Leids Universitair
Medisch Centrum

A genetic therapy for CADASIL: will it ever work?

Saskia Lesnik Oberstein

Dep. Clinical Genetics

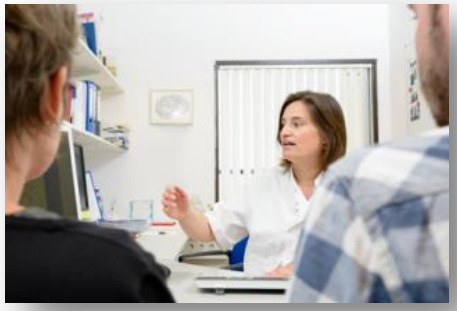
LEIDEN UNIVERSITY MEDICAL CENTER

Leiden University Medical Center
CADASIL referral centre

CADASIL clinic

CADASIL research lab
***NOTCH3* diagnostic lab**

Multidisciplinary clinic

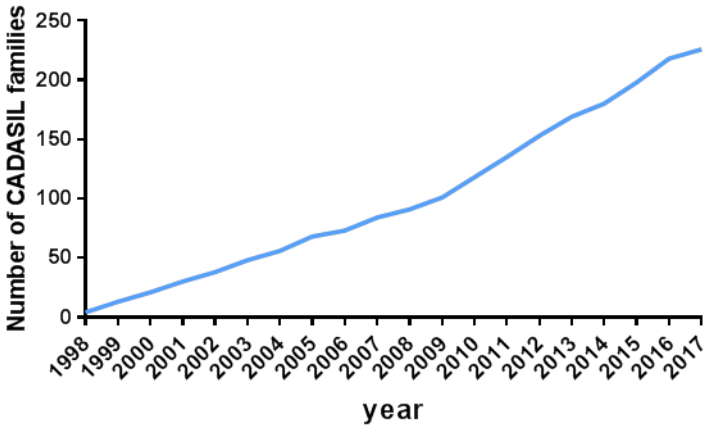


Research

Outline

- Introduction
- CADASIL: less rare than you might think
- Mutation position plays a role in disease severity
- Genetic therapy: where do we stand?

CADASIL in the Netherlands



7 ⟶ 226 Dutch families

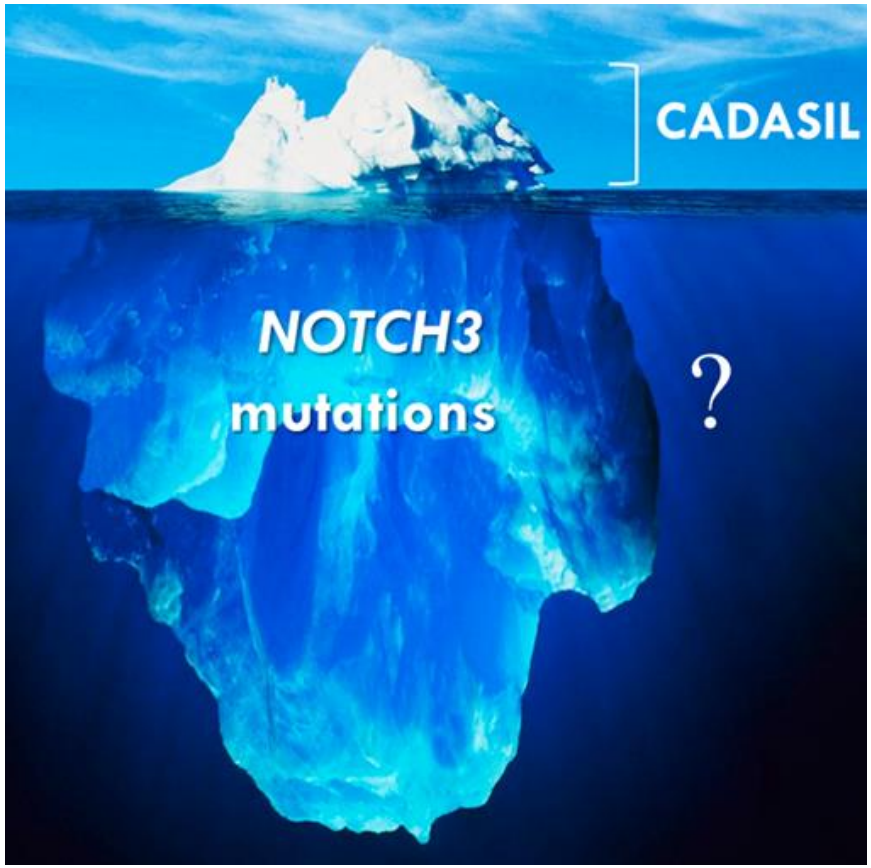
5 ⟶ 55 distinct mutations

How prevalent is CADASIL worldwide?

Database with DNA sequence of 138,632 individuals

Expected: 0-4 NOTCH3 mutations → 450 mutations!

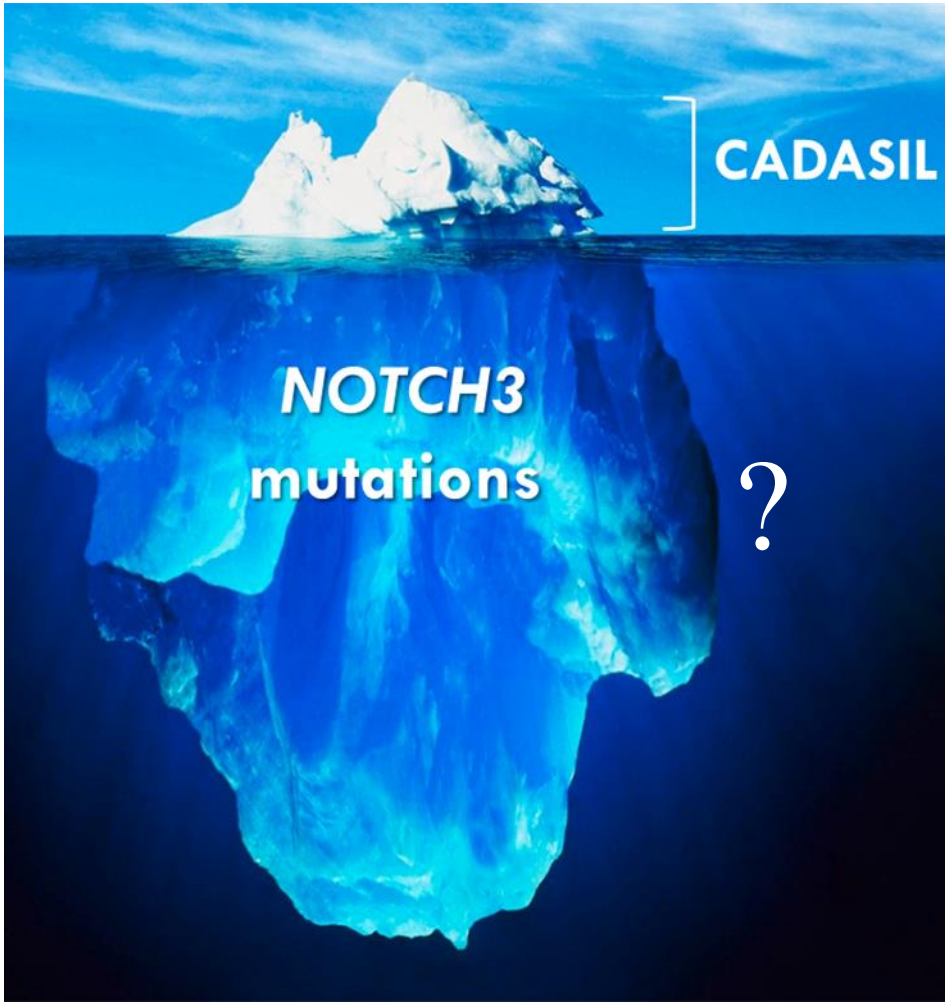
NOTCH3 iceberg…



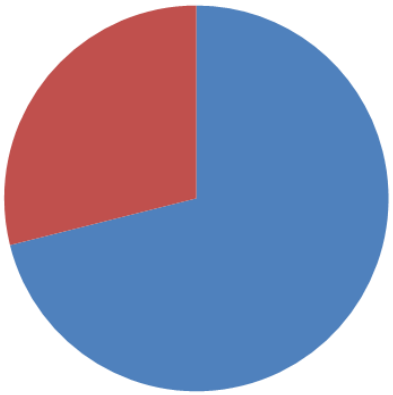
= 1% *NOTCH3* mutations

- Missed CADASIL diagnoses?
- Milder/later-onset?

Mutation position in NOTCH3 protein



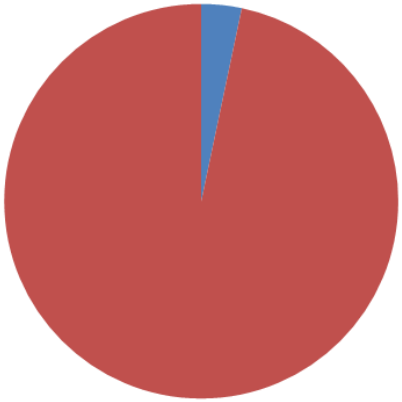
CADASIL



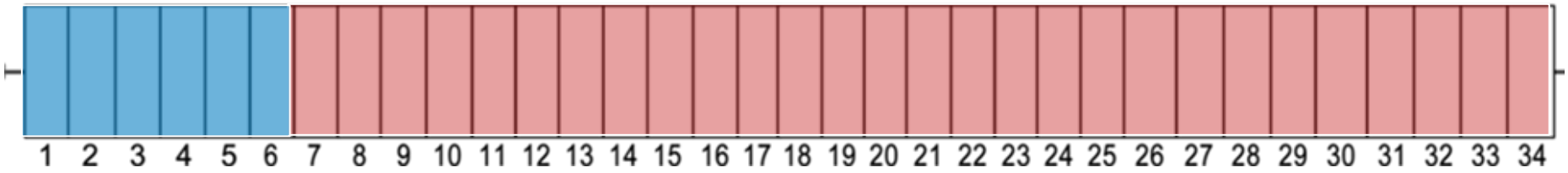
Mutation beginning protein

Mutation end protein

Population

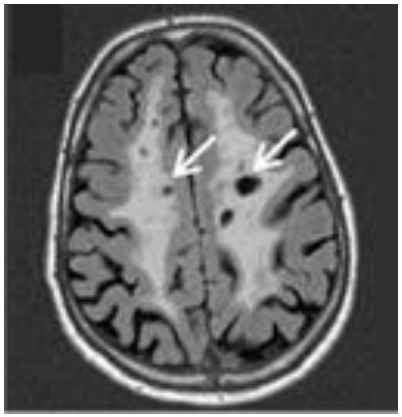


Effect of mutation position on disease severity

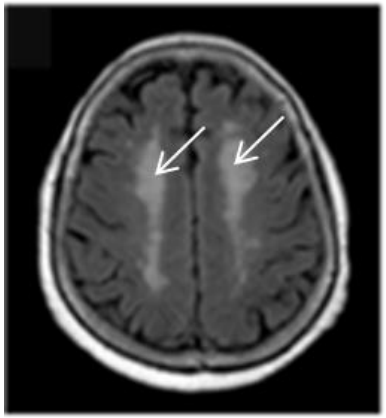


Severe CADASIL
Early onset stroke

Milder CADASIL
12 y later onset stroke



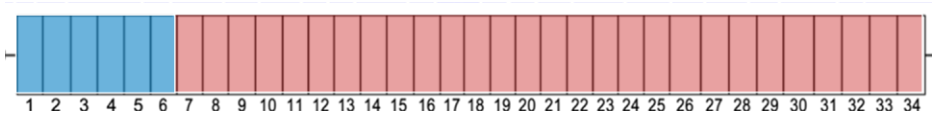
Other disease modifiers:
Smoking
High blood pressure



So…

- CADASIL: May be 100x more prevalent

- Mutations at beginning of protein predispose to more severe disease

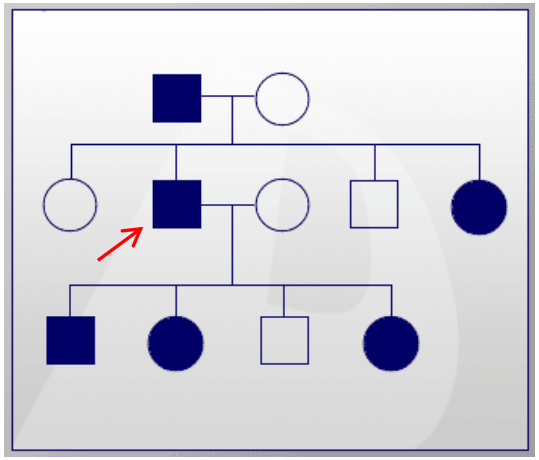


- Most patients have mutation at beginning of protein

- Therapy: target beginning of protein!

Urgency to develop a treatment for CADASIL

Young-onset strokes and dementia



Hereditary: 50% risk for offspring

No treatment options

Erfelijke hersenaandoening veroorzaakt dementie en beroertes

Ziekte Cadasil is niet zo zeldzaam

→

Increased interest of:

- Media
- Pharmaceutical companies
- Funding agencies

Developing a (genetic) treatment

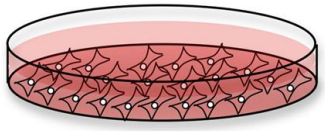
Understanding of: disease cause, course, variability, etc

Availability of: patient registries, disease models, markers etc

Pre-clinical phase

?

Idea

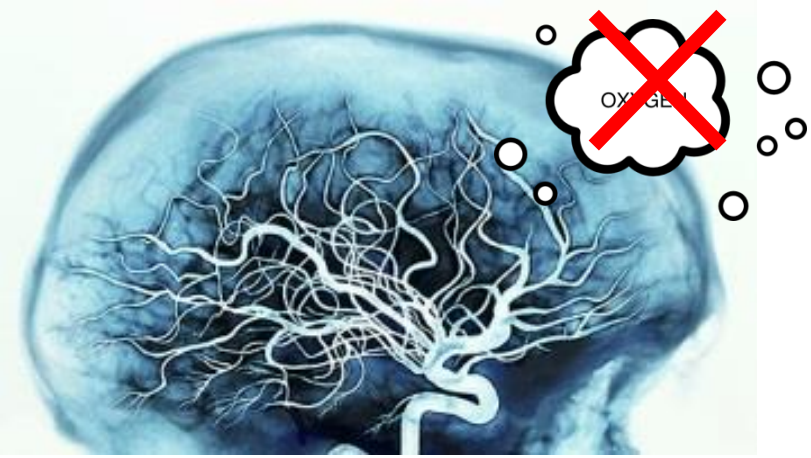


'in vitro' testing (cells)

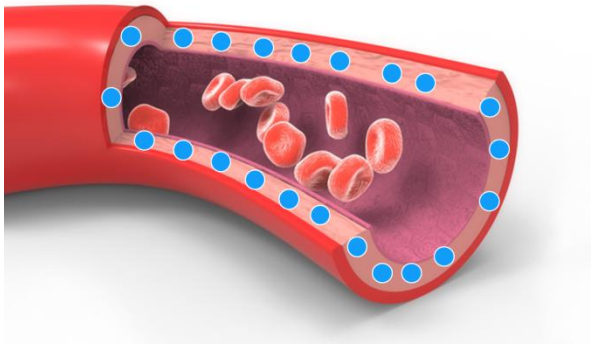
'in vivo' testing (mice)

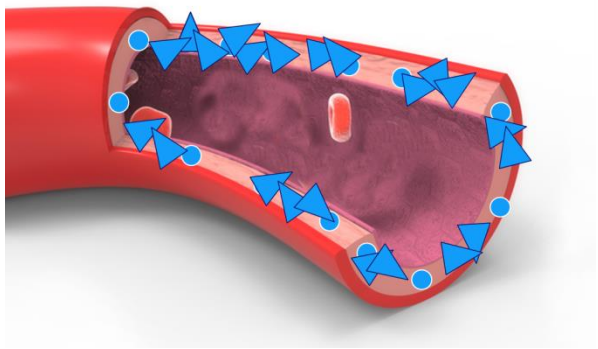
Patient trials

Problem: reduced blood flow to the brain



Cause: NOTCH3 protein deposits in blood vessels



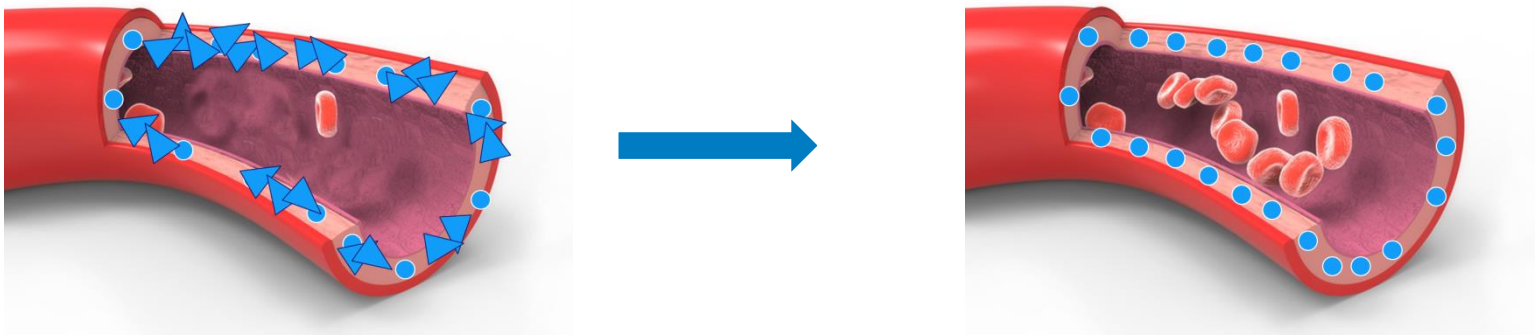


NOTCH3 protein

Defective NOTCH3 protein

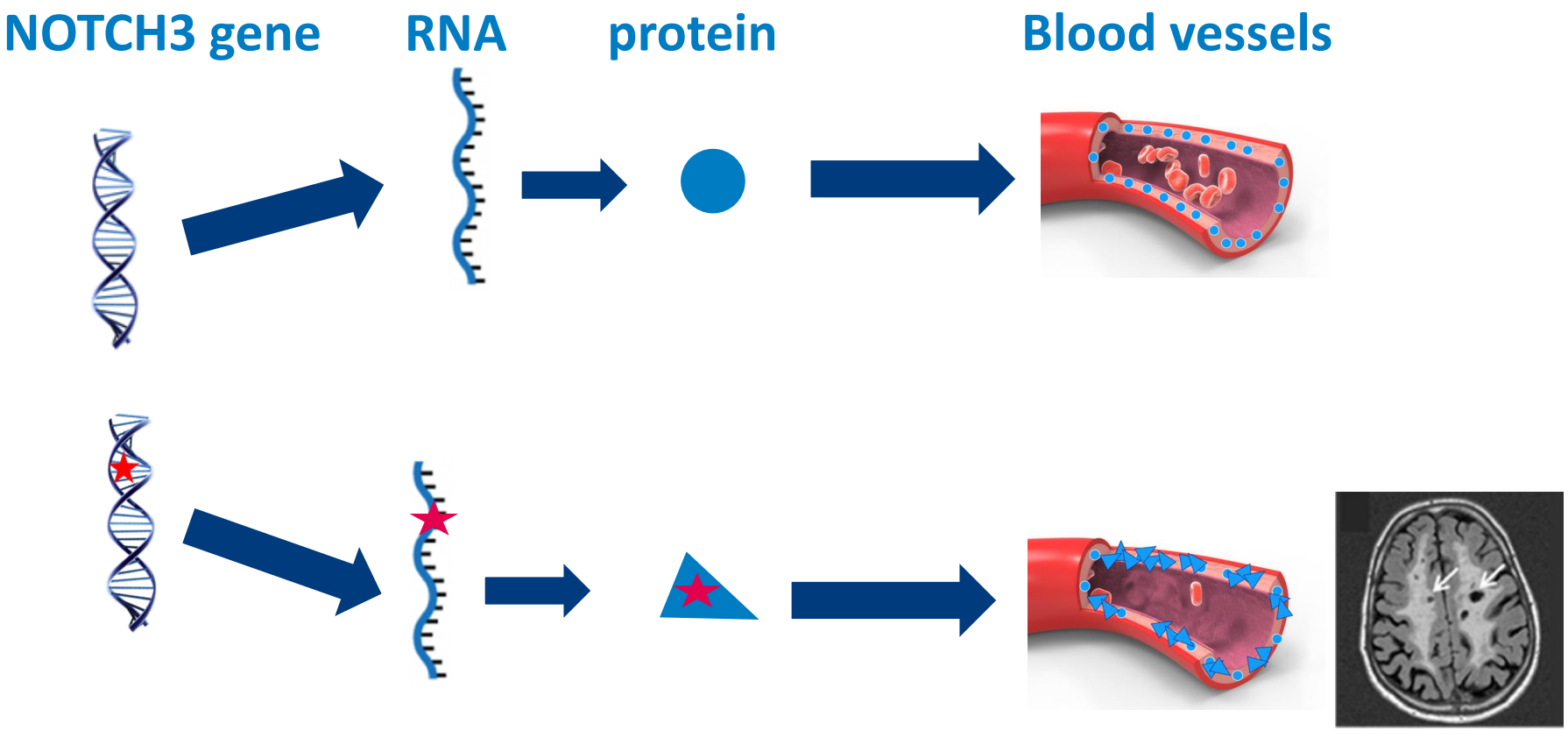
Idea therapeutic approach

Correct mutant NOTCH3 protein to reduce deposits



Maintain blood flow to the brain

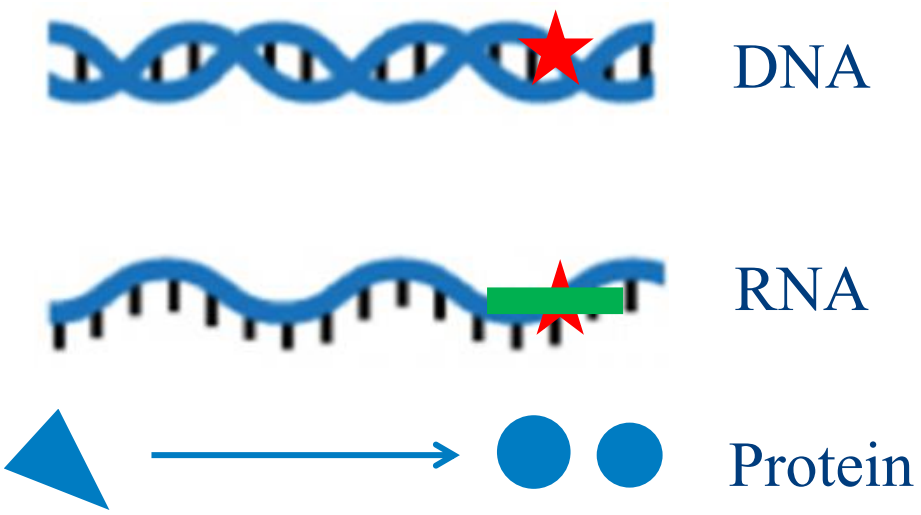
From gene mutation to defective protein



Idea: can we remove the mutation?

First approach:

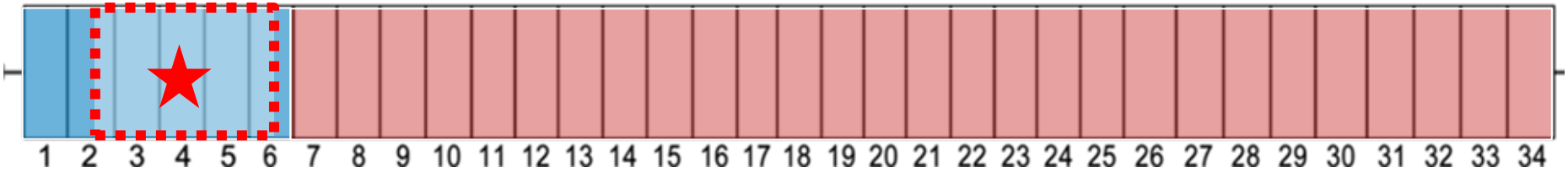
- ‘Hide’ the mutation in the RNA (= exon skipping)



Antisense **O**ligo **N**ucleotides (AON)

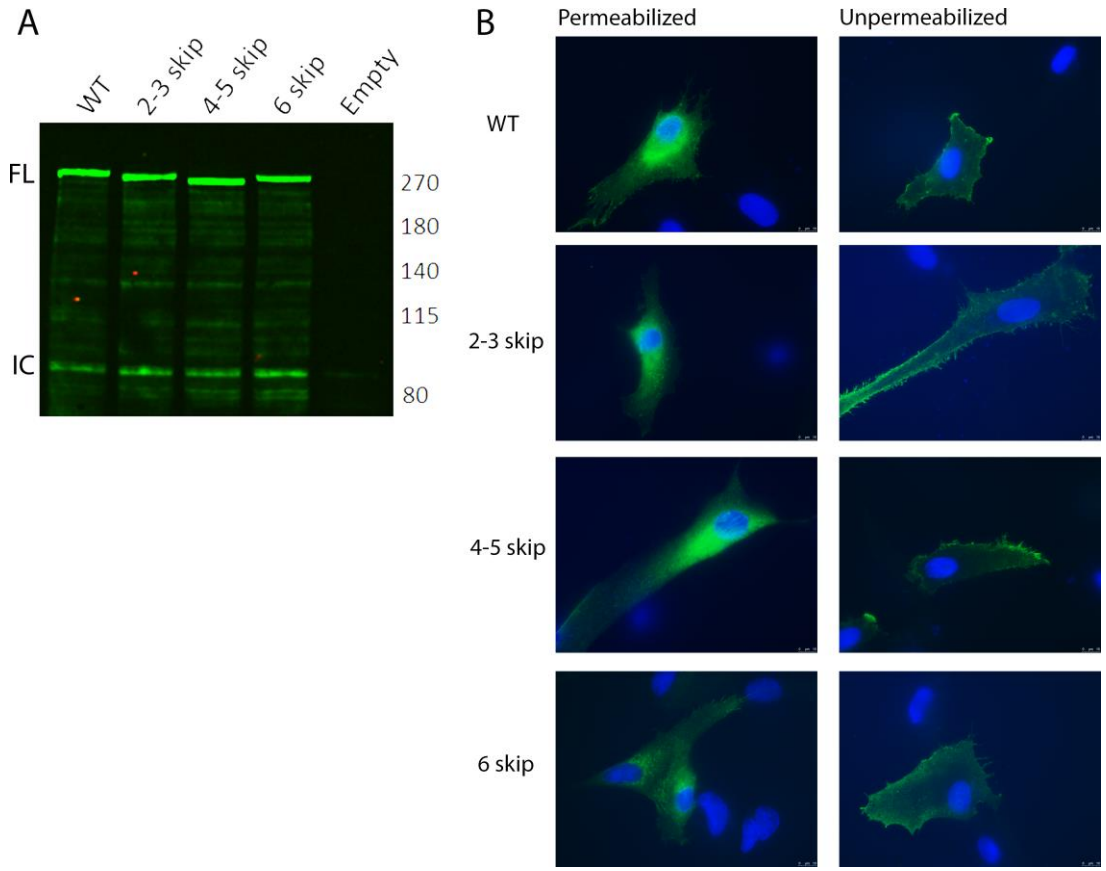
Exon skipping removes ~65% of mutations in patients

RNA with mutation hidden by AON



Shorter protein, mutation removed

NOTCH3 skip protein



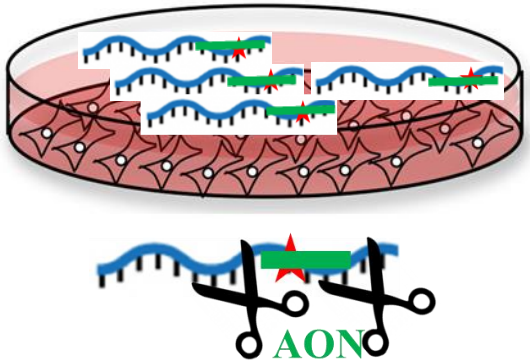
Shorter protein retains function

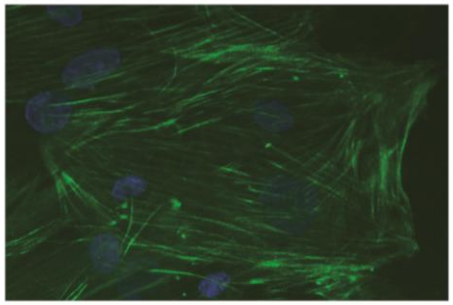
Normally located at cell membrane

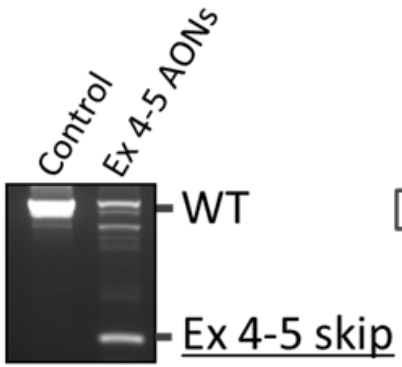
Testing exon skipping in cells

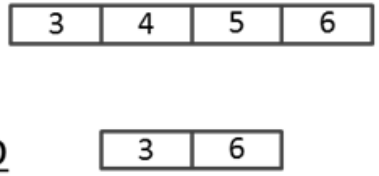
Patient cells:
RNA with
mutation

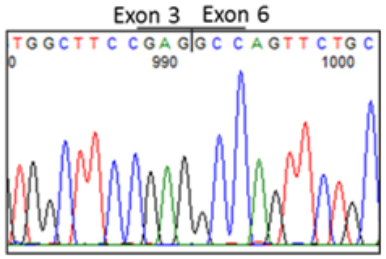
Treated cells:
shorter RNA, no
mutation









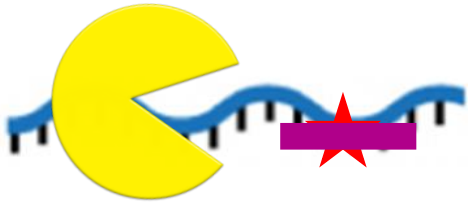


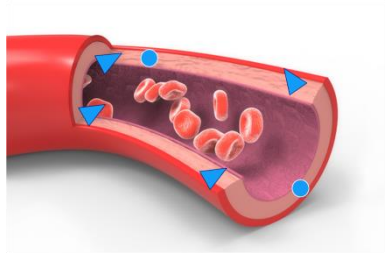
Second approach: NOTCH3 down regulation

1: 'Remove' the mutation (exon skipping)

Shorter protein
(no deposits)

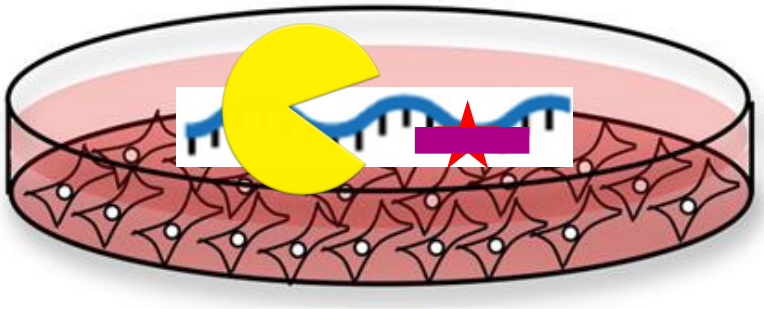
2: Reduce NOTCH3 protein production (downregulation)

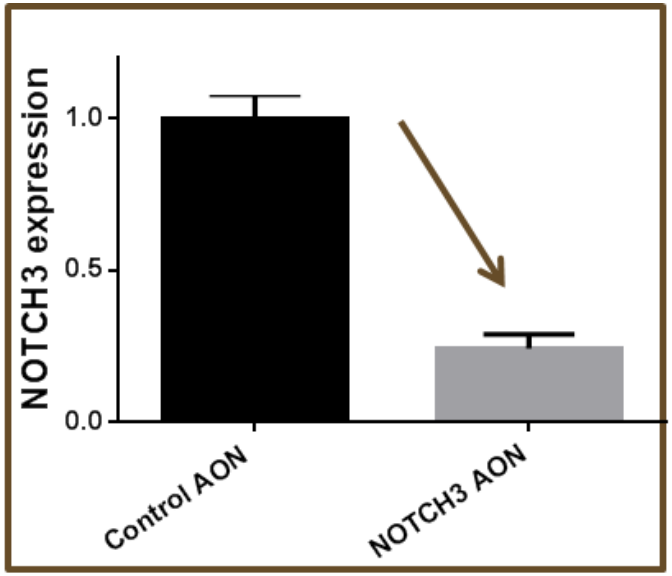




Less protein
deposits

Down regulation in cells: 75%



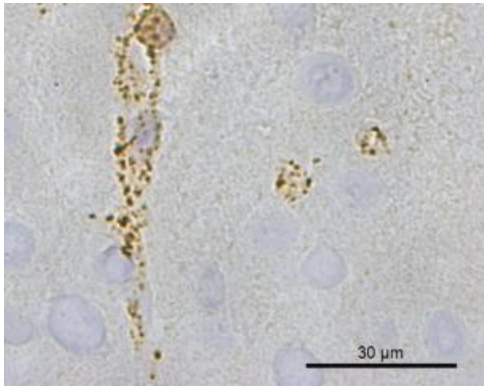


Next step: testing AONs in CADASIL mice

BAC 142,63 Kb (circular)

WT / c.544 C>T

SYDE1 **ILVBL** human *NOTCH3* **EPHX3** **BRD4**



20 months

Do AONs reach the brain vessels?
Are they effective?
Are they tolerated?

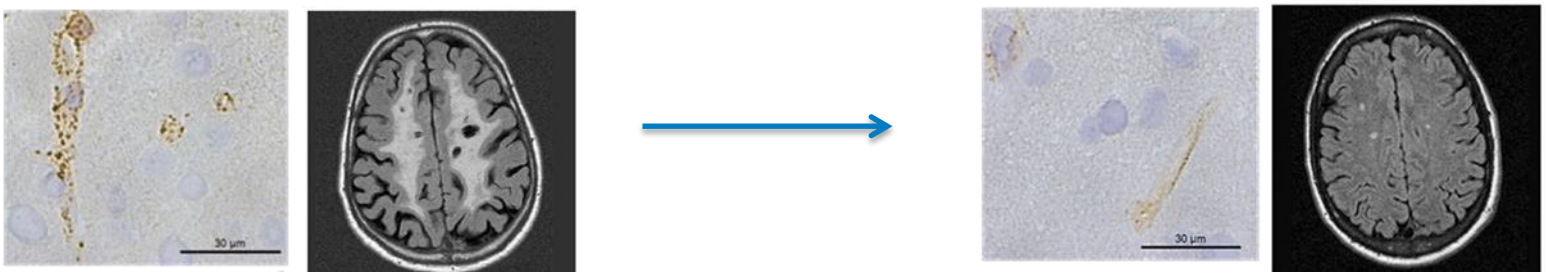
Rutten et al. ANC 2015

Current hurdles and steps to take

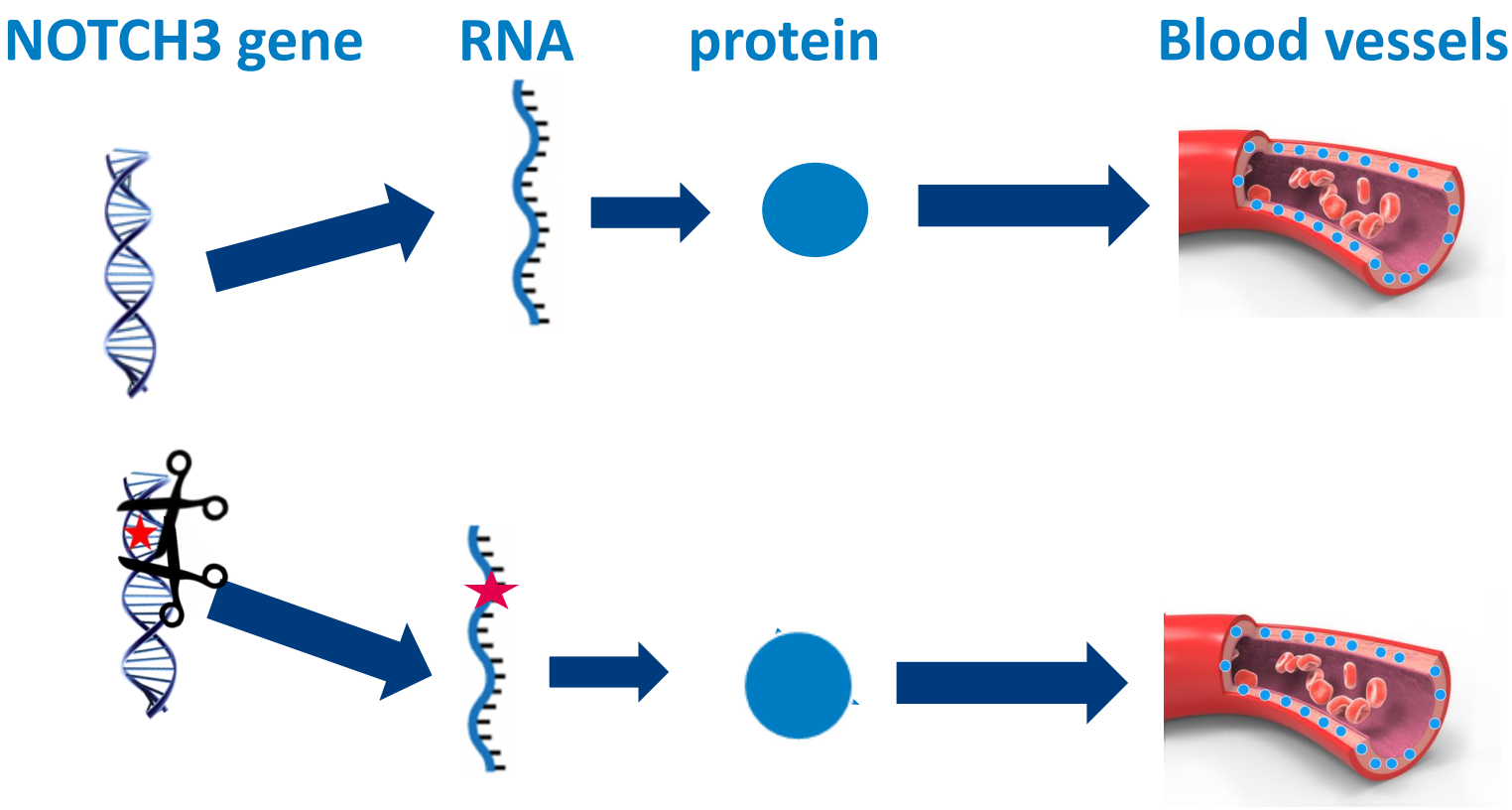
→ Delivery to brain vessels needs to be improved!

→ New types of NOTCH3 AONs (companies)

→ Only then we can test whether AONs reduce NOTCH3 deposits in mice (and eventually in patients)



CRISPR/Cas: removing the mutation from DNA



Other ideas: CADASIL in a dish

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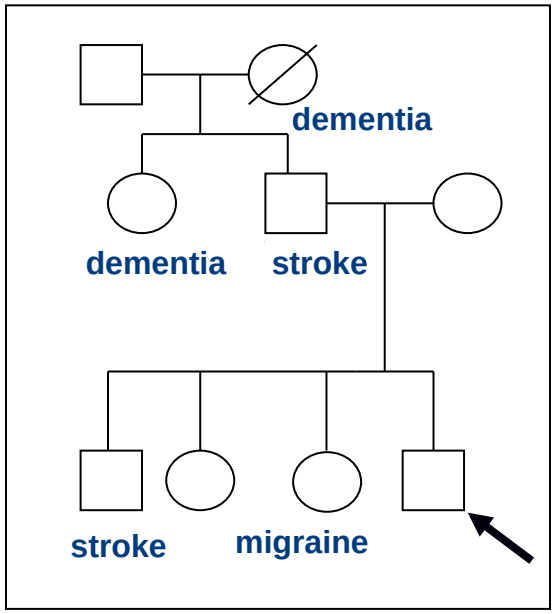
'in vivo' testing
(CADASIL mice)

Large scale screening of FDA approved drugs: do they reduce NOTCH3 deposits?

Goal: treat patients to delay or prevent strokes and dementia



NOTCH3 AONs

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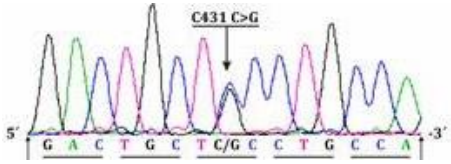
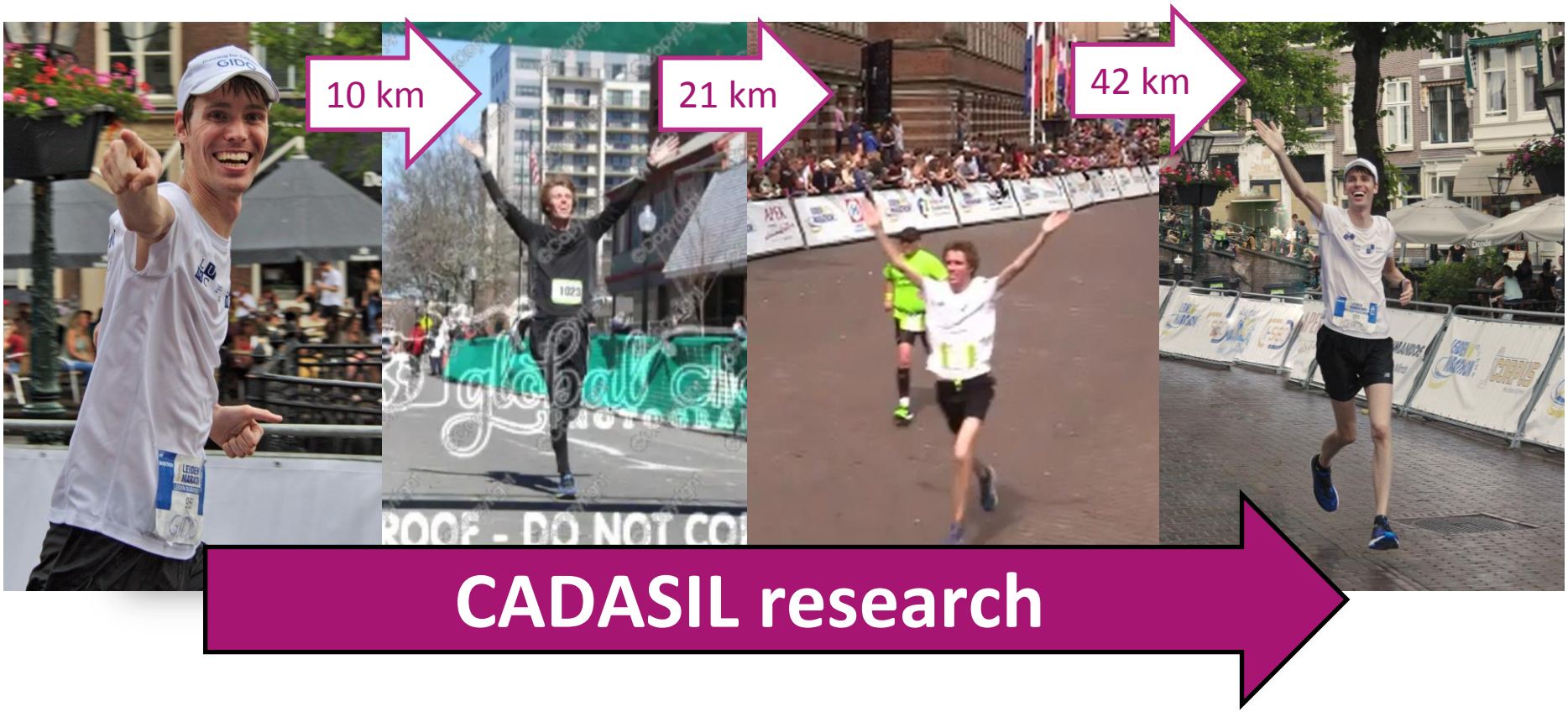


CADASIL PhD student Gido: from 5 km to the Marathon!



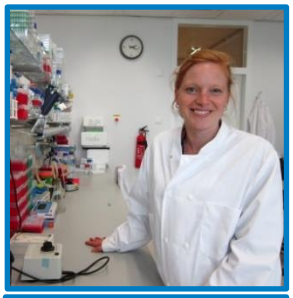
CADASIL research team

Annemieke Aartsma-Rus

Professor Translational Genetics

Remco Hack

PhD student



Julie Rutten

Clinical Geneticist in training/ post-doc researcher

Gido Gravesteijn

PhD student

Hans Dauwerse

Research technician

Maurice Overzier

Research technician

Platform CADASIL
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Hersenstichting
Brain Foundation of The Netherlands

ZonMw
The Netherlands Organisation for Health Research and Development