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Case report

Compound heterozygosity in *GPR56* with bilateral frontoparietal polymicrogyria

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Abstract

Polymicrogyria is caused by a diverse etiology, one of which is gene mutation. At present, only one gene (*GPR56*) is known to cause polymicrogyria, which leads to a distinctive phenotype termed bilateral frontoparietal polymicrogyria (BFPP). BFPP is an autosomal recessive inherited human brain malformation with abnormal cortical lamination. Here, we identified compound heterozygous *GPR56* mutations in a patient with BFPP. The proband was a Japanese female born from non-consanguineous parents. She presented with mental retardation, developmental motor delay, epilepsy exhibiting the feature of Lennox–Gastaut syndrome, exotropia, bilateral polymicrogyria with a relatively spared perisylvian region, bilateral patchy-white-matter MRI signal changes, and hypoplastic pontine basis. *GPR56* sequence analysis revealed a c.107G>A substitution leading to a p.S36N, and a c.113G>A leading to a p.R38Q. Although affected individuals with compound heterozygosity in *GPR56* have not been previously described, we presume that compound heterozygosity of these two mutations in a ligand binding domain within the extracellular N-terminus of protein could result in BFPP. In addition, we observed unusually less involvement of perisylvian cortex for polymicrogyria, and Lennox–Gastaut syndrome for epilepsy, which are likely common features in patients with BFPP caused by *GPR56* mutations.

Keywords: Polymicrogyria; Lennox–Gastaut; GPR56; Heterozygous mutation

1. Introduction

The autosomal recessive bilateral frontoparietal polymicrogyria (BFPP) is a well-characterized neuronal migration defect that shows bilateral polymicrogyria with an anterior to posterior gradient, bilateral patchy-white-matter MRI signal changes (without specific patterns), and brainstem or cerebellar hypoplasia. Patients with BFPP present with mental retardation, developmental motor delay, seizures, ataxia, and dysconjugate gaze [1]. The causative gene for BFPP is the G protein-coupled receptor 56 gene (*GPR56*) [2]. GPR56 is one of the adhesion G protein-coupled receptors (GPCRs). Like other members of the adhesion GPCRs, GPR56 has an unusually long N-terminal extracellular domain that contains a high percentage of serine and threonine residues and a GPCR proteolytic site domain just before the first transmembrane spanning domain [3,4]. The serine and threonine-rich region can serve as an O- and/or N-glycosylation site [3,4].

So far, multiple independent mutations have been identified in *GPR56*, all of which are homozygous germline mutations. To date, 25 mutations in *GPR56*, including nine N-terminal extracellular domain mutations, are

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reported in humans [1,2,5–8]. Here we report a BFPP patient carrying compound heterozygous mutations with a novel *GPR56* mutation, p.S36Q, and a previously reported mutation, p.R38Q. Additionally, we review previous reports and discuss the radiological and clinical features of BFPP patients.

2. Case report

The proband was a Japanese female born from non-consanguineous parents. She had normal prenatal and perinatal histories and normal head growth, but showed developmental delay within the first year of life. Complex partial seizures occurred at 2 months; carbamazepine was effective for the seizures. Since the age of two years, epileptic spasms in clusters have appeared and have been refractory to medications. Around the age of four years, she developed tonic seizures causing drop attacks, in addition to epileptic spasms.

The seizures persisted despite treatment with multiple antiepileptic drugs. Interictal electroencephalogram (EEG) showed generalized bursts of sharp waves and slow spike-wave discharges with anterior predominance (Fig. 1). Video-EEG monitoring (Grass Technology, West Warwick, RI, USA) at 6 years old revealed that she suffered from frequent atypical absence seizures and tonic seizures. She was diagnosed with Lennox–Gastaut syndrome by specific EEG features and seizures. At this age, she had moderate to severe developmental delay (developmental quotient = DQ: 33). She was unable to walk without help, and her speech was limited to a few isolated words. Neurologic examination revealed mild spasticity, hyperactive deep-tendon reflexes, poor coordination, and exotropia. She showed neither dysmorphic features nor other congenital anomalies.

Brain MRI at 6 years of age revealed bilateral polymicrogyria with an anterior to posterior gradient, in contrast to the relatively spared perisylvian regions, patchy signal change in the bilateral white matter, hypoplastic pons, and multiple small cysts in the corpus callosum (Fig. 2).

2.1. Mutation analysis

DNA was extracted from peripheral blood leukocytes obtained from the patient and her parents using standard methods, and after obtaining informed consent from the parents. We performed a mutation screening of for all coding exons and flanking introns of *GPR56* using the high-resolution melt analysis (HRM) or capillary sequencing. PCR samples showing an aberrant melting curve pattern were sequenced. PCR primers and conditions are available on request.

Mutation analysis revealed a compound heterozygous mutation in exon 2 of*GPR56,* (c.107G>A and c.113G>A), (which presumably leads to amino acid changes), (p.S36N and p.R38Q, respectively) in the extracellular N-terminus of the protein. Her father was heterozygous for the p.S36N mutation, and her mother carried the p.R38Q mutation, which indicates an autosomal recessive inheritance (Fig. 3). Both changes were not found in one individual among 80 Japanese controls.

3. Discussion

BFPP is an autosomal recessive polymicrogyria syndrome, which was frequently underdiagnosed before genetic testing and high-resolution neuroimaging were available.

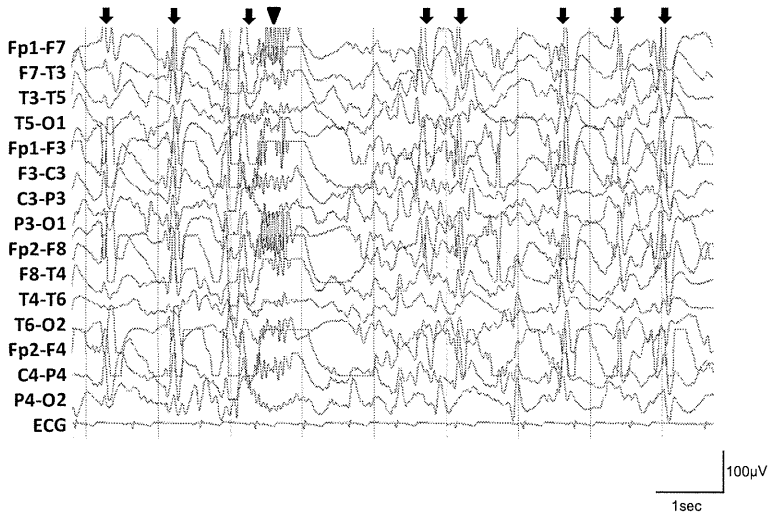

Fig. 1. Electroencephalography (EEG) finding. Generalized bursts of sharp waves (arrow head) and slow spike–wave discharges (arrows) with anterior predominance were seen during sleep.

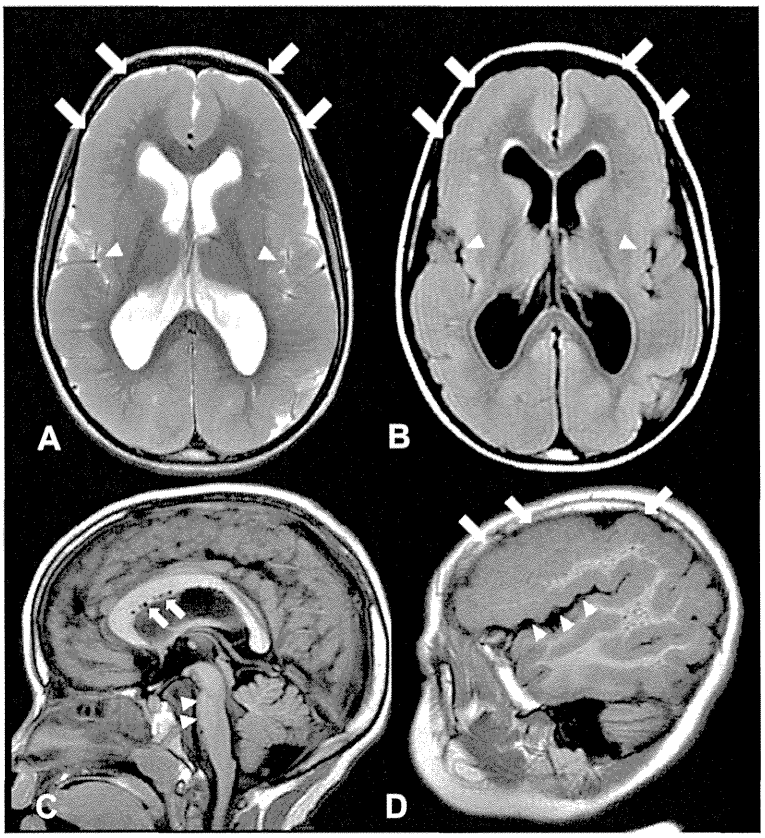

Fig. 2. Neuroimaging. Axial T2WI (A) and FLAIR (B) images demonstrate bilaterally symmetric thick cortex and irregular gyri compatible to polymicrogyria with anterior to posterior gradient (arrows) and, patchy high signals on both T2WI and FLAIR in the frontal subcortical white matter. Note the less involved insular cortex (arrow heads). Sagittal T1WI (C) shows flat pontine basis (arrow heads) and small cystic lesions in the corpus callosum (arrows). Sagittal T1WI (D) shows bilateral polymicrogyria with anterior to posterior gradient (arrows), in contrast with relatively spared perisylvian region (arrow heads).

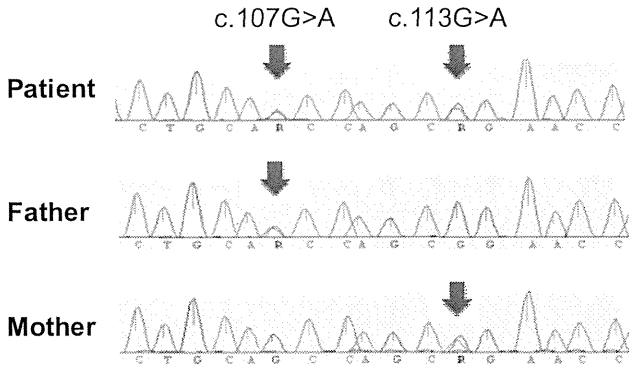

Fig. 3. Mutation analysis. Sequence chromatograms showing segregation of the compound heterozygous c.107G>A and c. 113G>A mutations in *GPR56* in the patient and her parents.

Piao, et al. [2] reported that the clinical phenotype of BFPP patients harboring *GPR56* mutations show considerable clinical homogeneity. This included five common clinical features and three typical MRI findings: (1) mental retardation of moderate to severe degree; (2) motor development delay; (3) seizures, most commonly symptomatic generalized epilepsy; (4) cerebellar signs, consisting of ataxia; (5) dysconjugate gaze, presenting variably as esotropia, nystagmus, exotropia, or strabismus; (6) bilateral polymicrogyria with anterior to posterior gradient; (7) bilateral patchy-white-matter signal changes without specific pattern; and (8) brain stem and cerebellar hypoplasia [1]. Thus, according to this description, our patient demonstrates the cardinal features of BFPP. Polymicrogyria typically has a predilection for the perisylvian cortex [9]. Our patient, however, shows less involvement of the perisylvian cortex, compared to the other regions where lesions were observed. Frontoparietal distribution of polymicrogyria is the most common feature of BFPP, but some patients show extensive distribution throughout the entire brain, as was observed with our patient [5,10]. Although other previous reports do not describe perisylvian cortex findings in detail, less involvement of perisylvian cortex might be a feature of BFPP caused by a GPR56 mutation.

Epilepsy is a common clinical problem in patients with BFPP caused by *GPR56* mutations [1,5–8,10]. Similar to our patient, four affected individuals from three families with BFPP caused by *GPR56* mutations had

Lennox–Gastaut syndrome [6]. Lennox–Gastaut syndrome manifestations can occur among patients with BFPP caused by GPR56 mutations.

Our patient had a compound heterozygous mutation of*GPR56* (p.S36N and p.R38Q), whereas other patients with BFPP have homozygous mutations of *GPR56* [1,2,5–8]. Both mutations are located in the ligand binding domain within the extracellular N-terminus of the protein, as was observed in the nine previously reported ligand binding domain mutations [1,2,5,6,8]. A homozygous p.R38Q mutation never has been reported with BFPP [1]. Moreover, a detailed analysis of the biochemical modifications by *GPR56* revealed that the disease-associated *GPR56* missense mutations in the tip of the N-terminal domain (p.R38Q) produced proteins with reduced intracellular trafficking and poor cell surface expression [11]. Similar to p.R38Q, p.S36N is another substitution and a novel mutation that locates a ligand binding domain within the extracellular N-terminus of the protein. Compound heterozygosity of these two mutations in the ligand binding domain could impair the subcellular trafficking of the GPR56 receptor, and reduce its cell surface expression, leading to BFPP.

Acknowledgments

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CHAPTER 12

Developmental Genetics

Introduction

We have introduced the concept of multiple congenital anomalies, and seen how the birth of an affected child can have a major impact on the entire family. Establishing a diagnosis for a child with congenital anomalies can be important for counseling of the family, regarding both future medical problems that may be anticipated and risk of recurrence in future offspring. Diagnosis has been historically based on clinical criteria, but advances in molecular genetics are rapidly revealing the genetic basis for congenital anomaly syndromes. This enables more precise diagnoses to be made, allows prenatal diagnosis to be offered, and in some cases predicts specific complications. It also reveals the underlying mechanisms of congenital anomalies, sheds light on normal development, and perhaps leads to future advances in prevention or treatment. In this chapter, we will consider the story of a child with multiple anomalies that include disparate systems. We will see how identification of the gene has improved the ability to provide diagnostic testing and counseling, and how the developmental mechanisms are coming to be understood. We will then look at the discipline of human dysmorphology, and glimpse some of the genetic systems that underlie human development.

Key Points

- The discipline of dysmorphology involves the use of clinical and laboratory approaches to establish the diagnosis underlying congenital anomalies.
- Establishing a diagnosis can provide a basis for counseling a family about the natural history and genetics of a disorder and may suggest specific approaches to management.
- Some congenital anomaly syndromes involve effects on widely varying organ systems. This may reflect the effects of disruption of multiple genes, or the possibility that a single gene participates in developmental processes common to various systems.
- Normal development is dependent on the activation or repression of genes under tight temporal and spatial control. Many of the genetic systems that underlie embryological development are coming to light.

Human Genetics and Genomics, Fourth Edition. Bruce R. Korf and Mira B. Irons.

Part I

Jane's pregnancy has proceeded uneventfully right through the time of delivery. Jane and Albert are offered ultrasound examination during the pregnancy, and one is scheduled at 18 weeks, far enough along so that fetal growth and structure can be best evaluated. The ultrasonographer spends quite a bit of time taking all of the necessary measurements and views of the fetus. After the initial scans are taken, a fetal radiologist comes into the room and does further imaging. She tells Jane and Albert that the fetus is smaller than expected, and she is concerned that the heart and kidneys are not normally formed. She tells them that instead of two separate kidneys, it appears as though the fetus has a single horseshoe kidney, and that the heart appears to have an atrioventricular canal defect.

A genetic counselor comes into the room, talks to Jane and Albert more about the findings, and recommends that an amniocentesis be performed for fetal chromosome analysis since two major structural abnormalities are present. Although Jane and Albert would not terminate a pregnancy regardless of the outcome, they decide to proceed with the amniocentesis so that they have all of the information that they might need to make appropriate decisions regarding the remainder of the pregnancy. An amniocentesis is performed, and chromosome analysis reveals a normal 46, XX chromosome pattern. Additional fluorescence in situ hybridization (FISH) testing for a 22q11.2 deletion is negative (normal). Jane and Albert are referred for additional fetal imaging, specifically a fetal echocardiogram performed by a pediatric cardiologist at 20 weeks. The cardiologist tells them that the fetus has a large atrioventricular canal defect that will require surgical repair early in life. They decide to deliver at a large tertiary care center, rather than their local hospital, so that the baby can be cared for shortly after delivery by neonatologists and pediatric cardiologists. Labor begins spontaneously. It is obvious at the moment of birth that there are problems. The baby, a girl, is noted to have abnormal ears and an unusual face. Although she breathes spontaneously in the delivery room, it is immediately apparent that she is having respiratory distress. She is intubated in the delivery room and brought to the neonatal intensive care unit. Jane and Albert barely have time to see her and are confused and frightened.

It is common to offer ultrasound examination as a component of routine prenatal care. In addition to helping one recognize fetal malformations, ultrasonography provides accurate assessment of gestational age. Many couples find that having information about their baby's health prenatally helps them to plan, even if they do not choose termination of pregnancy in the event of fetal problems. The stressful environment of the delivery room is not a good place in which to first learn that a baby is in trouble. This baby apparently has multiple congenital anomalies, including abnormalities that were not identified by the prenatal ultrasound, as well as respiratory distress.

Chromosome analysis is typically performed when fetal anomalies are detected prenatally, as malformations are seen more frequently in the presence of a chromosome abnormality, such as Down syndrome (trisomy 21), trisomy 18, trisomy 13, or many partial-trisomy or partial-monosomy syndromes. Many of these chromosome abnormalities can be detected on a standard karyotype with high-resolution banding. Other "submicroscopic" chromosome changes may need additional testing by FISH or multiplex ligation-dependent probe amplification (MLPA) techniques, as they may not be detectable on a standard karyotype because of their small size. One example of the latter is the chromosome 22q11.2 deletion syndrome (see Clinical Snapshot 6.3). Since specific cardiac and renal abnormalities are seen in this condition, prenatal testing for this microdeletion by FISH or MLPA is indicated when cardiac malformations are seen prenatally.

Part II

A geneticist is called to see the baby soon after her arrival at the medical center. She has been extubated, and although breathing on her own, is having some respiratory distress and feeding difficulty. On examination, she is small for gestational age and is noted to have several abnormal craniofacial findings, including a square face, a broad forehead, a small right eye, a prominent nasal bridge, tiny nasal openings, and short, wide, abnormally formed ears. Sloping shoulders are noted, and the geneticist also notices that the baby's face moves asymmetrically when she cries. The nurse tells her that a tube could not be passed down the nasal passages, indicating that they are smaller than normal. The cardiologists confirm the prenatal finding of an atrioventricular canal defect, and the baby is being closely monitored and treated for any possible cardiac problems that might occur prior to surgery.

The physical findings in this infant are indicative of a malformation syndrome that affects multiple organ systems. The clinical dysmorphologist tries to recognize patterns of abnormal development, with the goal of establishing an etiologic diagnosis. Major classes of anomalies are malformations, deformations, and disruptions. A malformation is the result of abnormal development of tissue. Developmental mechanisms

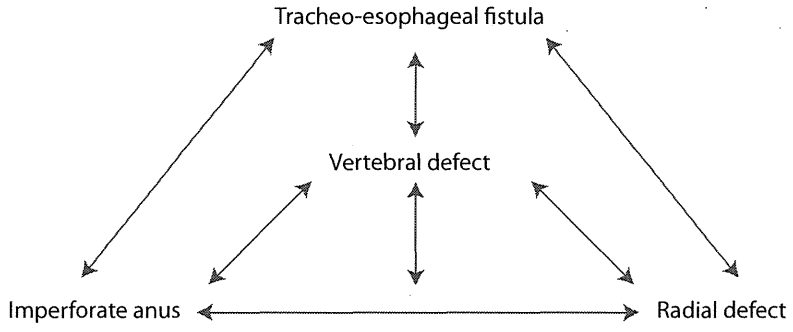

Figure 12.1 Diagram indicating association of tracheo-esophageal fistula, imperforate anus, vertebral defects, and radial defects. These malformations tend to occur together more often than might be expected due to chance. (Redrawn by permission from Quan and Smith 1973.)

somehow are interfered with, and the tissue does not form properly. Deformation is defined as the distortion of a normally formed tissue by extrinsic pressure. An example is asymmetry of the skull due to pressure from a benign uterine tumor called a fibroid. Disruption is the damage of a normally formed tissue. For example, a tear in the amniotic cavity can trap a limb, amputating part of the extremity. Disruptions tend to be asymmetric, whereas malformations are more often (but not always) symmetric.

Various patterns of malformations are seen. Some comprise syndromes, such as Down syndrome. These are sets of congenital defects that are the consequence of some defined, ultimate cause. Down syndrome results from having an extra copy of chromosome 21. Syndromes can also result from exposure to teratogenic agents such as thalidomide. Whatever the cause – abnormality of one gene or a group of genes, or exposure to a teratogen – the outcome is perturbation of multiple developing systems in a reproducible way.

A second pattern of malformations is called a sequence. A sequence generally arises as the consequence of a single primary event, for example underdevelopment of the lower jaw in the Pierre–Robin sequence. Other anomalies that comprise the sequence are secondary effects of the primary malformation. In the Pierre–Robin sequence, the tongue is too large for the small mouth and interferes with closure of the palate, resulting in cleft palate. Here, cleft palate is not a primary malformation but a secondary consequence of underdevelopment of the jaw.

The third pattern is referred to as an association. It has been observed that particular sets of congenital anomalies tend to occur together more often than expected due to chance. These do not comprise syndromes; the causes are unknown, and many associations are etiologically heterogeneous. Indeed, the majority occur sporadically. One example is the VACTERL association, the major components of which are *v*ertebral anomalies, *a*nal atresia, *c*ardiac anomalies, *t*racheo-*e*sophageal fistula, *r*enal anomalies, and *l*imb defects (Figure 12.1). Various combinations of these features occur in different babies, reflecting the nonrandom association of these malformations. It is not known why these associations occur. They may reflect processes that have some molecular or morphologic event in common or processes that all occur at the same time in development.

The medical practice of dysmorphology is partly science and partly art. A dysmorphologist examines for both subtle and major anomalies, looking for recognizable patterns. Correctly establishing a diagnosis can help one to provide anticipatory guidance: What does the future hold? Is the child likely to have additional anomalies that are not obvious on cursory examination? It also permits accurate genetic counseling. Sometimes the recurrence risk will be high; at other times, the family may be surprised to learn that recurrence is unlikely.

Part III

Albert tells the care team that he and Jane have decided to name the baby Lisa. The geneticist suggests that Lisa be seen by an otolaryngologist to evaluate for choanal atresia and to evaluate the abnormally formed ears better, and by an ophthalmologist for further evaluation of the small eye. She also recommends that a hearing test be performed as well as further imaging of the ears by computed tomography (CT) scan and the brain by magnetic resonance imaging (MRI). Bilateral choanal atresia is confirmed by the otolaryngologist and by CT scan, which also reveals abnormalities of the temporal bones and inner ear bones (Figure 12.2). The ophthalmologist confirms that the right eye is abnormally formed, and CT scan also reveals colobomata of the optic nerves bilaterally, as well as pontine and inferior vermian hypoplasia (Figure 12.3). More detailed imaging of the brain will need to be performed by MRI, which will have to wait until the respiratory problems resolve.

Lisa continues to have a problem breathing, which is felt to be due to her small nasal passages, and she requires tube feedings, as she is not able to feed comfortably by herself. Jane and Albert meet with the neonatologist later that evening, who explains that Lisa is sick but that no definite diagnosis has been made so far. The plan has been to provide maximum support as further information is collected. The other specialists have been called to see Lisa, but have not sent someone by yet.

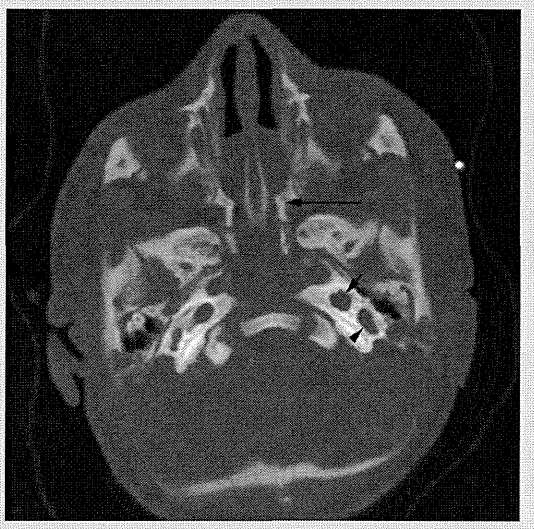

Figure 12.2 Temporal bone CT scan illustrating many features seen in CHARGE syndrome, including choanal atresia (long black arrow: lateral wall of the nasal cavity deviates medially and is opposed to the thickened vomer), abnormal cochlea (short black arrow: absence of internal cochlear structure and cochlear nerve canal), and hypoplasia of vestibule (black arrowhead: absence of semicircular canals). (Courtesy of Dr. Caroline Robson, Children's Hospital, Boston, and Harvard Medical School.)

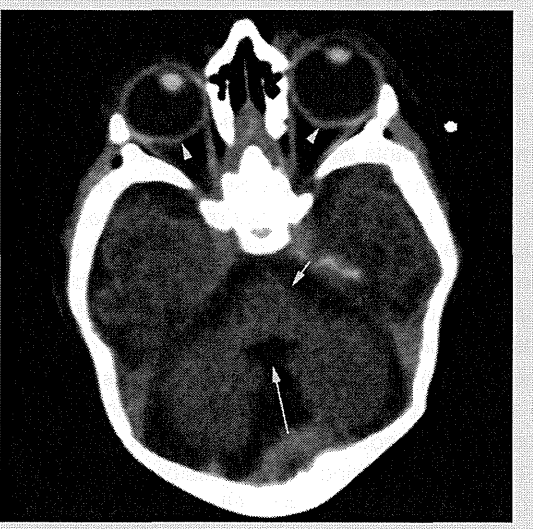

Figure 12.3 Brain CT scan illustrating pontine hypoplasia (short arrow) communication of the fourth ventricle with the retrocerebellar cerebrospinal fluid space due to inferior vermian hypoplasia (long arrow), in addition to small ocular colobomata (arrowheads). (Courtesy of Dr. Caroline Robson, Children's Hospital, Boston, and Harvard Medical School.)

The additional findings of choanal atresia and colobomata fit well with a rare condition called CHARGE syndrome (MIM 214800). This condition, first described in 1979, was initially called an association and is known by its acronym that stood for the physical abnormalities seen in the condition: *c*oloboma of the eye, *h*eart defects, choanal *a*tresia, *r*etarded growth and development, *g*enital hypoplasia, and *e*ar anomalies and deafness. CHARGE syndrome has an estimated prevalence of approximately 1 per 10 000–15 000 births (Figure 12.4).

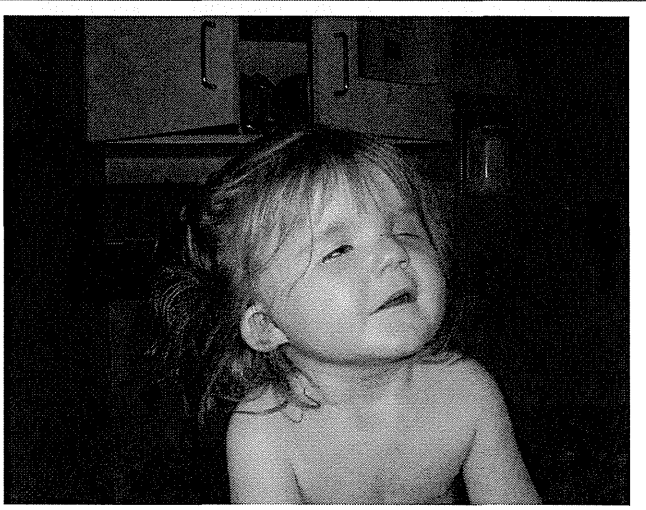

Figure 12.4 Picture of child with CHARGE syndrome that illustrates some of the craniofacial features of the condition, including a rectangular forehead, abnormally formed ears, and left microophthalmia. (Courtesy of Dr. Laurie Demmer, Tufts Medical School, Boston.)

Part IV

Lisa is now almost 48 hours old. The geneticist meets with Jane and Albert and explains that Lisa has the clinical findings of CHARGE syndrome. It is explained that this disorder is characterized by a variable pattern of clinical and developmental problems, though not all of the associated problems are seen in every affected individual. They are told that many infants with CHARGE syndrome have respiratory and feeding problems in the newborn period, as Lisa has. She may require a feeding tube to supplement her feedings, and she may be smaller than other children her age. Recurrent infections, especially upper respiratory, are common. Since Lisa has colobomata of the optic nerves, they are told that she will likely have some visual problems, in addition to some degree of hearing loss due to the inner ear abnormalities noted on her CT scan. A family history is obtained, revealing no prior history of similar problems. This is Jane and Albert's first child. Jane had one previous miscarriage at 8 weeks of gestation. Jane and Albert are not related to one another. The geneticist tells Jane and Albert that the syndrome is usually sporadic, although recurrence in families has been reported in rare instances.

Knowing the medical and developmental problems that can be associated with the condition can help guide diagnostic evaluation, as well as inform medical management and health surveillance as this child grows older. The other abnormalities that can be seen in CHARGE syndrome include cleft lip and/or palate, esophageal atresia, limb anomalies, and vertebral anomalies resulting in scoliosis and kyphosis. Genital hypoplasia, including micropenis or cryptorchidism in boys and labial hypoplasia in girls, can be seen at birth. Hypothalamic dysfunction may also be present and result in abnormal pubertal development. Renal anomalies can cause vesicoureteral reflux, hydronephrosis, or recurrent urinary tract infections. The prognosis for central nervous system (CNS) function has been more difficult to predict. A small number of infants with this disorder have CNS malformations, including agenesis of the corpus callosum, cerebellar hypoplasia, or holoprosencephaly. Hypoplastic olfactory bulbs causing anosmia are seen in the majority of affected individuals. All cranial nerves can be affected, and facial asymmetry (more easily noted when crying) can be seen in up to 50% due to facial nerve palsy. Brainstem dysfunction leading to feeding and/or respiratory problems and abnormalities in thermal regulation have been reported. Intellectual disability is variable and can range from severe cognitive impairment to normal intelligence.

Part V

Lisa continues to do well in the neonatal intensive care unit and is now breathing on her own and taking more of her feedings by mouth. The geneticist returns to talk to Jane and Albert about sending genetic testing to confirm the diagnosis of CHARGE syndrome in Lisa. The geneticist tells them that the gene responsible for CHARGE syndrome has recently been discovered, and confirmation of the diagnosis can be made in approximately 60–70% of affected individuals. Blood is drawn from Lisa and sent to a laboratory for testing.

The gene associated with CHARGE syndrome, *CHD7*, was first identified in 2004 using a microarray-based comparative genomic hybridization approach that identified a *de novo* microdeletion of 4.8 Mb on chromosome band 8q12.2. A previous patient with CHARGE syndrome had been found to have an apparently balanced translocation between chromosomes 6 and 8, which had originally drawn attention to these chromosomal regions. Further analysis of the genes within the deleted segment led researchers to the *CHD7* gene, which consists of 38 exons and codes for a protein made up of 2235 amino acids. Mutations of the *CHD7* gene in patients with CHARGE syndrome are spread across the coding regions and splice sites of the gene, and most are predicted to result in premature termination of translation of the protein, likely leading to haploinsufficiency. It is estimated that approximately 60–70% of individuals who meet diagnostic criteria for CHARGE syndrome have detectable mutations of the *CHD7* gene. Complete gene deletions, detectable by chromosome microarray analysis or MLPA, can be seen in additional patients.

CHARGE syndrome is an autosomal dominant condition that is usually sporadic, since most affected individuals represent new mutations. Recurrence risk is estimated at 2%, as both germline and somatic mosaicism have been identified in parents of affected children. Preimplantation genetic testing or prenatal diagnosis by means of chorionic villus sampling or amniocentesis is available for families with confirmed mutations of *CHD7*.

CHD7 encodes a chromodomain helicase DNA-binding protein and is part of the CHD family of proteins involved in chromatin remodeling and gene expression during early development. Analysis of *CHD7* expression patterns during early human development correlate well with the tissues affected in CHARGE syndrome (i.e., CNS, inner ear, and neural crest derivatives). It is believed that *CHD7* plays a critical role in chromatin remodeling, and its normal function is necessary for epigenetic control of target gene expression in mesenchymal cells derived from the cephalic neural crest.

Part VI

Jane and Albert continue to visit Lisa in the neonatal intensive care unit, meet with all of the specialists involved in her care, and learn more about CHARGE syndrome. The geneticist tells them that a mutation of the CHD7 gene was identified in Lisa, confirming that she has CHARGE syndrome on a molecular basis. Although Jane and Albert have no signs of this condition, they are also tested to see if either of them carries the CHD7 mutation. Both have normal testing. Although it appears that Lisa has CHARGE syndrome on a de novo, or sporadic, basis, they are told that they have an approximately 2% risk of having another child with the same mutation in a future pregnancy, as germline mosaicism has been reported in this condition. Since Lisa's mutation has been identified, preimplantation genetic testing is available to them, as well as prenatal testing during the pregnancy. Prenatal testing could determine if a future fetus has inherited the CDH7 mutation, predicting how the child would be affected would not be possible due to the clinical variability associated with this condition. Lisa continues to do well, has her corrective heart surgery, and recovers well postoperatively. After one month, Jane and Albert take Lisa home.

This case illustrates a number of issues commonly encountered in genetic counseling. First, the problems are complex and usually are well outside the knowledge of lay individuals. The parents have never heard of the syndrome and may have little or no understanding of the principles of inheritance, let alone molecular genetics. Counseling is complicated further by the emotional trauma of having a sick child, for whom life-and-death decisions need to be made at the present time, in addition to new concerns regarding whether the couple is at increased risk of having another child with the same condition. This is especially important in conditions such as CHARGE syndrome, where very mild clinical manifestations, such as asymmetry of the ears (believed to be a variation of normal or a retinal coloboma noted only upon further examination), have been reported to be present in a parent found to have the same mutation as his or her child, giving the couple a 50% risk of having another affected child. It is often helpful to introduce a family to others who have firsthand experience with a genetic syndrome. Although specific manifestations and severity can differ from individual to individual, such contact provides at least a glimpse of what life may be like for a person with the disorder in question.

Inborn Errors of Development

Approximately 3% of all pregnancies end with the birth of a child with a birth defect. Usually, these are isolated defects, such as cleft lip or a neural tube defect; the child is otherwise healthy, although he or she may suffer serious consequences from the presence of the malformation. Many of these malformations occur sporadically and are believed to have a multifactorial etiology. Others are determined by single genes or chromosomal abnormalities. Aside from isolated malformations, some babies are born with a complex of multiple congenital anomalies.

Until recently, the molecular basis of syndromes of abnormal development was largely unknown. This vastly limited the tools available for diagnosis. The identification of genes involved in both normal and abnormal development is rapidly changing this picture. Normal development involves processes of cell replication, migration, and differentiation, tightly controlled in a spatial pattern and temporal sequence. Major developmental events are initiated through cell–cell signaling, with binding of a receptor leading to transduction of a signal to the cell nucleus to initiate a program of transcription of specific genes.

Some of the first genes involved in development to be discovered were initially studied in the fruit fly, *Drosophila*. Over the years, a curious set of *Drosophila* mutants had been identified. *Drosophila* species have the typical arthropod body plan of three segments – head, thorax, and abdomen. Rare mutants, referred to as homeotic mutants (the result of **homeotic genes**), have distinctive and often bizarre disruptions of body segmentation. The mutant Antennapedia, for example, has legs protruding from the head where antennae should be. Other mutants are characterized by segmentation defects (e.g., conversion of a legless abdominal segment into a thoracic segment with legs).

Many of the genes responsible for these phenotypes have been cloned. It has been found that they share a region of homology that has come to be called the **homeobox (Hox)**, which consists of approximately 60 amino acids (corresponding with 180 base pairs of DNA) that have DNA-binding properties. Other genes involved in the regulation of development share a different DNA-binding domain called the **paired box (Pax)**. The Pax box consists of 128 amino acids. In *Drosophila*, there are five Pax genes, most of which appear to be involved in body segmentation.

There are several groups of Hox genes in *Drosophila* that share sequence homology. One group, referred to as the homeotic complex (HOM-C), consists of eight genes. These genes are expressed in an anterior–posterior pattern in the *Drosophila* embryo, in a spatial order that is the same as their arrangement on the chromosome (Figure 12.5). The gene at the 3′ end of the complex is expressed in the head region, and loss of function of this gene causes disruption of development of head structures. Genes located in a 5′ direction are expressed in progressively more posterior segments. Areas of expression tend