Gene therapy: Where are we now?

Graeme Black
Manchester
Genomics England, with the consent of participants and the support of the public, is creating a lasting legacy for patients, the NHS and the UK economy through the sequencing of 100,000 genomes: the 100,000 Genomes Project.

Genomics England was set up by the Department of Health to deliver the 100,000 Genomes Project. Initially the focus will be on rare disease, cancer and infectious disease.

Read more...
Can you find my faulty gene?

Inherited retinal disease

• 1 in 3,000
• All are genetic
• Dominant, recessive, X-linked
• Some associated with health complications
• 250+ different forms
• Clinically indistinguishable....
Gene therapy

‘Introduction, using vector, of nucleic acids into cells with the intention of altering gene expression to prevent, halt or reverse a pathological process’.

- Gene addition
- Gene knockdown
- Gene correction/alteration

Kay, 2011 Nature Reviews Genetics 12, 316-328
‘challenge areas’ – with considerable species-specificity
Successful and potential gene therapy targets

• The vectors can be administered *in vivo* or *ex vivo* (ie using autologous cells derived from individual patient).

• *Ex vivo*
  – Immunodeficiency (SCID) – *ex vivo*
  – Mucopolysaccharidosis – *ex vivo*

• *In vivo*
  – Retinal disease – *Subretinal injection*
  – Cardiac and Muscle disease – *in vivo* approaches

• Therapeutic DNA either integrates into host chromosomal DNA or exists as separate vector.
Adeno-associated viruses

- Small, single-stranded, nonpathogenic DNA viruses
  - require helper virus for replication and completion of their life cycle.
- Comprise 2 genes: *rep* (viral DNA replication) and *cap* (packages viral genome). Therapeutic expression cassette replaces both, leaving only viral inverted terminal repeats.
- Easily purified to high titres
- Transduce dividing & non-dividing cells.
- Insert size is restricted (to just over 4 kb).
- Prototype AAV2 vector based on a human-derived virus
  - Can be pseudotyped with various AAV capsids to alter specificity and transduction efficacy.
  - Not always concordance between *in vitro* and *in vivo* transduction
  - Improved vectors have been obtained by experimentally modifying capsid sequence
- Long-term risk for tumorigenesis in humans not known
Gene therapy for inherited eye disease

Loss of photoreceptor protein, peripherin

- No discs
- No outer segments

Retina has no electrical activity

Lose photoreceptors
Comparison of wildtype and *blind* mouse

+/-

No rho
Subretinal injection of virus encoding missing gene

Detection of protein (peripherin) 3 wks after injection

Detected only in upper half of retina – outer segments

CONFIRMS Tissue specific expression
Structural alteration confirmed

Electrical activity of retina confirms functional effect
Boy condemned to blindness has sight restored with gene therapy - giving hope to thousands with failing eyesight

By DANIEL BATES
Last updated at 08:28 28 April 2008

A team at London's Moorfields Eye Hospital has made the world's first attempt to treat a sight disorder using gene therapy.

They operated on Robert Johnson, a UK man born with a sight disorder which deteriorates with age.

At present Mr Johnson, who had genes inserted into his own cells, can see cutlery during the day, but little at night.

It will be several months before the researchers know whether their work has been a success.

If it is, they believe that it could be used to treat a range of inherited sight disorders.

How the gene therapy works

Mr Johnson's disorder is caused by a faulty gene in his eye.

This defect stops the layer of cells in the retina at the back of the eye from working.

 Usually, these are cells that detect light, but in Mr Johnson's case, they cannot.

The surgery required precision.
Vitamin A processing

Regeneration of light-sensing molecule or chromophore

Light cycle
Inherited retinal disease: a paradigm for gene-directed therapy?

Acland et al. (2001) Nat Genet

Bainbridge et al. (2008) NEJM
Vision not maintained long-term

Deteriorate after 6-12 months

Species differences between human and dog

Ali et al 2015; NEJM
Boy condemned to blindness has sight restored with gene therapy - giving hope to thousands with failing eyesight

By DANIEL BATES

Last updated at 08:28 28 April 2008
Choroideremia

X-linked

Progressive choroidal & retinal loss

No mouse model

Phase 1 clinical trial of retinal gene therapy for choroideraemia using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)

(McLaren, Oxford)
From a personal point of view, after about 3 weeks, I noticed an improvement in my colour perception, with a marked difference between the left (treated) and right eye. Looking through the right is like looking through a dirty spectacle lens compared to crystal clear vision through the left. I can't see more through the left, but I can see better.

The best moment came a couple of weeks ago when I was able to look up at the night sky and see stars for the first time in a long long time. It might not seem that earth-shattering, but it is something that I have missed dearly over recent years, and to be able to make them out again was wonderful.

I also seem to have a better tolerance to bright and low light, to the extent that the family complained they are living in a bat cave because I don't turn lights on if at all possible, whereas before it was necessary. I guess they have got used to having the lights on all the time.

My only hope now is that this continues and stays stable for the future.

---

**Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial**

Robert E MacLaren, Markus Groppe, Alun R Barnard, Charles L Cottrill, Tanya Tolmachova, Len Seymour, K Reed Clark, Matthew J During, Frans P M Cremers, Graeme C M Black, Andrew J Lotery, Susan M Downes, Andrew R Webster, Miguel C Seabra

**Summary**

**Background** Choroideremia is an X-linked recessive disease that leads to blindness due to mutations in the CHM gene, which encodes the Rab escort protein 1 (REP1). We assessed the effects of retinal gene therapy with an adeno-associated viral (AAV) vector encoding REP1 (AAV.REP1) in patients with this disease.
From a personal point of view, after about 3 weeks, I noticed an improvement in my colour perception, with a marked difference between the left (treated) and right eye. Looking through the right is like looking through a dirty spectacle lens compared to crystal clear vision through the left. I can't see more through the left, but I can see better.

The best moment came a couple of weeks ago when I was able to look up at the night sky and see stars for the first time in a long long time. It might not seem that earth-shattering, but it is something that I have missed dearly over recent years, and to be able to make them out again was wonderful.

I also seem to have a better tolerance to bright and low light, to the extent that the family complained they are living in a bat cave because I don't turn lights on if at all possible, whereas before it was necessary. I guess they have got used to having the lights on all the time.

My only hope now is that this continues and stays stable for the future.
Gene therapy

‘Introduction, using vector, of nucleic acids into cells with the intention of altering gene expression to prevent, halt or reverse a pathological process’.

- Gene addition
- Gene knockdown
- Gene correction/alteration

Kay, 2011 Nature Reviews Genetics 12, 316-328
Therapy
Mutation-specific siRNA for
*In vivo* therapy
Mutant-specific siRNA reverses cellular defect

K6a-wt/Tomato + K6a-mut/YFP

K6a-wt/Tomato + K6a-mut/YFP + TD101 siRNA
siRNA allele-specific screening using luciferase reporter assays

- Cloned WT and mutant CD gene into luciferase vector
- Using ENGENIS siRNA test system (Yorkshire Bioscience)
  - Compare wildtype and mutant reporter alleles with set of 19 allele-specific siRNAs
  - Use Renilla luciferase vector as an internal control for transfection efficiency.
- Design 19 mer (+2 nt overhang) siRNA duplexes to the target mRNA sequence incorporating the mutation at position 1 to 19 in the siRNA.
Screening for effective N171K siRNA inhibitors

K6A WT    GTGAACAGATCAAGACCCTCAACAACAAAGGTTTGCCTCCTTC
K6A N171K GTGAACAGATCAAGACCCTCAAAACAAAGGTTTGCCTCCTTC

Inhibitors:
K6a_513.1     ACAGAUCAAGACCCUCAAauu
K6a_513.2      CAGAUCAAGACCCUCAAaAuu
K6a_513.3       AGAUCAAGACCCUCAAaAAuu
K6a_513.4        GAUCAAGACCCUCAAaAACuu
K6a_513.5         AUCAAGACCCUCAAaAACAuu
K6a_513.6          UCAAGACCCUCAAaAACAAGuu
K6a_513.7           CAAGACCCUCAAaAACAAGUuu
K6a_513.8             AGACCCUCAAaAACAAGUUuu
K6a_513.9              GACCCUCAAaAACAAGUUUuu
K6a_513.10              ACCCUCUCAAaAACAAGUUUGuu
K6a_513.11               CCCUCAAaAACAAGUUUGCcuu
K6a_513.12                CCUCAAaAACAAGUUUGCCuu
K6a_513.13                   CUCAAaAACAAGUUUGCCUuu
K6a_513.14                      UCAAAaAACAAGUUUGCCUCuu
K6a_513.15                        CAAaAACAAGUUUGCCUCUuu
K6a_513.16                              AAaAACAAGUUUGCCUCCUuu
K6a_513.17                                           AaAACAAGUUUGCCUCCUUuu
K6a_513.18                                                  aAACAAGUUUGCCUCCUUCuu

Hickerson et al., J. Invest. Derm. 2008
Epidermolysis bullosa K6 Reporter gene (wild-type and N171K mutant)

- siRNA inhibitors

\[ \text{siRNA inhibitors} \rightarrow \text{CMV} \rightarrow \text{K6a} \rightarrow \text{YFP} \]

- CD luciferase vector, lipofectamine 2000, AD293 cells.
- Assays performed in triplicate.
- Data presented as % luciferase activity.
K6a/YFP reporter (WT and N171K)

Hickerson et al., J. Invest. Derm. 2008
K12-Luciferase siRNA reporter assay

CMV promoter

Luciferase → K12 cDNA

+/- Mutation

Figure 1: Screening of mutation K12 L132P specific siRNAs
Allele-Specific siRNA Silencing for the Common Keratin 12 Founder Mutation in Meesmann Epithelial Corneal Dystrophy

Edwin H. A. Allen,¹⁻³ Sarab D. Atkinson,¹⁻³ Haibui Liao,² Jonathan E. Moore,¹ Deena M. Leslie Pedrioli,² Frances J. D. Smith,² William H. Irwin McLean,² and C. B. Tara Moore¹,²

![Graph and images showing experimental results with untreated and transfected samples.](image-url)
Mutation-specific K6a siRNAs strongly inhibit reporter gene expression

Red represents highest luciferase expression
Purple represents lowest luciferase expression

Leachman et al., “First-in-human mutation-targeted siRNA treatment of an inherited skin disorder”
Gene therapy

‘Introduction, using vector, of nucleic acids into cells with the intention of altering gene expression to prevent, halt or reverse a pathological process’.

• Gene addition
• Gene knockdown
• Gene correction/alteration

Kay, 2011 Nature Reviews Genetics 12, 316-328