Molecular studies of intestinal malrotation Rare copy number variants contribute pathogenic alleles in patients with intestinal malrotation

Karin Salehi Karlslätt^{1,2}, Maria Pettersson³, Nina Jäntti^{3,4}, Przemyslaw Szafranski⁵, Tomas Wester^{6,7}, Britt Husberg⁸, Ulla Ullberg⁹, Pawel Stankiewicz⁵, Ann Nordgren^{3,4}, Johanna Lundin^{3,4}, Anna Lindstrand^{3,4}, Agneta Nordenskjöld^{1,6}

¹ Department of Women's and Children's Health and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden, ² Department of Pediatrics, Karolinska University Hospital, Stockholm, Sweden, ³ Department of Molecular Medicine and Surgery and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden, ⁴ Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden, ⁵ Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, ⁶ Department of Pediatric Surgery, Karolinska University Hospital, Stockholm, Sweden, ⁷ Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden, ⁸ Department of General Surgery, Ersta Hospital, Stockholm, Sweden, ⁹ Department of Pediatric Radiology, Karolinska University Hospital, Stockholm, Sweden, ⁹ Department of Pediatric Radiology, Karolinska University Hospital, Stockholm, Sweden, ⁹ Department of Pediatric Radiology, Karolinska University Hospital, Stockholm, Sweden, ⁹ Department of Pediatric Radiology, Karolinska University Hospital, Stockholm, Sweden, ⁹ Department of Pediatric Radiology, Karolinska University Hospital, Stockholm, Sweden, ⁹ Department of Pediatric Radiology, Karolinska University Hospital, Stockholm, Sweden

Aim

Our aim is to find the molecular cause of intestinal malrotation and map the genes needed for normal development of the intestines.

Intestinal development

During normal fetal development the intestines rotate 270 degrees counterclockwise to end up in its normal position. A disturbance in this process leads to intestinal malrotation. Intestinal malrotation stands for an incomplete rotation and fixation of the midgut during fetal development.

- We want to be able to give information on cause, recurrence risk, associated genetic diseases and also to decrease the feeling of guilt that can be experienced by parents when a child is born with a malformation. Increased knowledge on inheritance can lead to earlier diagnosis, which will lead to less suffering.
- This can contribute to an understanding in what genes are important in the normal embryonic development of the intestines and can be of importance in other fields.
- Further it can change the routines for genetic investigation in this patient group.





Normal rotation and fixation of the midgut during fetal development. Week 6-12.

Can cause



Intestinal malrotation

- familial cases reported
- caused by genetic and environmental factors

Midgut volvulus and Intestinal obstruction



Patient with Midgut volvulus

Midgut volvulus is a life-threatening condition due to strangulation of the blood supply to the intestines and requires emergency surgery.

Methods and Results

DNA from n=47 patients with symptomatic intestinal malrotaiton was analyzed with array comparative genomic hybridization (aCGH), a method to find copy number variants (CNVs), comparing the patients DNA to a reference DNA. We used a custom high-resolution oligonucleotide aCGH with a 2 \times 400K oligonucleotide probe design targeting 1,989 genes, including all genes in the cilia proteome and known malformation syndromes.

patients had other malformations except for patient 1.

Patient	Array result	Inheritance	Hg19 CNV Coordinates	Size (bp)	Affected genes/loci	Classification
1	Heterozygous deletion	Paternal	2p23.1 (31209654-31332990)¶	123 336	GALNT14	Likely benign
2	Duplication	De novo	7q11.23 (72634873-74142327)	1 507 454	7q11.23 microduplication 16p13.11 microduplication	Likely pathogenic
	Duplication	De novo	16p13.11 (15491492-16292218)	800 726		VOUS
3	Heterozygous deletion	NI	18q22.1q23 (64876751-78015117)	13 138 366	18q deletion syndrome	Pathogenic
4	Heterozygous deletion	De novo	Xq13.1q13.2 (71728056-72215882)	487 826	HDAC8 (Cornelia de Lange typ 5)	Pathogenic
5	Duplication	De novo	16q24 (86105735-86105696) ¶	1 488 327	FOXF1	Pathogenic

Table 1. Rare CNVs identified in five patients with intestinal malrotation.

Mol Genet Genomic Med 2019 Mar; 7(3):e549. DOI: 10.1002/mgg3.549

Conclusions

Future perspectives

Whole Genome Sequencing

The literature and our previous studies have given us a reason to hypothesise that there are other genetic alterations to find in patients with intestinal malrotation. With whole genome sequencing (WGS) we can find smaller and different kinds of alterations (single-nucleotide variations, insertions and deletions, translocations and large structural variants).

This is an ongoing part of the project where we perform WGS on patients with isolated intestinal malrotation (no other malformations). Panels with candidate genes (from the literature and our findings) will initially be used, followed by more detailed analysis.

Zebrafish model

Findings will be verified with Sanger sequencing and used in a zebra fish model. A zebrafish model is an appropriate model for this study as it exhibits left-right asymmetry of the intestines, it has a fast reproductive cycle and it covers a large part of the human genome, especially during fetal development.

In the present study, we identified rare CNVs contributing pathogenic or potentially pathogenic alleles in five patients with syndromic intestinal malrotation, suggesting that CNV screening is indicated in intestinal malrotation with associated malformations or neurological involvements. In addition, we identified intestinal malrotation in two known syndromes (Cornelia de Lange type 5 and 18q terminal deletion syndrome) that has not previously been associated with gastrointestinal malformations.





Karolinska Institutet

Karin Salehi Karlslätt • MD • Pediatric resident PhD-student • Department of Women's and Children's health Address: BioClinicum J10:20 • 171 64 Solna E-mail: karin.salehi@.ki.se Phone: 073-996 63 44 What inspires me to proceed with my project is my interest in fetal development and clinical research, all that I have learnt on the way and the findings we have done so far. I get to work with great colleagues, challenging and supporting me.

