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UNRAVELLING GENETIC MYSTERIES IN NEONATAL INTENSIVE CARE THROUGH PERIMORTEM TESTING: CASE SERIES AND LITERATURE REVIEW

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ABSTRACT
Principal constraints faced by physicians while providing terminal care for newborns without diagnosis are limited time for diagnosis due to rapid deterioration or sudden death, incomplete or inconclusive diagnostic tests and difficulty in obtaining samples. Also, emotional toll on physicians and healthcare teams with difficulty in communicating uncertain diagnoses to families along with grief and bereavement support challenges complicate this situation. The main strategy to combat this circumstance is offering integrated diagnostic approach (biochemical, imaging and genetic tests) to the dying infant. It can provide valuable answers for families experiencing newborn loss, alleviating uncertainty and promoting emotional healing. With imaging and biochemical tests yielding insufficient answers, perimortem genetic testing offers a last resort for diagnostic clarity.[1-3]

INTRODUCTION
Perimortem genetic testing in newborns refers to any type of genetic testing performed on deceased or critically ill newborns typically in the context of a life threatening condition or sudden death[4]. The primary goal of this testing is to identify the underlying genetic cause of the newborn's condition, providing valuable information for diagnostic clarity, counselling, guiding reproductive decisions and tailoring treatment strategies for affected / presymptomatic family members. This case series investigated the details of the infants who were subjected for perimortem genetic testing and analysed the genetic evaluation done for this population thereby evaluating its utility to diagnose genetic conditions, enhancing the clinician's understanding of neonatal mortality, and guiding for genetic counselling.

METHODS
Study Population
This study conducted a retrospective review of medical records from a South Indian tertiary care teaching institution's Neonatal Intensive Care Unit (NICU), focusing on deceased infants under one year old, admitted between January 2019 and September 2024. The Institutional Ethics Committee granted approval and waived informed consent due to the study's retrospective nature.
Case records of neonates whose status in the medical record was recorded as "deceased" from January 2021 till September 2024 were selected. From those records the shortlisting of case records were done by those babies who had high clinical suspicion of an underlying genetic aetiology, and underwent perimortem genetic testing (just before or immediately after death). For each infant, the data extracted from the case records included available demographic details, phenotypic features, laboratory data and genetic testing. The background for physician's decision for genetic testing, specimen type, type of genetic testing ordered were collected.
Genetic testing reports are interpreted using the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of sequence variants was used for interpretation of genetic tests. This classified the identified variants into five tiers (pathogenic, likely pathogenic, variant of uncertain significance [VOUS], likely benign, benign) based on evidence like population data, computational tools, and the patient's clinical context (symptoms, family history, referral reason) to determine if a variant causes disease or is just a normal variation[5]. If a pathogenic or likely pathogenic variant identified through testing that explained the infant's symptoms it is considered as a laboratory-confirmed genetic diagnosis. Variant of Uncertain significance were clarified by thorough review of the clinical summary and the treating physician's opinion. For confirmed genetic diagnoses, whether clinical diagnosis had been made prior to genetic confirmation was also noted.
The key expected outcomes were identification of the previously unknown genetic disorders, genotype – phenotype correlation and the influence of genetic testing on the family counselling.

RESULTS

This retrospective observational case series included nine neonates who were fulfilling the criteria. Characteristics of the study population is depicted in table 1. The study population comprised of term and late preterm neonates (born between 34 weeks and 40 weeks of gestation. Any degree of consanguinity was noticed among 4 families (44%). Strong family history of similar illness was present in only one case (11%). There were 7 males (78%) and 2 female (22%) in the study subjects. Dysmorphic features was present in 6 (67%). Among the remaining, one had unexplained lactic acidosis, one had mixed acidosis and another had erythroderma with acute renal failure.
Whole exome sequencing was the only test ordered for all the cases. The common indication for the testing was clinical ambiguity in diagnosis. Case records revealed that blood samples were sent in EDTA tubes. There were no tissue samples procured for this purpose. A repeat testing was not requested by the lab in any of the cases implying proper sampling.
The variants reported were enlisted in table 2. The classification of variants detected were likely pathogenic (3 out of 9; 33%) and VOUS (5 out of 9; 56%). In one case, no pathogenic variants were detected (case 4). Two diagnoses involved multiple VOUS variants (3 in case 2 and 2 in case 7). A combination with a likely pathogenic variant and a VOUS variant was detected in one neonate (case 3). The inheritance pattern of the encountered disorders were Autosomal recessive (6 out of 8; 75%) and Autosomal dominant (2 out of 8; 25%).
Similar clinical diagnosis prior to genetic testing was made in only one case (case 2 – Myopathy/dystrophy). Supportive lab data (excluding genetic evaluation) could lead a definitive diagnosis in three cases (Cases 1, 3 and 7). Case 7 is unique because the clinical diagnosis of myopathy was made but additionally urine GCMS revealed abnormal levels of Adenosine indicating coexistent Adenosine Deaminase (ADA) deficiency.
After the WES reports, parents were offered genetic counselling regarding the variant detected, zygosity and need for parental testing. Parental testing was needed in case 4 and advised for 8 couples. Four (50%) parents underwent single gene mutation analysis and found to be carriers for the affected gene (Case 1, 3, 5 and 7). The same was denied by 4 (50%) couples (Cases 2, 6, 8 and 9). Couples who underwent Sanger sequencing were offered genetic counselling regarding recurrence risks and prenatal diagnostic tests.

DISCUSSION

"Diagnostic odyssey" defined as the journey to a genetic diagnosis is often overlong and challenging.[6] More challenges in this journey will be imposed when it is necessitated in a dying neonate. During the perimortem period, the tasks encountered by the treating team are managing these babies, communicating with the families and simultaneously collecting and processing the appropriate diagnostic samples[7].
Molecular diagnostic tests in stillbirths and neonatal deaths often reveals an underlying cause, with chromosomal analysis having a 15% yield. Among the babies who expire in intensive care, 23.3% are due to genetic disorders, mostly chromosomal rather than genetic[8]. This was in the era before Next Generation Sequencing (NGS), resulting in imprecise encumbrance of genetic disorders. Data published till 2018 implied genetic syndromes contributed to 5% of neonatal mortality[9-11]. However these population were offered limited genetic testing which focussed on chromosomal analysis which led to Mendelian disorders being missed.
When a genetic disorder is suspected in a dying infant, a precise diagnosis needs to be established which enhances clarity in managing bereaved families. This can avoid potential tragedies in future pregnancies and guide targeted screening to decide presymptomatic treatment for family members. Next Generation Sequencing (NGS) has huge potential for diagnostic yields when compared to conventional tests. Whole exome / genome sequencing (WES / WGS) and Chromosomal microarray (CMA) are some of the NGS which are widely used.[12,13]
A promising inclusive and malleable diagnostic approach is being offered by WES / WGS particularly for perinatal and neonatal deaths. Either by analysing the exons or the whole genome, these tests enable the treating physicians to evade targeted gene panels and instead chose NGS.[14] In the reported case series all the neonates were offered WES and the diagnostic yield is 89%. Additionally WES have an advantageous expansive approach facilitating future revaluation of genomic data whenever needed[15,16]. This approach is particularly helpful when new genetic insights evolve over time.
While the bereaved families are being offered closure of diagnosis and guidance, NGS also influences clinical decisions regarding specific management or transferring the care towards palliation thereby enhancing the quality of life for the infants with life-limiting conditions.[17]
Prenatal diagnostic tests and perimortem genetic tests provides valuable information for both families and the health care workers. The information received by the families are understanding the cause of death, recurrence risks, decision on future pregnancies and preimplantation testing options. Additionally hidden carriers in the families can be identified with expanded genetic counselling involving family members. The health care providers will be equipped with augmented understanding of genetic disorders, inheritance patterns, institution of specific treatment, decision on palliative care and strategies to prevent catastrophes in future pregnancies[18-22]. Tailored personalised reproductive planning options can be offered to the individual affected families.

CONCLUSION

It is essential for both health care providers and families to attain a definitive diagnosis in a sick neonate receiving end of life care. If an underlying genetic disorder is suspected, offering NGS to these babies is vital. Prompt collection of biological samples with appropriate processing yields a valid report. This case series and review of the published data strongly supports the importance of perimortem genetic testing in providing essential information for diagnosis, clinical decision, reproductive planning and preventing future tragedies.

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Table 1: Characteristics and key lab findings of the reported neonates

Case number	Family history and consanguinity	Antenatal history	Birth details	Admission details	Examination findings	Abnormal Investigations
1	Nil significant; Non consanguineous	No complications	Term, male, AGA[≠]	Admitted on day 24; complaints of lethargy and poor feeding	Evidence of shock; systemic examination normal	Severe Lactic acidosis; abnormal urine GCMS[€]
2	Nil significant; third degree consanguinity	No complications	Term, male, SGA[¥], birth asphyxia	Admitted to intensive care for severe birth asphyxia	Dysmorphism – limb abnormalities, joint contractures, flat facies and generalized hypotonia	Metabolic acidosis
3	Nil significant; non consanguineous	Gestational hypertension	Term, AGA, male, born through meconium stained liquor	Treated elsewhere for meconium aspiration syndrome for first 21 days of life and referred for continuation of oxygen therapy. Had seizures on 27 days of life	No respiratory distress, long neck, overriding of fingers, generalized hypotonia	Hyperammonemia
4	Nil significant; non consanguineous	No complications	Term, female, AGA, cried at birth	Respiratory distress since birth	Progressive worsening of respiratory distress with normal systemic examination	Mixed acidosis
5	First pregnancy Still birth (cause unknown); non consanguineous	No complications	Late preterm (34 weeks), female, AGA, birth asphyxia	Severe birth asphyxia	Dysmorphic facies (depressed nasal bridge, low set ears, micrognathia), hypotonia, joint contractures	Nil
6	Third order birth with normal 2 elder siblings; third degree consanguinity	Fetal hydrops noted at 33 weeks of gestation	Late preterm (34 weeks), male, SGA	Severe birth asphyxia	Non immune fetal hydrops, facial dysmorphism; flat foot	-
7	Nil significant; third degree consanguinity	No complications	Early term (37 weeks), male, AGA	Neonatal seizures on Day 1	Central hypotonia, dysmorphic facies	Abnormal TMS[£]
8	Fourth order birth with 2 infantile deaths and one abortion; second degree consanguinity	Polyhydramnios	Late preterm, male, AGA	Neonatal seizures on Day 1	Central hypotonia, dysmorphic facies	

9	Nil significant; consanguineous	No complications	Late preterm (36 weeks), male, SGA	Extensive peeling of skin and whole body redness since birth	Neonatal Erythroderma, Acute renal failure	Elevated renal parameters

[≠] AGA- Appropriate for gestational age; [¥] SGA – Small for gestational age; [€] GCMS – gas chromatography mass spectrometry; [£] TMS – tandem mass spectrometry

Table 2: Whole exome sequencing reports of the cases (Variants reported with classification)

Case number	Gene & Transcript	Variant	Location	Zygosity	Disorder (OMIM)	Inheritance	Classification
1	NM_000155.3:c.442C>T	(p.Arg148Trp)	Exon 5	Heterozygous	Galactosemia	Autosomal Recessive	Likely pathogenic
2	RYR1 NM_000540.2	c.2348C>T (p.Ser783Leu)	Exon 19	Heterozygous	Minicore Myopathy with External Ophthalmoplegia (255320)	Autosomal Recessive	Uncertain Significance
	RYR1 NM_000540.2	c.4816C>A (p.Arg1606Ser)	Exon 33	Heterozygous	Minicore Myopathy with External Ophthalmoplegia (255320)	Autosomal Recessive	Uncertain Significance
	RYR1 NM_000540.2	c.4826A>G (p.Asn1609Ser)	Exon 33	Heterozygous	Minicore Myopathy with External Ophthalmoplegia (255320)	Autosomal Recessive	Uncertain Significance
3	PEX1 NM_000466.3	c.2548C>T (p.Gln850Ter)	Exon 15	Homozygous	Peroxisome biogenesis disorder 1B	Autosomal Recessive	Likely Pathogenic

	PEX1 NM_000466.3	c.2456G>A (p.Arg819His)	Exon 15	Homozygous	(NALD/IRD) (601539) Peroxisome biogenesis disorder 1B (NALD/IRD)	Autosomal Recessive	Uncertain Significance
Case number	**Gene & Transcript**	**Variant**	**Location**	**Zygosity**	**Disorder (OMIM)**	**Inheritance**	**Classification**
4	No pathogenic or likely pathogenic variants causative of the reported phenotype were identified						
5	SKI NM_003036.4	c.1619C>T (p.Ala540Val)	Exon 5	Heterozygous	Shprintzen-Goldberg syndrome (182212)	Autosomal Dominant	Uncertain Significance
6	*LZTR1* NM_006767.4	c.253G>A (p.Gly85Arg)	Exon 2	Heterozygous	Noonan syndrome-10 (616564) Noonan syndrome 2 (605275)	Autosomal Dominant/Autosomal Recessive	Uncertain Significance
7	ADA NM_000022.4 SCN4A NM_000334.4	c.1028T>Cp.Leu343Pro c.4624C>T p.Leu1542Phe	Exon 11 Exon 24	Homozygous Homozygous	SEVERE COMBINED IMMUNODEFICIENCY, AUTOSOMAL RECESSIVE, T CELL-NEGATIVE, B CELL-NEGATIVE, NK CELL-NEGATIVE, DUE TO ADENOSINE DEAMINASE DEFICIENCY:102700	Autosomal Recessive Autosomal Recessive	Uncertain Significance Uncertain Significance

					MYASTHENIC SYNDROME, CONGENITAL, 16; CMS16:614198 CONGENITAL MYOPATHY 22A, CLASSIC; CMYP22A:620351		
8	***CNTNAP1*** **(+)** (ENST00000264638.9)	c.1186G>A **(p.Gly396Arg**	Exon 8	Homozygous	Lethal congenital contracture syndrome 7 (OMIM#616286); Congenital hypomyelinating neuropathy-3 (CHN3)	Autosomal recessive	Uncertain Significance **(PM2,PP3)**
9	***SPINK5*** **(+)** (ENST00000256084.8)	c.1060A>T **(p. Lys354Ter)**	Exon 12	Homozygous	Netherton syndrome (OMIM#256500)	Autosomal Recessive	Likely Pathogenic **(PVS1, PM2)**