced *within* their organ of origin; in the case of the central nervous system, the most frequent example is heterotopic neurons within the white matter, usually due to incomplete migration. Neurons in the centrum semiovale are never ectopic. This problem of semantics is complicated by the fact that geneticists speak of "ectopic expression" of genes in the wrong neuromeres because the term "heterotopic" does not exist in the dictionary of molecular genetics. "Ectopic" thus is used somewhat differently in genetics than in reference to histology. It should be noted also, that this Greek-derived suffix "-ia" is correctly used as both the singular and plural forms. "Heterotopias" is a redundant form that is bad English and worse Greek ! It may be correctly used when writing in romance languages such as Spanish and Italian, where foreign terms are made to correspond to the grammar of those languages, but in English such words are not generally anglicized and they retain their original Latin or Greek grammatical forms.

In the new terminology of molecular biology, *upregulation* and *overexpression* are similar but not identical terms, and each has important semantic nuances. Upregulation is an increased amount of a normal gene product produced by a cell so that it has a stronger than expected influence on other cells that it is inducing. Overexpression conveys a similar meaning but, in addition to being due to upregulation, overexpression also may simply be loss of an antagonistic influence or genes of downregulation. A ventralizing effect on the dorsoventral axis of the neural tube thus may be due to upregulation of a ventralizing gene or due to downregulation of a dorsalizing gene with the normally expressed ventralizing gene appearing to have a stronger influence. In sum, *upregulation* is the more specific and *overexpression* the more general of this pair of term; *downregulation* and *underexpression* are another corresponding pair.

Neuromeres are the embryonic segments of the neural tube, but more specific terms designate neuromeres in

different parts of the tube: the hindbrain is composed of 8 *rhombomeres* (hindbrain neuromeres); the entire spinal cord is derived from rhombomere 8 and is not an intrinsically segmented structure. In addition, there is a *mesencephalic neuromere* and four *prosomeres*, two being diencephalic and two others, telencephalic. Many genes are expressed in a large number of neuromeres early in ontogenesis but later confine their expression to certain ones (*principle 2*).

Dysgenesis and *dysplasia* are terms that often have been used interchangeably, and this may have been satisfactory before the advent of molecular genetics. At present, *dysgenesis* generally implies a programmed genetic developmental defect, whereas *dysplasia* remains the more general term implying either a genetic or an acquired ætiology of defective development or abnormal growth of a structure. Hamartomas are restricted zones of dysplasia that either include abnormal cellular growth and differentiation, in addition to disordered tissue architecture, as in the case of subependymal and cerebral cortical lesions in tuberous sclerosis, or a variety of tissues of different lineage, such as the angiomyolipoma of the kidney in tuberous sclerosis. Other examples of hamartomas of the nervous system are hemimegalencephaly and dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos disease). Abnormal cells that may be classified as hamartomatous may have begun as normal cells but later changed in terms of growth and differentiation characteristics. An example is the "balloon cell" in focal cerebral dysgenesis of the Taylor type. These cells also are found in hemimegalencephaly, however, where they likely never were normal.

In midline malformations such as holoprosencephaly and septo-optic-pituitary dysplasia, the paramedian regions of the forebrain, diencephalon, midbrain or cerebellum (rhombencephalosynapsis) are continuous across the midline without an interhemispheric fissure, septum pellucidum, third ventricle or cerebellar vermis. Though such conditions are sometimes termed midline *fusion*, this term is not correct because fusion implies once normally formed, distinct paired structures that secondarily became adherent. The more correct term is *noncleavage*. □

References

1. Sarnat HB. The New Neuroembryology. Molecular genetic classification of CNS malformations. *J Child Neurol* 2000; 15: 675-87.

2. Sarnat HB, Flores-Sarnat L. A new classification of malformations of the nervous system. Integration of morphological and molecular genetic criteria. *Eur J Pæd Neurol* 2001; 5: 57-64.

3. Sarnat HB. *Cerebral dysgenesis embryology and clinical expression*. New York: Oxford University Press. 1992.

4. Norman MG, McGillivray GC, Kalousek DK, *et al. Congenital malformations of the brain. Pathological, embryological, clinical, radiological and genetic aspects.* London: Oxford University Press. 1995.

5. DeMyer W, Zeman W, Palmer GG. The face predicts the brain. Diagnostic significance of of median facial anomalies for holoprosencephaly (arhinencephaly). *Pediatrics* 1964; 34: 256-63.

6. Barkovich JA. *Pediatric Neuroimaging*. 3rd ed. Philadelphia: Lippincott Williams & Williams 2000.

7. Golden JA. Towards a greater understanding of the pathogenesis of holoprosencephaly. *Brain Dev* 1999; 21: 513-21.

8. Sarnat HB, Flores-Sarnat L. Neuropathological research strategies in holoprosencephaly. *J Child Neurol* 2001; 16: 918-31.

9. Fox JW, Lamperti ED, Eksioðlu YZ, *et al.* Mutations in *filamin-1* prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998; 21: 1315-25.

10. Eksioðlu YZ, Scheffer IE, Cardena P, *et al.* Periventricular heterotopia: an X-linked dominant epilepsy locus causing aberrant cerebral cortical development. *Neuron* 1996; 16: 77-87.

11. des Portes V, Pinard JM, Billuart P, *et al.* A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell* 1998; 92: 51-61.

12. Gleeson JG, Allen KM, Fox JW, *et al. Doublecortin*, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell* 1998; 92: 63-72.

13. Qin J, Mizuguchi M, Itoh M, Takashima S. Immunohistochemical expression of doublecortin in the human cerebrum: comparison of normal development and neuronal migration disorders. *Brain Res* 2000; 863: 225-232.

14. Dobyns WB, Reiner O, Carrozzo R, Ledbetter DH. Lissencephaly: a human brain malformation associated with deletion of LISd1 gene locate at chromosome 17p13. *JAMA* 1993; 270: 2838-42.

15. Lo Nigro C, Chong SS, Smith ACM, *et al.* Point mutations and an intragenic deletion in *LIS1*, the lissencephaly causative gene in isolated lissencephaly sequence and Miller-Dieker syndrome. *Hum Mol Genet* 1997; 6: 157-64.

16. Faina GT, Cardini FA, D'Incerti L, *et al.* Familial schizencephaly associated with *EMX2* mutation. *Neurology* 1997; 48: 1403-6.

17. Graf WD, Born DE, Sarnat HB. The pachygyria-polymicrogyria spectrum of cortical dysplasia in X-linked hydrocephalus. *Eur J Paediatr Surg* 1998; 8 (suppl 1): 10-4.

18. Sarnat HB. Central nervous system malformations: locations of known human mutations. *Eur J Paediatr Neurol* 2001; 4: 289-90.

19. McMahon AP, Joyner AL, Bradley A. McMahon JA. The midbrain-hindbrain phenotype of *Wnt-1-/Wnt-1-* mice results from stepwise deletion of engrailed-expressing cells by 9.5 days postcoitum. *Cell* 1992; 69: 581-95.

20. Wurst W, Auerback AB, Joyner AL. Multiple developmental defects in *Engrailed-1* mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. *Development* 1994; 120: 2065-75.

21. Joyner AL. *Engrailed, Wnt* and *Pax* genes regulate midbrain-hindbrain development. *Trends Genet* 1996; 12: 15-20.

22. Mastick GS, Fan C-M, Tessier-Lavigne M, *et al.* Early deletion of neuromeres in *Wnt-1(-/-)* mutant mice: evaluation by morphological and molecular markers. *J Comp Neurol* 1996; 374: 246-58.

23. Kuemerle H, Zanjani H, Joyner A, Herrup K. Pattern deformities and cell loss in *Engrailed-2* mutant mice suggest two separate patterning events during cerebellar development. *J Neurosci* 1997; 17: 7881-9.

24. Sarnat HB, Benjamin DR, Siebert JR, *et al.* Agenesis of the mesencephalon and metencephalon with cerebellar hypoplasia: putative mutation in the *EN2* gene – report of 2 cases in early infancy. *Ped Devel Pathol* 2002; 5: 54-68.

25. Casarosa S, Fode C, Guillemet F. *Mash1* regulates neuronogenesis in the ventral telencephalon. *Development* 1999; 126: 525-34.

26. Simon EM, Hevner R, Pinter JD, *et al.* Assessment of the deep gray nuclei in holoprosencephaly. *Am J Neuroradiol* 2000; 21: 1955-61.

27. Plawner LL, Delgado MR, Miller VS, *et al.* Clinical spectrum of holoprosencephaly: a clinical-neuroradiologic analysis. *Neurology* 2002; *in press*.

28. le Dourarin NM, Kalscheim C. *The Neural Crest*. 2nd ed. Cambridge: Cambridge University Press. 1999.

29. Hall BK. *The neural crest in development and evolution*. New York: Springer-Verlag. 1999.

30. Sarnat HB, Menkes JH. The New Neuroembryology: How to construct a neural tube. *J Child Neurol* 2000; 15: 110-24.

31. Hume CR, Dodd J. *Cwnt-8C*, a novel *Wnt* gene with a potential role in primitive streak formation and hindbrain organization. *Development* 1993; 119: 1147-60.

32. van Straaten HWM, Hekking JWM, Wiertz-Hoessels EJLM, *et al.* Effect of the notochord on the differentiation of the floor plate area in the neural tube of the chick embryo. *Anat Embryol* 1988; 177: 317-24.

33. Flores-Sarnat L. Hemimegalencephaly. 1. Genetic, clinical and imaging aspects. *J Child Neurol* 2002; 17: *in press*.

34. Flores-Sarnat L, Sarnat HB, Dávila-Gutiérrez G, Álvarez A, de León B. Hemimegalencephaly is a disturbance of cellular lineage, not of neuroblast migration. *J Neuropathol Exp Neurol* 2002; 61: 469 (abstract).

35. Flores-Sarnat L, Sarnat HB, Dávila-Gutiérrez G, Álvarez A. Hemimegalencephaly. 2. Neuropathological aspects. *J Child Neurol* 2002; 17: *in press*.

36. Takashima S, Chan F, Becker LE, Kuruta H. Aberrant neuronal development in hemimegalencephaly: Immunohistochemical and Golgi studies. *Pediatr Neurol* 1991; 7: 275-80.

37. Ryan AK, Blumberg B, Rodríguez-Estaban C, *et al. Pitx2* determines left-right asymmetry of internal organs in vertebrates. *Nature* 1998; 394: 545-51.

38. Saijoh Y, Adachi H, Mochida K, *et al.* Distinct transcriptional regulatory mechanisms underlie left-right asymmetric expression of *lefty-1* and *lefty-2*. *Genes Dev* 1999; 13: 259-69.

39. Casey B. Two rights make a wrong: human left-right malformations. *Hum Mol Genet* 1998; 7: 1565-71.

40. Ikenouchi J, Uwabe Ch, Nakatsu T. Embryonic hydromyelia: cystic dilatation of the lumbosacral neural tube in human embryos. *Acta Neuropathol* 2002; 103: 248-54.

41. Barkovich AJ, Kuzniecky RI, Dobyns WB, *et al.* Classification scheme for malformations of cortical development. *Neuropediatrics* 1996; 27: 59-63.

42. Barkovich AJ, Kuzniecky RI, Jackson GD, *et al.* Classification system for malformations of cortical development. Update 2001. *Neurology* 2001; 57: 2168-78.

43. Sarnat HB. Ectopic or heterotopic? An appeal for semantic precision in describing developmental disorders of the nervous system. *Pediatr Neurol* 1995; 13: 178-9.

44. Sarnat HB. Semantic precision for central nervous system malformations. *Acta Neuropediatrica* 1997; 3: 4-8.