EXOME SEQUENCING FOR FETAL PHENOTYPES: UNCOVERING NEW GENES AND STRETCHING LIMITS OF MENDELIAN INHERITANCE

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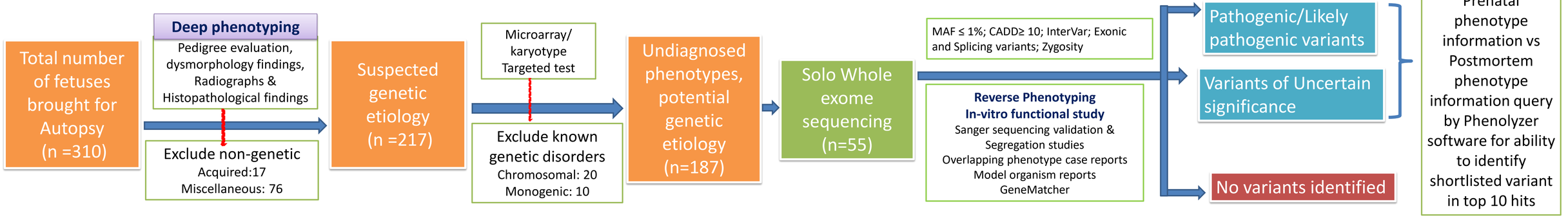
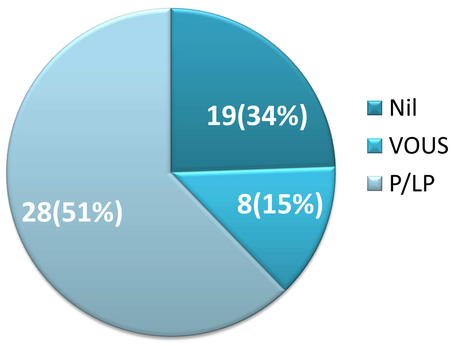
Introduction

Fetal phenotypes are likely to be enriched for genotypes with perinatal lethality or severe presentations that would be under-represented in postnatal cohorts. This study involved exome sequencing for a cohort of deeply phenotyped fetuses without a specific diagnosis with the aim to identify causative variants and novel genotype-phenotype associations.



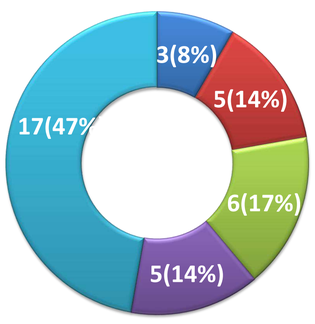
Yield of whole exome sequencing n=55



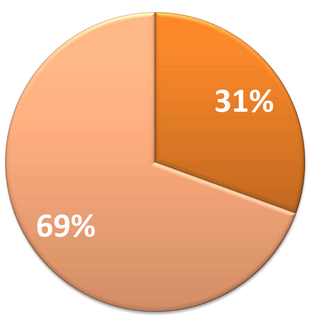
P/LP: Pathogenic/Likely Pathogenic variant
VOUS: Variant of Uncertain Significance

n= 36

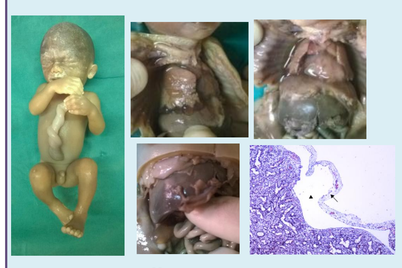
Autosomal recessive n=26 | Autosomal dominant n=10



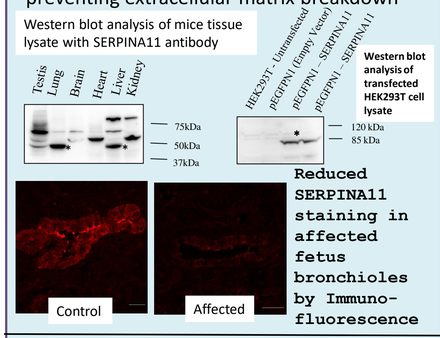
Phenolyzer aided study of importance of postmortem phenotype information

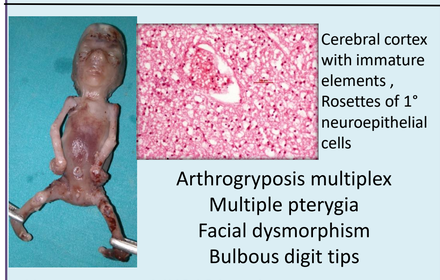


Novel Mendelian candidate genes



Serous cavity effusions;
Generalised blebs of gelatinous material on visceral surfaces
Disruption of collagen and elastin fibers in lungs
c.672C>A:p.Tyr224*
Homozygous variant in *SERPINA11*
SERPINA11- Function and expression not known. Predicted antiproteinase activity- preventing extracellular matrix breakdown

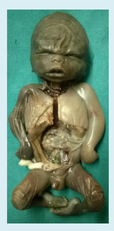




Cerebral cortex with immature elements , Rosettes of 1° neuroepithelial cells

Arthrogryposis multiplex
Multiple pterygia
Facial dysmorphism
Bulbous digit tips

Heterozygous *MYO18A* variant
NM_001346767:exon18:c.G2932A:p.A978
Zebrafish knockdown- myofiber abnormality
Important role in regulating epithelial cell migration



Arthrogryposis multiplex
Hydrops
Facial dysmorphism
Bulbous digital tips

Double heterozygous for
MYO1C exon3:c.G297C:p.Q99H
MYH3
exon40:c.A6248C:p.Q2083P

MYO1C: Candidate gene
Zebrafish knockdown- pericardial edema
Mouse knockout- abnormalities in skeletal muscle glucose transport

Novel Genotype-phenotype associations of known genes

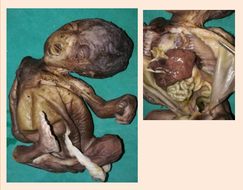
Excessive skin folds, emphysematous bullae on lung surface, Facial dysmorphism, distal joint contractures, internal hemorrhages
Aorta: Irregular elastic lamellae and degenerative disruptions of collagen, elastin and smooth muscle

LOX, c.70G>A; p.Val24Iso, Homozygous
Heterozygous variants cause thoracic aortic aneurysm in humans
Perinatal lethal due to biallelic *LOX* variants

Extensive network of dilated venous sinuses

Recurrent vein of galen malformation,hydrops

Both parents heterozygous for *ENG*
NM_000118.3 c.790G>A,Asp264Asn variant
Perinatal lethal phenotype of homozygous hereditary hemorrhagic telengiectasia



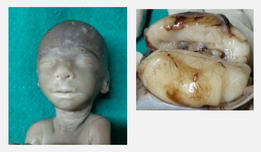
26 weeks IUD
Hydrops
Dysmorphism
Left CDH
Common atrium

Heterozygous c.185C>A:p.S62* variant in *CDK8*
Mutational hotspot of a dysmorphic syndrome
Missense variant(p.Ser62Leu) in 5/12 children with intellectual disability
CDH and lethality due to nonsense variant

Cleft lip, interhemispheric type holoprosencephaly,vermis hypoplasia
POMT1 c.1509_1510ins; p.H503fs homozygous

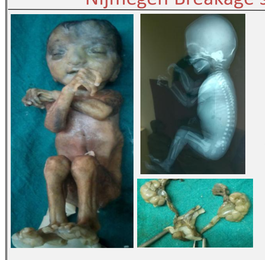
Lobar holoprosencephaly, vermis hypoplasia, recurrent
POMT1 c.G1417C:p.G473R homozygous

Expanded phenotypes of known disorders



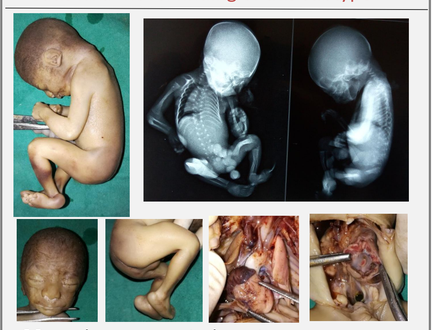
Microcephaly
Right renal hypoplasia
Corpus callosum agenesis

NBN c.T935A; p.Lys312*, Homozygous
Nijmegen Breakage syndrome



27 weeks IUD
Facial dysmorphism
Loose skin folds
Joint contractures
Hydro-uretronephroses
Cerebellar hypoplasia

ALG3 c.221A>G; p.Tyr74Cys, Homozygous
Congenital Disorder of Glycosylation 1d
Cutis laxa- novel finding of this subtype



20 weeks, recurrent phenotype
Truncus arteriosus, hydronephrosis
Platyspondyly with wafer thin vertebrae
Bent femur and tibiae,translucent ends

FGFR3 c.1183G>A:p.Val395Met, Homozygous
Novel phenotype of biallelic *FGFR3* variant

Novel finding of Focal cortical dysgenesis in Vici syndrome

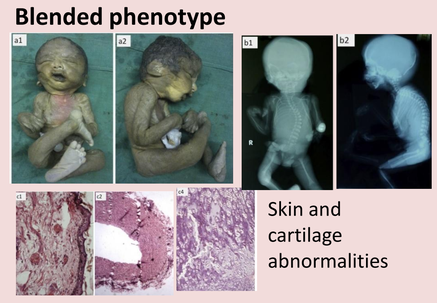
SLC6A5 c.G1759A:p.V587M,homozygous variant in a fetus with mild ventriculomegaly, recurrent in siblings
SLC6A5 variants cause hyperekplexia postnatally

Short bones
Cleft lip
Lobulated tongue
Vermis hypoplasia
Renal dysplasia,
Recurrent

NEK1 c.3253G>T ,p.Glu1085X,homozygous
Short rib polydactyly II- tongue lobules,vermis hypoplasia novel finding in this subtype

Oligogenic phenotypes

Blended phenotype

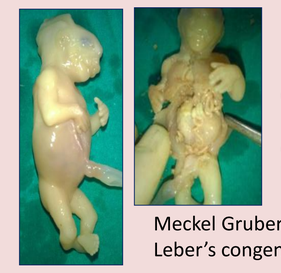


Skin and cartilage abnormalities

Multiple joint contractures, joint dislocations, arachnodactyly,micrognathia, scoliosis, loose skin folds

Double heterozygote for
c.6004C>T; p.Pro2002Ser variant *FBN1*
c.2945G>T; p.Cys982Phe variant *FBN2*
Blended Beals and Marfan syndrome

Additive phenotypes



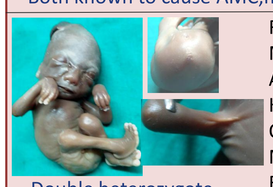
Encephalocele
Polydactyly
Cleft lip
Multicystic dysplastic kidneys

Meckel Gruber syndrome and Leber's congenital amaurosis

Double homozygous for variants
c.958G>A; p.Val320Ile in *MKS1*
c.900_906+14delTCAAGAGGTGAGTTGCCAT CA in *RPGRIP1L*

Microcephaly, hypertrichosis
Facial dysmorphism
Arthrogryposis,
Bulbous fingertips
Corpus callosum agenesis
Lissencephaly

Homozygous *ATP1A2* c.G1651C:p.G551R
Compound heterozygous *AP4M1*
c.G953A:p.R318Q & c.G1140A:p.M380I variant
Both known to cause AMC,microcephaly



Facial dysmorphism
Microcephaly
AMC
Kyphoscoliosis
Occult NTD
Multiple pterygia
Bulbous digit tips

Double heterozygote
RYR3 c. G13933A:p.A4645T, paternal
MYH3 c.C5555T:p.T1852M,maternal

MYH3 related arthrogryposis modifier effect of *RYR3* variant

Conclusions

- Deep phenotyping facilitates high diagnostic yield of exome sequencing in a fetal cohort and enables genotype-phenotype correlations
- Fetal cohorts are a goldmine for novel Mendelian gene discoveries, especially those related to lethal phenotypes underrepresented in postnatal cohorts
- Novel genotype-phenotype associations of known Mendelian disorders can present in fetal cohorts due to various mechanisms like biallelic mutations in dominant disorders or null mutations in disorders previously caused primarily by missense mutations
- Fetal exome sequencing also reveals expanded prenatal phenotypes of known Mendelian disorders- this indicates caution in use of postnatal phenotype for bioinformatic analysis
- Some fetal phenotypes are likely to represent phenomenon like oligogenic inheritance, with variants in more than one gene leading to early,severe presentation
- Postmortem/potnatal deep phenotyping is very important for bioinformatics analysis as many variants can be missed if only prenatal phenotype is available

References

1. Aggarwal et al. Exome sequencing for perinatal phenotypes: The significance of deep phenotyping. Prenat Diagn . 2020 Jan;40(2):260-273.
2. Aarabi et al. Importance of complete phenotyping in prenatal whole exome sequencing. Hum Genet. 2018;137(2):175-181.
3. Filges I, Friedman JM. Exome sequencing for gene discovery in lethal fetal disorders-harnessing the value of extreme phenotypes. Prenat Diagn. 2015;35(10):1005-1009.
4. Yang et al. Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. Nat Methods. 2015;12(9):841-843.

Acknowledgement

We acknowledge research grant received from Science and Engineering Research Board, Government of India, vide File No. EMR/2016/002478 which enabled performance of this work

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