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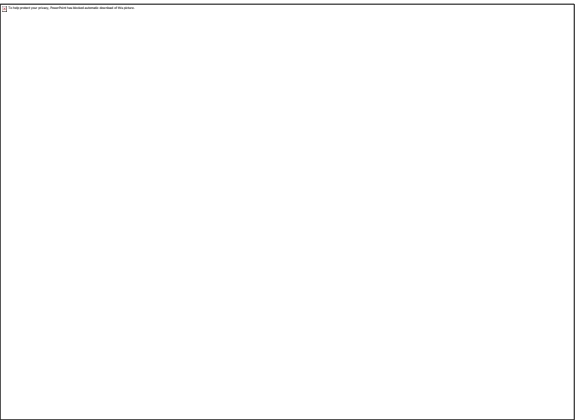
Examining copy number variants (CNVs) as a possible phenotype modifier in prion disease

Shashi Shetty, PhD

Grant Year: 2022



Nothing to disclose

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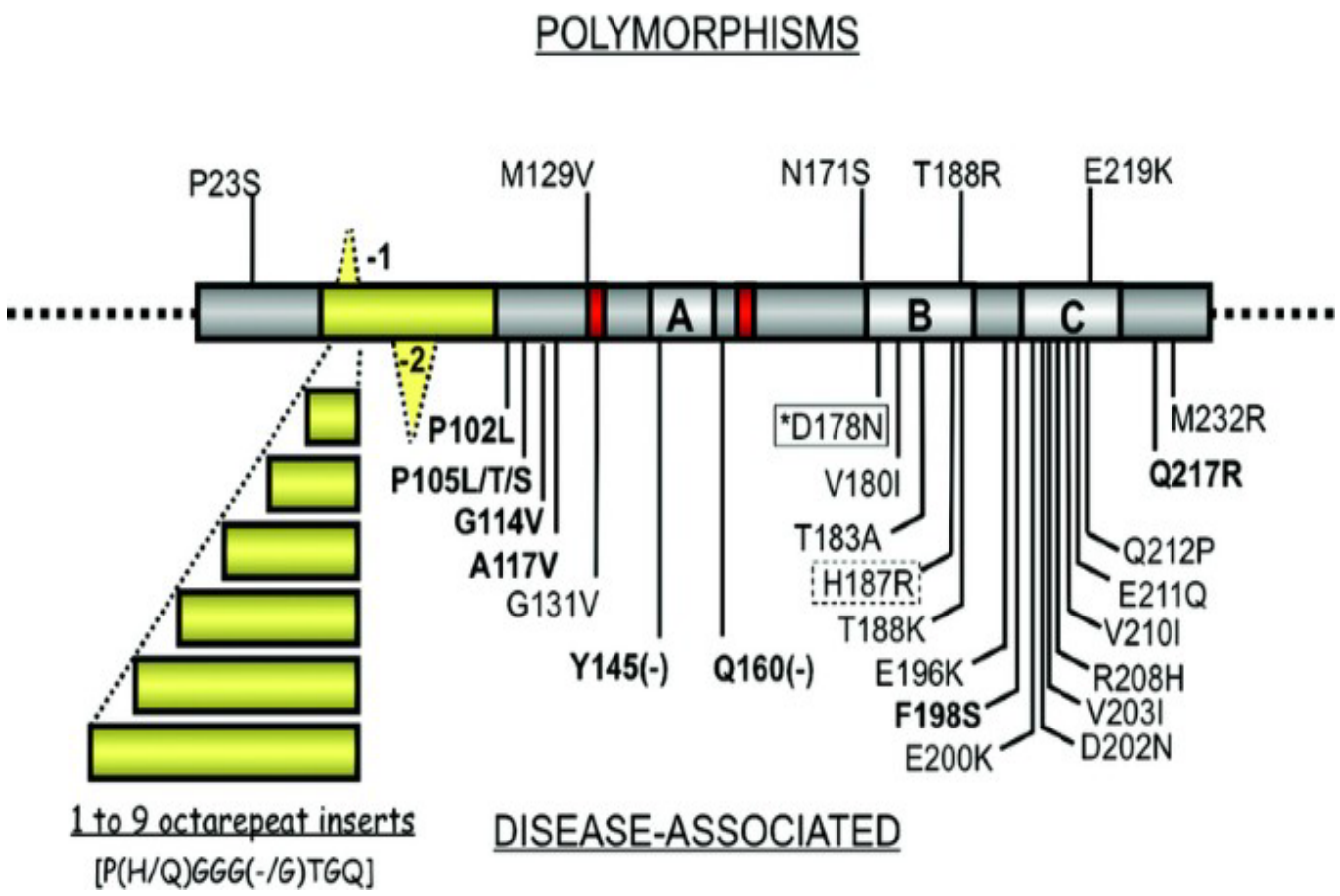
Genetic Prion Disease

- The ***PRNP* gene** is the only gene known to cause genetic prion disease
- There are at least **70 mutations** known to date in the *PRNP* gene
- The *PRNP* gene mutations is inherited in an ***autosomal dominant pattern***



Mastrianni, J. The genetics of prion diseases. *Genet Med* **12**, 187–195 (2010).

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Genetic Risk Factors or Modifiers

- The risk of prion disease onset and its expression is well-studied and is partially dependent on **codon 129** polymorphism
- It is perplexing that additional genetic variations have not been identified that explain the clinical heterogeneity (e.g., age at onset or penetrance)
- Common types of mutations include single nucleotide variants, **copy number variants**, chromosome abnormalities and mobile elements

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Why Copy Number Variants?

- In the human DNA, there are regions known as **copy number variants** (CNVs) that affect gene expression or function
- CNVs result in gain or loss of genetic material
- CNVs are also important for evolution and rare CNVs are known to cause disease
- In Alzheimer's disease and Parkinson's, the role of CNVs is well-studied
- In the context of prion disease, the information regarding CNVs contribution to disease is still evolving

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CNVs and Neurons

- Hundreds of CNVs have been linked to neurological phenotypes, including autism, schizophrenia, and bipolar disorder
- CNVs are seen in a mosaic state in 5-30% of the neurons
- Mosaicism can happen in the early embryonic phase (germline) or post conceptus (somatic)
- Neurons are non-dividing cells and depend on efficient DNA repair mechanisms to survive and function
- Age-related deterioration in DNA repair mechanisms could either result in loss or enrichment of CNVs in the neurons

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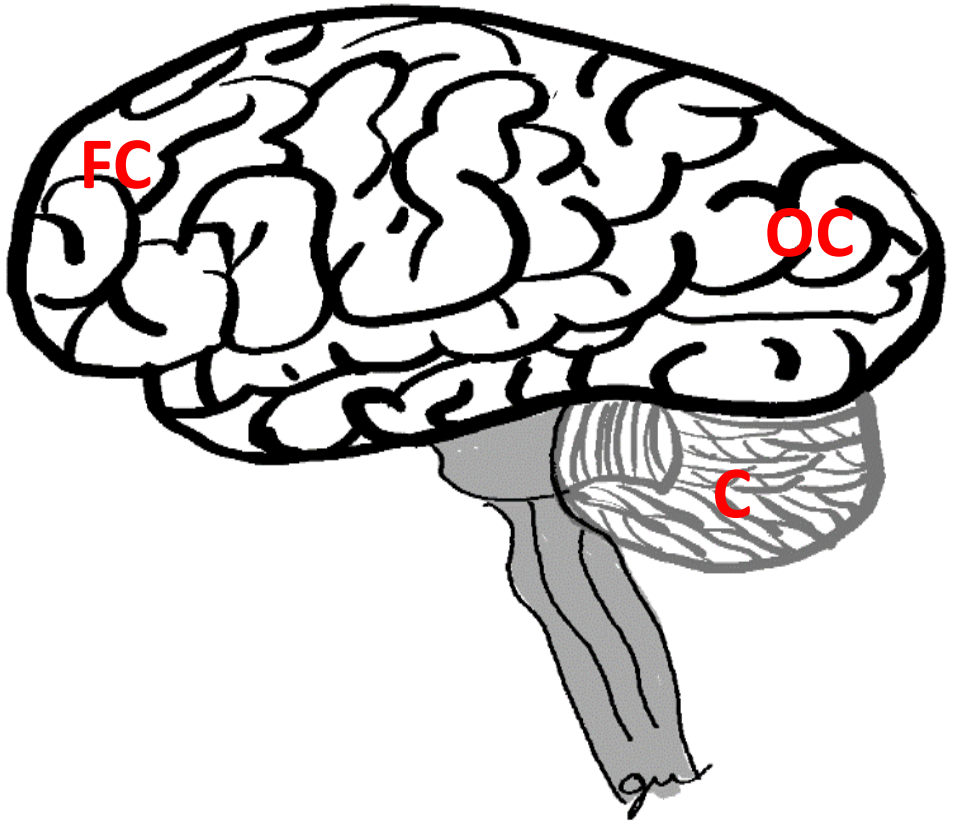
Distribution of Neurons



- Detection of mosaic CNVs can be challenging if present at a low-level in the neurons
- In the cortex, ~60 billion glia (dividing cells) and ~16 billion **neurons** (non-dividing cells) are present
- In the cerebellum, ~16 billion glia and ~69 billion **neurons** are present

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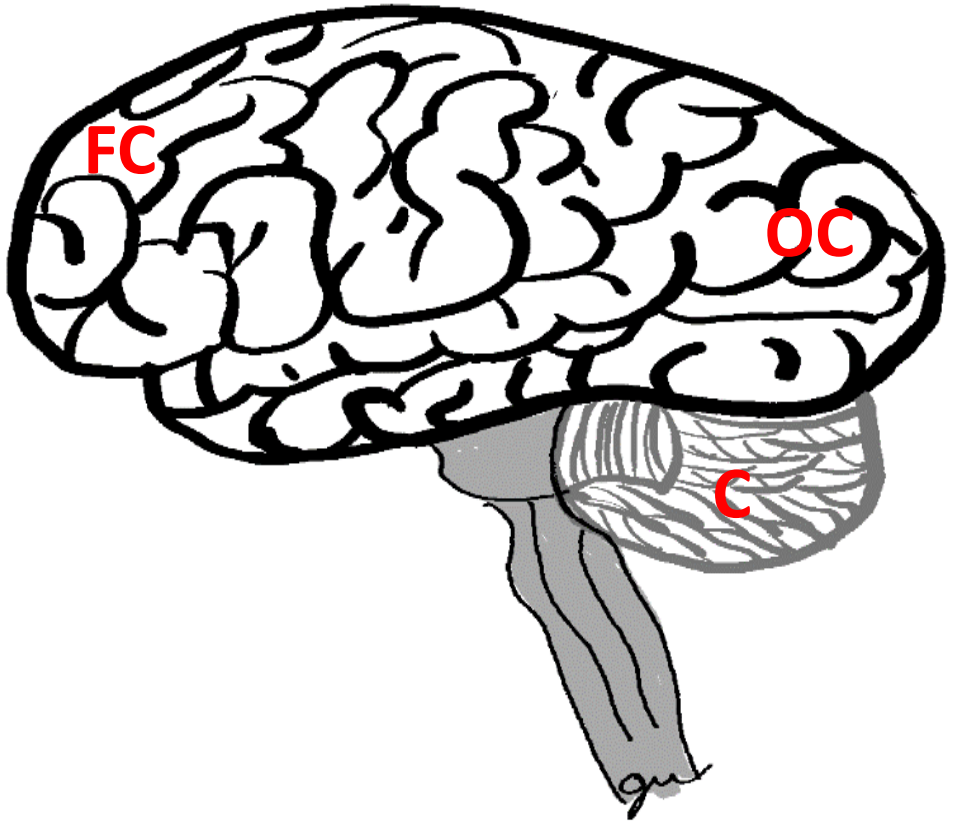
Distribution of Neurons



- Since neurons are present at a higher-level in the cerebellum, the chances of detecting mosaicism is greater
- We decided to study different regions of the brain to improve sensitivity
- For germline changes, blood DNA will be tested

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Question 1

*Are there unrecognized genetic factors that influence age of disease onset in **sCJD VV1 and sFI**?*

Sporadic forms of prion disease peak in the 6th to 8th decade of life. Young age at onset in certain sporadic prion diseases including sporadic CJD VV1 (sCJD VV1) and sporadic Fatal Insomnia (sFI) is similar to the inherited forms

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Question 2

What genetic determinants influence these differences in phenotype and age of onset and penetrance?

- Although some ***PRNP* pathogenic variants** are associated with specific neuropathologic phenotypes, evidence also suggests that the same pathogenic variant within a family can develop distinct clinicopathologic phenotypes.
- It is difficult to predict the impact on individuals within the same family carrying the identical *PRNP* gene mutation (e.g., age of onset or penetrance)

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Study aims

Aim 1: Examine the role of copy number variants (CNVs) in young age at onset sporadic forms of prion of disease like sCJD VVI or sFI

*Perform genome wide DNA single nucleotide polymorphism (SNP) array on **frontal cortex, occipital cortex and cerebellum** with age and sex matched controls*

Aim 2: Identify novel CNVs in genetic prion disease

*Perform genome wide DNA SNP array on **frontal cortex, occipital cortex and cerebellum** with age and sex matched controls*

Aim 3: Presence of CNVs in the brain will be confirmed by functional studies

Proteins encoded by genes identified by SNP array will be assessed for reduced or overexpression using immunohistochemistry and Western blot analyses

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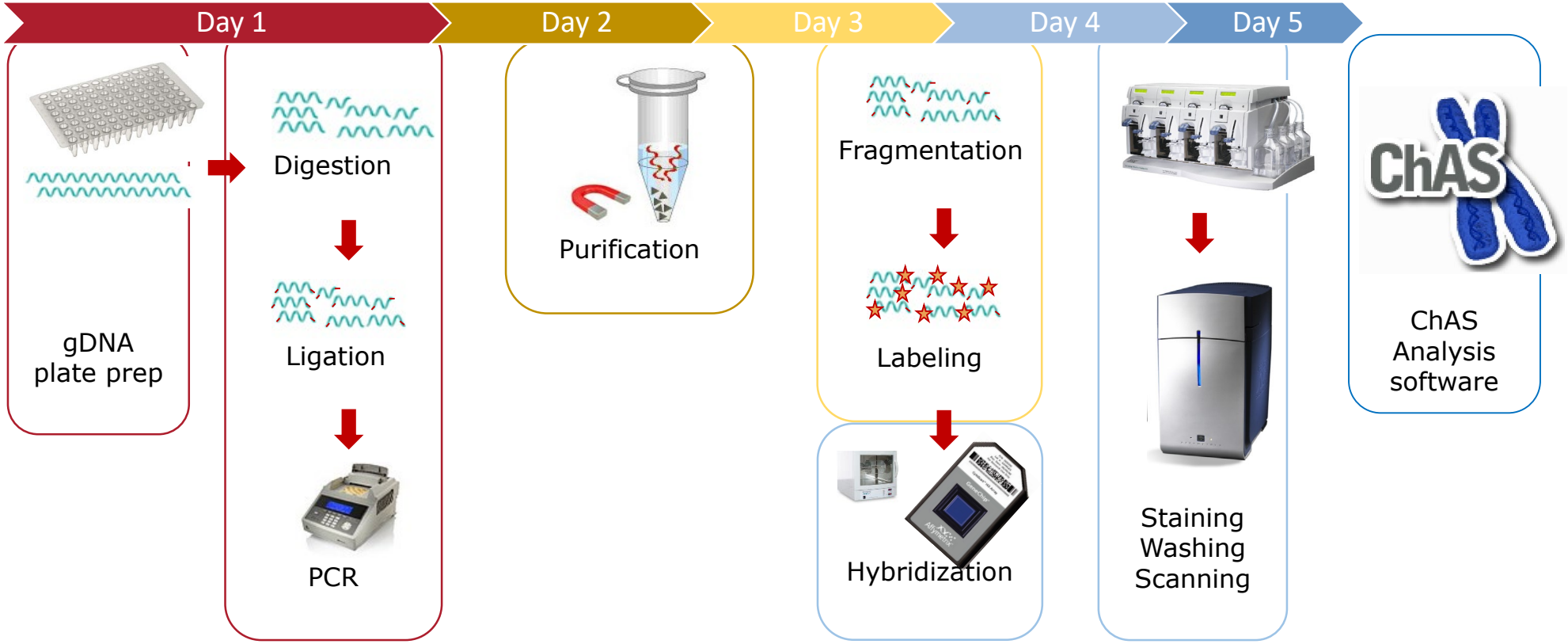
Genomic Microarray Technology



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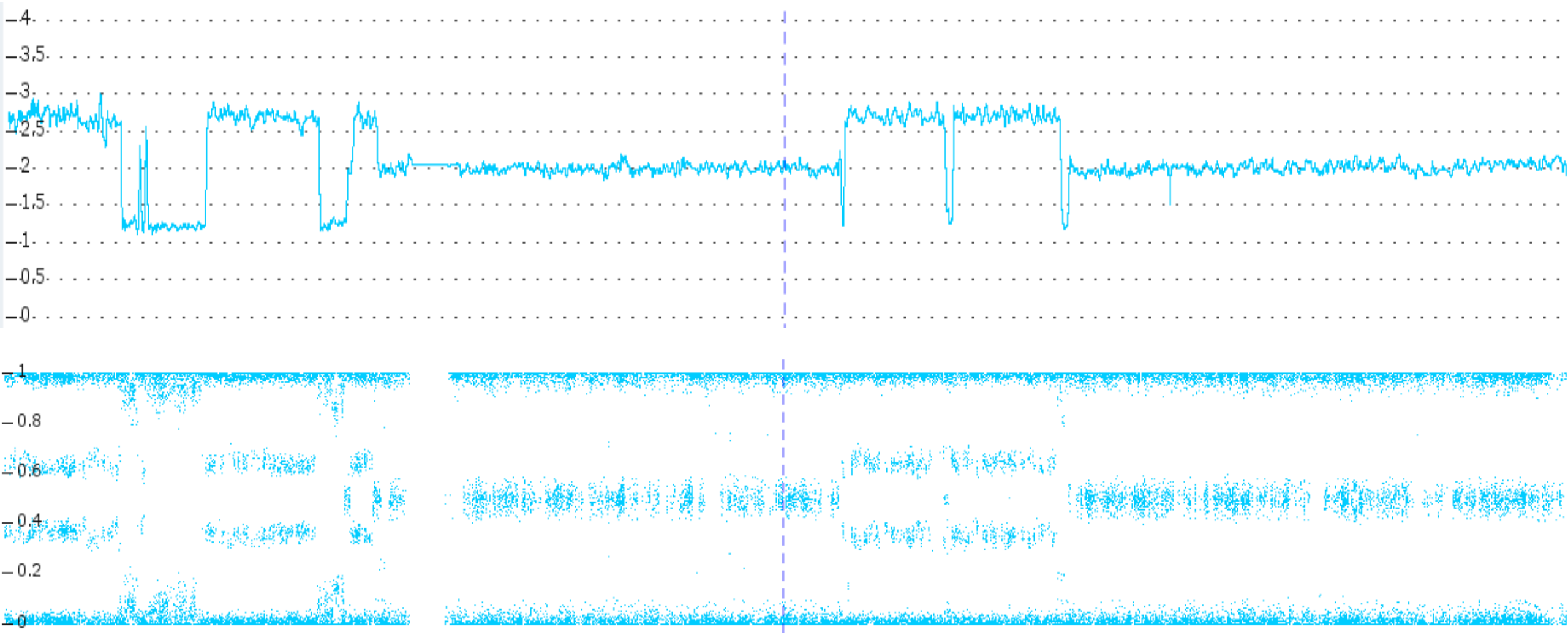
How to read the data?



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Cohort

- Method used: Affymetrix Chromosomal SNP microarrays
- Number of patients: 19, Age range: 13 to 75-year-old
- Regions of the brains: 35
  - frontal cortex: 8
  - occipital: 12
  - parietal: 1
  - cerebellum: 13
  - thalamus: 1
- Blood: 7/19 patients

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Preliminary data

Prion Disease	Sub type / age range	Number of cases	Sex
Sporadic	sCJD VV1 /[23-60 yo]	5	Male-4
			Female-1
	Fatal Insomnia (sFI) / [13-43 yo]	2	Male-2
			Female-0
Genetic	E200K MM / [57-66 yo]	3	Male-1
			Female-2
	E200K MV / [54-63 yo]	2	Male-2
			Female-0
Non- Prion Disease	Negative / [31-75 yo]	7	Male-3
			Female-4

Blood for germline = 7 and includes negative(3), SCJD VV1(2) , SFI(1), and E200K-MM(1)

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Preliminary result

- 17/19 cases were NORMAL (absence of rare copy number variants
- 2/19 cases showed abnormal findings
- Two genetic cases (E200K MM) showed a gain of the X chromosome

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Case 1

- Female
- Age of onset: 57-year-old
- Age of Death: 58-years-old
- Disease course: 8 months
- Subtype: E200K- MM
- Tissue tested: cerebellum

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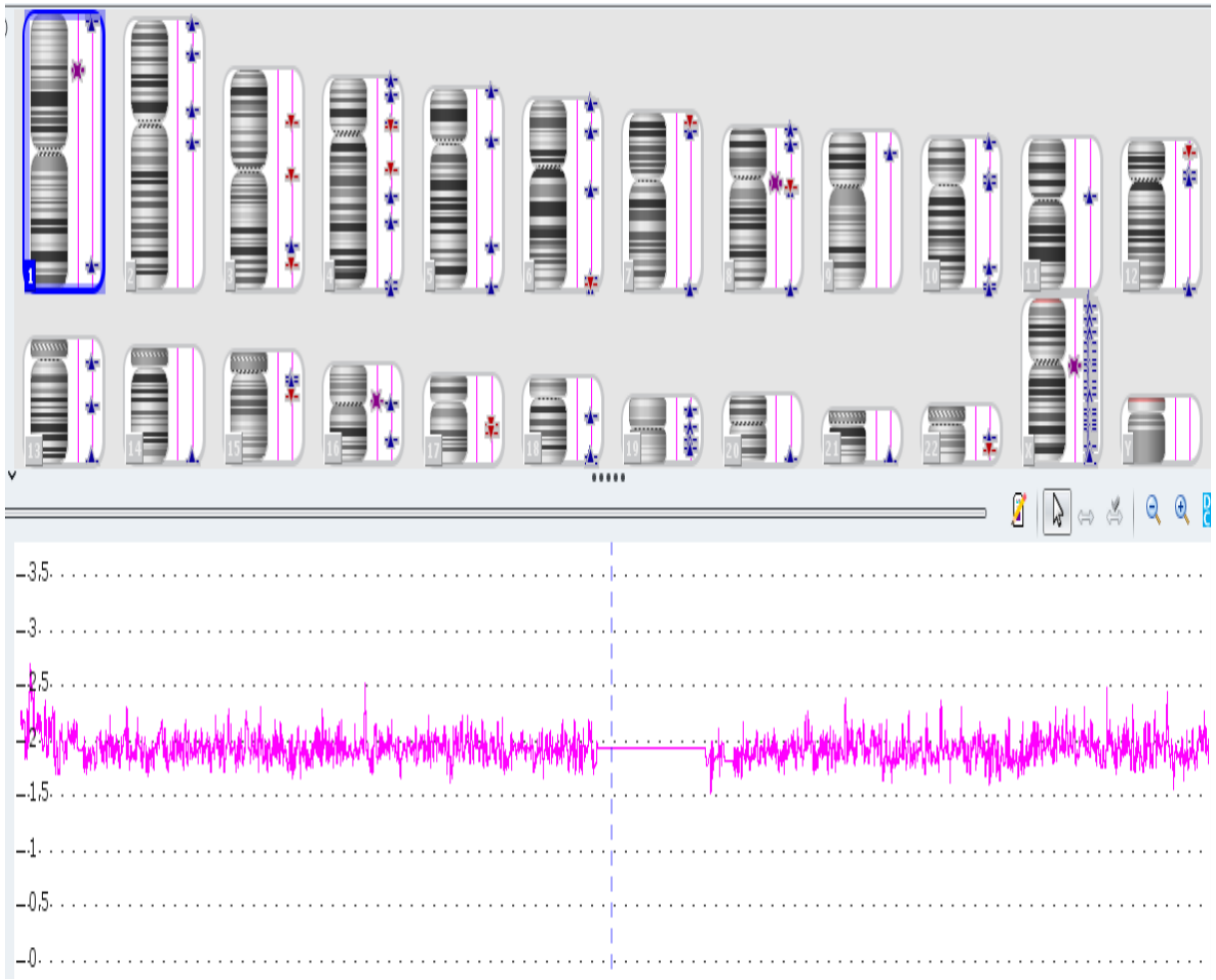
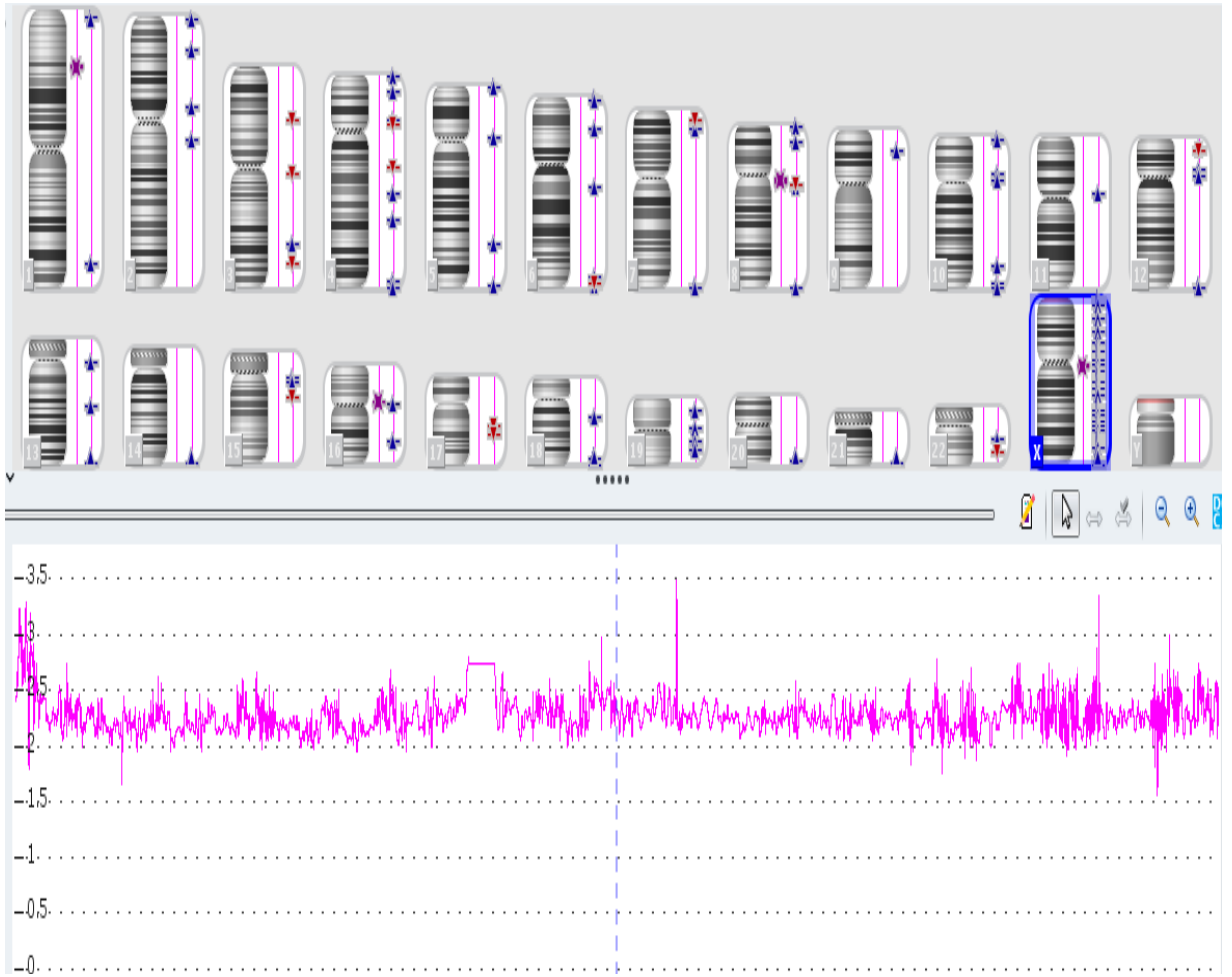
Cerebellum



Normal copy for chromosome 1



Gain of chromosome X

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Case 2

- Female
- Age of onset: 66 years
- Age of Death: 66 years
- Disease course: 1 month
- Subtype: E200K-MM
- Tissue tested: frontal cortex, occipital, cerebellum and blood

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Frontal Cortex

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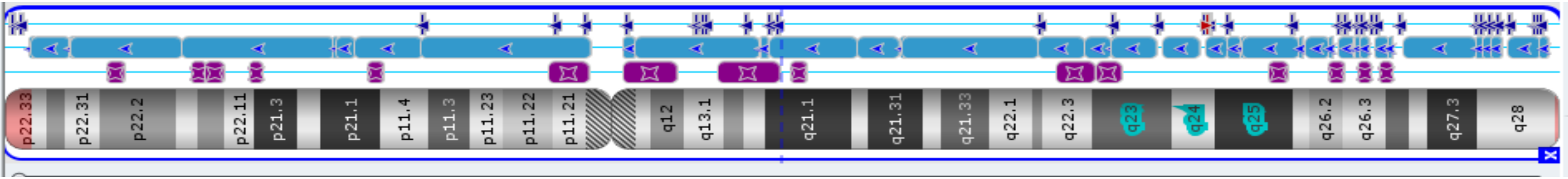
Occipital Cortex



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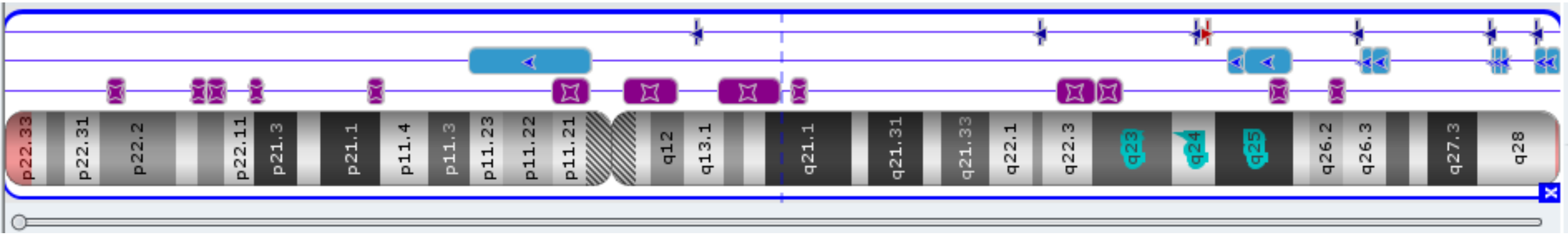
Cerebellum



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Blood

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Gain of the X Chromosome

- Is gain of the X chromosome age-related?
- Could it be associated with E200K subtype and modify the risks?
- Expression of genes on the X chromosome is reduced in neurodegenerative disorders
- Could a gain of the X chromosome increase expression of genes and have a protective effect?
- Interestingly, loss of Y was not seen in males in this cohort

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Work in Progress

Prion Disease	Sub type/ age range	Number of cases	Sex
Sporadic	sCJD VV1 [32 yo]	1	Male-0
			Female-1
	Fatal Insomnia (sFI) [18-37 yo]	3	Male-3
			Female-0
Genetic	E200K MM [42-50 yo]	3	Male-2
			Female-1
	E200K MV [45 yo]	1	Male-1
			Female-0
	E200K VV [46 yo]	1	Male-1
			Female-0
	Fatal Familial Insomnia (FFI) [24-48 yo]	5	Male-3
			Female-2
	Gertsmann Straussler Scheinker (GSS) [37-47 yo]	5	Male-2
			Female-3
Non- Prion Disease	Negative [29-39 yo]	2	Male-2
			Female-2

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Functional studies

Aim 3: Presence of CNVs in the brain will be confirmed by functional studies

- *Proteins encoded by genes identified by SNP array will be assessed for reduced or overexpression using immunohistochemistry and Western blot analyses*
- *Depends on data from specific aims 1 and 2*

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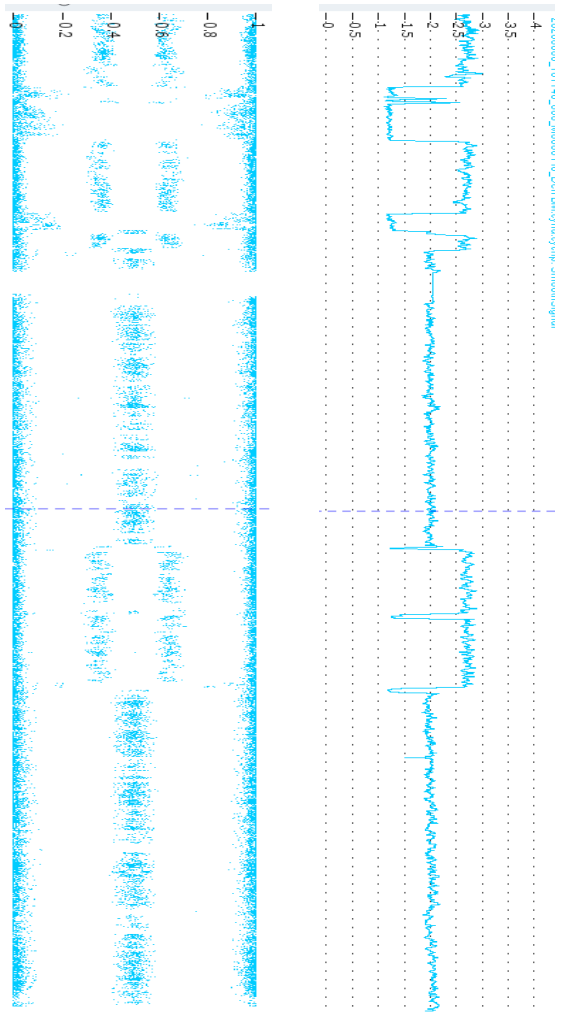

CNVs in genetic disease

SUMMARY

By using whole genome chromosomal SNP array, we can assess copy number variants (CNVs) in sporadic and genetic prion disease.

Future goals

- Test the impact of CNVs as a phenotype modifier in prion disease
- Test the impact of CNVs as a genetic risk factor in younger age-onset

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Genetics terminology

- Polymorphism (normal variation)
- Mutation or pathogenic variant (genetic change causing disease)
- Penetrance (extent to which gene is expressed)
- Complete penetrance (will show symptoms or develop disease)
- Incomplete / reduced penetrance (does not develop disease)
- Phenotype (observable properties)
- Genotype (genetic constitution)
- Mosaicism (genetically different cell types present; normal and abnormal)
- Germline (present at birth in germ cells and seen in all tissues)
- Somatic (develops later in life (post-conception)- can be very early or late and limited to specific tissue types)

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