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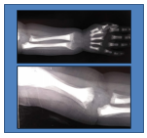

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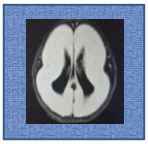

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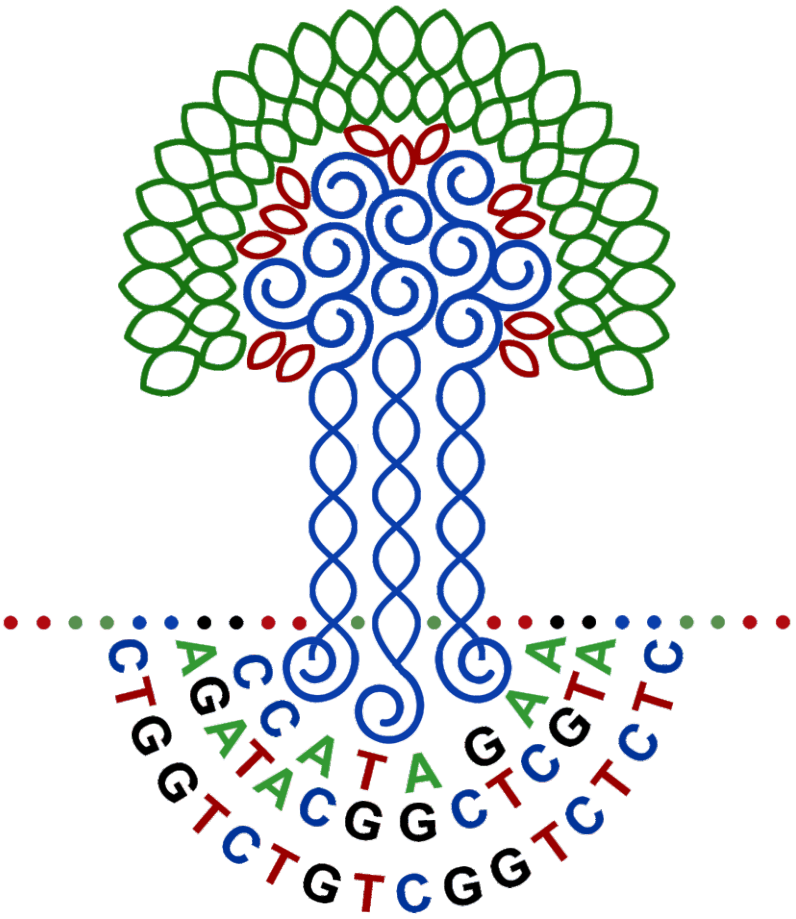

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From the Editor's desk

Editorial

I take great pleasure in presenting the 51st issue of Genetic Clinics. We have the honour of publishing in this issue, an invited editorial by Dr John Carey, former editor-in-chief of the *American Journal of Medical Genetics*. Dr Carey has summarized his long experience of syndrome delineation, which reflects the historical perspective of diagnosis of genetic syndromes. This is very relevant for the current generation of geneticists who have seen the 'exome first' strategy. The changing but important role of the clinician in deep phenotyping and reverse phenotyping is also highlighted in the articles reviewed in the GenExpress in this issue.

The year 2020 has been very important for Indian patients with genetic disorders due to the landmark guidelines for health insurance. The Insurance Regulatory and Development Authority (IRDA) has now disallowed exclusion of genetic diseases or disorders with effect from October 1, 2020. We recollect the position statement on 'Equal rights to health insurance and employment: Prevention of discrimination based on genetic information' issued in November 2019 by the SIAMG, and are happy about the positive developments in this area.

Happy new year!

Dr. Shubha Phadke
1st January, 2021

PhotoQuiz - 51

Contributed by: Dr Prajnya Ranganath

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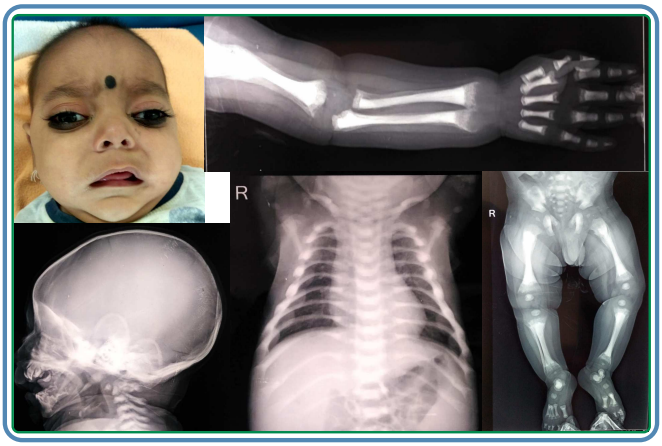
This 10 months-old male child was referred for evaluation of anemia with thrombocytopenia, hepatosplenomegaly, visual impairment with optic atrophy, and hearing loss. There was history of pathological fracture of the left femur at 6 months of age. Identify the condition.

Please send your responses to editor@iamg.in
Or go to http://iamg.in/genetic_clinics/photoquiz_answers.php
to submit your answer.



Invited Editorial: Reflections on Phenotype, Syndrome Delineation, and Six Decades of Medical Genetics

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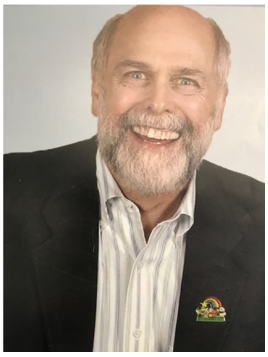

Dr John C Carey, Professor and formerly Vice Chair of Academic Affairs, Department of Pediatrics at the University of Utah, USA, is one of the most eminent medical genetics experts in the world. Throughout his brilliant career, spanning over 4 decades, he has made many significant clinical and academic contributions to the field of medical genetics particularly in the areas of syndrome delineation and congenital malformations. He was Editor-in-Chief of the *American Journal of Medical Genetics* from 2001 to 2016. We sincerely thank Dr Carey for accepting our request to write this invited editorial for Genetic Clinics.

Dedication: *I dedicate this Commentary to Dr Shubha Phadke, endearingly called "Madam" by her many devoted mentees, who first introduced me to the cultures and richness of India and welcomed me to the community of medical genetics in her beloved nation.*

One of the many benefits of getting older and remaining in a field is that you advance to an age where your colleagues allow you to compose a paper that is entitled "Reflections on." Another advantage is that you have the opportunity to have encountered and learned from the pioneers in the discipline. I have had that honor: I entered the field with a deep clinical focus in 1975 at a time before medical genetics was recognized as a specialty of US mainstream medicine. I worked with David W Smith, the perceived patriarch of dysmorphology; John Opitz, the founder of the *American Journal of Medical Genetics* and a champion in *syndrome delineation* as well as *phenotype analysis*; and Victor McKusick, the recognized founder of Medical Genetics. And along this journey I have had the privilege to observe the landmark events in real time. These years of observation and participation ideally position me to "reflect on" where we have been and where we are going in the analysis of phenotype.

In this Commentary, I would like to present one person's view of the field of Medical Genetics, its rich history, and what I perceive to be the future directions in regards specifically to our study of phenotype and syndrome delineation. I will highlight the key landmarks in Human and Medical Genetics and will propose that since 1960 we have witnessed four *Eras* (see Table 1). My aim in this discourse is to help us ponder the future challenges in the analysis of phenotype within the context of the remarkable advances that have led to what we now call *genomics* and its clinical outcome, *genomic medicine*.

Any student of Human Genetics is immediately familiar with the historic landmarks that united human genetics and medicine: Mendel's seminal work of 1865 rediscovered in the early 20th

The key terms in the essay are placed in italics.

Table 1 The Four Eras of Medical Genetics and Genomics.

Era I: The Golden Era of Phenotype and Syndrome Delineation 1960-2010
Era II: Cloning Disease Genes by the Mapping Approach 1980-2010
Era III: Emergence of Genomic Medicine 2005-present
Era IV: The Modern Era of Phenotype Analysis 2015-present

century; Watson and Crick's recognition of the structure of DNA in 1953; the characterization of the correct number of human chromosomes as 46 in 1956; the recognition that Down syndrome is due to an extra #21 chromosome (1959), and the subsequent rapid emergence of clinical cytogenetics during the 1960s (the key references to these landmarks and those below can be found in Jorde et al., 2019). The observation and description of Edwards syndrome/trisomy 18, Patau syndrome /trisomy 13, and the classical monosomy syndromes (4p-, 5p-, 18p-, and 18q-) paralleled the recognition of now well-established multiple congenital anomaly syndromes during the 1960s (e.g., Smith-Lemli-Opitz syndrome, rediscovery of the de Lange syndrome). Over the ensuing 5 decades cytogenetics technology would advance from various banding types to high resolution banding to the application of florescent DNA probes for specific conditions (e.g., Prader-Willi syndrome) and subtelomeric regions to the current platforms of cytogenomic microarray. I would consider Medical Genetics to have emerged as a medical discipline during that period of the 1960s and 1970s and set the stage for 4 Eras. The first of those eras is what I entitle the "Golden Era of Phenotype and Syndrome Delineation." Syndrome recognition and description represents, in my opinion, one of the 2 major cornerstones in those early decades of human genetics as it entered mainstream medicine and expanded to medical (and clinical) genetics. Pioneers such as David Smith, John Opitz, Victor McKusick, Judith Hall, and their trainees recognized distinct entities in their clinic settings, described them, and facilitated the process of syndrome delineation. This truly was a time of novel disease discovery; it occurred often within the context of Pediatrics, and would later be referred to as the *phenotype-first* model. During these years Dr. Smith introduced the term *"dysmorphology"* (Smith, 1966) hoping that it would replace the potentially pejorative term, *teratology*, as the study of abnormal structure or altered morphogenesis.

This "Golden Era" proceeded-in my view- from the 1960s until about 2010 when whole exome sequencing introduced the *"gene-first"* model and significantly changed genetics testing and the methodology of syndrome delineation. I also suggest that the application of *exome sequencing* (ES) to research, gene discovery, and the clinical setting was not truly a paradigm shift or scientific revolution (Kuhn,1974) because it was the logical extension of the central tenet of biology, i.e., *DNA→RNA→protein*. In the late 1970s clinical genetics (while still loosely connected to its parent discipline of pediatrics) and biochemical genetics merged together in the USA under the aegis of Medical Genetics and together with laboratory geneticists (cytogenetics and biochemical) initiated the process of certification of professionals and accreditation of training programs (i.e., the American Board of Medical Genetics). Canada and some countries in Europe established a similar approach.

The 1970s witnessed the rise of prenatal diagnosis by amniocentesis and fetal chromosome analysis as a routine component of obstetric medical care in pregnancy. This continued through the 1970s and into the 1980s until chorionic villus sampling and other prenatal screening modalities, i.e., the triple screen and quadruple screen, emerged and expanded prenatal genetic screening in standard prenatal practice.

Paralleling these advances in syndrome delineation (and prenatal diagnosis) was the emergence of *genetic counseling* as the other cornerstone of Medical Genetics. While the term had been used long before the 1970s (actually in the 1940s), it was at that time that the development of Master's level training programs blossomed in the US. A consensus ASHG definition of genetic counseling was proposed in 1975, and soon after, the National Society of Genetic Counselors gained prominence in the field; genetic counseling as a discipline and profession was cemented. By the early 1990s the American Board of Genetic Counseling was established to certify practitioners and accredit training programs.

The second Era began in 1978 with the Alta Conference in Utah led by Drs Botstein, White and Skolnick, who predicted that libraries of DNA polymorphisms could map genes to their chromosome (published in 1980) culminating in 1983 with the mapping of the gene for Huntington chorea to chromosome 4. At first the term "reverse genetics" was used since the approach was the "reverse" of what had existed before, i.e., recognition of genes through their biochemical structure. But in fact, this approach was really forward genetics: the collection of families, the application of technologies to map a gene, and the (then) time-consuming process of gene identification through the convergence of various approaches (animal models, chromosome rearrangements in patients, and gene linkage). Early on, the benefits of gene identification were recognized as clarification of recurrence risks in pregnancies, the application to the genetic counseling process, and the understanding of pathogenesis (hopefully) leading to treatment. This cloning "by the mapping approach" led during the 1980s to the mapping and then recognition of genes for important and classical genetic disorders, i.e., Duchenne muscular dystrophy, cystic fibrosis, neurofibromatosis type 1 (NF1), and Marfan syndrome. By the 1990s, genes for over 2000 diseases had been mapped or cloned, and the explosion in recognition of cancer genes (e.g. *BRCA1* and *2*) occurred that propelled the rise of *cancer genetics* as a bone fide area of the field. These advances often occurred in the context of the *Human Genome Project*, which was initiated in 1990, and by 2001 a 90% draft of the human genome had been completed. Soon, it was common for review articles to display a map where one could see the location and gene identification of genes for various syndrome types, e.g., malformation syndromes, skeletal dysplasias, and more. Certainly, the stories of the mapping and then identification of a gene are rich narratives, each having their own lessons, and all worth retelling. Each story contains its own bends and turns often involving rich collaborations and at other times secrecy and press releases. NF1 is a particularly interesting example (Carey, 2017): using the approach that was widely available in mid 1980s, the gene for NF1 was mapped by 2 different sets of collaborators in 1986. At the time that the mapping located the causative gene to the pericentric region of 17q11, two patients with NF1, who had balanced translocations involving 17q11 were identified and helped lead to the sequencing of the full-length cDNA that was labeled *NF1* with its encoded protein, the NF1 peptide. At that point in history, the excitement surrounding finding the patients with these translocations cannot be underemphasized. Similar stories where translocations in patients seen in clinic settings (e.g., Waardenburg, van der Woude, Sotos) helped identify other disease genes are commonly known in the field. The promise, of course, in recognizing *NF1* and its peptide would be treatment. But it took another 15 years for clinical trials surrounding the treatment of malignant peripheral nerve sheath tumors and cognitive disabilities to come forward. This is not the correct forum to review all that has come about in regards to treatment of genetic disease because my focus here is primarily on phenotype and syndrome delineation, but clearly the importance of treatment and gene therapies (and now with gene editing) cannot be overstated.

By the late 1990s, mainstream medicine in the US witnessed the creation of the American Board of Medical Genetics (eventually American Board of Medical Genetics and Genomics) the American College of Medical Genetics (1991), the American Board of Genetic Counseling (1993), and Medical Genetics Residencies by the early 2000s. Similar austere organizations emerged as well in Canada and Europe.

The third Era is one that I label *Genomic Medicine*. In this light I cite the famous Chinese proverb "May you live in interesting times." The emergence of genomic medicine in the last decade or so certainly would qualify us as living in "interesting times." Genomic medicine overlaps what others later refer to as personalized healthcare or individualized healthcare. Genomic medicine is defined by the NIH in the US as an emerging "discipline that involves genomic information about the individual as part of clinical care". Various components would include the systematic implementation of studying Mendelian disease, pharmacogenomics, and the application of clinical ES and whole genome sequencing to clinical care. Certainly, ES and genome sequencing (*Next generation sequencing, NGS*) are unparalleled in this observer's mind to any approach or technique that had entered the field since cytogenetics. In the early part of the 2010 decade, the ability to identify the gene for a condition using ES became remarkably rapid and depended on having only a few patients with the condition. This advance led to an explosion in gene discovery in known phenotypes (e.g., Miller acrofacial dysostosis, Kabuki syndrome). It was

followed closely by the identification of novel syndromes by applying the approach to various clinical presentations (e.g., intellectual disability, multiple congenital anomalies); the "gene first" model had emerged. What occurred then was unprecedented: a condition was identified first through its etiology and the characterization of its full phenotypic spectrum and its clinical variability would follow with additional reports. The concern at the time by traditional clinical geneticists (including myself) was that trainees and junior practitioners would rely solely on the molecular testing and lose the clinical skills needed to characterize phenotypic signs, generate a relevant differential diagnosis, establish a clinical diagnosis, and interpret the variants. As wisely foreseen by Hennekam and Biesecker (2012), that scenario has **not** turned out to be the case. I would concur: delineation of the phenotype now depends on modern phenotypic analysis. It is my contention that we then entered Era IV, the Modern Era of Phenotype Analysis.

The current Era is indeed exciting and interesting. I recently suggest in the review article on phenotype that, "... there has likely never been a more exciting time in the history of medicine" as phenotype analysis in all medical specialties has become paramount. In the review (Carey, 2017) I highlight the principles, history, definition, and current strategies to define phenotype and point to the advent of a "Human Phenome Project". Approaches (such as Phenotips in the clinical setting and electronic medical record), facial recognition technologies (such as Face2Gene), and online resources that link clinicians, families, and researchers (such as MyGene2) have changed the landscape in which clinical geneticists practice medical genetics and characterize syndrome delineation. These approaches have heralded a modern era in *phenotype analysis*. Comprehensive characterization of phenotype, in my view, is increasingly becoming valued by research scientists, laboratory geneticists, and clinicians of all specialties. In the interpretation of NGS, the importance of curating both the phenotypic findings and the DNA variants in making sense of the results and inferring causation is widely recognized (Friedman et al., 2020).

To conclude, these milestones make this observer optimistic about the future directions of phenotype analysis and syndrome delineation. The continuation, however, of this current trajectory will depend on the wholehearted acceptance of medical genetics as a vital component of mainstream medicine.

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Clinical Vignette

Hystrix-like Ichthyosis and Deafness Syndrome in a Toddler

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Abstract

Hystrix-like ichthyosis and deafness (HID) syndrome is characterized by ichthyosis, erythrokeratoderma, alopecia and deafness in varying degrees of severity. The clinical manifestations are present since birth, evolve and gradually worsen. It occurs due to a single known mutation in the *GJB2* gene. Early diagnosis and management and genetic counseling require a high index of suspicion for an underlying genetic basis in such skin disorders.

Introduction

Hystrix-like ichthyosis and deafness (HID) syndrome (OMIM#602540) was first described in a patient in 1977 who presented with icthyosis-hystrix and bilateral hearing loss (Schnyder et al., 1977). Its initial name was 'ichthyosis hystrix gravior, type Rheydt' after the city of origin of the patient, located near Dusseldorf, Germany, with the word 'hystrix' indicating spiky porcupine-like skin changes [Konig et al., 1997]. Traupe H suggested including deafness in the nomenclature, naming it hystrix-like ichthyosis with deafness, or HID syndrome (Traupe, 1989). The molecular basis of the HID syndrome has been found to be a heterozygous pathogenic variant (p.Asp50Asn) in the *GJB2* gene. Pathogenic variants in *GJB2* are more commonly known to cause non syndromic deafness [autosomal recessive (AR) or autosomal dominant (AD)]. A phenotypic variant to the HID syndrome is the keratitis icthyosis deafness (KID) syndrome. KID syndrome patients have keratitis (inflammation of the cornea) that can cause photophobia, scarring and vision loss. They also have palmoplantar keratoderma in addition to erythrokeratoderma, ichthyosis and deafness which is seen in the HID syndrome. About 100 cases of HID have been reported to date in literature (Avshalumova et al., 2014). Here we present a rare case of the HID syndrome.

Case Report

The patient is a 17-month-old girl born to non consanguineous parents. She was born preterm at 36 weeks of gestation, appropriate for gestation with a birth weight of 2.5 kg. She had required admission in the neonatal intensive care unit (NICU) for 4 weeks in view of respiratory distress. Soon after birth she developed redness and peeling of the skin involving the face, arms, trunk and dorsum of hands and feet which persisted at the time of discharge (Figures 1A and 1B). She was treated for congenital pneumonia and seborrheic dermatitis during her NICU stay. However, the skin lesions were persistent and difficult to treat. She received multiple courses of topical steroids, antifungal and antibiotic ointments in view of a possibility of seborrheic dermatitis or atopic dermatitis along with recurrent skin infections. Over a period of time, she developed diffuse thickening of the skin and hyperkeratotic plaques over the arms and legs. The eyebrows were absent and hair on the scalp and body was sparse and lightly pigmented (Figure 1C). There was relative sparing of the skin of the palms and soles. A lack of sweating was also observed. There was no significant developmental delay. Eye evaluation did not reveal any significant finding. Her immunoglobulin profile and blood counts were normal. The patient was the sole affected family member and the only child, with an ongoing pregnancy in the mother. The family desired a definitive diagnosis for the child and genetic counseling for the ongoing pregnancy. With a possibility of congenital ichthyotic disorder or

Figure 1 Clinical photographs of the child, in the neonatal period (A, B) showing facial rash and alopecia, and at 18-months of age (C) showing slight skin rash, and ichthyosis especially on dorsum of hands.

ectodermal dysplasia further definitive genetic testing was planned. Next generation sequencing, for genes related to ichthyosis related disorders revealed the heterozygous pathogenic variant c.148G>A (p.Asp50Asn/ p.D50N) in the *GJB2* gene, which has been previously reported with HID syndrome. Although the parents did not complain of any significant hearing impairment in the child, and her speech appeared appropriate for age, a formal hearing test (auditory steady state responses) showed mild to moderate and moderately profound to severe hearing loss in the right and left ear, respectively. Sanger sequencing further confirmed the presence of the mutation in the child. It was noted to be a de-novo mutation as it was not present in the parents (Figure 2). This confirmed the overall low risk of recurrence for the ongoing pregnancy (~1% due to gonadal mosaicism). The parents chose against prenatal testing of the fetus and continued the pregnancy. For the affected child, the parents were provided with appropriate dermatological referral and antikeratolytic, antibiotic and emollient topical treatment. They were advised to discuss the need for hearing aid or cochlear implant in the future, with an otolaryngologist.

Discussion

HID is a genetic disorder occurring due to a mutation in the *GJB2* gene, which belongs to the family of gap junction proteins. Connexin 26 is a 225- amino acid- long protein encoded by the *GJB2* gene located on chromosome 13. Gap junction channels are made from a family of proteins called connexins. Their main function is to allow passage of small molecules between adjacent cells, coupling them both metabolically and electrically. The function of the various connexin channels is distinct in terms of their gating, conductance and permeability characteristics (Avshalumova et al., 2014). Connexin 26 is involved in intercellular communication and differentiation of cells in the epithelium of cornea, cochlea, palmoplantar epidermis, hair and sweat glands. The *GJB2*

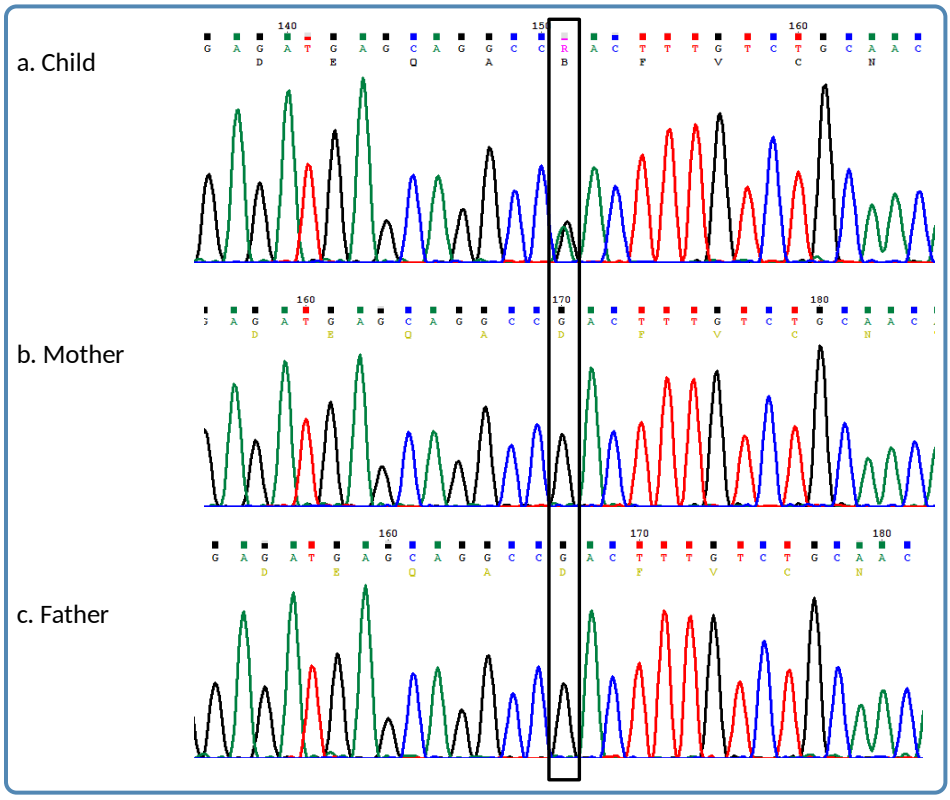

Figure 2 Sanger sequencing analysis of *GJB2* gene (A) child heterozygous for the c.148G>A variant; (B & C) Mother and father negative for the c.148G>A variant.

gene has been more commonly implicated with non-syndromic deafness, both autosomal recessive and autosomal dominant types. It has been identified as the most common cause of non-syndromic deafness – DFNB1 accounting for up to 50% of congenital severe-to-profound autosomal recessive non-syndromic hearing loss in many countries (Smith et al., 2016).

GJB2 has also been studied to cause five syndromic forms of deafness that include skin disease. The syndromic deafness disorders are very rare, and can be divided into two broad groups. The first group includes Bart-Pumphrey syndrome, Vohwinkel syndrome, and Palmoplantar keratoderma with deafness, presenting with palmoplantar keratoderma along with deafness. Specifically, patients with the Bart-Pumphrey have nail involvement in the form of leukonychia and growth on the knuckle pads while, constriction bands and auto amputation have been reported in the Vohwinkel syndrome (Srinivas et al., 2018).

Hystrix-like ichthyosis deafness syndrome (HID) and keratitis ichthyosis deafness (KID) syndrome make up the second group. HID manifests shortly after birth with erythematous patches. By the age of 1 year, spiky and cobblestone-like greyish brown to red hyperkeratotic masses involve the entire skin including the scalp and face. The palms and soles are usually mildly affected. Scarring alopecia can also occur. Histopathologic features resemble those of lamellar ichthyosis with reduction of tonofibrils and abundance of mucous granules and are not diagnostic. There is associated bilateral neurosensory hearing loss. Our patient had all the clinical characteristics of HID syndrome.

HID and KID are identical at the molecular level and the difference is mainly clinical. Some basic differences between the two are: KID can present at birth in the form of hyperkeratotic erythroderma which resolves spontaneously only to recur later but never involves the trunk. Scaling typical of ichthyosis (seen in HID) is not seen, so it is not a true ichthyosis. In addition, palms and soles are severely affected and eye manifestations are typically seen in KID, although few case reports have mentioned mild keratitis in patients of HID

(Van Geel at al., 2002; Avshalumova et al., 2014). Both AD (*GJB2* gene) and AR (*AP1B1* gene) types of KID are known (Boyden et al., 2019). In the AD variety of KID, the p.Asp50Asn accounts for ~80% of mutations but other mutations have also been described. KID also has increased morbidity and chance for disfigurement along with the risk for squamous cell carcinoma (SCC) as compared to HID which generally has a good prognosis. KID is the only connexin-related skin disorder described with SCC. HID begins as erythematous patches soon after birth and evolves to ichthyosis involving the scalp and face. Palms and soles are less affected (differentiating it from KID). A mild punctate keratitis has also been described in some HID patients. There is only one known mutation for HID (p.Asp50Asn). The electron microscopy features are now known to not be diagnostic for either disorder, contrary to the previous notion, and include excess formation of mucous-containing granules and reduction of tonofibrils (Avshalumova et al., 2014). Thus, HID and KID may represent a spectrum of the same disorder at the molecular level with HID being less severe. As suspected with other genes with a wide spectrum of disease severity, possible causes include gene-gene interactions, polymorphisms in other genes expressed in the skin, environmental modifiers and other epigenetic mechanisms.

A genotype-phenotype correlation has been suggested among the KID patients. The *GJB2* p.Asp50Asn mutation-associated patients of KID syndrome live into adulthood despite vision loss and high risk for developing squamous cell carcinoma, while the *GJB2* p.Gly45Glu and p.Ala88Val mutation-associated patients have higher chances of dying in childhood due to septic complications (Srinivas et al., 2018).

The other skin disorders reported with connexin mutations are erythrokeratoderma variabilis (EKV), involving mutations in *GJB3* and *GJB4*, Clouston syndrome (a.k.a. hidrotic ectodermal dysplasia), involving mutations in *GJB6*, and oculodentodigital dysplasia (ODDD) caused due to mutations in *GJA1* (Avshalumova et al., 2014). Confirmation of diagnosis in skin disorders has implications for accurate counseling and management. HID syndrome is a condition that requires regular skin care throughout life. Patients with the KID phenotype need to be monitored for possibility of developing life-threatening SCC. Accurate diagnosis helped to pick the additional symptom of hearing loss in our patient, which may have gone unnoticed until significant speech impairment might have appeared.

Although this is an autosomal dominant disorder, one study reported 64% of cases to be sporadic while 36% cases were familial, many with unaffected parents (Mazereeuw-Hautier et al., 2007). Hence, germline mosaicism is high for this disorder, like most skin disorders and this is a challenging point in counselling.

Acknowledgements: The authors thank the parents of the child for their participation, and colleagues for their guidance.

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Clinical Vignette

Lysinuric Protein Intolerance Presenting with Hepatosplenomegaly and Pancytopenia

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Abstract

Lysinuric protein intolerance (LPI) commonly presents with hyperammonemic encephalopathy and failure to thrive. We report a case of a three-and-a-half-year old boy who presented with failure to thrive, recurrent respiratory tract infections, anemia, thrombocytopenia, hepatosplenomegaly and fracture on trivial trauma, indicating towards differential diagnosis of lysosomal storage diseases like Gaucher disease, Niemann-Pick disease, saposin deficiency etc. A homozygous pathogenic variant identified in the *SLC7A7* gene, through next-generation sequencing-based exome sequencing of the child, suggested the diagnosis of lysinuric protein intolerance (LPI). We conclude that LPI should be considered as a differential diagnosis in a patient with presentation of hepatosplenomegaly with pancytopenia.

Introduction

Lysinuric protein intolerance (OMIM #222700) is a primary inherited aminoaciduria with an autosomal recessive mode of inheritance predominantly reported in Finland (Simell et al., 2002). The incidence of the disorder is around 1 in 60,000 new-borns in Finland. There are many cases reported from Japan also. where the prevalence of the disorder is around 1:60,000. There are two cases reported from India, where the patients presented with recurrent hyperammonemic encephalopathy, diagnosed through molecular genetic testing and/or metabolic profile (Bijarnia-Mahay et al., 2016; Moosa et al., 2005). Absence or reduced levels of ornithine and arginine lead to functional deficiency in the urea cycle, causing increased levels of ammonia and may precipitate encephalopathy. The presentation in our patient was totally different with the patient following up with the hematologist for hepatosplenomegaly, pancytopenia and recurrent infections. It is only after detailed analysis and molecular genetic testing that we could confirm the diagnosis.

Case report

A three-and-a-half-year-old boy, born to a consanguineously married couple, presented with distension of abdomen since the age of 6 months and history of recurrent infections and hospitalization since 6 months. Distension of abdomen was generalized and gradually progressive. Patient required hospitalizations at 12 months, 15 and 18 months of age for lower respiratory tract infections. Child required packed cell transfusion once for severe anemia. Child had a fracture of the left arm 10 days prior to visit to the Genetics OPD. Child was born at term gestation, with birth weight of 3.25 kg. Developmental milestones were normal for age. Dietary history revealed strong aversion to proteins as the child was not eating any non-vegetarian foods and pulses and only preferred rice and vegetables.

On examination, anthropometric parameters revealed: weight 10.7 kg (between -2 to -3 SD), height 83 cm (-2 to -3 SD) and head circumference 47 cm (-2 to -3 SD), according to WHO growth charts. Child was pale and had hepatosplenomegaly. Investigation of complete blood counts (CBC) revealed pancytopenia with hemoglobin of 6.2 gm/dl, total leucocyte count of 3,400/cu mm and platelet count of 40,000/cu

mm. Hemoglobin electrophoresis was normal. Bone marrow biopsy was unremarkable with few hemophagocytes being seen. Serum ferritin was normal (97 ng/mL; reference 17.9-464 ng/mL) and other clinical features did not support the diagnosis of hemophagocytic lymphohistiocytosis. Liver function tests including ALT of 55 IU/L (0-60 IU/L) and total bilirubin of 1.3 mg/dl, were normal. In the serum lipid profile, cholesterol was 170 mmol/dl and triglycerides were 230 mmol/dl; which were both elevated above the normal range.

Considering pancytopenia with hepatosplenomegaly, failure to thrive, recurrent lower respiratory tract infections and fracture humerus, differential diagnosis of lysosomal storage diseases like Gaucher disease or Niemann-Pick disease type B was considered. Though bone marrow biopsy did not reveal any storage cells, absence of storage cells cannot rule out these diseases. We wanted to rule out small molecule diseases based on history of aversion to protein rich foods. Initial tandem mass spectroscopy (TMS) did not show any abnormality. Because of an unclear phenotype, NGS-based exome sequencing was done for the child which showed a homozygous, 'likely pathogenic' one-base pair duplication c.110dupT in exon 3 of the *SLC7A7* gene (transcript id ENST00000397532) that results in frameshift and premature truncation of the protein at codon 38 (p.Ser38LeufsTer4). This frameshift variant is not reported in the 1000 Genomes (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes), gnomAD (https://gnomad.broadinstitute.org) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases. This variant is predicted to be 'disease-causing' by the MutationTaster software (www.mutationtaster.org). After receiving the genetic report, we performed plasma amino acids assay and urine amino acid analysis. Plasma high performance liquid chromatography (HPLC) of amino acids revealed low levels of arginine (2.67 nmol/ml; normal range 10-140 nmol/ml) and glutamine (226 nmol/ml; normal range 254-823 nmol/ml). Urine amino acids assay by HPLC revealed increased excretion or levels of ornithine (124 nmol/ml; normal range 2-91 nmol/ml), lysine (4565.89 nmol/ml; normal range 34-894 nmol/ml), arginine (1044 nmol/ml; normal range 7-133 nmol/ml), and citrulline (283 nmol/ml; normal range 0-90 nmol/ml).

The child was started on levocarnitine, low dose citrulline and lysine supplements and a protein-restricted diet. He showed marked symptomatic improvement and gained 400 grams of weight after 2 months of follow up. Urinalysis for proteinuria and a chest radiograph and serum ammonia were normal on follow-up.

Discussion

Lysinuric protein intolerance (OMIM# 222700) is a rare metabolic disorder also known as dibasic aminoaciduria, caused by a defective membrane transport of cationic amino acids (CAA) like lysine, arginine and ornithine. This leads to decreased circulating plasma CAA levels and increased excretion in urine (Simell et al., 1975).

Massive urinary excretion of dibasic amino acids, especially lysine, and poor intestinal absorption of these amino acids leads to deficiency of these amino acids (Simell et al., 2002). Protein malnutrition and deficiency of the essential amino acid lysine contribute to the patient's failure to thrive (Nunes & Niinikoski, 2006). Biallelic pathogenic variants in the *SLC7A7* gene are responsible for this disorder. Although more than 65 mutations have been detected in patients across the world, as per the Human Gene Mutation Database (HGMD), only one mutation has been reported from India. Excess intracellular arginine, because of trapping (due to block in transport) may trigger an overproduction of nitric oxide, leading to dysfunction of monocytes and macrophages dysfunction (Nunes & Niinikoski, 2006). This explains the association of this disorder with immunodeficiency features, hemophagocytic lymphohistiocytosis, pulmonary alveolar proteinosis and renal disease seen in older untreated patients with LPI. Though bone marrow showed few hemophagocytes, ferritin was normal in our patient. Our patient's predominant presentation of hepatosplenomegaly with pancytopenia and fracture of the humerus added differential diagnosis of lysosomal storage disease like Gaucher disease, saposin C deficiency etc. (Nunes & Niinikoski, 2006). The bone marrow examination did not reveal any storage cells. Therefore, with an unclear phenotype, exome sequencing was performed which revealed the diagnosis. Reverse phenotyping revealed excess excretion of lysine in the urine, confirming the diagnosis. Our patient also had strong aversion to proteins, eating only cereal based food and minimal vegetables. This patient could really get misdiagnosed to have a malabsorption syndrome, storage disorder or immunological disease,

considering such a variable phenotype. Without NGS-based diagnostic testing, it would have been difficult to identify this as a case of small molecule-associated inborn error of metabolism. The blood ammonia levels were not elevated in our patient, but it is important to counsel regarding the life-threatening phenomenon of hyperammonemia in these patients, so that it can be recognized and treated early enough to be able to prevent further neurological damage and other complications. The standard treatment for LPI involves low protein diet, ammonia-lowering nitrogen scavengers like sodium benzoate (100-250 mg/kg/day in 3 divided doses), levocarnitine (100 mg/kg/day in three divided doses) and low-dose citrulline and lysine supplementation. The metabolic derangements are readily treatable with dietary modifications, but some complications like interstitial lung disease, proximal renal dysfunction, hypercholesterolemia, hemophagocytic lympho-histiocytosis, pancreatitis, and growth hormone deficiency add to the morbidity and mortality (Nunes & Niinikoski, 2006). As LPI is an autosomal recessive disease, there is 25% chance of recurrence in future offspring of parents of the index case. Thus, genetic counselling will help these families and prenatal genetic testing can be done if the pathogenic variant(s) is/are identified in the proband.

In conclusion, next generation sequencing is a boon to genetic disease diagnostics, especially for diseases like lysinuric protein intolerance which has multisystemic presentation mimicking various conditions as described above, but deep and reverse phenotyping is an irreplaceable tool to aid the molecular diagnostic results.

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GeneVerse

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An Ode to Duchenne Muscular Dystrophy

There are two threads X and Y
And on the X-thread, an error arrives
Mutating the 'DMD' gene
Causing a condition progressive and mean
07 September is the day to spread
awareness about the same
A condition we know as Duchenne
Muscular Dystrophy, by name

A young boy hasn't walked up to
18 months
Your radar needs to beep!
Super-raised blood CPK may be the
first clue
We need to delve deep

EMG and gene test may follow the line
To cross the first hurdle
And detect this condition in time

Screen their mums early, not late!
Be wary,
A single mutant copy can dilate their hearts,
And cause a second child with DMD
A matter of unease!

Monitor the kids up-and- close
Allow good physiotherapy,
Delay the muscles getting contracted and sore
Timely steroids and breathing exercises
Flu shots and more...

And how can we not mention
Exon skipping, ataluren, CRISPR-Cas9
and gene therapies ...
Oh! The magical world of genomic
possibilities!
Wingardium Leviosa, Abra-ca-dabra
'HOPE', we clinch on to you!
Why let their bodies be wheelchaired?
Let their dreams fly far!

Spread the word,
The condition isn't all-that-rare
We all need to stand up for these kids
And show that we indeed,
Dare to Care!

GeNeViSTA

Cortical Developmental and Neuronal Migration Disorders: An Overview

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Abstract

Cortical developmental and neuronal migration disorders are a diverse group of disorders that are common causes of neurodevelopmental delay and seizures. Cortical malformations and neuronal migration abnormalities result from molecular disruption of normal cerebral cortex development. This article focusses on the pathogenesis, neuroimaging characteristics, and genetic basis of these disorders.

Normal cerebral cortical development

Cerebral cortical development is a complex dynamic embryonic process, which involves integration of multiple cellular processes like neural stem cell proliferation, migration, differentiation and organization. Abnormalities of fetal brain development including neuronal migration disorders could be due to non-genetic causes like in utero exposure to infections, maternal smoking, maternal comorbidities such as uncontrolled diabetes, untreated phenylketonuria and untreated hypothyroidism, and hypoxic-ischemic injury, or they could be due to multifactorial or genetic etiologies (Gressens, 2000; Gressens et al., 2001; Auso et al., 2004; Debillon et al., 2000).

Normal cortical development starts with formation of neuronal progenitor cells (projection neurons and interneurons) from stem cells in the pseudostratified columnar neuroepithelium of the ventricular zone (germinal zone/matrix) in the first stage. Progenitor cells in the ventricular zone give rise to progenitor cells in the subventricular zone. Subventricular zone contains two major types of progenitors: the basal progenitors and the multipotential progenitors.

In the second stage, projection neurons and interneurons migrate in a radial and tangential fashion respectively towards the pial surface and settle in an inside-out pattern within the cortical plate between the 6^{th} and 7^{th} week of gestation which peaks between the 11^{th} and 15^{th} week and is completed by around 24 weeks of gestation. Structural barriers of the pial surface and molecular signals arrest neuronal migration. Projection neuronal cells developing in the germinal zone of the lateral ventricles give rise to glutaminergic neurons of the neocortex as well as the neurons in the medial and lateral ganglionic eminence, and projection neuronal cells developing in the walls of the third ventricle give rise to neurons of the basal ganglia and GABA neurons. Interneurons developing in the rhombic lips in the roof of the fourth ventricle give rise to neurons of the cerebellar cortex.

In the third stage, final organization takes place into the typical six layers of the cortex. In this stage, the six-layered laminar structure of the cerebral cortex is formed. However, the development of the cortex and late migration from the germinal matrix into the cerebral cortex continues postnatally till the age of 5 months. Any disturbance in this process leads to cortical malformations and abnormal cerebral function in the form of cognitive and motor impairment and seizures as most frequent consequences. (Gressens, 2000; Letinic et al., 2002; Nadarajah et al., 2002).

Genetic mechanisms involved in cortical developmental disorders

Pathogenic variants in genes which affect neurogenesis cause microcephaly by altering regulation of transcription, cell cycle, mitotic

Table 1 Classification and genetic basis of microcephaly.

Microcephaly		Associated Genes and Conditions
i. Microcephaly with normal or mildly simplified gyri	Microcephaly vera	• *MCPH1, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, ZNF335, PHC1, CDK6, CENPE, SASS6, MFSD2A, ANKLE2, CIT, WDFY3, COPB2, KIF14, NCAPD2, NCAPD3, NCAPH, NUP37, MAP11* – Non syndromic
	Microcephaly with seizures developmental delay	• *PNKP* - Early epileptic encephalopathy 10
	Microcephaly with short stature	• *ATRX* - Seckel syndrome • *PCNT* - Microcephalic osteodysplastic primordial dwarfism type II (MOPD2)
ii. Microcephaly with simplified gyri (Microlissencephaly)		• *NDE1, TUBA1A, TUBA3E, TUBB2B, TUBB3, TUBG1* - Non syndromic • *RELN* - Norman–Roberts syndrome • *RNU4ATAC* - Microcephalic osteodysplastic primordial dwarfism type I (MOPD1)
iii. Microcephaly with other brain malformations		• *LIS1, DCX, DYNC1H1, KIF5C, TUBA1A, TUBB2B, TUBB3, TUBG1* - Non syndromic

spindle disruption, centrosome duplication and maturation, and DNA repair. Pathogenic variants in genes involved in specific pathways that control cell proliferation like the mTOR pathway are involved in the pathogenesis of megalencephaly and focal cortical dysplasias and tubers. Abnormal neuronal migration results from pathogenic variants in genes regulating the organization and stability of microtubules, cytoplasmic dynein function, conversion of nascent post-mitotic neurons to multipolar pre-migratory cells and conversion of multipolar to bipolar migratory cells that facilitate neuronal motility (Lee, 2017). Pathogenic variants in genes involved in the regional patterning of the cerebral cortex during early stages of development i.e. during the proliferation and migration phase, mainly lead to malformations of cerebral cortical development.

Main malformations of cerebral cortical development

(Guerrini & Parrini, 2010; Barkovich et al., 2012; Najm et al., 2018; Spalice et al., 2009; Desikan et al., 2016; Kim et al., 2019; Oegema et al., 2020; Barkovich et al., 2004; Abdel Razek et al., 2009)

1. Microcephaly:

Microcephaly is defined as occipitofrontal (head) circumference (OFC) less than third centile compared to the normal standards for age, sex and ethnicity. It is caused by decreased proliferation or increased apoptosis of neuronal glial cells. See Table 1.

Clinical presentation

Microcephaly vera usually presents with mild to moderate intellectual disability, rarely seizures. and those with microlissencephaly are encephalopathic at birth and have global developmental delay (GDD) or may have normal neonatal and infantile development followed by seizures. The syndromic forms present with dysmorphic features, congenital anomalies, short stature and global developmental delay.

MRI findings

In microcephaly with simplified gyri, the cortex is of normal thickness with reduced sulcation and in microlissencephaly the cortex is abnormally thick with reduced sulcation.

2. Macrocephaly and Megalencephaly

Macrocephaly is defined as occipitofrontal (head) circumference greater than 97th centile

Table 2 Classification and genetic basis of macrocephaly and megalencephaly.

Macrocephaly/ Megalencephaly		Associated Genes and Conditions
i. Megalencephaly with or without other cerebral anomalies	With short stature	• *FGFR3* - Achondroplasia
	With gigantism	• *NSD1, NFIX, APC2* - Sotos syndrome 1,2,3 • *EZH2* - Weaver syndrome • *PTEN* - Bannayan-Riley-Ruvalcaba syndrome • *GPC3* - Simpson-Golabi-Behmel syndrome • *PIK3CA* - Congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal/spinal anomalies (CLOVES) syndrome
	Metabolic	**Lysosomal storage disorders** • *HEXA* - Tay-Sachs disease • *HEXB* – Sandhoff disease
		Leucoencephalopathies • *ASPA* - Canavan disease • *GFAP* - Alexander disease • *MLC1, HEPACAM* - Megalencephalic leukoencephalopathy with subcortical cysts
		Organic acidurias • *GCDH* - Glutaric aciduria type 1 • *D2HGDH* – D-2-hydroxy glutaric aciduria
ii. Megalencephaly with polymicrogyria		• *PIK3R2* - Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) • *PIK3CA* - Megalencephaly-capillary malformation syndrome (MCAP)
iii. Hemimegalencephaly		• *FGFR3, PIK3CA, HRAS* - somatic mutations • *AKT1*(somatic mutation)- Proteus syndrome • *PIK3CA* (somatic mutation)- Klippel-Trénaunay-Weber syndrome • Hypomelanosis of Ito

compared to age-matched and sex-matched normal standards. Megalencephaly refers to an abnormally large size of the brain and is defined as brain weight greater than average for the age and gender of the child. These are caused secondary to increased proliferation or decreased apoptosis of neuronal glial cells. See Table 2.

Clinical presentation

These cases present with features related to the specific syndrome/disorder. Hemimegalencephaly presents with contralateral hemiparesis, epilepsy, and intellectual disability.

MRI findings

The enlarged hemispheres usually show gyral abnormalities in the form of agyria/pachygyria or polymicrogyria, and the lateral ventricle is often enlarged, boundaries of gray and white matter may be blurred, and gray matter heterotopias may be found.

3. Periventricular nodular heterotopias

Periventricular nodular heterotopias are formed due to abnormal neuronal migration. See Table 3.

Clinical presentation

Around 90% of patients present with various types of seizures, mostly in adolescence. Females affected with X-linked PNH typically present with epilepsy, commonly generalized tonic-clonic or complex partial seizures and dyslexia, with usually normal intelligence.

Table 3 Classification and genetic basis of periventricular nodular heterotopias.

Periventricular nodular heterotopias	Associated Genes/ Chromosomal Loci and Conditions
i. X-linked periventricular heterotopia	• *FLNA* - Non syndromic
ii. Autosomal recessive periventricular heterotopia with microcephaly	• *ARFGEF2* - Non syndromic
iii. Heterotopia due to chromosomal aberration	• 1p36 deletion - 1p36 del syndrome • 4p deletion - 4p del syndrome (Wolf-Hirschhorn syndrome) • 5p deletion - Cri du chat syndrome • 7q11.2 deletion - Williams syndrome • 22q11.2 deletion - DiGeorge syndrome • Xp22.3 deletion – associated with Steroid sulfatase deficiency

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Table 4 Classification and genetic basis of lissencephaly.

Lissencephaly	Associated Genes and Conditions
i. Classic lissencephaly/ Subcortical band heterotopia	• *LIS1 –LIS1*- associated non-syndromic lissencephaly; with chromosome 17p13.3 deletion - Miller-Dieker syndrome • *ACTB, ACTG1* - Baraitser-Winter syndrome • *DCX* – Non syndromic X-linked lissencephaly 1 • *ARX* - X-linked lissencephaly 2 with abnormal genitalia • *TUB1A - TUB1A* associated lissencephaly • *TUBA1A, TUBB2B, RELN, CASK, VLDLR, WDR81, CA8, ATP8A2* - Lissencephaly with cerebellar hypoplasia
ii. Cobblestone lissencephaly	• *POMT1, POMT2, CRPPA, FKTN, FKRP, LARGE1* - Walker-Warburg syndrome • *POMGNT1* - Muscle eye brain disease • *FKTN* - Fukuyama congenital muscular dystrophy

MRI findings

The heterotopias appear as round or oval nodules in the wall of the ventricle which project into the ventricular lumen or may be in the periventricular white matter.

4. Lissencephaly spectrum

Classic lissencephaly is due to abnormal neuronal migration. Cobblestone lissencephaly is due to abnormal over-migration of neurons through breaches in the pial surface, and gliovascular proliferation. See Table 4.

Clinical presentation

Patients with classic lissencephaly tend to be neurologically abnormal from birth, with hypotonia initially followed by hypertonia and early onset of epilepsy and global developmental delay. Those who are less severely affected may achieve normal developmental milestones but develop epilepsy in late infancy or in childhood and those with cobblestone lissencephaly present with hypotonia at birth, generalized muscle weakness, and joint contractures of variable degree.

DCX mutations cause classic lissencephaly with mental retardation in hemizygous males and a milder phenotype with seizures and subcortical band heterotopia in females, sometimes in the same family. Affected females usually have normal cognitive function.

MRI findings

In classic lissencephaly (Figure 1) sulcation is completely absent (agyria) or few broad, flat gyri separated by a few shallow sulci (pachygyria)

with abnormally thick cerebral cortex may be present. The frontal and temporal opercula are not developed, leading to a characteristic 'figure of 8' appearance of the brain on axial images. In most females with *DCX* mutations and in patients with missense mutations in *LIS1*, subcortical band heterotopias are seen which is characterized by the presence of nodules or nodular curvilinear bands of gray matter that extend from the ventricular wall to the cerebral cortex as a thin layer of white matter between 2 layers of gray matter. In patients with *DCX* mutations, the band heterotopia are located in the frontal region and in *LIS1* mutations they are located in the parieto-occipital region. In *ARX*-related lissencephaly, the corpus callosum is always completely absent and the basal ganglia are either hypoplastic or dysplastic or completely absent.

Figure 1 T1-weighted MRI brain axial image of classic lissencephaly.

Cobblestone lissencephaly is seen in dystroglycanopathies. The findings in Walker-Warburg syndrome are thin cortex with few sulci, unmyelinated white matter, hydrocephalus/severe ventriculomegaly, thin/hypoplastic corpus callosum, hypoplastic cerebellum and vermis, and small and dysplastic ocular globes. Muscle-eye-brain disease has a less dysmorphic appearance of the cerebral cortex with slight irregularity of the inner and outer surfaces of the cortex, hypomyelination, hypoplastic cerebellum and vermis with abnormal folial pattern and multiple cysts in the cerebellum below the surface of the cortex, and small ocular globes with subretinal fluid collections. Fukuyama congenital muscular dystrophy has appearance of cortex which resembles that of Walker-Warburg syndrome with polymicrogyria in the frontal cortex. The myelination pattern looks similar to that seen in muscle-eye-brain disease.

5. Polymicrogyria and schizencephaly

These malformations are due to abnormal post-migrational organization. See Table 5.

Clinical presentation

Clinical presentation of these patients includes global developmental delay, refractory seizures, and bilateral pyramidal and cerebellar signs, depending on the pattern of distribution of polymicrogyria. Unilateral polymicrogyria presents in infancy with congenital hemiplegia. In addition, features related to specific syndromes are noted in syndromic presentations.

Closed-lip schizencephaly often presents with hemiparesis or motor delay. Open-lip schizencephaly may present with seizures, hemiparesis or motor delay.

MRI findings

Polymicrogyria (Figure 2) involves almost any area of the cerebral cortex but those adjacent to the sylvian fissures are preferentially involved than other parts of cortex. In the neonatal period, the affected cortex appears very thin and irregularly undulating. After complete myelination, the cortex becomes thicker and smoother with irregular, bumpy inner and outer cortical surfaces, broad gyri, and shallow sulci. The MRI appearance of schizencephaly shows cerebrospinal fluid extending from the subarachnoid space into the lateral ventricle, and the walls of this cleft are lined by dysmorphic gray matter. The shape of at least a part of the lateral ventricle is seen even in large bilateral clefts which helps in differentiating this from hydranencephaly.

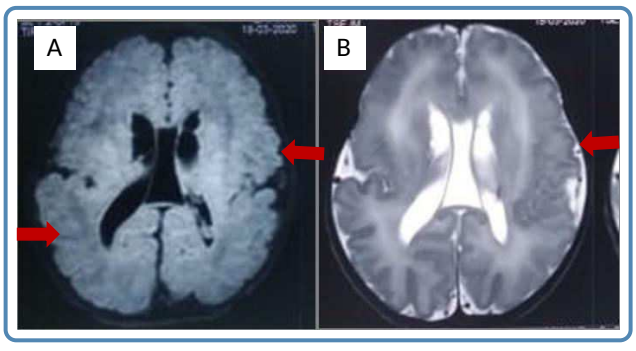

Figure 2 A. T1-weighted MRI brain axial image with extensive polymicrogyria, and B. T2-weighted axial image with shallow sulci in the frontoparietal region.

Table 5 Classification and genetic basis of polymicrogyria and schizencephaly.

Polymicrogyria/ Schizencephaly	Associated Genes and Chromosomal Loci and Conditions
i. Polymicrogyria (classic) with trans-mantle clefts (schizencephaly-closed lip/type 1 or open lip/type 2) or calcification	• *EMX2* - Non syndromic • *OCLN* – Pseudo-TORCH syndrome 1
ii. Polymicrogyria without clefts or calcifications classified by location	More than 40 genes associated with the following groups of disorders: • mTORopathies • Tubulinopathies • Alpha dystroglycanopathies • Laminopathies • Congenital disorders of glycosylation
iii. Syndromes with polymicrogyria	• *PIK3R2* - Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) • *PIK3R2* - Megalencephaly-capillary malformation syndrome (MCAP) • *KIAA1279* - Goldberg-Shprintzen megacolon syndrome • *INPP5E, TMEM216,* & more than 30 other genes - Joubert syndrome • *RAB3GAP1* – Micro syndrome • *COL3A1* - Polymicrogyria with or without vascular-type Ehlers-Danlos syndrome
iv. Polymicrogyria due to chromosomal aberration	• 22q11.2 deletion- DiGeorge syndrome • Deletion of 1p36,4q21, 6q26, 13q3, 18p11, and 21q2 • Duplication of 2p13

6. Focal cortical dysplasia (FCD)

FCD type I and Type III are due to abnormal post-migrational organization and FCD type II is due to abnormal proliferation and differentiation of neuronal glial cells. See Table 6.

Clinical presentation

Patients usually present with partial epilepsy which may generalize and typically becomes clinically apparent during the first decade of life or sometimes as early as in the early neonatal period. The epilepsy is often refractory to medication.

MRI findings

Focal cortical thickening and blurring of the cortical-white matter junction with abnormal signal intensity can be identified extending from the cortical-white matter junction to the superolateral margin of the lateral ventricular surface (Figure 3). The signal intensity of this abnormality varies with the age of the patient. In neonates and infants, it is bright on T1-weighted images and dark on T2-weighted images. In late childhood and adults, it is seen as T2 hyperintensity. Single photon emission computed tomography (SPECT) or positron emission tomography (PET) are sometimes needed for identifying the anomaly as the dysplasia may not be identified in standard MRI images.

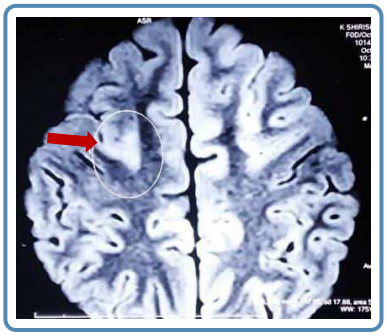

Figure 3 MRI brain showing focal cortical dysplasia type 1b in the frontoparietal region.

Table 6 Classification and genetic basis of focal cortical dysplasia.

Focal cortical dysplasia (FCD)		Associated Genes and Conditions
i. Focal cortical dysplasia type I	FCD Ia - Focal cortical dysplasia with abnormal radial cortical lamination	-
	FCD Ib - Focal cortical dysplasia with abnormal tangential cortical lamination	-
	FCD Ic - Focal cortical dysplasia with abnormal radial and tangential cortical lamination	-
ii. Focal cortical dysplasia type II	FCD IIA - Focal cortical dysplasia with dysmorphic neurons	*MTOR, DEPDC5, PIK3CA*
	FCD IIB -Taylor type- Focal cortical dysplasia with dysmorphic neurons and balloon cells	*MTOR*, *DEPDC5*, *NPRL3*
iii. Focal cortical dysplasia type III	FCD IIIa - Cortical lamination abnormalities in the temporal lobe associated with hippocampal sclerosis	-
	FCD IIIb - Cortical lamination abnormalities adjacent to a glial or glioneuronal tumor	-
	FCD IIIc - Cortical lamination abnormalities adjacent to vascular malformation	-
	FCD IIId - Cortical lamination abnormalities adjacent to any other lesion acquired during early life, e.g., trauma, ischemic injury, encephalitis	-

7. Dysgyria

Dysgyria is described as variable cortical thickness with an abnormal gyral pattern characterized by abnormalities in depth or orientation of sulci and does not meet the classic features of any of the above mentioned main cortical malformations.

Approach to cerebral cortical malformations (Oegema et al., 2020)

The following clinical and investigative approach is recommended for evaluation of patients with cerebral cortical malformations and neuronal migration disorders.

i. History (including family history):

- Prenatal history of maternal fever or rash to rule out infectious etiology; maternal comorbidities like uncontrolled hypothyroid and diabetes; ultrasound abnormalities; reduced fetal movements to rule out dystroglycanopathies.
- Detailed three-generation family pedigree, history of consanguinity and family history of congenital anomalies, global developmental delay and seizures.

ii. Clinical assessment; common clinical findings include:

- **Symptoms -** Seizures/epilepsy; feeding difficulties; breathing difficulties; global developmental delay; visual defects
- **Signs -** Microcephaly or macrocephaly; dysmorphic features; congenital malformations; hypotonia or hypertonia

iii. Genetic evaluation:

The genetic test to be done would depend on the clinical diagnosis. Whole exome sequencing (preferably patient-parents trio) would be preferred for evaluation of monogenic conditions, while chromosomal microarray would help detect chromosomal copy number variations. For clinically suspected specific microdeletion syndromes such as Miller-Dieker syndrome, targeted testing through fluorescence in situ hybridization (FISH) or multiplex

ligation-dependent probe amplification (MLPA) may be done.

iv. Additional tests (as applicable): Ophthalmological evaluation; hearing evaluation; serum creatine phosphokinase and electromyography; metabolic testing

v. Management: There is no disease-specific treatment available at present for the cortical malformations and neuronal migration disorders. Management includes symptomatic and supportive care including physiotherapy, occupational therapy, and antiseizures medication. Surgical intervention is often considered for patients with refractory epilepsy as in cases with focal cortical dysplasia.

Conclusion

Malformations of cortical development have been increasingly recognized by MRI Brain. With the availability of next-generation sequencing based molecular genetic testing over the last few years, the exact etiological diagnosis of cortical malformations is being made in more and more cases, and novel associated genes are being identified. Identification of the exact disease-causing mutation in the index child helps in appropriate counseling of the family, ascertaining the pattern of inheritance, the recurrence risk in future offspring and in definitive prenatal testing of their subsequent conceptions. The exact understanding of molecular pathways and causative genes of normal cerebral cortical development may facilitate early therapeutic options/interventions in the near future.

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GeNeXpreSS

Next-Generation Phenotyping in the Next-Generation Sequencing Era

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Next-generation phenotyping using computer vision algorithms in rare genomic neurodevelopmental disorders (van der Donk et al., 2019)

The authors used a novel algorithm by combining two computer algorithms: the Clinical Face Phenotype Space (CFPS) for facial dysmorphism and OpenFace for facial recognition. Using them, they detected the facial gestalt in three novel intellectual disability syndromes involving the genes *PACS1*, *PPM1D*, and *PHIP*. Significant facial similarity for all three syndromes was found. Hence information contained in the face can be used to delineate genetic entities including in novel ID syndromes with no previously known knowledge of a facial phenotype.

Evaluating Face2Gene as a tool to identify Cornelia de Lange syndrome by facial phenotypes (Latorre-Pellicer et al., 2020)

This study explored the sensitivity of artificial intelligence by means of Face2Gene technology for facial recognition in a group of 49 patients with molecularly confirmed Cornelia de Lange syndrome with mutations in *NIPBL*, *SMC1A*, *HDAC8* and *RAD21* genes. Cornelia de Lange, which can be diagnosed clinically but has features that vary widely in range and severity, was the first diagnosis in 41/49 patients and one of the top five diagnosis in 47/49 cases giving a sensitivity of 83.7% and 97.9% respectively. The other top five diagnoses were KBG syndrome, CHARGE syndrome, Rubinstein–Taybi syndrome and Moebius syndrome, with frequencies of 44.89% (22/49), 36.7% (18/49), 34.7% (17/49), and 18.4% (9/49), respectively. Although substantial difference in sensitivity regarding the age at which facial images were taken was not present, the sensitivity differed with the affected gene and presence of classical features with high sensitivity noted in patients with *NIBPL* variants (97%) and those with the classical phenotype (88.8%). Thus, each gene presented a different pattern recognition and this can be utilized for studying the genotype–phenotype correlations and to differentiate between genetic subtypes. For example, it has been described that thicker eyebrows are suggestive of a variation in *SMC1A* or *SMC3*, and females containing variants in *HDAC8* tend to have hypertelorism and a slightly bulbous nasal tip. Patients with *NIPBL* variants show pronounced facial features, compared to patients with *RAD21* variants who have less prominent features.

Computer-aided facial analysis in diagnosing dysmorphic syndromes in Indian children (Narayanan et al., 2019)

This study used Face2Gene to assess its utility in predicting the diagnosis in 51 Indian children with obvious facial dysmorphism and a definite molecular or cytogenetic diagnosis. A correct diagnosis as the first suggestion was found in 26 patients (50.9%) and as a part of the top ten suggestions was obtained in 37 patients (72.5%). This study highlights that the results of the software can change based on the ethnicity as the software was unable to provide a diagnosis in easily recognizable syndromes like Turner syndrome, Waardenburg syndrome and Wolf-Hirschhorn syndrome. Since Face2Gene learns from every solved case, its sensitivity is

likely to improve further with increasing use particularly in non-Caucasian populations.

PEDIA: prioritization of exome data by image analysis (Hsieh et al., 2019)

This paper assessed the value added by computer assisted image analysis (DeepGestalt) to the diagnostic yield on a cohort consisting of 679 individuals with 105 different monogenic disorders. For every case, scores from DeepGestalt were used along with the clinical features and CADD score of the causative variant and a PEDIA score was generated. The additional information from the photographs pushed the correct disease gene to the top 10 in 99% of all PEDIA cases from less than 45% when only CADD scores were used. The accuracy rate for the top one gene rose from 36–74% without DeepGestalt scores to 86–89% when artificial intelligence was used. The results were not affected by the ethnicity of the patients, however low accuracy was seen in very rare disease due to limited training for those particular genes.

Precision medicine integrating whole–genome sequencing, comprehensive metabolomics, and advanced imaging (Hou et al., 2019)

A cohort of 1190 adult volunteers underwent whole genome sequencing followed by deep phenotyping by metabolomics, advanced imaging, and clinical laboratory tests in addition to family/medical history. Integrating the results of WGS with deep phenotyping, 11.5% individuals had a pathogenic variant, thereby providing a plausible genetic cause for abnormal physiological measurements at the individual level of analysis. A high percentage of genotype phenotype correlation was observed for dyslipidemia, cardiomyopathy and arrhythmia, and diabetes and endocrine diseases. With deep phenotyping, heterozygous carriers of autosomal recessive diseases were also found to exhibit detectable phenotypic changes. Sixty-nine (5.8%) individuals had pathogenic/ likely pathogenic variants but did not have associated family history, medical history, or phenotypes detected in tests. This could be because of reduced penetrance, variable expressivity, or late onset of disease presentation.

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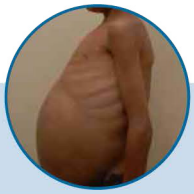
Answers to CrossWord - 01

The solution to Crossword - 01 of the previous issue is given online and can be accessed through this link:
http://www.iamg.in/genetic_clinics/crossword_01_answers.pdf

Correct Responses to Crossword - 01 were given by:

1. Dr Beena Suresh, Mediscan Systems, Chennai
2. Dr Ravneet Bhatia

Are you suspecting a **Lysosomal Storage Disorder (LSD)** in your patient?



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