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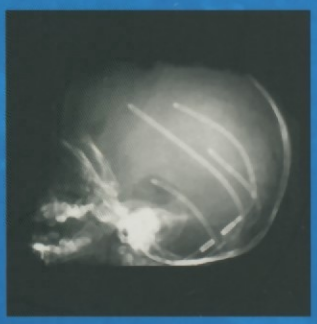
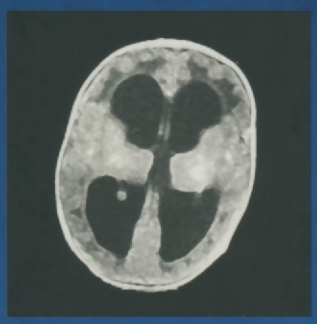
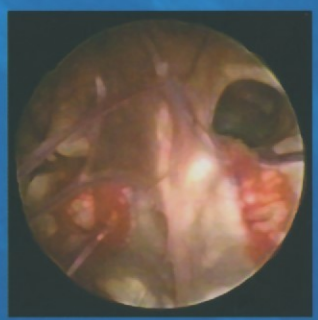
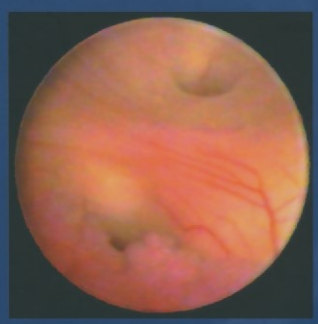
(Eds.)

Pediatric Hydrocephalus









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To Fabrizia, Francesco, and Maria Allegra,
the most beautiful gifts of my life,
to thank them for their patience and support
in all the moments that this book has stolen from us

To my Mother, who loved and supported me all my life,
and to my Father, who accompanied me at every step
but allowed me to find the way

GC

To my parents and Harriott, who is inspiration
for me in all things

WM

To Federica, Elise, and George

CSR

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G. Cinalli · W.J. Maixner · C. Sainte-Rose (Eds)

Pediatric Hydrocephalus

Springer

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Preface

This book was conceived as a result of the weekly staff meetings and daily clinical rounds at the Department of Neurosurgery, Hôpital Necker–Enfants Malades in Paris. The understanding and management of pediatric hydrocephalus has been part of our routine work for years. As so often happens to those who are thus preoccupied, the first proposition for the book came not from the neurosurgical unit, but from our colleagues, in this instance Jean Aicardi in 1994. He understood that it was a period of "revolution" for pediatric hydrocephalus particularly as it pertained to neuroendoscopy. He therefore petitioned Christian Sainte-Rose to write a book on pediatric hydrocephalus addressed to pediatric neurosurgeons, pediatric neurologists, and pediatricians.

The gestational phase was long, but serendipitous for the project. This phase witnessed the publication of the final results of the shunt trial (1998 and 2000) and the evolution and definition of neuroendoscopy. In 1999, the time finally became ripe to look back and try to define the real impact of these two events in the treatment of pediatric hydrocephalus. Thus, the book was born.

We are deeply indebted to Springer-Verlag Italia, who agreed to publish the book, and we are particularly grateful to Dr. Donatella Rizza and to the whole editorial team for their patience, their professionalism and for the quality of their work.

It is necessary also to thank in this page all the people who have directly or indirectly contributed to the ideas that are included within – to all the permanent and temporary neurosurgical staff at Necker–Enfants Malades, the residents, the chefs de clinique, the students, and the consultants whose questions, criticisms, and comments have molded our thoughts. We are and always will be grateful to Jean-François Hirsch for his curiosity, his teaching, and his support. We thank our nurses for their care of our patients. Most of all, we thank our little patients, all of them, for bearing the consequences of our decisions.

January 14, 2004

Giuseppe Cinalli
Wirginia Maixner
Christian Sainte-Rose

Foreword

by Maurice Choux

Yet another book on hydrocephalus! That was my first thought when I received a copy of the manuscript of the present book. It is true that the problems related to hydrocephalus and their importance seem more limited now than they were a few years ago. The reason for this apparently reduced interest is the belief that hydrocephalic patients have become significantly fewer in recent years. The development of prenatal diagnosis and the drop in the birth rate in developed countries may explain this.

I believe this impression needs to be corrected. As pointed out by Hanlo et al. in Chap. 7, "The actual importance of hydrocephalus as a neurological disorder is severely underestimated. The incidence of congenital and infantile hydrocephalus is reported to be 0.48 to 0.81 per 1000 live births and stillbirths .... Cases of secondary hydrocephalus are seldom included in the incidence and prevalence figures". That is true in developed countries and, moreover, in developing countries where prenatal diagnosis is not widespread and where shunt costs significantly limit the number of shunt implantations. In developing countries, the total number of hydrocephalus cases has not only not diminished, but is increasing with the higher birth rate. Consequently, the questions raised by its management remain fundamental.

The main recent changes in the field of hydrocephalus relate to modern radiological investigations and modern ways of management, since shunts are no longer the exclusive treatment for a child with hydrocephalus. We are in need of a new approach to hydrocephalus, and that is why this book, edited by highly esteemed experts in hydrocephalus, is so welcome and promises to be such a great success.

This is a modern and new presentation of an old disease. Thus, it is significant that the opening chapter is devoted to genetics, and that the chapters dedicated to endoscopy are longer than those on shunts. Some aspects of hydrocephalus that were ignored in most previous books on the topic are extensively developed here: examples are growth and puberty in hydrocephalus, and hydrocephalus and epilepsy. Classical concepts, such as the development of the cerebrospinal fluid (CSF) pathways, CSF hydrodynamics, the pathophysiology of hydrocephalus, or the classification and definition of hydrocephalus, are covered in a new way and will change our previous certainties.

The perfect or ideal shunt is not described in this book, but we may dream with Ginsberg and Drake when they say, in Chap. 20, "We envision a future where a patient receives only one shunt in his/her lifetime, which is sophisticated enough to control intracranial pressure within normal physiological limits, can be adjusted and monitored noninvasively, and treated minimally invasively for shunt obstruction".

Another aspect of hydrocephalus which is of central importance at present and is not commonly discussed is the cost of the different treatments. Garton and Steinbok treat of this crucial question in Chap. 31, in a cost analysis of shunt surgery versus endoscopic surgery.

This book on pediatric hydrocephalus is indispensable not only for neurosurgeons but also for those interested in all aspects of hydrocephalus: pediatricians, radiologists, endocrinologists, pathologists, and geneticists. They will all discover that the best management of hydrocephalus is definitely not shunting, and alternatives to shunts, such as external drainage, endoscopic procedures, and management of the causes, are always preferable. Interestingly, four chapters of this book are dedicated to tumoral hydrocephalus, underlining how managing the mass lesion first, without inserting a shunt, will avoid a definitive shunt in most cases. Faced with a hydrocephalic patient, the first question will no longer be "What type of shunt is needed", but, "Does the child need a shunt?" This book also devotes space to the question of shunt malfunctions, with special attention to their management – which is not always shunt revision. Alternatives to shunt revisions also exist, and endoscopic techniques may not only avoid shunt replacement, but also allow shunt

removal. I am pleased to see that Dr. Hammer's old motto, "once a shunt, always a shunt", is no longer valid in 2004.

I am glad to congratulate the editors and all the contributors to this excellent book, and to deeply thank Giuseppe Cinalli, Wirginia Maixner, and Christian Sainte-Rose for giving me the great honour and pleasure of recommending this major modern contribution in the field of hydrocephalus to its public.

January 2004

Foreword

by Giuseppe Maggi

A book published at the beginning of the third millennium offering a thorough review of the diagnosis and treatment of pediatric hydrocephalus has been long awaited by many neurosurgeons. This is by far the most commonly observed pathology in the everyday clinical practice of pediatric neurosurgeons. Its prognosis has changed dramatically since the introduction of the devices for the diversion of cerebrospinal fluid, and the last ten year have witnessed a very significant breakthrough in treatment with the advent of neuroendoscopy. Pediatricians and pediatric neurologists and neurosurgeons nowadays need to be aware of the existence of two alternative and sometimes complementary treatments, with different indications, diagnostic and therapeutic implications, and different follow-up schedules. The main objective of putting them in this position is well achieved in this book, all the chapters of which are by experts recognized as among the best in the world in the various fields of hydrodynamics, pathophysiology, diagnosis, and treatment.

But I think that the most important quality of this book is its clear, detailed, and attentive guide for the reader in the choice of the best surgical strategy for the treatment of the most challenging forms of hydrocephalus, such as the post-meningitic, loculated, tumoral, and Dandy-Walker-related forms. This is probably the first, real organic attempt at a valid organization of the knowledge in this field of neurosurgery.

Neuroendoscopic techniques are critically described and their effectiveness thoroughly evaluated. The complications, and their avoidance and management are depicted in detail and are illustrated progressively, following the single individual steps of every procedure, with wonderful pictures that powerfully support the text.

In conclusion, I am very grateful to the editors for the high quality of their work, for this titanic attempt to share their knowledge with their colleagues all over the world, and for giving me the opportunity to introduce this very unique book in the crowded ballroom of the international neurosurgical library.

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Table of Contents

List of Contributors

Genetics of Hydrocephalus

PETER B. DIRKS

Introduction

Due to the complexity of organization and function of the human brain, a large proportion (estimates are >50%) of the 40 000 or so genes in the human genome are expected to be involved in the formation and function of the brain [20]. As hydrocephalus is a frequent manifestation of a variety of human neurological diseases, we are at a threshold for improved understanding of the molecular pathogenesis of hydrocephalus and its associated diseases. This review discusses the genetics of CNS disorders associated with hydrocephalus. In these disorders hydrocephalus is usually not the only clinical manifestation of a genetic defect, but is seen in the context of a more broad CNS malformation or syndrome. In this chapter I intend to focus on disorders with hydrocephalus that have been significantly characterized from studies of human subjects and from studies of animal models. These animal studies, mainly using mice, have led to exciting discoveries that will prove to be breakthroughs for understanding the molecular pathogenesis of many disorders with hydrocephalus. This chapter covers vast ground and a complete discussion of all the literature is not possible, but important other reviews from the literature are cited to allow the reader to explore the area in more detail.

Genetics of Hydrocephalus: Overview

Congenital hydrocephalus has an incidence of 0.5-2.5:1000 total births [54, 115]. Congenital hydrocephalus is a heterogeneous group of developmental disorders. For the purposes of this chapter hydrocephalus secondary to intracranial infection, hemorrhage, vascular malformations (such as vein of Galen malformation), and neoplasms will not be considered, even though these diseases or the response to the primary insult may have an important genetic component. Excluding these etiologies, hydrocephalus may occur as a primary malformation or associated with other complex brain malformations such as neural tube defects, X-linked hydrocephalus (part of the clinical spectrum of CRASH syndrome), or Dandy-Walker syndrome. Hydrocephalus also occurs as a feature of genetic syndromes with multiple systemic malformations (such as occurring with chromosomal abnormalities or other mendelian disorders). Chromosomal abnormalities associated with congenital hydrocephalus are most commonly trisomy 13, trisomy 18, and trisomy 9. The number of genetic conditions where hydrocephalus is described as a clinical feature is enormous; this review will focus on human conditions with hydrocephalus with known genetic association.

Determining the genetic cause for hydrocephalus is critically important in determining outcome and plays an important role in genetic counseling. The recurrence risk of congenital hydrocephalus varies widely depending on the etiology. For non-NTD and non-X-linked hydrocephalus, the recurrence risk has been estimated at 1-4% for subsequent children [115]. These risks will be modified with increasing understanding of genetic causes of hydrocephalus and improved genetic testing. The clinical presence of additional congenital malformations or cytogenetic abnormalities adversely affects prognosis for intellectual development in hydrocephalus patients despite treatment with shunting.

X-Linked Hydrocephalus

X-linked hydrocephalus is a neurological disorder characterized by hydrocephalus due to aqueductal stenosis (see Table 1). Male preponderance in congenital hydrocephalus is due in part to this condition. X-

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Table 1. Features of X-linked hydrocephalus

	Clinical genetics	Key clinical features	Gene	Gene mutations in disease
X-linked hydrocephalus	1:30000 male births About 25% of males with definite aqueductal stenosis have this disease Chromosome Xq28	Spectrum of abnormalities part of the "CRASH" syndrome Aqueductal stenosis with hydrocephalus Spasticity and hypoplasia of corticospinal tracts Adducted thumbs	L1CAM A neural cell surface adhesion molecule	Large variety of different mutations Mutations involving cytoplasmic portion of the molecule may have less severe hydrocephalus

linked hydrocephalus is thought to represent about 2-5% of all nonsyndromal congenital hydrocephalus [115]. This condition has been recognized since the early 1960s [32, 33]. X-linked hydrocephalus is the most common genetic form of congenital hydrocephalus and occurs in about 1:30 000 male births. Transmission of the disease gene is from mothers to sons. The neurological abnormalities can be variable, and in addition to a varying severity of hydrocephalus, there may be hypoplasia of the corticospinal tracts (characterized by absence of medullary pyramids), corpus callosal agenesis, hypoplasia of the anterior cerebellar vermis and fusion of the thalami [132]. Other neurological abnormalities can occur. Patients present with hydrocephalus, mental retardation, spastic paraparesis, and a characteristic adduction deformity of the thumbs ("clasped" thumbs are present in about 50% of cases) [52]. There is a generally poor intellectual outcome in children despite adequate treatment of hydrocephalus related to the intrinsic brain abnormalities [52]. Non-X-linked hydrocephalus has a better intellectual prognosis.

It has been estimated that about 25% of males with definite aqueductal stenosis have X-linked hydrocephalus [8, 52]. This information is critically important in counseling parents of a male fetus with hydrocephalus. Knowing the sex of the fetus with hydrocephalus is obviously essential. X-linked hydrocephalus should be strongly considered if there is a maternal history of spontaneous abortion or early death of previous males in the family. However, these historical features are frequently not present as two-thirds of cases of X-linked hydrocephalus are sporadic. Female carriers can be mildly affected [54]. The risk for a male child for the condition is 50% if the mother is a carrier. Identification of associated clinical features in the suspected fetus is also important to consider in determining risk. In a male with obstructive hydrocephalus without the clinical findings of adducted thumbs and without hypoplasia of medullary pyramids, the estimated risk for a subsequent male is 4% and that for a female is 2% [52].

In the early 1990s linkage analysis studies of families with X-linked hydrocephalus established that the disease gene was located at chromosomal region Xq28 [65, 133]. Another syndrome known as the MASA syndrome (mental retardation, adducted thumbs, spasticity, and aphasia) was also localized to this region, suggesting that it may be caused by the same disease gene and that the two syndromes could be variations of the same disorder. It has now been established that mutations in the neural cell adhesion molecule known as L1 (also called L1CAM) causes both disorders [38, 39, 106, 124]. In fact, it is apparent that spastic paraplegia type 1 and X-linked agenesis of the corpus callosum are also caused by L1 alterations. Because of overlap and similarity between these disorders and common molecular genetic etiology, these groups of conditions have been grouped as the CRASH syndrome (corpus callosal hypoplasia, mental retardation, adducted thumbs, spastic paraplegia, and hydrocephalus) [38].

L1 is a transmembrane glycoprotein belonging to the immunoglobulin superfamily of cell adhesion molecules that mediate cell-cell adhesion [39, 68]. The protein has a large extracellular domain, a single transmembrane domain, and short cytoplasmic tail [5]. L1 is predominantly expressed in the nervous system and is thought to play a role in neuron-neuron adhesion, axonal elongation, and axonal pathfinding [71]. It has been functionally implicated in learning and memory. The molecular mechanism of activation and signaling of L1 is poorly understood. Many different molecules bind to the extracellular domain of L1, including L1 itself, and the significance of these different ligands is unclear. The intracellular signaling pathways have not yet been completely defined, but the cytoplasmic domain interacts with components of the sub-plasma membrane cytoskeleton [71]. L1-L1 interactions have been shown to activate fibroblast growth factor receptors leading to activation of calcium channels at the sites of axonal elongation, called growth cones [71].

The gene for L1 is large, having 28 exons over 15 KB of genomic DNA. A large number of different mutations have been described with each family having their own unique mutation. The mutations reported including missense, splicing, frameshift, nonsense, duplication, and deletion, meaning that they can have extremely variable effects on L1 genotype and possibly phenotype [41]. Mutations causing severe extracellular domain truncation or absence of protein seem to have more severe clinical phenotypes [41]. Mutations involving the cytoplasmic domain seem to cause less severe phenotypes, particularly less severe hydrocephalus [71, 137]. It has been speculated that these cytoplasmic mutations cause less severe phenotypes because they do not disrupt L1 extracellular adhesive function [71].

Mouse models have been generated to understand the function of L1 and to understand the pathogenesis of the CRASH syndrome. Complete loss of L1 in transgenic mice causes abnormalities in the brain similar to those of patients with the disease [16, 40, 68]. Homozygous L1 deletion (L1–/–) in mice leads to hydrocephalus, abnormal cortical pyramidal neurite extension, small hippocampi, small corpus callosum due to failure of crossing of axons, corticospinal tract hypoplasia, and cerebellar vermis aplasia – features strikingly similar to those seen in humans with L1 mutations. Animals have defects in learning. Hydrocephalus severity seems to depend on the particular mouse strain used to create the knockouts, suggesting that modifier genes play a role in the disease phenotype. The mechanism by which L1 causes hydrocephalus or aqueductal stenosis is not understood.

Because of the very wide variety of mutations causing the CRASH syndrome and the large size of the gene, molecular genetic analysis of the whole gene as prenatal work-up in previously unaffected families is a substantial undertaking [35, 36]. Detection of carriers can be determined through analysis of markers at Xq28 if other male patients are alive and if the family is large enough [115]. Mutational analysis can be performed by a method known as single-strand conformational polymorphism analysis (SSCP) of polymerase chain reaction (PCR)-amplified genomic DNA or reverse transcriptase PCR from RNA samples from amniotic fluid cells or chorionic villus samples. It is important to identify clinical features in family members in order to assess risk. Females in the family should be examined for adducted thumbs and learning difficulties. Perhaps with increased sophistication of MRI, abnormalities of the corticospinal tracts can be detected in the brainstems of affected individuals to help with prenatal diagnosis. Prenatal ultrasound has detected morphological abnormalities characteristic of the syndrome early in pregnancy [95, 121]. Because of the extremely wide spectrum of mutations in a very large gene and uncertain genotype-phenotype correlation, the identification of a mutation does not necessarily predict the severity of the disease in the affected individual.

Autosomal Recessive Hydrocephalus

Hydrocephalus inherited as an autosomal recessive disorder is rare and has a greater chance of being manifest in children born to consanguineous parents. Candidate genes for hydrocephalus inherited in this manner have not been isolated.

Dandy-Walker Malformation

Dandy-Walker malformation (DWM) is characterized by hydrocephalus, partial or complete absence of the cerebellar vermis, and a large posterior fossa cyst continuous with the fourth ventricle. It occurs with an estimated incidence of 1:25000 births. There may be a slight female preponderance [74, 92]. Dandy-Walker malformation is responsible for less than 5% of hydrocephalus cases. The etiology of this malformation is poorly understood. It has been associated with mendelian genetic disorders, chromosomal abnormalities, and teratogenic exposure (see Table 2).

Table 2. Conditions associated with Dandy-Walker syndrome (see [13, 86])

Mendelian disorders	Chromosomal abnormalities	Teratogens
Walker-Warburg syndrome (AR)	Trisomy 8, 9, 13, 18, 21	Maternal insulin-dependent diabetes
Meckel-Gruber syndrome (AR)	Triploidy 69	Alcohol
Joubert syndrome (AR)	Deletions 2q, 3q, 6p	Cytomegalovirus infection
Aicardi syndrome (XL)	Duplications 5p, 8p, 8q, 17q, 22	Rubella infection
Many others	Many others	Warfarin
		Valproic acid
		Vitamin A

AR, autosomal recessive; *XL*, X-linked

DWM is part of a heterogeneous group of disorders occurring as an isolated CNS abnormality or as part of a malformation syndrome involving other parts of the CNS or other organ systems. The list of mendelian disorders and chromosomal abnormalities associated with DWM is very extensive and has been previously detailed in the report by Chitayat et al. [13]. DWM occurs in the context of autosomal recessive conditions such as Walker-Warburg syndrome, Meckel-Gruber syndrome, and Joubert syndrome, as well as others. The genes for these three disorders remain unknown. Trisomy 18, trisomy 13, and triploidy are the most common associated chromosomal abnormalities. Recurrence risk has been estimated at 1-5% when not associated with Walker-Warburg syndrome or Meckel syndrome [86].

The pathogenesis of DWM has been debated for years, but it is probably generally accepted now that there is a primary defect in cerebellar development as opposed to atresia of the outlets of the fourth ventricle. The cerebellar vermis begins to form in the 9th week of human development beginning superiorly and is completed inferiorly by the 15th week [10]. The cerebellum emerges rather late in development. It begins as a proliferation of cells in the alar aspect of the first rhombomere of the hindbrain (also called the metencephalon) [66]. The most numerous cells in the cerebellar cortex, the granule neurons, arise along the edge of the fourth ventricle in a region known as the rhombic lip [82]. A critical region, known as the "isthmus," between the developing mesencephalon and metencephalon, acts as an organizer for the proper regional development of the brain stem and cerebellum. Cerebellar hemispheres develop from the metencephalon and the vermis develops from the mesencephalon adjacent to the isthmus. Ectopic transplantation of the isthmus more caudally in the rhombencephalon in mice leads to ectopic cerebellar formation [66]. Molecular analysis of the midbrain-hindbrain organizer region has lead to the discovery of a number of genes encoding for transcription factors or secreted factors involved in the proper development of the cerebellum (Otx1, Otx2, Gbx2, Engrailed 1, Engrailed 2, Pax2, Pax5, fibroblast growth factor 8). The discussion of the midbrain-hindbrain organizer and molecular control of cerebellar development is beyond the scope of this chapter, but the reader is referred to a number of excellent reviews [66, 82, 98, 134]. DWM cannot be diagnosed before 18 weeks as it has been estimated that the vermis is still open in 4% of fetuses at 17.5 weeks [74].

Walker-Warburg syndrome (cerebro-oculomuscular syndrome) is characterized by severe hydrocephalus, type II lissencephaly, cerebellar or posterior fossa malformation, eye and retinal abnormalities (retinal dysplasia, retinal detachment), and congenital muscular dystrophy [30]. The posterior fossa abnormality consists of DWM or occipital encephalocele. A diagnosis of Walker-Warburg syndrome can be made with clinical features in the context of lissencephaly on MRI, elevated muscle enzymes, and abnormal muscle biopsy. Prognosis is very poor [97]. Joubert syndrome is characterized by hypotonia, ataxia, mental retardation, characteristic facies, and ocular abnormalities. Hypoplasia of the cerebellar vermis occurs and in some patients there is DWM [97]. Meckel-Gruber syndrome consists of a variety of features, most importantly cystic kidney dysplasia, liver fibrosis, polydactyly, and CNS abnormalities including DWM and encephalocele [15, 138]. This disease has been mapped to chromosome 17q21-24 [91].

A posterior fossa malformation consistent with DWM also can occur in the context of Aicardi syndrome, which is characterized clinically by infantile spasms, agenesis of the corpus callosum, and pathognomonic retinal abnormalities (chorioretinal lacunae) [1, 31, 63, 88]. There can also be enlarged ventricles, choroid plexus cysts, and cerebral heterotopias [14, 123]. Spinal vertebral anomalies and cleft palate can also occur. The intellectual prognosis is generally very poor [80]. Visual prognosis is also poor and dependent on the location of the chorioretinal lacunae. Aicardi syndrome is considered to be an X-linked dominant disease, lethal in hemizygous males. All reported cases occur in females (except one male with a 47XXY karyotype). The disease has been mapped to chromosome Xp22, but the gene is unknown [31, 88].

It is clear that genetic analysis of affected persons with DWM has shed little light on the pathogenesis of the malformation. In the past decade, however, a staggering amount of knowledge has been accumulated about the genes involved in development of the brainstem and cerebellum (as discussed above). The use of mouse models in which these genes have been knocked out or misexpressed has led to some exciting discoveries that may now illuminate the pathogenesis of this complex malformation. Other animal models with cerebellar malformations are being screened to identify the gene responsible for cerebellar defects [82]. A recent transgenic mouse model suggests that misexpression of a homeodomain transcription factor called Engrailed-1 (En-1) leads to a posterior fossa malformation reminiscent of DWM [109]. The animals have hydrocephalus involving all ventricles associated with a cyst in the posterior cerebellar vermis contiguous with the fourth ventricle. Interestingly, the timing of development of hydrocephalus in the mouse resembles the course in humans, presenting in the postnatal period. Hemo-

siderin deposits in the walls of the posterior fossa cyst suggest that, as in humans, hemorrhage may contribute to the hydrocephalus observed postnatally.

This transgenic mouse model involves expression of En-1 driven under the control of an enhancer element of a gene from the Wingless developmental signaling pathway, Wnt-1. Both Wnt-1 and En-1 have been shown to be essential genes for cerebellar development, as knockouts for these genes in mice lead to absence of cerebellar development. The expression of Wnt and Engrailed genes in the developing human fetal brain is similar to that in the mouse. Normally, Wnt-1 is expressed in a slightly more rostral position at the midbrain-hindbrain junction than En-1. Increased gene dosage of En-1 therefore in a more rostral position during development may cause abnormal cell fate determination, abnormal cell migration, or inappropriate cell death, thereby altering cerebellar development, leading to a Dandy-Walker-like malformation. Obviously there is a great leap between finding out that misexpression of a particular gene leads to a Dandy-Walker-like malformation and understanding how that perturbation in function explains the abnormal brain morphology. It is clear, however, that such models promise to bring forth understanding far beyond the stalemate that exists.

Holoprosencephaly

Holoprosencephaly (HPE) is characterized by incomplete cleavage of the forebrain into two hemispheres, including telencephalon, diencephalon, and olfactory and optic bulbs and tracts [84, 104, 128]. In its most severe form, there is a single ventricle and single forebrain, without an interhemispheric fissure. Brain malformations are associated with facial malformations including nasal proboscis, single nostril, and cleft lip. Hydrocephalus is often associated. HPE is a spectrum of brain abnormalities but is classified as alobar (most severe, no midline separation), semilobar (partial interhemispheric fissure), or lobar (interhemispheric fissure except for ventral frontal lobes) [47]. There is a wide spectrum of clinical manifestations of HPE, varying from cyclopia with single hemisphere in alobar HPE to subtle facial anomalies such as absence of a superior labial frenulum in lobar HPE [4, 77]. All HPE patients are not expected to have a sense of smell [4]. Mortality and developmental delay correlate with the severity of CNS anomalies. Patients with cyclopia die shortly after birth, and most other patients with alobar HPE do not live beyond 6 months [4]. The incidence of HPE is about 1:16000 births, although the incidence in conceptuses is 1:250, suggesting high lethality in utero. Most cases of HPE are sporadic and there is variable severity even within families, suggesting incomplete penetrance of disease genes.

HPE is caused by disruption of development of the ventral forebrain and midline facial structures [84]. It has been known for nearly 100 years that experimental removal of the prechordal mesoderm, specialized mesodermal tissue that lies in the midline of the developing embryo ventral to the developing forebrain, can cause HPE. These experiments suggested that the prechordal mesoderm supplies a factor for induction of proper ventral forebrain development. The etiology of this disorder is heterogeneous, with environmental and genetic factors playing a role in its pathogenesis. Numerous teratogens have been implicated, including maternal diabetes, vitamin A, alcohol, low cholesterol and solvents. About 25% of HPE cases are due to a single gene mutation. Chromosomal abnormalities such as trisomy 13 and 18 are associated with HPE [105]. HPE is more commonly seen as a sporadic disease than a familial one. There are five genetic loci known so far to be associated with HPE, but there may be as many as a dozen [105]. The HPE1 locus is unknown. The genes at the other HPE loci include sonic hedgehog (Shh) (HPE3 locus, chromosome 7q36), Zic2 (HPE5, chromosome 13q32), Six3 (HPE2, chromosome 2p21), and TGIF (HPE4, chromosome 18p11). These genes associated with HPE represent the minority of familial and sporadic cases. There is a much longer list of genes associated with HPE in model organisms such as mice or zebrafish, but mutations in human homologues have not yet been found [105]. Shh is the best characterized of these genes and has emerged as a critically important gene for pattern formation of tissues in the developing embryo. HPE also occurs in the context of other disorders such as Smith-Lemli-Opitz syndrome, Pallister-Hall syndrome, and Rubenstein-Taybi syndrome. In these three disorders a genetic mutation is thought to perturb a component of the Shh signaling pathway.

The Shh gene was initially defined in *Drosophila* (Hedgehog, Hh). It is a secreted protein that undergoes post-translational cleavage and lipid modification by cholesterol and palmitate. These latter lipid modifications are important for determining its extracellular diffusion and tissue targeting. Shh acts on adjacent cells and exerts its effects through alteration of gene expression. A discussion of the signaling effects of Shh is beyond the scope of this chapter (see [48, 126]). Shh is required for proper dorsal-ventral patterning of the entire CNS. It is secreted by midline axial mesoderm, either prechordal mesoderm in the head for development of the telencephalon or by the notochord or specialized ventral cells in the neural tube for the brainstem and spinal cord. Elimination of Shh in homozygous knockout mice produces holo-

prosencephaly [48, 126]. A large number of different mutations in the Shh gene have been discovered in humans [105]. Loss of a single copy of the gene in humans is sufficient to cause HPE in humans [103, 105]. In an HPE family, individuals with the same mutation may be affected very differently.

Neural Tube Defects

Neural tube defects (NTDs) are amongst the most common human congenital malformations. The incidence is about 1:1000 live births, with geographical and racial variations, being highest in Ireland, the United Kingdom, and Mexico [29]. The birth incidence in NTDs is falling in many developing countries. NTDs are thought to be due to failure of elevation of the neural folds with subsequent failure of fold fusion, leading to anencephaly, myelomeningocele, and craniorachischisis. These three forms of NTDs are respectively caused by failure of anterior neuropore closure, failure of posterior neuropore closure, or complete failure of neural fold fusion, resulting in an open nervous system [53]. Encephalocele is thought to be a postneurulation defect arising from a protrusion of brain and meninges through the skull due to an abnormal opening in the skull or a failure of separation of cutaneous and neural ectoderm [53]. Spina bifida occulta is also a secondary failure of mesenchymal tissues to completely cover a closed neural tube [29].

The etiology of NTDs has long been thought to be heterogeneous, due to gene mutations, chromosomal abnormalities, and environmental factors such as teratogens and dietary deficiencies. Genetic etiology is suggested by a familial incidence of about 3% and risk to subsequent offspring of parents with an affected child. Genetic susceptibility to environmental factors may be very important. An understanding of the etiology is also confused somewhat by terminology and the heterogeneous phenotypes. Consider the following that can make discussion of NTDs confusing: open versus closed neural tube defects, anencephaly versus myelomeningocele versus craniorachischisis, isolated NTD versus syndromic NTD, defects of primary neurulation versus secondary neurulation, neurulation defect versus postneurulation defect. This review will focus on myelomeningocele, anencephaly, and craniorachischisis, with particular emphasis on myelomeningocele as it is the typical entity seen by clinical specialists. There are likely common and unique mechanisms genetically involved in this phenotypic spectrum of NTDs. With recent tremendous progress made in understanding the molecular genetic mechanisms of the dorsal-ventral and anterior-posterior (rostral-caudal) patterning of the nervous system during development, our knowledge of the pathogenesis of NTDs will rapidly increase. The human genome sequence with cataloguing of individual DNA sequence variations will hopefully lead to identification of the constellation of multiple genes that are involved together with environmental factors to produce these complex defects.

Etiologies of NTDs have also been elusive because of the fact that neurulation is an extremely complex process controlled by coordinated expression of many different genes throughout the developing nervous system. Errors in many different genes are likely to cause similar disease phenotypes. However, NTDs also consist of several phenotypes, with different sets of genes involved in different processes leading to different phenotypes. It is likely that a significant number of genes involved in the process of neurulation are involved in the same biochemical or molecular pathway, and therefore dysfunction of one of a large number of different genes in a single pathway may be sufficient to produce an NTD. Also, for many important biological processes there exist redundant pathways enabling the organism to compensate for error; therefore, the effects of a single gene defect may not be readily apparent and may depend on interaction with other modifier genes or on environmental factors. This means that the gene defect will not be manifest unless these other contributing genetic or environmental conditions are also present.

A major breakthrough in recent years in understanding the pathogenesis of NTDs has been the discovery that periconceptual folic acid supplementation can decrease the risk of having a child with a NTD. Another important breakthrough has been the creation of multiple mouse models with NTDs, and new molecular insights gained into mice that spontaneously get NTDs. In a transgenic mouse, an exogenous gene is introduced into a fertilized egg, causing widespread or tissue-specific expression of the gene in the developing mouse. In a knockout mouse, a gene of interest is targeted for elimination in cultured mouse embryonic stem cells. These manipulated embryonic stem cells are subsequently implanted into a recipient pseudopregnant mouse for embryonic development. These founder mice are then bred so progeny are either heterozygous for the targeted allele or homozygous for the targeted allele in every cell of the organism. These models have resulted in NTDs occurring as a result of manipulation of some rather unexpected genes.

Current mouse models now number more than 50 different natural or genetically engineered mice with NTDs (see Table 3) [53, 67]. It is rather surpris-

Table 3. Partial list of mouse models of neural tube defects (see [29, 53, 67])

Name	Genetics	Features of NTD	Response to nutritional supplementation
Splotch mouse (spontaneous mouse mutant)	Pax3 homeodomain transcription factor 32 base pair deletion results in protein truncation Normally expressed in dorsal half of developing neural tube	Waardenburg syndrome in humans is caused by Pax3 mutations in one allele Cranial and caudal NTDs	Folic acid or thymidine administered to pregnant mice reduces the frequency of NTDs in splotch homozygote offspring
Loop tail (spontaneous)	Gene unknown, mechanism unknown	Cranioraschischisis	Unknown
Cart1 knockout	Cart1, homeodomain transcription factor	Cranial NTD	Folic acid
p53 knockout	P53, transcription factor, cell cycle and apoptosis regulator	Cranial NTD	Unknown
Crooked tail (spontaneous)	Unknown gene	Cranial NTD	Folic acid
Open brain (spontaneous)	Unknown gene	Cranial NTD	Unknown
Curly tail (spontaneous)	Unknown gene deficiency in retinoic acid signaling from hindgut	Mainly caudal NTDs	Inositol (water soluble vitamin)
Axd, axial defects mouse (spontaneous)	Unknown gene	Caudal NTDs	Methionine

NTD, neural tube defect

ing how several of these animal models clinically parallel the human disease; for example, several mouse models for NTDs ("splotch," "crooked," "*Cart1*") have a significant reduction in the risk of the NTD following folic acid administration [67]. The genes involved in mouse NTDs also display a surprising diversity of function (transcription factors, signaling molecules, enzymes, and cell surface receptors), suggesting that a great variety of mechanisms can produce an NTD [67]. The hypothesized mechanisms by which a gene defect causes an NTD include: (1) abnormal ventral bending of the embryo, leading to disturbed neural fold fusion dorsally; (2) lack of supporting mesenchyme, causing failure of fold elevation; (3) defective basal lamina of surface ectoderm, leading to lack of support in forcing adjacent neural fold to elevate; (4) abnormally broad notochord and floor plate; (5) excessive neuroepithelial cell death; and (6) delayed/failed elevation of neural folds [53].

NTDs can occur in isolation or in the context of complex congenital malformations. A minority of myelomeningoceles has an identified chromosomal abnormality by karyotyping by traditional G-banding techniques. A myelomeningocele occurring in isolation, with a Chiari II malformation and hydrocephalus only, is associated with a 2.6% chance of a variety of different chromosomal abnormalities by karyotyping [70]. A 38% chance of a chromosomal abnormality exists in the context of myelomeningocele associated with other prenatal ultrasound-identified abnormalities [70]. Occasionally a chromosomal abnormality is also detected in a parent, suggesting a significant increased risk for subsequent pregnancies. Anencephaly is more common in females, but myelomeningocele occurs equally in males and females.

Only rarely are human myelomeningoceles seen associated with a single gene defect. Waardenburg syndrome type I is an autosomal dominant disorder clinically characterized by a wide nasal bridge, skin pigmentation abnormalities, and deafness [25, 83]. Patients with this syndrome occasionally get NTDs [56, 83]. Waardenburg syndrome is caused by mutation in the Pax3 gene [58, 120]. Pax3 mutations may occasionally also be associated with familial NTDs. Pax3 is a one of a family of homeodomain transcription factors that plays a crucial role in development of the nervous system. In the early developing neural tube Pax3 expression defines the dorsal half of the spinal cord. Genetic studies in mouse models reveal that Pax3 is critically important for proper spinal cord development. The splotch mouse is naturally mutant for Pax3, and these animals have NTDs predominantly affecting the lumbosacral region but also anencephaly (in 50%) [34, 83]. Mice that are homozygous for the Pax3 mutation (loss of both Pax3 alleles) die during embryogenesis. Restoration of Pax3 expression to the dorsal neural tube in splotch mice using transgenic techniques rescues homozygotes from NTDs, underlying its importance in the proper formation of the neural tube [76]. Interestingly, administration of folate to mice pregnant with splotch homozygotes substantially reduces the incidence of NTDs [37]. As the splotch mouse model illustrates, animal models can be surprisingly similar to the human condition. Other animal models of NTDs are also responsive to nutritional supplementation [67].

NTDs can be prevented by folic acid [2, 19, 23, 24, 96]. The mechanism is not clear, as maternal serum and red cell folate levels are normal or only mildly deficient [19]. Fetal genetic defects in enzymes involved in folate metabolism and maternal placental folate transport defects are now being intensively studied. One enzyme receiving a significant amount of attention is methylenetetrahydrofolate reductase (MTFHR) [3, 19]. There is a thermolabile variant of this enzyme that has been associated with some NTD patients and their family members, but definitive association with NTDs has not been established.

Encephalocele

Encephaloceles are thought to be caused by a postneurulation defect, either from a mesenchymal abnormality involving the skull or a problem with the normal separation of the neural ectoderm from the cutaneous ectoderm [46, 59, 139]. Encephaloceles of all types have an incidence of 0.1-0.5 per 1000 live births. Posterior encephaloceles are more common in Western countries and anterior encephaloceles are more commonly seen in Asia, particularly Thailand [93]. The etiology of encephaloceles is probably multifactorial, with interaction between genetic and environmental factors playing a role in their pathogenesis [93]. Almost nothing is known about which gene or genes may be involved in this malformation. Encephaloceles occur in the context of systemic or brain malformation syndromes of unknown etiologies such as Meckel-Gruber syndrome, Walker-Warburg syndrome, Dandy-Walker syndrome, and others [63]. Chromosomal aberrations are also associated with encephaloceles [135].

Achondroplasia

Achondroplasia is the most common cause of short-limbed dwarfism and is an autosomal dominant disorder (see Table 4). The incidence is about 1 in 15 000 births [63]. Most cases are sporadic and are caused by new mutations. Penetrance is complete. Achondroplasia is associated with increased morbidity and mortality in all ages. The disease is characterized by short stature with a long narrow trunk and short extremities, especially proximal segments (rhizomelia) [60]. Macrocephaly with frontal bossing is common, and there is hypoplasia of the midface. Specific head circumference charts for achondroplastic children have been developed and can be found in Smith's textbook [63]. Macrocephaly is caused by megaencephaly or is due to ventriculomegaly from venous stenosis of the jugular foramina [94, 112, 119]. Ventriculomegaly without hydrocephalus is common. Neurologically, there is diffuse hypotonia and delayed development of motor milestones but generally normal intelligence. There is risk of sudden death within the 1st year of life due to stenosis at the craniocervical junction, but prophylactic treatment of patients without clinical symptoms and signs of craniocervical compression is controversial. Standard measurements of the size of the foramen magnum have been published [55] and together with MR imaging and neurophysiological studies may identify those asymptomatic individuals at risk of neurological deterioration from cervicomedullary stenosis [69]. Spinal stenosis with neurological deterioration in leg function is also relatively common in adulthood. The pathological substrate for the disease is believed to be an impaired rate of endochondral ossification, causing reduced bone elongation. Longitudinal growth of the skeleton occurs because of endochondral ossification at the ends of long bones. The growth plate of patients with achondroplasia demonstrates severe disorganization.

Table 4. Features of achondroplasia

	Clinical genetics	Key clinical features	Gene	Gene mutations in disease
Achondroplasia	1:15000 births Many sporadic cases due to new mutations Chromosome 4p16	Short stature Short proximal limbs Macrocephaly Ventriculomegaly without hydrocephalus Midface hypoplasia Craniocervical junction stenosis Spinal stenosis	FGFR3 fibroblast growth factor receptor type 3 Receptor Tyrosine kinase	Mutation at nucleotide 1138 of the cDNA causes characteristic glycine to arginine amino acid substitution (G380R) in the transmembrane part of the receptor Mutation causes inappropriate activation of the FGFR3 Genetic diagnosis is straightforward because of stereotypical mutation, G380→R

The genetic locus of this disease was initially localized to chromosome 4p16 by linkage analysis study of affected families. In 1994, a mutation in the fibroblast growth factor receptor 3 (FGFR3) was found to be the genetic cause of the disease [108, 117]. In almost every case of achondroplasia a mutation results in a glycine-to-arginine amino acid change in the transmembrane portion of the receptor protein (G380→R). Prenatal genetic diagnosis is possible and more straightforward because of the stereotypical genetic abnormality in the context of ultrasound findings [81]. Reverse transcriptase PCR of small cDNA fragments derived from RNA followed by sequencing will determine the mutation. Homozygotes for the mutation are extremely severely affected and die shortly after birth, and heterozygotes survive with characteristic skeletal abnormalities [27]. Thanatophoric dysplasia is another form of dwarfism with neonatal lethality that also has a mutation in the extracellular or intracellular portion of FGFR3. Hypochondroplasia is a less severe form of chondrodysplasia without craniofacial abnormalities that also has a mutation in FGFR3.

The FGFR3 belongs to a family of protein receptor tyrosine kinases (FGFR1-4 in humans) that have high affinity binding to fibroblast growth factors (FGF1-10) [27]. Binding of an FGF ligand by an FGFR in conjunction with a cell-surface heparin sulfate proteoglycan results in clustering of the receptors and autoactivation of the receptor by phosphorylation. The phosphorylation occurs on the intracellular portion of the receptor and serves to active the kinase activity of the receptor, leading to further phosphorylation and activation of downstream signaling molecules. Phosphorylation of the receptor also creates docking sites for other signaling molecules, allowing recruitment of a variety of intermediates to the site of an activated receptor. Activation of the receptor causes pleotropic effects on the cell. FGFs and FGFRs play a role in cell proliferation, cell differentiation, cell survival, and cell motility. The effect of FGFR activation depends on the cell type and the developmental stage of that cell. FGFR3 has high expression in the nervous system and in prebone cartilage. The characteristic mutation in FGFR3 that occurs in achondroplasia results in constitutive activation of receptor signaling, even in the absence of appropriate FGF ligand [87, 130]. The mutation is thought to cause inappropriate activation of the receptor by stabilization of clustering of receptors.

Mouse models for achondroplasia suggest that FGFR3 normally acts as a negative regulator of chondrocyte proliferation. Mice completely devoid of FGFR3 expression (knockout mice) demonstrate enhanced endochondral bone growth, with an enlarged growth plate and increased chondrocyte proliferation [18, 28]. Bones of these mice are longer and thicker than normal. The achondroplasia mutation (G380→R) actually results in "gain of function" for the FGFR3 gene, leading to more potent inhibition of chondrocyte proliferation. Mice genetically engineered to have the human achondroplasia mutation have dwarfism and craniofacial abnormalities analogous to the human condition [129].

Neurofibromatosis

The neurofibromatoses consist of a group of autosomal dominant inherited disorders characterized by abnormalities of the neural crest, particularly tumor formation (see Table 5) [78, 99]. The two classical forms, neurofibromatosis I (NF1; formerly von Recklinghausen neurofibromatosis) and neurofibromatosis II (bilateral acoustic neuromas) will be the focus of discussion in this segment. Other so-called NF variants, with some clinical characteristics similar to NF1 but also with other manifestations, have been described but are not clearly distinct genetic entities (so-called neurofibromatosis types 3-6; including segmental neurofibromatosis, familial café au lait spots, familial spinal neurofibromatosis) [9, 110].

The genes whose mutations are responsible for NF1 and NF2 were discovered 10 years ago. These discoveries have led to remarkable advances in the understanding of these diseases, especially with respect to an understanding of mechanisms of tumor formation. However, despite these genetic advances, the diagnosis of NF1 and NF2 remains a clinical one. Relatively little is still known about the potential normal role of these genes in the normal development of the central and peripheral nervous systems.

Neurofibromatosis 1

NF1 is one of the most common autosomal dominant inherited diseases, with an estimated incidence of 1 in 4000 individuals [9, 42, 110]. It occurs in all races and in all countries. The disease has 100% penetrance but there is a remarkable variable degree of expressivity. Affected individuals within a single family, where all the affected persons carry the same genetic mutation, can have extremely variable clinical manifestations, with some individuals being mildly affected and others severely affected. About 50% of NF1 patients do not have a family history of the disease, and these cases are therefore caused by new mutations that occur in the developing gametes of the father (usually) or the mother. Reasons for the high new mutation rates in the NF1 gene are unknown.

The disease characteristically involves neural crest tissues largely, but not exclusively. Most manifestations of the disease increase in frequency with age, so that it may be relatively difficult to make a certain clinical diagnosis in infancy or early childhood, but the disease is usually apparent in affected individuals by age 8 and in 100% by age 20 [50]. There are well-established clinical criteria developed by the United States' National Institute of Health (NIH) for making the diagnosis of NF1 [85, 90]. These criteria are most

Table 5. Neurofibromatosis and tuberous sclerosis

Disease	Clinical genetics	Gene, protein	Gene function	Gene mutations in disease
Neurofibromatosis type 1	1:4000 births autosomal dominant variable severity in families 50% of cases new mutations	*NF1*, Neurofibromin Chromosome 17q.11	Ras-GAP downregulates the function of the proto-oncogene Ras	Huge variety of different mutations (no hotspots), poor genotype-phenotype correlation
Neurofibromatosis type 2	1:40000 births autosomal dominant 50% new mutations	*NF2*, Schwannomin (merlin) Chromosome 22q12	ERM protein, acts as a molecular bridge between plasma membrane and cell cytoskeleton	Protein truncation mutations more common and are more easily screened, truncation mutations associated with more severe disease
Tuberous sclerosis	1:10000 births autosomal dominant new mutations in 2/3 of patients (mostly *TSC2* gene related)	*TSC1*, Hamartin Chromosome 9q34 50% of tuberous sclerosis	Bind to ERM proteins and is therefore involved in plasma membrane and cell cytoskeletal function	Protein truncation mutations mainly
	TSC2 patients may have more severe intellectual disability Most sporadic cases have *TSC2* mutations	*TSC2*, Tuberin Chromosome 16p13 50% of tuberous sclerosis	Rap-GAP, Rab-GAP downregulates the function of these other Ras-like small molecules, analogous to *NF1* function	Wide variety of mutations

reliable in adults [26]. Almost all NF1 patients have two or more cardinal clinical features at age 8, but under age 1, 30% have only one of the features. The usual order of appearance of clinical features is café au lait macules (present at birth or developing by 2 years), axillary or inguinal (skin fold) freckling (starts at age 2-3, in 80% by age 6), Lisch nodules, and dermal neurofibromas (usually by preadolescence) [50]. Patients with NF1 can have learning difficulties but are usually without mental retardation [50, 110].

Tumors of the central and peripheral nervous systems are characteristic. Optic nerve gliomas are usually diagnosed within the first 5 years of life and affect up to 20% of patients [110]. These lesions can be asymptomatic or present with diminished vision; characteristically, clinical progression is uncommon even if visual symptoms are present. Gliomas occur in other locations, particularly involving the brainstem, and most are low-grade. Malignant astrocytomas can occur [6]. Macrocephaly is also common (independent of hydrocephalus), and aqueductal stenosis causing hydrocephalus has been reported, possibly from proliferation of subependymal glial cells with aqueductal obstruction [57, 110, 116]. Dermal neurofibromas (very common) and plexiform neurofibromas (in about 25% of patients) also occur, and the latter but not the former can become malignant (in about 10%). NF1 patients can also get neoplasms outside the nervous system, such as myeloid leukemia, pheochromocytoma, and rhabdomyosarcoma. The lifelong risk of a malignant neoplasm has been estimated at 5% [50].

NF1 patients are also at increased risk of cerebrovascular disease including moyamoya disease and cerebral arterial dysplasia with aneurysms [72, 110, 114, 118]. Hypertension also occurs due to pheochromocytomas and renal artery abnormalities [50, 110].

Modification of NIH criteria has been suggested to improve the ability to make the diagnosis in children; such modifications might include short stature, macrocephaly, or unidentified bright objects (UBOs). UBOs are present in 50%-75% of children with NF1 and can be seen as lesions without enhancement on T2-weighted MRI scans of the brain [26]. The presence of UBOs has been associated with learning difficulties. These lesions are most commonly seen in the basal ganglia, thalamus, cerebellum, and brainstem, and seem to be more frequently observed in children, suggesting that they may tend to disappear [110]. An infant with multiple café au lait spots most likely has NF1, but the NIH criteria for the diagnosis will not be established until the child is older [50].

The clinical disease of NF1 is now known to be caused by mutations in one copy of the *NF1* gene (meaning patients are heterozygotes) that resides on chromosome 17q [9]. The *NF1* gene was originally cloned in 1990 (Cawthon 1990, Wallace 1990). Every somatic cell acquires the mutation, but it is not clear why the disease is manifest predominantly in neural crest tissues. The gene is normally expressed in all tissues but has highest expression in the adult central and peripheral nervous system. Some of the clinical NF variants (such as segmental neurofibromatosis) may be caused by somatic mosaicism of the *NF1* mutation, meaning that only a portion of the individual's cells were heterozygous for the *NF1* mutation [110].

The *NF1* gene is a very large gene spanning 300 kb of genomic DNA with 60 exons and a large protein with 2818 amino acids. The large size of this gene has made it technically laborious and difficult to routinely determine mutations in individuals, especially in the case of those without a family history. About 5% of affected individuals have complete deletion of one allele, and these patients are thought to have more severe intellectual impairment, facial dysmorphism, and greater numbers of plexiform and dermal neurofibromas [110]. Aside from this group, there is poor correlation between genotype and disease phenotype.

Screening for gene mutations is difficult due to the large size of the gene, high rate of spontaneous mutations, and lack of clustering (or hotspots) of mutations in segments of the gene. The variability in alterations in the gene necessitates multiple different screening strategies that are costly. No single test is currently available to determine the mutation. Also, identification of a mutation may tell an individual if he/she is affected but will not be able to predict severity. In families, screening for alterations in the *NF1* gene involves analysis of linkage of DNA markers associated with the disease trait. Screening for sporadic *NF1* gene alterations can be done using fluorescence in situ hybridization (FISH) with intragene probes. Lack of a signal using the probe implies deletion of that segment of the gene. PCR techniques, which amplify fragments of DNA, can also be used to screen for gene loss or truncation by using two complementary probes (primers) chosen to flank all or part of the gene. Detection of point mutations use PCR and a technique called SSCP (single-strand conformational polymorphism analysis) that detects alterations in migration of fragments of the gene based on single base pair alterations (these regions of DNA with aberrant migration in gel electrophoresis are then confirmed by direct DNA sequence analysis). These techniques will probably improve and become more efficient in the future.

The protein product of the *NF1* gene, neurofibromin, is a guanosine triphosphatase (GTPase)-*a*ctivating *p*rotein, known as a GAP [49]. Neurofibromin is thought to restrain the activity of a molecule called *ras*, a potent proto-oncogene associated with the cell membrane that is activated by growth factor and other cell signaling events. Neurofibromin enhances the natural GTPase function of *ras*, converting it from an activated form as-

sociated with GTP (*ras*-GTP) to an inactive form associated with GDP (*ras*-GDP). *NF1* therefore helps to regulate the activity of *ras* that acts as a molecular switch shuttling between active GTP-bound forms and inactive GDP-bound forms. *ras*-GTP activates intracellular signaling pathways leading to cell proliferation. Mutant (oncogenic) forms of *ras* are predominantly associated with GTP and have been found in many cancers, contributing to aberrant intracellular signaling and proliferation. Loss of neurofibromin is thought to lead to increased levels of *ras*-GTP (activated form), causing inappropriate activation of cell proliferation and tumor formation. One copy of *NF1* is sufficient to specify production of neurofibromin, but a second hit to the intact allele, leading to complete absence of neurofibromin expression, is thought be an important step leading to tumor formation. A distinct hit to another important gene in cells heterozygous for the *NF1* mutation can also contribute to tumor formation (see below).

These mechanisms have been identified through studies of knockout mice for the *NF1* gene [7, 12, 62, 127]. Mice homozygous for *NF1* deletions die early in embryogenesis, precluding an analysis of tumor formation but suggesting that the *NF1* gene plays a critical role in normal mouse development. Mice heterozygous for mutations in *NF1* do not exactly mimic the human disease as they do not get neurofibromas, but acquire instead other *NF1*-associated tumors, pheochromocytomas, and leukemias [62]. However, studies in mice performed with somatic inactivation of the second allele through creation of chimeric mice (so 20% of the cells in the mouse have a homozygous *NF1* deletion) have led to formation of neurofibromas and malignant peripheral nerve sheath tumors [12]. These studies demonstrate that a complete loss of the expression of neurofibromin is associated with the development of the most common *NF1*-associated tumor. Human peripheral nerve tumors from NF1 patients have also been identified to completely lack neurofibromin expression, suggesting inactivation of the second *NF1* allele [17, 113], but other events are also thought to contribute to neurofibroma formation [111]. Heterozygous inactivation of *NF1* with heterozygous inactivation of the *p53* tumor suppressor gene (which is a tumor suppressor gene involved in cell proliferation and cell death regulation) causes malignant peripheral nerve tumors in mice, suggesting alternative and cooperative mechanisms between two genes leading to neoplastic transformation [12].

Neurofibromatosis 2

Neurofibromatosis 2 is also inherited in an autosomal dominant fashion but is considerably less common than NF1, with an incidence of about 1 in 40 000 live births [78, 110]. Penetrance is nearly 100%. About half the cases show no family history and are due to new mutations. There is a maternal effect on disease severity, with earlier onset of disease in offspring of affected females. The clinical hallmark of this disease is deafness caused by bilateral vestibular schwannomas, but affected individuals are also prone to develop schwannomas of other cranial nerves, spinal dorsal roots, and peripheral nerves, as well as meningiomas and glial tumors, particularly ependymomas [50, 78, 110]. Other characteristic features are hairy cutaneous plaques (a skin tumor), café au lait spots (less frequent and fewer in number than in NF1 patients), and posterior lens opacities (juvenile cataracts). NF2 presents later than NF1, usually in late adolescence or in early adulthood. Only 10% of cases present before age 10 [50].

Patients with NF2 inherit a mutant copy in the *NF2* gene. This gene is located on chromosome 22 and was identified in 1993. The gene spans 110 kb of genomic DNA and has 17 exons, specifying a protein product of 595 amino acids [107, 122]. The protein product is called merlin or schwannomin. The name merlin came from recognition that the *NF2* gene product relates structurally to the moezin-, ezrin-, radixin-like protein family (called ERM proteins). These proteins interact with cell membrane glycoproteins at their amino termini and with the actin cytoskeleton at their carboxy termini, suggesting that these proteins mediate actin cytoskeletal organization in response to extracellular signals. ERM proteins are particularly localized to dynamic actin structures such as membrane ruffles [51]. There are some distinct differences in merlin structure compared to ERM proteins, suggesting unique functions. Very little is presently definitely known about merlin's function, but it is suspected to play an important role in cell motility, cell adhesion, cell proliferation, and membrane trafficking. Integration of extracellular adhesion to cell proliferation may be the most critical function. Mice heterozygous for an *NF2* mutation develop a surprising variety of cancers including osteosarcoma, lymphoma, fibrosarcoma, and lung adenocarcinoma as well as schwannomas [45, 79]. Most of these tumors have loss of the second *NF2* allele and are particularly metastatic [79]. In transgenic mice expressing *NF2* mutations in Schwann cells, Schwann cell tumors arise, suggesting that the mutant protein may act to interfere with retained wildtype allele function [44].

Although many of the same arguments apply with genetic testing for NF2 as NF1, mutational analysis of patients with NF2 suggests that most *NF2* mutations result in protein truncations with altered function [140]. This fact means that genetic screening is more feasible. Study of NF2 patients over the past 8 years in

light of the gene discovery suggests that there may be two different clinical subgroups with the disease, with particular genotype-phenotype correlation [110]. In some families the disease is relatively more severe, with symptoms appearing before age 25 with multiple tumors with progressive growth, and in others the disease is milder, presenting at older ages with a smaller number of more slowly growing tumors [110]. The more severely affected families have mutations in the *NF2* gene that result in protein truncations, and in more mildly affected families, missense mutations are more common, resulting possibly in a milder dysfunction of the protein. Screening is possible for protein truncation mutants, using PCR techniques to amplify fragments of the gene.

Tuberous Sclerosis

Tuberous sclerosis (TSC) is an autosomal dominant syndrome affecting multiple body systems including the brain, eye, skin, kidneys, and heart (see Table 5) [21, 22]. Less frequently, other organ systems such as the lungs, skeleton, and endocrine systems are involved. The incidence is 1:10 000 births. There is a significant incidence of new mutations so there may not be a family history. Some apparently unaffected parents of affected children could have somatic or gonadal mosaicism. The parents with somatic mosaicism may not demonstrate typical features of the disease as only a proportion of their cells have the mutation, and parents with gonadal mosaicism will not have any features, as the mutation is only present in germ cells. Future children would be at risk if mosaicism were present in a parent.

The characteristic lesions of TSC are hamartomas comprised of tissues that are derived from all three germ layers. In the CNS these lesions include cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas. These latter lesions can cause hydrocephalus by obstruction of the foramen of Monro. As with NF, exciting discoveries have been made in the understanding of TSC since the discovery of the two genes *TSC1* and *TSC2* [21, 22]. In particular, parallels between NF1 and TSC seem to be striking as the respective genes *NF1* and *TSC2* encode for proteins that regulate small *ras*-related molecules involved in intracellular signaling and both diseases are associated with benign nervous system tumors.

Clinical diagnosis of TSC can be made on the basis of clinical and radiologic criteria that have been divided into major and minor features [21, 100-102]. The disease has extremely variable clinical features although the penetrance of the disease within families is very high. A definitive diagnosis can be made with one major feature. Major features include facial angiofibromas (formerly adenoma sebaceum), ungual fibromas (seen in older children and adults), retinal hamartomas, more than three hypopigmented macules (ash leaf spots, often present early in life), shagreen patch, renal angiofibroma (usually multiple and bilateral), cardiac rhabdomyoma (usually congenital but often regressing), pulmonary lymphangiomatosis, cortical tubers, subependymal nodules, and giant cell astrocytomas. Minor features include dental pits, bone cysts, renal cysts, gingival fibromas, and hamartomatous rectal polyps. Common clinical problems with TSC include epilepsy, mental retardation, and behavioral problems. Guidelines for investigations of individuals suspected of having TSC can be found in a recent report [102].

The *TSC2* gene, located on chromosome 16p13, was identified in 1993 [61]. The gene contains 41 exons and spans 43 kb of genomic DNA. The gene product is a 1807-amino-acid protein called tuberin. The *TSC1* gene was discovered in 1997 and is found at chromosome 9q34 [125]. The *TSC1* gene product is called hamartin and has 1164 amino acids. Familial TSC has been linked to *TSC1* in 50% and *TSC2* in 50% of cases [64]. Up to two-thirds of TSC patients do not have a family history and therefore bear new mutations. Eighty percent of these sporadic cases have *TSC2* mutations. *TSC2* gene mutations comprise a wide variety of different DNA alterations, and combined with the large size of the gene make routine screening currently impractical. *TSC1* mutations are more uniform, resulting in protein truncation, making screening potentially easier for this gene. Mutations in each of the TSC genes result in variable and overlapping patterns of disease, although patients with *TSC2* mutations may have more severe intellectual disability than patients with *TSC1* mutations [64]. Diagnosis remains predominantly based on clinical and imaging features.

Both genes have been classified as tumor suppressor genes as loss of heterozygosity or mutations have been identified in the remaining wildtype allele in tumors from TSC patients. Sporadic tumors in non-TSC patients have been identified with *TSC2* gene alterations. An animal model, the "Eker rat," which has a natural *TSC2* mutation in one allele, develops tumors characteristic of TSC, including subependymal giant cell tumors, providing further supporting evidence of the role for this gene in tumor suppression [73]. Tumors in these mice lose the normal *TSC2* allele and lack tuberin expression. Eker homozygotes die in midgestation due to brain malformations, with papillary overgrowth of neuroepithelium, reminiscent of hamartomas. The Eker rat models the human disease well as there is also variable severity of disease between

different strains of rats, possibly as a result of influences of modifier genes which have differing impacts in the different strains on the loss of tuberin function.

Tuberin (TSC2) has GTPase-activating protein (GAP) function, similar to neurofibromin, and acts on distinct small molecule GTPases that share 50% amino acid identity with *ras*, called Rap1 and Rab5 [21, 131, 136]. These molecules play roles in cell proliferation and cell endocytosis. Loss of tuberin function through mutation leads to unregulated Rap1/Rab5 activity. It is presently unclear how this activity contributes to tumor formation. Rap1 has been shown to have a role in the promotion of DNA synthesis (and therefore progression through the cell division cycle) [21]. Rab5 plays a role in endocytosis, a process that has been shown to be critical in regulation of growth factor receptor signaling [21]. Loss of tuberin therefore could lead to inappropriate cell cycle progression and inappropriate trafficking or processing of growth factor receptors, leading to increased activity of these molecules and also to increased stimulation of cell cycle progression. Hamartin (TSC1) is a protein of unique structure and unclear function associated with the cell membrane and the cell cytoskeleton. It associates with members of the ERM family (see section on NF2, p. 12) [43, 75]. Interestingly, both TSC proteins may function in a single growth regulatory pathway, as shown by experiments which have found that these two proteins bind together, possibly enabling one protein to regulate the function of the other [89].

Conclusion

A genetic understanding of hydrocephalus and diseases associated with hydrocephalus has made remarkable progress in the past decade. With the human genome now sequenced, we stand to accelerate our knowledge of human CNS diseases even more rapidly. A basic understanding of the genetics of hydrocephalus is essential for the neurosurgeon to care for and to counsel his or her patients and their families. With improved prenatal diagnosis, neurosurgeons are being called upon with greater frequency to provide prenatal counseling for expectant parents and it will be their obligation to keep up with an understanding of the molecular genetics of CNS malformations.

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