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Study of the genetic heterogeneity of the Filaggrin gene

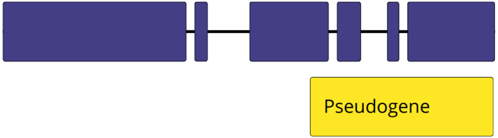
Victor Grentzinger

Laboratory of Human Genetics
Promotor : Pr. Vincent Bours

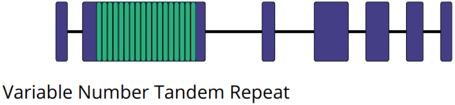
GIGA-MCB seminar 06/06/24

Challenging medically relevant genes

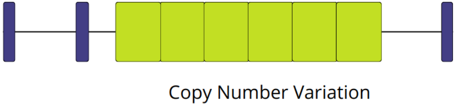
Genes with high-impact variants for genetic diseases.



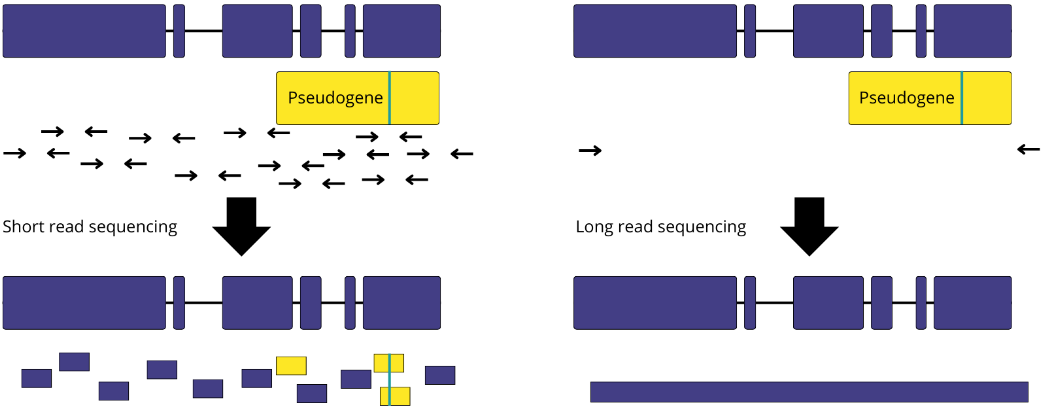
Highly similar to other DNA sequences within same genome.



Homologous regions within CMRG are really complex to characterize by short read sequencing.



Variant identification for CMRG



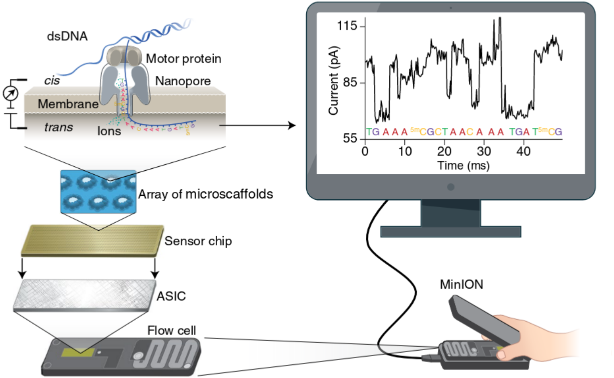
Oxford Nanopore Technologies sequencing

DNA strand passes through a nanopore.

The sequencer records electrical current changes.

Read length: +10k bp.

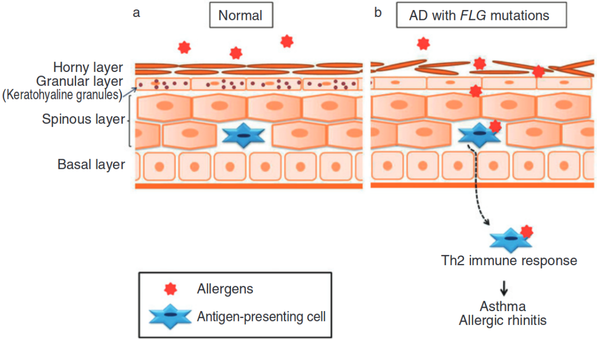
Read Until: Select only reads from given region(s) of interest.



Y. Wang et al. (2021)

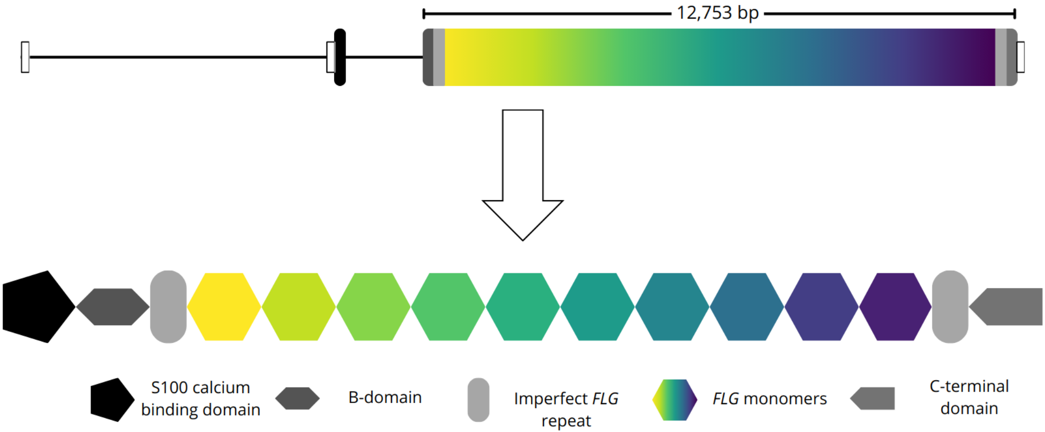
Atopic Dermatitis (AD)

J. Heilman (2010)

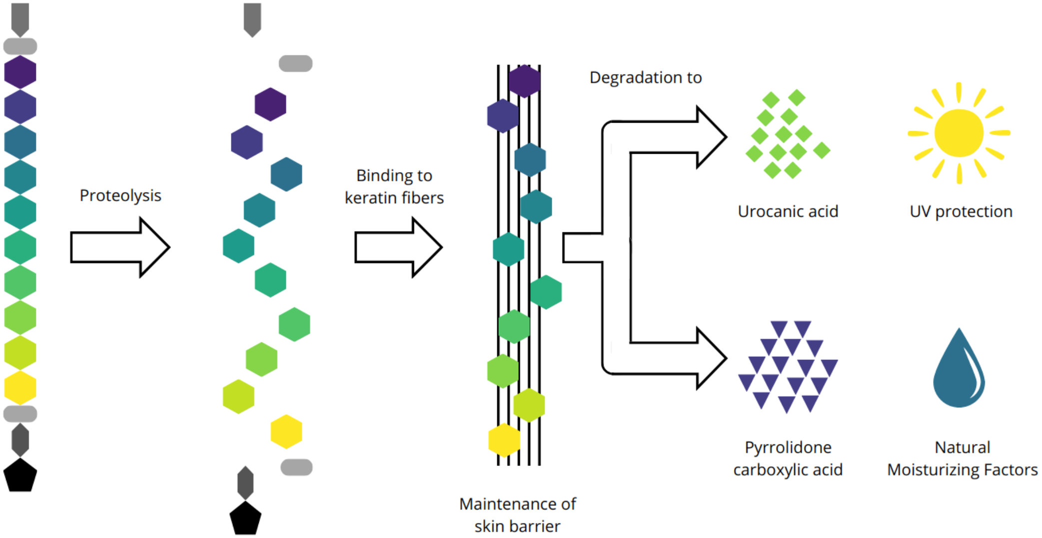


R. Osawa, M. Akiyama and H. Shimizu (2011)

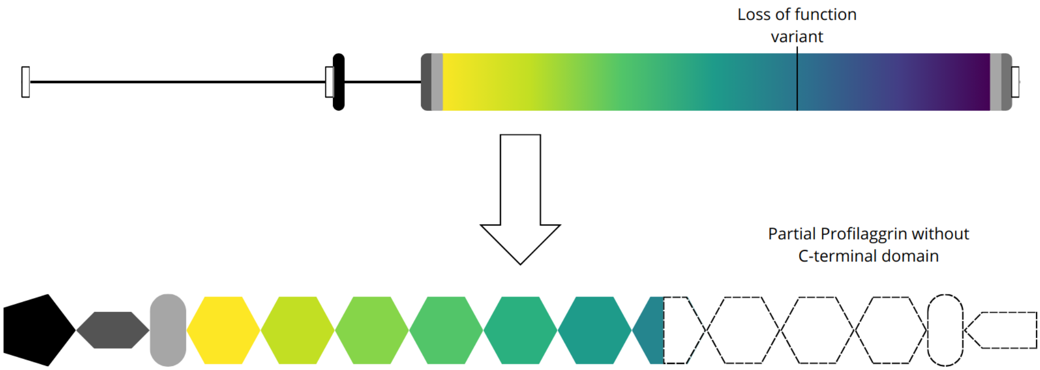
The Filaggrin gene (*FLG*)



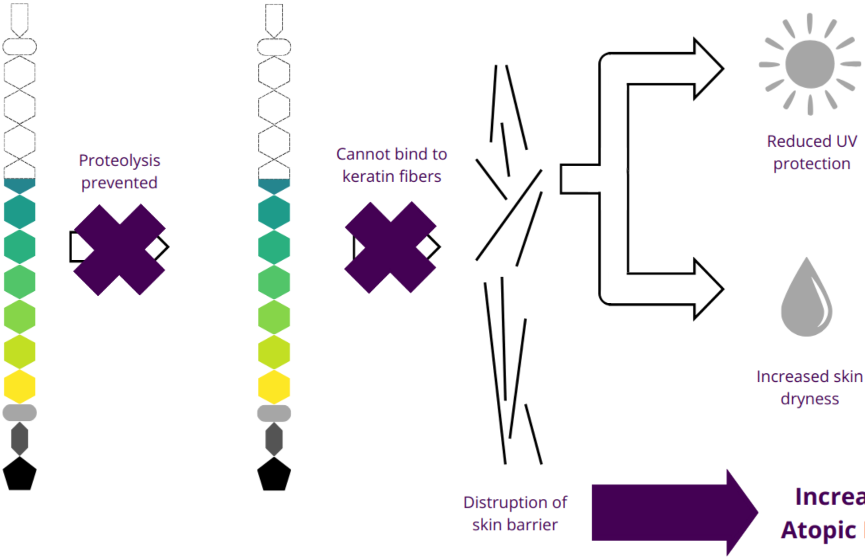
Role of *FLG*


Proteolysis
Binding to keratin fibers
Degradation to
Urocanic acid
UV protection
Pyrrolidone carboxylic acid
Natural Moisturizing Factors
Maintenance of skin barrier

LOF variants of *FLG*



LOF variants prevents proteolysis



Proteolysis prevented

Cannot bind to keratin fibers

Reduced UV protection

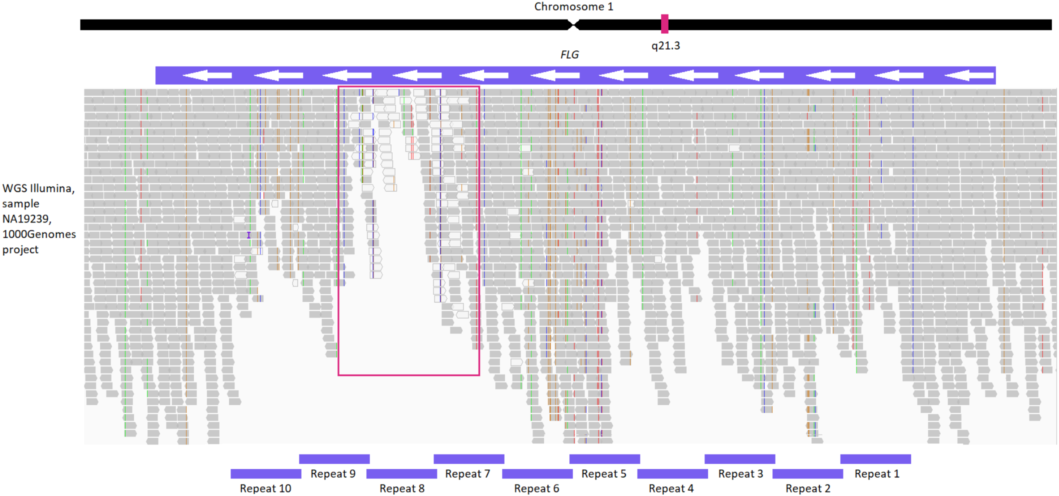
Increased skin dryness

Distruption of skin barrier

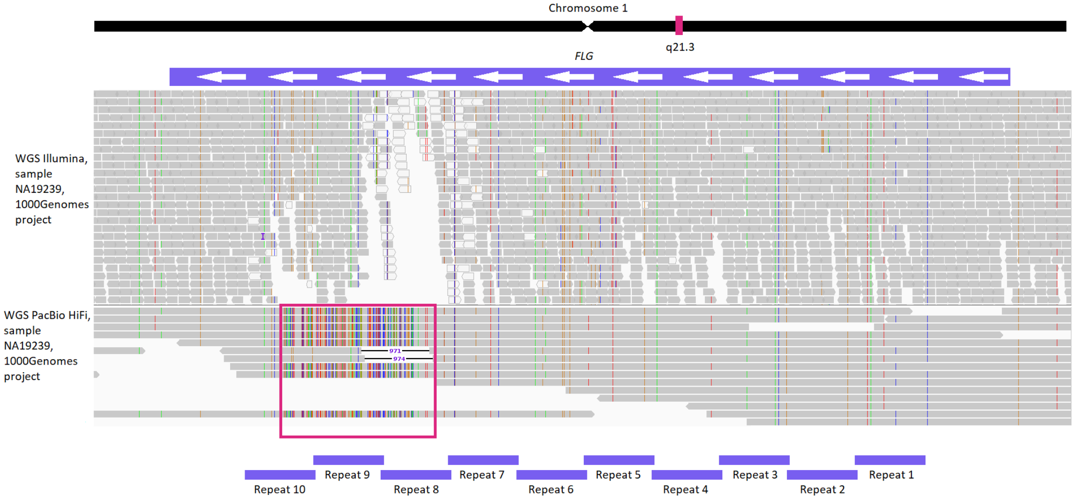
Increase risk of Atopic Dermatitis

Multiple known alleles of *FLG*

Short read sequencing limitation



Discovery of recombinant allele (REC)



FLG research project

Capture the genetic variability of the *FLG* gene in a catalog of alleles and infer new pathogenic variants by using long read sequencing technologies.

Multi-ethnic cohort

Origin	Control samples	AD patients	Total
Africa	55	68	123
Asia	45	0	45
Europe	96	43	139
Latin America	19	0	19
Total	215	111	326

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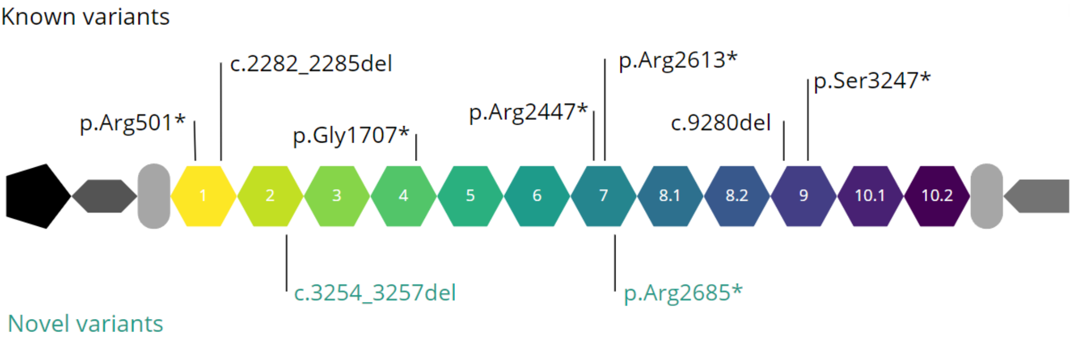
Identification of new LOF variants



Known variants

p.Arg501*

c.2282_2285del

p.Gly1707*

p.Arg2447*

p.Arg2613*

c.9280del

p.Ser3247*

c.3254_3257del

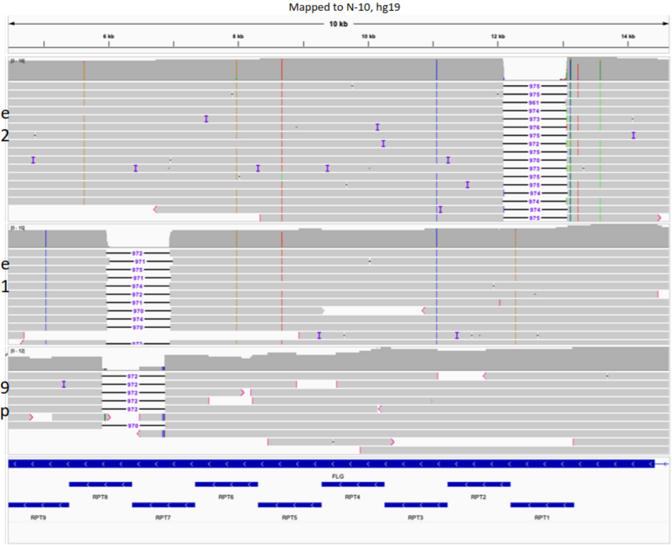
p.Arg2685*

Novel variants

	Control	AD patient
Variants frequencies	14.5%	39.5%

Identification of REC alleles

Recombinant alleles with 9 repeats



REC-9.2 1000 Genome HG02502-HP2

REC-9.1 1000 Genome HG02502-HP1

REC-9.3 EurCase-39 Adaptive samp

Characterization of breakpoints

Recombinant allele with 10 repeats

REC-10

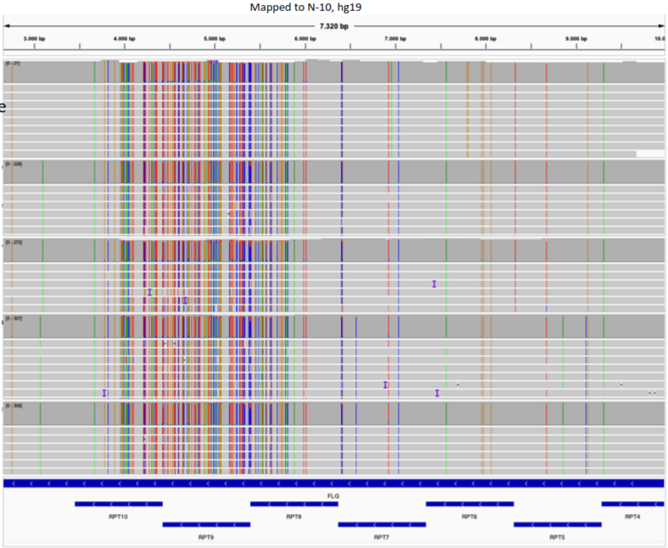
1000 Genome HG02282

EurCase-14 Exon3 PCR2

EurCase-14 Exon3 PCR1

RwaCase-45 Exon3 PCR1

RwaCase-45 Exon3 PCR2

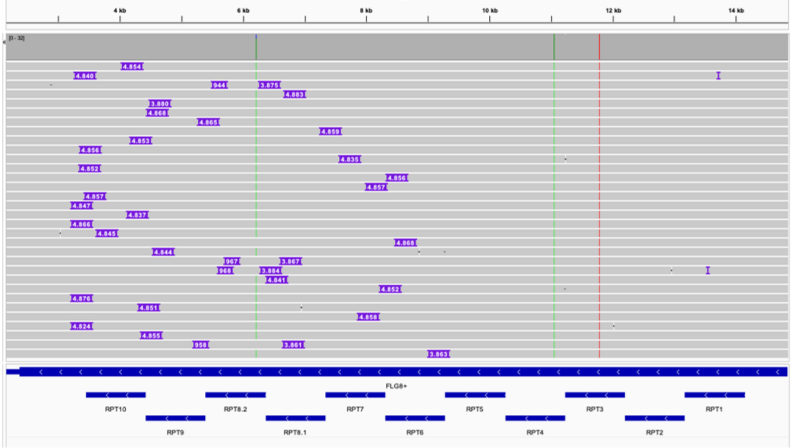


One special case: a 16 repeat allele

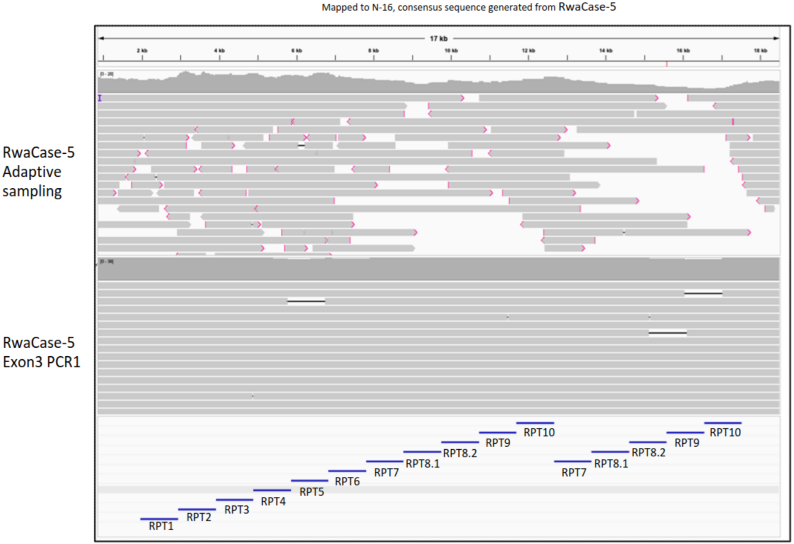
**Recombinant allele
with 16 repeats**

REC-16
RwaCase-5
Exon3 PCR1

Mapped to N-11, HG002 T2T PAT genome

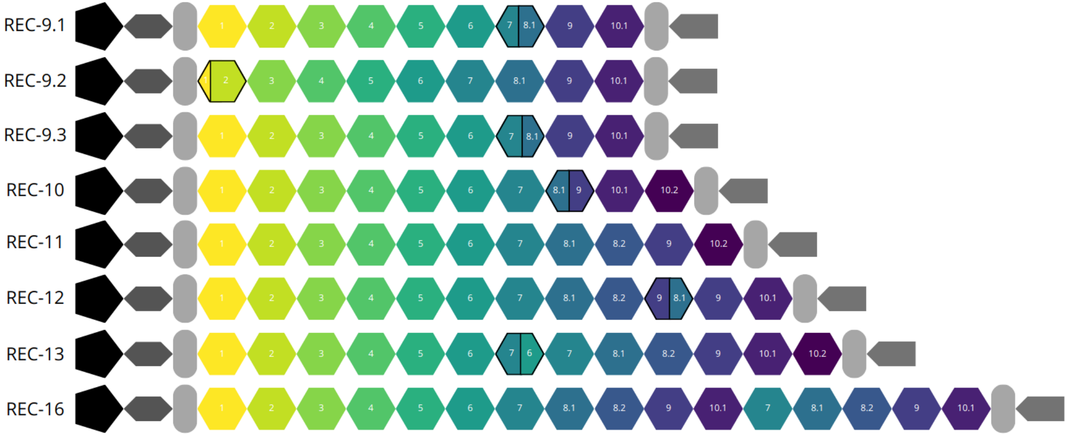


Understanding *FLG* repeat order



Mapped to N-16, consensus sequence generated from RwaCase-5

Catalog of novel REC alleles



Frequencies of REC alleles

Allele	Africa	Asia	Europe	Latin America
N-10	46	16	91	5
N-11.1	71	22	**147**	15
N-11.2	0	3	0	0
N-12	11	***47***	29	***17***
REC-9.1	7	0	0	0
REC-9.2	1	0	0	0
REC-9.3	0	0	1	0
REC-10	***92***	0	4	0
REC-11	0	2	1	1
REC-12	4	0	2	0
REC-13	13	0	1	0
REC-16	1	0	0	0

Frequencies of REC alleles

Allele	Africa	Asia	Europe	Latin America
N-10	46	16	91	5
N-11.1	71	22	**147**	15
N-11.2	0	3	0	0
N-12	11	***47***	29	***17***
REC-9.1	7	0	0	0
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REC-9.3	0	0	1	0
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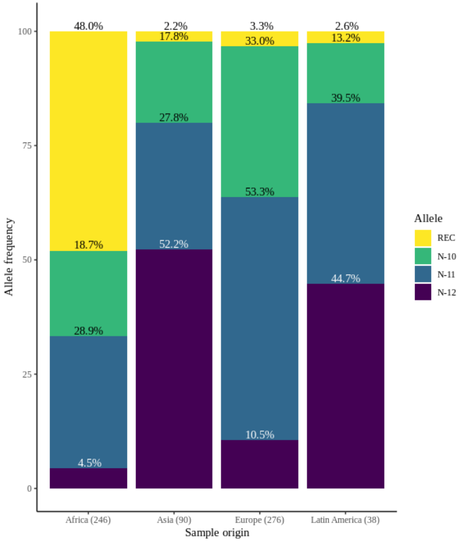
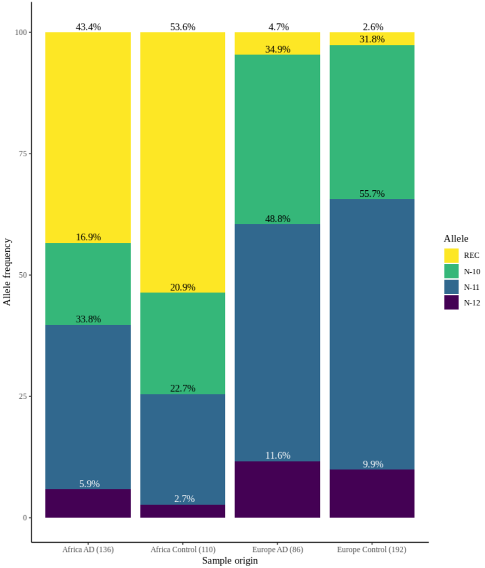
REC-12	4	0	2	0
REC-13	13	0	1	0
REC-16	1	0	0	0



Frequencies comparison AD/controls

Allele	Africa		Europe	
	AD	Control	AD	Control
N-10	23	23	30	61
N-11.1	46	25	42	105
N-11.2	0	0	0	0
N-12	8	3	10	19
REC-9.1	2	5	0	0
REC-9.2	0	1	0	0
REC-9.3	0	0	1	0
REC-10	46	46	2	2
REC-11	0	0	0	1
REC-12	4	0	1	1
REC-13	6	7	0	1
REC-16	1	0	0	0



FLG project current status

- Samples from AD patients: routine analysis implemented using Nanopore sequencing.
- Novel REC alleles identified are added to the catalog to improve diagnosis.

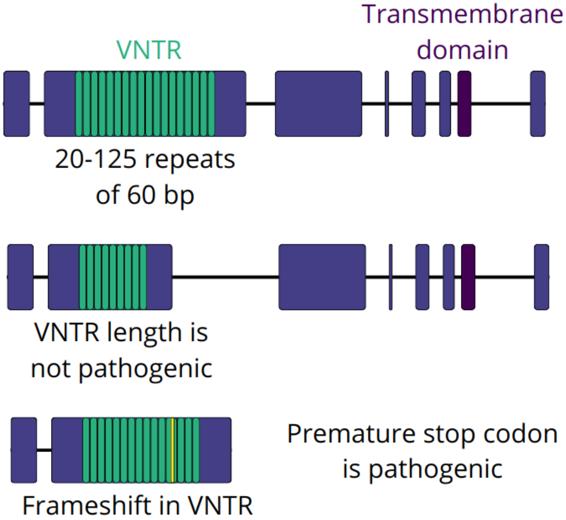
Other genes involved in AD

Two genes of the same family as *FLG*.

FLG2: sequenced for AD patients, no pathogenic variant found.

HRNR: VNTR region too big for PCR amplification.

MUC1: Medullary Cystic Kidney Disease type 1



20-125 repeats of 60 bp

VNTR length is not pathogenic

Frameshift in VNTR

Premature stop codon is pathogenic

Acknowledgement

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