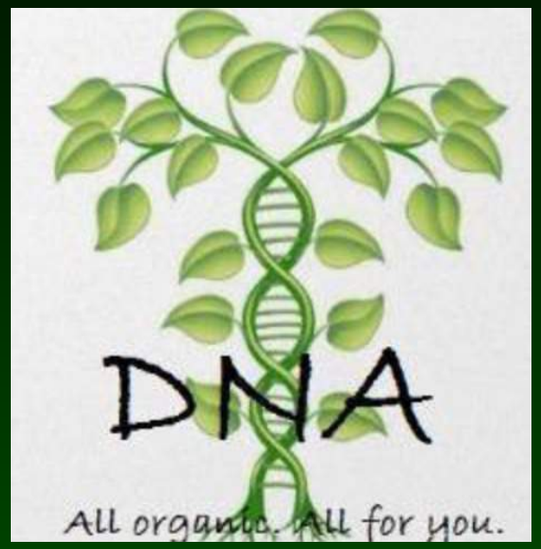
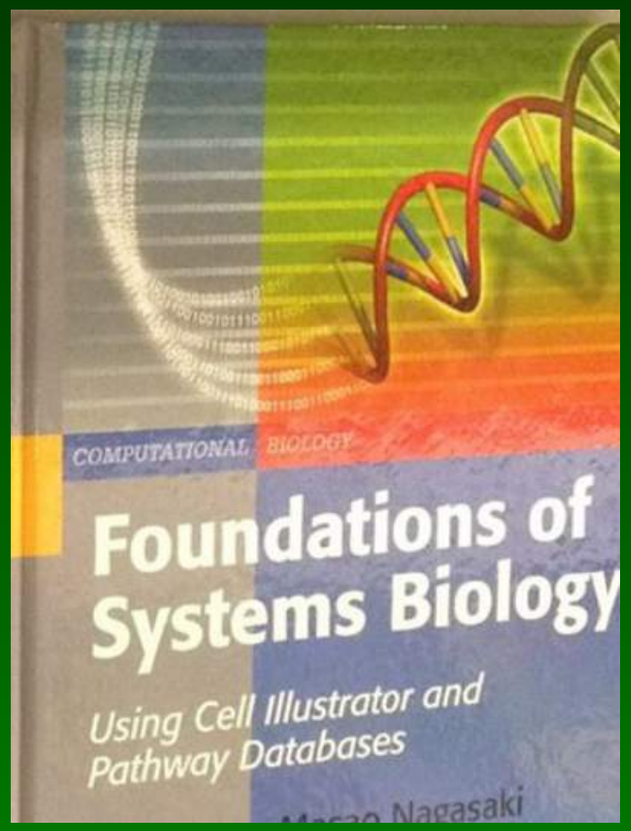
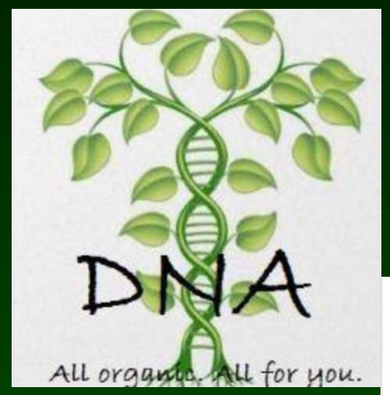


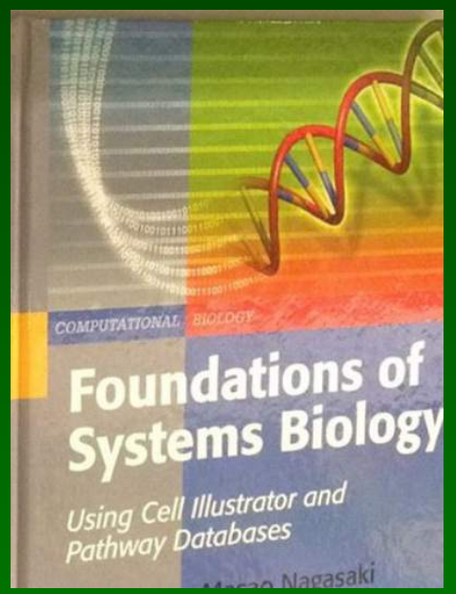


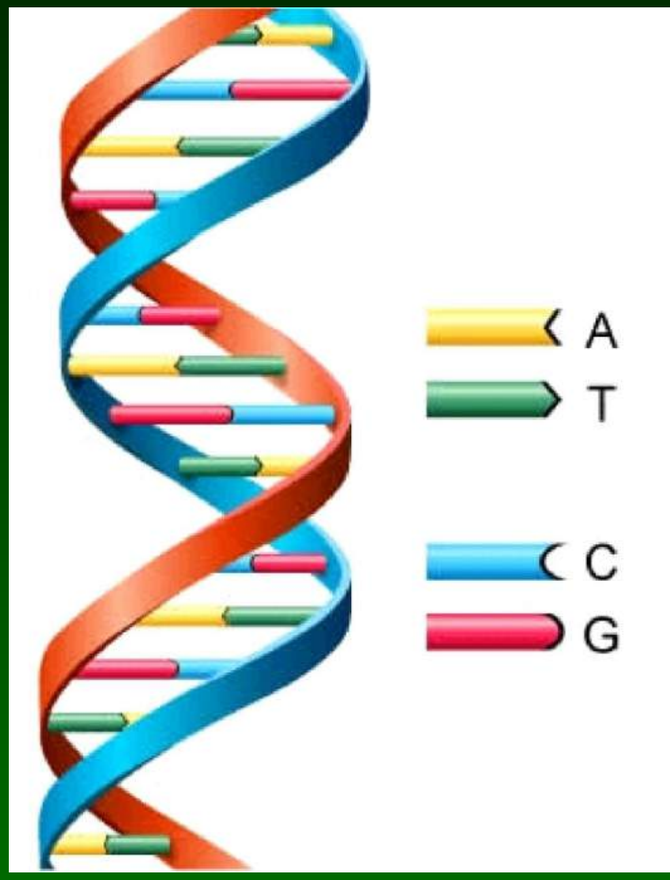
DNA turns…

advertisement

did you pay attention at school?







correct !

Functional assays

confirm predicted consequences

tinyurl.com / VEPTC2018-19

Johan den Dunnen

The question

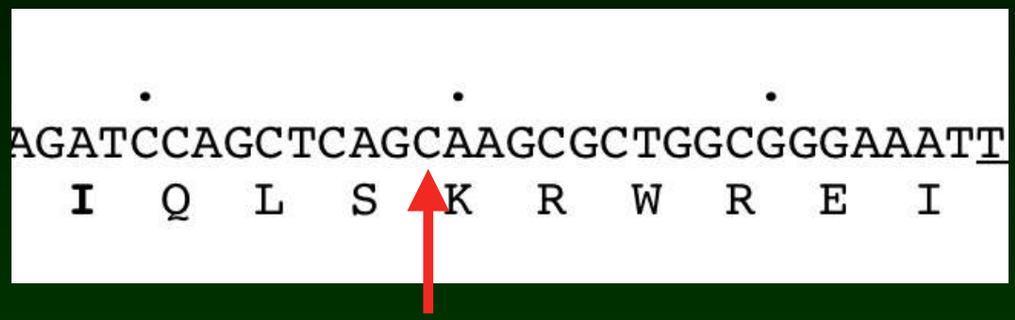
I found a variant and want to know whether it may be related to the health problems I see in the individual

What can I try in the lab ??

(not computer predictions)

Share !!

DMD gene



c.5859C>T r.(?) p(Ser1953=)

found in diagnosis

prenatal
at risk family muscular dystrophy
no definite diagnosis

found in diagnosis

WES, trio analysis
male parent
45y, healthy
one of many variants

you may have life saving information, did you realize ?

Database submission 1st

first example,

many should follow

VOLUME 46 | NUMBER 2 | FEBRUARY 2014 188

Loss-of-function mutations in *MICU1* cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling

nature genetics

Clare V Logan[1,15], György Szabadkai[2,3,15], Jenny A Sharpe[2,15], David A Parry[1], Silvia Torelli[4], Anne-Marie Childs[5], Marjolein Kriek[6], Rahul Phadke[4,7], Colin A Johnson[1], Nicola Y Roberts[1], David T Bonthron[1], Karen A Pysden[5], Tamieka Whyte[4], Iulia Munteanu[4], A Reghan Foley[4], Gabrielle Wheway[1], Katarzyna Szymanska[1], Subaashini Nata… Helen Roper[8], Gijs W E Santen[6], Erik H Niks[9], W Ludo… Diego De Stefani[3], Johan T den Dunnen[6], Yu Sun[6], Ieke Gin… sario Rizzuto[3], UK10K Consortium[14], Michael R Duche… ,16

LOVD Leiden Open Variation Database

Leiden Muscular Dystrophy pages

Mitochondrial calcium uptake 1 (MICU1)

Curator: Johan den Dunnen

Home | Variants | Submitters | Submit | Documentation

LOVD - Variant listings

About this overview [Show]

Patient data (#0030375)	
Phenotype	dystrophy, muscular, limb-girdle
Phenotype additional	-
Reference	2-generation family, brother-1, Netherlands:Leiden
Remarks	-
Geographic origin	Netherlands
Ethnic origin	-
Gender	M
Inheritance	familial, autosomal recessive
Consanguinity	no
Fam_Pat	1 (2)
# reported	1
CK level	-
Protein data	-
Submitter	Marjolein Kriek

Date created
2013-05-21 21:46:21

Variant data	
Allele	Paternal (confirmed)
Reported pathogenicity	Probably pathogenic
Concluded pathogenicity	Unknown
Exon	8i
DNA change	c.741+1G>A (View in UCSC Genome Browser, Ensembl)
Var_pub_as	-
RNA change	r.741_742ins741+1_741+155{741+1g>a}
Protein change	p.Val248Thrfs*9
DB-ID	MICU1_00001
Variant remarks	whole exome sequencing; fibroblast RNA; NOTE: causality MICU1 variants not absolutely proven, yet highly likely
Genet_ori	germline (inherited)
Segregation	yes
Reference	-
Template	DNA, RNA
Technique	RT-PCR, SEQ, NGS-I

Topics

- share first, then...
- analyse cells / tissue
- RNA consequences
- protein consequences
- animal model
- …
- *(not computer predictions)*

The sample

- **blood**

DNA collected, sample also contains...

RNA

protein

cells

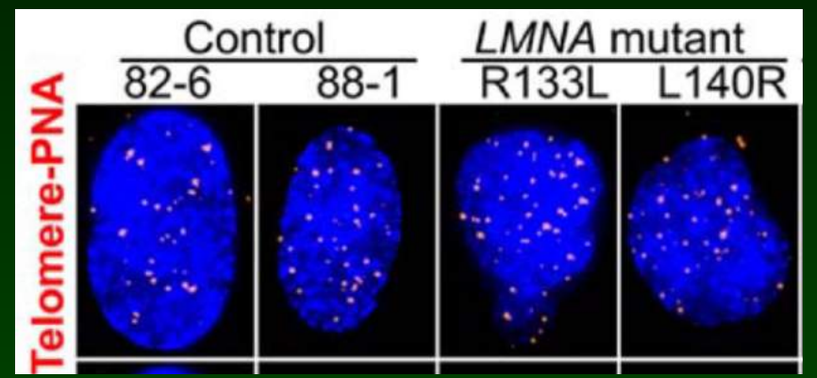
-use the opportunity

-get new sample difficult

-store

Tissues

- **analyse tissue from the patient**
  works for cancer, rare in other cases
  e.g muscle biopsy



- **look**
  abnormal structures
  e.g. LMNA irregular shaped nucleus
  ...required for expression cloning

- **stain**
  protein *(antibody)*
  abnormal amount / location / …
  RNA *(probe)*
  abnormal amount / location / …

Tissues

- **effects can be direct or indirect**

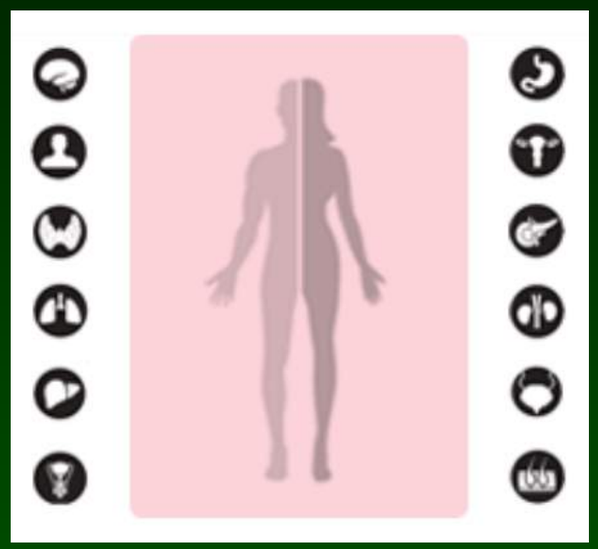
- **effect is full-genome**

influenced by all other variants in genome

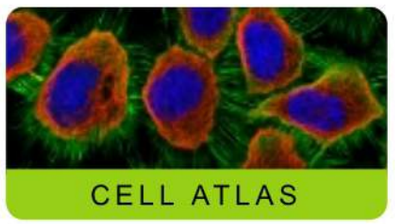
> proof by adding 1 variant to known genome

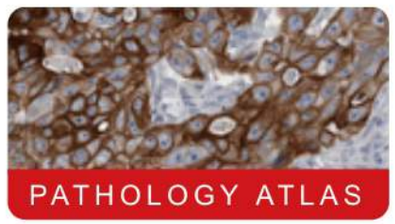
Proteins

THE HUMAN PROTEIN ATLAS



www.proteinatlas.org





antibodies

Tissues / cells

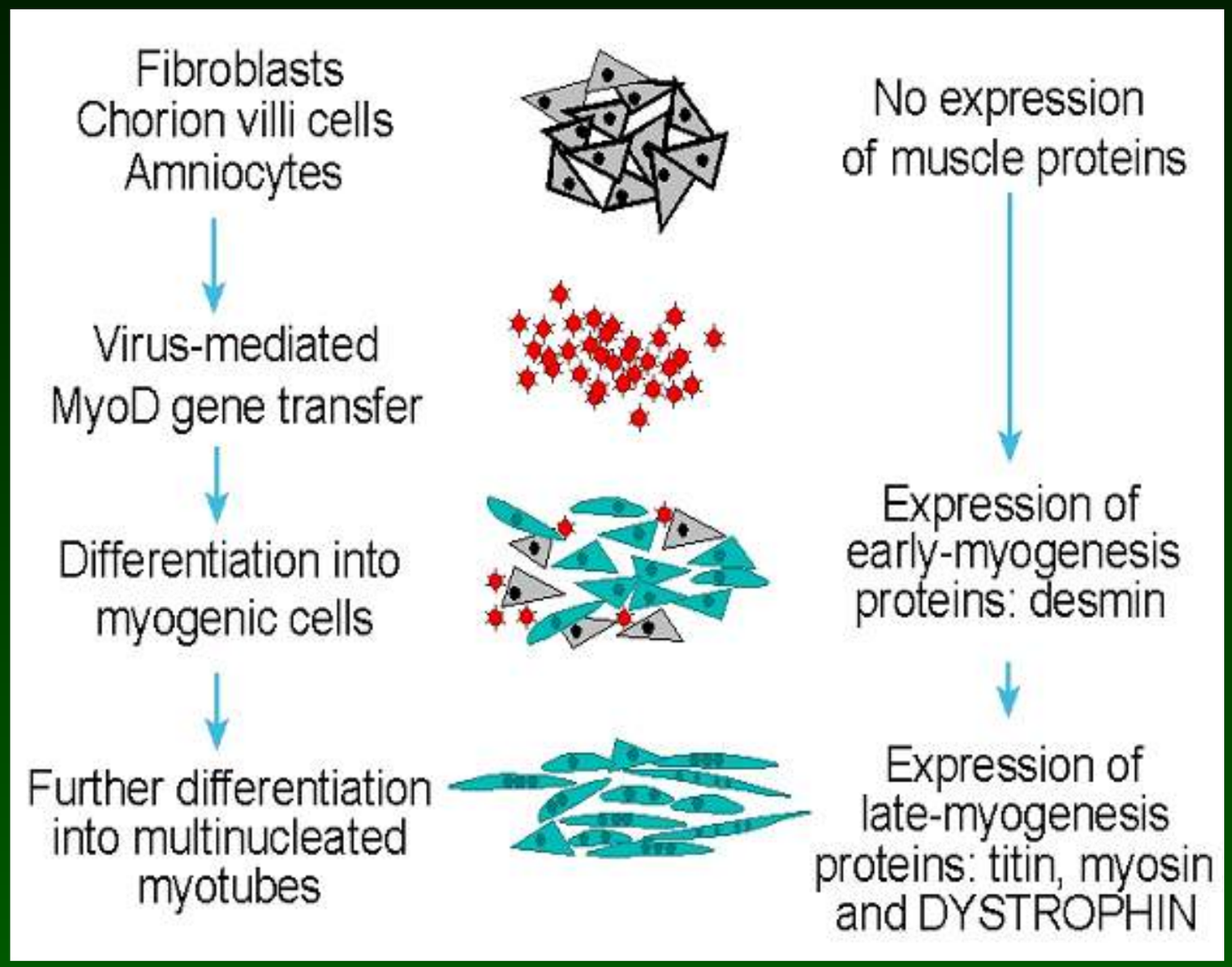
- **isolate DNA, RNA, protein**
- **cell extract / purified protein**
  measure activity (enzyme)
- **protein**
  detect amount / size *(antibody)*
  (co-)immunoprecipitation, …
- **RNA**
  detect amount / size *(probe)*
  study expression profile
  Protein Truncation Test = make protein

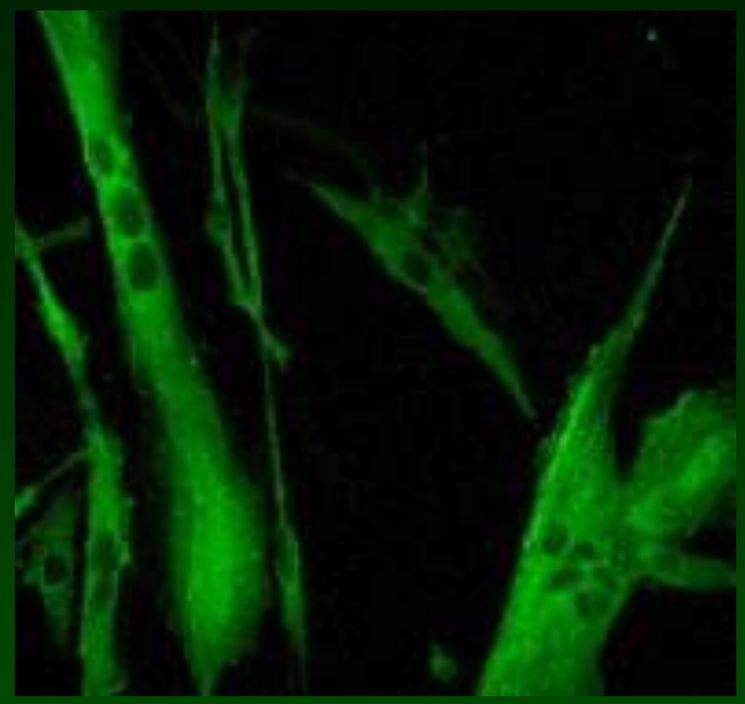
Patient cells

- **cell culturing**
  grow under specific conditions, test function
- **complementation**
  fuse cells, problem remains/solved ?
  Fanconi anemia, peroxisomal genes, …
- **expression cloning**
  express wt sequence in cell: normalised?
- **...tissue specific expression**
  reprogram cell
  myoD transfection > muscle cell
  IPS > lineage specific culturing

MyoD differentiation

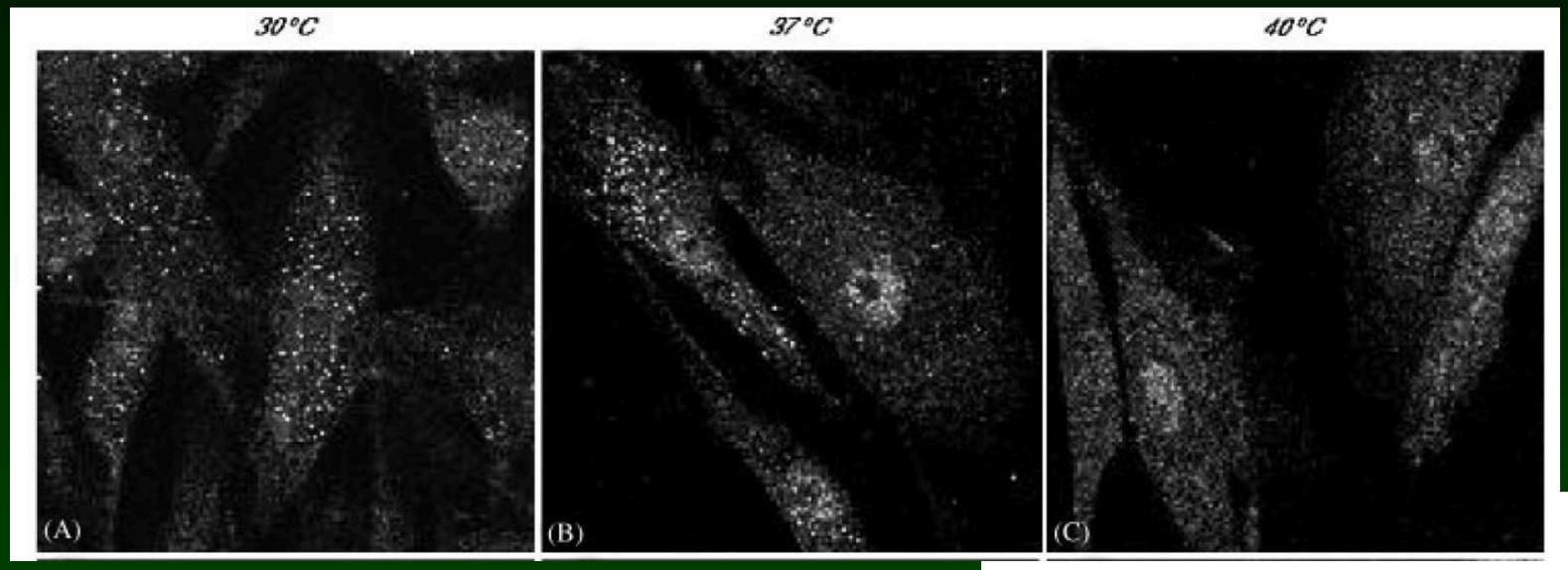
make 'muscle' cells from any cell





DMD/BMD diagnosis

T-sensitive



low T: dampens
high T: exaggerates

HUMAN MUTATION 24:130–139 (2004)

RESEARCH ARTICLE

Identification of the Molecular Defect in Patients With Peroxisomal Mosaicism Using a Novel Method Involving Culturing of Cells at 40°C: Implications for Other Inborn Errors of Metabolism

Jeannette Gootjes,[1] Frank Schmohl,[1] Petra A.W. Mooijer,[2] Conny Dekker,[2] Hanna Mandel,[3] Meral Topcu,[4] Martina Huemer,[5] M. von Schütz,[6] Thorsten Marquardt,[7] Jan A. Smeitink,[8] Hans R. Waterham,[2] and Ronald J.A. Wanders[1,2*]

Normal cells

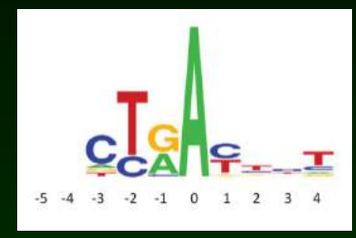
- **expression cloning variant**
  - ***dominant effect***
  - ***endogenous gene not expressed***
    - ***overexpression (recessive effects)***

- **destroy normal gene** (CRISPR/Cas)
  - ***effect detectable ?***
  - ***express variant; compensation ?***

- **express specific protein domain only**
  - ***simpler expressed***
    - ***e.g DNA binding domain BRCA1 protein***

RNA

- **effect on structure / integrity**
  - ***pre mRNA splicing***
    - ***splice site (SD, SA), branchpoint, ESE / ESS / ISE / ISS***



- **altered stability / turnover**
  - ***cap-site,***
  - ***polyA adddition (signal, site)***
  - ***folding, miRNA binding***

- **altered translation dynamics**
  - ***codon usage (rare/frequent codon)***
    - ***effect on co-translational protein folding***

UUU F 0.46	UCU S 0.19	UAU Y 0.44	UGU C 0.46
UUC F 0.54	UCC S 0.22	UAC Y 0.56	UGC C 0.54
UUA L 0.08	UCA S 0.15	UAA * 0.30	UGA * 0.47
UUG L 0.13	UCG S 0.05	UAG * 0.24	UGG W 1.00
CUU L 0.13	CCU P 0.29	CAU H 0.42	CGU R 0.08
CUC L 0.20	CCC P 0.32	CAC H 0.58	CGC R 0.18
CUA L 0.07	CCA P 0.28	CAA Q 0.27	CGA R 0.11
CUG L 0.40	CCG P 0.11	CAG Q 0.73	CGG R 0.20
AUU I 0.36	ACU T 0.25	AAU N 0.47	AGU S 0.15
AUC I 0.47	ACC T 0.36	AAC N 0.53	AGC S 0.24
AUA I 0.17	ACA T 0.28	AAA K 0.43	AGA R 0.21
AUG M 1.00	ACG T 0.11	AAG K 0.57	AGG R 0.21
GUU V 0.18	GCU A 0.27	GAU D 0.46	GGU G 0.16
GUC V 0.24	GCC A 0.40	GAC D 0.54	GGC G 0.34
GUA V 0.12	GCA A 0.23	GAA E 0.42	GGA G 0.25
GUG V 0.46	GCG A 0.11	GAG E 0.58	GGG G 0.25

[Codon/a.a./fraction per codon per a.a.]
Homo sapiens data from the Codon Usage Database

RNA

suggested reading, before starting

RESEARCH ARTICLE

Human Mutation

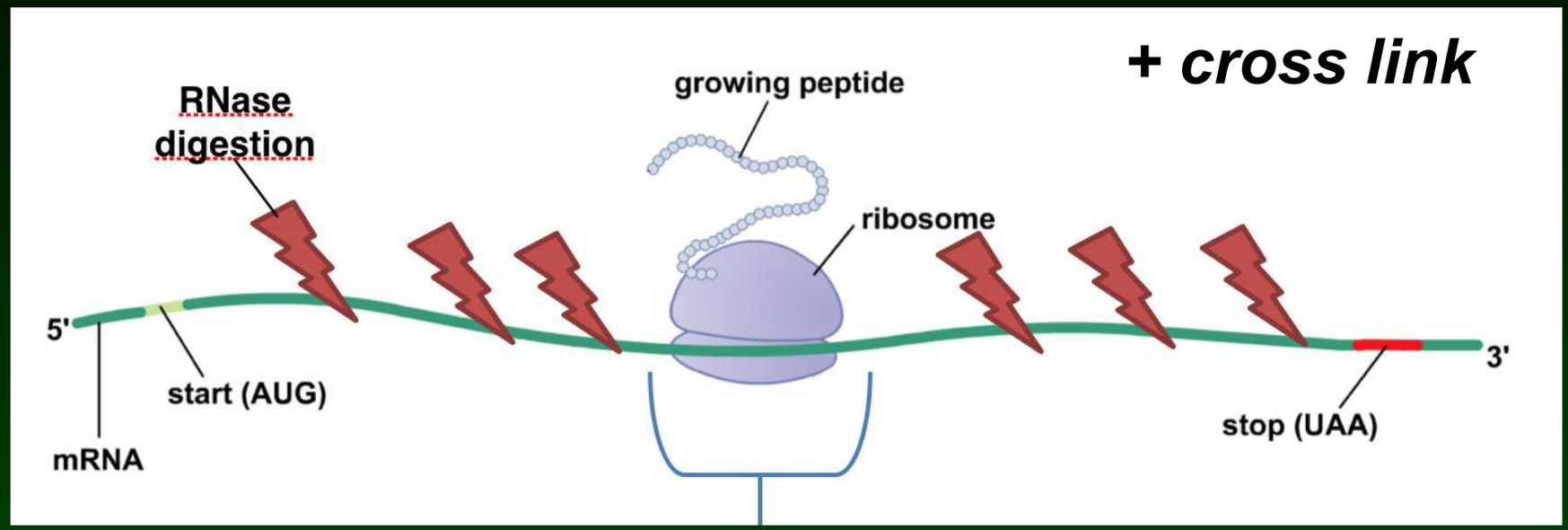
OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Guidelines for Splicing Analysis in Molecular Diagnosis Derived from a Set of 327 Combined *In Silico/In Vitro* Studies on *BRCA1* and *BRCA2* Variants

Claude Houdayer,[1*] Virginie Caux-Moncoutier,[1] Sophie Krieger,[2] Michel Barrois,[3] Françoise Bonnet,[4] Violaine Bourdon,[5] Myriam Bronner,[6] Monique Buisson,[7] Florence Coulet,[8] Pascaline Gaildrat,[9] Cédrick Lefol,[10] Mélanie Léone,[11] Sylvie Mazoyer,[7] Danielle Muller,[12] Audrey Remenieras,[3] Françoise Révillion,[13] Etienne Rouleau,[10] Joanna Sokolowska,[6] Jean-Philippe Vert,[14] Rosette Lidereau,[10] Florent Soubrier,[8] Hagay Sobol,[5] Nicolas Sevenet,[4] Brigitte Bressac-de Paillerets,[3,15] Agnès Hardouin,[2] Mario Tosi,[9] Olga M. Sinilnikova,[7,11] and Dominique Stoppa-Lyonnet[1,16]

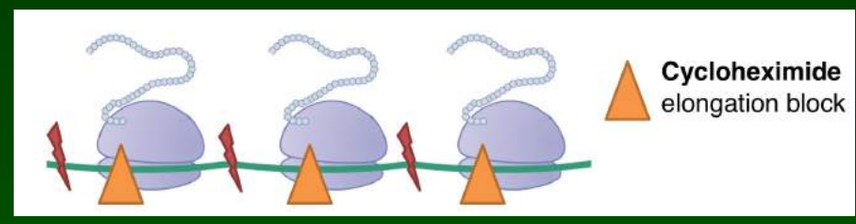
culture cells with/without cycloheximide (inhibit NMD)

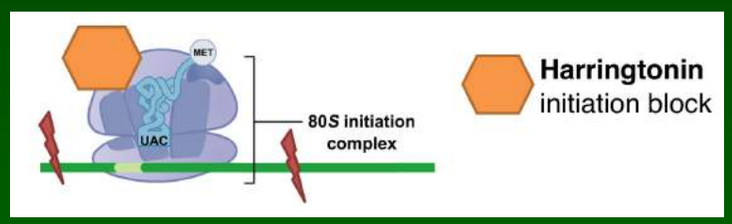
Ribosome profiling



©Eleonora de Klerk

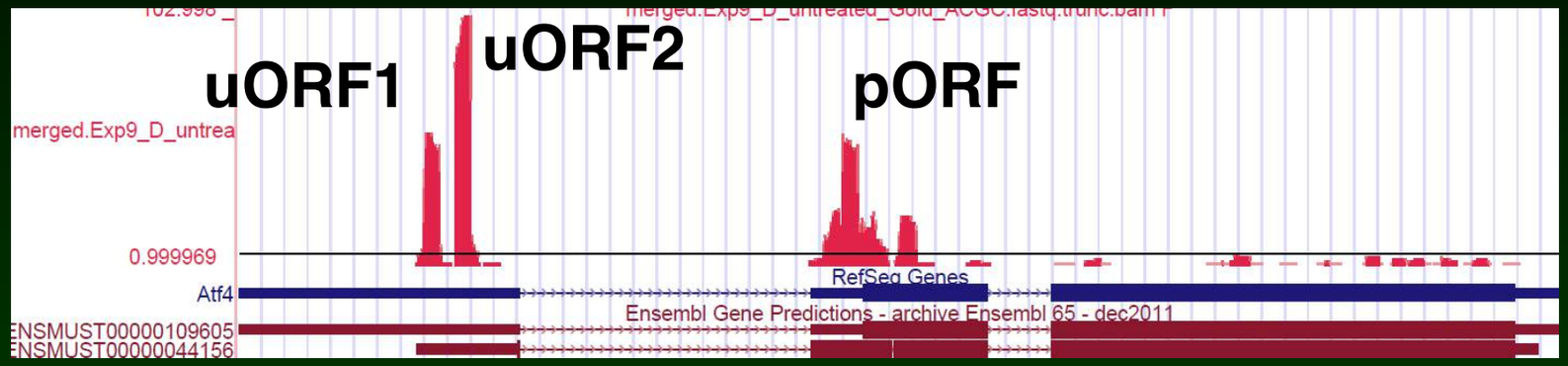
- **isolate ribosomes**
  - ***measure actively translated RNA***
  - ***+/- antibiotics***
    - ***cycloheximide, block elongation***
    - ***harringtonin, blocks initiation***



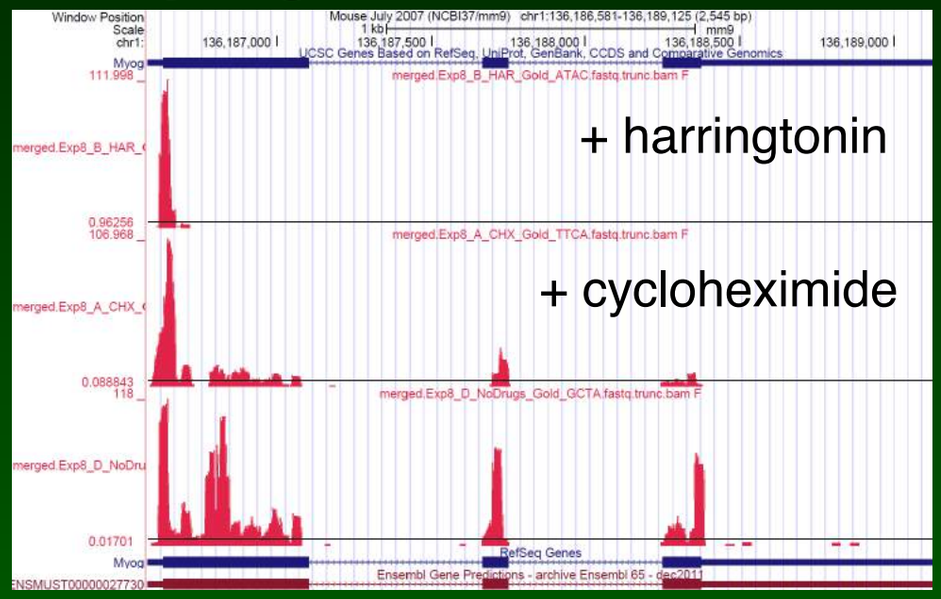


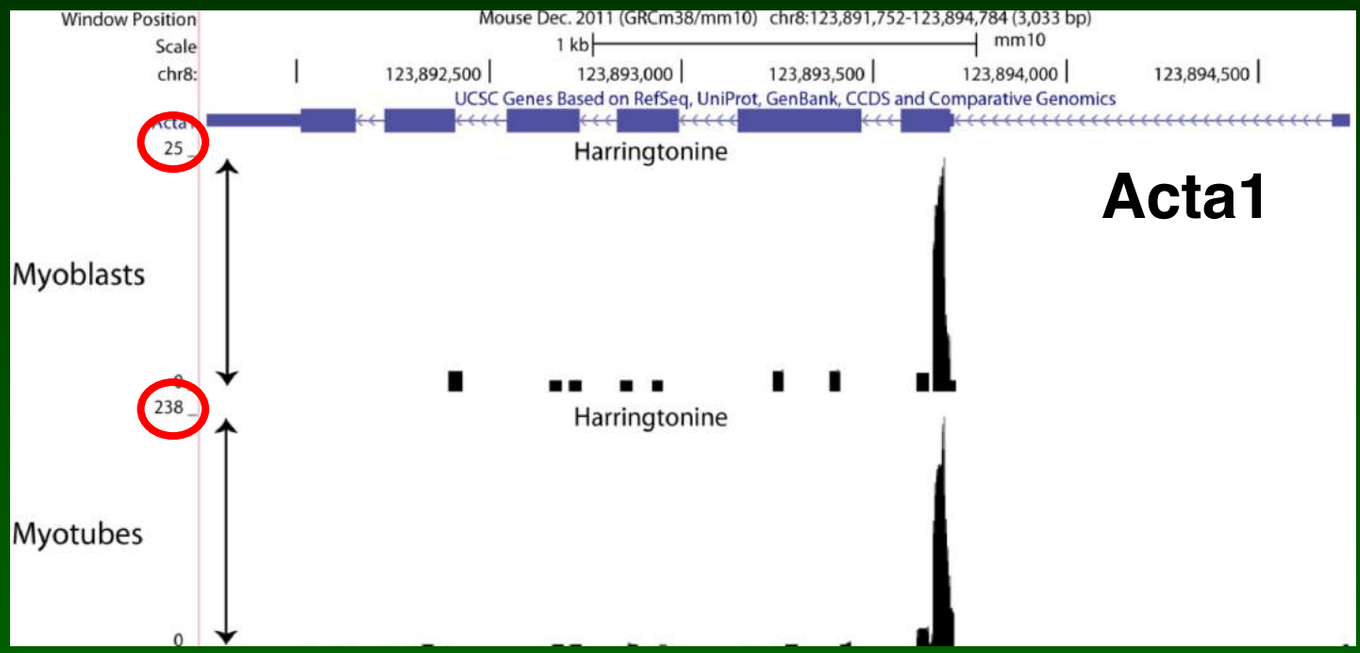
Ribosome profiling

Atf4



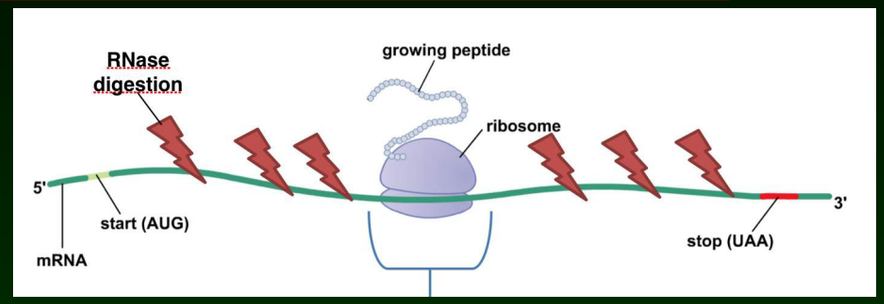
Myog





expression stable,
translation upregulated 10x

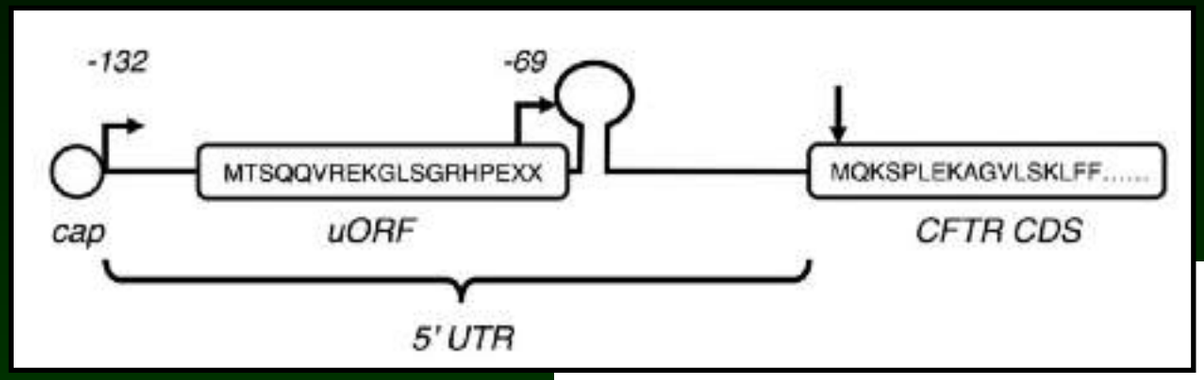
Ribosome profiling



- **discovery** *(gene annotation)*
  - ***define start codon***
  - ***determine protein coding regions***
  - ***determine reading frame used***
  - ***is RNA translated ?*** *(identify ncRNA)*
  - ***detect uORFs***
  - ***equals RNA expression protein expression?***

- **analysis** *(diagnostics)*
  - ***ATG variants (creating or destroying)***
  - ***uORF variants (creating or destroying)***

Ribosome profiling



Human Molecular Genetics, 2015, Vol. 24, No. 4 **899–912**
doi:10.1093/hmg/ddu501
Advance Access published on September 30, 2014

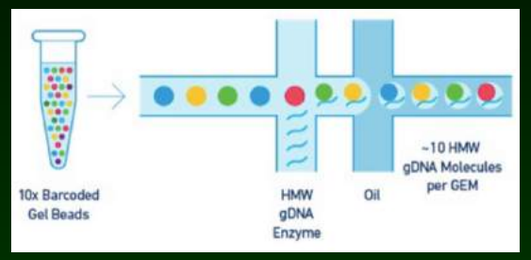
CFTR mRNA expression is regulated by an upstream open reading frame and RNA secondary structure in its 5′ untranslated region

Samuel W. Lukowski[1,2,†,*], Joseph A. Rothnagel[1] and Ann E. O. Trezise[1,2]

influence process > increase protein expression

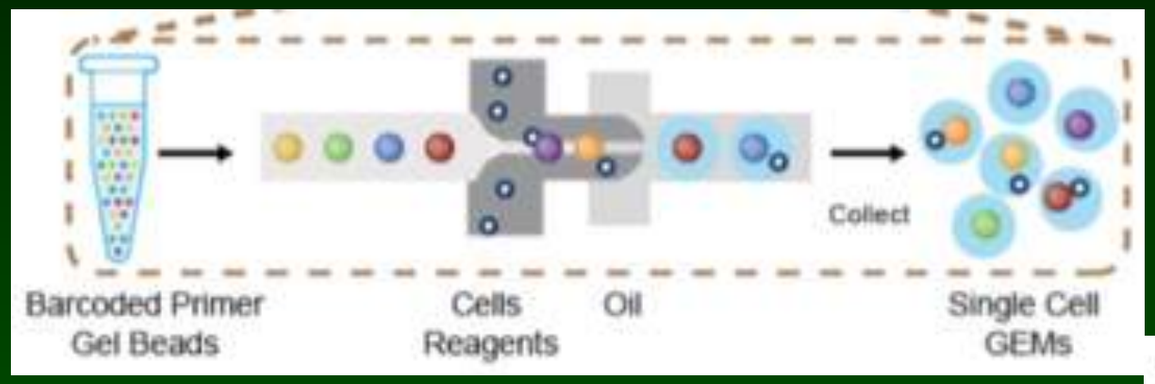
> treat CF-patients

Single cell

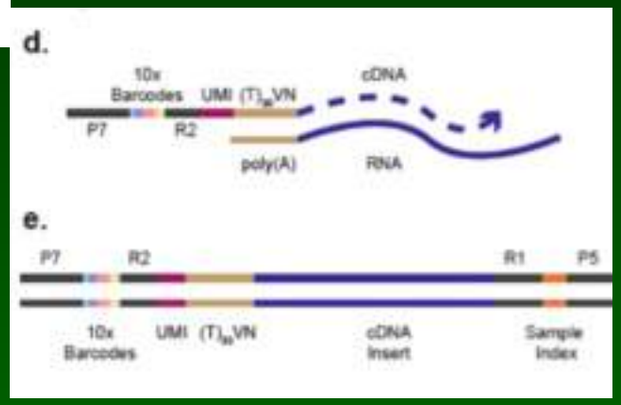


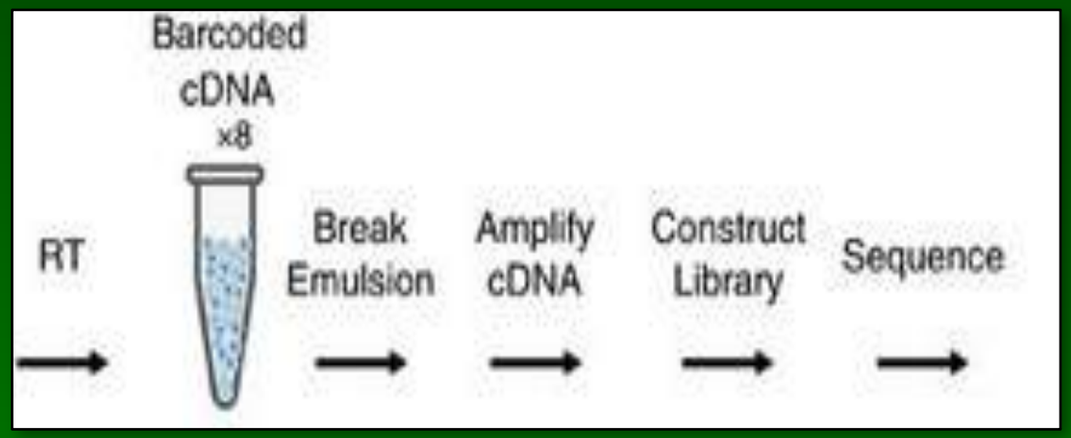
mix DNA + unique barcodes

mix single cells + unique barcodes



RT-PCR





amplify, pool, sequence

same barcode = same cell

First experiment

CELL RANGER

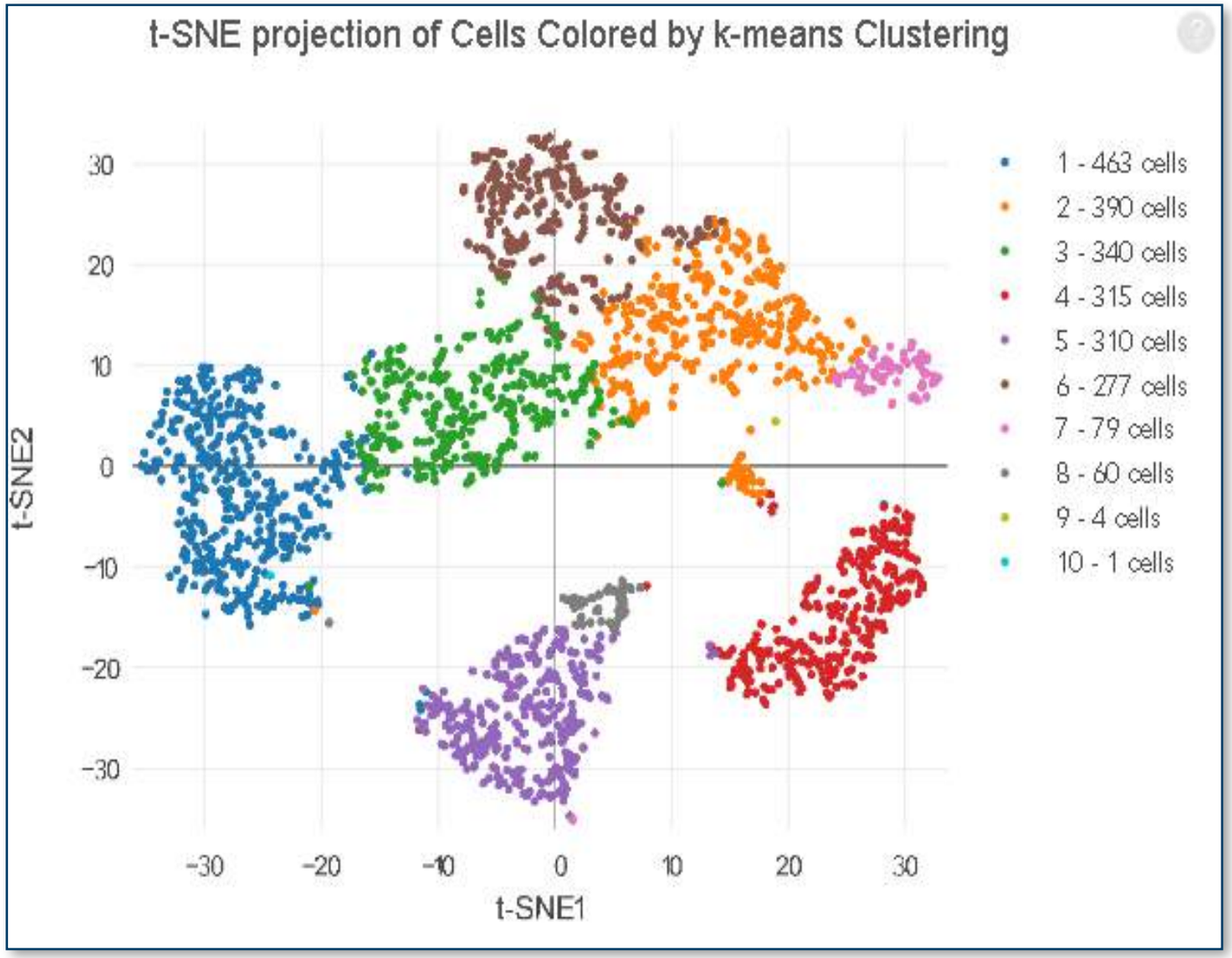
Using the Cell Ranger R package cluster cells based on differential gene expression patterns

Estimated Number of Cells
2,239

Mean Reads per Cell	Median Genes per Cell
10,480	1,128

Sequencing

Number of Reads	23,464,741
Valid Barcodes	93.0%
Reads Mapped Confidently to Transcriptome	68.9%
Reads Mapped Confidently to Exonic Regions	72.9%
Reads Mapped Confidently to Intronic Regions	7.8%
Reads Mapped Confidently to Intergenic Regions	2.6%
Sequencing Saturation	22.5%
Q30 Bases in Barcode	75.5%
Q30 Bases in RNA Read	76.6%
Q30 Bases in Sample Index	85.9%
Q30 Bases in UMI	78.4%



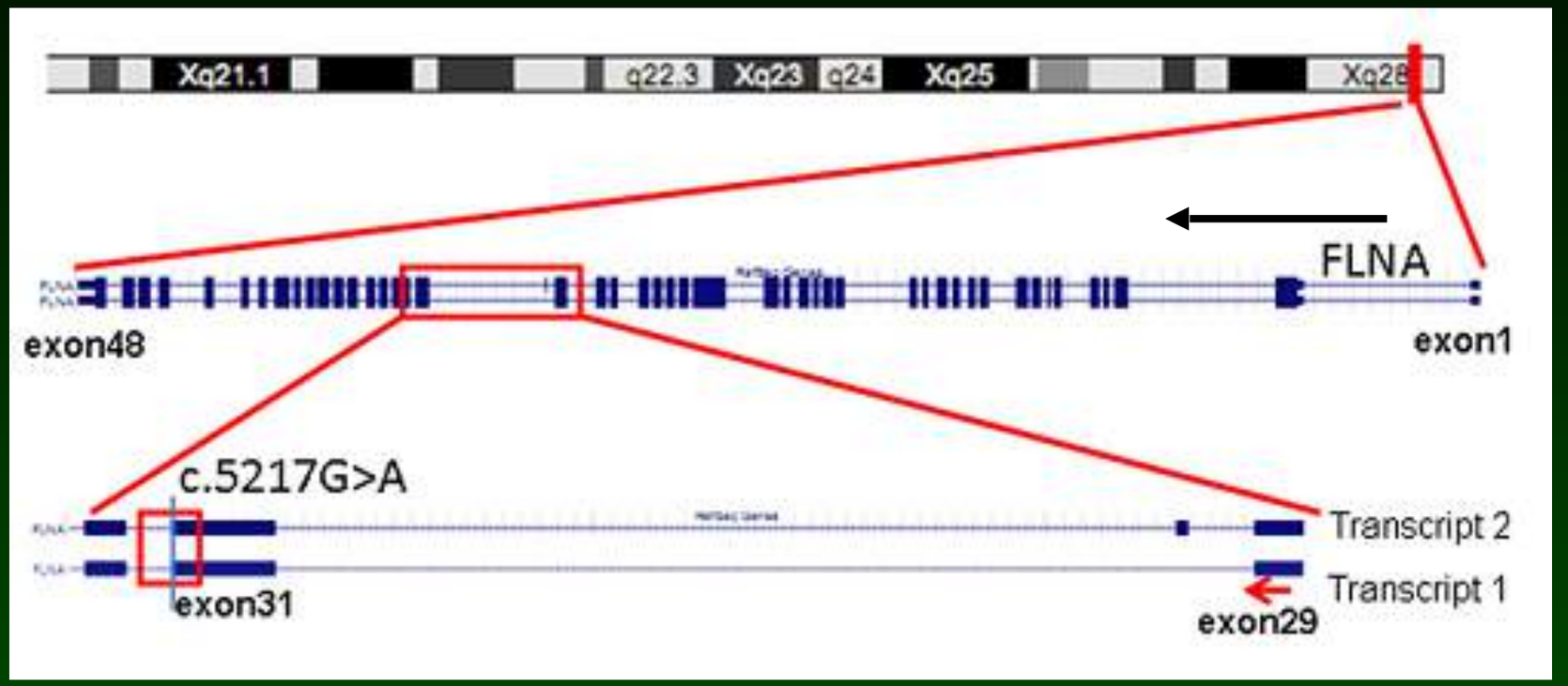
RNA splicing

- **expression cloning**
  clone exon(s) in splice construct
  transfect and express (cell line)
  analyse splice products generated

- **NOTE** *TEMPERATURE*
  artificial situation
  not complete gene/intron
  expression in other cell type (tissue specificity)

- **RNA of patient preferable**
  cell line, biopsy, …

TOD X-exome$_2$

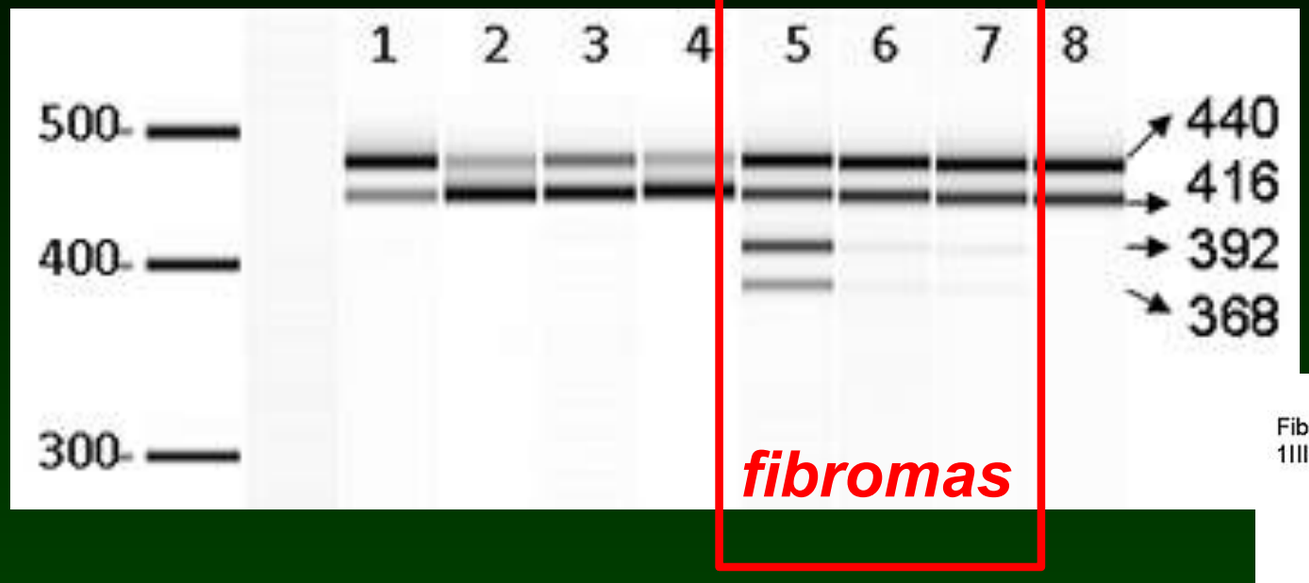


- **variant last nucleotide exon**
  alters splicing !?

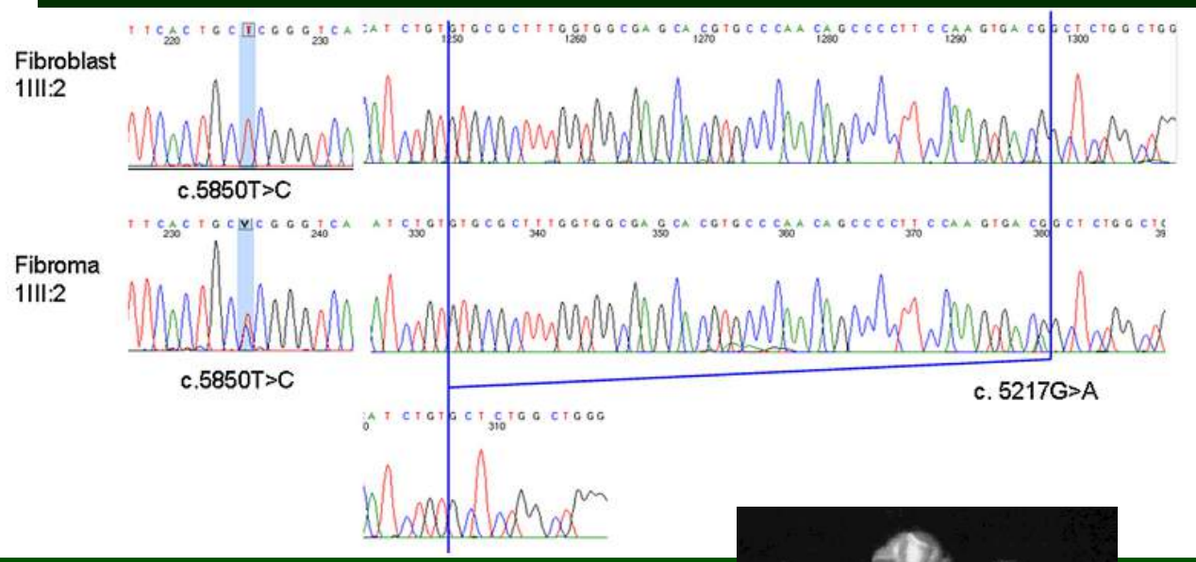
- **RNA expression**
  cultured cells / blood
    100% X_i, only normal allele expressed
  X_i always "affected chromosome"

Sun et al. 2010
Am.J.Hum.Genet. 87: 146

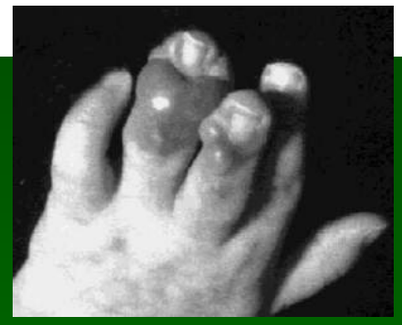
TOD X-exome$_3$



RNA



- **archived fibroma tissue**
  (15 year old)
  both alleles expressed
  activated cryptic exonic splice site



Sun et al. 2010 Am.J.Hum.Genet. 87: 146

X-linked TOD[4]

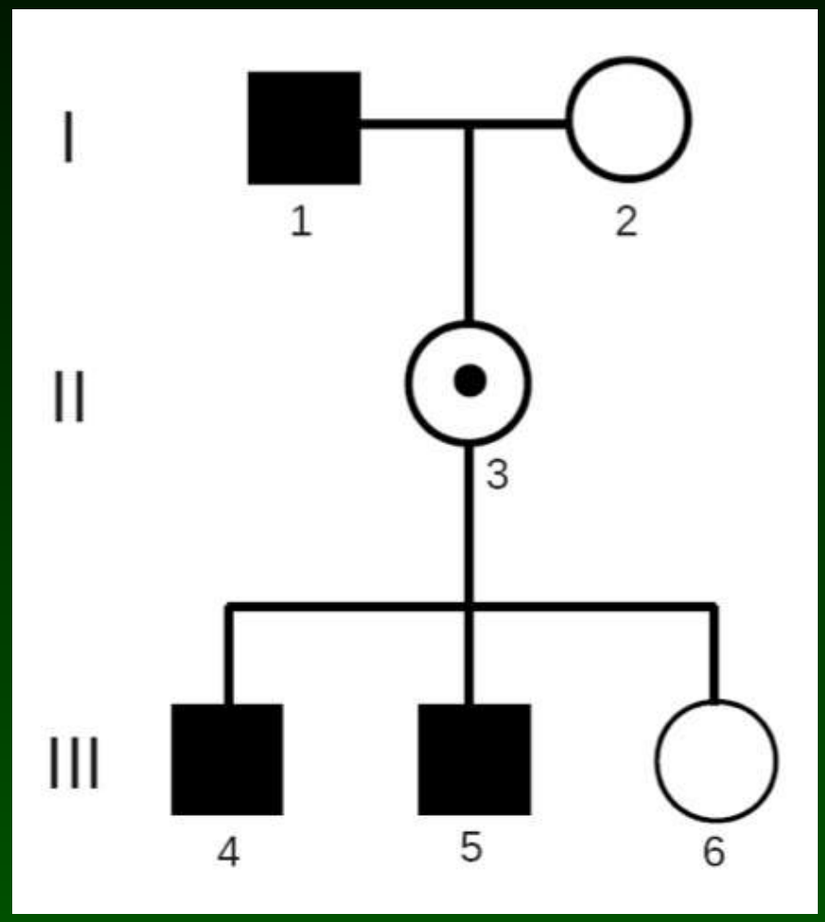
- **FLNA gene a surprise ?**
  - ***first FLNA mutations published***
  - ***the obvious candidate for TOD***
    - ***phenotypic overlap***
- **2004 gene analysed**
  - ***sent to expert > nothing reported back***
  - ***c.5217G>A was detected***
    - ***in vitro splice test negative***
- **case could be solved 6 years before**

We need to share & report ALL variants, immediately

Aarskog-Scott syndrome

- **Aarskog-Scott syndrome**
  FGD1 gene screened
  > no variants

- **whole exome capture**
  no obvious variants
  lower thresholds

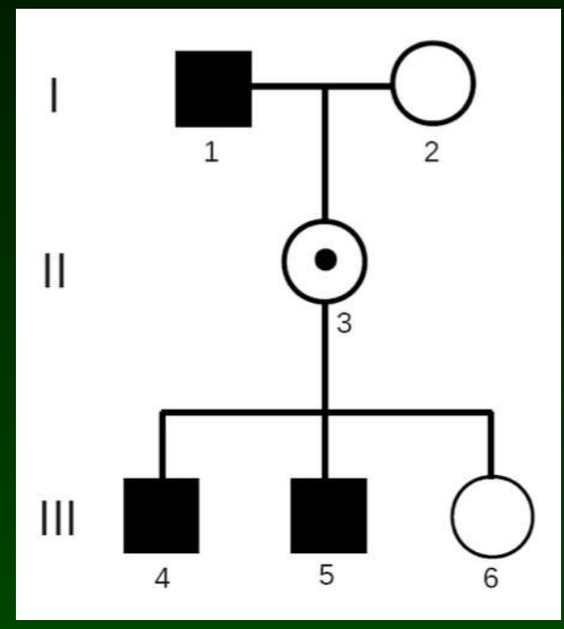


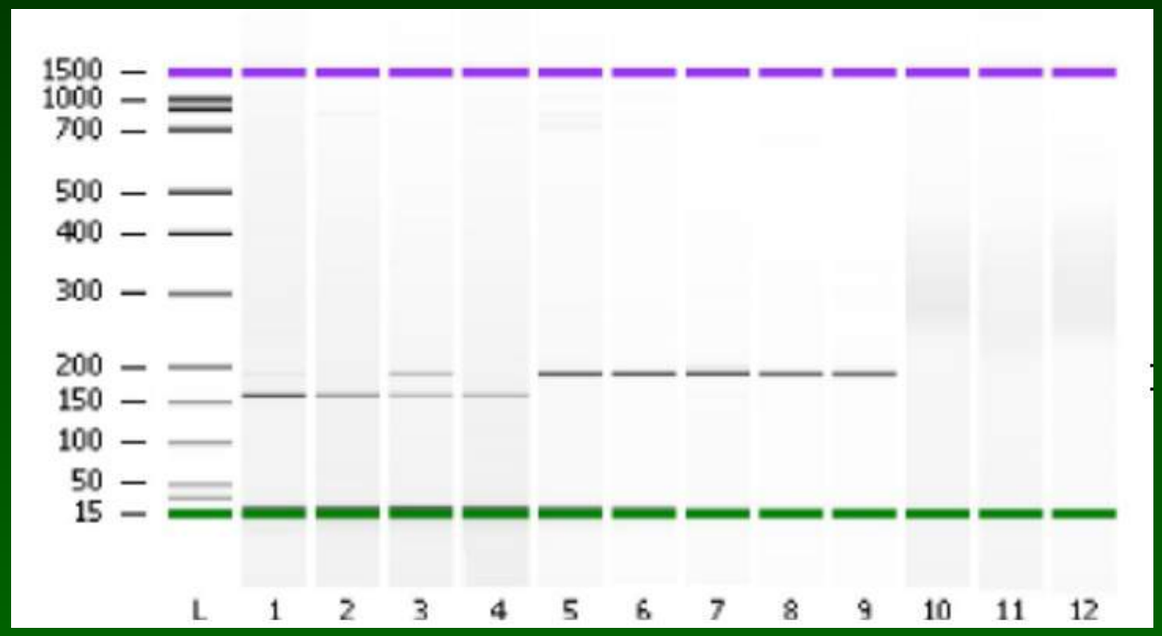
intron -35delA variant

©Yu Sun
Emmelien Aten

Aarskog-Scott syndrome

- **FGD1 intron -35delA variant**
  predicted branch site
- **RNA analysis**
  expressed in blood / fibroblasts





P P C P controls

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Emmelien Aten

Aarskog-Scott syndrome

- **why FGD1 variant missed ?**
  - *primer on variant site*
    - *not standard to screen to -50*
- **exome capture**
  - *lower coverage into intron*
  - *variant filtering to -10*
    - *many additional variants*
    - *difficult to confirm*
- **few branch site variants**
  - *rare, easily missed,*
  - *difficult to proof*


BRIEF REPORT

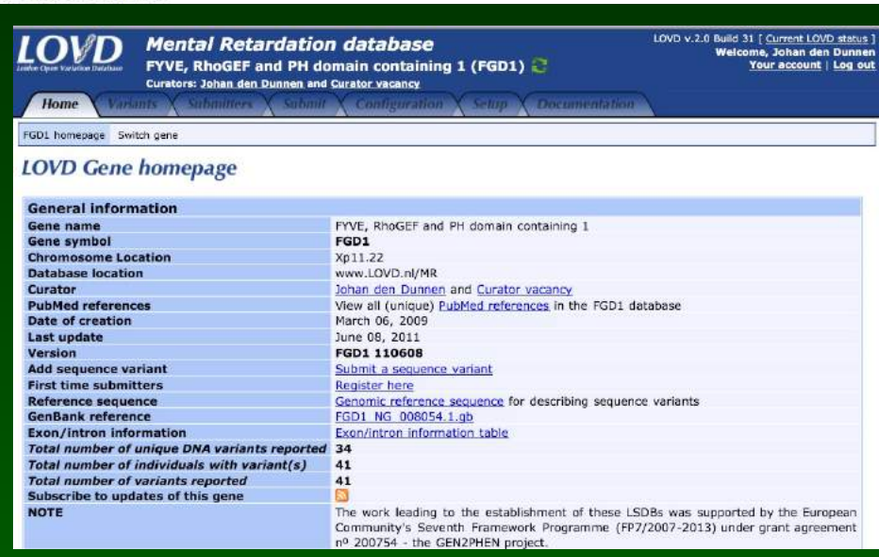
Human Mutation

OFFICIAL JOURNAL

HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Exome Sequencing Identifies A Branch Point Variant in Aarskog–Scott Syndrome

Emmelien Aten,[1†] Yu Sun,[1†] Rowida Almomani,[1] Gijs W.E. Santen,[1] Tobias Messemaker,[1] Saskia M. Maas,[2] Martijn H. Breuning,[1] and Johan T. den Dunnen[1*]



www.LOVD.nl/FGD1

exome performed, RNA analysis would be simpler ...& much cheaper

Animal models

- **time consuming & costly**
  - *mouse / rat*
  - *zebra fish*
  - *Drosophila*
  - *C.elegans*
  - *yeast*
  - *...*
- **existing models ?**
  - *large international projects KO all genes*

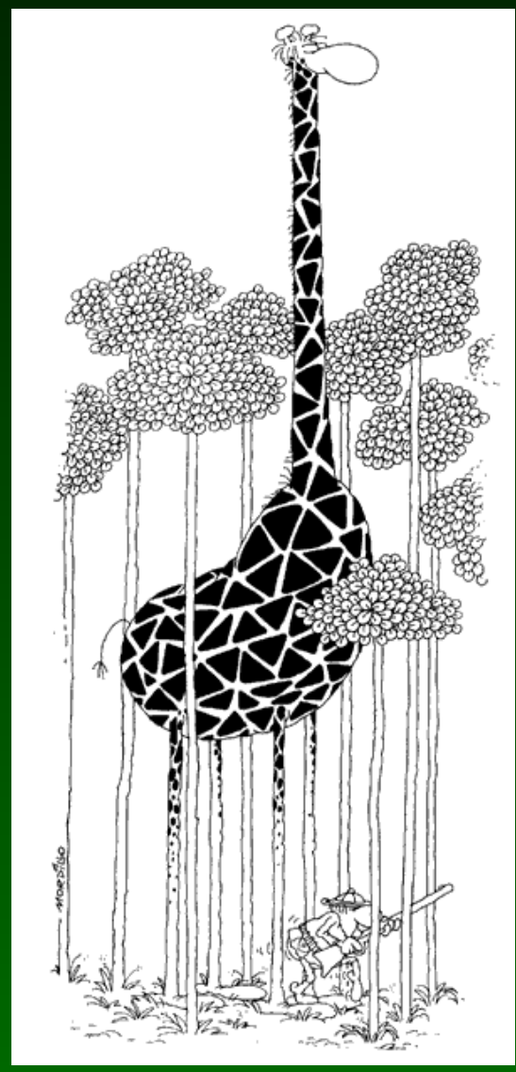
Mouse model

- **existing model > ”no” phenotype**
  - ***realise not everything can be tested***
  - ***patient data > specific phenotyping***

NATURE GENETICS VOLUME 44 | NUMBER 12 | DECEMBER 2012

Loss-of-function mutations in *IGSF1* cause an X-linked syndrome of central hypothyroidism and testicular enlargement

Yu Sun[1,20], Beata Bak[2,20], Nadia Schoenmakers[3,20], A S Paul van Trotsenburg[4,20], Wilma Oostdijk[5], Peter Voshol[3], Emma Cambridge[6], Jacqueline K White[6], Paul le Tissier[7,8], S Neda Mousavy Gharavy[7], Juan P Martinez-Barbera[7], Wilhelmina H Stokvis-Brantsma[5], Thomas Vulsma[4], Marlies J Kempers[4,9], Luca Persani[10,11], Irene Campi[10,12], Marco Bonomi[11], Paolo Beck-Peccoz[10,12], Hongdong Zhu[13], Timothy M E Davis[13], Anita C S Hokken-Koelega[14], Daria Gorbenko Del Blanco[14], Jayanti J Rangasami[15], Claudia A L Ruivenkamp[1], Jeroen F J Laros[1], Marjolein Kriek[1], Sarina G Kant[1], Cathy A J Bosch[1], Nienke R Biermasz[16], Natasha M Appelman-Dijkstra[16], Eleonora P Corssmit[16], Guido C J Hovens[16], Alberto M Pereira[16], Johan T den Dunnen[1,17], Michael G Wade[18], Martijn H Breuning[1], Raoul C Hennekam[4], Krishna Chatterjee[3,21], Mehul T Dattani[19,21], Jan M Wit[5,21] & Daniel J Bernard[2,21]



Not like this

Human Molecular Genetics, 2012, Vol. 21, No. 4 **811–825**
doi:10.1093/hmg/ddr512
Advance Access published on November 7, 2011

Modeling the human *MTM1* p.R69C mutation in murine *Mtm1* results in exon 4 skipping and a less severe myotubular myopathy phenotype

Christopher R. Pierson[1,2,4,*], Ashley N. Dulin-Smith[1], Ashley N. Durban[1], Morgan L. Mar Jordan T. Marshall[1], Andrew D. Snyder[1], Nada Naiyer[1], Jordan T. Gladman[1], Dawn S. Chandler[1,3,4], Michael W. Lawlor[5,†], Anna Buj-Bello[6], James J. Dowling[7] and Alan H. Beggs[5,*]

- **unclear missense effect**
  - ***mouse model generated***
  - ***> no protein in mouse***
  - ***> RNA analysis shows splice effect***
  - ***> confirmed in human***

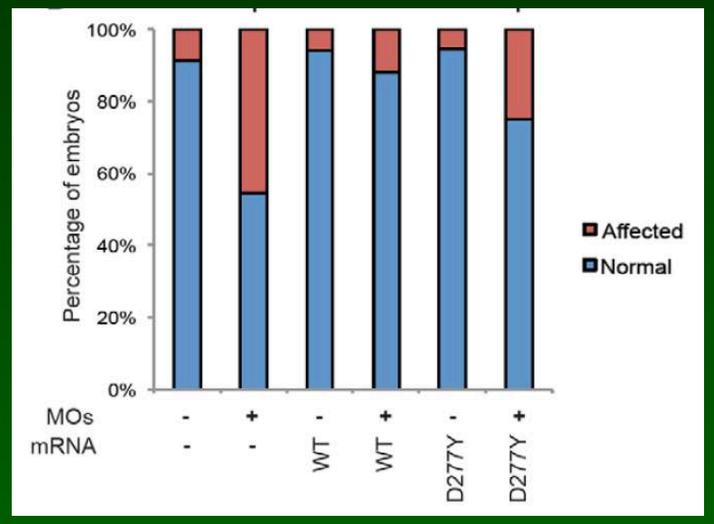
Zebrafish

- **gene KO**
- **embryos**
  morpholino injection
  downregulate expression
  co-expression gene (RNA)

(*Circ Cardiovasc Genet*. 2015;8:544-552.

Loss of Function Mutations in *NNT* Are Associated With Left Ventricular Noncompaction

Matthew N. Bainbridge, PhD*; Erica E. Davis, PhD*; Wen-Yee Choi, PhD; Amy Dickson, BS; Hugo R. Martinez, MD; Min Wang, PhD; Huyen Dinh, PhD; Donna M. Muzny, MS; Ricardo Pignatelli, MD; Nicholas Katsanis, PhD; Eric Boerwinkle, PhD; Richard A. Gibbs, PhD; John L. Jefferies, MD



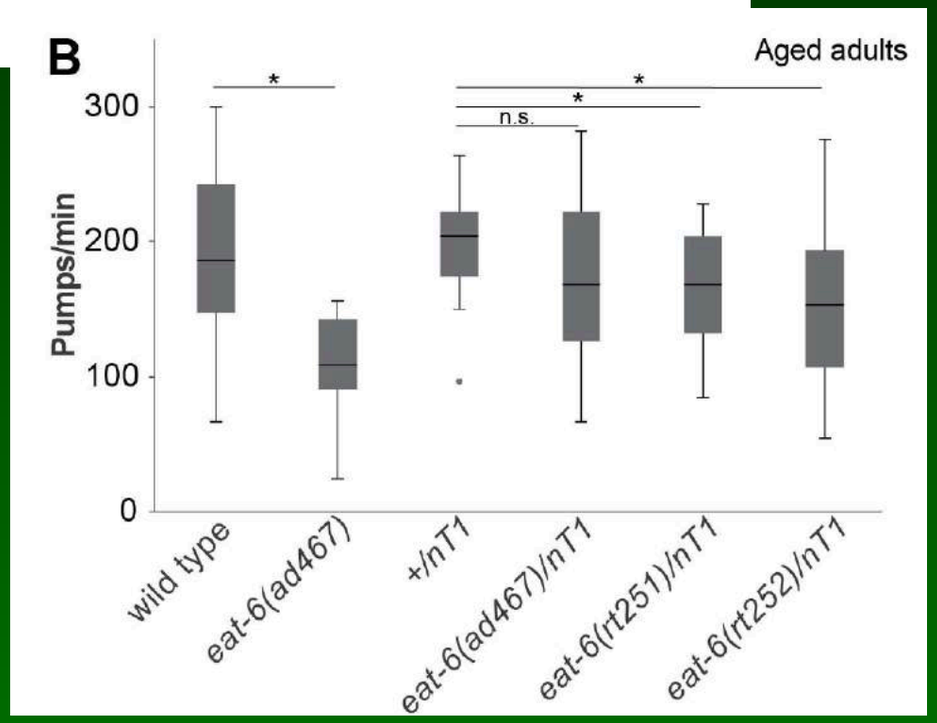
C. elegans

- **knock-out / knock-in**
  normal development?
  measure specific features

PLoS One. 2016 Dec 9;11(12):e0167963. doi: 10.1371/journal.pone.0167963. eCollection 2016.

In Vivo Modelling of ATP1A3 G316S-Induced Ataxia in C. elegans Using CRISPR/Cas9-Mediated Homologous Recombination Reveals Dominant Loss of Function Defects.

Sorkaç A[1], Alcantara IC[1], Hart AC[1].



Databases

LOVD 3 Shared database

Leiden Open Variation Database

SPINK5 (serine peptidase inhibitor, Kazal type 5)

LOVD is supported by: interactive biosoftware

Curator: LOVD-team, but with Curator vacancy

Genes | Transcripts | Variants | Individuals | Diseases | Screenings | Submit | Documentatio

- View all genomic variants
- View all variants affecting transcripts
- View unique variants in gene SPINK5
- View all variants in gene SPINK5
- Full data view for gene SPINK5

All genes

22977 entries on 230 pages. S

100 per page | « Firs | 11 ... | Next › | Last »

Symbol	Gene			Transcripts	Variants
A1BG	alpha-1-B glycoprotein	19	q13.43	1	12
A1BG-AS1	A1BG antisense RNA 1	19	q13.4	1	0
A1CF	APOBEC1 complementation factor	10	q21.1	2	0
A2LD1	AIG2-like domain 1	13	q32.3	1	1

Chromosome	X
Allele	Unknown
Affects function (reported)	Affects function
Affects function (concluded)	Not classified
DNA change (genomic) (Relative to hg19 / GRCh37)	g.74291351A>C
DNA change (hg38)	-
Published as	1200T>G, I400M
ISCN	-
DB-ID	ABCB7_000001 See all 3 reported entries
Variant remarks	cloned in yeast ATM1 (V365M), partial loss of function (reduced growth rate iron limiting conditions)
Reference	PubMed: Allikmets 1999, OMIM:var0001
dbSNP ID	-
Germline/Somatic/De novo	In vitro (cloned)

Germline/Somatic/De novo

in vitro

In vitro (cloned)

In vitro (cloned)

All variants affecting transcripts

3281 entries on 33 pages. Showing entries 1 - 100.

100 per page | Legend | « First | ‹ Prev | 1 2 3 4 5 6 7 8 9 10 11 ... | Next › | Last »

Gene	Transcript	Chr	Allele	DNA change (genomic) (hg19)		
ABCB7	NM_004299.3	X	Unknown	g.74291351A>C	-	1200T>G, I400M
ABCB7	NM_004299.3	X	Unknown	g.74290268C>T	-	G1305A, E433K
ACTC1	NM_005159.4	15	Unknown	g.35082659T>C	-	-
ACTC1	NM_005159.4	15	Unknown	g.35083364C>T	-	-
ACTC1	NM_005159.4	15	Unknown	g.35082750C>G	-	-
ACTC1	NM_005159.4	15	Unknown	g.35084729G>C	-	-
ADAMTSL4	NM_019032.4	1	Both (homozygous)	g.150526871dup	-	c.1162dupG
AMHR2	NM_020547.2	12	Unknown	g.53818072C>A	-	-

Gene	ABCB7
Transcript ID	NM_004299.3
Affects function (reported)	Affects function
Affects function (concluded)	Not classified
Exon	9
DNA change (cDNA)	c.1203T>G
Class.	-
RNA change	-
Protein	p.Ile401Met

Colleagues

- **specialists on genes / pathways**
  database curators, reviews

- **functional test**
  frequently used, expert analysis
  diagnostic lab may change from detect variants to test variants
  unpublished data

Hum Mutat. 2012 Mar;33(3):488-94. doi: 10.1002/humu.22000. Epub 2011 Dec 29.

A rapid and cell-free assay to test the activity of lynch syndrome-associated MSH2 and MSH6 missense variants.

Drost M[1], Zonneveld JB, van Hees S, Rasmussen LJ, Hofstra RM, de Wind N.

- **saturation mutagenesis**
  massive mutagenesis gene / gene region
  test all variants *(functional test)*

Functional tests ?

- ...