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Copenhagen Studies on Asthma in Childhood

FACULTY OF HEALTH SCIENCES
UNIVERSITY OF COPENHAGEN

Genetics of childhood asthma with severe exacerbations

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Copenhagen Prospective Study on Asthma in Childhood
Denmark

Programme

- A registry-based genetic study on childhood asthma with severe excerbation
- A future clinical follow-up study

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From skin barrier defect to childhood asthma and allergy

- Filaggrin
  - A skin barrier protein

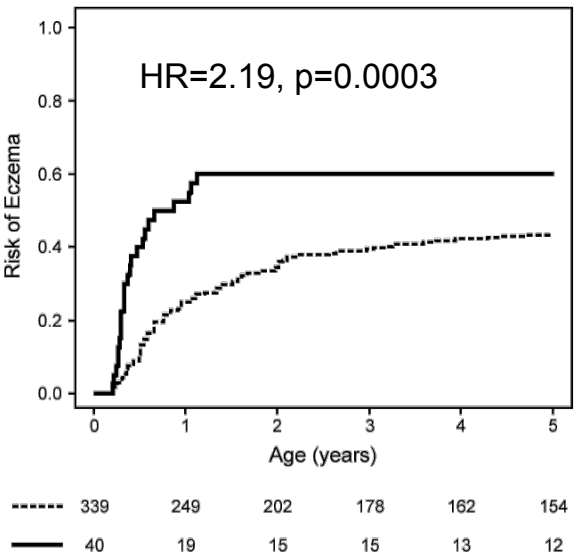
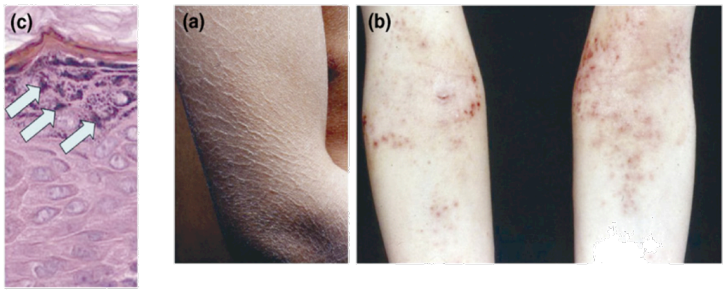


Figure 1. Kaplan-Meier Estimates of Cumulative Risk of Eczema in the COPSAC Cohort with and without FLG Mutation



- Filaggrin and eczema
  - $COPSAC_{2000}$

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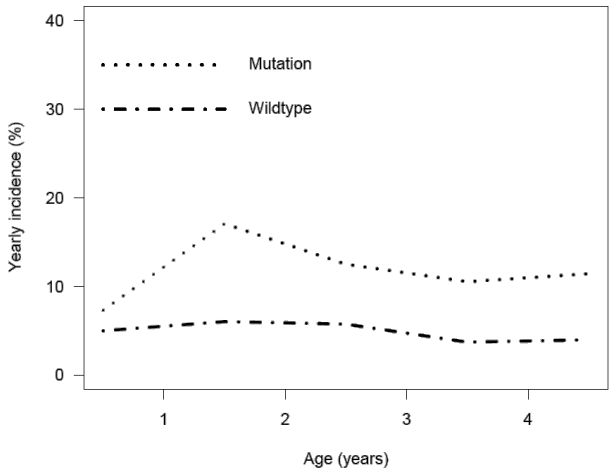
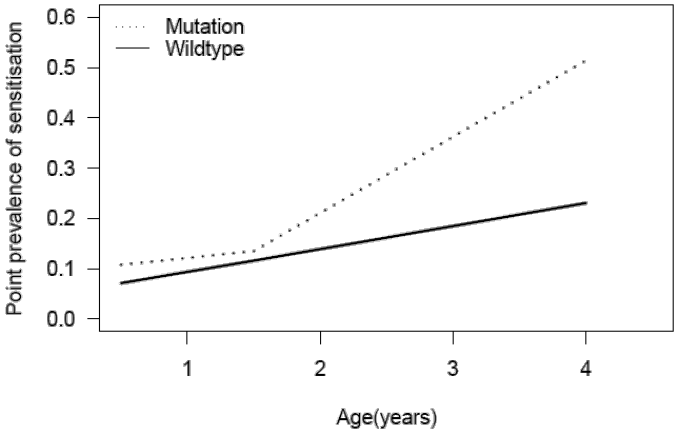
Filaggrin defined atopic asthma phenotype in early childhood

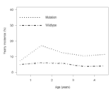
Asthma exacerbations



Allergic sensitization



Bønnelykke et al. PAI 2010



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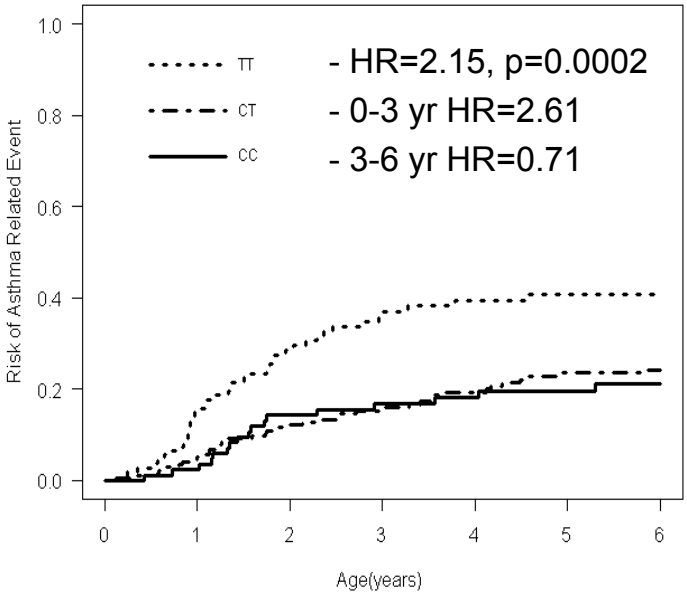
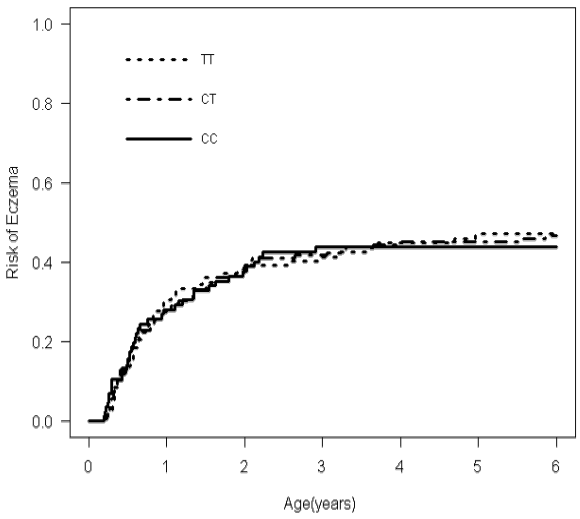
ORMDL3-defined early non-atopic asthma

Asthma



No association with eczema



Bisgaard et al. AJRCCM 2009

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The ”black box” of pre-school asthma

- A highly heterogeneous syndrome
- Viral-induced episodic symptoms
- Neutrophilic airway inflammation (?)
- Lack of objective measurements
- Etiologies largely unknown
- Poor treatment response

$COPSAC_{CASE}$- a registry-based cohort on asthma with severe exacerbations

- Genetic discovery
- Focusing on early childhood asthma with recurrent severe exacerbations
  - Clinical relevance
  - Highly heritable phenotype
  - Specific endotype (?)

Bønnelykke et al. Nature Genetics 2014

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Design

- Asthma cases identified from hospital registries
  - 1,173 cases with recurrent asthma hospitalizations (2-6 years)
  - 2,500 controls
- Blood from the neonatal screening biobank
- DNA-amplification
- Genome-wide association study (125,000 SNPs)

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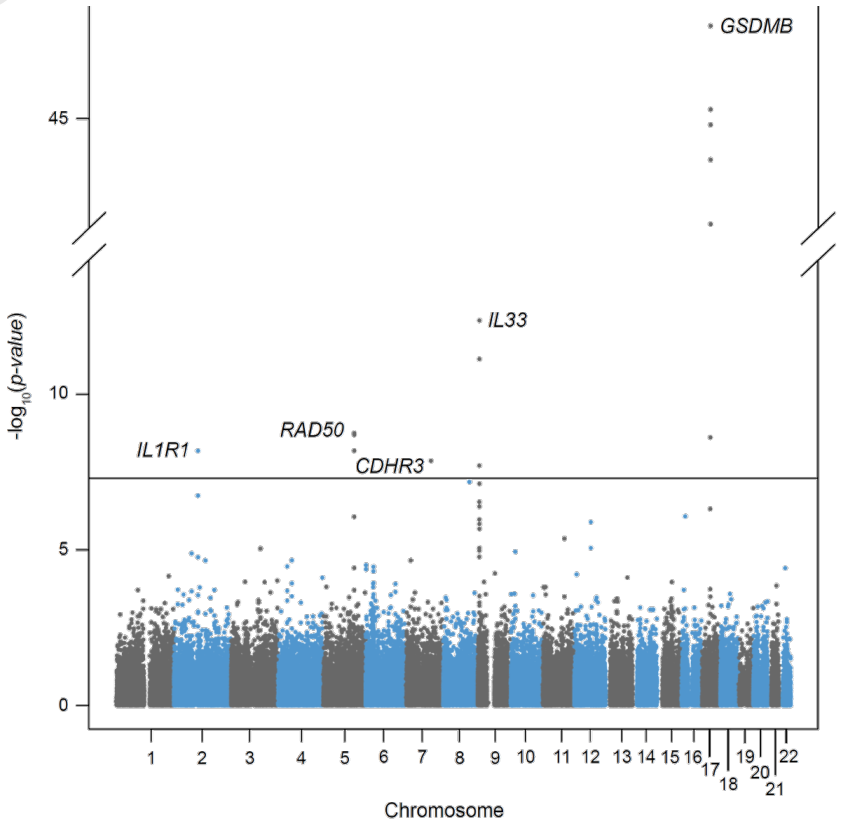
Results



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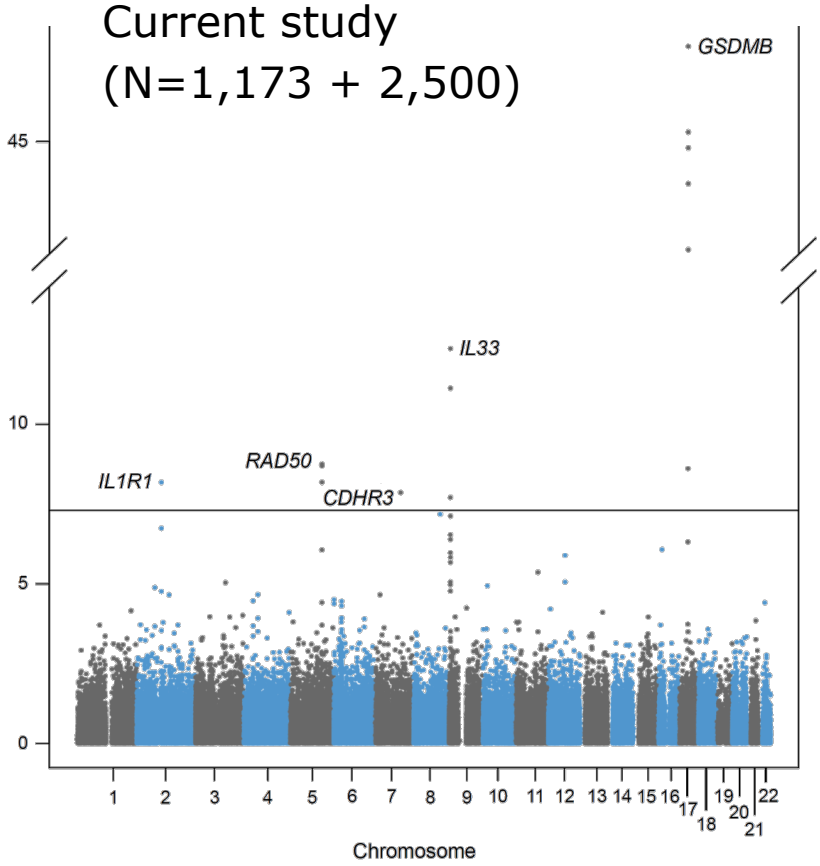
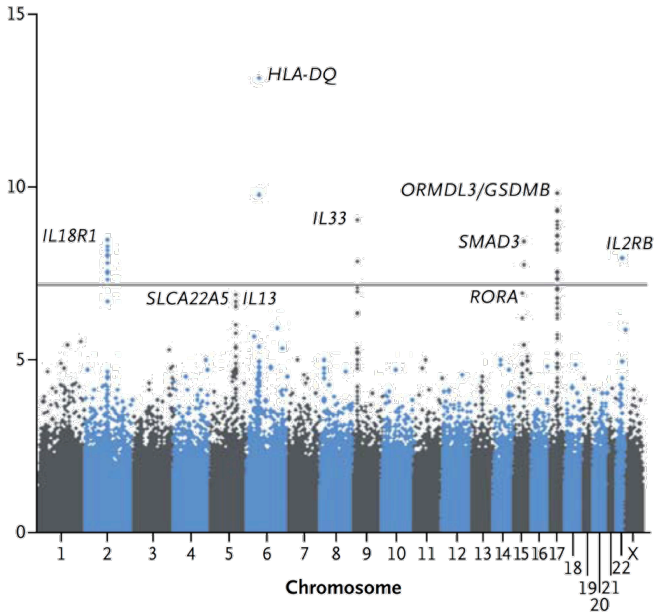
Results compared with largest published GWAS

Current study
(N=1,173 + 2,500)



Previous (GABRIEL)
(N=10,000 + 10,000)



Increasing effect size by increasing severity

				Current GWAS stratified on number of asthma hospitalizations			
		Previous (Gabriel)	**Current study**	**2**	**3**	**4-5**	**6 or more**
	Cases	N=10,365	N=1,173	N=272	N=228	N=277	N=358
GSDMB	OR	**1.17**	**2.28**	**1.87**	**2.24**	**2.24**	**2.72**
	P-value	4.6E-09	1.3E-48	1.5E-10	2.1E-13	1.7E-15	3.5E-27
IL33	OR	**1.20**	**1.50**	**1.32**	**1.22**	**1.47**	**1.91**
	P-value	8.7E-12	4.2E-13	0.005	0.07	8.5E-05	6.2E-14
RAD50	OR	**1.15**	**1.44**	**1.31**	**1.26**	**1.45**	**1.58**
	P-value	1.4E-08	1.8E-09	0.01	0.05	3.6E-04	1.3E-06
IL1R1	OR	**1.15**	**1.56**	**1.53**	**1.20**	**1.32**	**2.19**
	P-value	3.5E-12	6.6E-09	0.002	0.20	0.04	3.2E-08
CDHR3	OR	**1.18**	**1.45**	**1.23**	**1.37**	**1.42**	**1.63**
	P-value	3.0E-06	1.4E-08	0.07	0.01	0.003	1.6E-06

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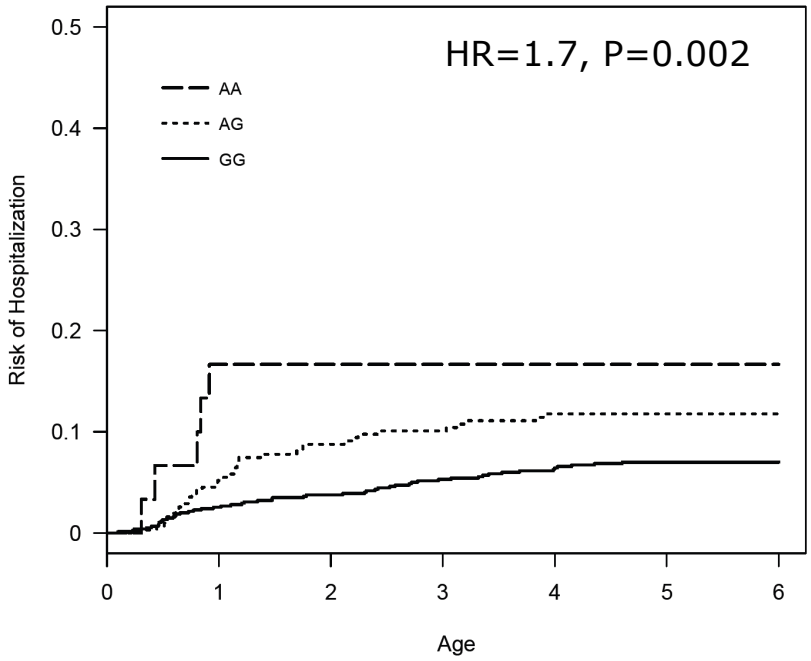
CDHR3 replication in birth cohorts (COPSAC + MAAS)

Acute hospitalization for asthma



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CDHR3 subanalyses in birth cohorts

	Meta-analysis COPSAC$_{2000}$+MAAS+ Generation R	
	OR (CI)	*P* value
Asthma ever 0-6 yrs	1.40 (1.16-1.67)	**3.2 E-04**
Asthma/recurrent wheeze ever 0-3 yrs	1.35 (1.16-1.57)	**7.5 E-05**
Asthma with exacerbation (0-6 yrs)	1.68 (1.21-2.34)	**0.002**
Asthma without exacerbation	1.36 (0.97-1.91)	0.08
Number of exacerbations (0-6 yrs) >=2 exacerbations	1.60 (1.08-2.38)	**0.02**
1 exacerbation	1.33 (0.89-1.98)	0.16
Eczema ever 0-6 yrs	0.93 (0.82-1.06)	0.30
Allergic sensitization by age 5/6 yrs	1.22 (0.97-1.54)	0.09*

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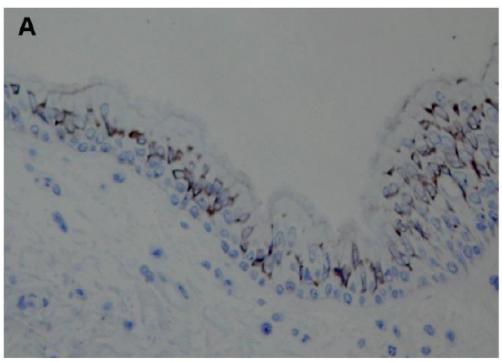
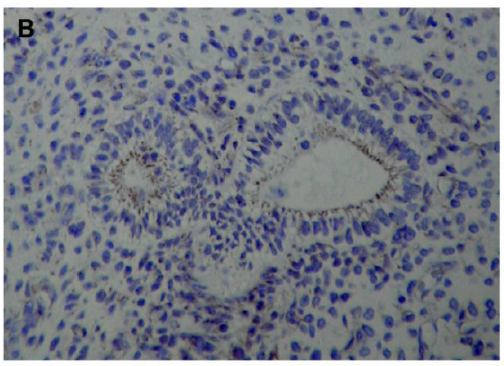
CDHR3 – a lung epithelial protein

- Cadherins involved in cell adhesion, epithelial polarity and signalling
  - Family members associated with asthma (E-cadherin, PCDH1)
- CDHR3 predominantly expressed in respiratory epithelium and upregulated during epithelial differentiation





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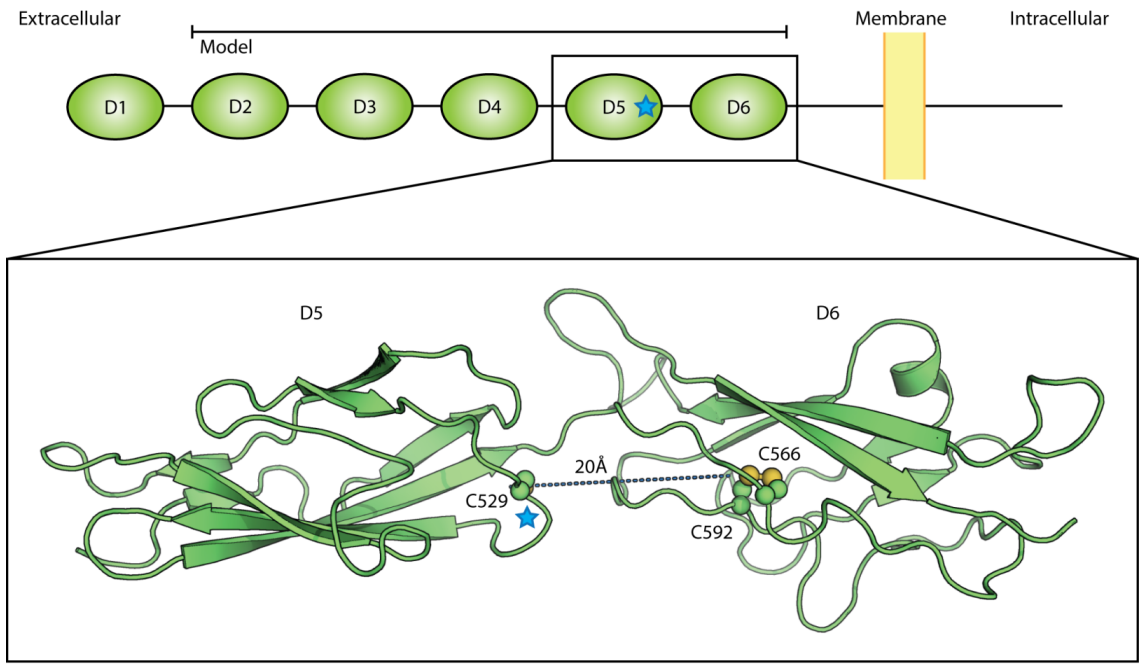
CDHR3 protein model



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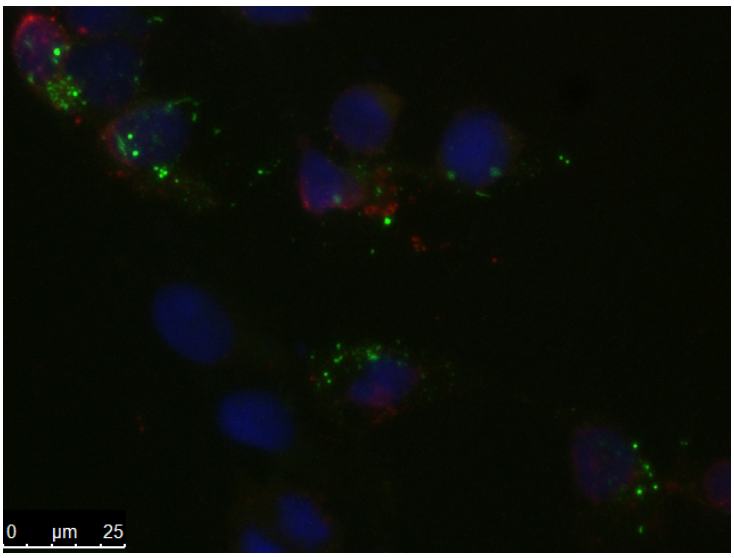
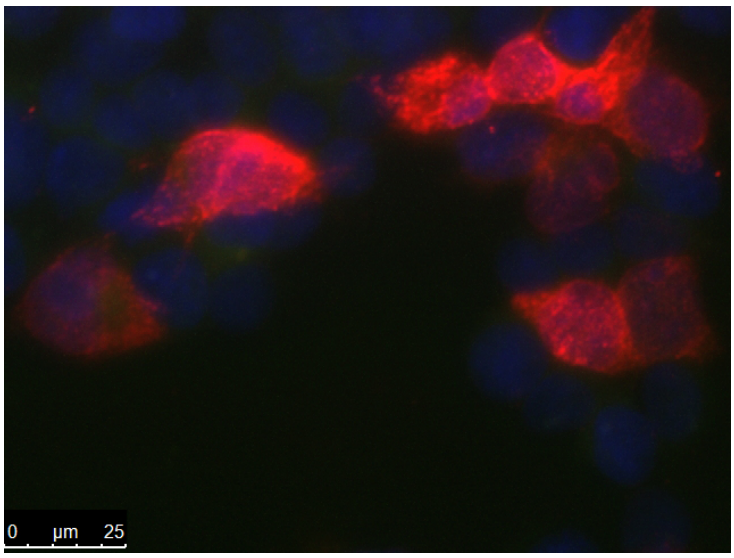
The risk variant affects surface expression of the protein

Risk variant (surface expression)



Wild type (intracellular expression)



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Species	Common name	Group	Order	529	Ind.
Homo sapiens	Human	Mammals	Primates	C (app 65% homozygous)	-
Pan troglodytes	Chimpanzee	Mammals	Primates	Y	1
Pan paniscus	Bonobo	Mammals	Primates	Y	1
Gorilla gorilla	Gorilla	Mammals	Primates	Y	3
Pongo abelii	Sumatran orangutan	Mammals	Primates	Y	1
Nomascus leucogenys	Northern white-cheeked gibbon	Mammals	Primates	Y	1
Papio anubis	Olive baboon	Mammals	Primates	Y	1
Saimiri boliviensis	Black-headed squirrel monkey	Mammals	Primates	Y	2
Callithrix jacchus	Common marmoset	Mammals	Primates	Y	1
Otolemur garnettii	Greater galago	Mammals	Primates	Y	1
Ornithorhynchus anatinus	Duck-billed platypus	Mammals	Monotremeta	Y	1
Monodelphis domestica	Gray short-tailed opossum	Mammals	Didelphimorphia	F	1
Sarcophilus harrisii	Tasmanian devil	Mammals	Dasyuromorphia	F	1
Mus musculus	House mouse	Mammals	Rodentia	H	2
Cricetulus griseus	Chinese hamster	Mammals	Rodentia	H	1
Rattus norvegicus	Brown rat	Mammals	Rodentia	H	1
Spermophilus tridecemlineatus	Thirteen-lined ground squirrel	Mammals	Rodentia	Y	2
Cavia porcellus	Guinea pig	Mammals	Rodentia	Y	1
Odobenus rosmarus	Walrus	Mammals	Carnivora	Y	1
Ailuropoda melanoleuca	Giant panda	Mammals	Carnivora	Y	1
Canis familiaris	Dog	Mammals	Carnivora	Y	1
Felis catus	Cat	Mammals	Carnivora	Y	1
Equus caballus	Horse	Mammals	Perissodactyla	H	1

Conclusions

- Identification of *CDHR3* as a novel asthma gene
  - Early asthma with severe exacerbations
  - Highlights the importance of the airway epithelial barrier
  - May identify a novel disease mechanism
- Strong results for known asthma genes
  - Proves strength of specific phenotyping
  - Powerful approach for identifying phenotype specific as well as general disease mechanisms

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Mechanisms of Childhood Asthma with severe exacerbations ($COPSAC_{CASE}$)

- A (future) clinical cohort of children with recurrent severe exacerbations
- Aiming to understand disease mechanisms
  - Genetic regulation and disease pathways
  - Subtypes of disease
  - Prediction of exacerbations
- Combining
  - **global assessments of genetics, epigenetics and gene expression**
  - clinical and immune assessments

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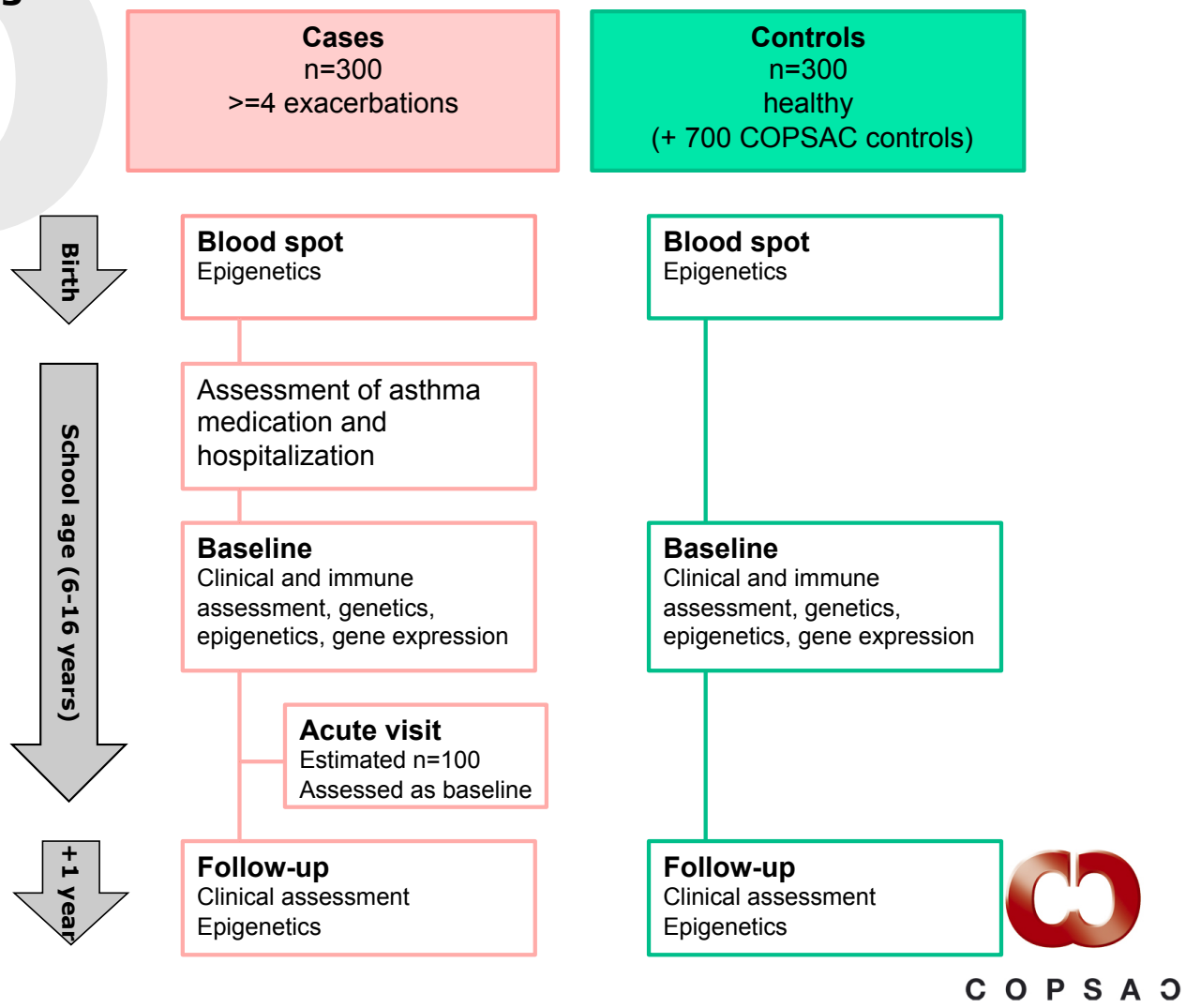
Study design



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Genetic regulation and disease pathways (WP1)

- Integrative analyses of genetics, epigenetics and gene expression
- Focus on epigenetics
  - Increasing evidence of large role in asthma and allergy
  - Explain characteristics of asthma
    - ➔ “Missing heritability”
    - ➔ Early programming
  - Mediate environmental factors

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- Methodology
  - Genome-wide arrays (epigenetics as methylation)
  - Analyses
    - Longitudinal assessments
    - Blood vs. target organ (nasal respiratory epithelium)
    - Environmental factors
    - Post-doc project (bioinformatician)

- Perspectives
  - Identification of novel targets for prevention and treatment

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Functional subtypes of disease (WP2)

- Heterogeneity is a main reason for poor treatment response
- Methodology
  - Combining symptom characteristics, intermediate traits, allergy assessment, immune data and susceptibility genes/pathways
  - PhD-study supervised by bioinformatician
- Perspective
  - Improved treatment response by tailored management

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Prediction of exacerbations (WP3)

- Prediction based on clinical assessment is inadequate
- Methodology
  - Combining clinical and immune phenotyping with data on genetics, epigenetics and gene expression
  - (Metabolomic analyses of blood and exhaled air)
  - PhD-study supervised by bioinformatician
- Perspective
  - Prevention of severe asthma attacks by optimized treatment

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Assessments and biobank	Birth	School-age baseline	School-age + 1 year	Acute visit
Clinical assessments				
Symptom burden		▼	▼	▼
Lung function, airway reactivity, airway inflammation		▼	▼	▼
Allergy measurement (skin prick test + specific IgE)		▼		
Immunology				
Mucosal imprint for cyto-, chemokines		▼	▼	▼
Stimulated PBMCs		▼	▼	▼
Epigenetics				
Airway epithelium (nasal)		▼	▼	▼
Peripheral blood	▼	▼	▼	▼
Gene expression				
Airway epithelium (nasal)		▼	▼	▼
Peripheral blood	▼	▼	▼	▼
Metabolomics				
Exhaled air (Volatile Organic Compounds)		▼	▼	▼
Peripheral blood	▼	▼	▼	▼
Microbiome				
Nasal swab		▼	▼	▼
Fecal Sample cultures		▼	▼	▼
Exposures				
Vitamin D levels	▼	▼	▼	▼

▼ samples collected for future studies (outside the current proposal)

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Collaboration

- Genetic and epigenetic analyses (University of Chicago)
- Bioinformatics (LIFE Sciences)
- Blood spot analyses (Statens Serum Institut)
- Replication
  - $COPSAC_{2000}$ + $COPSAC_{2010}$ birth cohorts
  - Consortium of birth cohorts (EAGLE)
- Experimental studies (University of Southampton)
- IMI, EU-projcet
  - Severe childhood asthma (N=200), breathomics
- “Global” meta-analysis on allergic rhinitis

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Perspectives

- Improve understanding of genetic regulation and disease pathways of severe childhood asthma
- Identify functional subtypes of severe disease
- Improve prediction of exacerbations
- Potential to improve prevention and treatment of disease
- Provide a valuable cohort for further studies of asthma etiologies

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