The Rapid Development of NeuroGenetics: Things you should know about!

Daniel Woo, MD, MS

Professor of Neurology

Objectives

- Describe basic terms and terminology used in genetic research
- Describe advances being made in diagnosis and phenotyping of disease
- Describe advances being made in gene therapy

Basics

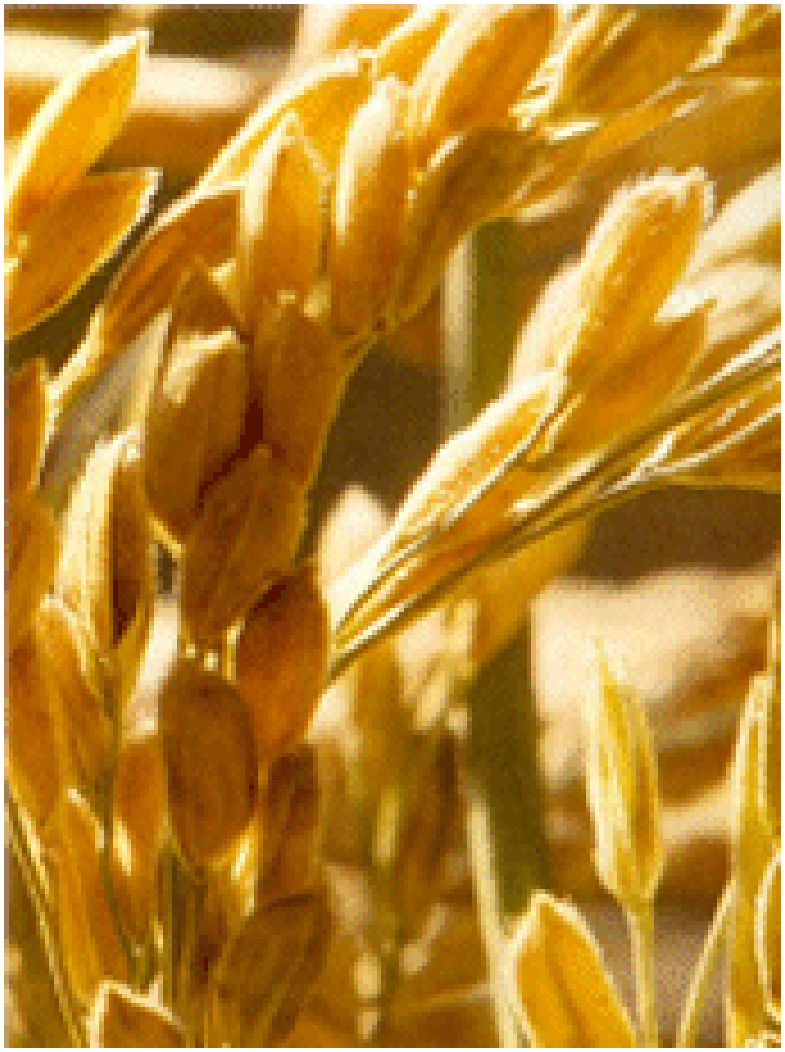
- DNA→ RNA →Protein
  - Each Human cell contains ~**$6.6X10^9$** bases (A,C,G or T) or ~ 5 feet of DNA
  - Adult body contains ~10 trillion cells
  - (5 feet)(10 trillion) is enough DNA to reach from the Earth to the Sun ~90 times
- 1-2% of our DNA codes for proteins

Number of Genes

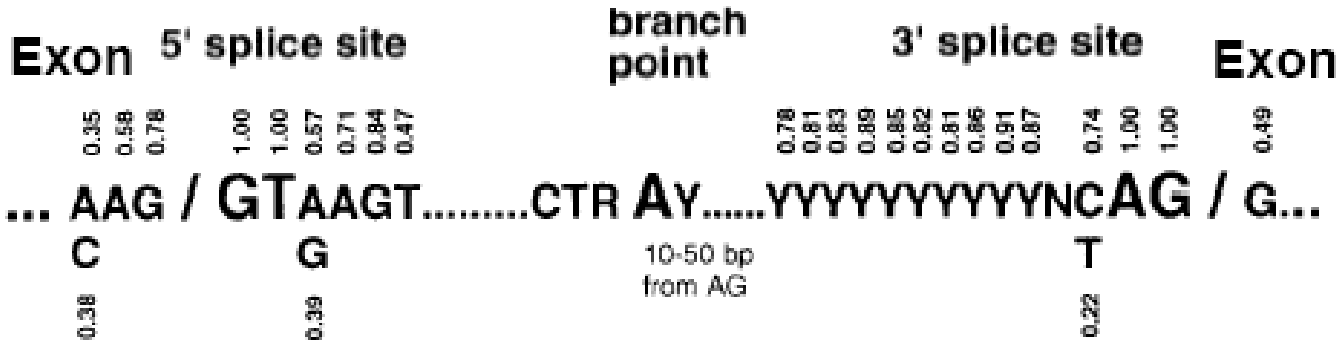
- 13,000 – Fruit Fly
- 19,000 – Roundworm
- 25,000 – Mustard Weed
- 30-40,000 – Humans

Oryza Sativa

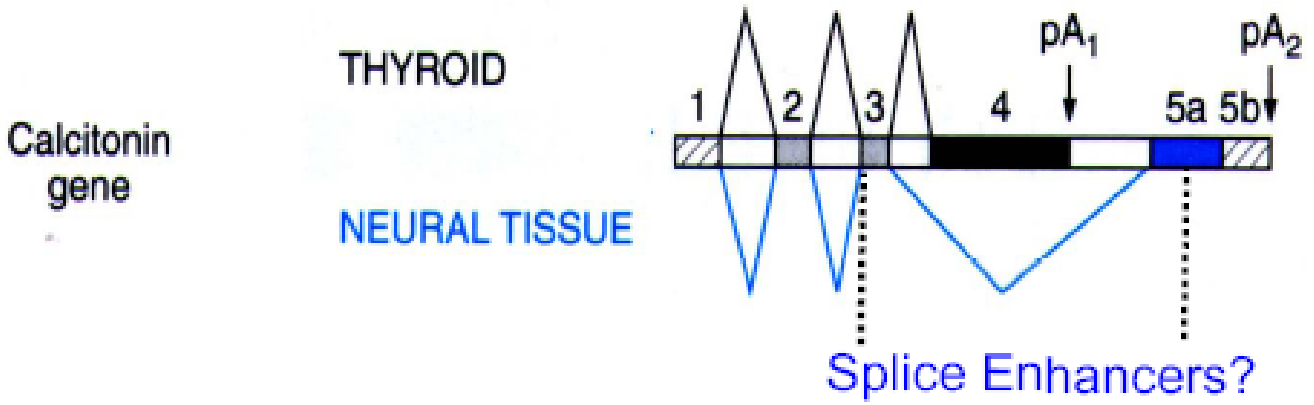
- 2005 Genome Sequence
- 56,000 GENES!
- Humans gain complexity through regulation and splicing



mRNA Splicing: Consensus Sequences



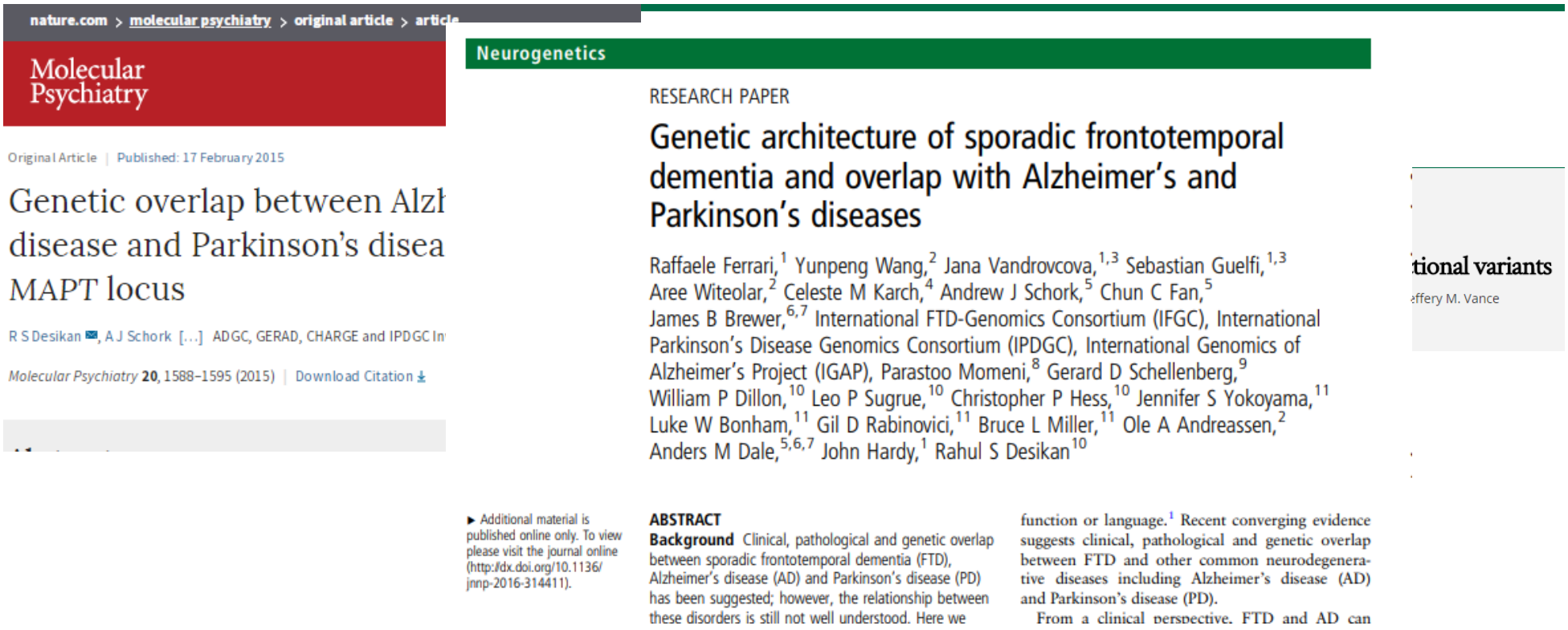
Alternative Splicing: Splicing Enhancers



How Genetics is Changing Diagnoses and Phenotypes

- Disease versus syndrome
  - A disease is a disorder of structure or function
  - A syndrome is a group of symptoms that consistently occur together or a condition characterized by a set of associated symptoms.
- Is Parkinson's a disease or a syndrome?
  - Bradykinesia, resting tremor, rigidity
  - Lewy body deposition in substantia nigra
  - Diffuse lewy body disease
  - Progressive supranuclear palsy
  - Parkinson's with dementia

Genetic Overlap Alzheimer's and Parkinson's



nature.com > molecular psychiatry > original article > article

Molecular Psychiatry

Original Article | Published: 17 February 2015

Genetic overlap between Alzł disease and Parkinson's disea MAPT locus

R S Desikan, A J Schork [...] ADGC, GERAD, CHARGE and IPDGC In

Molecular Psychiatry **20**, 1588–1595 (2015) | Download Citation

Neurogenetics

RESEARCH PAPER

Genetic architecture of sporadic frontotemporal dementia and overlap with Alzheimer's and Parkinson's diseases

Raffaele Ferrari,[1] Yunpeng Wang,[2] Jana Vandrovcova,[1,3] Sebastian Guelfi,[1,3] Aree Witeolar,[2] Celeste M Karch,[4] Andrew J Schork,[5] Chun C Fan,[5] James B Brewer,[6,7] International FTD-Genomics Consortium (IFGC), International Parkinson's Disease Genomics Consortium (IPDGC), International Genomics of Alzheimer's Project (IGAP), Parastoo Momeni,[8] Gerard D Schellenberg,[9] William P Dillon,[10] Leo P Sugrue,[10] Christopher P Hess,[10] Jennifer S Yokoyama,[11] Luke W Bonham,[11] Gil D Rabinovici,[11] Bruce L Miller,[11] Ole A Andreassen,[2] Anders M Dale,[5,6,7] John Hardy,[1] Rahul S Desikan[10]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2016-314411).

ABSTRACT

Background Clinical, pathological and genetic overlap between sporadic frontotemporal dementia (FTD), Alzheimer's disease (AD) and Parkinson's disease (PD) has been suggested; however, the relationship between these disorders is still not well understood. Here we

function or language.[1] Recent converging evidence suggests clinical, pathological and genetic overlap between FTD and other common neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD).

From a clinical perspective, FTD and AD can

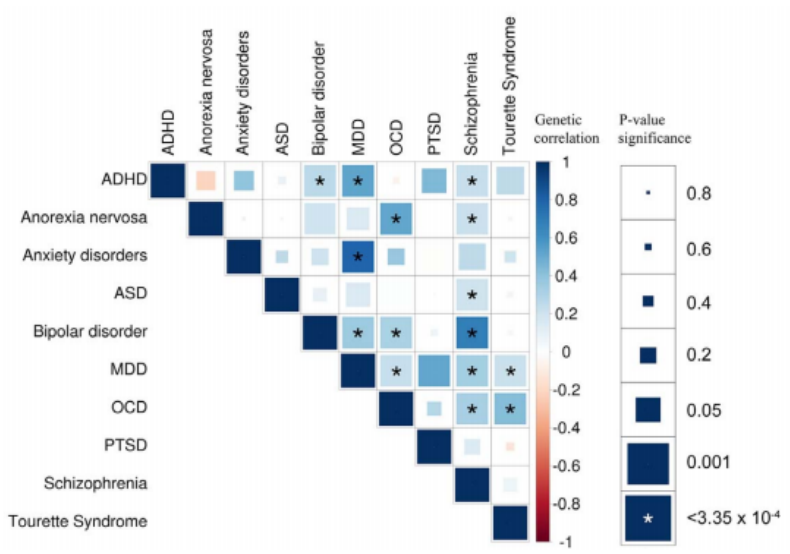
tional variants

ffery M. Vance

Brainstorm Consortium

- 200,000 patients with 25 brain-associated disorders and 17 phenotypes
- June, 2018

Table 1. Brain disorder phenotypes used in the Brainstorm project. Indented phenotypes are part of a larger whole (e.g., the epilepsy study contains the samples from both focal epilepsy and generalized epilepsy). "Anxiety disorders" refers to a meta-analysis of five subtypes (generalized anxiety disorder, panic disorder, social phobia, agoraphobia, and specific phobias). References are listed in table S1 and data availability in table S13. PGC-ADD2, Psychiatric Genomics Consortium (PGC) Attention Deficit Disorder Working Group; PGC-ED, PGC Eating Disorder Working Group; ANGST, Anxiety Neuro Genetics STudy; PGC-AUT, PGC Autism Spectrum Disorder Working Group; PGC-BIP2, PGC Bipolar Disorder Working Group; PGC-MDD2, PGC Major Depressive Disorder Working Group; PGC-OCDTS, PGC Obsessive Compulsive Disorder and Tourette Syndrome Working Group; PGC-PTSD, PGC Posttraumatic Stress Disorder Working Group; PGC-SCZ2, PGC Schizophrenia Working Group; IGAP, International Genomics of Alzheimer's Project; ILAE, International League Against Epilepsy Consortium on Complex Epilepsies; ISGC, International Stroke Genetics Consortium; METASTROKE, a consortium of the ISGC; IHGC, International; Headache Genetics Consortium; IMSGC, International Multiple Sclerosis Genetics Consortium; IPDGC, International Parkinson's Disease Genomics Consortium. " indicates same as above.



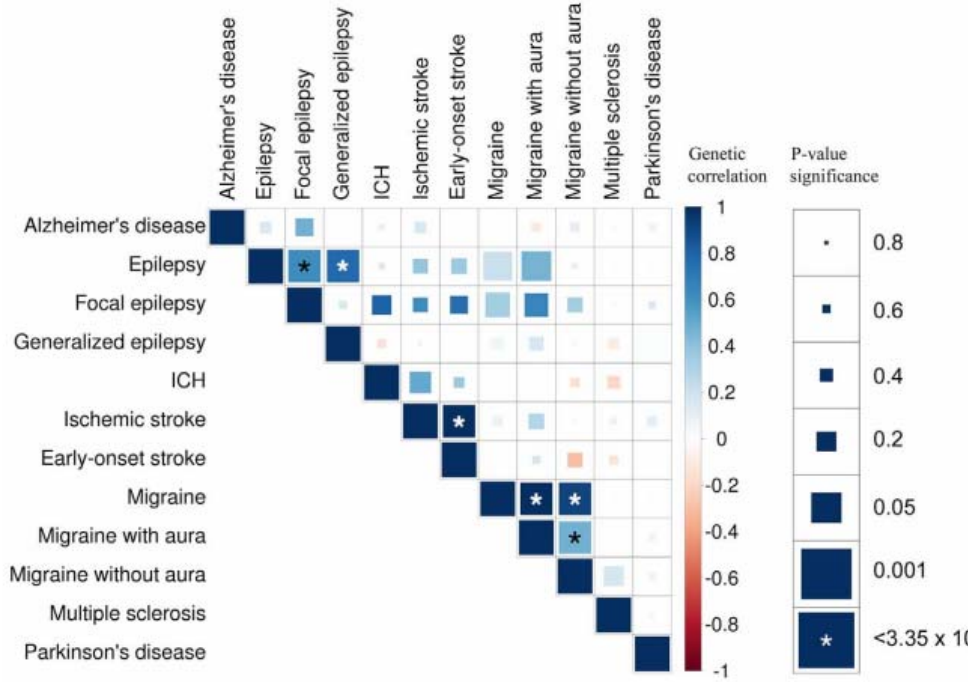


Fig. 1. Genetic correlations across psychiatric phenotypes. The color of each box indicates the magnitude of the correlation, and the size of the box indicates its significance (LDSC), with significant correlations filling each square completely. Asterisks indicate genetic correlations that are significantly different from zero after Bonferroni correction.

Brainstorm Consortium

- Most disorders did not have a common genetic overlap but some overlaps observed
  - Migraine and Epilepsy
  - Focal Epilepsy with Intracerebral Hemorrhage
  - Attention deficit and depression
  - Schizophrenia with most psychiatric illnesses

Neurology/Psychiatry Overlap?

- Anxiety disorders and ICH?
  - Recent paper that found stress was an independent risk factor for ICH
- Anxiety with Epilepsy
- Schizophrenia with ischemic stroke?
- Migraine with Major Depressive Disorder and Anxiety and ADHD

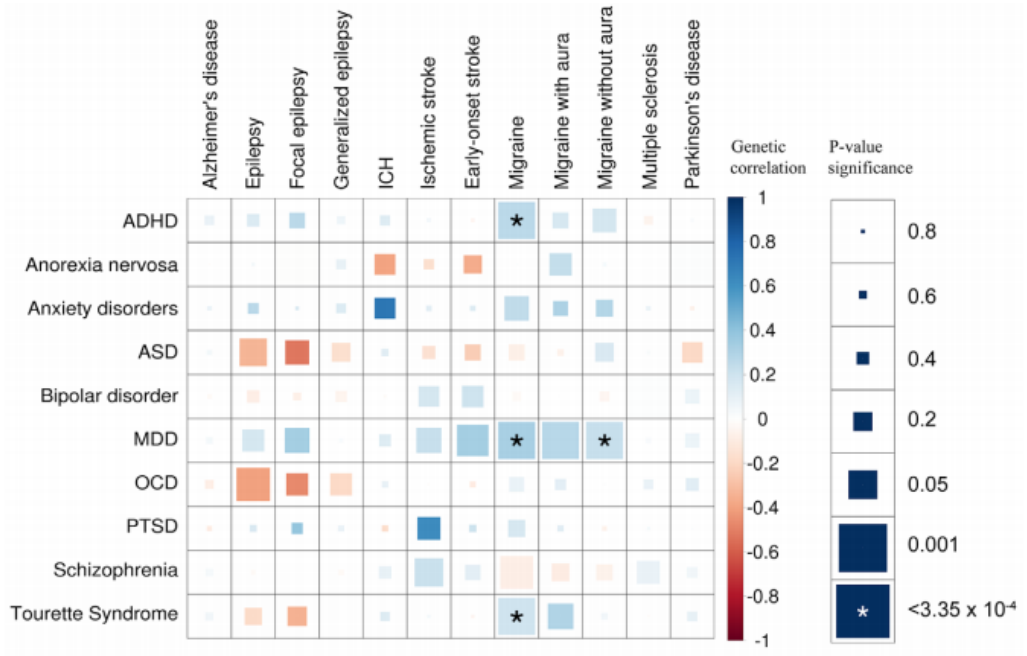


Fig. 3. Genetic correlations across neurological and psychiatric phenotypes. The color of each box indicates the magnitude of the correlation, and the size of the box indicates its significance (LDSC), with significant correlations filling each square completely. Asterisks indicate genetic correlations that are significantly different from zero after Bonferroni correction.

Chronic Traumatic Encephalopathy and Lou Gehrig's Disease?

Chronic trauma to the neurologic system is a risk factor for a progressive decline in memory, cognition, depression, parkinsonism, poor impulse control and eventually dementia

In some patients, it can lead to chronic encephalomyelopathy appearing similar to ALS.

Could there be a common mechanism?

New Phenotypes?

- Epidemiologically, trauma is a risk factor for chronic traumatic encephalopathy, Alzheimer's disease, Amyotrophic lateral sclerosis
- Head injury triggers multiple biocehemical events that may lead to neurodegeneration.

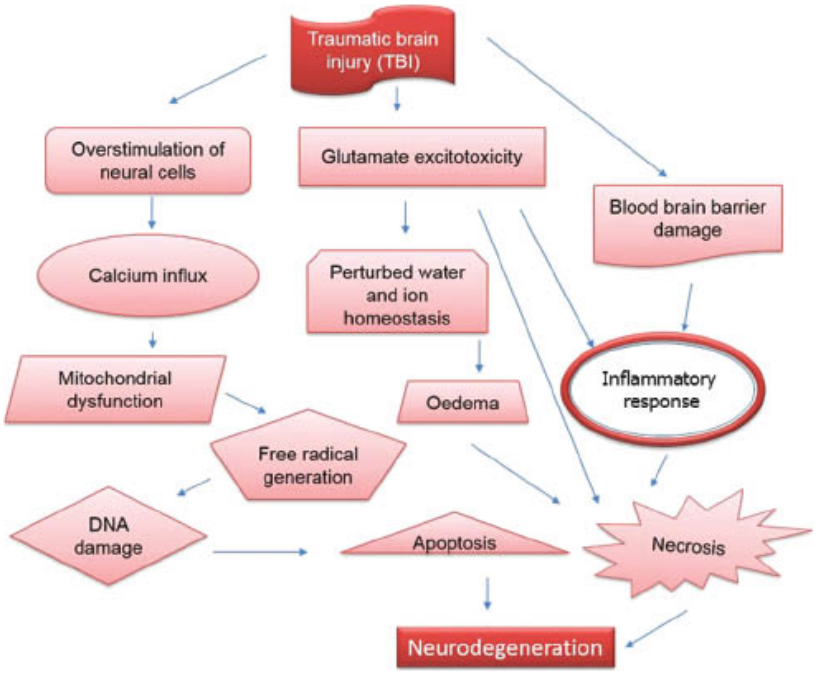
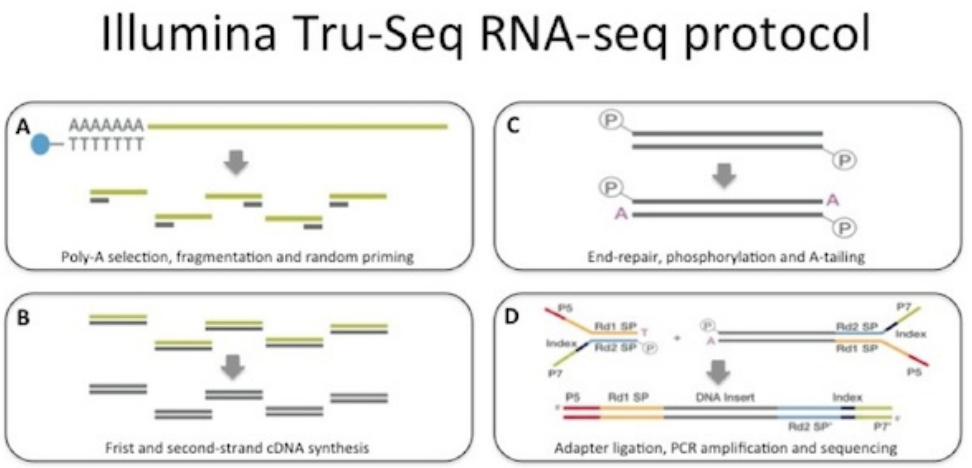


Figure 1:
TBI-induced biochemical reactions involved in secondary neuronal damage.

RNA Sequencing

- Whole transcriptome screening (all of the mRNA)
- Plus all of the micro RNA
- Methylation (gene silencing)
- Can identify normal genes that are abnormally transcribed/expressed



Library prep begins from 100ng-1ug of Total RNA which is poly-A selected (A) with magnetic beads. Double-stranded cDNA (B) is phosphorylated and A-tailed (C) ready for adapter ligation. The library is PCR amplified (D) ready for clustering and sequencing.

EBioMedicine 16 (2017) 184–194

Contents lists available at ScienceDirect

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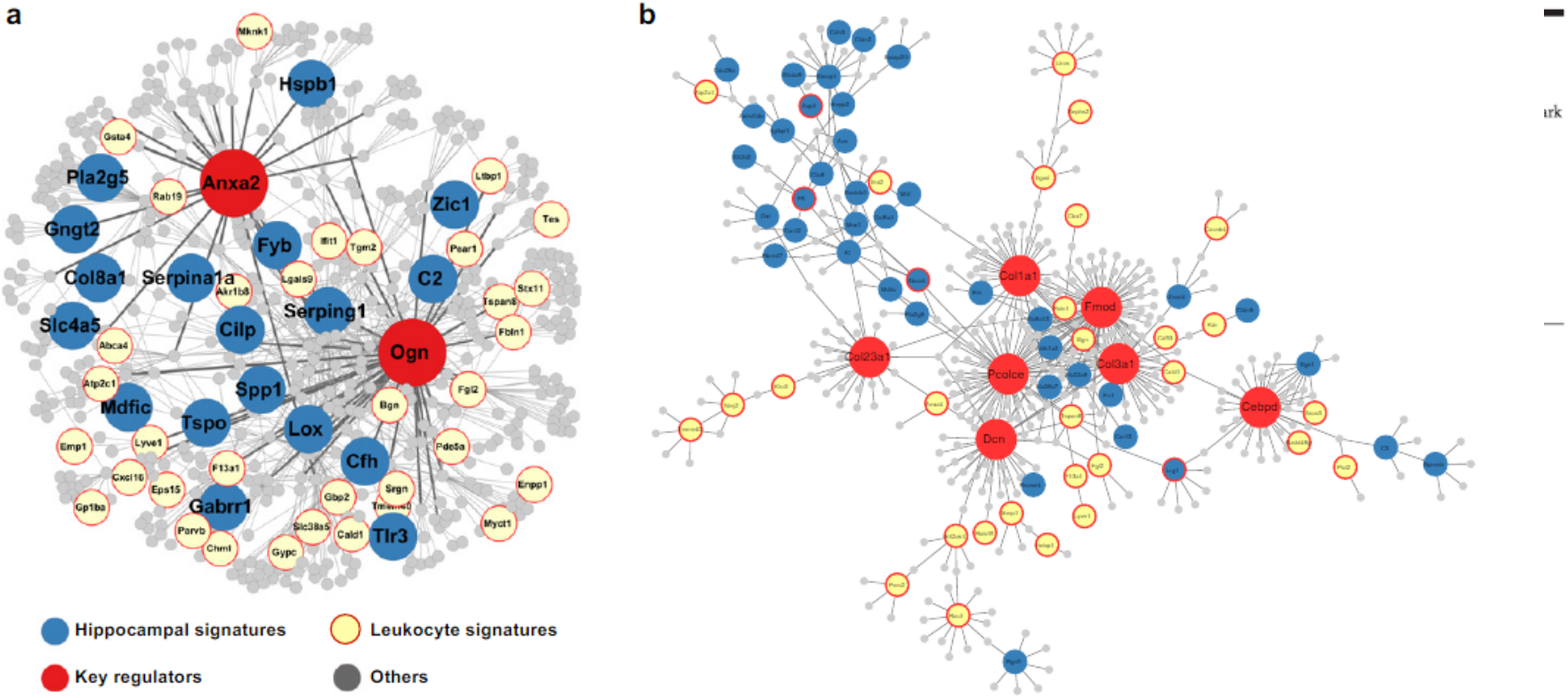


Fig. 4. Shared subnetworks between hippocampal and leukocyte signature genes. A) A subnetwork centered at shared key drivers (KDs) *Anax2* and *Ogn*. B) A subnetwork centered at shared KDs *Fmod*, collagen genes, and *Cebpd*. Larger red nodes are the KDs; blue and yellow nodes denote hippocampal and leukocyte signature genes, respectively. Grey nodes are network genes in the neighborhood of KDs that are not affected by TBI.

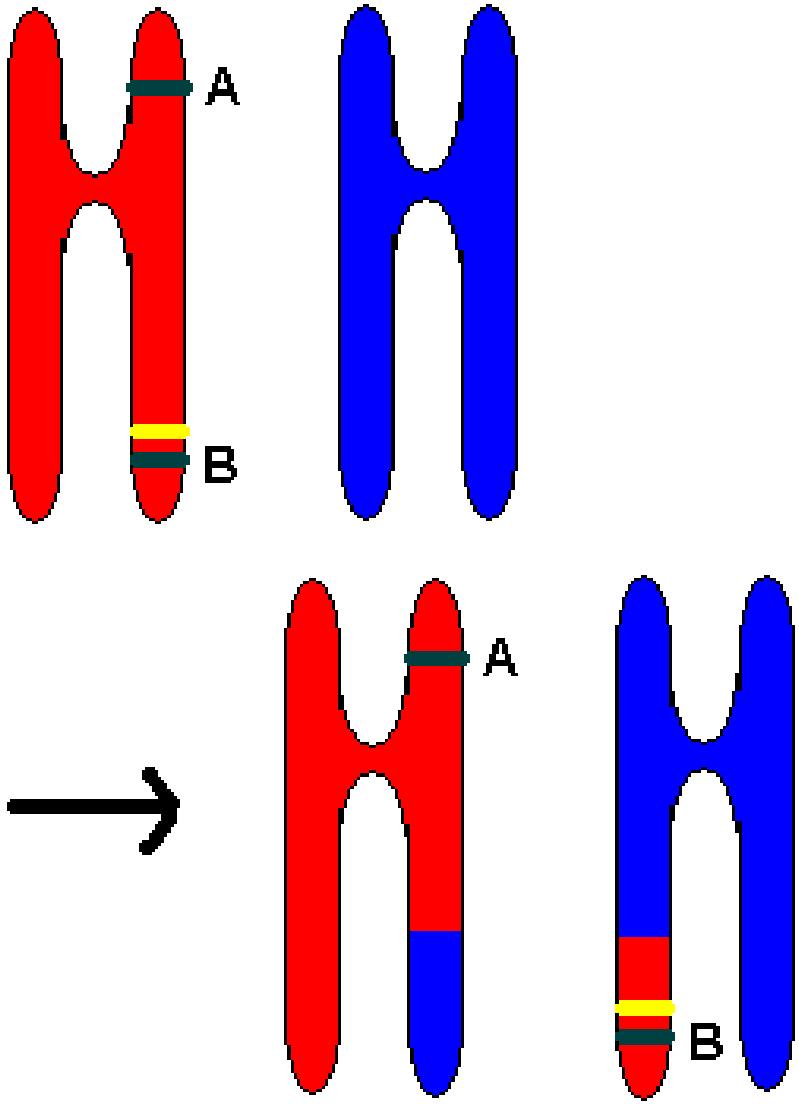
Summarizing Phenotypes

- What we understand about a specific phenotype may be the end product of a wide variety of different pathophysiologic mechanisms
- These different mechanisms may share pathways with other diseases and conditions allowing ‘orphan drugs’ or treatments to have a broader effect
- Personalizing medical treatment to the specific susceptibility a patient or disease process may have

- How do we find these mechanisms?

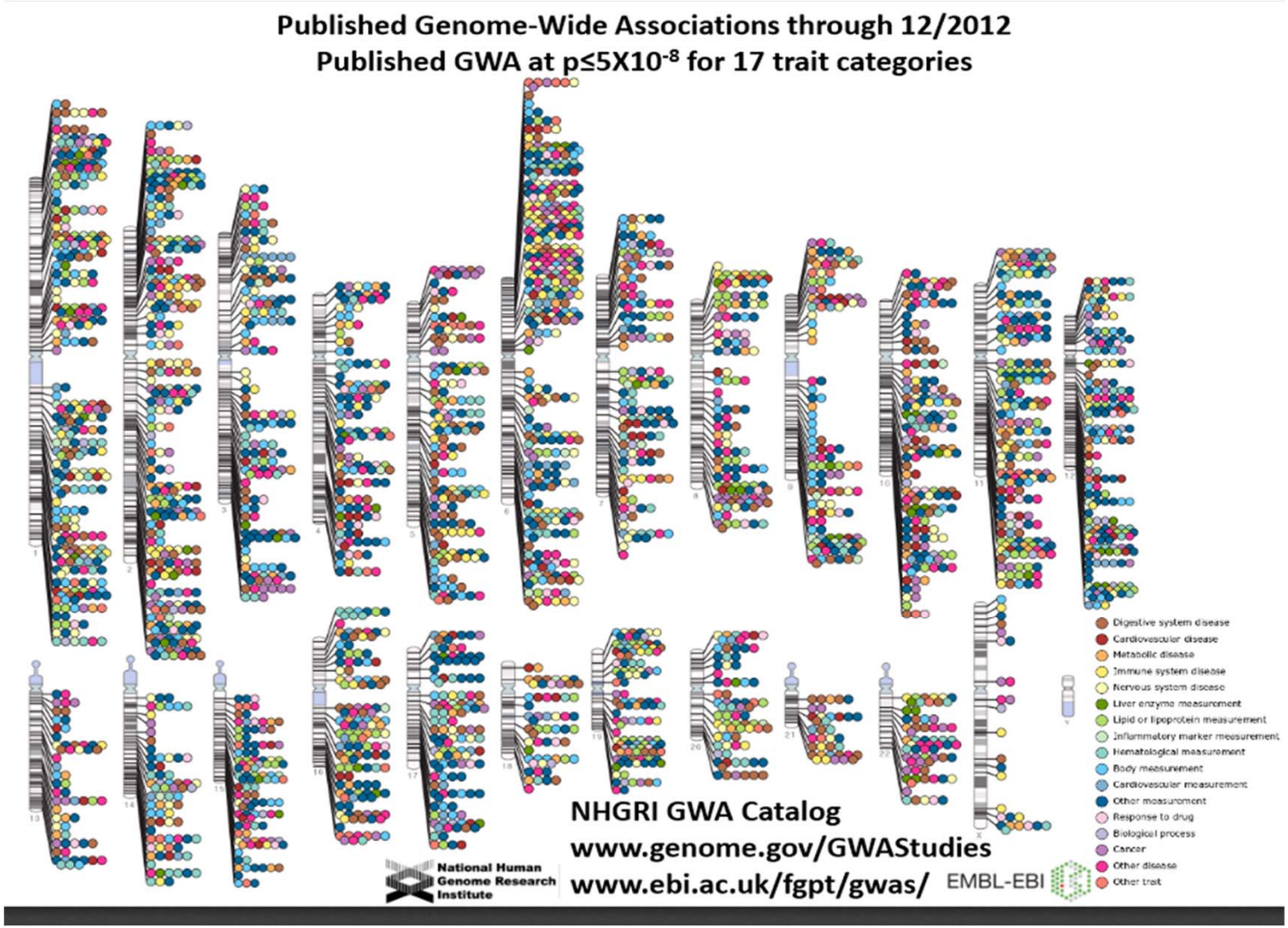
Linkage

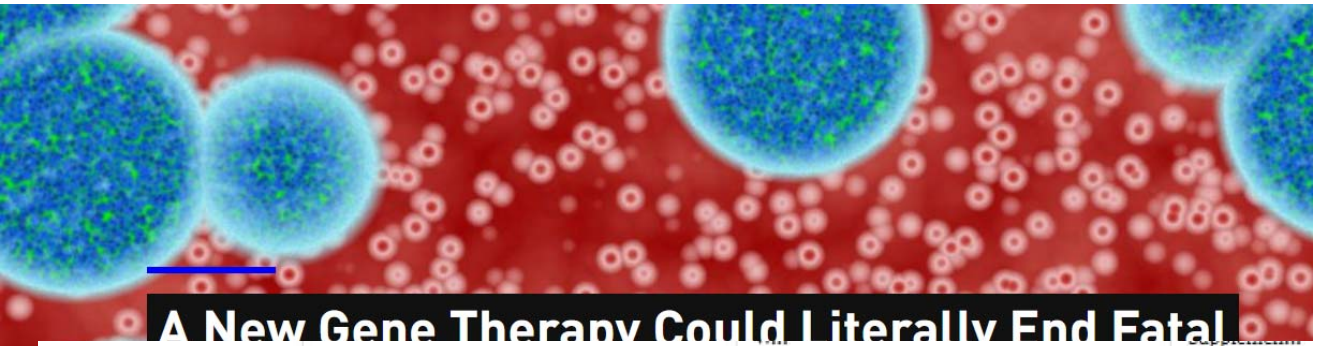
- The closer two places are on the chromosome, the more likely they will be inherited together.
- A recombination means that two places were NOT inherited together.
- The ratio of recombinations to non-recombinations is the recombination frequency


A
B
A
B

Genome Wide Association Study (GWAS)

- Look at hundreds of thousands to a million markers across the genome
- Because of linkage, there is more than 95% coverage of the genome depending on race/ethnicity
- Looks for association with disease (could be a risk or protective)
- If $p<0.05$ is considered statistically significant but we have a million markers, we would have 50,000 positive hits by chance alone
- Bonferroni correction of a million markers = 0.05/1 million= 5E-8 is 'genome wide significant'


Published Genome-Wide Associations through 12/2012
Published GWA at p≤5X10-8 for 17 trait categories
Digestive system disease
Cardiovascular disease
Metabolic disease
Immune system disease
Nervous system disease
Liver enzyme measurement
Lipid or lipoprotein measurement
Inflammatory marker measurement
Hematological measurement
Body measurement
Cardiovascular measurement
Other measurement
Response to drug
Biological process
Cancer
Other disease
Other trait
NHGRI GWA Catalog
www.genome.gov/GWAStudies
www.ebi.ac.uk/fgpt/gwas/
National Human Genome Research Institute
EMBL-EBI



A New Gene Therapy Could Literally End Fatal

ORIGINAL ARTICLE

Hematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy

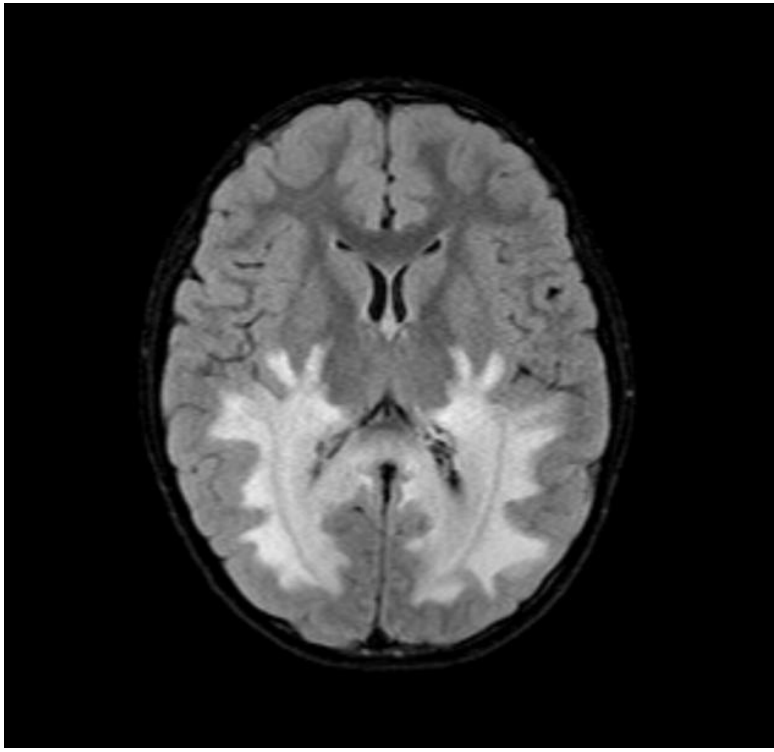
Florian Eichler, M.D., Christine Duncan, M.D., Patricia L. Musolino, M.D., Ph.D., Paul J. Orchard, M.D., Satiro De Oliveira, M.D., Adrian J. Thrasher, M.D., Myriam Armant, Ph.D., Colleen Dansereau, M.S.N., R.N., Troy C. Lund, M.D., Weston P. Miller, M.D., Gerald V. Raymond, M.D., Raman Sankar, M.D., et al.

October 26, 2017
N Engl J Med 2017; 377:1630-1638
DOI: 10.1056/NEJMoa1700554
Chinese Translation 中文翻译

Brandon Rojas, left, who has ALD, with his mother, Liliana, and father, Paul Rojas, in their home in Dover Plains, N.Y. His younger brother, Brian, received treatment in a clinical trial whose results show that gene therapy can stave off the disease with no side

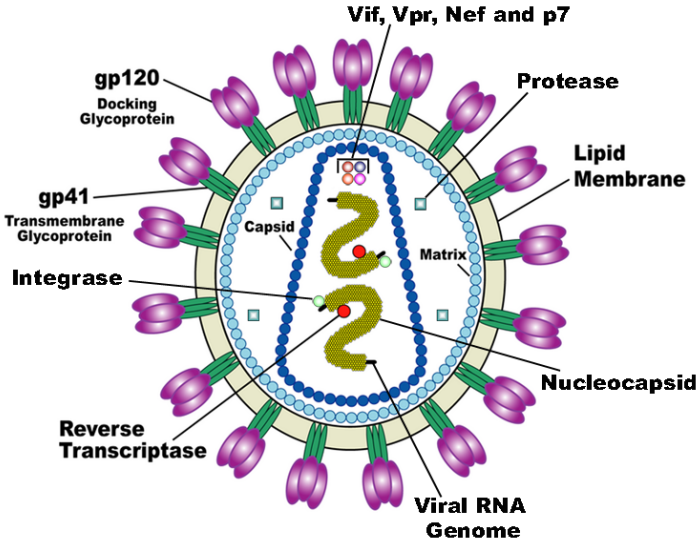
Lorenzo's Oil

- X-linked Adrenoleukodystrophy affects boys typically younger than 12 years of age; progressive white matter disease leading to dementia and eventually unresponsiveness;
- Universally patients die within a decade of diagnosis unless treated
- Transplant is the only effective therapy and must be performed at an early stage of degeneration
- ABCD1 Gene produces the Adrenoleukodystrophy protein (ALDP)
  - Spontaneous mutations
  - Over 500 different but private mutations identified
  - Perioxosomal ATP-binding cassette
  - Leads to accumulation of very long chain fatty acids



Lentiviruses

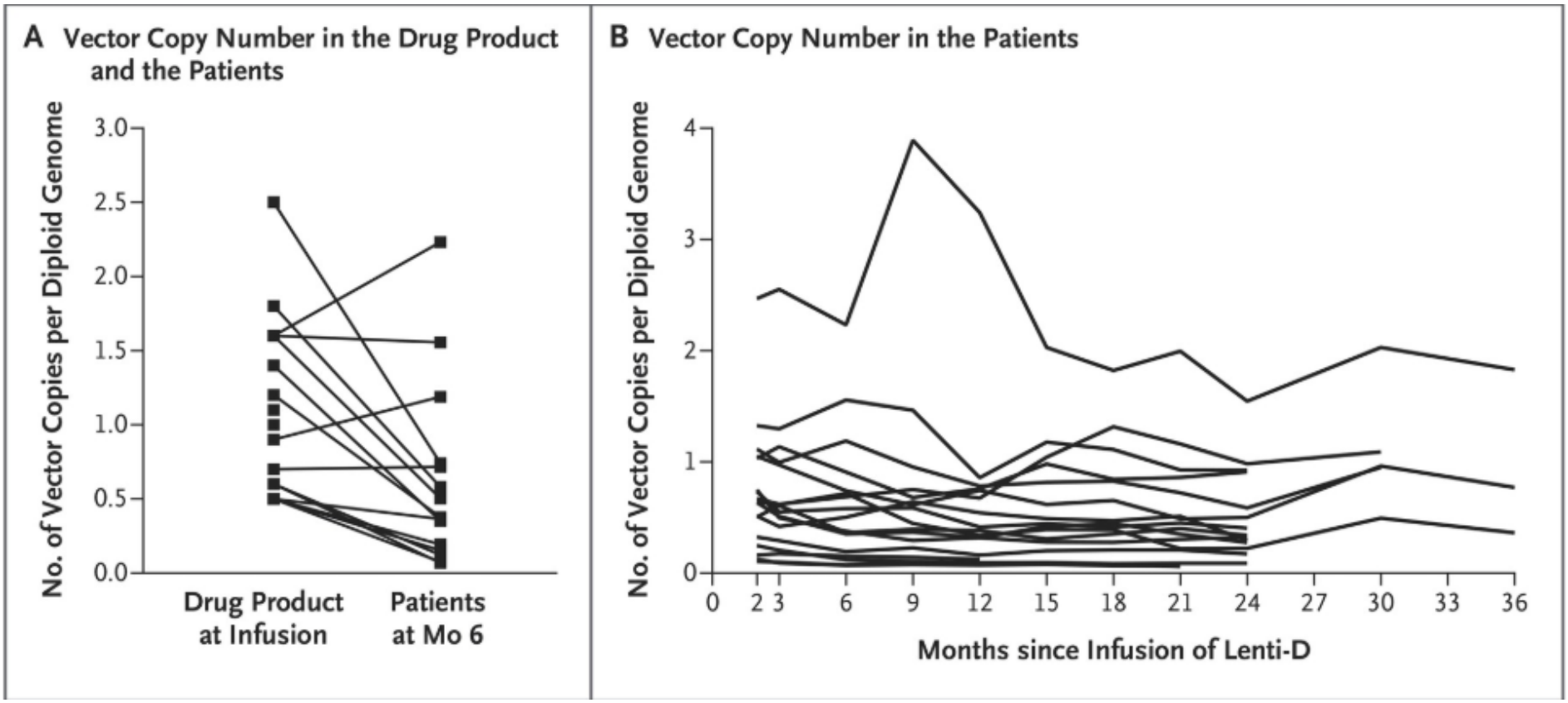
- HIV is the best known lentivirus
- CD4 protein is a receptor for the virus
- Retrovirus, RNA genome utilizing reverse transcriptase to make a complementary DNA copy
- Small viruses, only 5 major structural proteins and 3-4 non-structural proteins.



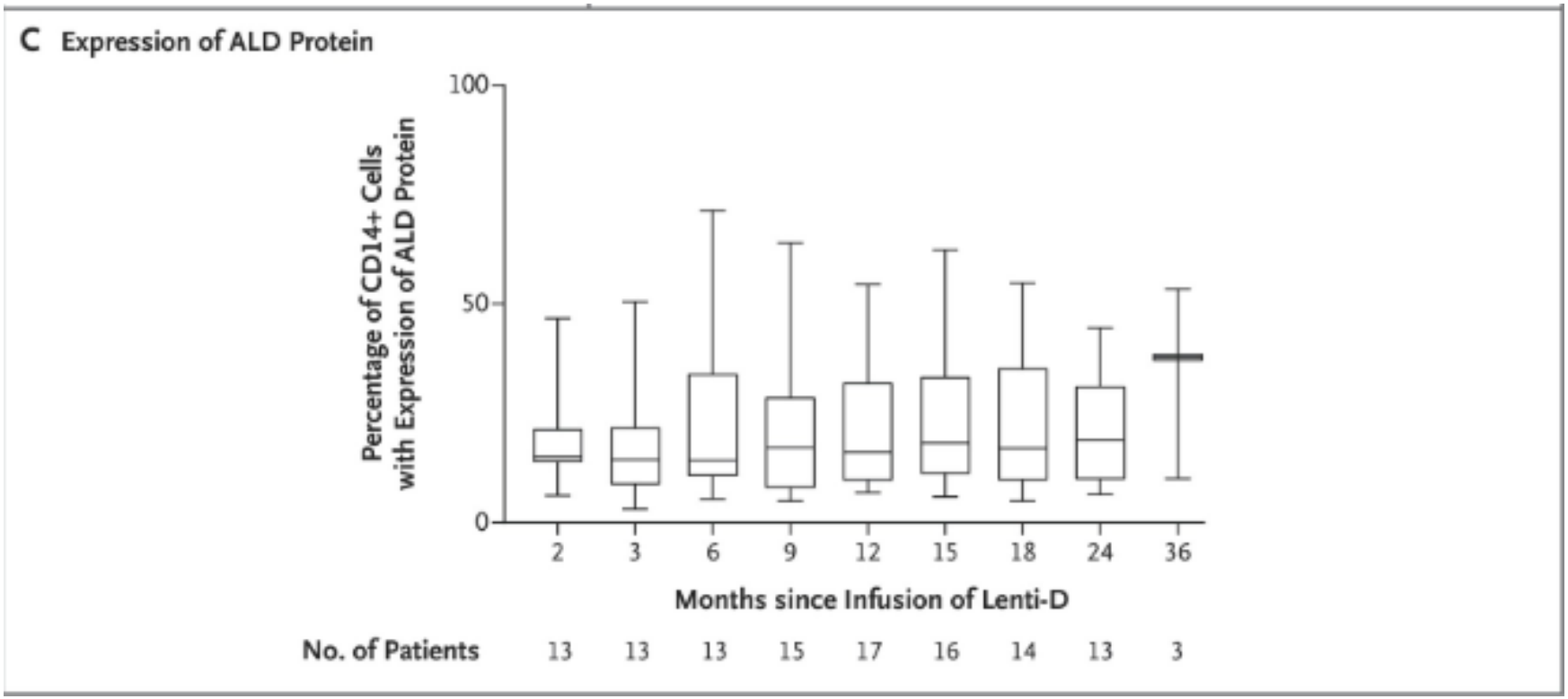
Gene Therapy

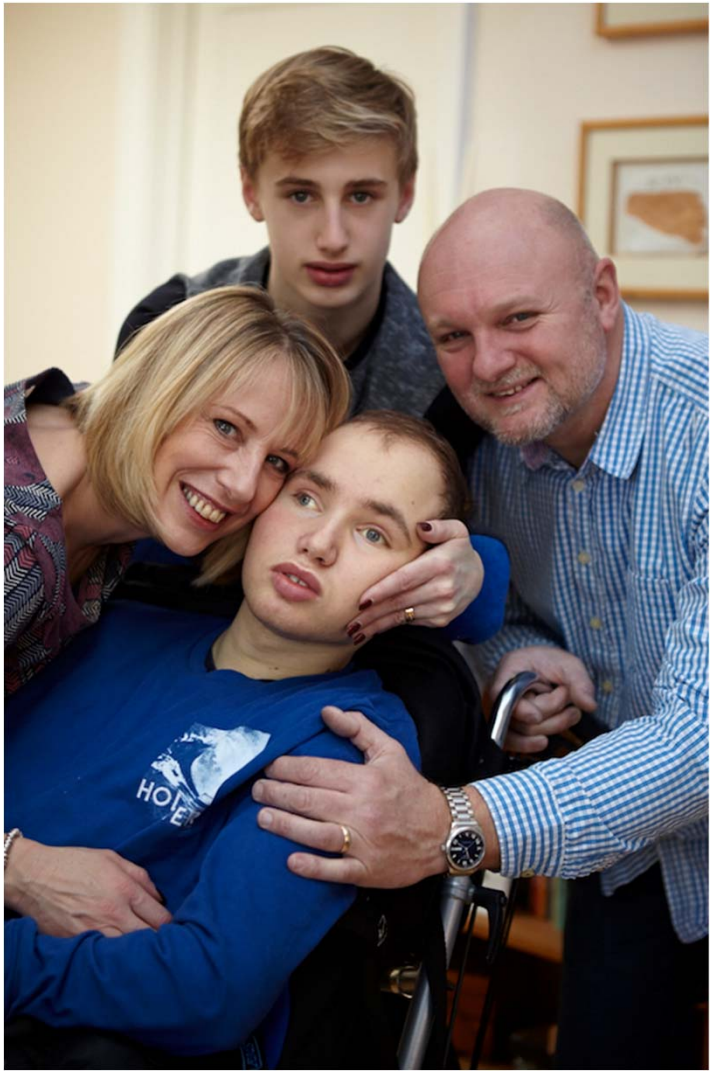
- The HIV virus infects primarily immune cells but can also penetrate into the brain and infect microglial cells within the brain
- ALD is largely an immune mediated disease with inflammation against myelin
- Lentivirus has a tiny single stranded RNA
  - If you used DNA, it'd be unstable as a single strand thus you'd need to have helicase, topoisomerase and then transcribe to RNA
  - By using a single strand of RNA with reverse transcriptase, it creates a complementary single stranded DNA which will then transcribe into RNA
  - Most of Lentivirus products are made to create more virus but you can limit how much virus is made by simply removing those DNA
- Used Apheresis to gather the patient's own immune cells and then to transfect with the lentivirus
- Then they undergo allegenic stem cell transplantation (wipe out the existing cells and transfect with these new ones)

Results


A Vector Copy Number in the Drug Product and the Patients
No. of Vector Copies per Diploid Genome
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Drug Product at Infusion
Patients at Mo 6
B Vector Copy Number in the Patients
No. of Vector Copies per Diploid Genome
4
3
2
1
0
0 2 3 6 9 12 15 18 21 24 27 30 33 36
Months since Infusion of Lenti-D

Results


C Expression of ALD Protein
Percentage of CD14+ Cells with Expression of ALD Protein
100
50
0
2
3
6
9
12
15
18
24
36
Months since Infusion of Lenti-D
No. of Patients
13
13
13
15
17
16
14
13
3



So…Can we do this again?

- An ideal scenario
  - Cells of interest were blood cells
  - Virus which targets these cells and is a reverse transcriptase
  - Herpes and adenovirus
  - Did not require removal of the abnormal DNA

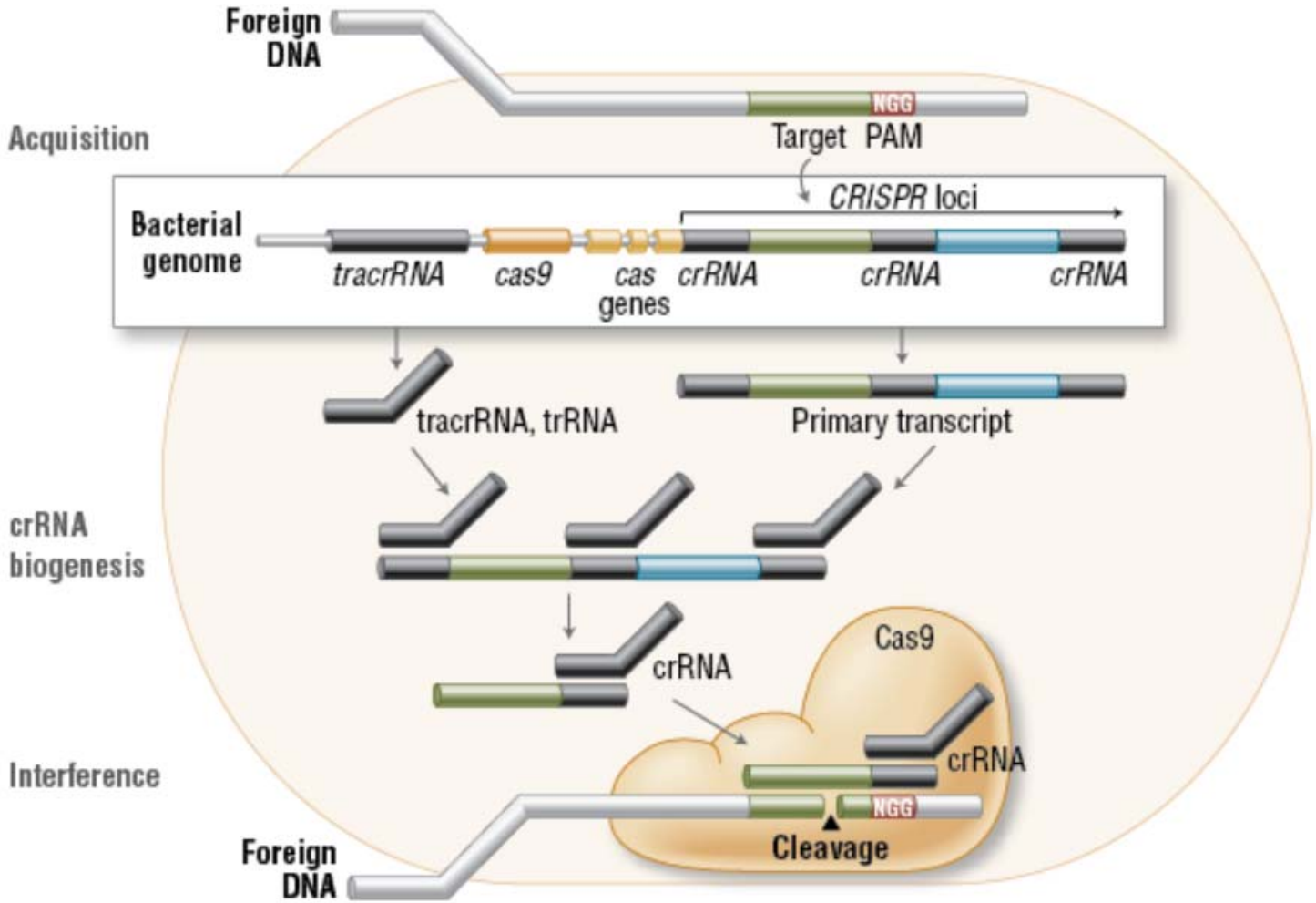
- Wouldn’t it be great if we could just send the right DNA to the right cells wherever we want to?....

Yogurt and Y

- *Streptococcus*
  produce yogur
  viral 'phages' v
  yogurt and che
- The resistant o
  worked better
  piece of each r

CRISPR/Cas9

- Clusters of Regularly Interspaced Short Palindromic Repeats (CRISPR)
- For every cell there are about 10 viruses; these viruses likely are predators but also facilitators of genetic exchange between species
- Bacteria use a system to remove offending viral DNA called CRISPR/Cas9; Each CRISPR is essentially a library of foreign viral DNA that it will identify. New spacers are added for each virus the bacteria is exposed to.
- It will transcribe the foreign DNA but shielded by the spacers and then the Cas9 recognizes the spacers and degrades any protein that matches it throughout the cell that has the spacers (including the offending viral DNA).


Foreign DNA
NGG
Target PAM
Acquisition
CRISPR loci
Bacterial genome
tracrRNA
cas9
cas genes
crRNA
crRNA
crRNA
tracrRNA, trRNA
Primary transcript
crRNA biogenesis
Cas9
crRNA
crRNA
Interference
NGG
Cleavage
Foreign DNA

How do we go from killing viruses to gene editing?

- Now imagine instead of viral DNA, we want to edit the genome in a very specific spot? We replace the viral phage DNA with the sequence that we want to replace.
- CRISPR RNA is then created which binds to our host DNA at the location of the mutation and then CAS9 cuts it right there and only there
- We also introduce the correct version and our own cellular repair mechanisms, seeing the cut, will then edit in the correct version.
- Three types of CRISPR systems, type II systems are the ones most commonly used for gene editing

Example

- CCTGAGGAGAAGGAAA

- We make a guide RNA to that DNA sequence: GGACTAATCTTCCTTT
- That RNA will only bind to the exact place in the genome where the mutation occurs.
- Once it binds, then CAS9 sees a double stranded DNA with the target sequence and cuts it out.
- Introduce a normal DNA sequence: CCTGAGAAGAAGGAAA
- And our body's own repair mechanism transcribes the piece

2012, Edit any DS-DNA with CRISPR

Science Home News

CURIOUS2018 FUTURE INSIGHT CONFERENCE

TECHNOLO BREAKTHR OF THE YE COMPELLING DRIVEN BY CU

SHARE

RESEARCH ARTICLE

A Programmable in Adaptive Bact

Martin Jinek[1,2,*], Krzysztof Chylinski

+ See all authors and affiliations

Science 17 Aug 2012:
Vol. 337, Issue 6096, pp. 816-821
DOI: 10.1126/science.1225829

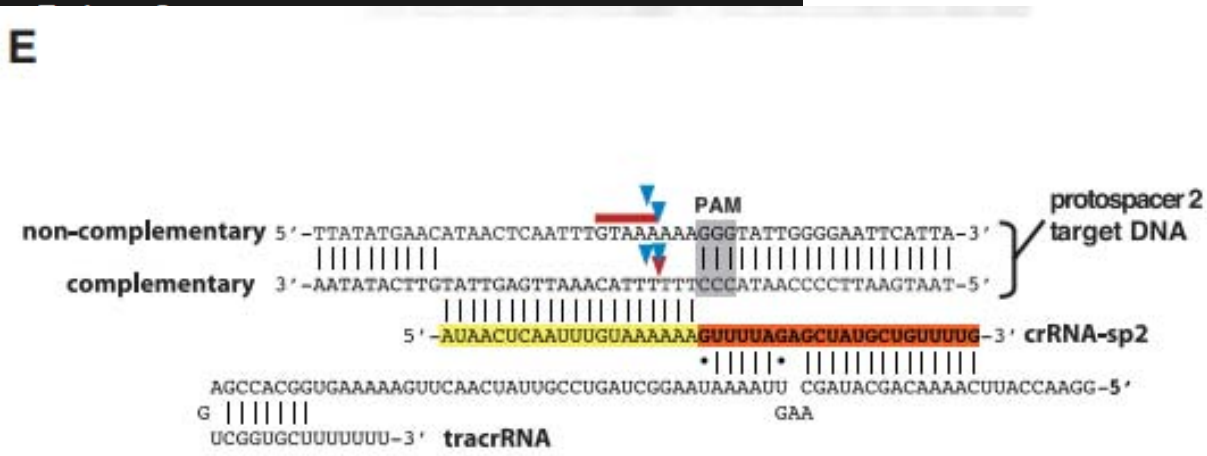


Fig. 1. Cas9 is a DNA endonuclease guided by two RNA molecules. (**A**) Cas9 was programmed with a 42-nucleotide crRNA-sp2 (crRNA containing a spacer 2 sequence) in the presence or absence of 75-nucleotide tracrRNA. The complex was added to circular or XhoI-linearized plasmid DNA bearing a sequence complementary to spacer 2 and a functional PAM. crRNA-sp1, specificity control; M, DNA marker; kbp, kilo–base pair. See fig. S3A. (**B**) Cas9 was programmed with crRNA-sp2 and tracrRNA

2015 - Shortening Trinucleotide Repeats

- Fragile X syndrome – FMR1 trinucleotide repeats
- Using CRISPR to target and snip out sections of repeats in specific genes (repeats occur all over the DNA)
- Ultimately removed 200 CGG repeats!

In 2017, Edit Muscle Cells

- CRISPR insertion using adeno-associated virus to put a normal dystrophin gene into muscle cells with Duchenne's in a mouse model and muscle stem cells

In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar[1,2,*], Kexian Zhu[1,3,*], Jason K. W. Cheng[1], Wei Leong Chew[2,4], Jeffrey J. Widrick[5], Winston X. …
+ See all authors and affiliations

Science 22 Jan 2016:
Vol. 351, Issue 6271, pp. 407-411
DOI: 10.1126/science.aad5177

Article | Figures & Data | Info & Metrics | eLetters | PDF

You are currently viewing the abstract. **View Full Text**

Editing can help build stronger muscles

Much of the controversy surrounding the gene-editing technology called CRISPR/Cas9 centers on the ethics of germline editing of human embryos to correct disease-causing mutations. For certain disorders such as muscular dystrophy, it may be possible to achieve therapeutic benefit by editing the faulty gene in somatic cells. In proof-of-concept studies, Long *et al.*, Nelson *et al.*, and Tabebordbar *et al.* used adeno-associated virus-9 to deliver the CRISPR/Cas9 gene-editing system to young mice with a mutation in the gene coding for dystrophin, a muscle protein deficient in patients with Duchenne muscular dystrophy. Gene editing partially restored dystrophin protein expression in skeletal and cardiac muscle and improved skeletal muscle function.

Science, this issue p. **400**, p. **403**, p. **407**

CRISPR Convert ApoE4 to E3

- ApoE4 is the most prevalent genetic risk factor for late onset Alzheimer's disease. It has putative roles in amyloid beta-42 secretion as well as uptake of amyloid beta.
- On May 31, 2018 in *Neuron*, Tsai et al demonstrated using CRISPR/CAS9, the ability to convert ApoE3 cells into ApoE4 cells to determine the activity of the change
- Yet they also were able to CRISPR/CAS9 ApoE4 cells back to ApoE3 cells! Doing so attenuated the multiple Alzheimer's disease pathologies
  - Using ApoE4/E4 cells from an Alzheimer's patient, they used CRISPR/Cas9 Approach to replace the aberrant sequence variant back to ApoE3.
  - This led to enhanced ability of microglia to take-up amyloid beta-42

F
Aβ42-555
Hoechst / Iba1 / Aβ42-555
Parental line (APOE4)
Isogenic line (APOE3)
Cellular Aβ intensity (normalized to APOE4)
*
0
2
4
6
8
APOE4
APOE3
G
Hoechst / Aβ / MAP2
Parental line (APOE4)
Isogenic line (APOE3)
Number of Aβ puncta (a.u.)
***
0
200
400
600
800
1000
APOE4
APOE3

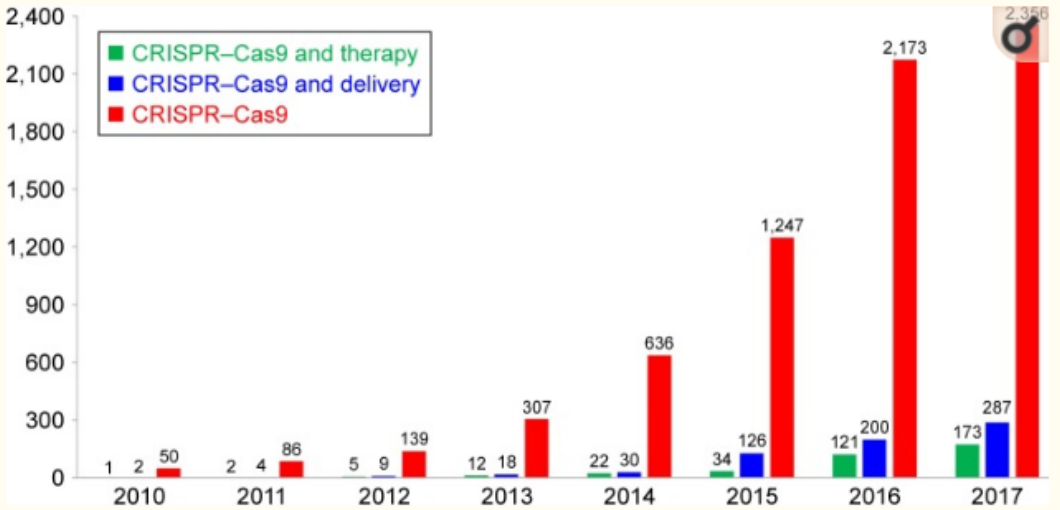


Figure 2

Articles were searched with NCBI Global Cross-database, including PubMed, PMC, Gene, PubChem, UpToDate, among others, as well as Google Scholar by using "CRISPR–Cas9", "CRISPR–Cas9 and therapy", and "CRISPR–Cas9 and delivery" in title/abstract for each year.

Abbreviations: Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats.

Layla and Leukemia

- A one year old with leukemia refractory to all other treatments
- Extracted t-cells from a healthy donor and deactivated immune genes that would cause the donor cells to attack when injected into a leukemia patient.
- Used as a bridge until a matched donor could be found

Leukaemia success heralds wave of gene-editing therapies

One-year-old girl treated as plans to inject DNA-cutting technology directly into patients' bodies take shape.

Sara Reardon

05 November 2015 | Updated: 06 November 2015, 10 November 2015

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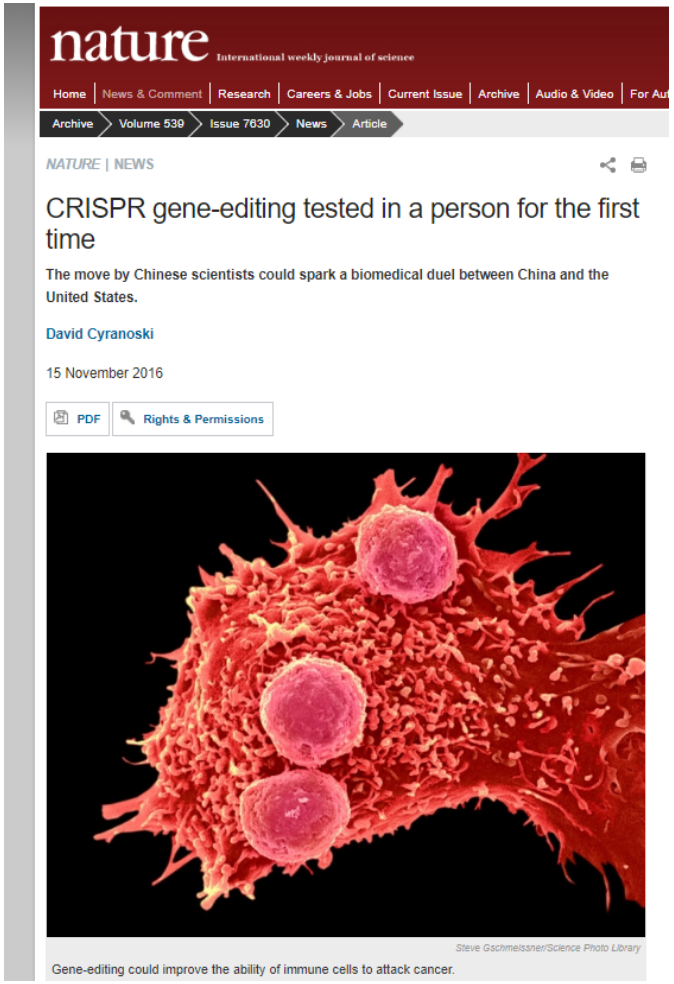
Sharon Lees/ Great Ormond Street Hospital

Layla received gene-edited immune cells from a healthy donor.

2016 - Nature

- Removed immune cells from recipients blood and disabled a gene using CRISPR-Cas9 for PD-1 which stops a body's immune response to cancer
- Cultured the edited cells and reinfused back into the patient.



Trial in Humans?

- NIH sponsored trial for CPD-1 knockout engineered T cells for metastatic non-small cell lung cancer
- 15 participants, August 2016 start
- Peripheral blood lymphocytes will be collected and programmed with a cell death protein specific to the lung cancer cells and then infused back into the body.

July 11, 2018 - Nature

- Elec
- Usir
  mo
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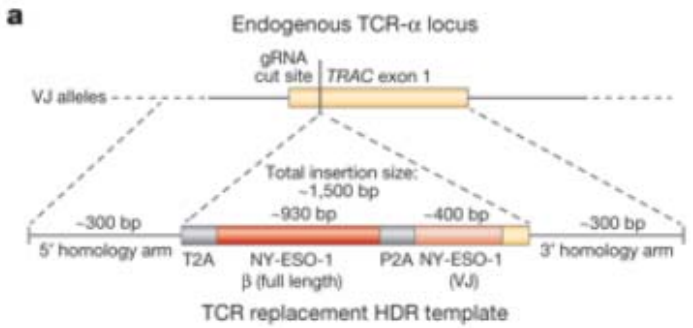
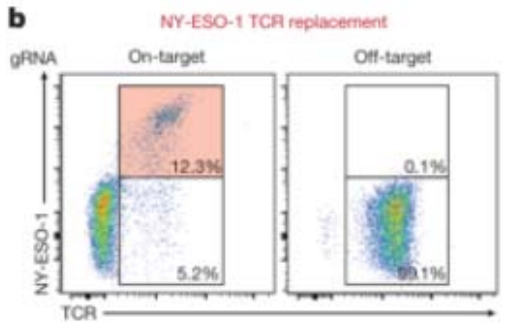
  - F

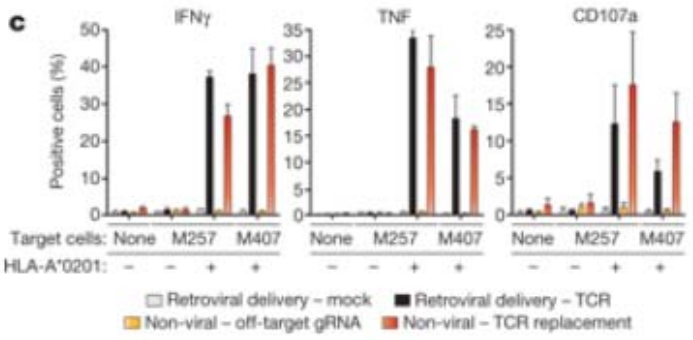
g mechanism

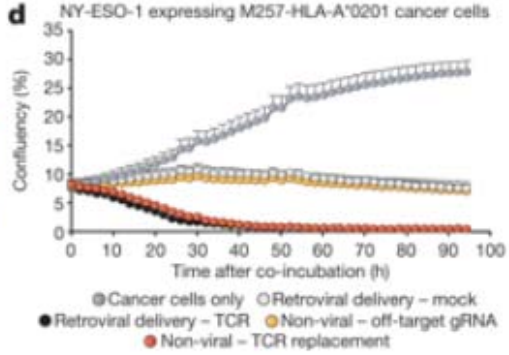
ignant melanoma

Fig. 4: Replacement of the endogenous TCR by non-viral genome targeting.









The ‘Oops’ Factor

- Unfortunately, the CRI
  ‘accidental’ editing of
  documented phenome
- Classically a 20 nucleo
  genome and is the bas
  genotyping technique
- However, CRISPR/Cas9
  because it’s not quite
  some substitution

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doi: 10.1093/nar/gku402

CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences

Yanni Lin[1], Thomas J. Cradick[1], Matthew T. Brown[1], Harshavardhan Deshmukh[1], Piyush Ranjan[2], Neha Sarode[2], Brian M. Wile[1], Paula M. Vertino[3], Frank J. Stewart[2] and Gang Bao[1,*]

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RNA Interference

- RNAi refers to a complementary strand to the mRNA of a disease causing gene. It binds to the mRNA preventing or lowering the ‘dose’ of the protein in question. It can be specific to mRNA that is ‘bad’ while leaving normal mRNA untreated.

- With RNAi therapeutics,
  - very little pharmacologic ‘side effects’,
  - no metabolism issues as the RNAi is metabolized using normal cellular mechanisms
  - Very specific gene targeted therapy

RNA Interference in Lung Transplant Patients

- 2012 - In lung transplant patients, lower respiratory tract infections due to respiratory syncytial virus (RSV) leads to marked morbidity
- Randomized double blind placebo controlled trial to receive standard of care or aerosolized small RNA inhibitor to a nucleocapsid gene critical to RSV replication.
- The study rec
from 50% to

RNA Interference Therapy in Lung Transplant Patients Infected with Respiratory Syncytial Virus

Martin R. Zamora[1], Marie Budev[2*], Mark Rolfe[3], Jens Gottlieb[4], Atul Humar[5], John DeVincenzo[6], Akshay Vaishnaw[7], Jeffrey Cehelsky[7], Gary Albert[7], Sara Nochur[7], Jared A. Gollob[7], and Allan R. Glanville[8]

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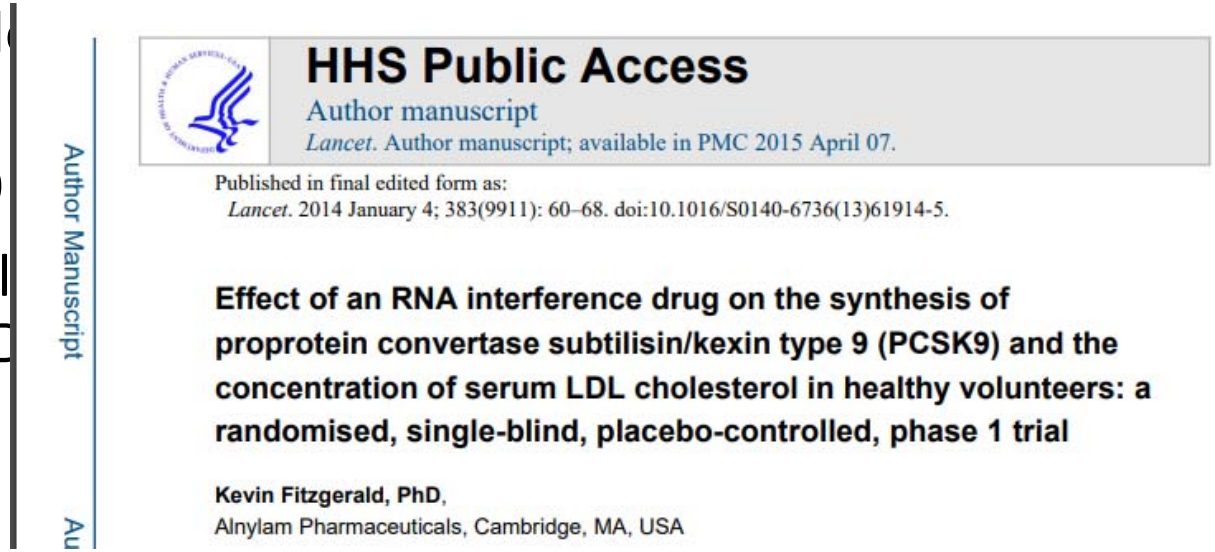
PCSK9

- Proprotein convertase subtilism/kexin type 9 or PCSK9 – Gain of function mutation in *PCSK9* in familial hypocholesterolemia. It binds to hepatocyte LDL receptors leading to degradation and loss of function mutations l
healthy.
- Antibodies to PCSK9
- In 2014, the RNAi pil
markedly lowered LD



Published in final edited form as:
Lancet. 2014 January 4; 383(9911): 60–68. doi:10.1016/S0140-6736(13)61914-5.

Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial

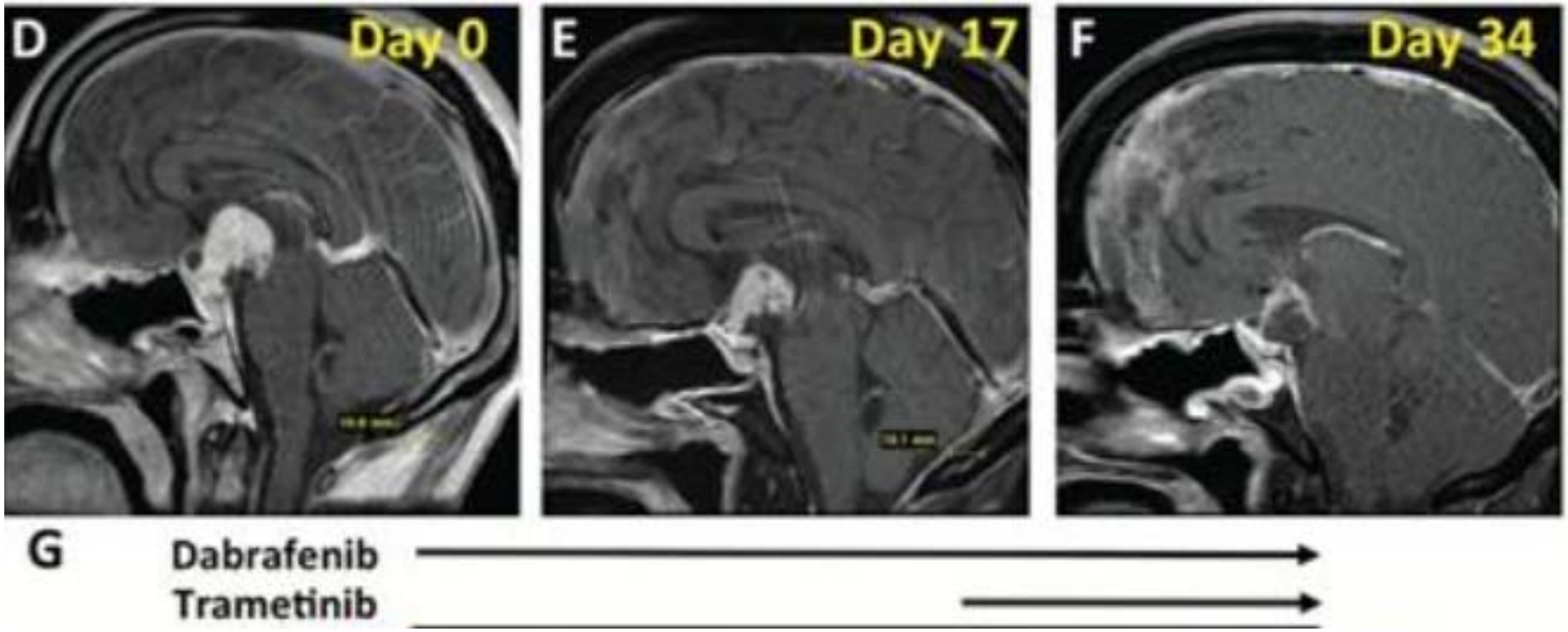
Kevin Fitzgerald, PhD,
Alnylam Pharmaceuticals, Cambridge, MA, USA

Inhibitor RNA Therapy

- Viral therapy – hepatitis B cure? Influenza,
- Cancer treatment – Targeted cancer therapy
- Treatment of heart failure
- Patent to use for treating Alzheimer's disease by silencing presenilin 1, 2 and beta secretase
- Blocking oncogenes before someone gets cancer

Pharmacologic libraries

- Many failed drugs for one indication may affect many other indications
- 39m presents with craniopharyngioma requiring emergent decompression; re-presented 7 months later requiring urgent decompression, again at 8.5 months and 9 months and 9.25 months each time requiring resection.
- Investigators had recently reported in 2016 that BRAFV600E alterations were the cause of nearly all papillary craniopharyngiomas. And, there was a BRAF inhibitor already being used in melanoma, colon and other cancers with moderate success
- So, they gave it to the patient!


D
Day 0
E
Day 17
F
Day 34
G
Dabrafenib
Trametinib

- Now in phase 2 trials for all craniopharyngiomas
- Genome sequencing is now possible for only $1000
- An evaluation of brain mets from lung and breast cancer found that 53% of these mets had therapeutic targets already developed for other diseases perhaps suggesting sensitivity to them!

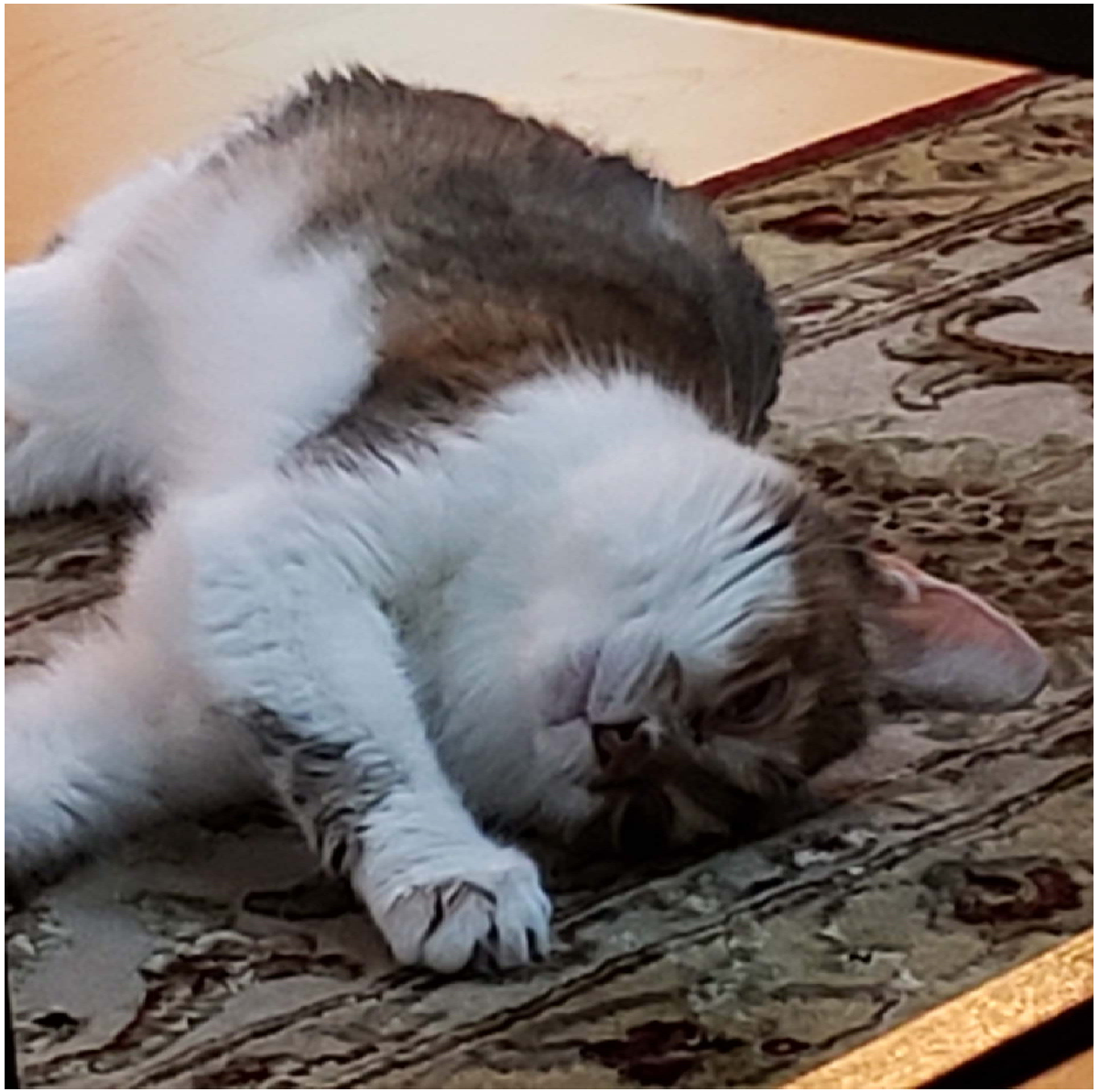
Therapy Summary

- Gene therapy may not work for every disease
  - Viral therapies must infect the correct cell type
  - CRISPR can make errors occur and best in germline
  - RNAi must be able to get to diseased regions
- But gene therapy is going to break out in the next 10 years
  - Both in identifying already existing agents that may work on target diseases, reprogramming cells and redirecting the immune system

A new future in treatment?

- Geneticists understand genetics well
- Neuroscientists understand our diseases well
- We need scientists that can bridge the gap between neurosciences and genetics to rapidly translate these advances into therapies

Genetics has reached a new tipping point and is about to break out!



Thanks!