

REVIEW ARTICLE

INHERITED NEURODEVELOPMENTAL BRAIN DISEASES: APPLICATIONS OF HOMOZYGOSITY MAPPING TO IDENTIFY NEW GENETIC CAUSES OF DISEASE.

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Abstract

Objective

The last two decades have seen major advancements in our understanding of some of the most common neurodevelopmental disorders in the field of child neurology. However, in the majority of individual patients, it is still not possible to arrive at a molecular diagnosis, due in part to lack of knowledge of molecular causes of these tremendously complex conditions. Common genetic disorders of brain development include septo-optic dysplasia, schizencephaly, holoprosencephaly, lissencephaly and hindbrain malformations. For each of these disorders, a critical step in brain development is disrupted. Specific genetic diagnosis is now possible in some patients with most of these conditions. For the remaining patients, it is possible to apply gene-mapping strategies using newly developed high-density genomic arrays to clone novel genes. This is especially important in countries like Iran where large family size and marriage between relatives makes these strategies tremendously powerful.

Keywords: Inherited Neurodevelopmental disease, Microcephaly, Joubert Syndrome

Introduction

Remarkable progress in our understanding of the genetic, molecular, and pathogenic mechanisms underlying congenital structural or functional brain defects has paralleled progress in developmental and molecular neurobiology. The extraordinary information about the sequence of molecular and structural events controlling brain development, and likewise, the effects of gene dysregulation in the pathogenesis of many well-recognized but poorly understood malformations, continues to emerge at an exciting pace. Three of the most important contributions that have advanced this knowledge are the capability to perform detailed neuroimaging in patients with MRI, the draft of the human genome sequence, and the ability to perform high-density genomic testing in patients and families. Imaging has provided more refined correlations between clinical symptomatology, progression of disease, while the genomic evaluations has fueled the identification of the causes of these diseases, which has resulted in improved diagnosis, increased prognostic information, and in some cases, led to the development of clinical trials of new treatments.

Normal embryonic development

Knowledge of nervous system embryology is essential for understanding developmental abnormalities (Table 1). Nervous system development begins with

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Table 1. Simplified scheme of brain development in humans.

Critical Event in Brain Development	Developmental Window (Week of Gestation)	Example of Major Human Diseases
Neural tube closure	3-6 weeks	Schizencephaly, Spina bifida
Dorsal cleft formation	5-6 weeks	Holoprosencephaly
Ventral specification	5-6 weeks	Septo-optic dysplasia, Agen CC
Neuronal proliferation	8-16 weeks (majority)	Microcephaly
Neuronal migration movement	10-20 weeks	Lissencephaly
Cerebellar development	20-40 weeks and beyond	Dandy-Walker, Joubert Sx

induction of the neural plate, which then undergoes a morphological change toward the neural tube. Failure of neural tube closure anywhere along the neural axis gives rise to several disorders, including spina bifida and anencephaly, and may underlie other conditions such as encephaloceles and schizencephaly. Next, the neural tube is divided into three major axes: rostral-caudal, dorsal-ventral, and medial-lateral. One of the examples of a disease that represents failed axis formation is holoprosencephaly, in which the dorsal cleft fails to form and as a result there is failure to divide the brain into the two distinct hemispheres. Next, previously unspecified nervous system tissue is endowed with certain anatomical and cell-type specific identities. This process is termed segmentation or specification; for instance, the base of the forebrain is specified to hypothalamic tissue. Examples of diseases of this class are septo-optic dysplasia and agenesis of the corpus callosum. Next, neural stem cells located along the lining of the ventricle undergo cell division to produce neurons that populate brain structures. The prime example of a disease of this class is microcephaly. Next, neurons initiate migration from the site birth to towards the developing gray matter in a process termed neuronal migration. Next is the development of the cerebellum, using some of the same mechanisms involved in development of the cerebral cortex. The major disorders of cerebellar development are Dandy-Walker malformation and Joubert syndrome. For the purposes of this review, we will focus on recent advances in two of these conditions: Microcephaly and Joubert syndrome.

Primary Microcephaly: A disorder of neuronal proliferation

Microcephaly is usually defined as an occipitofrontal circumference (OFC) that is < 2 SD below the mean for patient’s age and gender (1). When congenital microcephaly is the only abnormality, then the disorder has been designated primary microcephaly or microcephaly vera, and is divided into borderline microcephaly for those -2 to -3 SD or severe microcephaly for those below -3 SC. The clinical manifestations associated with microcephaly are very heterogeneous. In general, below-average intelligence is the major finding. Although intellectual impairment is seen in only about 11% of patients with borderline microcephaly, it is seen in over 50% of patients with severe microcephaly (2).

In most patients with severe primary microcephaly, brain imaging reveals characteristic abnormalities, designated “microcephaly with simplified gyral pattern” (3). At its most severe, the gyral pattern becomes so simplified as to appear lissencephalic (i.e. lacking gyri and sulci in some places), which is designated “microlissencephaly” (3). Common associated abnormalities include hypoplastic frontal lobes, thin corpus callosum and mild hypoplasia of the brainstem and cerebellum.

Primary microcephaly is divided into two clinical groups. The first group is composed of children with severe microcephaly but only moderate neurological problems, usually with only mild to moderate mental retardation without spasticity or epilepsy. The second group is composed of children that additionally display adverse neurological phenotypes including severe spasticity and epilepsy. These children have abnormal neonatal reflexes,

poor feeding, recurrent vomiting and failure-to-thrive (4). Additional abnormal MRI findings may be present in this second group to include ventriculomegaly, white matter signal changes or thickened cortical mantle. Because the prognosis is so dramatically different between these two phenotypes, it is important to distinguish these as early as possible using careful assessment as well as genetic evaluations when beneficial.

One of the most exciting recent developments is the delineation of Seckle syndrome and microcephalic osteodysplastic primordial dwarfism, unique conditions characterized by dwarfism, microcephaly, retardation and seizures (5). In Seckel syndrome, there is severe microcephaly akin to that seen in primary microcephaly, but in addition the body of the patients is comparably reduced in size. This suggests that there is a class of disease that particularly affects brain size, and another disease that affects both brain and body size. Thus it is important to assess height and weight percentiles when evaluating a child with microcephaly, to distinguish between primary microcephaly and Seckel syndrome.

Six distinct genetic loci for primary microcephaly have been identified (MCPH1-6) with the genes MCPH1, CDK5RAP2, ASPM, and CENPJ causing MCPH1, 3, 5, and 6 respectively (Table 2) (6). These proteins encoded by these critical genes play roles in regulating cell cycle and in DNA damage response signaling. The two genes for Seckel syndrome to be cloned so far ATR and PCTN, encode proteins that also function in cell cycle arrest and DNA damage repair in response to DNA damage (7,8). Thus it appears that many of the microcephaly

conditions are probably due to cell cycle regulation in regards to perturbed DNA damage response and in the regulation of mitotic entry.

Joubert syndrome: Commonest inherited congenital cerebellar ataxia

Human congenital ataxias comprise a group of conditions that present in the first few years of life with motor disability, muscular hypotonia and incoordination, and impaired development. Characteristically, such patients display some degree of cerebellar dysplasia that typically involves the vermis, and which may accompany other developmental brain abnormalities and other non-CNS developmental abnormalities.

Cerebellar hypoplasia may be encountered in several different conditions, but additional brain malformations or systemic features may help to differentiate these various conditions. One of the most helpful signs is the “molar tooth malformation” (MTM) of the midbrain-hindbrain junction, which is apparent on axial brain MRI section just above the pons. The MTM is a specific malformation of the brainstem, cerebellum and the cerebellar peduncles, which gives the appearance of a tooth-like shape in axial MRI images (9). The appearance of the MTM is a result of (1) cerebellar vermis hypoplasia that produces enlargement of the IV ventricle; (2) a deep interpeduncular fossa and; (3) upwardly rotated and elongated superior cerebellar peduncle. The MTM is pathognomonic for Joubert syndrome and related disorders (JSRD), and its presence can differentiate

Table 2. Genetic basis of primary microcephaly syndromes involved in DNA damage response.

Condition	Clinical features	Gene	Proposed function
MCPH1	Primary microcephaly	MCPH1	DNA damage response and cell cycle regulation
MCPH2	Primary microcephaly	unknown	
MCPH3	Primary microcephaly	CDK5RAP2	Centrosome organization in neurons
MCPH4	Primary microcephaly	unknown	
MCPH5	Primary microcephaly	ASPM	Spindle microtubule nucleation at centrosome
Seckel1	Primordial dwarfism with microcephaly	ATR	G2/M checkpoint defect
Seckel2	Primordial dwarfism with microcephaly	PCTN	ATR-dependent checkpoint signaling

JSRD from other cerebellar disorders such as Dandy-Walker malformation, pontocerebellar hypoplasia and isolated vermis hypoplasia (Fig. 2).

Joubert syndrome is a recessively inherited condition that is characterized clinically by congenital ataxia, hypotonia, episodic breathing dysregulation, and mental retardation (10). The signature feature of this disease is the “molar tooth” sign. It has been recognized that many patients with Joubert syndrome identified by brain imaging studies, also display retinal dystrophy and nephronophthisis (renal fibrocystic disease) (11), two conditions that are proposed to relate to defective function of primary cilia, cellular appendages of unclear function. Several recent proteomics and bioinformatics studies have identified lists of candidate ciliafunctioning proteins, together with availability of large, consanguineous families segregating the condition, has sped the discovery of several of the

responsible genes.

Currently, there are seven genetic loci that have been mapped for the various subtypes of Joubert syndrome, and the first five genes have been identified. These include Ableson-helper integration-1 (AHI1), Nephrocystin-1 (NPHP1), Centrosomal protein-290 (CEP290), Transmembrane protein 67 (TMEM67) and Retinitis pigmentosa GTPase regulator-interacting protein-like (RPGRIP1L). Each of the genes encodes a modular scaffolding protein without clear enzymatic domains, but sharing several protein-interaction domains of unknown function, suggesting that they may be part of a signaling complex (12-20). The next few years should see the elucidation of the molecular pathways regulating cerebellar development, and hopefully some of these ideas can be translated into new therapies for these patients.

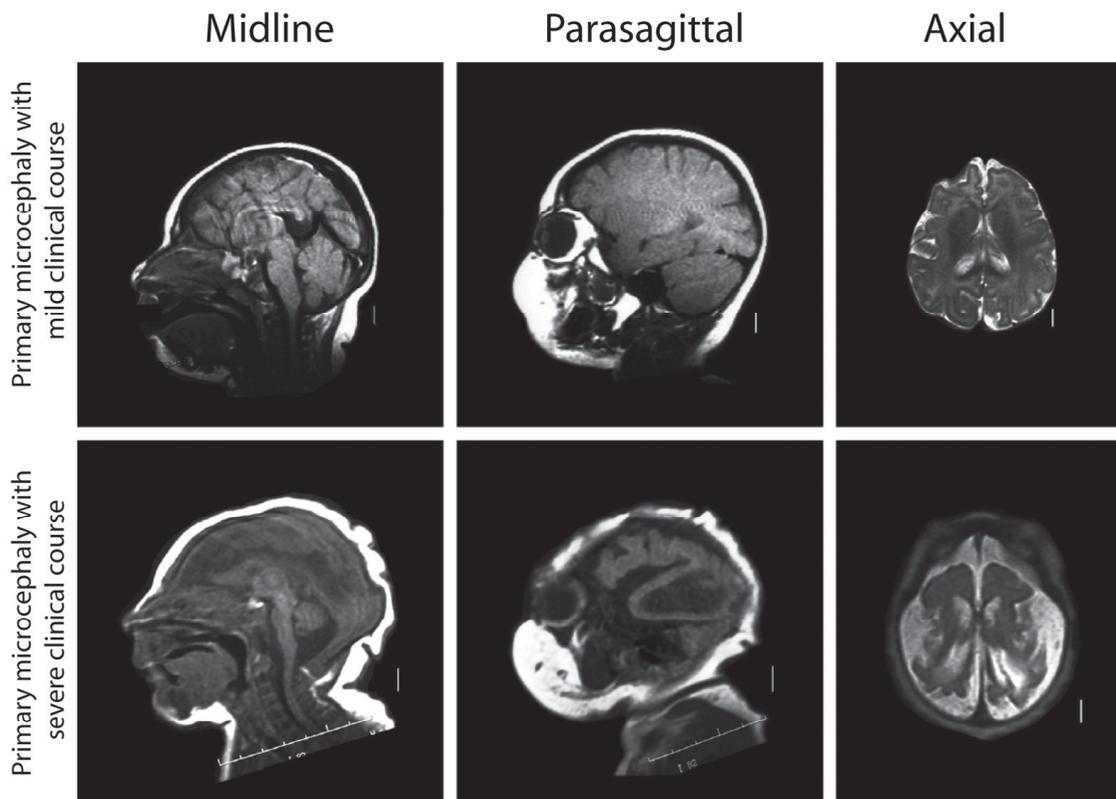


Figure 1. **Subtypes of severe congenital microcephaly.** Top row is from a patient with primary microcephaly and simplified gyral pattern. Bottom row is from a separate patient with primary microcephaly with markedly enlarged extra-axial spaces and lateral ventricles, also with white-matter changes and cerebellar hypoplasia. The patient at bottom display severe spasticity and intractable epilepsy. Adopted from [21] with permission.

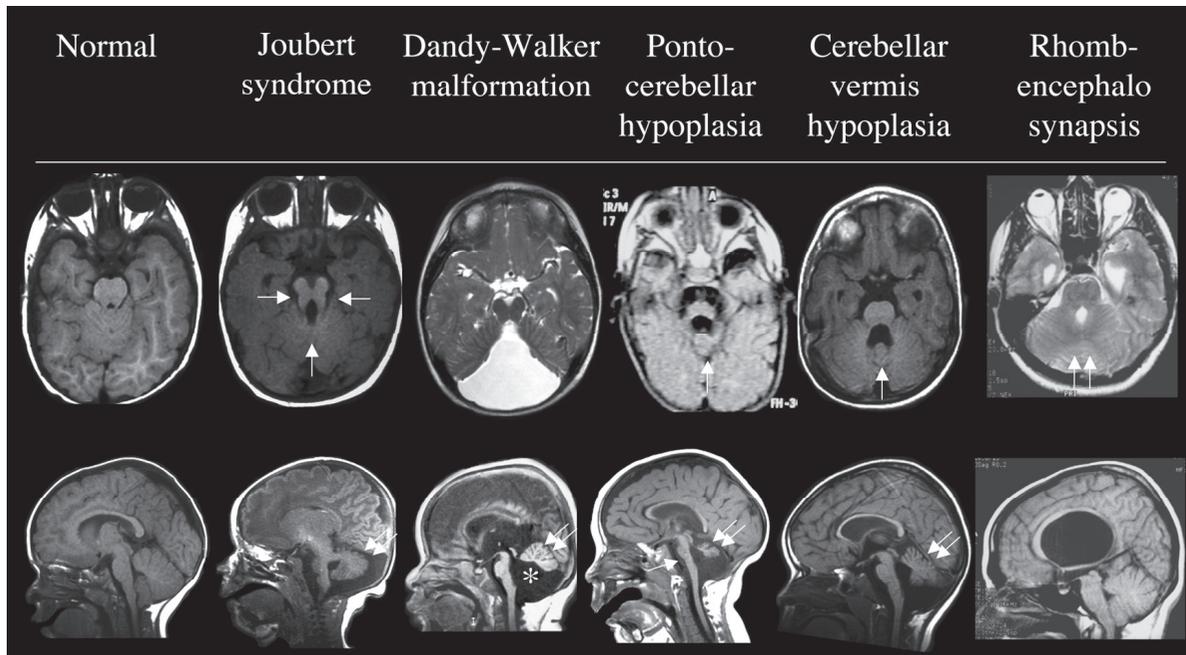


Figure 2. **Spectrum of human cerebellar malformations.** Top row: Axial at the level of the midbrain-hindbrain junction (isthmus). Bottom row: Midline sagittal. In normal, isthmus shape is relatively circular, and cerebellar vermis is full. In all of the conditions except rhombencephalosynapsis the cerebellar vermis is hypoplastic (double arrows on bottom row). In Joubert syndrome the isthmus takes the shape of a “molar tooth” (arrows). In Dandy-Walker malformation, the posterior fossa is full of fluid (white field) and bottom shows the cystic dilatation of the fourth ventricle (*), which is rotated anteriorly. In Pontocerebellar hypoplasia the brainstem is also involved. Note small isthmus and vermis (arrow, top) and reduced brainstem volume (arrow, bottom). Cerebellar vermis hypoplasia shows reduced vermis without other associated features. Rhombencephalosynapsis shows fusion of the two cerebellar hemispheres, and the vermis is replaced by this fusion. (double arrows, top).

Future prospects

In the field of child neurology, one of the main ways that we can help improve knowledge of the conditions that we treat is to help uncover the cause of disease through collaborative research study. In the field of genetics, this takes the form of helping to identify new genetic forms of disease and aiding to identify the genetic cause of disease. Gene mutations underlie a majority of the cases that we treat in our clinics, and it is the astute clinician that can recognize when a particular patient or family can help advance research in the field.

For recessive diseases, one of the main ways to help advance knowledge is by identifying families that can be

used for gene evaluation. For example, our Neurogenetics Laboratory at the University of California is working to identify the genetic causes of recessive disease in Iran and the Middle East using a gene mapping strategy. In this approach, we work to identify large families with documented consanguinity in which there are several children displaying the same disease. The parents in such an ideal family are typically related to one another through cousin marriage, or are from the same small town. Also, it is ideal if cousins with the same disorder are identified as well, which helps to increase the likelihood of finding a diseased chromosomal interval. It is key to have such ideal, albeit rare, families invited

to participate in research studies that can help uncover new causes of disease. The information from the study can be helpful to the family, because of identification of carriers in the family as well as the mutation that causes the disease, which can impact diagnosis and prognosis.

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