Walking the walk:

Renal genetic evolutionary stories

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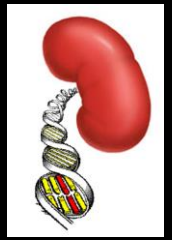
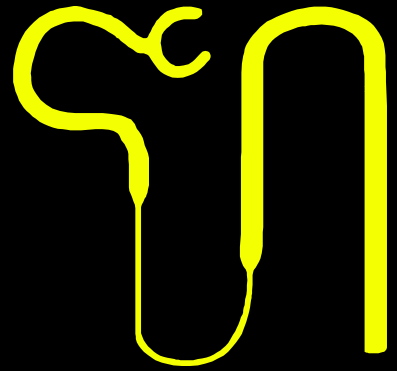
University of Cambridge

CIMR

UNIVERSITY OF CAMBRIDGE



Declaration: founding director, Kalium Health Ltd



CKD
BP and/or electrolyte disorders
Acidosis/alkalosis
Stones
Proteinuria

Anaemia
Bones
Accelerated vascular disease
Multiorgan disease

Renal function:
- A million little sieves
- Multiple functions
- Specialised cell types

Depends on normal
- Development and structure
- Immunology

d. 1884	Mendel	(of Bright's disease)
1871	DNA	Miescher
1878	Chromosomes	Flemming
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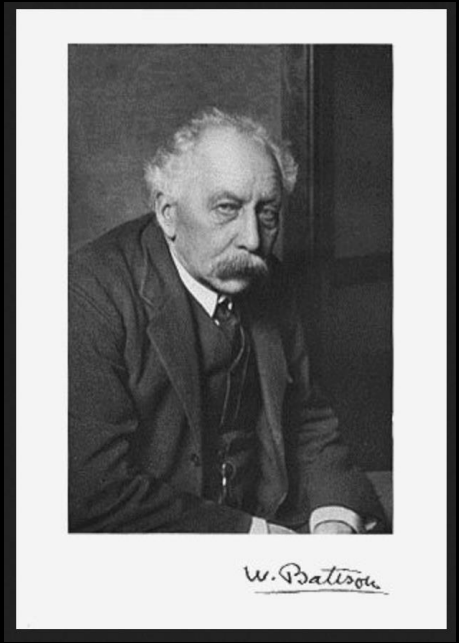
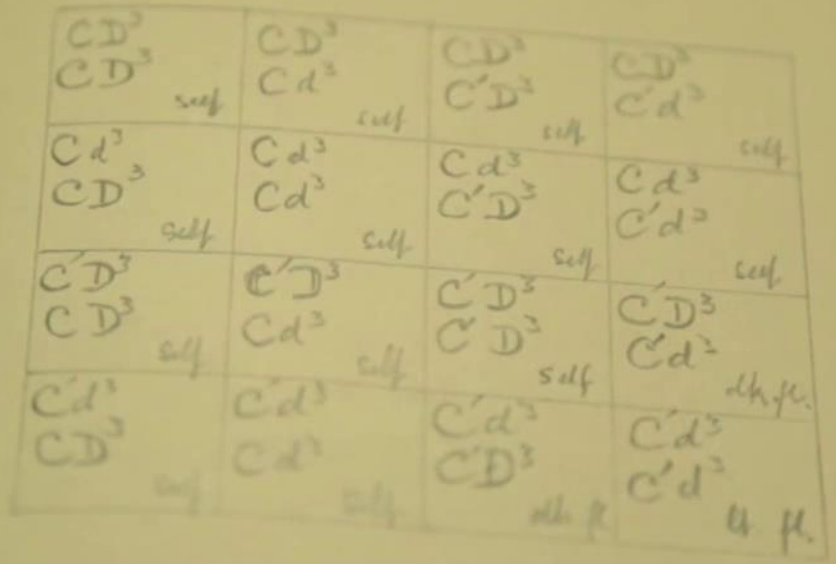
Early 1980s	PCR (Kary Mullis)	and chromosome walking

William Bateson, FRS (1861-1926) – arguably the father of genetics



with Reginald Punnett

Mainly worked on pigeons (cf. Darwin) and brassicas
Showed that traits remained true over generations (ie biometricians were wrong)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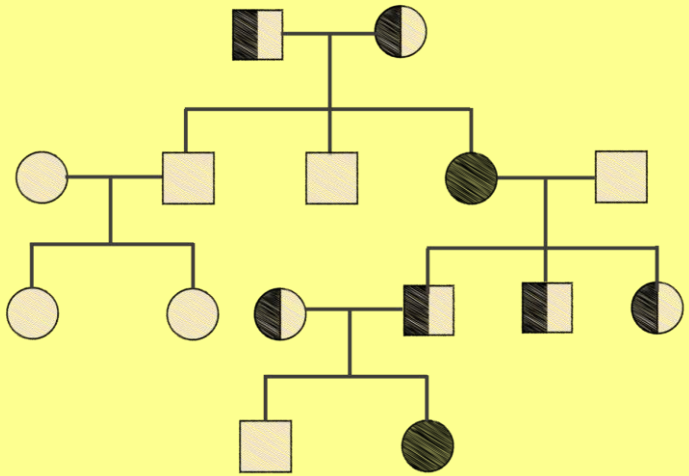
Edith Saunders

Worked with Bateson and Punnett

Mainly on sweet peas, and showed independently the same results obtained by Mendel (of whom no-one in England had heard!)

This work unified the laws of inheritance across animals and plants

The first suggestion explaining recessive conditions in man



(In reference to traits skipping a generation)

"That marked individual peculiarities fail to appear in the immediate offspring, but may reappear in a subsequent generation, has been often observed, and the fact has taken a great hold on the popular imagination. ... we have now in the phenomenon of dominance a fact which may possibly be a real element in the causation of such appearances, and those who are familiar with statistics of inheritance, in man for example, might usefully study them with the possibility in view."

Saunders, E. R. (1902) "The facts of heredity in the light of Mendel's discovery." Reports to the Evolution Committee of the Royal Society, I. pp. 125–160

Bateson coined the term 'genetics', and…

epistasis
homozygous
allele
heterozygous
dominance

Bateson, Saunders *et al*'s research team comprised mainly women students

However, while these women could attend classes (and take exams), they could not attain degrees

In 1897 a vote was held by the University (all degree holders could vote – all men and had to vote
in person)

Natural Science group photograph 1889,
including 2nd-4th year students and demonstrators

The result stood until 1948

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DNA

Friedrich Miescher, Basel in 1871
‘Nuclein’ (= DNA + scaffold proteins)

- worked with human pus - isolated from used hospital bandages

- precipitate from lymphocytes

- waited to publish until results reproduced by another lab member

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RNA

1889: Richard Altmann described for the first time nuclear substances in the cell nucleus that were later identified as ribonucleic acid (RNA); also mitochondria

1909: Phoebus Levene determined by hydrolysis that RNA is composed of ribose, phosphate and nitrogenous bases

1938: Marjory Stephenson isolated RNA from the spirochete responsible for syphilis
First female FRS, 1945

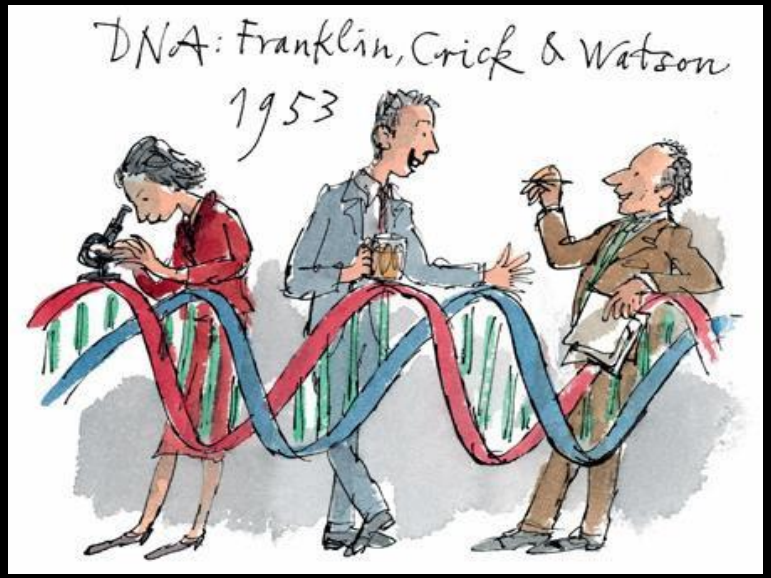
1958: Francis Crick proposed the hypothesis of the RNA “adaptor”, which plays an important role in the translation of RNA into proteins

1960: Sydney Brenner, François Jacob, Matthew Meselson discover messenger RNA

The double helix

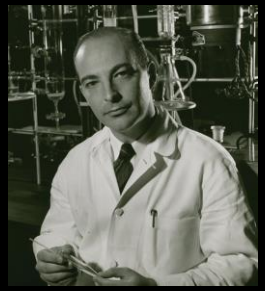
(Watson, Crick and….) 1953

Rosalind Franklin



Quentin Blake1999

1958



Kornberg

DNA polymerase from bacteria; within a year successfully synthesized DNA *in vitro* for the first time.

1967: Discovery of DNA ligases: considered pivotal, because they are essential for the repair and replication of DNA in all organisms.
Underpins a host of DNA manipulations, creating transgenes etc.

1967: Howard Temin and David Baltimore discover reverse transcriptase, the enzyme that enables the synthesis of DNA from RNA

1968: Restriction enzymes: Werner Arber noticed that certain bacterial strains fought off bacteriophage infection by chopping off the phage DNA

Now, > 4,000 different restriction enzymes that collectively recognize more than 360 different recognition sequences have been isolated from a wide variety of bacterial strains

1977: Frederick Sanger: radioactive chain-termination sequencing

1987: Leroy Hood and Michael Hunkapiller –
fluorescent and automated sequencing

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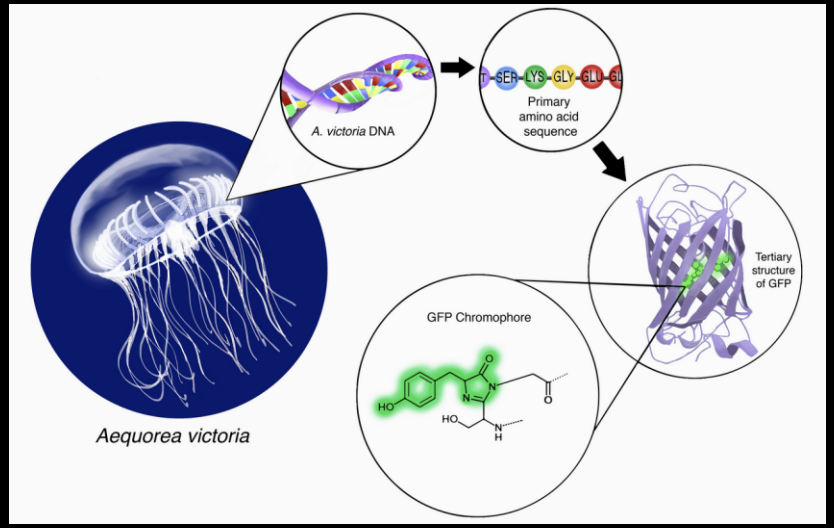
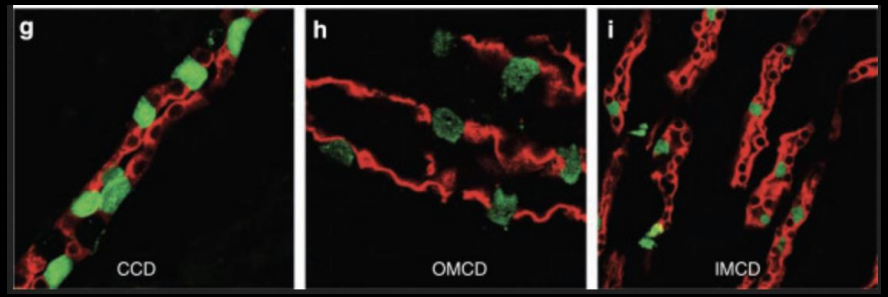
More molecular tools……

1962: Shimomura and colleagues. Green fluorescent protein (GFP) isolated. Naturally present in the *Aequorea Victoria* jellyfish and fluoresces with a green light when exposed to blue wavelength



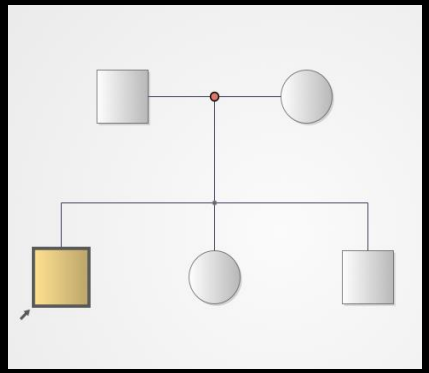


Green is *ATP6V1B1 promoter fused to YFP*

Counterstain (red) is AQP2

Miller et al KI 2009

Mechanisms of Inheritance



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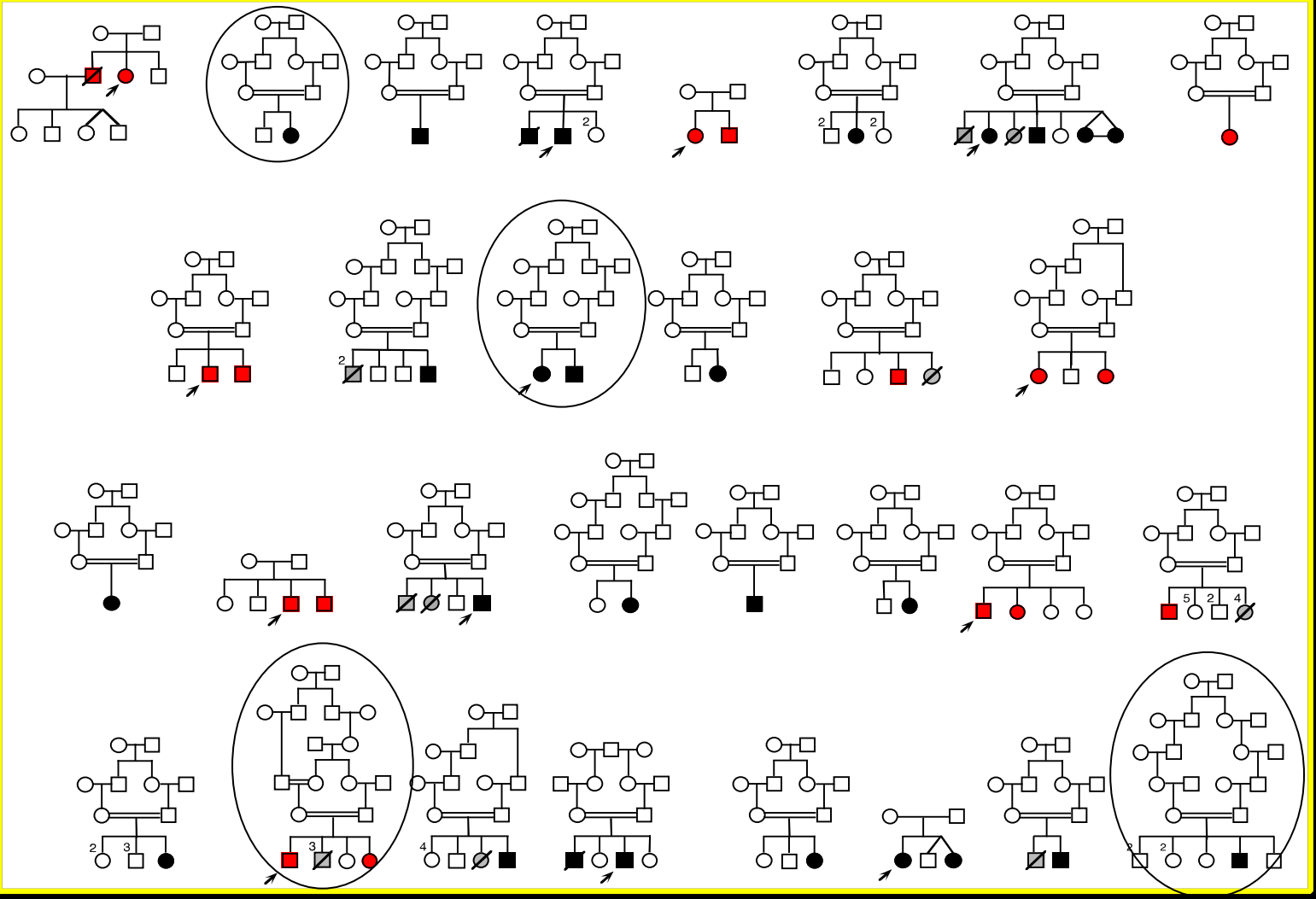
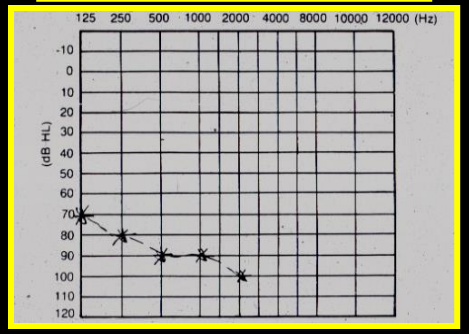




Distal Renal Tubular Acidosis

Lightwood, 1935 Butler *et al*. 1936

Metabolic acidosis + hypokalemia
nephrocalcinosis
rickets

Primary: autosomal dominant
autosomal recessive
± deafness

Secondary: autoimmunity *e.g.* Sjogrens
drugs / toxins *e.g.* vanadate
nephrocalcinosis
pregnancy

Functional failure of urine acidification
Treated with oral alkali

Acid-base normality is important for:

Electrolyte stability

Bone density

Cardiac conduction

Renal tract calcium solubility

Identifying causes of single gene disorders provides a window into normal physiology

Multiple skills needed – ‘gene jockeying’ followed by protein characterisation, cell biology, animal models, ‘organ-on-a-chip’ (etc.) studies

Ever more sophisticated gene expression work (single cell atlases, epigenetics)

May lead to drug discovery / new therapeutic approaches

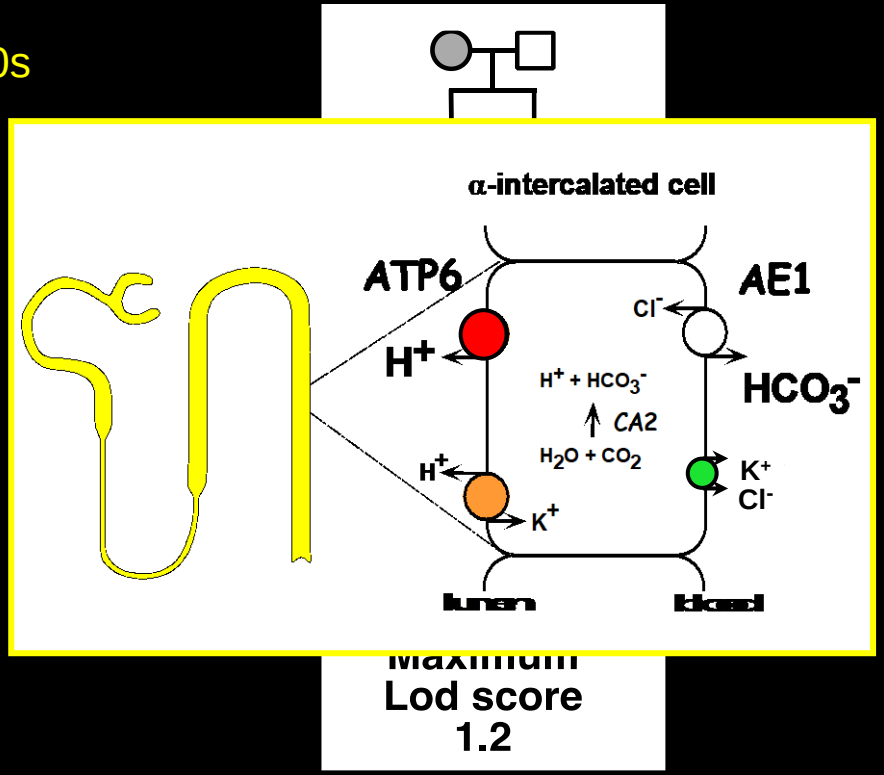
Approaches to recessive dRTA families: 1990s

- Screen candidate genes/loci
  - proton pump subunits
  - anion exchangers
  - carbonic anhydrases etc.

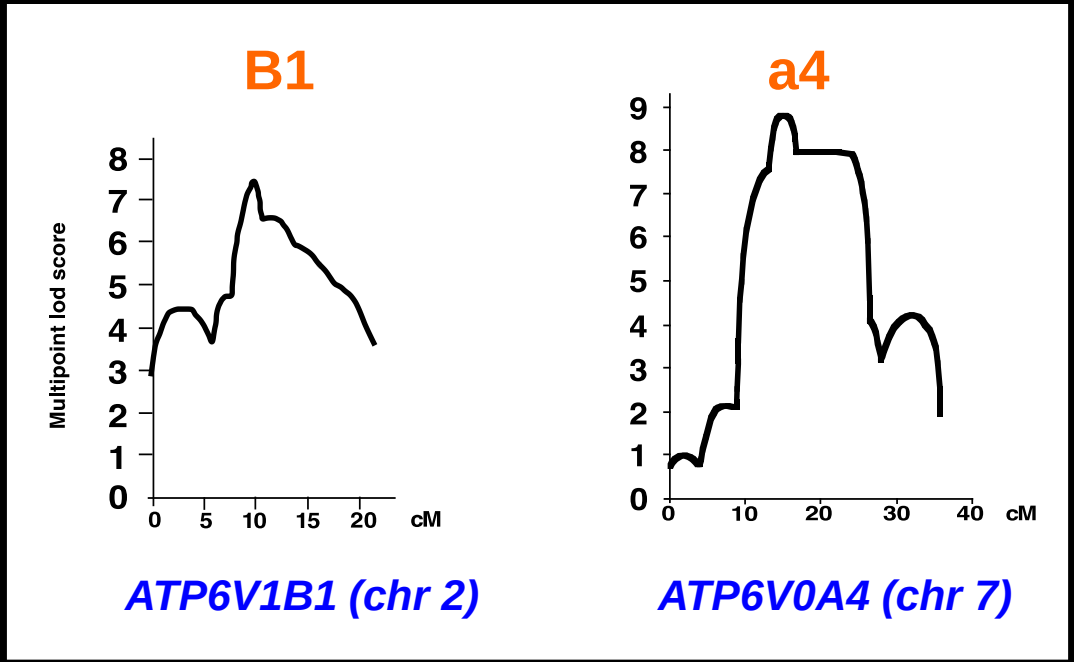
  X too many
  X not all mapped or characterized

- General linkage - homozygosity mapping in consanguineous kindreds

  incorporates models of heterogeneity
  powerful



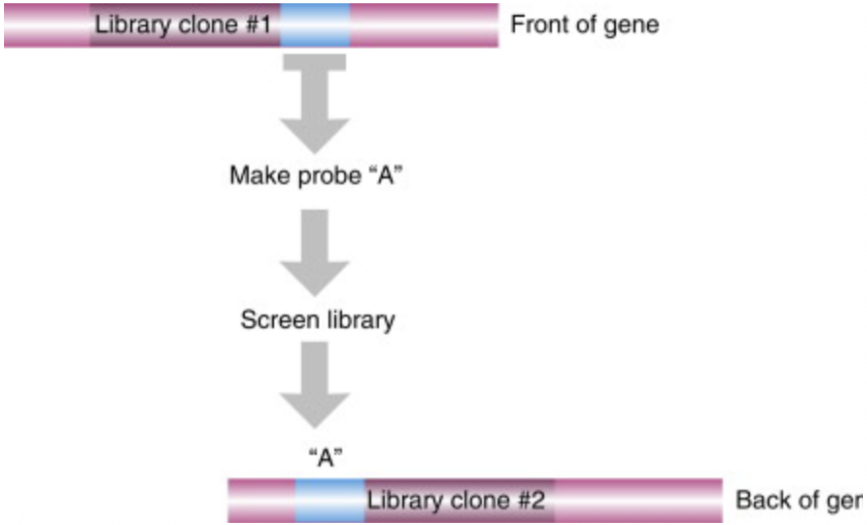
- Today would be followed by whole exome sequencing of linked region(s) or replaced by whole genome sequencing

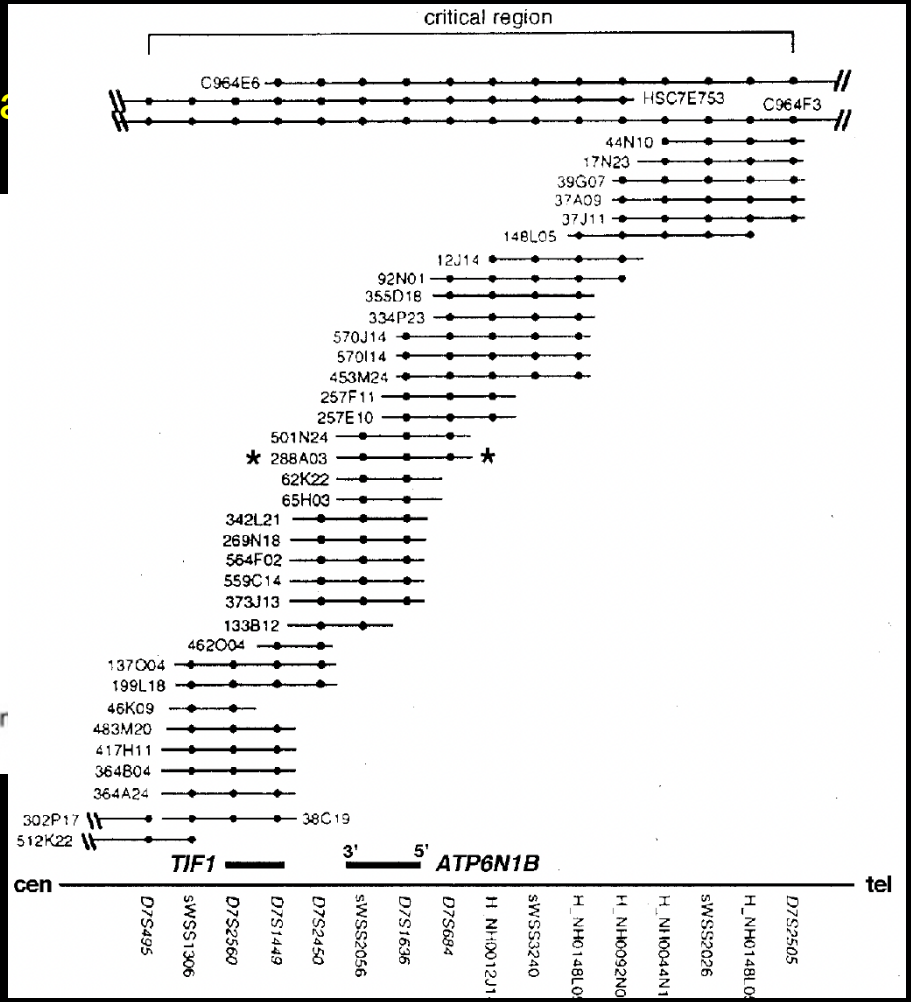


Karet et al, *Nat Genet* 1999

Smith et al., *Nat Genet* 2000

Chromosome walking…using BACs a



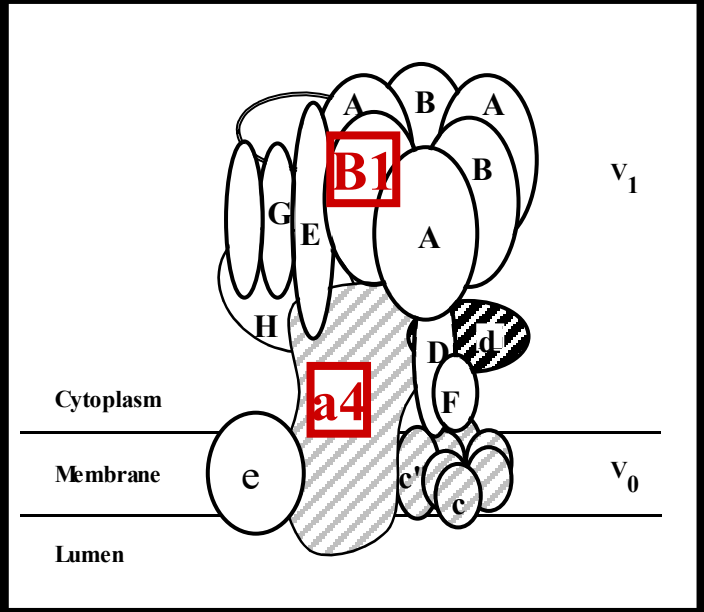


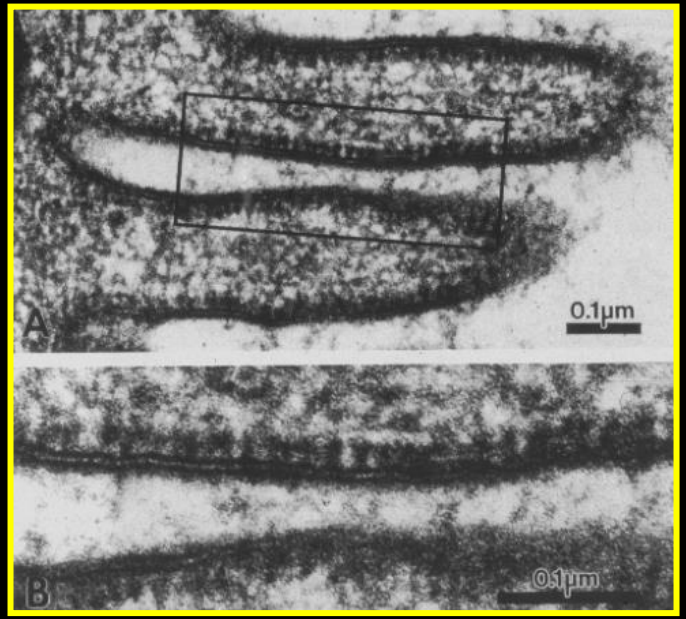
100 kb chromosome region per month?

The distal nephron H^+ATPase

B1 and a4 : defects cause recessive disease +/- deafness

(B2 and a1: ubiquitous)

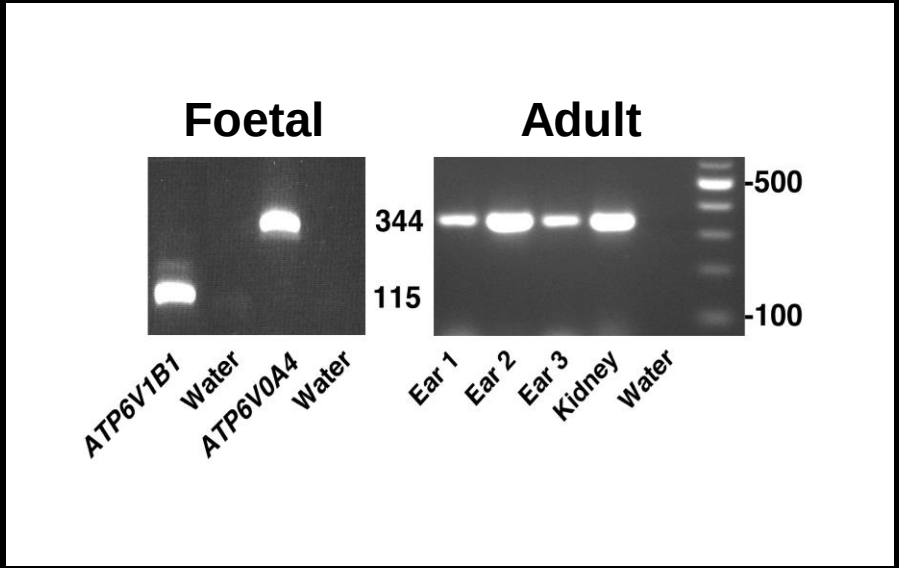




Steinmetz *et al. 1996*

Some a4 mutant-bearing humans DO become deaf

- milder
- older onset



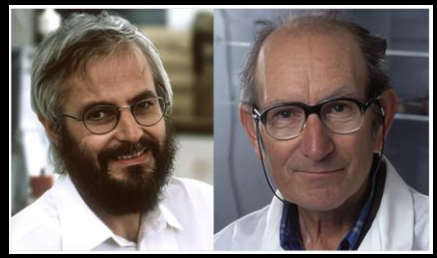
Stover et al J Med Genet 2002

1975: Kohler and Milstein – monoclonal antibodies

Began independently: Kohler created cells that produced very specific antibodies, but were short-lived;
Milstein (in Cambridge) had antibodies with unknown specificity, but that multiplied and grew forever.

By combining, they were able to make monoclonal antibodies with amazing precision, that divided and continued to do so - and survive - forever

Revolutionised lab science, diagnostics and immunology treatments



Michael Neuberger uncovered the role of DNA deamination in immunity by explaining how cytosine deamination drives the somatic hypermutation and class-switch recombination of antibody-encoding genes

MAbs now in widespread use for experimental work and therapies (eg in vasculitides and cancer)

1985	Recombinant vaccine for HepB
1987-93	CRISPR
1996	Dolly the sheep
1999	First chromosome sequence
2001	Imatinib
2003	Human genome
2006	IPSCs
2010	The World's First Synthetic Life Form
2020	Human single cell RNA atlas
2021	Spatial transcriptomics

1998: Shankar Balasubramanian and David Klenerman:
Solexa massively parallel fluorescent sequencing (start of ‘NGS’)

DNA fragments immobilised on a chip; fluorescently sequenced and then decoded

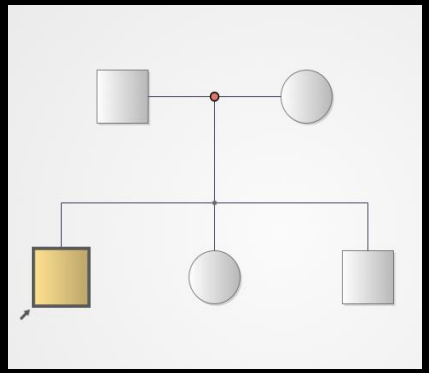
1990-2003: full human genome

Collaborative effort included Sir John Sulston (Sanger Centre)

Now the basis for majority of genetic diagnostics (but some exomes, some specific tests eg GRA)

Tests are nationally funded in the UK

Mechanisms of Inheritance



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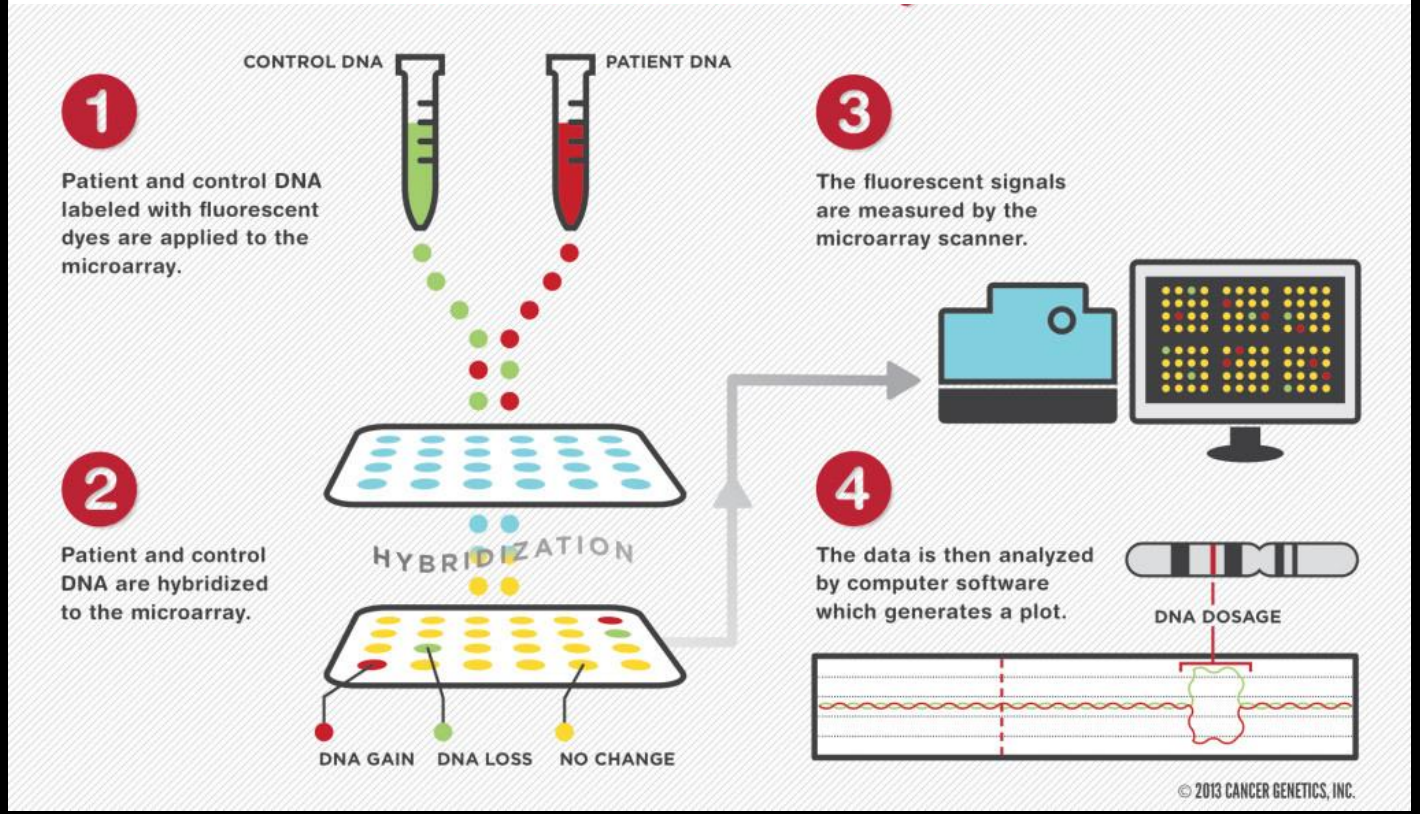
Now feasible to investigate singletons genetically

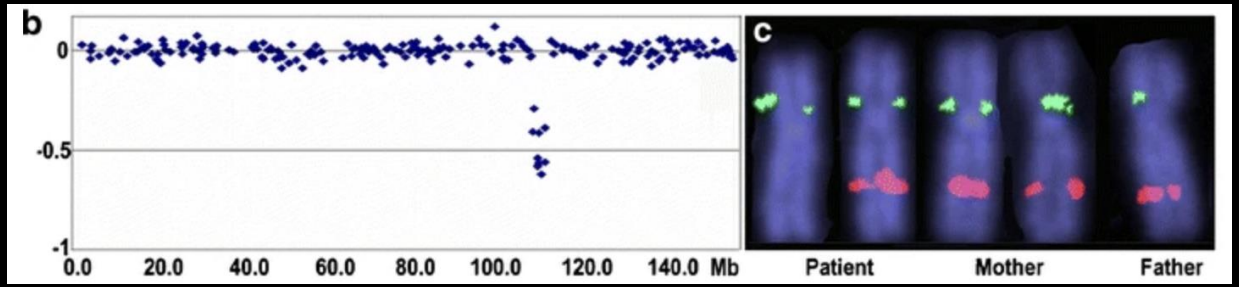
Gene dosage: Array CGH



Xq22.3-q23 deletion syndrome

14 y old female: haematuria; proteinuria; hearing loss; mental retardation; dysmorphism; epilepsy; double cortex; neuronal migration disorder.

Deletion: *de novo*, uniallelic and 3.3 Mb in size.



Genes: *COL4A5*, MR genes *FACL4* and *PAK3*, and parts of the X-chr lissencephaly gene *DCX* (assoc. with double cortex formation in girls and epilepsy)

Hoischen *et al. Pediatr Nephrol* 2009

Potential for genetic therapies:

- Mitochondrial donation
  - Ethically approved in UK; trials ongoing

Gorman et al, Lancet 2018

- CRISPR-Cas9
  - Example: Alport syndrome, >40% normalisation in podocyte lines

Daga et al EJHG **2**2020

- RNA manipulation
  - e.g. anti miRNA-21 oligo (lademirsen) study in Alport's
  - Terminated early after futility analysis - mouse model had been encouraging

Gale et al CJASN 2024

  - 'Readthrough' to bypass stop codons (ELX-02, a synthetic aminoglycoside)
  - Alports, ADPKD and cystinosis

- Antisense oligos
  - Exon-skipping (Alports)
  - Knock down (ADTKD)
  - Potentially suitable for dominant disorders

Yamamura et al Nature Comms 2020

From single gene disorders to population genetic risk factors…..

e.g. different severities of CKD progression in different populations,

- quantified since the 1970s, but initially thought to be socioeconomic
- even correcting for diabetes and hypertension

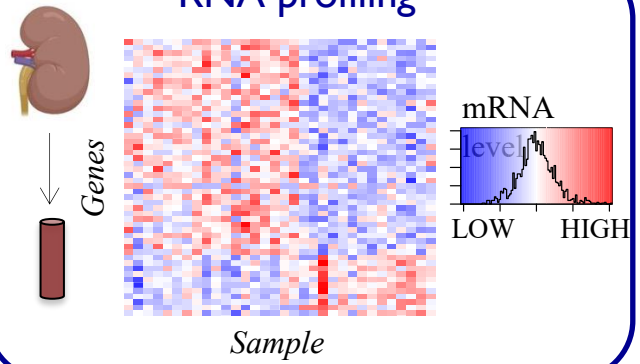
Now individual risk alleles can be discerned, e.g.

APOL1 genotype is now a known risk factor and treatments are being developed:

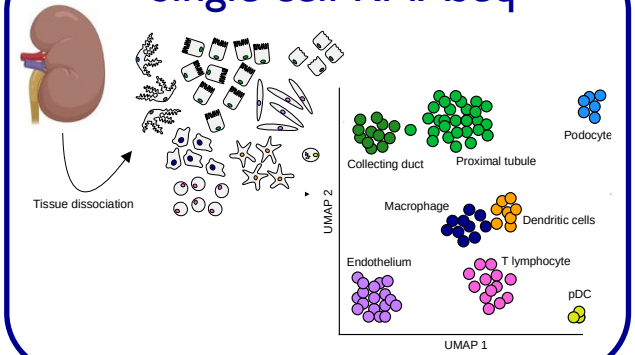
- inaxaplin (a Vertex Pharmaceuticals injectable small molecule) diminished proteinuria in transgenic mice, and in a pilot human study.

Next steps… from tissue to single cell and single nuclei atlases
From histopathology to functional pathology

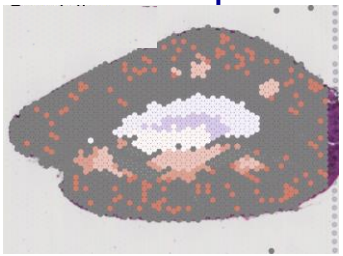
RNA profiling



Single cell RNAseq



Spatial transcriptomics



Future integration into routine clinical diagnoses and personalized medicine

Stewart BJ, Ferdinand JF, Clatworthy MR. ***Nat Rev Neph*** 2020

Richoz N *et al.* ***JCI Insight***. 2021

Conclusion

- We owe William Bateson a great deal
- We've come a long way in well under 200 years!
- If anything, we now have even more ethical issues to face than ever before

Thanks to:

- ❖ Clinical and lab colleagues
- ❖ Menna Clatworthy, Lucy Raymond, Paul Kimmel
- ❖ Patients and their families and their physicians and scientists