MENDELIOME SEQUENCING AS A PRENATAL DIAGNOSTIC INVESTIGATION AFTER DETECTING FETAL ANOMALIES DURING ULTRASOUND SCREENING

Kai Muru[1], Sander Pajusalu[1,2], Konstantin Ridnõi[2,3], Hanno Roomere[1], Riina Žordania[4], Elvira Kurvinen[4], Mari Sitska[1], Tiia Reimand[1,2,5]

[1] Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia; [2] Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia; [3] Women's Clinic, East-Tallinn Central Hospital, Tallinn, Estonia; [4] Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tallinn, Estonia; [5] Department of Biomedicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

INTRODUCTION

- **Fetal anomalies are detected in about 3% of pregnancies during routine ultrasound (US) screening.**
- **The prognosis and long–term outcome is vary variable, depending of type of fetal anomaly, whether the anomaly is isolated or not, and the underlying genetic etiology.**
- **Certain congenital anomalies have been associated with chromosomal aneuploidies, while other findings may indicate monogenic disorders.**

The aim of our study was to evaluate the efficacy of using large next generation sequencing (NGS) panel in diagnostic settings in fetuses with prenatally diagnosed anomalies.

MATERIAL AND METHODS

Our study sample consisted of 17 prenatal cases (Table 1) in whom TruSight One (TSO, Illumina) panel covering 4813 genes was sequenced between July 2015 and December 2016 (18 months).
In all cases trisomies were excluded prior to TSO panel sequencing.

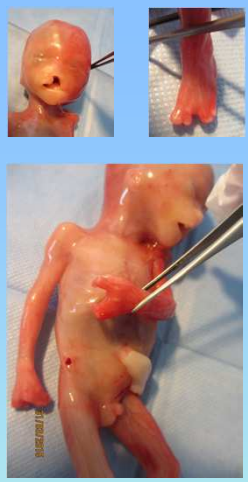
Table 1. Clinical description of patients and test results

	CLINICAL FINDINGS	TSO PANEL RESULTS	DIAGNOSIS
1	contractures of extremities, multiple pterygiums, club foot, hypospadia	negative	
2	*spina bifida apertae*, trigonocephaly, exophtalmia	negative	
3	unilateral clef lip and palate, hand-feet anomaly (ectrodactyly)	NM_003722.4(TP63):c.1028G>A p.(Arg343Gln)	EEC3 syndrome
4	malformation of the right arm, single umbilical artery	negative	
5	pulmonary hypoplasia, CCAM, unilateral agenesis of left kidney, multicystic malformation of right kidney, Meckel`s diverticulum, anal atresia, single umbilical artery	negative	
6	short extremities, hypochondroplasia (?)	NM_000316.2(PTH1R):c. 448C>T p.(Arg150Cys)	Ollier` disease (?)
7	short extremities	NM_000088.3(COL1A1):c. 649A>T p.(Met217Leu)	Osteogenesis imperfecta (?)
8	agenesis of kidneys	negative	
9	meningomyelocele, abnormal genitals, anal atresia, exopthalm	negative	
10	Fallot´ tetrad, hydropericardium, ACC, anal atresia, *spina bifida occultae*, sort femur	negative	
11	asymmetrical IUGR, malformation of left arm, anal atresia, pulmonary malformation, intestinal malrotation, duodenal atresia, bilateral dilatated ventricles, single umbilical artery	negative	Clinical diagnosis of VACTERL-H association
12	hypoplastic left heart syndrome, rhabdomyoma?	NM_007194.3(CHEK2):c.1100del p.(Thr367Metfs*15) – unclear significance	
13	agenesis of vermis, adrenal cortical pseudofollicular degeneration	negative	
14	IUGR, short extremities	negative	
15	IUD at the 23th week of pregnancy	negative	
16	IUGR, hydrocephaly	negative	Parvovirus B19 infection (?)
17	short extremities, contractures of extremities, bilaterally six finger, ascites	NM_001377.2(DYNC2H1):c.4267C>T p.(Arg1423Cys); NM_001377.2(DYNC2H1):c.5176C>T p.(Arg1726*)	Short-rib thoracic dysplasia (Majewski syndrome)

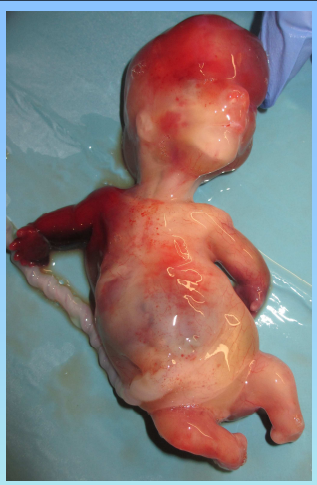
RESULTS

- **During the study period 17 fetuses were investigated with TSO panel (Table 1).**
- **A certain genetic etiology was confirmed in two cases:**
  - **First, heterozygous mutation in *TP63* gene was detected. Pathogenic *TP63* mutations are known to cause following disorders:**
    - **Ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC syndrome)**
      Rapp-Hodgkin syndrome
    - **Acro-dermo-ungual-lacrimal-tooth syndrome (ADULT syndrome)**
    - **Ectrodactyly, ectodermal dysplasia, cleft lip/palate syndrome 3 (EEC3)**
    - **Limb-mammary syndrome**
    - **Split-hand/foot malformation type 4 (SHFM4)**
    - **Isolated cleft lip/cleft palate (orofacial cleft 8)**

    The fetus presented with the phenotype of EEC3 syndrome (Picture 1): split-hand/foot deformation with cleft lip/palate. It was confirmed as a *de novo* mutation. Thus, recurrence risk is low.
  - **Second, compound heterozygous mutations were identified in *DYNC2H1* confirming the diagnosis of short-rib thoracic dysplasia, Majewski syndrome. The fetus showed (Picture 2) short extremities, bilaterally six short fingers, club foot. Both parents were carriers of one mutation. Recurrence risk is 25%.**
- **In two fetuses, possibly pathogenic mutations were detected. Both cases presented with shortening of long bones during the second trimester US screening:**
  - **paternally inherited heterozygous *COL1A1* mutation. Mutation in *COL1A1* are associated with *osteogeneis imperfecta.***
  - **heterozygous *PTH1R* mutation (not inherited from mother, paternal carrier status unknown). *PTH1R* mutations are associated with Ollier´ disease.**



Picture1. Fetus with EEC3 syndrome



Picture 2. Fetus with Majewski syndrome

CONCLUSION

Our study cohort consisted of 17 fetuses with US anomaly in whom NGS panel sequencing was performed.

- **NGS facilitated a definite diagnosis in two cases (2/17).**
- **In another two, possible pathogenic mutations were detected (2/17).**
- **Thus NGS is a feasible method for detecting genetic etiology of fetal anomalies. At the same time ethical questions remain regarding reporting variants of unclear clinical significance.**

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