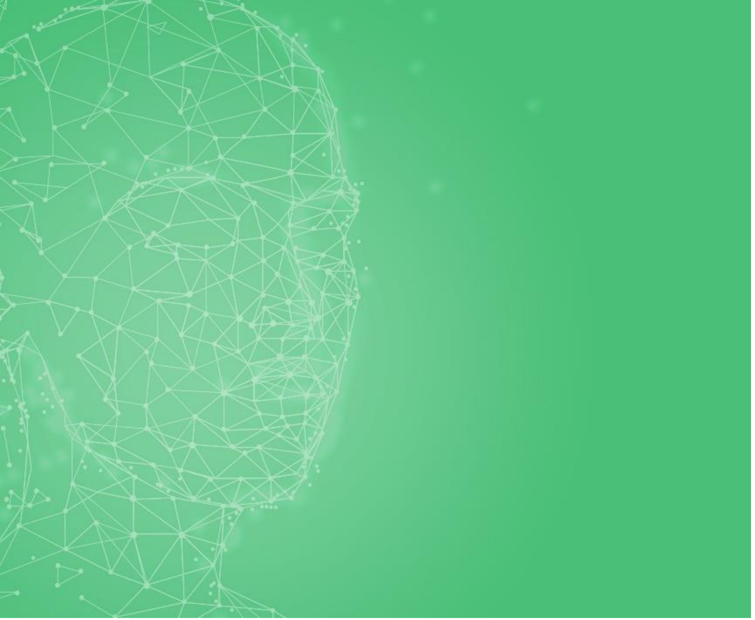


Understanding health and disease by leveraging the potential of large national biobanks and health registries

Bjarke Feenstra, Statens Serum Institut, Denmark

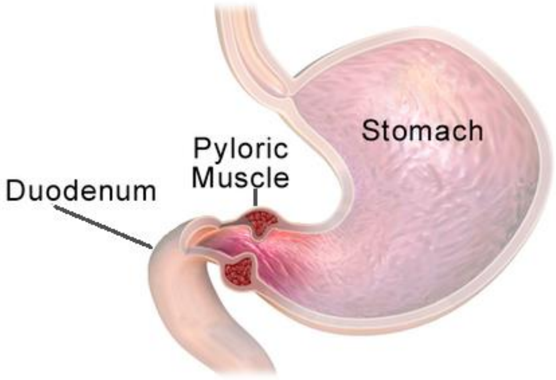
Nordic Biobank Conference 2022

Conflict of interest

No Disclosures

Focus

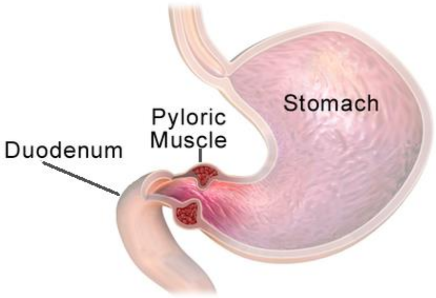
- An early childhood disease: Infantile hypertrophic pyloric stenosis (IHPS)
- Exemplifies how diverse sample types and detailed register information can lead to robust discoveries and generate hypotheses about etiology

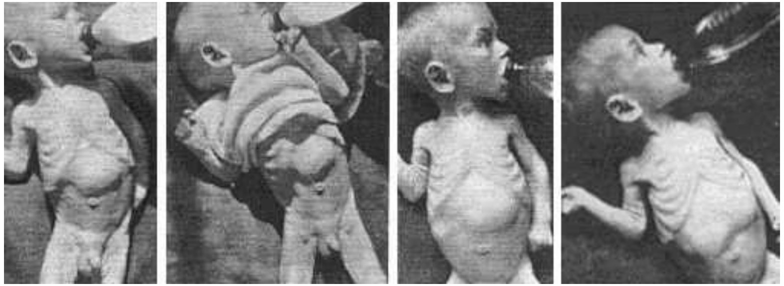



A. M. HIRSCHSPRUNG & SÖNNER
Nº 6
Nº 6
Store of Segars and Tobacco
Wholesale and detail
Dépôt de Cigarres et Tabacs
Engros & en detail

Infantile hypertrophic pyloric stenosis (IHPS)

- Occurs at age ~2-8 weeks in otherwise healthy newborns
- Caused by hypertrophy of the pyloric sphincter smooth muscle
- Symptoms: vomiting, dehydration, electrolyte imbalances
- Surgical treatment: pyloromyotomy
- Male excess, 4:1 affected boys to girls





Family Relations Database
National Patient Register

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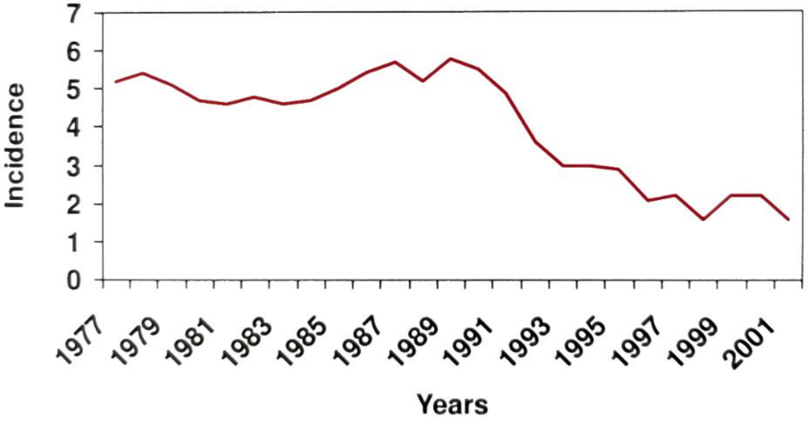
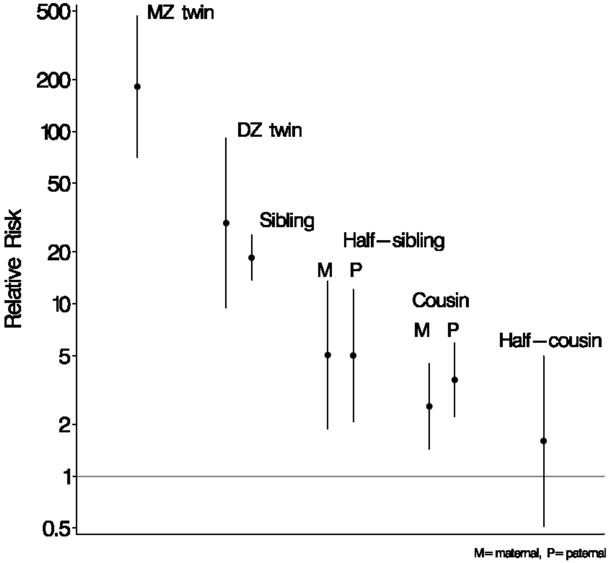
Genes and environment

High familial recurrence risks → genetic factors matter

Dropping incidence → modifiable environmental factors matter



Krogh 2013. PhD Dissertation



Krogh et al. *JAMA*. 2010

Family Relations Database
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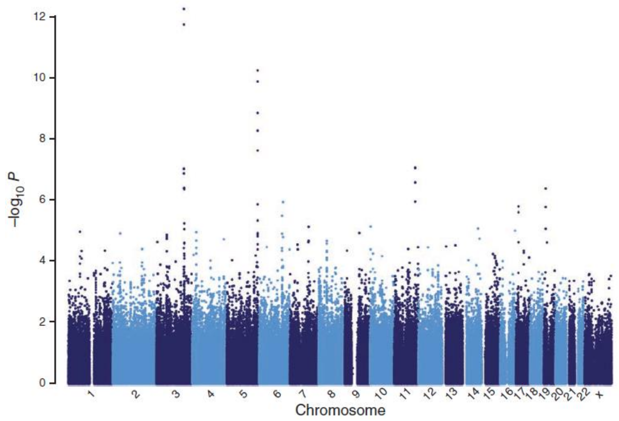
First biobank-based GWAS of IHPS

- Cases defined by surgery code for pyloromyotomy in National Patient Register
- Dried blood spot samples from neonatal screening
- Discovery ~1000 cases and ~2400 controls
- Replication in DK, SE, US (~1600 cases and ~2300 controls)

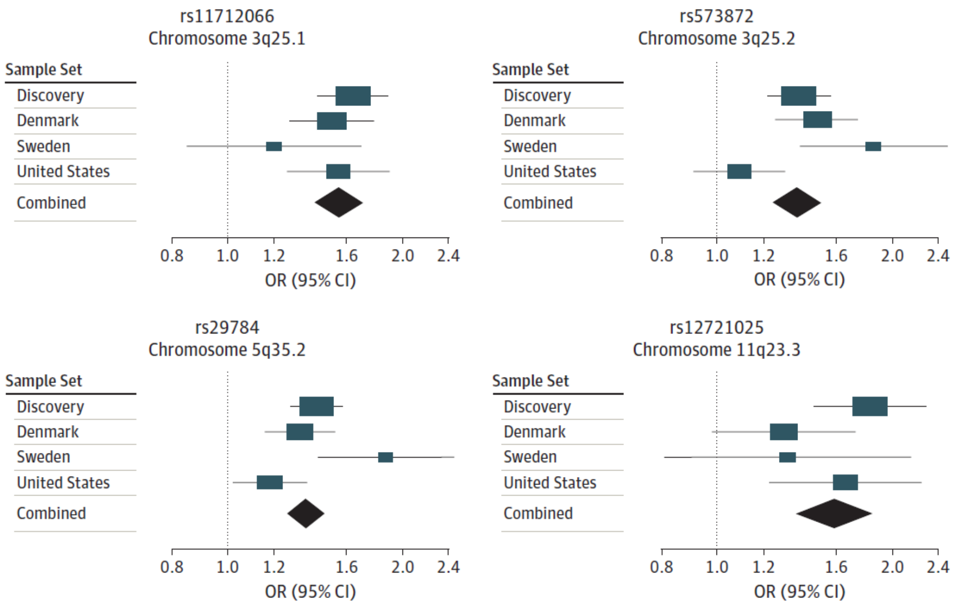
DANMARKS NATIONALE BIOBANK

Initial findings

- 4 robustly associated loci
- Effect sizes around 1.4 - 1.6
- Explain ~2% of variance in liability



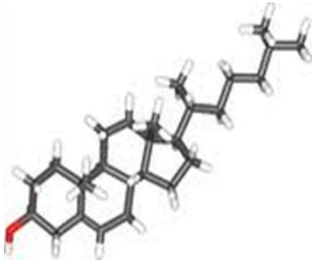
Feenstra et al. *Nat Genet* (2012)

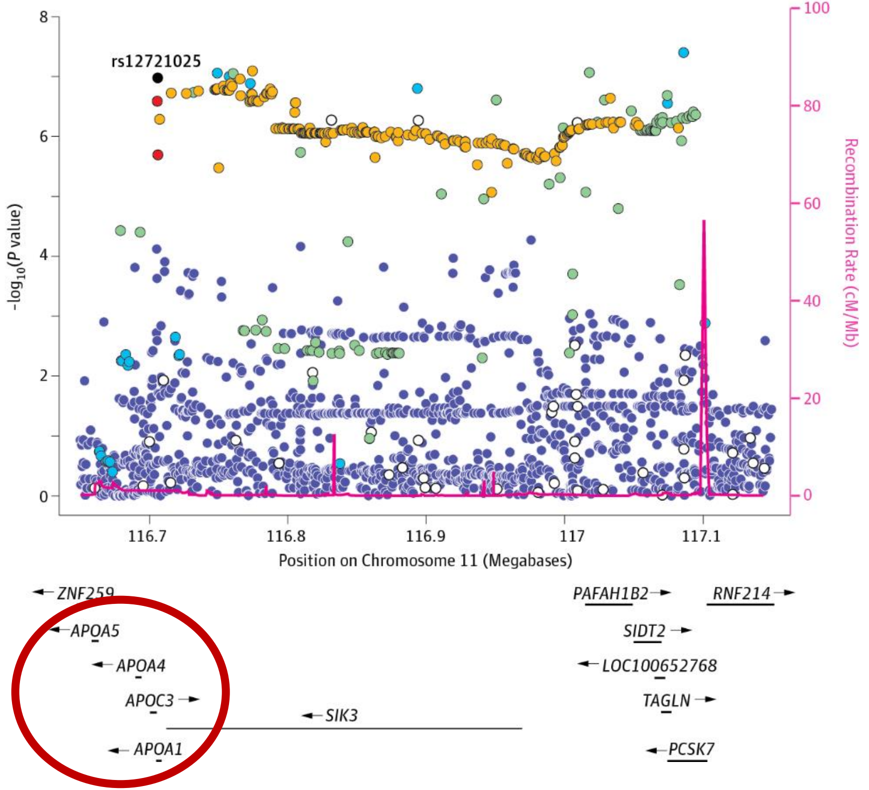


Feenstra et al. *JAMA* (2013)

A link to lipids

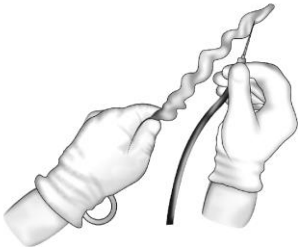
- Apolipoprotein gene cluster
- Known association with blood lipid levels
- Hypothesis: low levels of lipids in newborns are associated with increased risk of IHPS



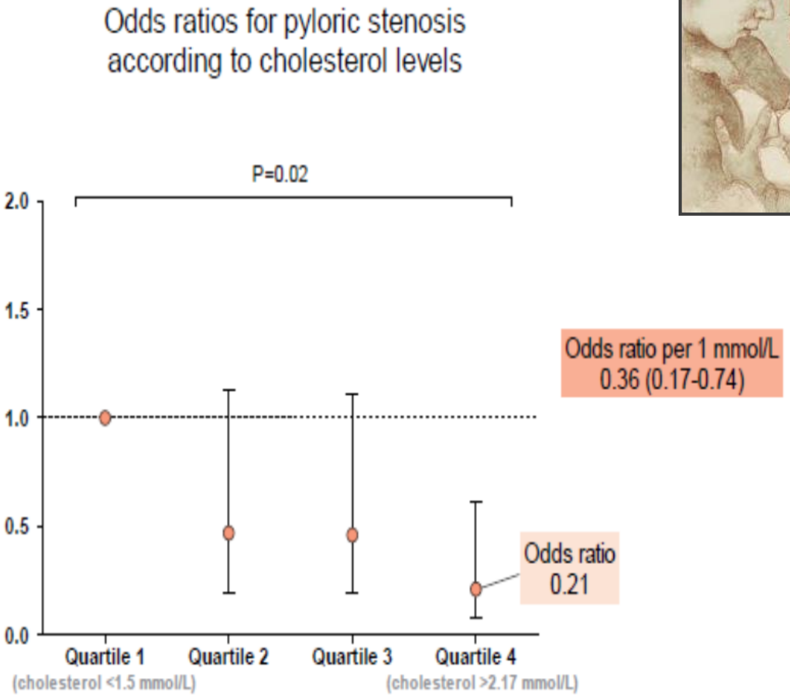


Feenstra et al. *JAMA* (2013)

A new trip to the biobank



- Lipid measurements in umbilical cord blood samples from Danish National Birth Cohort
- 46 newborns with later IHPS and 189 matched controls
- Highest risk of pyloric stenosis in newborns with lowest levels of total cholesterol



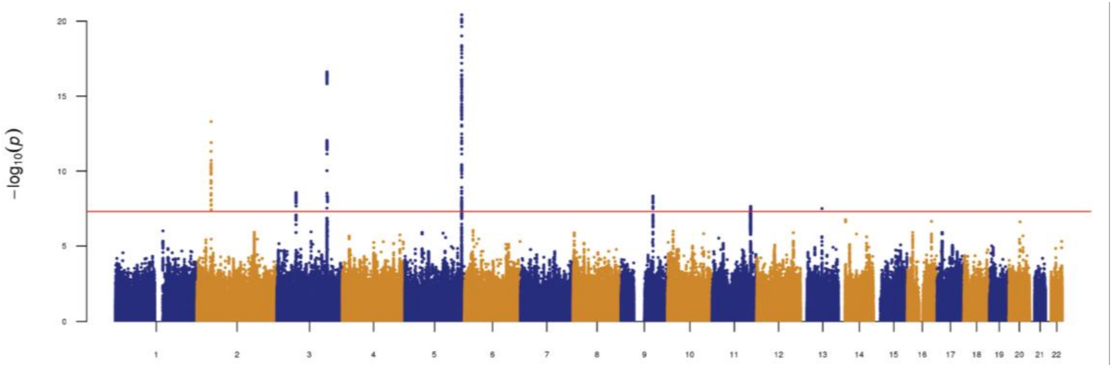
Feenstra et al. *JAMA* (2013)


Family Relations Database
National Patient Register

New round of GWAS

- Discovery: 1395 cases and 4438 controls
- Replication in SE, US: ~1800 cases, 1900 controls
- 2 new robustly replicated loci
- SNP heritability 30%
- Genetic correlations to lipids:

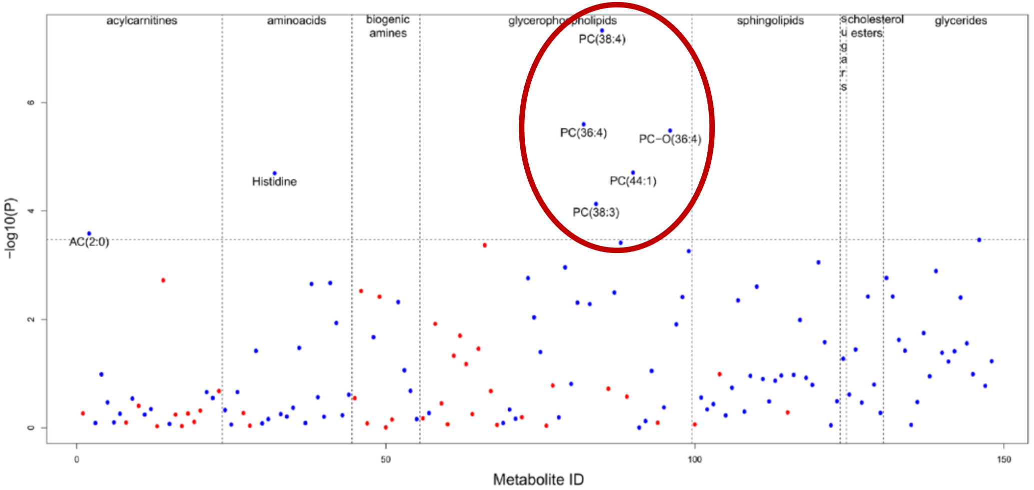
  Total cholesterol, free cholesterol, phospholipids



Fadista et al. *HMG* (2019)

Metabolites measured on filter paper

- Dried blood spot samples from neonatal screening
- 267 matched case/control pairs
- Biocrates AbsoluteIDQ p400 kit: data for 148 metabolites
- Newborns with later IHPS had lower levels of glycerophospholipids

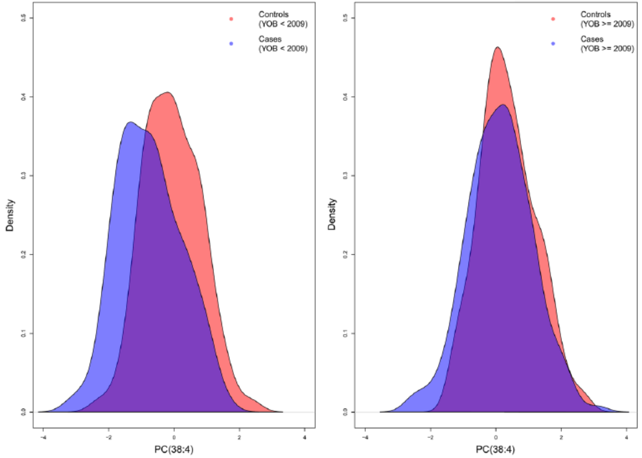


Fadista et al. *Metabolomics* (2021)

Age at sampling matters

- Neonatal screening in DK
  - before 2009 median age = 6 days
  - from 2009, median age = 2 days
- Association much stronger with age at sampling ~6 days

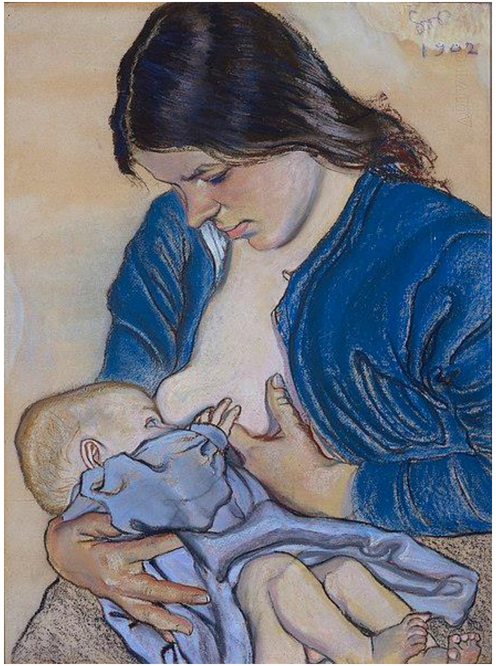
Metabolites	98 IHPS case/control pairs (YOB < 2009; median age = 6 d)			169 IHPS case/control pairs (YOB ≥ 2009; median age = 2 d)		
	Beta	SE	P	Beta	SE	P
PC(38:4)	– 0.71	0.12	**1.28E–08**	– 0.25	0.09	9.35E–03



Fadista et al. *Metabolomics* (2021)

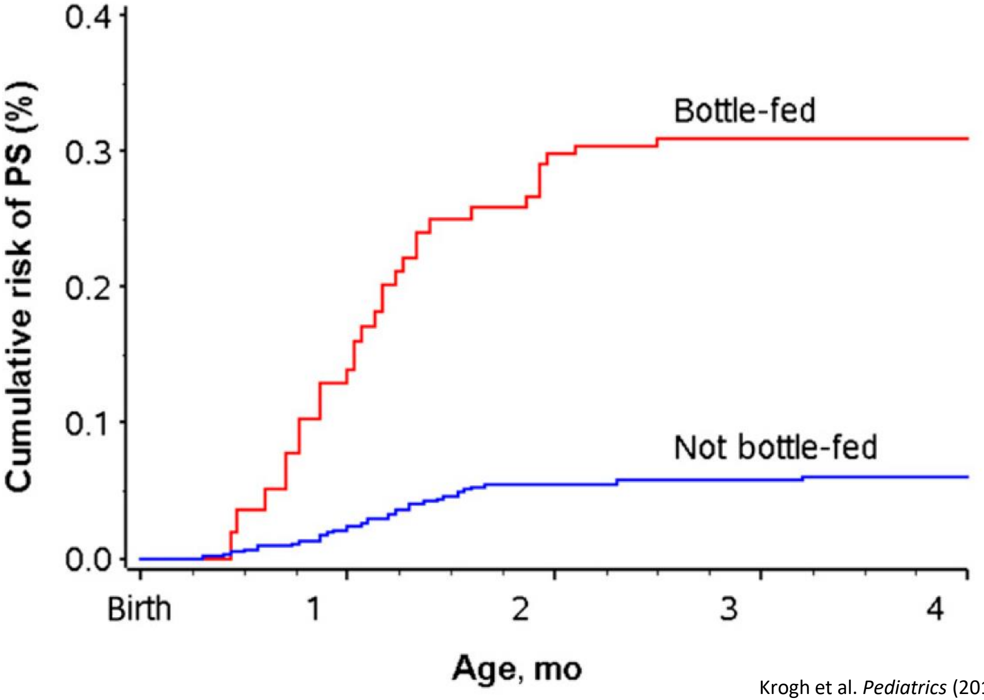
A link to breast feeding?

- At 6 days after birth, more opportunity for differences in feeding practice to have an effect
- Phospholipids known to be at higher levels in blood from breast-fed infants compared to bottle-fed infants
- More IHPS cases (n = 17) than controls (n = 4) had the ICD-10 code P92.5 (neonatal difficulty in feeding at breast; OR, 4.46; $P = 6.15 \times 10^{-3}$)



Stanisław Wyspiański 1902: Motherhood

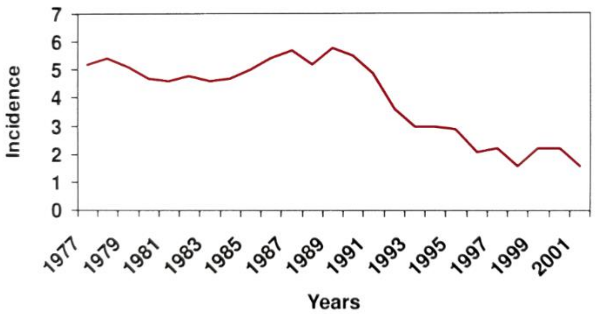
Bottle feeding associated with IHPS risk



Krogh et al. *Pediatrics* (2012)

Lipids and epidemiological observations

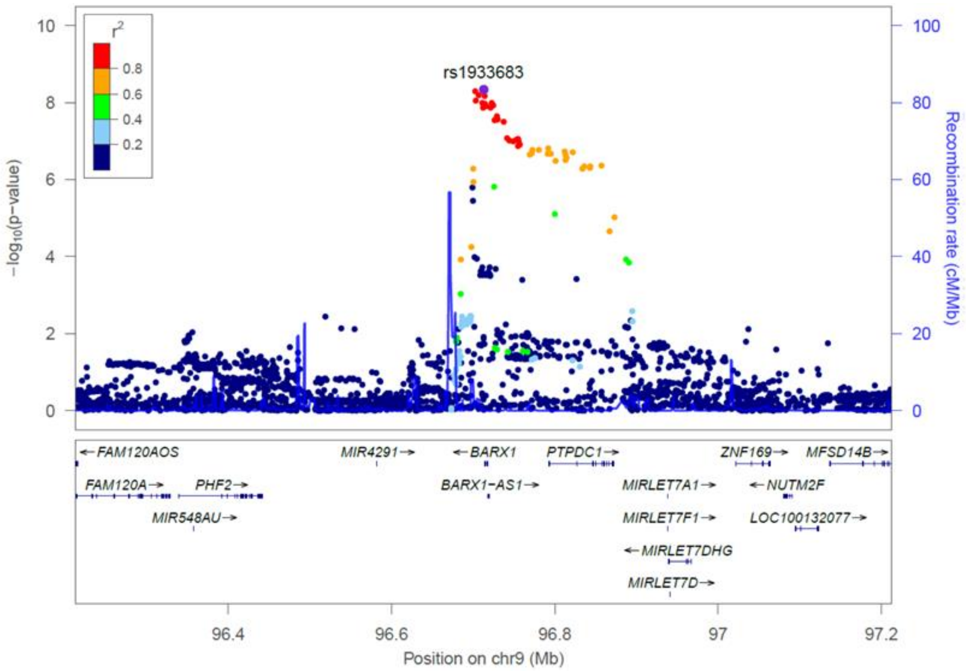
- 4:1 Boys vs Girls… and lower average lipid levels in boys at birth
- Bottle-fed babies at 5x increased risk… and bottle-feeding leads to lower levels of circulating lipids
- Decreasing incidence in the 1990s… while breast feeding incidence (and thereby average lipid levels in infants) increased



- Pyloric stenosis seen in 10-15% of patients with Smith-Lemli-Opitz syndrome, a rare inborn defect of cholesterol biosynthesis

Chromosome 9q22.32 locus

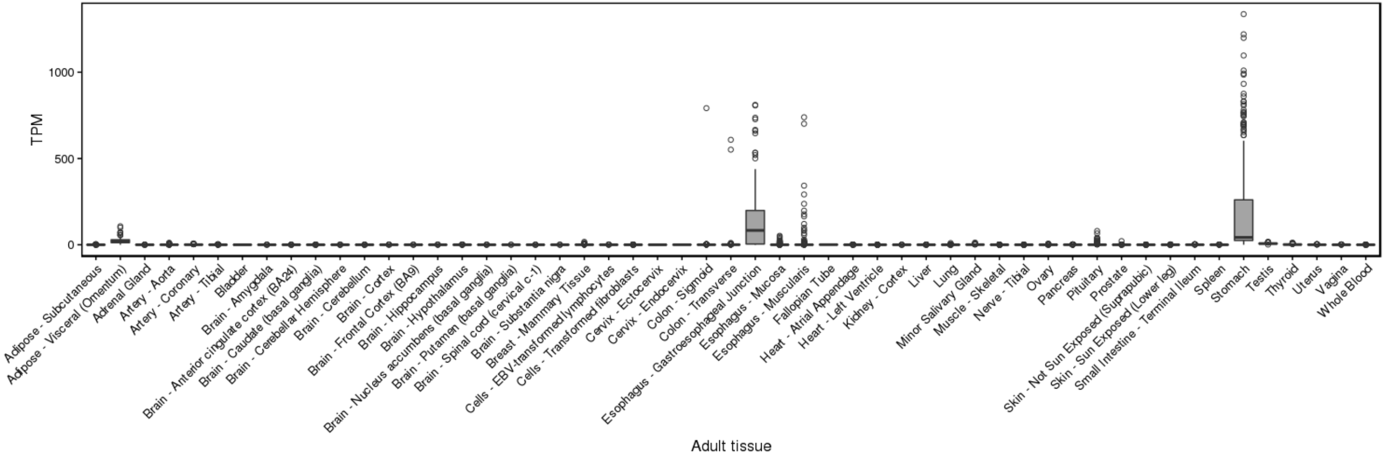
- Top associated variants cover the *BARX1* gene
- Encode a homeodomain (hox) transcription factor
- Expression of hox genes crucial for normal limb and organ development

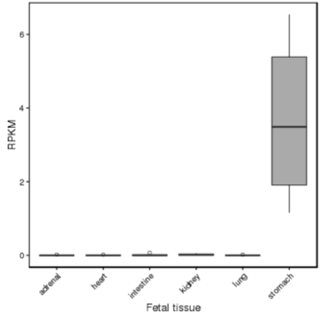


Fadista et al. *HMG* (2019)

BARX1

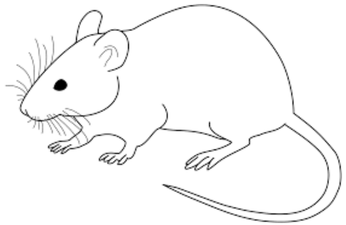
- Expression in adult tissues: Stomach and gastroesophagal junction
- Expression in developing fetus: Stomach





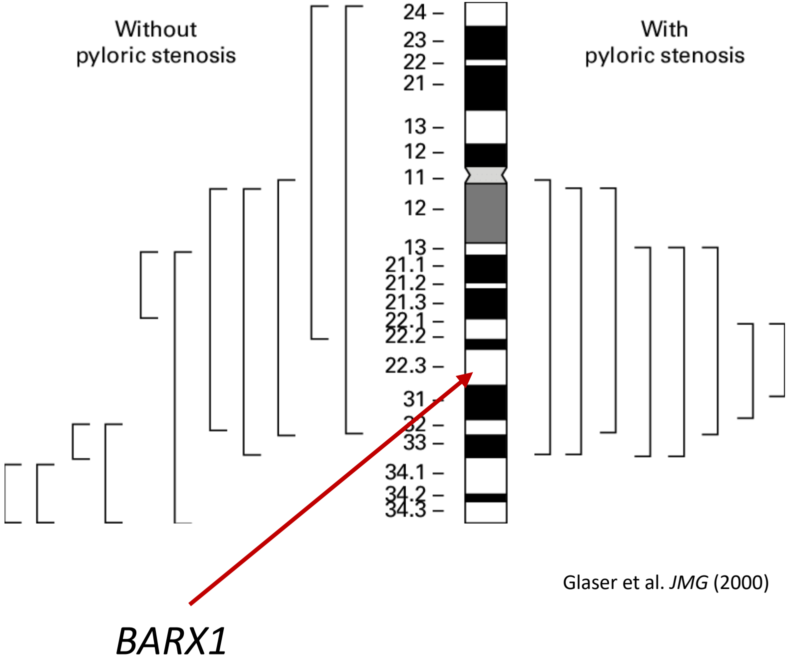
Barx1 in mice

- Essential for the formation of the stomach in mouse embryo
- Pyloric sphincter is absent in *Barx1* null mice
- Ectopic expression of *Barx1* in developing intestine induces smooth muscle of stomach type



9q duplication syndrome

- Region harbors the *BARX1* gene
- Syndrome often includes IHPS as part of clinical presentation
- Example of common variant analyses of isolated cases helping to identify candidate gene involved in a specific condition in a syndrome associated with a larger genomic region



Glaser et al. *JMG* (2000)

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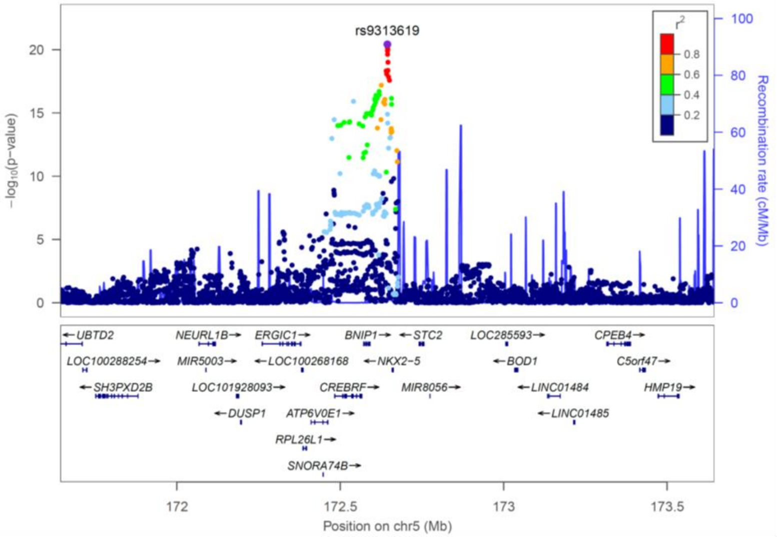
NKX2-5 locus

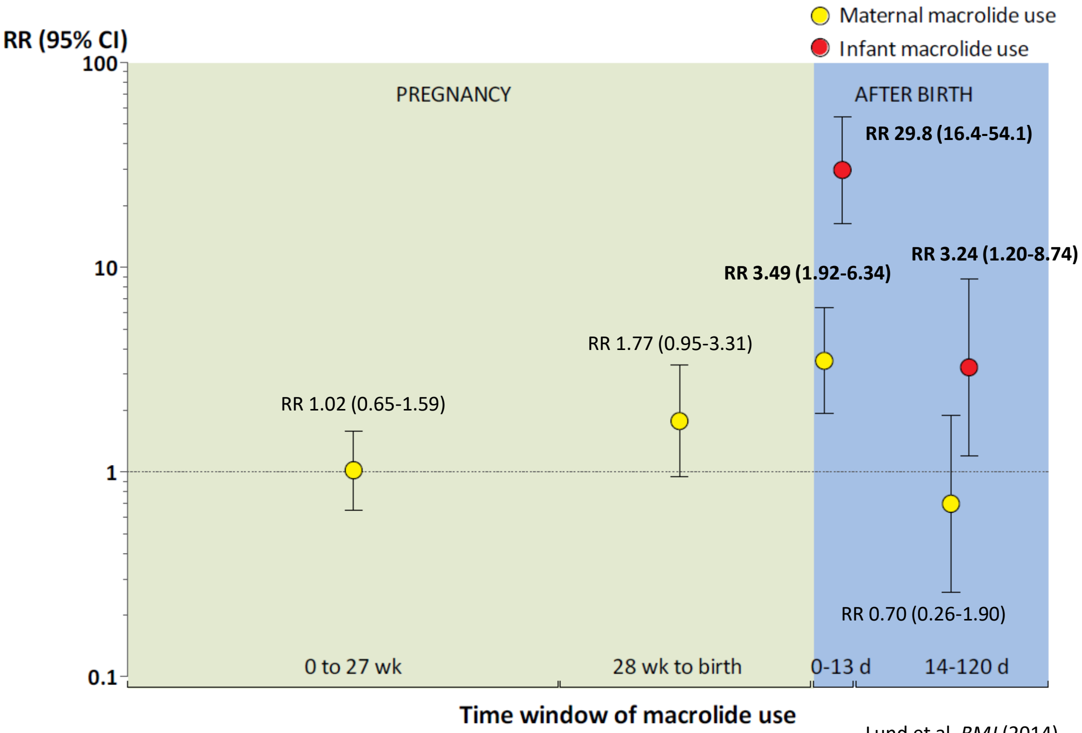
- In chicken and mouse, *NKX2-5* expression occurs in a sharply defined ring between the foregut and midgut on specific days of embryonic development
- Repression of *NKX2-5* results in loss of the pyloric sphincter endodermal phenotype
- Conversely, formation of pyloric sphincter–like epithelium in other parts of the stomach can be induced by ectopic expression of *NKX2-5* via a retroviral vector



Fadista et al. *HMG* (2019)

Exposure to macrolide antibiotics

- Exposures information from Danish National Prescription Registry
- Strongest environmental risk factor
- Most vulnerable period first 2 weeks after birth
- Not many infants exposed, but 24 excess cases per 1000 exposed
- Effect on pyloric sphincter muscle contraction



Lund et al. *BMJ* (2014)

Conclusions

- Different types of biobanked samples + health registers + 'omics technologies provide new pieces of the puzzle
- Strong modifiable risk factors: bottle-feeding, macrolide exposure
- Robust genetic associations → hypotheses about mechanisms
- Causal link between lipids and IHPS?
- Importance of embryonic and early post-natal development of gastrointestinal tract

The Continuing Enigma of Pyloric Stenosis of Infancy

A Review

Brian MacMahon

MacMahon *Epidemiology* (2006)

Perspectives

- Possible to protect newborns in families with a history of IHPS through dietary supplementation?
- Recommendations for nutritional composition of milk formula
- Importance of breast feeding
- Further research:
    - Functional follow-up studies
    - Unified studies of genetics, metabolomics, feeding and macrolide exposure

Inheritance of Congenital Pyloric Stenosis

C. O. CARTER and K. A. EVANS

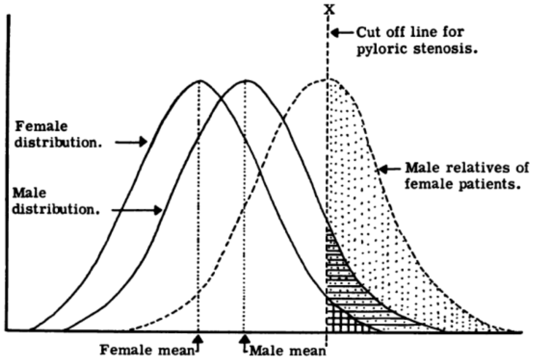


FIG. Hypothetical distribution of multifactorial genotype contributing to pyloric stenosis, in males, females, and male relatives of female index patients.

Carter & Evans *JMG* (1969)

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Acknowledgements

João Fadista
Line Skotte
Frank Geller
Camilla Krogh
Marie Lund
Mads Melbye

STATENS SERUM INSTITUT

DANMARKS NATIONALE BIOBANK

DET FRIE FORSKNINGSRÅD
DANISH COUNCIL FOR INDEPENDENT RESEARCH

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CARLSBERGFONDET

NATIONAL INSTITUTES OF HEALTH

THE UNIVERSITY OF IOWA

Karolinska Institutet

OAK FOUNDATION

Thank you!

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