Myotonia congentia

Muscle relaxation disorder and infertility

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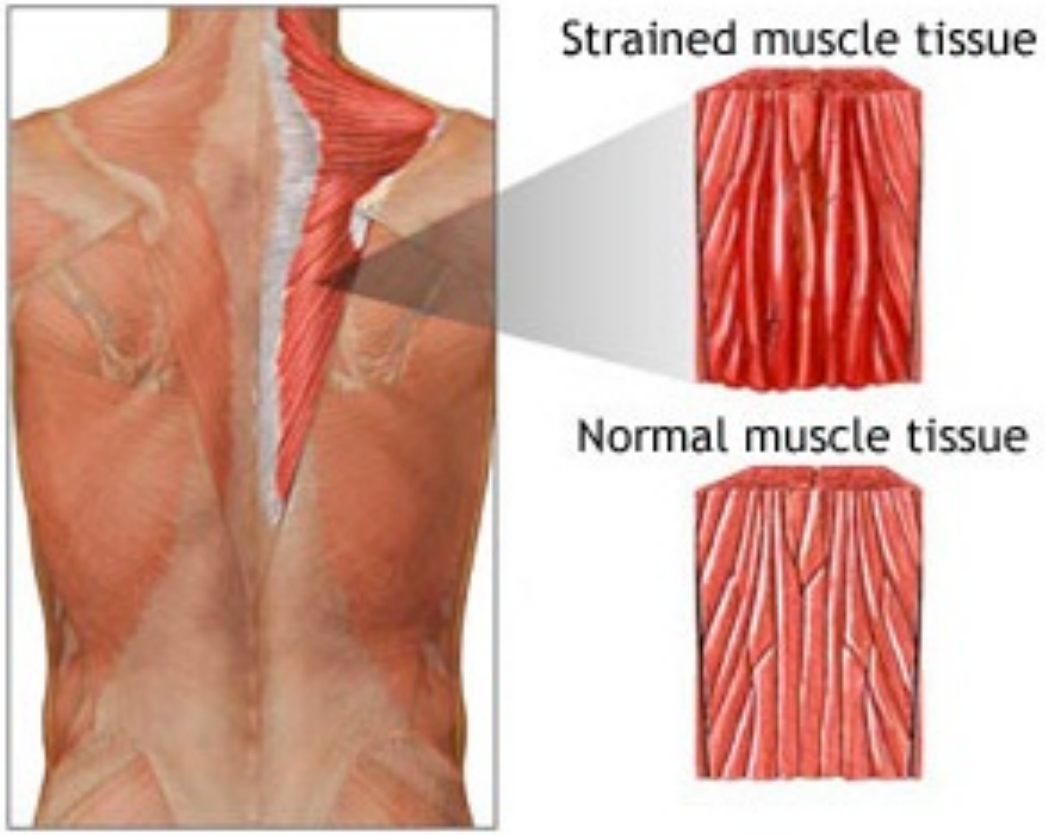
….So what is Myotonia congentia?

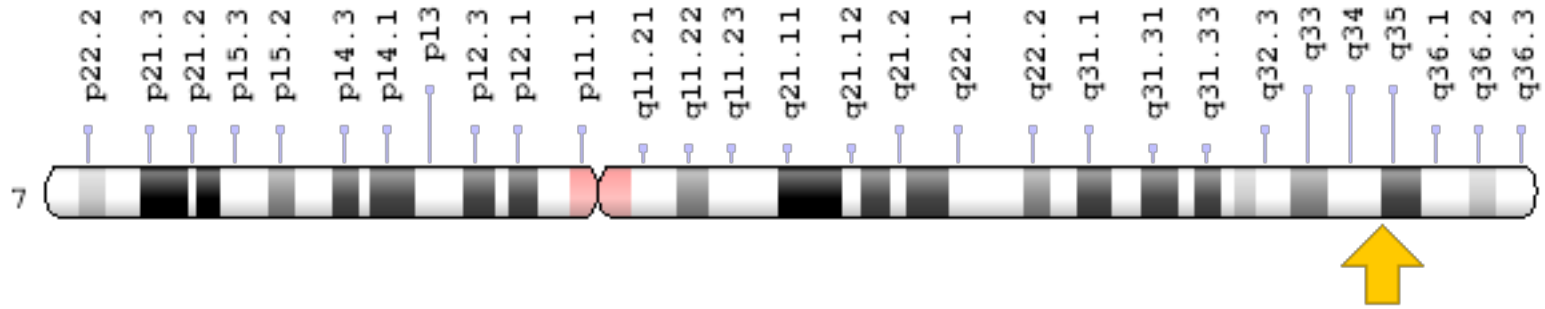
Characterised by the inability of skeletal muscle to relax

What are the symptoms of Myotonia Congentia?



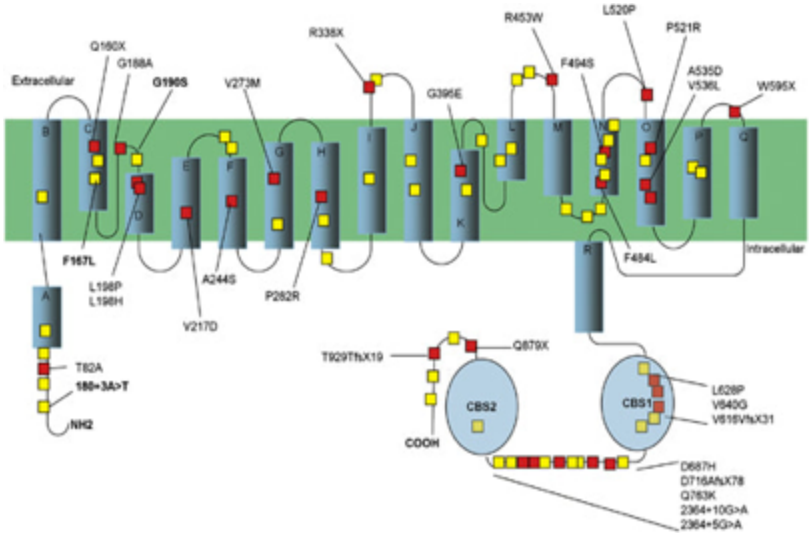
Painless muscle stiffness with several repetitions of the same movement

It’s all about CLCN1…



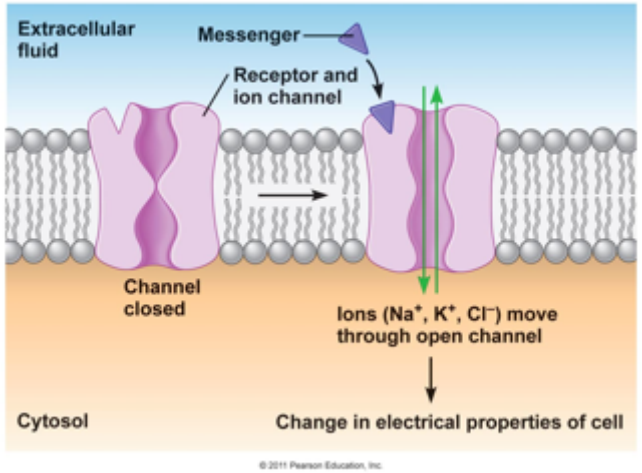
Voltage-dependent chloride channel genes

Molecular function



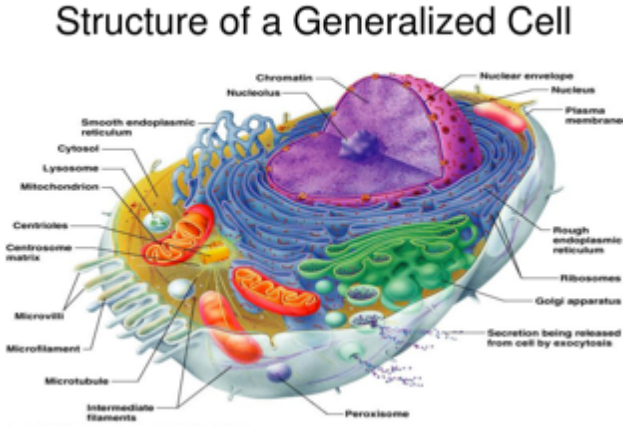
Chloride ion transport

Biological Process



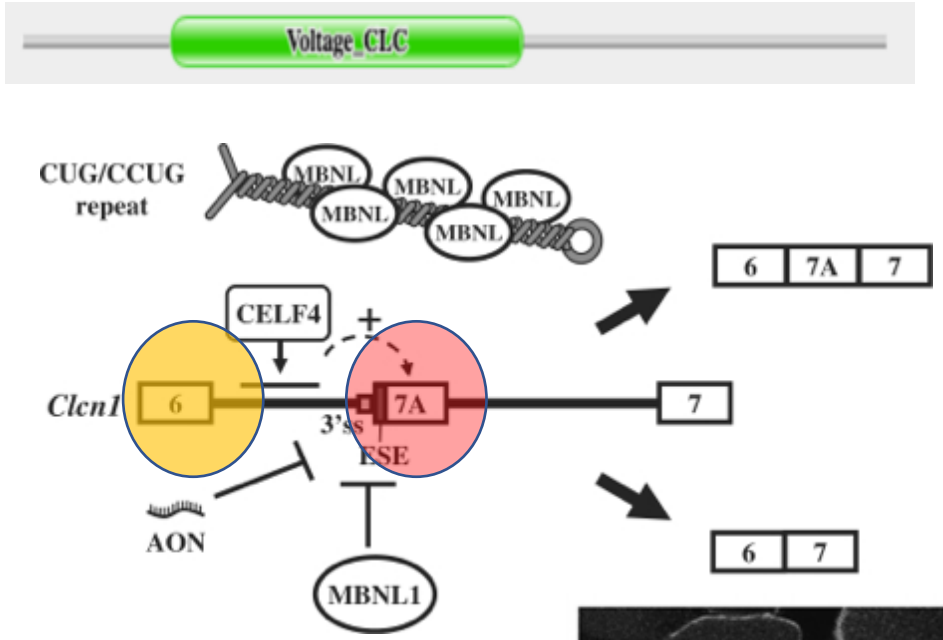
Equalisation of charge

Cellular component



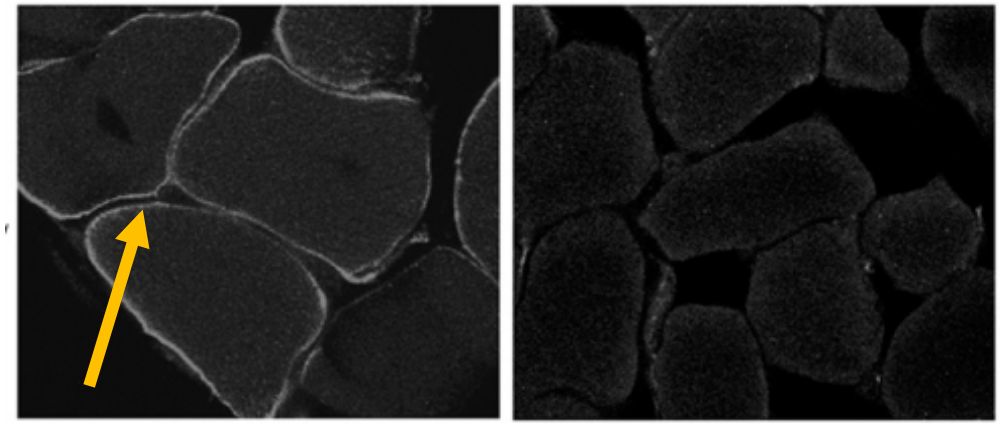
Skeletal Muscle

What is known about CLCN1?



Expression of CLCN1 in skeletal muscle is driven by alternative splicing

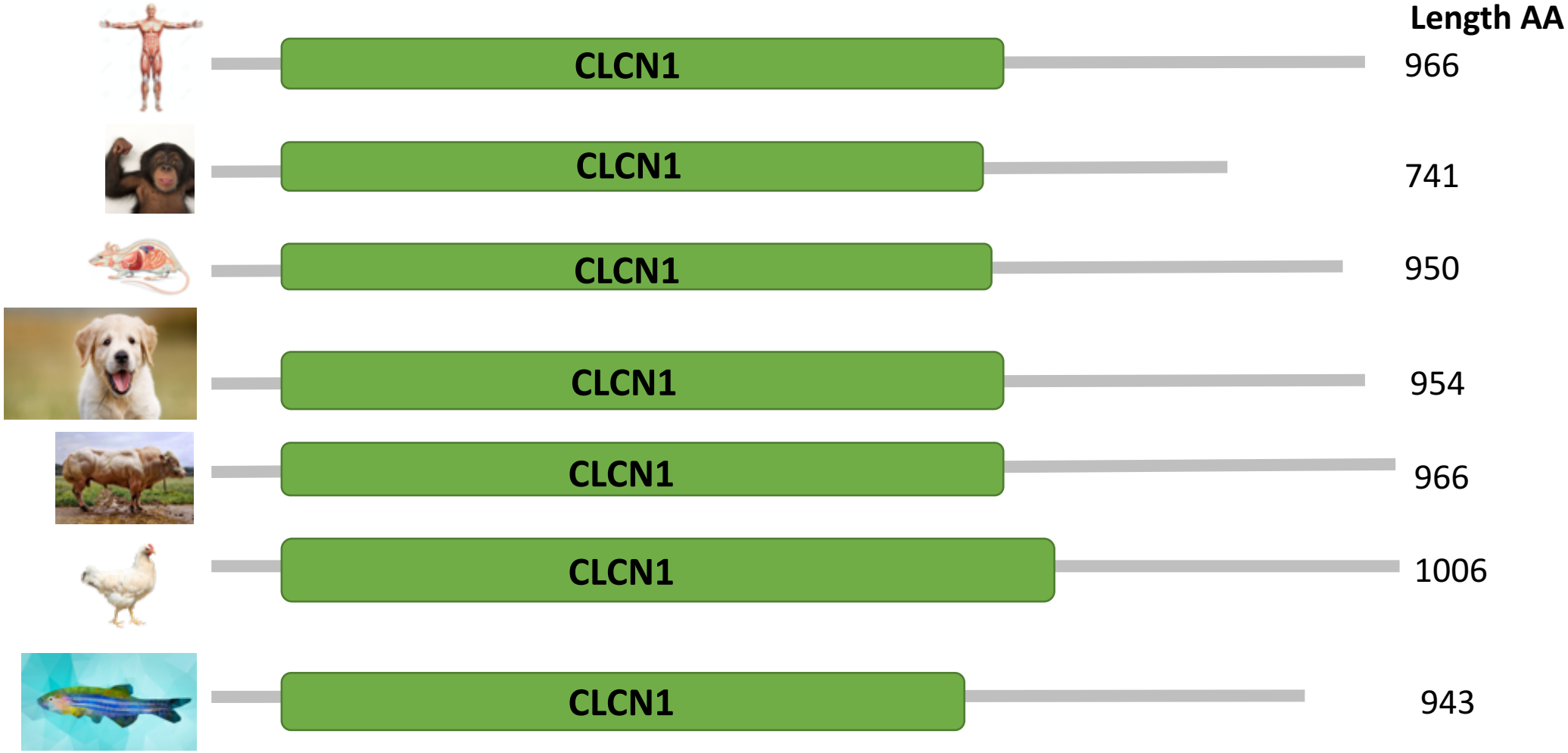
Myotonia is caused by the expansion of CTG or CCTG repeat



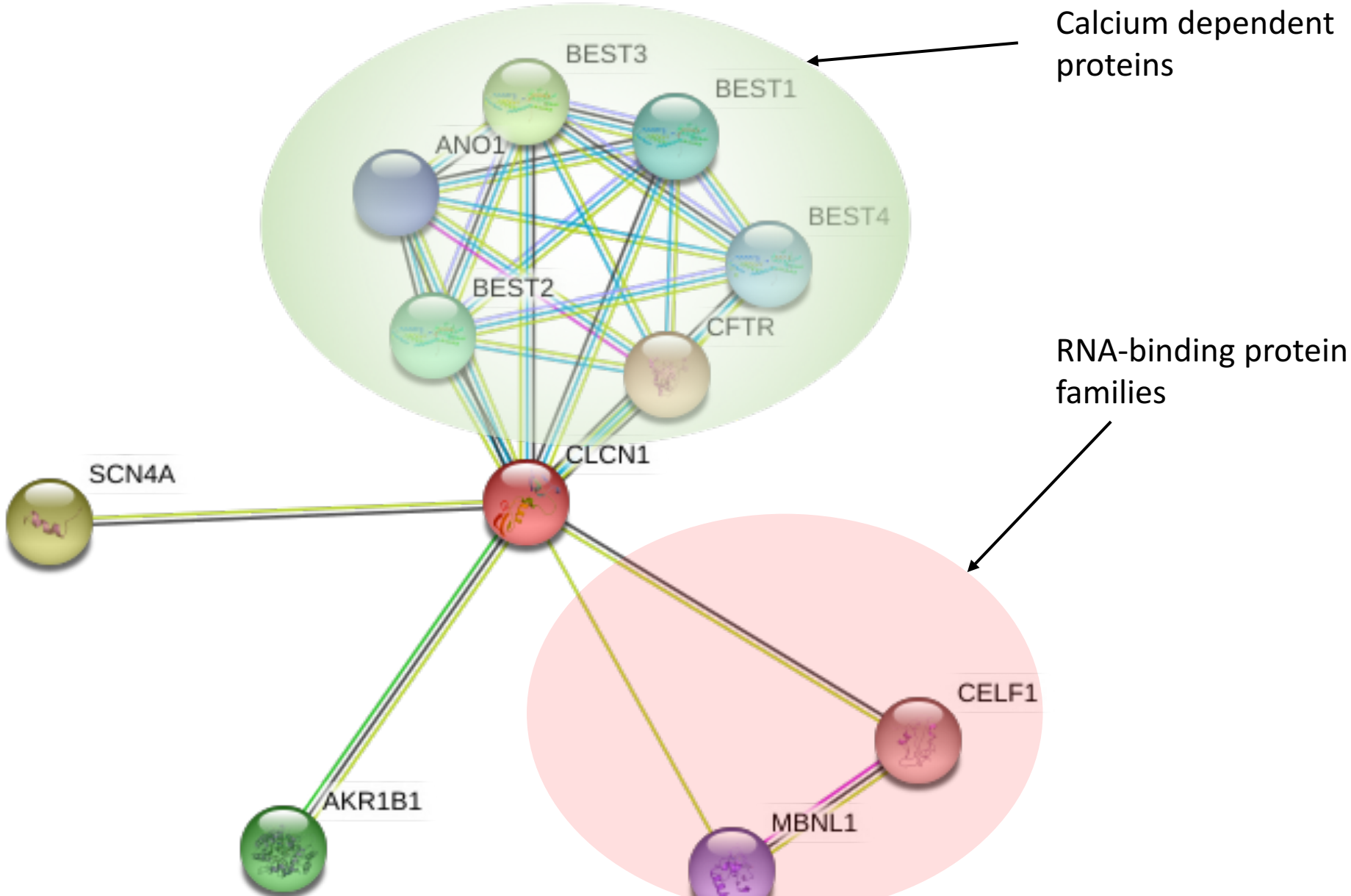
Normal

Myotonia

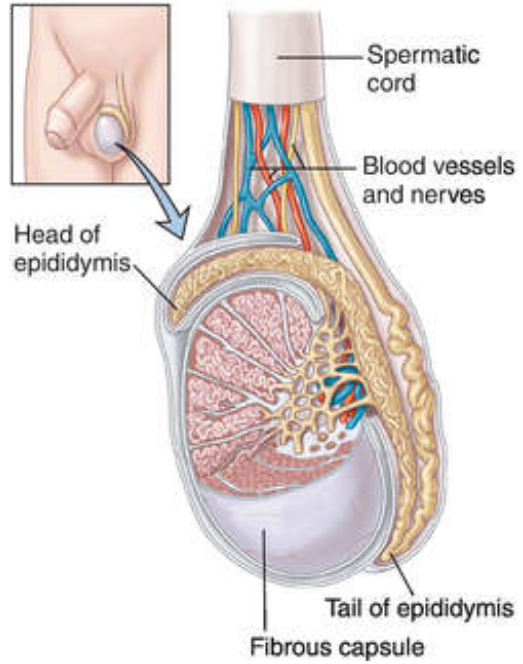
How conserved are CLCN1 homologs?



How CLCN1 interacts?

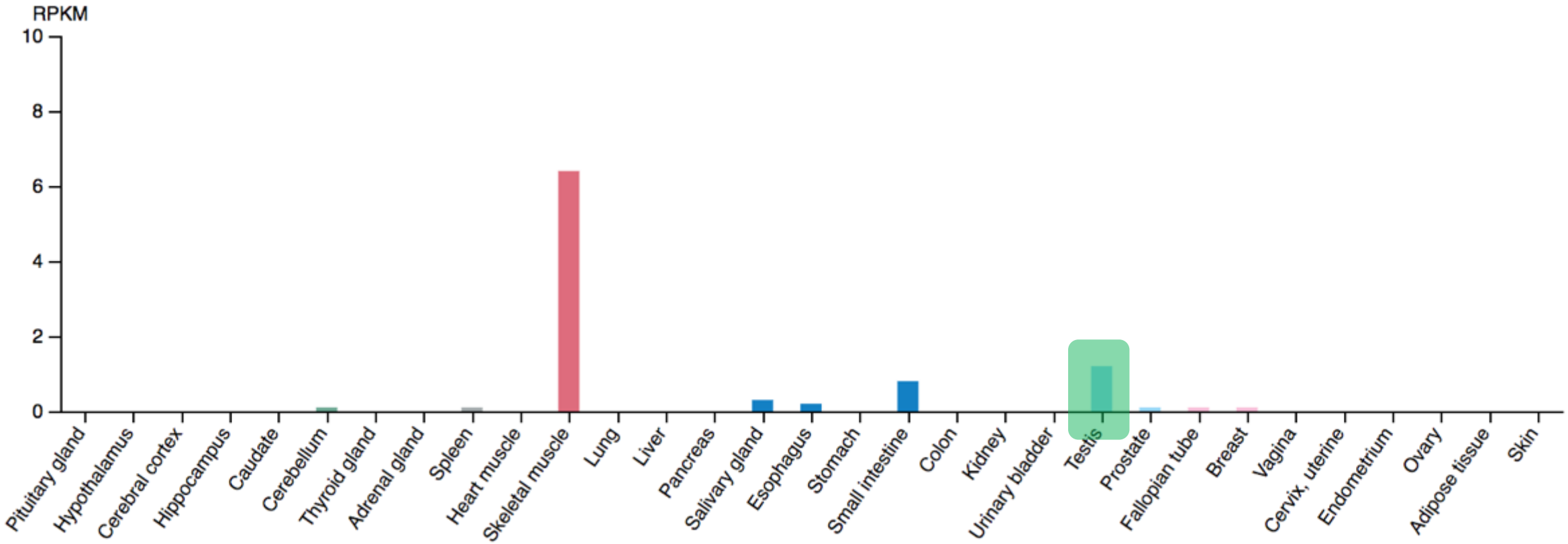


What is the gap in knowledge?



My primary goal is to determine how the CLCN1 protein regulate chloride ions and its interaction with other proteins in testis

CLCN1 is not only found in muscle



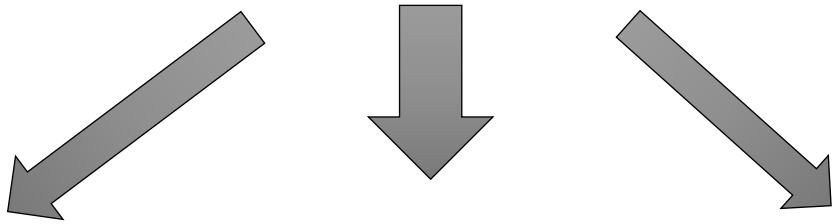
Why mouse?

Similar biology to humans

Reproductive system has structural similarities to human

Relatively cheap compare to other model organisms used in previous studies

Aims



Aim 1: Determine conserved amino acids in the protein that mediate proper sperm function?

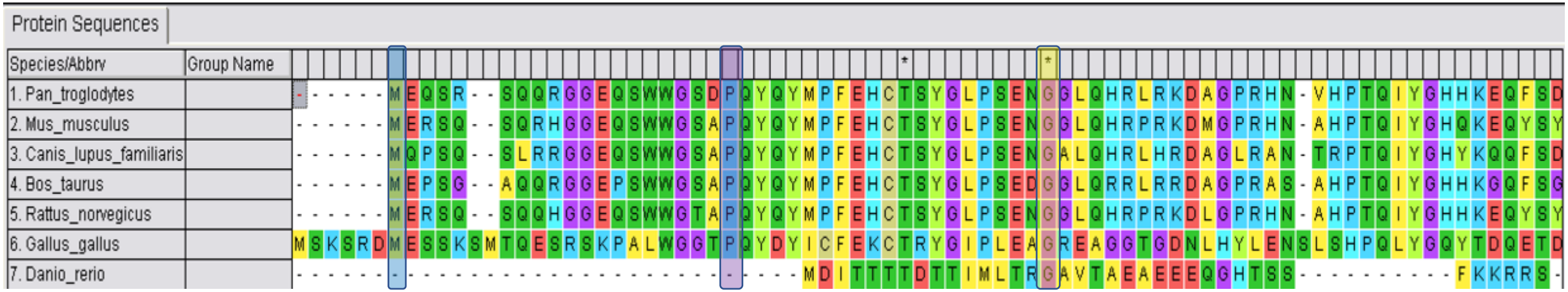
Aim 2: Determine gene expression changes in testis necessary for fertility.

Aim 3:Identify novel CLCN1 protein interactions important for sperm development

Hypothesis is that mutations in the CLCN1 gene decreases the transmembrane transport of chloride ions in turn affecting the electrochemical gradient causing sperms to be immobilised.

Aim 1: Determine conserved amino acids in the protein that mediate proper sperm function?

MEGA → CRISPR-Cas9 → Screen



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MEGA → CRISPR-Cas9 → Screen

WT

Infertile mutants

Aim 1: Determine conserved amino acids in the protein that mediate proper sperm function?

MEGA → CRISPR-Cas9 → Screen

Hypothesis: I expect alleles with mutations will cause infertility in males.

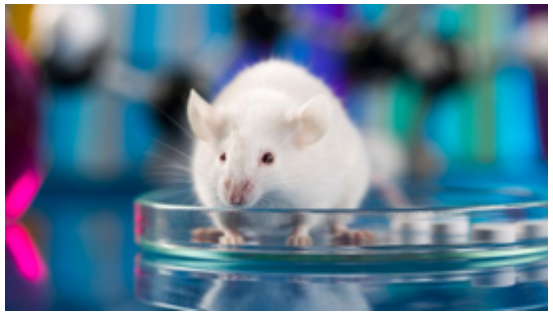
Aim 2: Determine gene expression changes necessary for fertility in CLCN1.

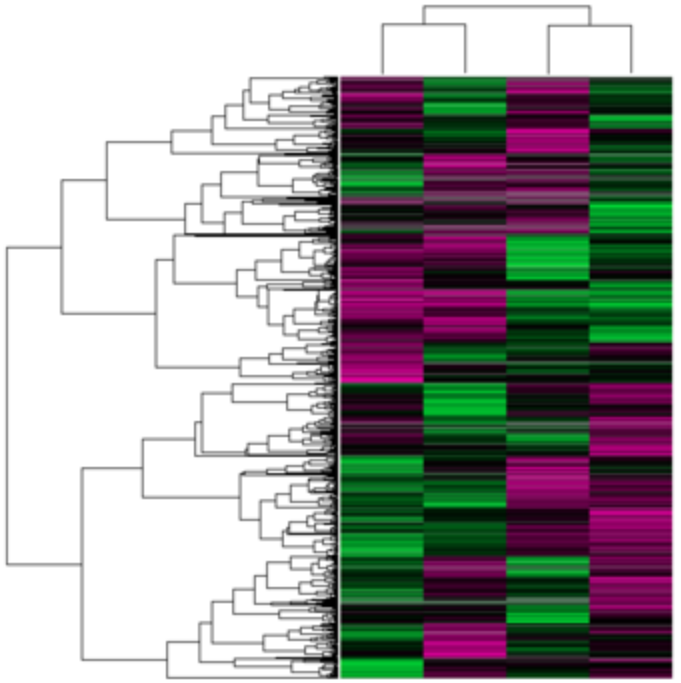
Single cell RNA sequencing

GO

WT

Mutant





Hypothesis: Mutations in CLCN1 will result in decreased expression of chloride channel within the testis

Aim 3: Identify novel CLCN1 protein interactions important for sperm development

Known protein-protein interactions → AP-mass spec → Top down

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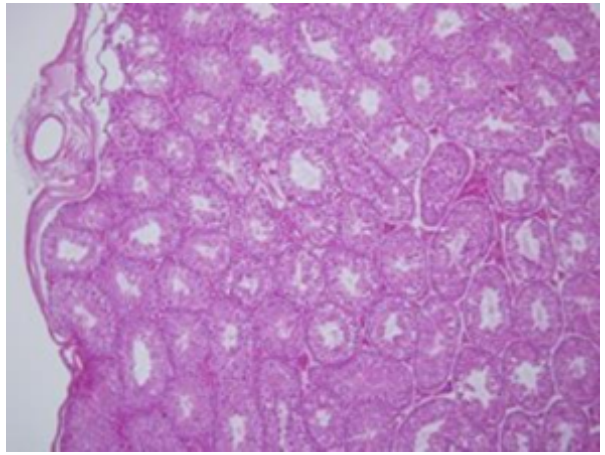
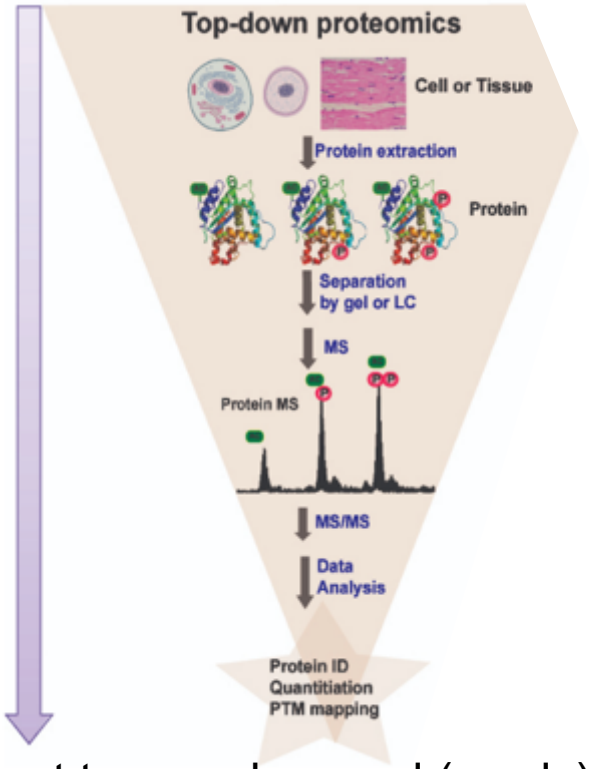
Known protein-protein interactions → **AP-mass spec** → Top down



Aim 3: Identify novel CLCN1 protein interactions important for sperm development

Known protein-protein interactions → AP-mass spec → Top down

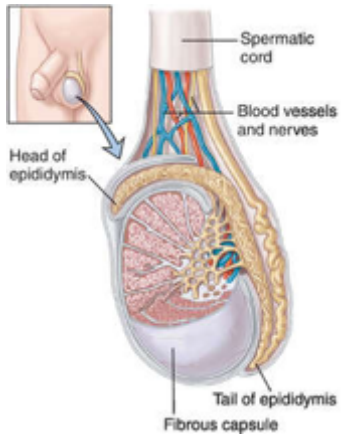


Hypothesis: I expect to see abnormal (newly) protein interactions in the CLCN1 knockout mice.

Conclusion

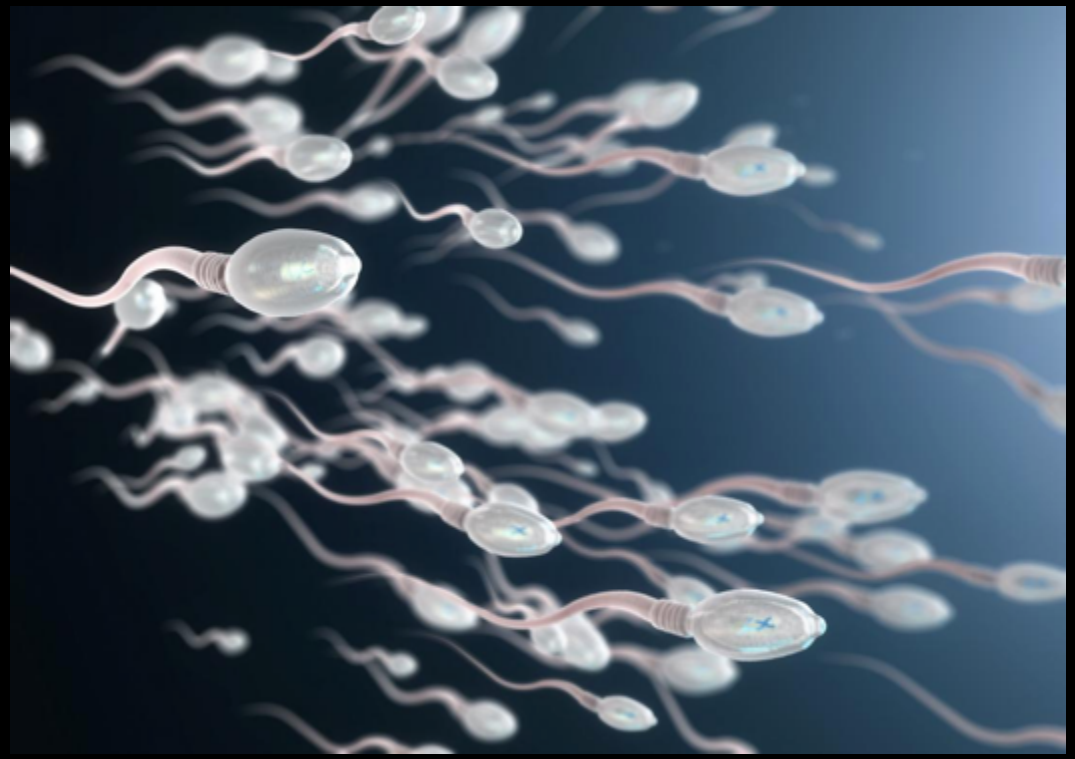
Myotonia Congenita is characterised by the inability of skeletal muscle to relax after voluntary movement

Mutations within CLCN1 is associated with infertility



Understanding CLCN1's mechanism in testis may lead to ways to stop infertility associated with myotonia congentia

Future directions

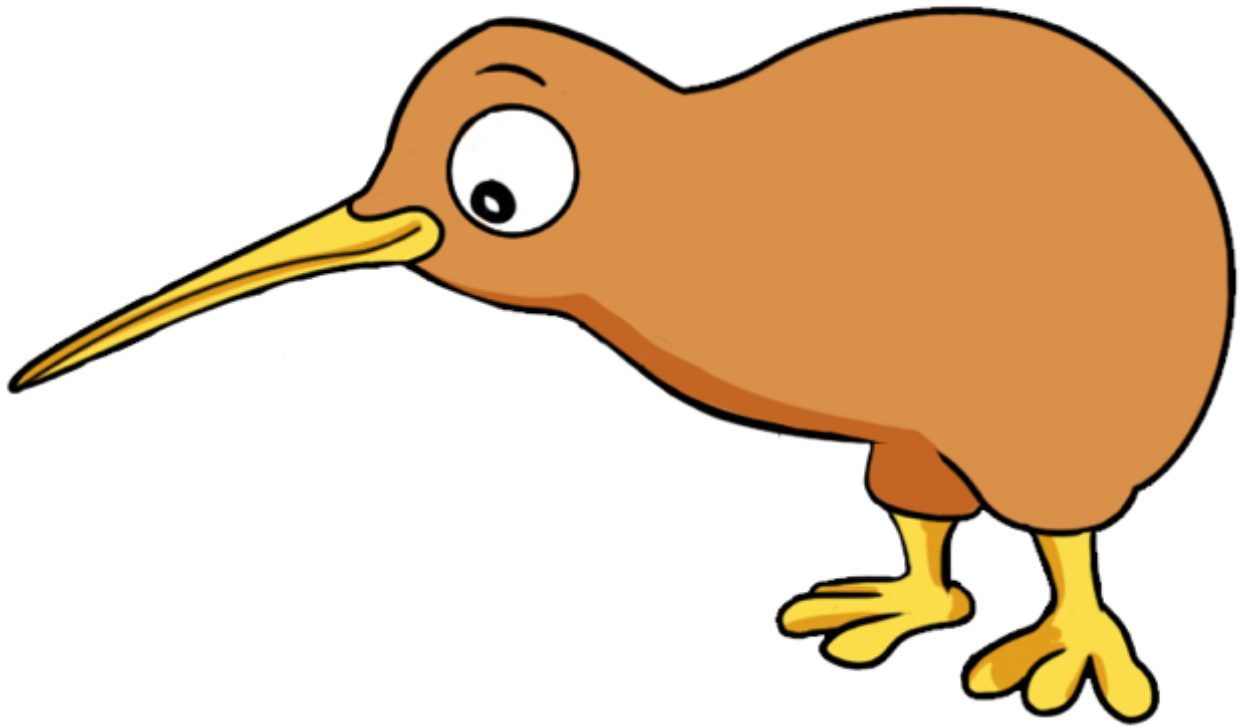


Develop drugs/therapy for fertility for both men and women

It is estimated to affect 1 in 100,000 people worldwide

More common in northern Scandinavia – 1 in 10,000

Questions?



References

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