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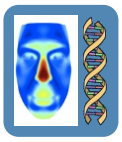

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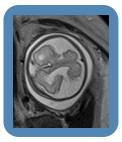

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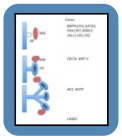

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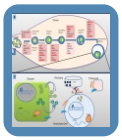

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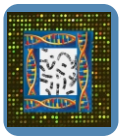

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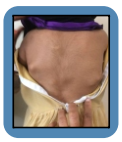

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# GeNeEvent - Manipal Genetics Update V on Genomics of Neurodevelopmental Disorders

## 9 – 10 February, 2018

Manipal Genetics Update V on Genomics of Neurodevelopmental Disorders, organized by the Department of Medical Genetics, Kasturba Medical College, Manipal Academy of Higher Education, was held on 9th and 10th of February, 2018 at Manipal, Karnataka. The event brought together international and national experts in the field of neurodevelopmental and neurogenetics research. The first day of the event commenced with inauguration and the John M Opitz Award Presentation to Dr Gandham SriLakshmi Bhavani by Dr John Carey, Editor-in-Chief Emeritus, American Journal of Medical Genetics. The researchers deliberated in detail about the current standards for best practices in diagnosis, treatment, support services and prevention of neurodevelopmental disorders. The conference served as a platform for the delegates to become acquainted with the research findings in the field and exchange ideas and perspectives on current challenges in the diagnosis and management of these disorders. The event also provided an excellent opportunity to establish strong collaborations for research on neurogenetics.

## GeneVerse

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### *Musings of a Fetus*

Confined for nine months in this dark cocoon,
Hoping to come out into the bright world outside really soon.
Thank you for nourishing me, my beloved life-giving mother!
On seeing me, don't break my little heart by wishing I were someone other.

GeNeDit

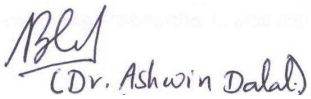
# 'Face to Face' with our Genes

## Editorial

Human face is the mirror to our brains. Face is an important part of the body. We identify ourselves and others based on the facial characteristics. Human face gives identity to an individual. The embryonic development of human face involves complex interplay of large number of developmental events. Hence it is but obvious that face development would be affected in dysmorphic syndromes resulting from mutations in developmental genes. Most of the human dysmorphic syndromes have typical facial characteristics as an important component. The classic text on dysmorphic syndromes by Gorlin has been suitably titled as "Syndromes of Head and Neck". Most descriptions of dysmorphic syndromes include subjective information provided by individual investigators and this leads to lot of confusion for use in clinical practice. There have been many efforts to standardize the terms used in dysmorphology including the special issue of American Journal of Medical Genetics on "Elements of morphology". However the 'subjectiveness' in the assessment is always a confounding factor. Hence there have been efforts towards introducing 'objectiveness' in dysmorphic feature reporting. The initial attempts involved use of two dimensional photographs of individuals. Landmarks were plotted on the photographs at identifiable sites like 'corner of mouth', 'tip of nose' etc. and then a complex statistical analysis would be used to differentiate between 'normal' and 'dysmorphic' facies. Later the investigators used three dimensional images and landmark acquisition which further refined this technique. Recently a mobile application called Face2Gene has been extensively used for facial recognition of various dysmorphic syndromes. Facial gestalt recognition is an art perfected over years of practice but beginners can be significantly benefitted by the use of such apps/software which can aid in diagnosis as well as plan for genetic investigations.

The ability to include 'objectiveness' in face recognition has opened a Pandora's box of possibilities. Face recognition has been used in mobile phones as passwords as well as by law enforcement agencies to track and find criminals. The idea that study of human genetic makeup could help in prediction of 'human face' appears to be science fiction but the same has been recently demonstrated in an article published in Proceedings of National Academy of Sciences (PNAS), USA. The authors have used genetic information from whole genome sequencing data to predict the facial phenotype as well as other demographic details of an individual like eye colour, skin colour, ethnicity, etc with considerable accuracy. This has resulted in renewed discussions about privacy of human genomic data and possible misuse of such techniques for unlawful activities as well as for planning of 'designer babies' with beautiful facial characteristics. As is true with any new breakthrough in science, it remains to be seen how the technology shapes itself in the future.

As clinicians and medical geneticists, it is our responsibility to shape such discussions in future so that the new developments are used for better care of patients and families. 'Genetic clinics' is an effort towards achieving this goal through dissemination of useful and accurate information from the complex world of human genetics to the clinicians in practice.



Dr. Ashwin Dalal
Assistant Editor
1st April, 2018

# Fetal Schizencephaly Associated with Complex Cardiac and Limb Defects

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## Abstract

We report a case of prenatally diagnosed schizencephaly in association with cardiac and limb defects. The diagnosis was made by antenatal ultrasound and fetal echocardiography at 28 weeks of gestation and corroborated by fetal MRI. There was unilateral open lip schizencephaly along with limb defects and cardiac anomalies such as common arterial trunk and large ventricular septal defect. The newborn delivered at term expired immediately after birth. Schizencephaly is rarely diagnosed in prenatal life with most cases recognized after birth. As prognosis is related to the extent of the lesion and associated malformations, prenatal diagnosis of schizencephaly should prompt a thorough evaluation for the same.

## Introduction

Schizencephaly is a rare congenital anomaly characterized by deep clefts in the brain surrounded by heterotopic gray matter and may extend from ependyma of the ventricles to the pia mater. It is rarely diagnosed prenatally with most cases recognized only after birth. We report a case of schizencephaly with associated cardiac and limb anomalies in a fetus and propose a model for the hypothesized theory of vascular insult in the pathogenesis.

## Case report

A 23-year-old second gravida at 28 weeks of gestation with one previous normal child, was referred in view of multiple anomalies in the fetus. Other than Rh incompatibility and previous Caesarean section, there was no relevant antenatal or family history. Routine antenatal investigations including the blood sugars and thyroid function tests were normal. Antenatal ultrasound revealed a unilateral deep clefting of the Sylvian fissure in the left cerebral hemisphere extending up to the lateral ventricle. Fetal MRI showed the extent of the cleft and confirmed the open type, large, unilateral schizencephaly (Figure 1 A & B). On fetal echocardiography, there was a large ventricular septal defect and a common arterial trunk (Figure 1C). Amniocentesis followed by fetal karyotyping and fluorescence in situ hybridization (FISH) for 22q deletion were done, which were reported to be normal. Poor fetal outcome was explained to the parents in view of multiple major anomalies. A male baby, weighing 2.8 kg was delivered at term, who expired soon after birth. An external examination of the baby revealed ectrodactyly and no other obvious malformation. Fetal autopsy for internal examination could not be done as parents declined the same. We propose that the schizencephaly in this fetus could have been the result of an early vascular insult secondary to major cardiac defects.

## Discussion

Schizencephaly is defined as a fluid-filled cavity in the fetal brain, which may communicate with the lateral ventricle and subarachnoid space. The incidence is estimated to be 1.54 in 100,000 live births (Howe et al., 2012). Schizencephaly is thought to be secondary to multiple factors leading to a final common manifestation of abnormal neuronal migration. It could be the result of a congenital malformation of cortical development or follow a destructive process with various factors implicated in pathogenesis such as toxins, maternal warfarin, cytomegalovirus infection, genetic factors and vascular injury and can occur in association with various syndromes (Montenegro et al., 2002).

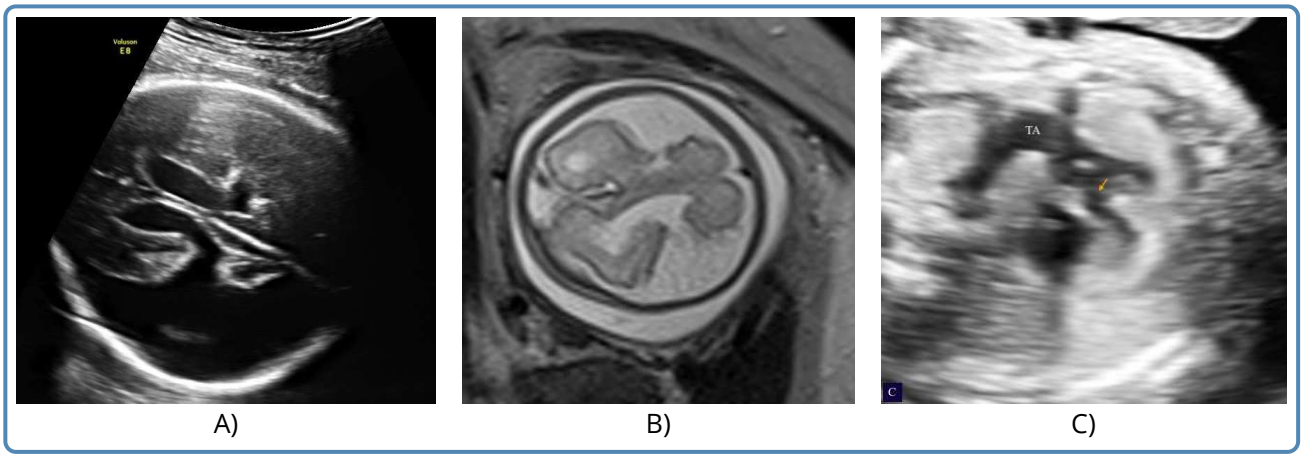


**Figure 1** A) Fetal sonogram showing unilateral deep clefting of the Sylvian fissure in the left cerebral hemisphere extending up to the lateral ventricle. B) Fetal MRI showing the open type, large, unilateral schizencephaly. C) Fetal echocardiogram showing a common arterial trunk and ventricular septal defect (indicated with the yellow arrow).

Familial cases have been reported, suggesting a possible genetic origin and heterozygous mutations of the *EMX2* gene have been associated with schizencephaly (Granata et al., 1997). The only significant and probably etiological factor in our case was the presence of complex cardiac defects which could have resulted in an early vascular injury.

The clefts of fetal schizencephaly may extend through the hemispheres from the ventricles to the pial surface. It can be unilateral or bilateral and of the closed-lip or open-lip variety. Closed-lip schizencephaly is characterized by gray matter–lined lips that are in contact with each other (type 1) and open-lip schizencephaly has separated lips and a cleft filled with cerebrospinal fluid (CSF), extending to the underlying ventricle (type 2) (Yakovlev & Wadsworth, 1946). Schizencephaly is a rare diagnosis in prenatal medicine and most cases have been diagnosed after 28 weeks of gestation or after birth (Howe et al., 2005). Two-dimensional ultrasonography is the main prenatal diagnostic tool; however, the clefts are often not easily identified on ultrasound and it may be necessary to use other imaging modalities such as magnetic resonance imaging (MRI). MRI can also aid in differentiating schizencephaly from other possible diagnosis and in delineating associated brain anomalies. Various CSF containing lesions of the brain can be confused as schizencephaly which include developmental lesions like arachnoid cyst, mono ventricle in holoprosencephaly, agenesis of corpus callosum with an interhemispheric cyst and destructive lesions such as porencephalic cyst, ventriculomegaly and hydranencephaly (Oh et al; 2005). The prenatal ultrasonogram and fetal MRI in this case revealed a deep cleft in the region of Sylvian fissure extending all the way from the surface of brain up to the left lateral ventricle. Thus, a diagnosis of unilateral (left) schizencephaly of open-lip type 2 variety was made.

The clinical phenotypes associated with schizencephaly vary widely, depending on the size of the defect, unilateral versus bilateral defect, and open versus closed-lip defect. Affected individuals most often present with varying degrees of developmental delay, motor impairment and seizures. Open-lip clefts usually result in the most significant impairment, while unilateral clefts have a less severe clinical phenotype. If there is a small, unilateral closed-lip cleft without involvement of the motor cortex, the patient is usually normal except for seizures (Guerrini & Carrozzo, 2001; Liang et al., 2002). Schizencephaly can be associated with other anomalies including facial malformations, ventriculomegaly, polymicrogyria, pachygyria, heterotopias and lissencephaly, absence of the cavum septum pellucidum, agenesis of the corpus callosum and septo-optic dysplasia (Yakovlev & Wadsworth, 1946). Prenatal diagnosis of schizencephaly should therefore prompt a thorough search for other anomalies and counseling should be based on the extent of lesion, gestational age at diagnosis and associated anomalies. In our case, there was a large, unilateral open cleft in the brain along with complex major cardiac defects. Hence, parents were counselled about the expected poor outcome which was later evident with the early death of the

baby.

## Conclusion

Prenatal diagnosis of schizencephaly is possible by ultrasound and fetal MRI can aid in further characterization of the extent and type of cleft. As no postnatal therapeutic options exist at present, accurate delineation of the extent of lesion and a thorough search for associated malformations should be done to help in counseling the parents about the prognosis, outcome and further management.

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GeNeViSTA

# Genetics of Congenital Abnormalities of Kidney and Urinary Tract (CAKUT)

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## Abstract

Congenital Abnormalities of Kidney and Urinary Tract (CAKUT) are amongst the most common malformations in humans. Most CAKUT are sporadic in origin though single gene mutations have been identified in syndromic and some non-syndromic CAKUT. This article briefly reviews the recent advances in the genetics of CAKUT.

## Introduction

Congenital Abnormalities of Kidney and Urinary Tract (CAKUT) encompass a wide range of structural malformations, which occur due to a defect in the morphogenesis of the kidney and urinary tract. CAKUT are among the most common birth defects, accounting for 20-30% of all birth defects (Loane et al., 2011). The estimated incidence of CAKUT is 3 to 6 per 1000 live births (Nicolaou et al., 2015). CAKUT account for 40-50% of children with chronic kidney disease (Vivante et al., 2014). CAKUT are the most frequent malformations detected by antenatal ultrasound scan (Wiesel et al., 2005)

The phenotypes included under CAKUT are structural abnormalities like renal agenesis, renal hypoplasia (renal length less than 2SD below the mean for age with normal architecture), renal dysplasia (malformation of tissue elements), multicystic dysplastic kidneys, horse shoe kidney, vesicoureteric reflux (VUR), megaureter, duplex collecting system, ectopic ureter, ureterocele and posterior urethral valve. These anomalies can exist as single entities or can occur concurrently as in VUR and duplex collecting system. These abnormalities may be seen in an orthotopic kidney or ectopic kidney. Around 50% of CAKUT have associated lower urinary tract abnormalities and uretero-pelvic junction obstruction is the most common phenotype seen in 20% (Capone et al., 2017). Most of these abnormalities arise due to a disruption of normal nephrogenesis either due to environmental factors or due to dysfunction of the genes involved in that process.

Understanding the genetics of CAKUT is essential for early diagnosis and early initiation of treatment and for prevention of end stage renal disease, especially in children. This brief review summarizes the classification of CAKUT, genetic approaches used to identify genes implicated in CAKUT and the genes causing syndromic and non-syndromic CAKUT.

## Classification

CAKUT can be classified into syndromic and non-syndromic types, based on whether systems other than the renal system are involved. CAKUT can occur as part of a known genetic syndrome with congenital abnormalities outside the urinary tract. For example, renal agenesis can occur as part of the Townes-Brocks syndrome (OMIM #107480), Kallmann syndrome (OMIM #308700) or Branchiotorenal syndrome (OMIM # 113650). Some of the common syndromic causes for CAKUT along with the implicated genes are listed in Table 1. In non-syndromic CAKUT structural abnormalities are limited to the kidney and urinary tract.

## Evidence for a genetic etiology in CAKUT

Genetic basis for CAKUT was suspected because of familial segregation of renal anomalies like renal agenesis, renal hypodysplasia and multicystic dysplastic kidneys. Many such families have been described in literature, suggesting autosomal dominant inheritance with reduced penetrance (Monn & Nordshus, 1984; McPherson et al.,1987; Kalpan et al., 1989). Known syndromes with CAKUT and other extra-renal manifestations with a single gene

Table 1 Some syndromic causes for CAKUT and their renal phenotypes.

| Syndrome | Genes | Renal phenotype |
|---|---|---|
| Alagille syndrome | *JAG1, NOTCH2* | Cystic kidneys |
| Branchiootorenal syndrome | *EYA1, SIX5* | Unilateral or bilateral renal agenesis, hypoplasia, collecting system abnormalities |
| Campomelic dysplasia | *SOX9* | Hydronephrosis |
| Fraser syndrome | *FRAS1, FREM2, GRIP1* | Renal agenesis or hypoplasia |
| Kallmann syndrome | *KAL1, PROKR2* | Renal aplasia |
| Meckel-Gruber syndrome | *MKS1, TMEM216, TMEM67, CEP290, TMEM231, TMEM107* | Renal agenesis, cystic kidneys, duplicated ureter, hypoplastic bladder |
| Pallister-Hall syndrome | *GLI3* | Renal ectopia, renal dysplasia |
| Papillorenal syndrome/ Renal coloboma syndrome | *PAX2* | Renal hypoplasia, cysts, Multicystic dysplastic kidneys, VUR |
| Townes-Brocks syndrome | *SALL1, DACT1* | Ectopic kidney, hypoplastic, multicystic dysplastic kidneys |

etiology also point to the existence of a genetic basis for CAKUT. Monogenic mouse models, which show a CAKUT phenotype, also indicate a genetic basis for these disorders.

## Etiopathogenesis of CAKUT

The etiology of CAKUT is complex with environmental, genetic and epigenetic factors playing a role in disease causation.

- Environmental factors: Renal agenesis was shown to have a significant association with pregestational maternal diabetes mellitus. A fetus with an early exposure to diabetes in utero has an increased risk of CAKUT (Dart et al., 2015) and it has been recommended that maternal diabetes should be included in the evaluation of CAKUT. Maternal intake of angiotensin converting enzyme inhibitors (ACEI) during the first trimester is associated with an increased risk of renal dysplasia in the fetus (Cooper et al., 2006). In utero exposure to cocaine and alcohol have been linked to a higher occurrence of CAKUT in fetuses. (Yosipiv., 2012)

- Epigenetic factors: Whole exome sequencing, exome data-based copy number variants (CNV) analysis and bisulphite sequencing were done on a pair of monozygotic twins, who were discordant for congenital renal agenesis. The analysis showed 514 differentially methylated regions with no differential single nucleotide polymorphism or CNV, suggesting that epigenetic modification can be an explanation for environmental factors causing CAKUT (Jin et al.,2014). Data suggest that epigenetic phenomena could influence nephrogenesis, predetermine disease susceptibility and account for the variable penetrance seen in CAKUT.

- Genetic factors: Before the advent of molecular diagnostic techniques, the classical anatomy theory, which highlighted the importance of the position of ureteric bud, was used to describe the etiopathogenesis of CAKUT. But with evidence from mouse models, newer insights to the molecular mechanism of development of CAKUT have been obtained.

**Nephrogenesis:** Nephrogenesis can be divided into the following stages:

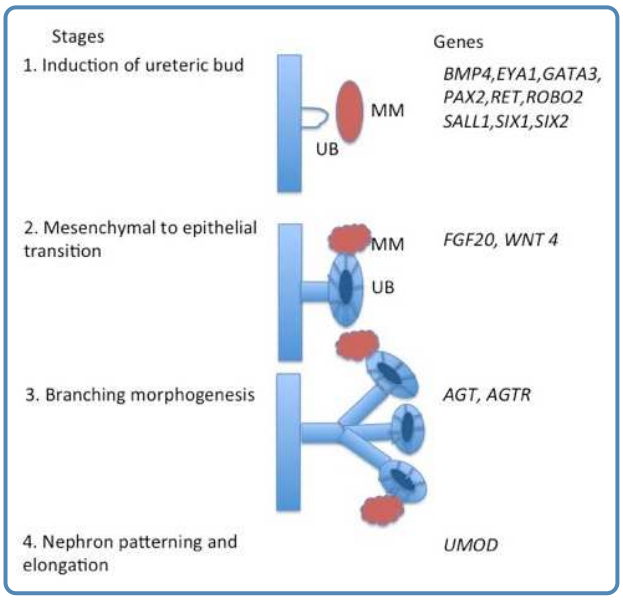
- ureteric bud induction
- mesenchymal-to-epithelial transition (MET)
- renal branching morphogenesis
- nephron patterning and elongation (which include proximal and distal tubule morphogenesis and glomerulogenesis)

This complex process is controlled by a large number of genes and signaling pathways, and alterations in these genes have been identified to cause CAKUT. The various genes implicated in the different developmental stage of nephrogenesis in mice models and human beings are shown in Figure 1 (Vivante et al., 2014).

**GDNF/RET pathway:** (Figure 2)

GDNF/RET is the most frequently studied pathway to understand the pathogenesis of CAKUT

(Capone et al., 2017). During embryonic development, Glial Derived Neurotropic Factor (GDNF) is expressed in metanephric mesenchyme along the length of the mesonephric duct. Tyrosine kinase receptor, RET and co receptor, GDNF alpha 1 (GDNFA1) are expressed in the mesonephric duct and when GDNF binds to RET and GDNFA1, ureteric bud is formed (Puri et al., 2011). Transcription factors like PAX2 (paired box gene 2), GATA3 (trans-acting T- cell-specific transcription factor GATA 3), EYA1 (eyes absent homolog 1), SI X1 (sine oculis-related homeoBox 1 homolog protein SI X1), SALL1 (Sal-like 1) and HOX11 (homeoBox 11) act as positive regulators of GDNF. The expression of the protein WNT11 in the epithelial tip of the ureteric bud propagates mesenchymal GDNF signaling.




UB: Ureteric bud; MM: Metanephric mesoderm

**Figure 1** Nephrogenesis and human genes implicated in various stages.

GDNF activity is restricted by transcription factors like FOXC1/FOXC2 (forkhead box C1 and C2) transcription factors and the SLIT2–ROBO2 (slit homolog 2–roundabout homolog 2). The receptor tyrosine kinase antagonist Sprouty1 (SPRY1) negatively regulates GDNF/RET signaling. Negative regulation of GDNF is important to ensure a single ureteric bud and mutations involving negative regulators have shown to cause multiple ureteric buds in mouse models. Transforming growth factor $\beta$1 (TGFB1) and bone morphogenic protein 4 (BMP4) are endogenous inhibitors of the GDNF/RET signaling pathway and restrict the outgrowth of the ureteric bud to a single location. FGFR2, independent of the GDNF/RET pathway, stimulates ureteric budding and WT1 also induces ureteric bud formation in an independent manner. Angiotensin type II receptor (AGT2R) is essential for early stages of ureteric bud morphogenesis.

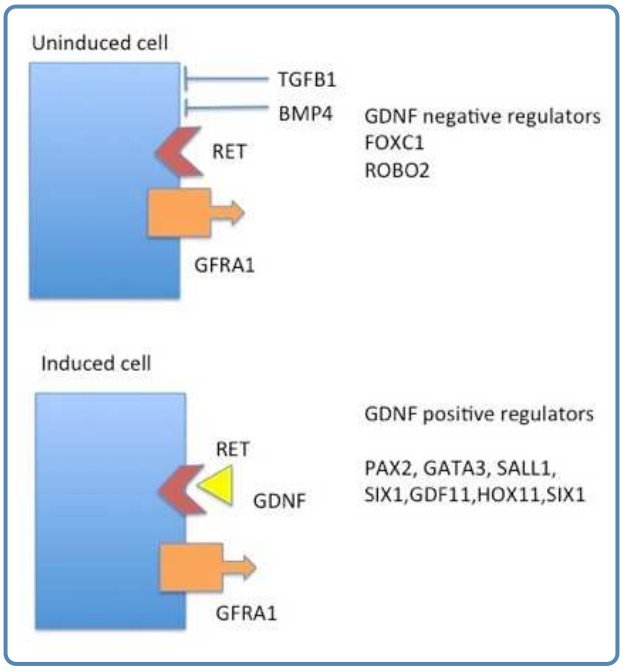



**Figure 2** GDNF- RET pathway.

## Strategies used to study genetic susceptibility

*i. Candidate gene studies:* Even though the genes identified for non-syndromic CAKUT are lesser in number, knock out mice models have enabled to identify some of the candidate genes which were validated in human beings. Candidate gene approach has enabled identification of causative heterozygous gene mutations in 6-20% of patients. (Nicolaou et al., 2015). Although different studies have identified mutations in genes like *BMP7, CHD1L, CDC5L, EYA1, GATA3, RET, ROBO2, SALL1, SIX2, SIX5, FRAS1* and *FREM2*, functional evidence is still lacking to prove causality. Even though functional characterization was done for variants in some genes like *WNT4* and *RET*, some of those variants were identified in unaffected parents of patients, indicating incomplete or lower degree of penetrance or even multifactorial etiology for CAKUT.

*ii. Linkage analysis:* This approach has been tried

in familial VUR and 60% of the 12 families with VUR showed linkage in chromosome 12p11- q13, even though no specific gene was identified (Weng et al., 2009).

*iii. Genome wide association studies:* Association studies done in sporadic cases of CAKUT like VUR have been limited by the sample size to attain statistical significance. (Capone et al., 2017)

*iv. Targeted next generation sequencing:* By massive parallel sequencing of multiple genes, the cause of CAKUT was elucidated in around 10%. (Capone et al., 2017).

*v. Whole exome (Bekhernia et al., 2017) and whole genome sequencing:* Many new genes have been implicated and the need for functional analysis of variants in various genes is on the rise.

*vi. Analysis of copy number variants by chromosomal microarray:* Nephrogenesis is highly sensitive to gene dosages and it has been shown that 16% of CAKUT are due to CNV's (Capone et al., 2017). De novo microdeletions of chromosome 17q12 (which contains *HNF1B*) have been implicated in patients with CAKUT with or without diabetes mellitus.

## Genetics of non–syndromic CAKUT

Identification of genes in non-syndromic forms of CAKUT has been difficult. The first single gene defect identified as causing CAKUT was a deletion in *PAX2* gene in a family with optic coloboma, renal hypoplasia and VUR (Nicolaou et al., 2015). The second gene to be implicated in CAKUT was *HNF1B* in a family with two children with diabetes and renal cysts. *PAX2* and *HNF1B* were later identified as the two most common genes implicated in CAKUT, contributing to 15% of all patients with CAKUT (Nicolaou et al., 2015). The ESCAPE study, which analyzed the renal developmental genes like *PAX2, HNF1B, SALL1, EYA1 and SIX1* in a large cohort of children with renal hypodysplasia, showed a high prevalence of mutations in *PAX2* and *HNF1B* (Capone et al., 2017).

Mutations have been identified in patients with CAKUT in *BMP4, RET, DSTYK, WNT4* and *SIX2*. Though *UMOD* gene has been implicated in familial juvenile hyperuricemic nephropathy (FJHN), glomerulocystic kidney disease (GCKD) and autosomal dominant medullary cystic kidney disease 2, *UMOD* mutations were not identified in patients with isolated CAKUT, implying that it may represent a very rare etiology for this condition. In a study by Hwang et al., mutations in known CAKUT-causing genes were identified in 16% of the total 749 patients and the most commonly mutated genes were *SALL1, HNF1B* and *PAX2* (Hwang et al., 2014). Nicolaou et al, analysed the largest number of genes (a total of 208 candidate genes) by targeted NGS in phenotypically heterogeneous 453 patients with CAKUT and found only five disease causing variants (in *HNF1B, PAX2, SIX5* and *UMOD*) and concluded that many of the previously implicated genes contributed to the pathogenesis much less than what was expected (Nicolaou et al., 2016).

All these findings imply that the majority of the causes of CAKUT are still unknown, while novel variants in genes like *FRAS1, FREM2, GRIP1, ITGA8* and *TRAP1* are being identified and need functional validation. Recessive mutations in these genes have been previously characterized and could imply autosomal recessive inheritance in some cases of CAKUT.

## Clinical presentation

Clinical outcomes of CAKUT are highly variable and range from asymptomatic to chronic kidney disease requiring renal replacement during a period ranging from newborn period to adulthood. With the widespread use and a sensitivity of around 80%, many malformations of kidney and urinary tract are recognized as early as 18 to 20 weeks of gestation by antenatal ultrasound scan. Oligohydramnios and altered morphology of kidney or urinary tract could indicate CAKUT. In the newborn period, CAKUT can present as part of other malformation syndromes or as a palpable abdominal mass or as respiratory distress in a newborn due to pulmonary hypoplasia. Abnormalities of the outer ear and single umbilical artery are associated with an increased risk of CAKUT.

## Genetic evaluation of a patient with CAKUT

If a patient is suspected to have any syndrome, which is associated with CAKUT, specific investigation and molecular diagnosis for that particular syndrome can be attempted. In non-syndromic CAKUT, chromosomal microarray may be done to look for CNVs. Since most disease-causing variants have been identified in *PAX2* and *HNF1B*, these two genes may be screened for pathogenic variants. With the advent of NGS-based technology, targeted sequencing of previously implicated genes, whole exome sequencing or whole genome sequencing may be done to look for pathogenic variants,

but the yield of such investigations still remains low and whether such investigations need to be advised routinely for patients with non-syndromic sporadic CAKUT is debatable.

## Genetic counseling of families with CAKUT

Majority of CAKUT are sporadic and cannot be explained by monogenic inheritance. The empirical recurrence risk of CAKUT has been shown to range from 4-20% in various studies (Capone et al., 2017). If a specific pathogenic variant has been identified in a proband, the family should be counseled appropriately regarding the risk of recurrence and availability of prenatal diagnosis. Antenatal ultrasound scan can be used to detect renal structural abnormalities as early as 18 to 20 weeks of gestation.

Renal abnormalities are seen in asymptomatic close relatives in 10% of patients with CAKUT. Close relatives may be screened by ultrasound scan for renal structural abnormalities.

## Conclusions and future prospects

CAKUT are complex malformations, which occur due to interplay of genetic, environmental and epigenetic factors. *PAX2* and *HNF1B* are the two most commonly implicated genes in non-syndromic CAKUT. However more evidence is needed to establish the monogenic inheritance of CAKUT. Seven miRNAs with a potential role in CAKUT have been identified and await functional validation before defining the precise role of miRNAs as biomarkers for diagnosis and prognosis of CAKUT. With the decreasing cost of sequencing and increasing collaboration among researchers, in the near future, it would be possible to delineate the genetics of CAKUT and devise a method for predicting the severity and prognosis of the renal phenotype based on the genotype.

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GeNeViSTA

# Genetics of Premature Ovarian Failure

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## Introduction

The term premature ovarian failure (POF) refers to the cessation of menses in a woman prior to the age of forty years. It is characterized by amenorrhea (either primary or secondary) and hypergonadotropic hypogonadism (level of serum follicle stimulating hormone (FSH) >40mIU/ml) (Coulam et al.,1982). The general prevalence of POF is approximately 1% (Coulam et al., 1986). It is an etiologically heterogeneous disorder with autoimmune, infectious, iatrogenic, environmental and genetic causes being implicated. Yet, in nearly half of these cases, the cause remains unknown and is proposed to be multifactorial. Nevertheless, approximately 10%–15% of these idiopathic cases have an affected first-degree relative suggesting a strong genetic component (Van Kasteren et al., 1999). Overall, cytogenetic, cyto-genomic and whole genome approaches have shown that approximately 20 to 25% cases of premature ovarian insufficiency are of genetic etiology (Qin et al., 2015).

The term primary ovarian insufficiency (POI) has been suggested as a more appropriate term to encompass the biochemical abnormalities, menstrual and fertility issues, thus representing a disease continuum from insufficiency to failure (Welt et al., 2005). Online Mendelian inheritance in man (OMIM) uses the term POF to denote the causative genes/loci .

## Pathophysiology of Premature Ovarian Failure

In POF, the lack of ovarian function is a result of either a primary ovarian defect due to streak gonads or dysgenetic gonads, or a normal ovary with depleted follicles or impaired function such as steroidogenesis or a receptor defect. Premature depletion of the ovarian reserve is attributed to either a low initial primordial follicle pool or to accelerated follicular atresia. Altered maturation/recruitment of primordial follicles are other factors causing ovarian dysfunction (Persani et al., 2010). Several important prenatal and postnatal events impact the overall function of the ovary and its endowment with primordial follicles. Therefore, understanding the key processes involved in ovarian development and function and their regulation provides insight into the pathophysiology of premature ovarian failure (Fig.1).

## Genetic Causes of POF

The genetic causes of POF may be broadly classified as cytogenetic and single gene causes which are further subdivided into syndromic or non syndromic POF.

## Cytogenetic abnormalities implicated in POF

Chromosomal abnormalities are a frequent cause of POF, with an estimated prevalence of 10 –13% from large population studies (Lakhal et al., 2010; Dalpr et al., 2011; Jiao et al., 2012; Kalantari et al., 2013). In ovarian failure presenting as primary amenorrhea, approximately 21% will be associated with an abnormal karyotype compared to approximately 13% of women with secondary amenorrhea (Jiao et al., 2012; Kalantari et al., 2013). A 'critical region' for normal ovarian function is defined on the long arm of the X chromosome, corresponding to the Xq13-q26 interval (Thermen et al., 1990). Epigenetic down-regulation of oocyte-expressed autosomal genes are proposed to be controlled by this region (Toniolo et al., 2007). Multiple POF genes (*POF1B, DIAPH2*, *DACH2*, *DACH2*, *CHM, PGRMC1, COL4A6, NXF5* etc.) have been identified by X- autosomal translocations studies. Cytogenetic abnormalities of the X chromosome account for 5-10% of cases of POF (Goswami et al.,2005). Table 1 lists the various X chromosome abnormalities and their key characteristics. These include numerical X chromosome defects

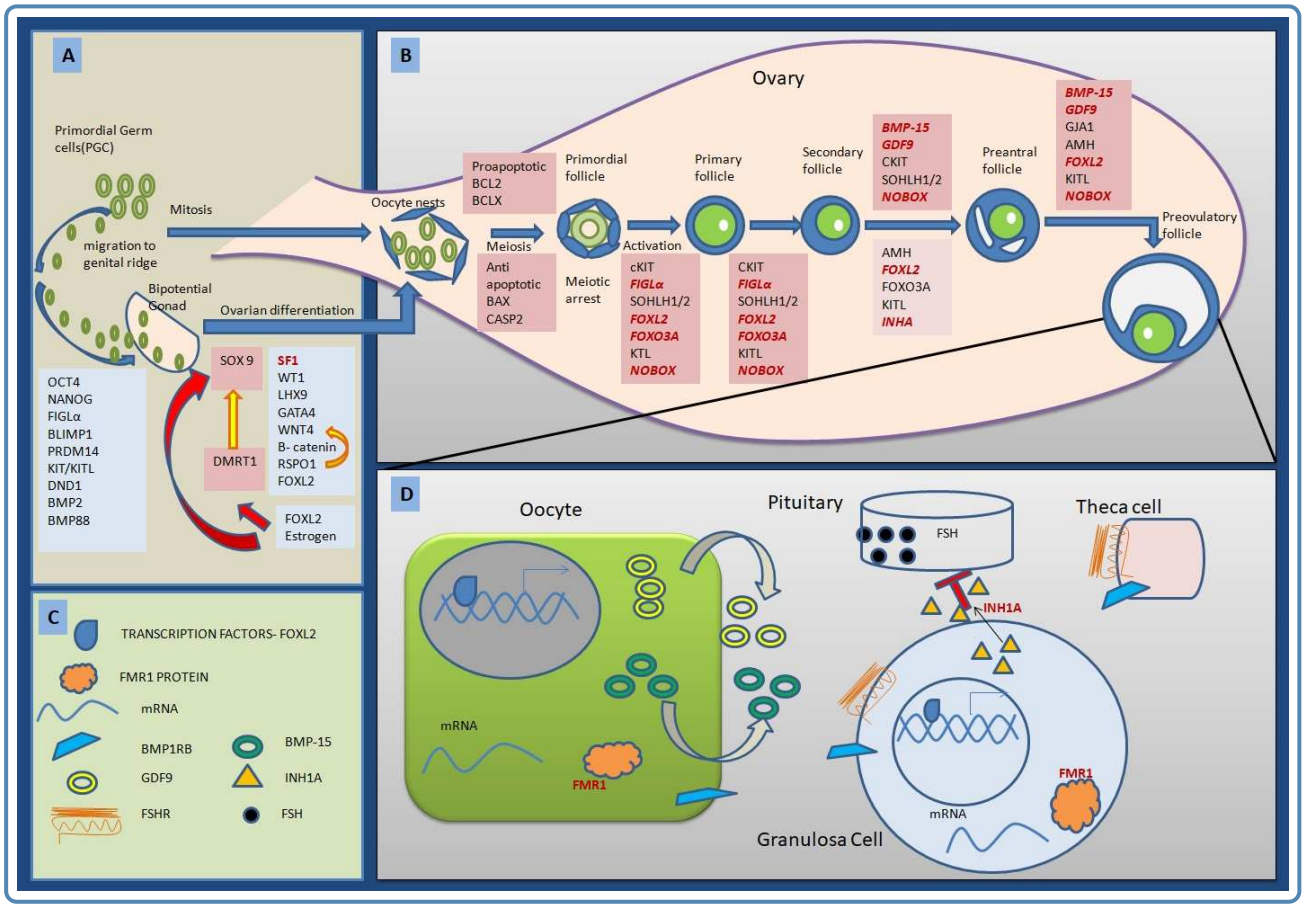


**Figure 1** Schematic representation of the genes and signalling factors involved in the genetic control of ovarian function. Panel A depicts migration of the primordial germ cell (PGC) to the bipotential gonad specified by the BMP pathway. Gonadal differentiation into the ovary occurs in the absence of SRY together with the antagonistic effects of FOXL2 and estrogen on SOX9 and DMRT1 and the agonistic effects of the Wnt signalling molecules such as Rspo1 and Wnt4 and various other factors. The yellow arrows depicted in the figure represent an agonistic effect and the red arrows indicate an antagonistic effect. Panel B shows the morphogenesis of follicles from the arrival of primordial germ cells (PGC) in the nascent ovary to secondary follicles along with the genes and signalling factors involved in each transition. Some of the known single gene defects have been highlighted in red and bold. Panel D illustrates the site of expression of some of the genes involved in the pathogenesis of POF. Panel C lists the key to the depictions in Panel D.

(monosomy X; X chromosomal mosaicism; 47,XXX), structural rearrangements (X-autosome translocations, X-isochromosomes and others), X-deletions and other abnormalities such as 46,XY.

## Monogenic defects

### Syndromic monogenic defects

- Perrault syndrome: Perrault syndrome is characterized by 46,XX ovarian dysgenesis and bilateral prelingual onset sensorineural deafness in females. The spectrum of ovarian dysfunction extends across a continuum from mild to severe. Perrault syndrome is known to be caused by biallelic pathogenic variants in one of four genes: *HARS2, HSD17B4, LARS2,* or *CLPP* and in the majority of cases, the molecular basis is unknown. Mental retardation, ataxia, and cerebellar hypoplasia may be associated features. HSD17B4/D-bifunctional protein is a multifunctional peroxisomal enzyme involved in fatty acid $\beta$-oxidation and steroid metabolism, while *LARS2* which encodes a mitochondrial leucyl-tRNA synthetase and *HARS2* which codes for histidyl tRNA synthetase are mitochondrial genes.

- Galactosemia: Classical galactosemia is an inherited inborn error of galactose metabolism caused by galactose-1-phosphate uridyltransferase

Table 1 X Chromosome abnormalities in premature ovarian failure.

| X chromosome abnormality | Phenotype | Genetic mechanism |
|---|---|---|
| Turner syndrome (Monosomy X) | Female with dysmorphism, structural cardiac defects (one third cases), skeletal abnormalities, hearing loss (50%), hypothyroidism (10%), short stature. Milder phenotype in mosaic Turner | Non-disjunction event (meiotic or post zygotic), haploinsufficiency of *SHOX* gene, accelerated prenatal oocyte apoptosis |
| 47,XXX (Trisomy X) | Small proportion experience POF and may have genitourinary abnormalities. | Nondisjunction errors in meiosis I or II in oogenesis. |
| Xq deletions | Terminal deletions originating at Xq13 are associated with primary amenorrhea and absent secondary sexual development. Primary amenorrhoea is not a feature of terminal deletions arising at Xq25 or Xq26, and more distal deletions having a milder phenotype (Simpson et al.,1999). | |
| Xp deletions | Approximately 50% of delXp11 cases show primary amenorrhea and 45% show secondary amenorrhea (Ogata et al.,1995). Deletion of only the most telomeric portion of Xp (Xp22.3 → Xpter) does not result in amenorrhea (Thomas et al., 1999). | |
| X autosome translocations | Primary or secondary amenorrhoea. Turner stigmata if translocation occurs within the critical region of Xq13-q26 | Haploinsufficiency or disruption of critical genes in these regions, positional effect on contiguous genes or non-specific defective meiotic pairing |
| 46,XY gonadal dysgenesis | Female internal and external genitalia, minimal breast enlargement, propensity for malignant transformation of the gonads (20-30%,) | Mutations in *SRY* (15% of cases), *SOX9, GATA4, FOG2, NR5A1, WT1, DHH, CBX2, ATRX, MAP3K1* and *FGF9*.<br>Deletions encompassing DMRT1 (9p) or EMX2 (10q)<br>Duplication of Xp21 (DAX1/NROB1) |

(GALT) deficiency and premature ovarian failure is the most common long-term complication experienced by girls and women with this condition, with more than 80% being affected despite neonatal diagnosis and careful lifelong dietary restriction of galactose. The presence of a homozygous Q188R mutation is associated with a 16-fold increased risk of POF.

- Others syndromic causes: A variety of genetic disorders (Table 2) have been described in which POF is a commonly occurring feature, and the genes implicated in these cases may therefore have a role in ovarian failure. In some of these conditions the onset of POF is related to gonadal insult or endocrine dysfunction resulting from the disease process, while in others the mechanism of ovarian failure remains unknown.

## Non–syndromic Monogenic Defects

There are many X-linked genes and autosomal genes implicated in non-syndromic presentations of POF.

- Fragile X premutation: The *FMR1* premutation occurs in the critical gene for Fragile X mental retardation gene located at Xq27.3. In females with POF, the risk of having a premutation allele

is 3–4% when she is the only affected individual in the family, but 12–15% if a second female in the pedigree is affected with POF. The pathology is caused by expansion of the CGG repeat in the gene's 5' untranslated region to a premutation state of between 56 and 199 repeats which leads to an increased production of the fragile X mental retardation protein (FMRP), an RNA-binding protein which is highly expressed in germ cells of the foetal ovary Its accumulation is believed to impair the expression of genes required for oocyte development and have toxic effects leading to follicle atresia. The risk of having POF appears to increase with increasing premutation repeat size between 59 and 99. The risk plateaus or decreases for women with repeat sizes of 100. FMR1 premutations carried by women are unstable and can expand in the next generation to transmit fragile

Table 2 Syndromes with premature ovarian failure.

| Syndrome | Gene | Prominent associated findings |
|---|---|---|
| Aromatase deficiency | *CYP19A1* | Maternal virilization during pregnancy due to absence of placental aromatase |
| Ataxia telangiectasia | *ATM* | Cerebellar ataxia, telangiectasias, immune defects, a predisposition to malignancy, premature aging, genome instability |
| Autoimmune polyendocrine syndrome, type 1/Autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (APECED) | *AIRE* | Adrenal insufficiency, hypoparathyroidism, chronic mucocutaneous candidiasis <60% of patients have ovarian failure |
| Autoimmune polyendocrine syndrome, type 2 | Unknown | Adrenal insufficiency, type 1 diabetes mellitus, autoimmune thyroid disease 3-10% of APS type II patients have POF |
| Bassoe syndrome | Unknown | Muscular dystrophy and infantile cataract |
| Blepharophimosis -ptosis- epicanthus inversus syndrome (BPES) Type I | *FOXL2* | Autosomal Dominant condition. Complex eyelid malformation. |
| Bloom syndrome | *BLM* | Premature aging, a predisposition to malignancy, genome instability |
| Cerebellar ataxia with hypergonadotropic hypogonadism | Unknown | Ataxia, sensorineural deafness with vestibular hypofunction, peripheral sensory impairment |
| Congenital adrenal hyperplasia due to 17-alpha hydroxylase deficiency | *CYP17A1* | Hypertension, hypokalemic alkalosis |
| Congenital disorder of glycosylation, type 1A | *PMM2* | Neonatal encephalopathy, hypotonia, psychomotor retardation, cerebellar hypoplasia, retinitis pigmentosa |
| Demirhan syndrome | *BMPR1B* | Severe limb malformation, genital anomalies |
| Fanconi anemia | *FANCA, FACA, FA1, FA, FAA* | Anemia, leucopenia, thrombocytopenia; cardiac, renal and limb malformations; dermal pigment changes |
| Fryns syndrome | *PIGN* & additional unknown loci | Intellectual disability, craniofacial dysmorphism |
| GAPO | *ANTRX1* | Growth retardation, alopecia, pseudoanodontia, and optic atrophy |

| | | |
|---|---|---|
| Leukoencephalopathy with vanishing white matter | *EIF2B2, EIF2B4, EIF2B5* | Encephalopathy with leukodystrophy |
| Lipoid congenital adrenal hyperplasia | *STAR* | Congenital adrenal insufficiency, testis function is more severely affected than ovarian function |
| Malouf syndrome | Unknown | Cardiomyopathy |
| Marinesco-Sjogren syndrome | *SIL1* | Cerebellar ataxia, congenital cataracts, retarded somatic and mental maturation |
| Mental retardation, X linked | *FRAXE* | Intellectual disability |
| Progressive external ophthalmoplegia with mitochondrial DNA deletions | *POL G* | Adult onset weakness of external eye muscles and exercise intolerance |
| Proximal symphalangism (SYM1) | *NOG* | Symphalangism |
| Pseudohypoparathyroidism(PHP) type Ia | *GNAS* | Elevated parathyroid hormone (PTH) with low/normal calcium, high thyrotropin (TSH) with normal thyroid hormone levels, growth hormone deficiency and high gonadotropins in patient with delayed puberty and skeletal abnormalities (Albright osteodystrophy) |
| Rapp-Hodgkin syndrome | *TP73L* | Ectodermal dysplasia, cleft lip, cleft palate |
| Werner syndrome | *WRN* | Premature aging, a predisposition to malignancy, genome instability |
| Woodhouse-Sakati syndrome | *DCAF17* | Alopecia, diabetes mellitus, intellectual disability, extrapyramidal syndrome |

X syndrome to male offspring, especially if women have more than 100 repeats. As women with POF have an 5% chance of conceiving, these women are at risk of having a child with fragile X syndrome. As per the American College of Medical Genetics (ACMG), carrier screening of *FMR1* premutation is recommended for women who are experiencing reproductive or fertility problems associated with elevated follicle stimulating hormone (FSH) levels, especially if they have: (a) a family history of premature ovarian failure, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation (Sherman et al., 2005).

- Other isolated gene defects: Several of the isolated gene defects and their prevalence is listed below in Table 3.

## Genomic aberrations, copy number variants (CNVs)

In rare cases, microdeletions and microduplications in known POF genes (*SYCE1, CPEB1*), genes involved in meiosis (*PLCB1, RB1CC1, MAP4K4, RBBP8, IMMP2L, FER1L6, MEIG1*) and possible candidate genes for POF and ovarian dysfunction involving DNA repair, or folliculogenesis have been identified.

## Micro RNAs (MiRNAs)

MiRNAs are a class of small (18-22 nucleotides in length) noncoding RNAs which cause negative regulation of target genes by mediating post-translational gene silencing (He et al., 2004). Dicer, a pre-miRNAs processor is shown to be important for folliculogenesis, maturation of oocytes, and follicle recruitment (Murchison et al., 2007). Polymorphisms in *XPO5* (Exportin), a premiRNA transporter are associated with an increased risk of POI (Rah et al., 2013). Differentially expressed miRNAs are involved in various ovarian processes and have been associated with POI (Yang et al., 2012).

In many instances, candidates genes that have been found in experimental or natural animal models showing ovarian failure have shown no variants in the corresponding human orthologue.

Table 3 Isolated gene defects associated with premature ovarian failure.

| Gene | Prevalence in POF cohorts |
|---|---|
| **TGF-B family** | |
| *BMP 15* | 1.5-12% |
| *GDF9* | 1.4% |
| *INHA* | 0–11% |
| **Gonadotropin receptors** | |
| FSH/LH resistance (*FSHR* and *LHCGR*) | 0–1% |
| **Transcription factors** | |
| **Nuclear Proteins** | |
| *NR5A1(SF1)* | 1.6% |
| **Oocyte specific transcription factors** | |
| *NOBOX* | 0–6% |
| *FIGLA* | 1–2% |
| **Forkhead like transcription factors** | |
| *FOXL2* | Rare |
| *FOXO3* | 2.2% |
| **Progesterone receptor membrane component 1** | |
| Progesterone receptor membrane component 1 *(PGRMC1)* | 1.5% |
| *LHX8* | Rare |
| **DNA replication/meiosis and DNA repair genes variants** | |
| *DMC1*, *MSH4*, *MSH5*, *SPO11*, *STAG3*, *SMC1β*, *REC8*, *POF1B*, *HFM1*, *MCM8*, *MCM9*, *SYCE1*, *PSMC3IP*, *NUP107*, *FANCA*, *FANCC*, *FANCG* | Unknown |

## Clinical presentation

The lack of ovarian function leads to absence of production of ovarian hormones leading to low estradiol levels. The resulting effects represent the consequences of hypoestrogenism and also vary depending on the age at which the ovarian failure occurred. Failed development of the gonads, prenatal or prepubertal depletion of the ovarian follicles and ovarian dysfunction result in primary amenorrhoea with poor/absent secondary sexual development. The age limit for defining primary amenorrhea is 13 years of age in the absence of secondary sexual development or 15 years of age in the presence of normal secondary sexual characteristics. Secondary amenorrhea (as absence of menstruation for three normal menstrual cycle or four months period) and well developed secondary sexual characteristics are features of post-pubertal events. A preceding history of infertility, recurrent pregnancy loss or irregular cycles is usually elicitable in such cases. Other symptoms of POF are the typical manifestations of climacterium such as palpitations, heat intolerance, flushes, night sweats, irritability, anxiety, depression, sleep disturbance, decreased libido, hair coarseness, vaginal dryness, fatigue. These symptoms are uncommon among women with primary amenorrhea who never received estrogen. Over 75% of women with POI will have at least menopausal intermittent symptoms including hot flushes, night sweats, and emotional lability. Moderate hirsutism may be seen due to the action of androgens originating from the adrenal glands. In addition to these common manifestations, some have additional specific features associated with specific syndromes or etiologies (see Table 2).

## Clinical workup

The clinical assessment of a woman with premature ovarian failure is aimed at finding etiological clues. These include determining the age of onset of amenorrhea, the sexual maturity rating (SMR), anthropometry, dysmorphological assessment, systemic examination for associated features such as cardiac abnormalities or signs of endocrinological disturbances and examination of the external genitalia. A comprehensive three generation pedigree for history of familial POF and for members affected with Fragile X syndrome or ataxia, and maternal menarcheal and menopausal age may provide vital information. A sonographic evaluation of the pelvis helps to delineate the pelvic anatomy, presence of female internal genital organs, and uterine and ovarian morphology. Relevant imaging and laboratory tests may then be undertaken to further aid in establishing the diagnosis and optimize patient management. In every woman of reproductive age with amenorrhea pregnancy should be ruled out. Serum FSH levels of greater than 40 mIU/ml are diagnostic of POF and this is confirmed with a repeat value after four weeks. About half of the cases of primary amenorrhea are due to ovarian dysgenesis, which is revealed by the finding of streak ovaries accom-

panied by uterus hypoplasia at ultrasound. In the other patients, follicles (<10 mm) may be found on histological evaluation, such as in the case of *FSHR* gene mutations.

## Genetic testing

A routine karyotype is performed in all cases with premature ovarian failure regardless of the age of onset. Besides detecting X chromosome abnormalities, a karyotype will help identify any Y chromosome material which necessitates gonadectomy due to the associated risk of gonadal tumors. The ACMG 2005 guidelines and the American College of Obstetrics and Gynaecology (ACOG) 2010 committee opinion recommend Fragile X premutation screening in women with unexplained premature ovarian insufficiency (Sherman et al.,2005; ACOG committee opinion., 2006). All identified premutation carriers should be counseled regarding the risk to their offspring of inheriting an expanded full-mutation Fragile X allele and also the importance of cascade screening of at-risk female relatives. Currently there are no recommendations for the routine testing of other candidate genes associated with POF in cases with a normal karyotype. Testing of specific disease-associated genes can be considered if a particular syndrome is suspected.

## Role of Next Generation Sequencing

Strategies to identify POF candidate genes have included studies in animal models, study of X chromosome deletions and X-autosome translocations, linkage analysis, comparative genomic hybridization (CGH) array, genome-wide association studies (GWAS), and recently, next generation sequencing (NGS) based approaches. Of these, NGS has several advantages and is a powerful diagnostic tool. The mitochondrial gene linked to Perrault syndrome *LARS2*, which codes for a mitochondrial leucyl-tRNA synthetase, was identified by exome sequencing in two POF families (Pierce et al., 2013). Studies involving whole exome sequencing(WES) have identified pathogenic variants in genes implicated in DNA repair and genomic stability such as stromal antigen 3 (*STAG3*), synaptonemal complex central element 1 (*SYCE1*), minichromosome maintenance complex component 8 and 9 (*MCM8*, *MCM9*) and ATP-dependent DNA helicase homolog (*HFM1*) genes in consanguineous families with non-syndromic POF (Carburet et al., 2014; Wood-Trageser et al., 2014; Al Asiri et al., 2015; Wang et al., 2014; de Vries et al., 2014). A multigene panel study by Fonseca et al. in 12 unrelated women with POF identified two plausible candidate genes in POF, namely *ADAMTS19* and BMP receptor 2 (*BMPR2*) genes with POF pathogenesis (Fonseca et al., 2015). Candidate gene discoveries are important to enhance the knowledge of the underlying molecular mechanisms of POF and thereby increase the prospects of development of definitive treatment.

## Emerging Concepts

Recently, the concept of POF being a purely monogenic disorder has been questioned with digenic findings in several cases and the synergistic effects of several mutations have been suggested to underlie the POI phenotype (Bouilly et al., 2016). Research in POF besides being directed towards identifying causative gene defects, involves exploring therapeutic options to restore ovarian function. In vitro activation of dormant primordial germ cells and grafting are being studied as a potential infertility therapy for POF patients who have residual follicles (Kawamura et al., 2013; Suzuki et al., 2015). The online Ovarian Kaleidoscope Database (http://ovary.stanford.edu) provides information regarding the biological function, expression pattern and regulation of genes expressed in the ovary. It also contains information on gene sequences and is a useful resource of knowledge on ovarian genetics.

## Conclusion

Currently there are no proven predictive tests or biomarkers to identify women who will develop POF, unless a mutation known to be related to POI is detected, and there are no established POI preventing measures. The development of multigene predictive panels may enable the identification of women at risk for early menopause or premature ovarian failure. The availability of target therapies is the ultimate goal for the immense ongoing research in unravelling the intricacies of the ovarian function.

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GeNeXpreSS

# Whole Genome Sequencing as a Diagnostic Tool: Utility and Challenges

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## A whole new way to see!

(Ellingford et al, 2016)

With the recent technical developments in analyzing big data, whole genome sequencing has unlocked a whole new way in clinical genomics to evaluate various rare diseases, including the highly heterogenous group of inherited retinal diseases (IRD). Illumina Hiseq sequencing has given it a new direction through its high coverage and accuracy to identify and validate a particular variant more confidently than the ABI SOLiD platform. Whole Genome Sequencing (WGS) was performed in 46 individuals out of 562 patients with IRD for whom diagnostic Next Generation Sequencing (NGS) did not identify any mutation. The study also compared the sensitivity and specificity of WGS and diagnostic NGS in detecting Single Nucleotide Variations. By using WGS, it was possible to detect disease-causing variants in 11 individuals for whom a molecular diagnosis was not made previously. The authors concluded that WGS reported a higher rate of mutation detection as WGS had a more powerful pipeline to detect structural variants and variants in noncoding regions. The deletions identified with the pipeline ranged from <1.7 Kb to >520 Kb in size, including the identification of break points in noncoding regions. This study highlights the benefit of WGS as a superior tool compared to diagnostic NGS for evaluation of IRD patients if the cost factor can be minimized.

## WGS about WGS: What General public Surmise about Whole Genome Sequencing (Roberts et al, 2017)

A randomized controlled trial was done to examine the use of WGS and family health information in cardiology and primary care settings compared to the use of family health information alone. A total of 202 patients were surveyed before and 6 months after the disclosure of the WGS results. Compared to patients for whom family health information alone was used, the patients for whom WGS results were combined with family health information were generally able to understand key facts about the sequencing trial, had less decisional regret and a good level of satisfaction about the utility of WGS findings. Due to the higher cost of the service, the patients had a preset expectation about the outcome of the service. Hence a decent communication between the physician and the patient to explain the utility of the WGS and its results in a clear and effective manner is very important. For better understanding of people with lower health literacy, it is suggested to have enhanced consent forms, informative videos and brochures and extended discussion schedules by physicians. A successful implementation of WGS in clinical settings is critical to guide future practice.

## WGS unveils human faces

(Lippert et al, 2017)

Of the various promises of genome sequencing technology, this study challenged its ability to associate genotype to physical traits. The target was to utilize individual level of genotype obtained from WGS to match with the predictive model data created from the various physical features like facial structure, voice, eye color, skin color, height, weight and BMI. A model to estimate the age of a person was also generated. A sample size of 1061 individuals from African, European, Latino, East Asian, and South Asian ethnicity was considered for the study. $R^2_{cv}$ value ($R$-squared evaluates how much of the variability in the actual

values is explained by the model, *cv* - coefficient of variation) was calculated between observed and predicted models to assess the influence of each covariate on predictive accuracy. Although the success rate was not uniform for all the models, the prediction accuracy for face, voice and age supported the efficiency of the model. Statistical significance will be higher if it can be applied on a larger sample size. In the long run, WGS could be helpful for genomic forensic sciences studies.

## A Drop of Blood Suffices!

(Bassaganyas et al, 2017)

Dried Blood Spot (DBS) specimens represent an unparalleled resource to investigate rare genetic disorders in newborn, such as inborn errors of metabolism. The current work proposed a successful protocol to do WES and WGS from DBS samples without whole genome amplification prior to library preparation. The authors attributed the success of this method to factors such as preservation of the cards at -20°C under desiccation, keeping DNA intact, automated DNA extraction protocol yielding a good quantity of DNA, smaller amount of DNA needed for the newer exome capture kits and a shorter DNA shearing time. The study proved that even very low amounts of genomic DNA from DBS could generate acceptable exome coverage. The successful implementation of a protocol to produce high quality exome and genome sequences from newborn DBS offers an unparalleled opportunity to advance the understanding of rare disease associated and polymorphic variants in various populations.

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# PhotoQuiz - 40

*Contributed by:* Dr. Prajnya Ranganath

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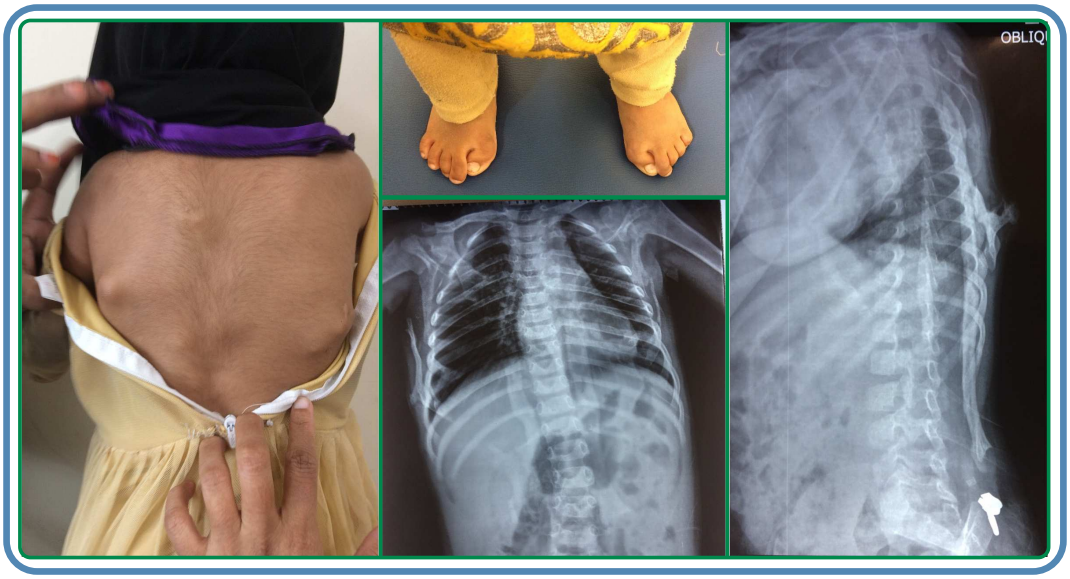
This 2.5 years-old female child, the fourth offspring of non-consanguineous parents, presented with history of painful, hard swellings on the back. Her developmental milestones were normal. There was no significant family history. Identify the condition.

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




## Answer to PhotoQuiz 39

### Freeman-Sheldon syndrome (Distal arthrogryposis type 2A) (OMIM # 193700)

Freeman-Sheldon syndrome is a distal arthrogryposis syndrome characterized by contractures of hands and feet (arthrogryposis), oropharyngeal abnormalities, and a distinctive face which includes a very small oral orifice, puckered lips, and an H-shaped dimpled chin. Hence it is also called the 'whistling face syndrome'. It is an autosomal dominant disorder caused by mutation in the *MYH3* gene.

**Correct Responses Were Given By:**

1. Beena Suresh, Chennai
2. Manjeet Mehta, Mumbai
3. Kalpana Gowrishankar, Chennai
4. Sheetal Sharda, Bengaluru
5. Sangeeta Khatter, New Delhi
6. Mohandas Nair, Kozhikode
7. Poonam Singh Gambhir, Kanpur
8. Alka Ekbote, Aurangabad
9. Prashant Kumar Verma, Jaipur
10. M L Kulkarni, Davangere
11. Jayarekha Raja, Chennai
12. Niby J Elackatt, Bengaluru

# GeNeEvent - PediGen 2018

## 23 – 25 February, 2018


PediGen2018, the third of the three-yearly national Pediatric Genetics conferences was organized jointly by Deenanath Mangeshkar Hospital & Research Center, Pune and the Pune chapter of the Indian Academy of Pediatrics between February 23rd-25th 2018, in Pune. Pediatric growth disorders and Genodermatoses were the main themes along with a half-day session on the basic principles of Medical Genetics for pediatricians followed by case discussions with enthusiastic audience participation. The growth disorders session included various aspects of normal and abnormal growth and addressed the genetic etiology underlying low birth weight. Genodermatoses covered common clinical disorders and the session was also attended by eminent dermatologists from Pune. Prof Karen Temple, Professor of Genetics at Southampton University requires a special mention for her talk on "Imprinting" which was very well appreciated. A special program to commemorate the Rare Disease Day celebration was held on February 24th in which families with lysosomal storage disorders came to share their experiences and hear about new developments in the field. PediGen2018 delegates were encouraged to complete a Genetics crossword, present an e-poster as well as participate in a PG Quiz. Dr Pallavi Vats from MAMC, New Delhi was the winner of both the quiz and poster competitions. SIAMG president Prof. Madhulika Kabra kindly presented the compilation of reviews from Genetic Clinics (*'Genetics Update for the Next Generation Clinician'*) to PediGen2018 delegates which was highly appreciated by the attending pediatricians.

**Join SIAMG**

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**Submit your article to Genetic Clinics**

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

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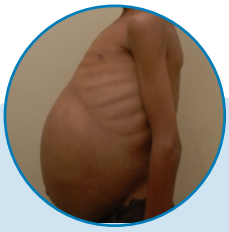
*Dried Blood Spot Enzyme Assay & Mutation Analysis for low/subnormal enzyme level on DBS samples.

## Patients with the following signs and symptoms may have a

# Lysosomal Storage Disorder...



### GAUCHER DISEASE

- Enlarged liver and spleen
- Delayed or stunted growth in children
- Easy bruising and bleeding
- Anemia and Thrombocytopenia
- Unexplained Bone pains
- Unexplained Avascular necrosis of femur

Cerezyme®# imiglucerase

### POMPE DISEASE

- "Floppy" appearance in infants or young children
- Unexplained Cardiomyopathy
- Progressive respiratory muscle weakness or insufficiency
- Progressive Limb-girdle muscle weakness (in late-onset cases)

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### MPS I DISEASE

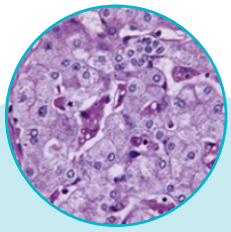
- Coarse facial features
- Early onset joint stiffness/ claw-hand deformities/ contractures
- Corneal clouding (leading to light sensitivity or impaired vision)
- Recurrent respiratory infections (including sinuses & ears)
- History of recurrent hernia repair in young age

ALDURAZYME®# (LARONIDASE)

### FABRY DISEASE

- Severe burning pain in hands & feet
- Intolerance to heat & cold
- Inability (or decreased ability) to sweat
- Red, purple spots on skin (angiokeratomas)
- Evidence of early renal involvement (nephropathy)
- History of stroke in young age

Fabrazyme®# agalsidase beta



### NIEMANN PICK - B DISEASE

- Enlarged liver & spleen
- Bleeding manifestations
- Skeletal abnormalities & Growth delays

Olipudase-α$

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