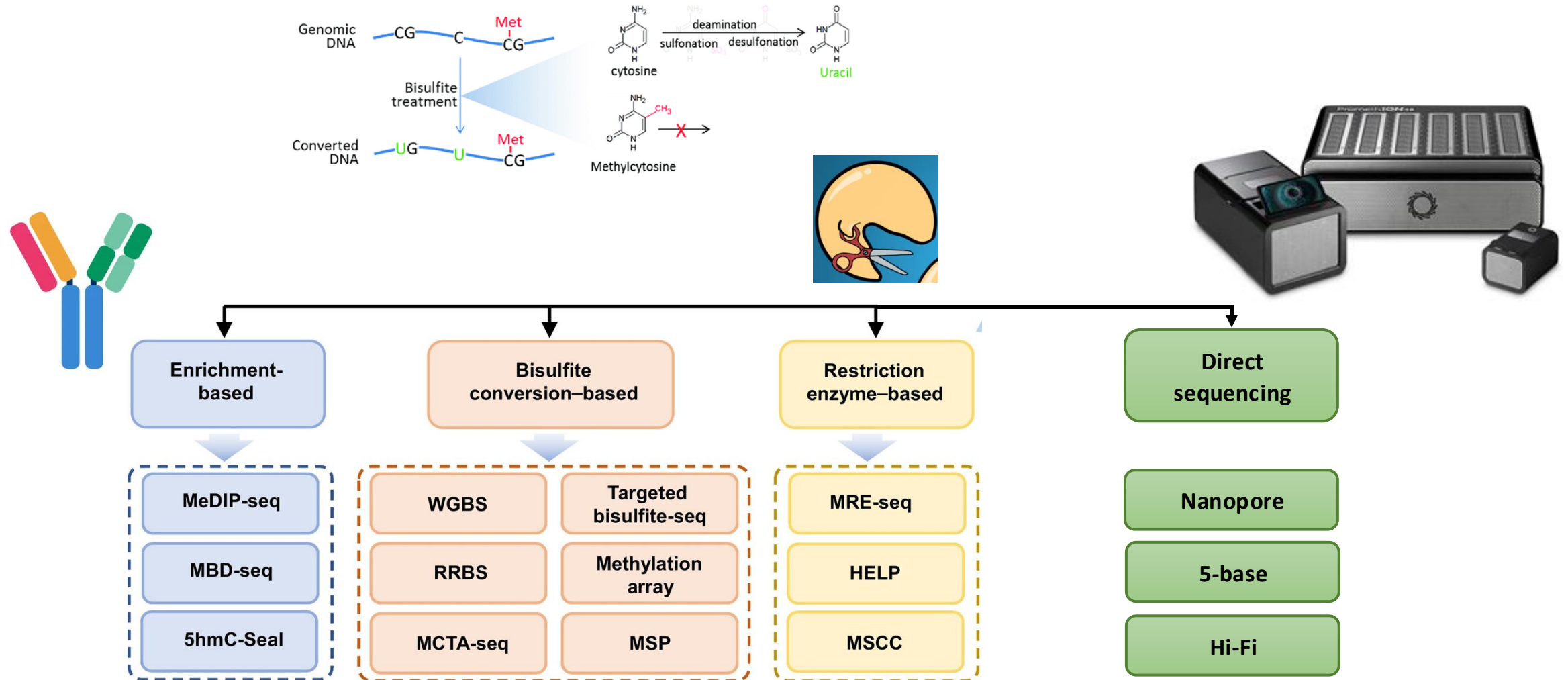


# DNA methylation profiling for diagnosing rare genetic disorders

**Siddharth.Banka@manchester.ac.uk**

# Technologies for studying DNA methylation status



# Episignatures as diagnostic biomarkers for VUS

~28 million CpGs in human genome

~1 million probes on Illumina Epic array

Few thousand differentially methylated points in disease v control

Few dozens to hundreds DMPs selected for developing episignature

Test patient sample with VUS

Positive

Intermediate

Negative

NHS  
Manchester University  
NHS Foundation Trust

Our Hospitals The Trust Case Studies

Whole genome  
methylation  
profiling for rare  
disease

Age, sex, ethnicity, cellular composition of the tissue (blood), number of samples (disease v controls), types of variants

Episignatures for >150 diseases have been defined so far. Several available diagnostically.

## Advantages of using DNA Methylation Signature as a diagnostic biomarker for VUSs

Biomarkers tend to be -

- **Not available** for a vast majority of genes
- **Invasive** and/or **expensive**
- **Not validated** diagnostically
- Require **different lab equipment/experimental set-ups/expertise**

- **Episignatures**

- **Non-invasive** (stored DNA extracted from peripheral blood can be used).
- **Single platform for ~100 disease-genes** currently (and the list is growing).
- **Reasonably high specificity and sensitivity** (awareness of background and limitations).

# Episignatures

Positive

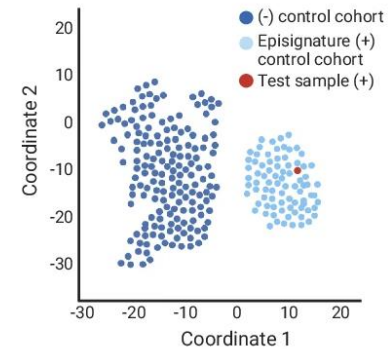
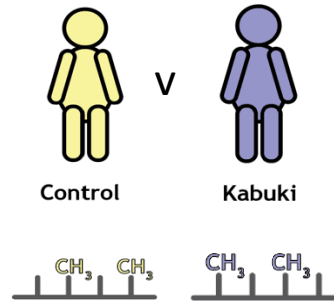
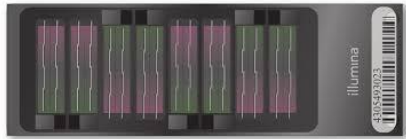
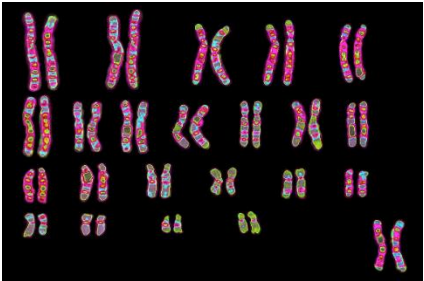
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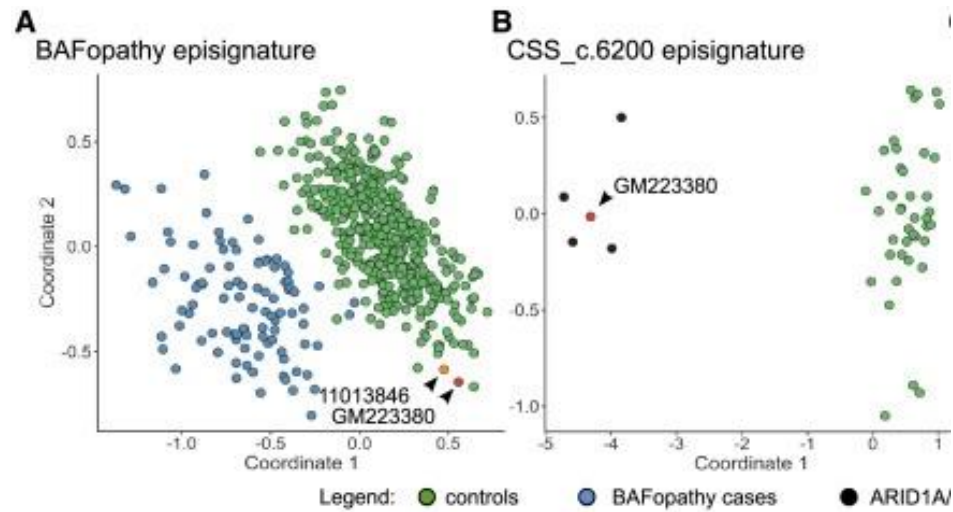


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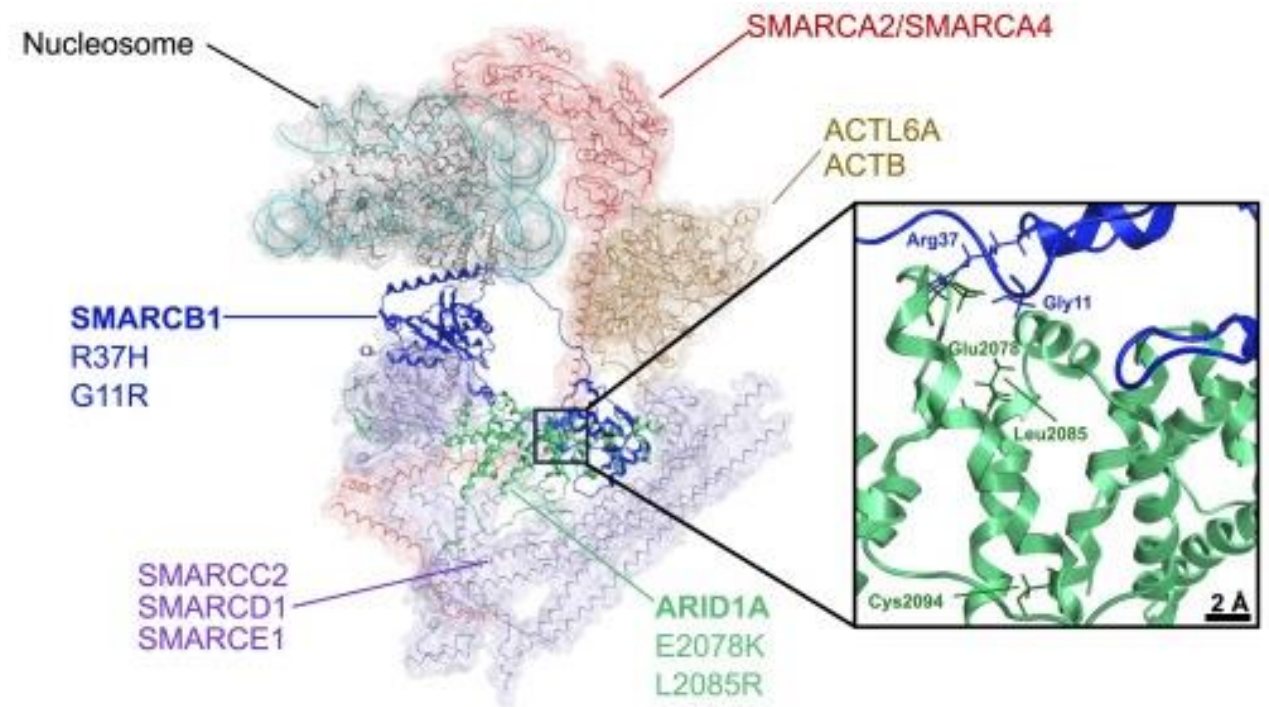
# Positive episignature but not as expected



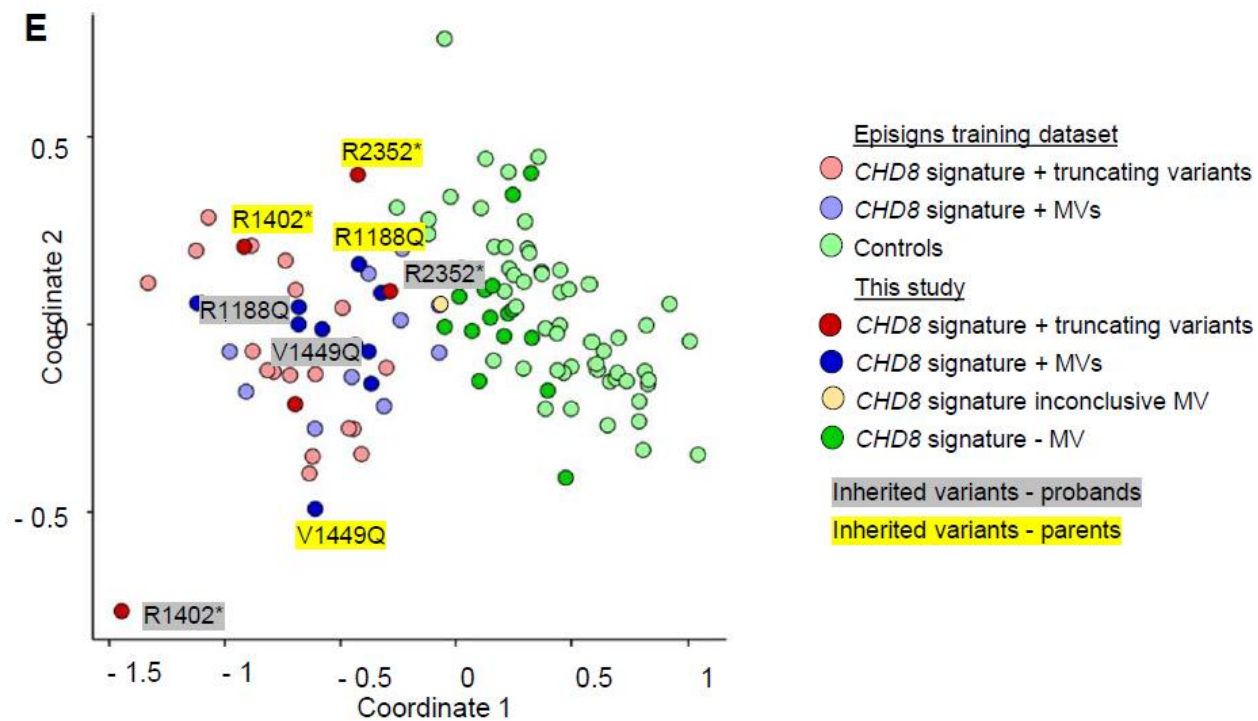
GM223380



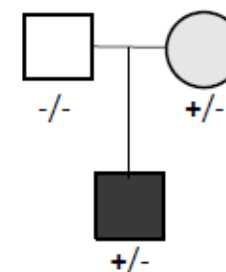
*SMARCB1*  
p.(Arg37His)



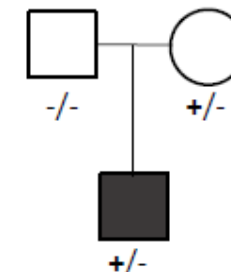
# CHD8-IDDAM DNAm cannot predict non-pentrance



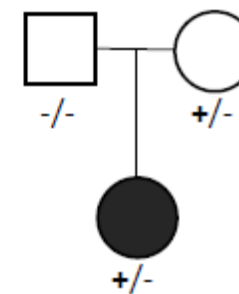
c.4346T>A p.(Val1449Glu)



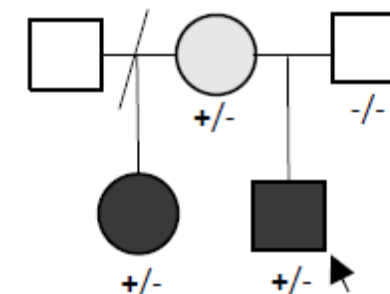
c.3563G>A p.(Arg1188Gln)



c.7054C>T p.(Arg2352Ter)



c.4204C>T p.(Arg1402Ter)



**) Disease causing LoF variants**



# Episignatures

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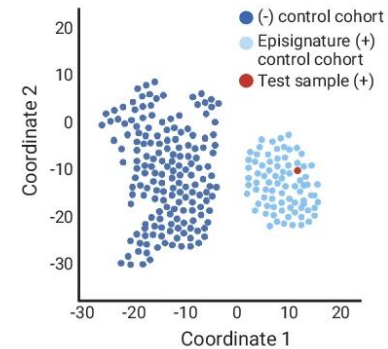
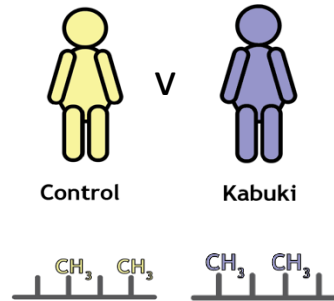
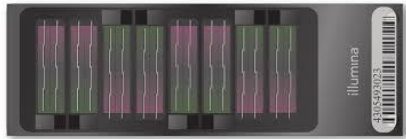
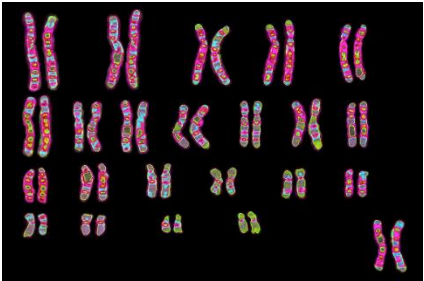
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## A Discovery Cohort



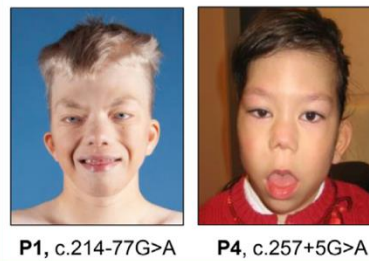
## B Validation Cohort



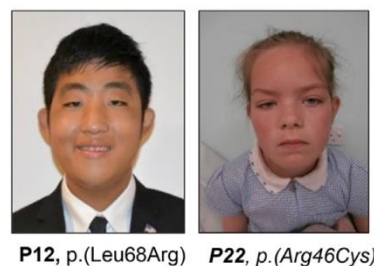
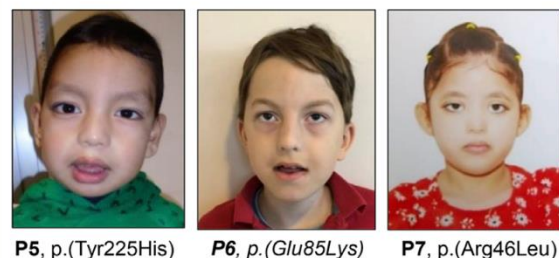
## F



## C Test Cohort – Positive DNAm signature

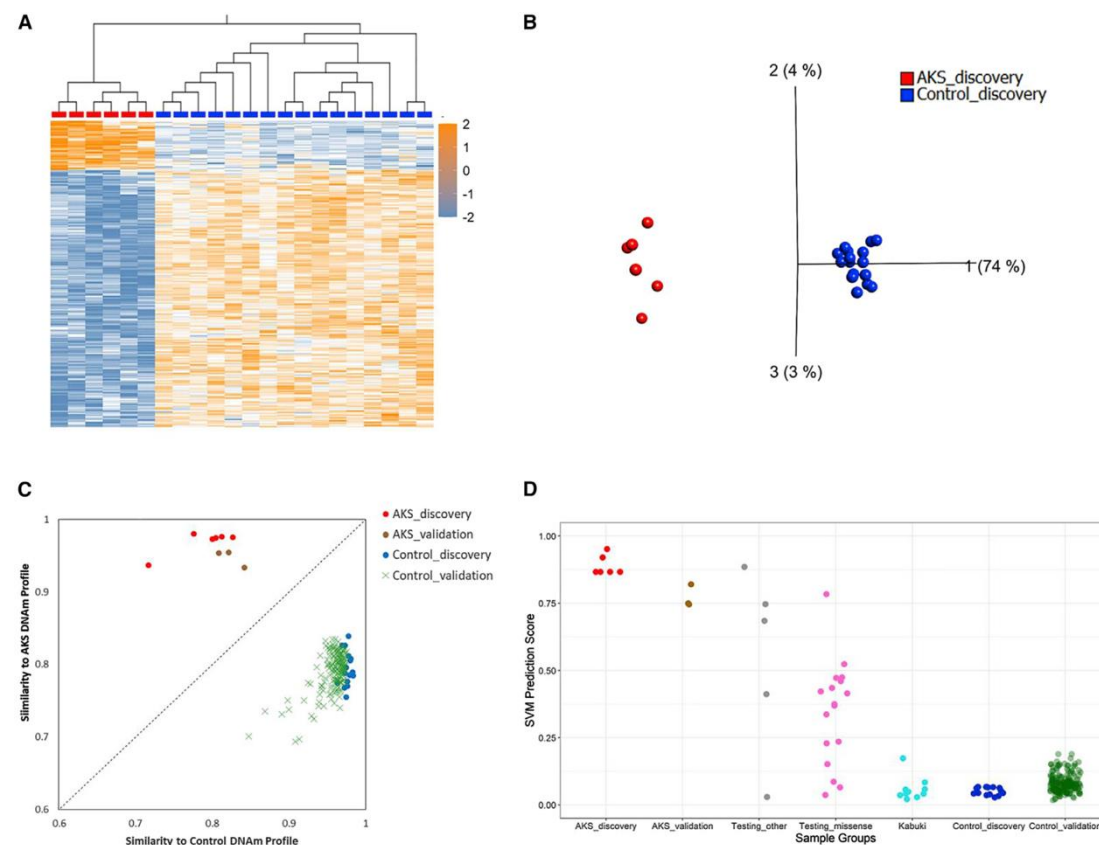


## D Test Cohort – Intermediate DNAm signature



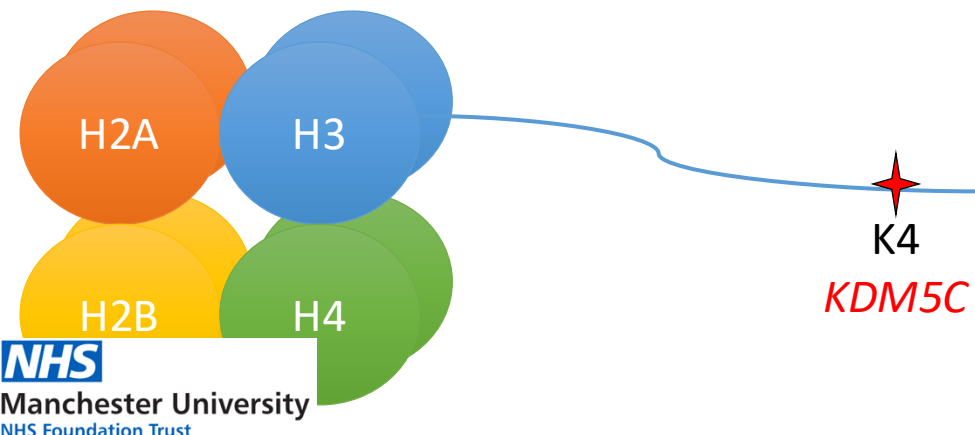
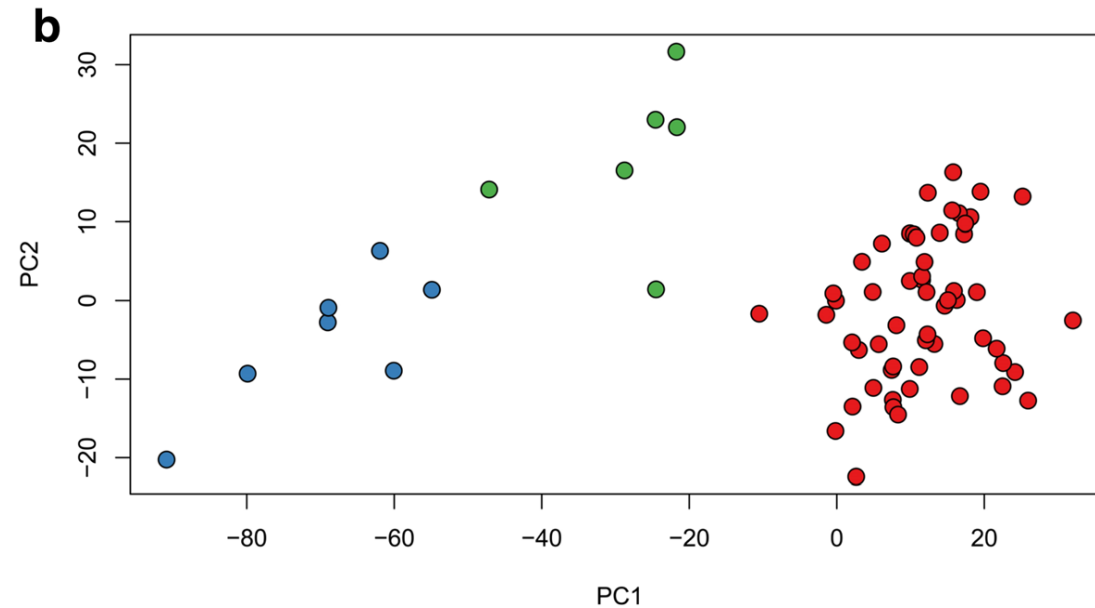
# An *HNRNPK*-specific DNA methylation signature makes sense of missense variants and expands the phenotypic spectrum of Au-Kline syndrome

Sanaa Choufani,<sup>1,48</sup> Vanda McNiven,<sup>2,3,48</sup> Cheryl Cytrynbaum,<sup>1,2</sup> Maryam Jangjoo,<sup>1</sup> Margaret P. Adam,<sup>4</sup>



What are other possible scenarios where one could get an 'intermediate' episignature?

# Intermediate signatures can be found in carriers of some diseases



# Episignatures

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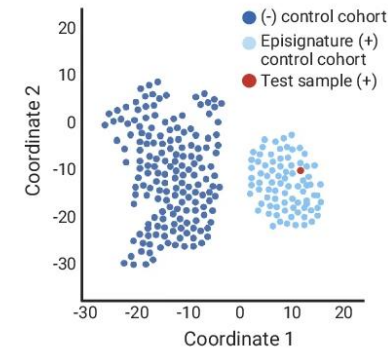
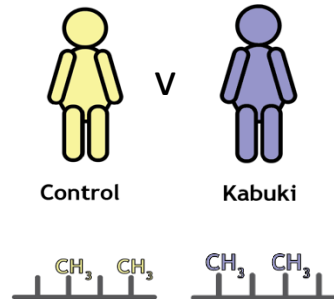
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Negative

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Whole genome  
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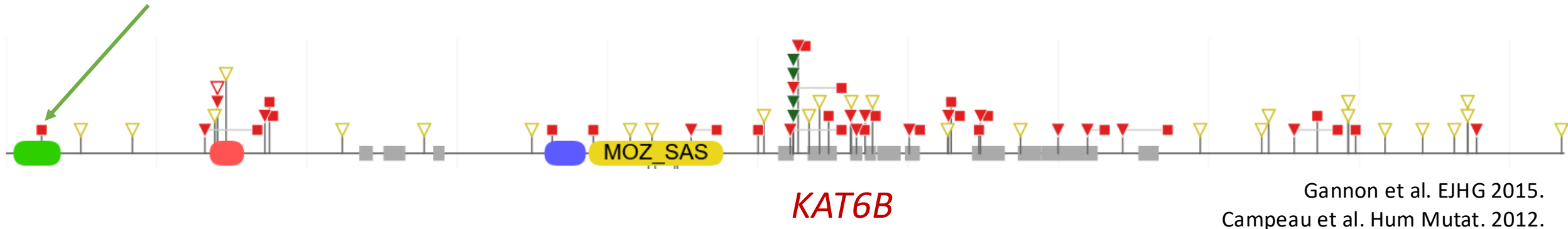


What are possible scenarios where one could get an unexpected 'negative' episignature?

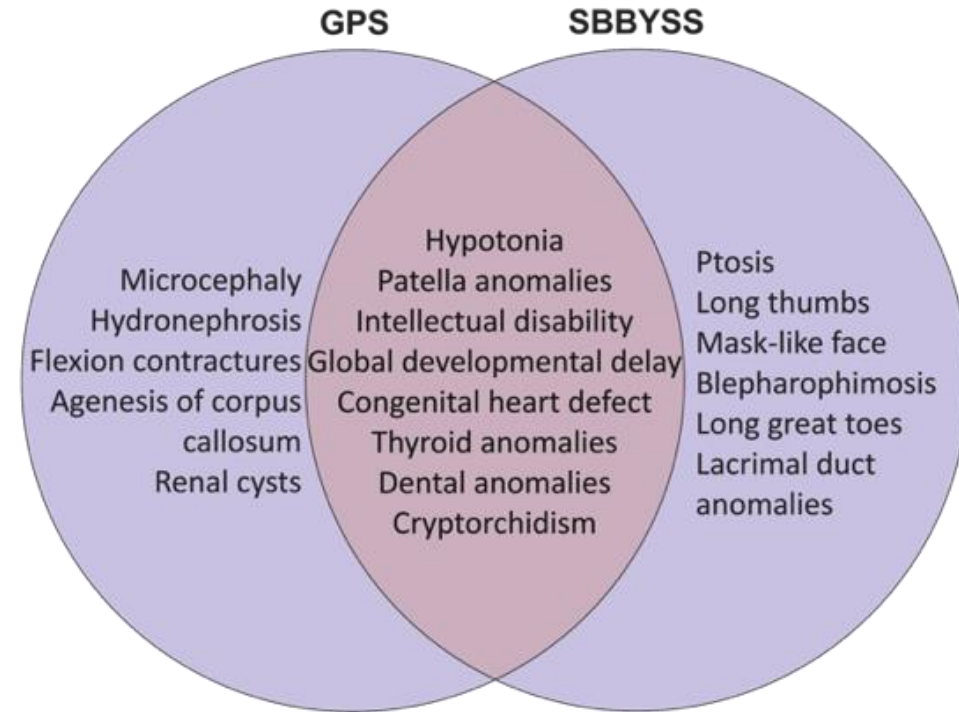
Which syndrome is associated with KAT6B?

Does her phenotype match the syndrome?

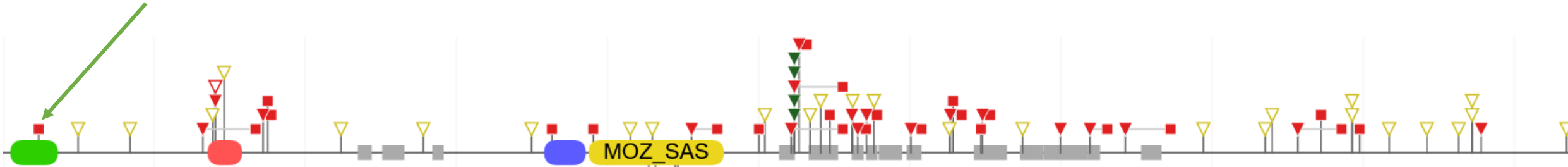
*De novo* Glu49LeufsTer



Gannon et al. EJHG 2015.  
Campeau et al. Hum Mutat. 2012.



*De novo* Glu49LeufsTer

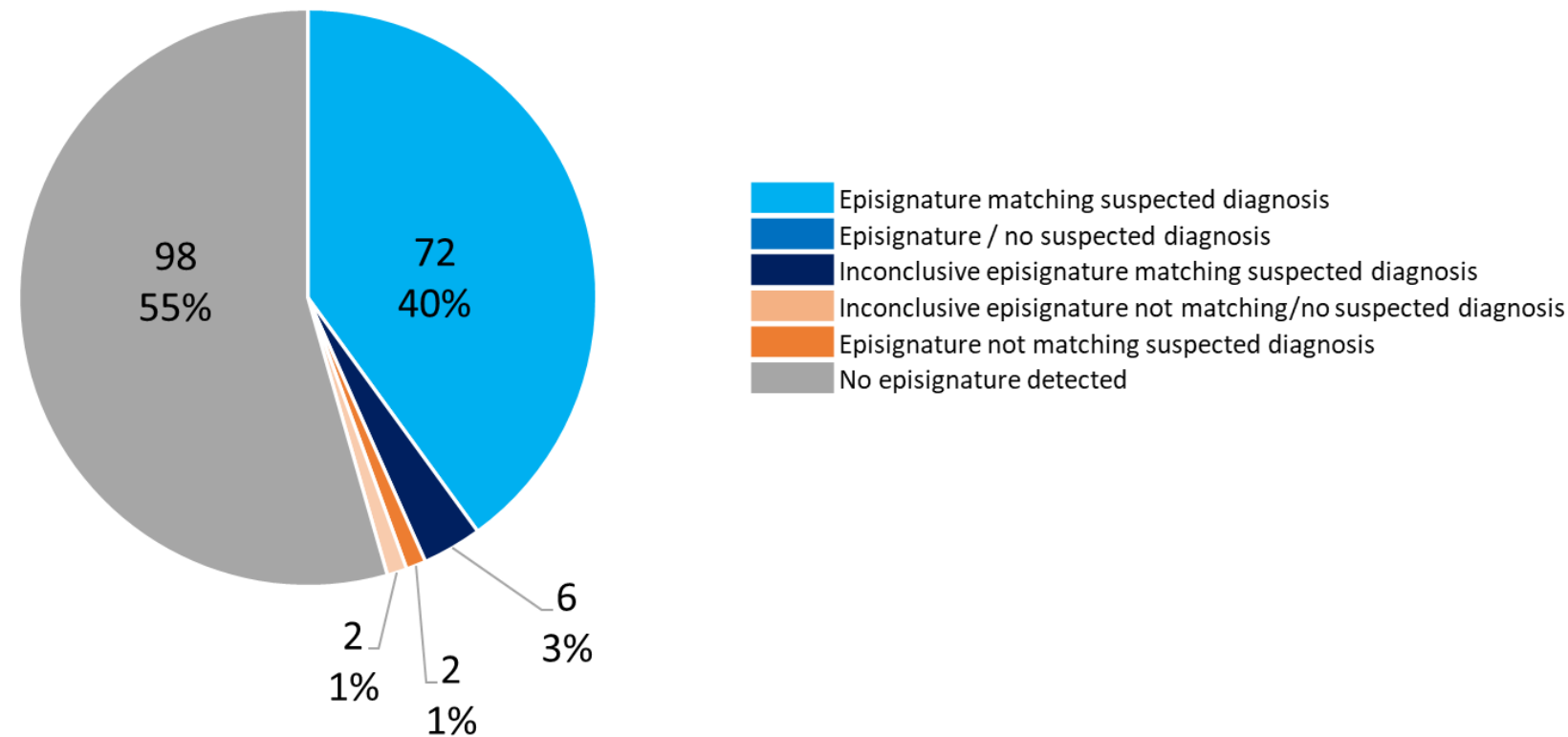


**KAT6B**

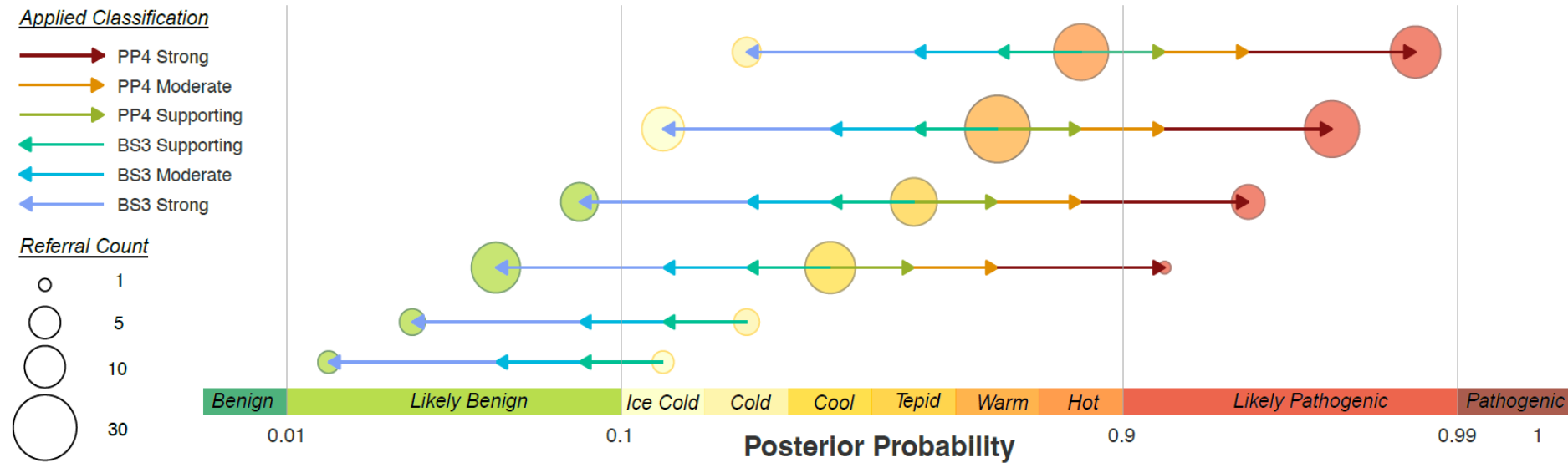
Gannon et al. EJHG 2015.  
Campeau et al. Hum Mutat. 2012.

# DNA methylation based diagnostic testing improves diagnosis of rare diseases

NHS referral with VUS declared (n=180)

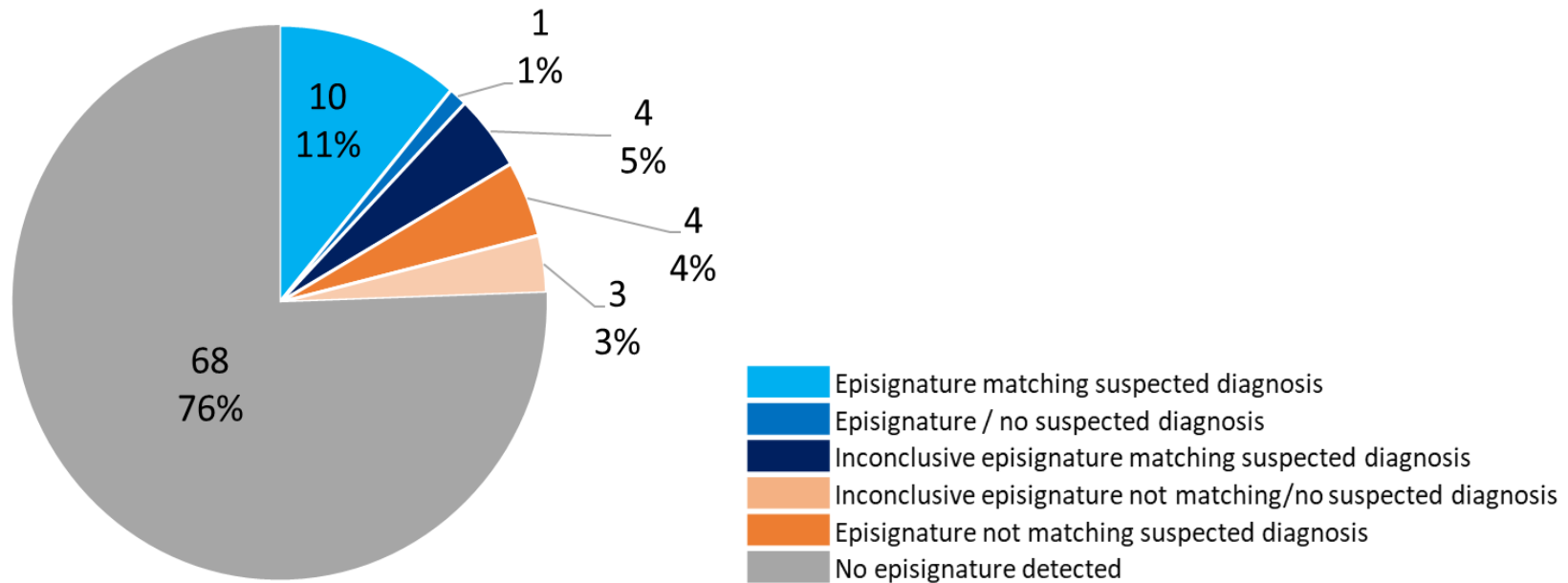


# Guidelines and pathway for incorporating episignatures in VUS interpretation

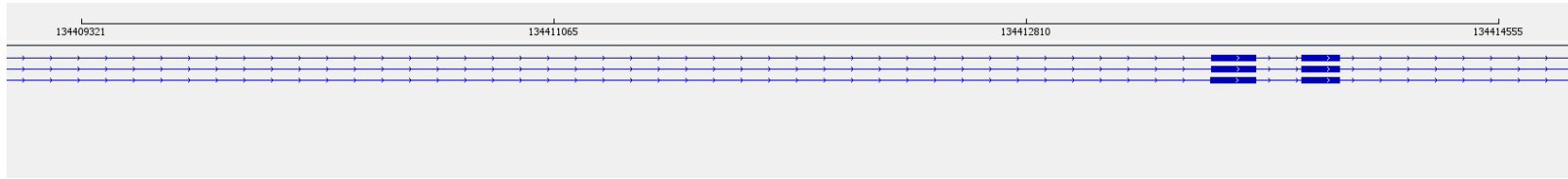
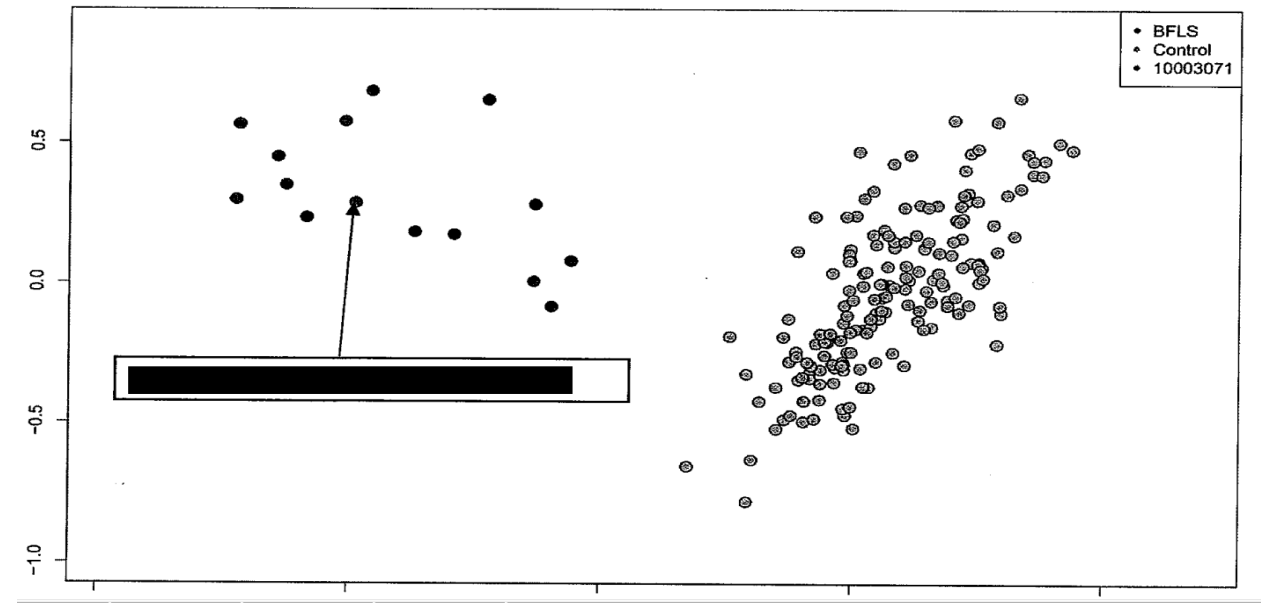


# Episignatures can also be useful for 'Non-VUS cases'

NHS referral without VUS (n=90)

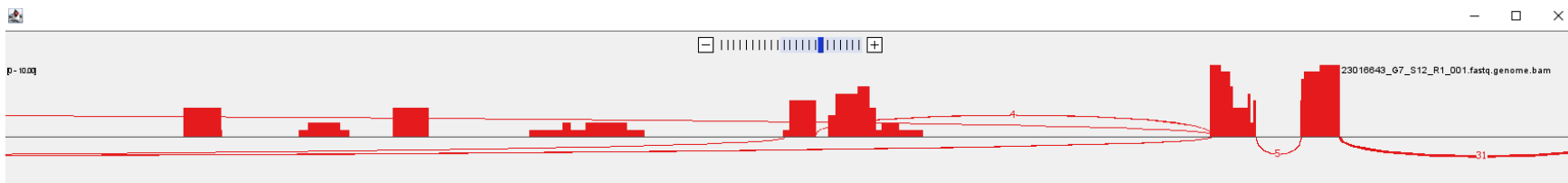


- Developmental delay
- Muscle hypotonia, lower limb rigidity/spasticity, muscle wasting over thighs, flexion contractures of left knee
- Nystagmus
- Short stature, treated with growth hormone and testosterone – resulted in scoliosis
- Gastro-oesophageal reflux
- Negative trio exome via Deciphering Developmental Disorders Study and negative 100,000 Genomes Project results

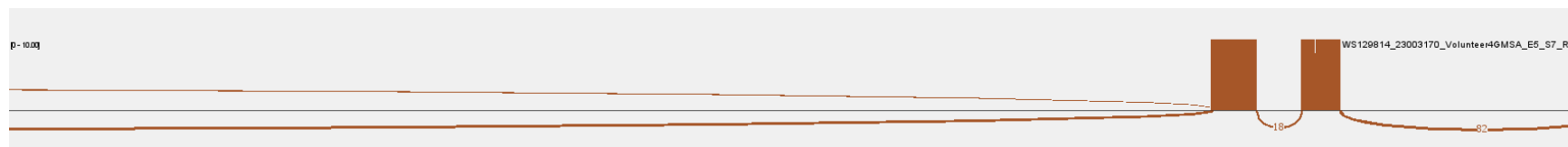


De novo *PHF6*:c.419-1454C>T  
detected on targeted reanalysis

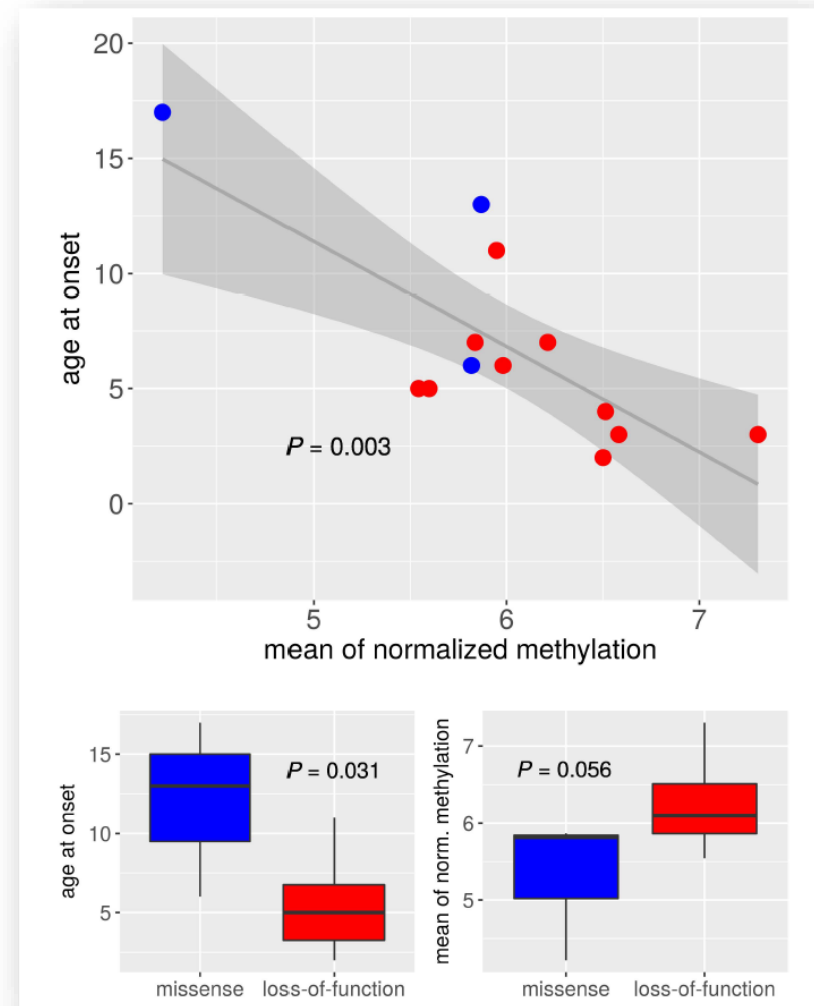
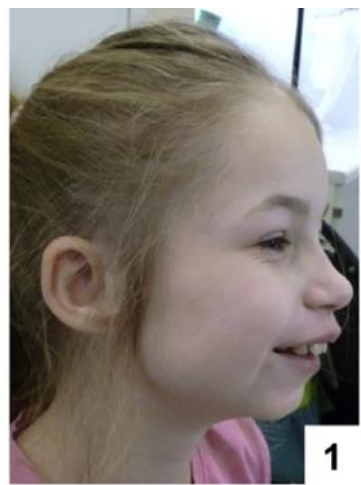
Patient



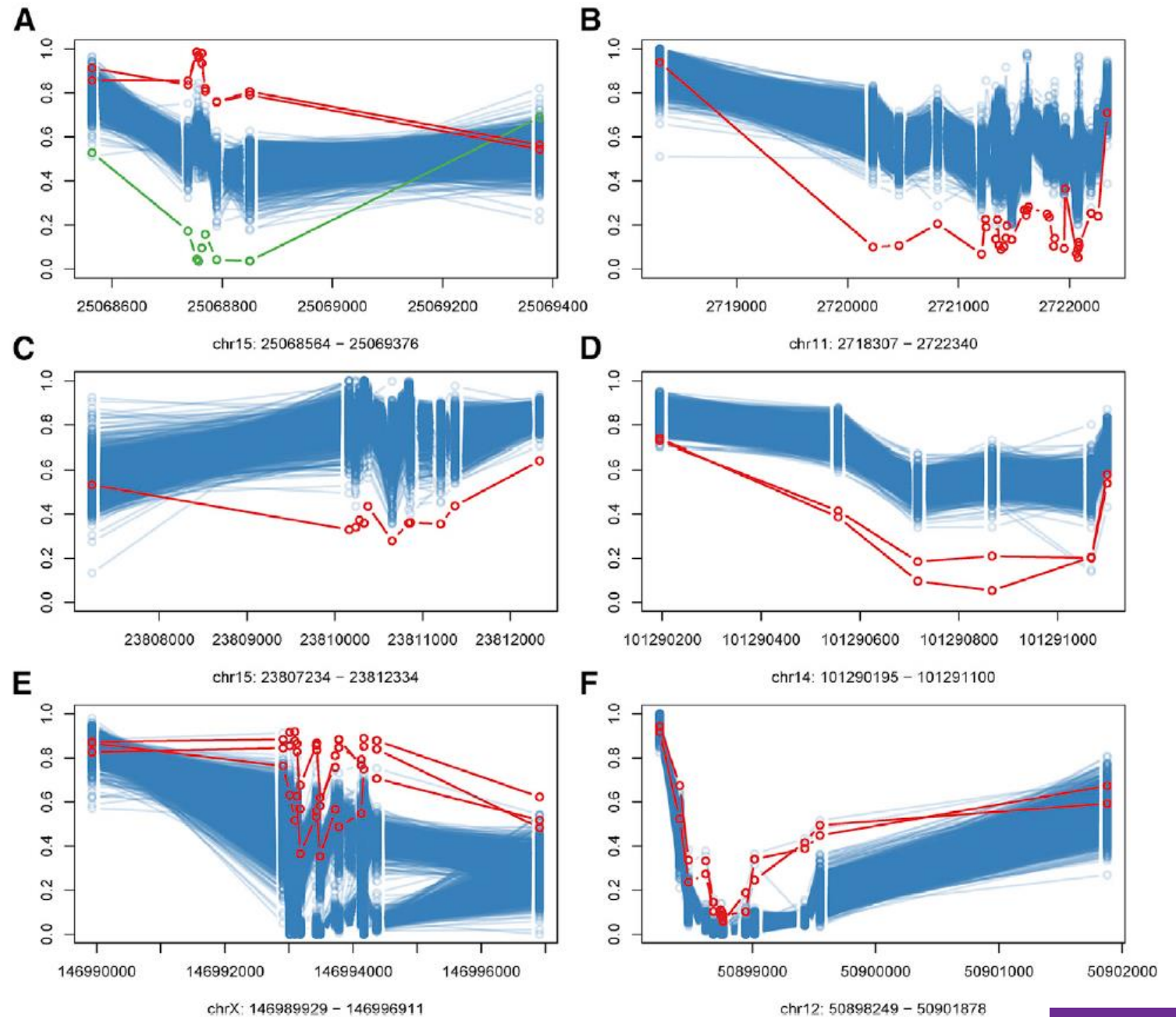
Control

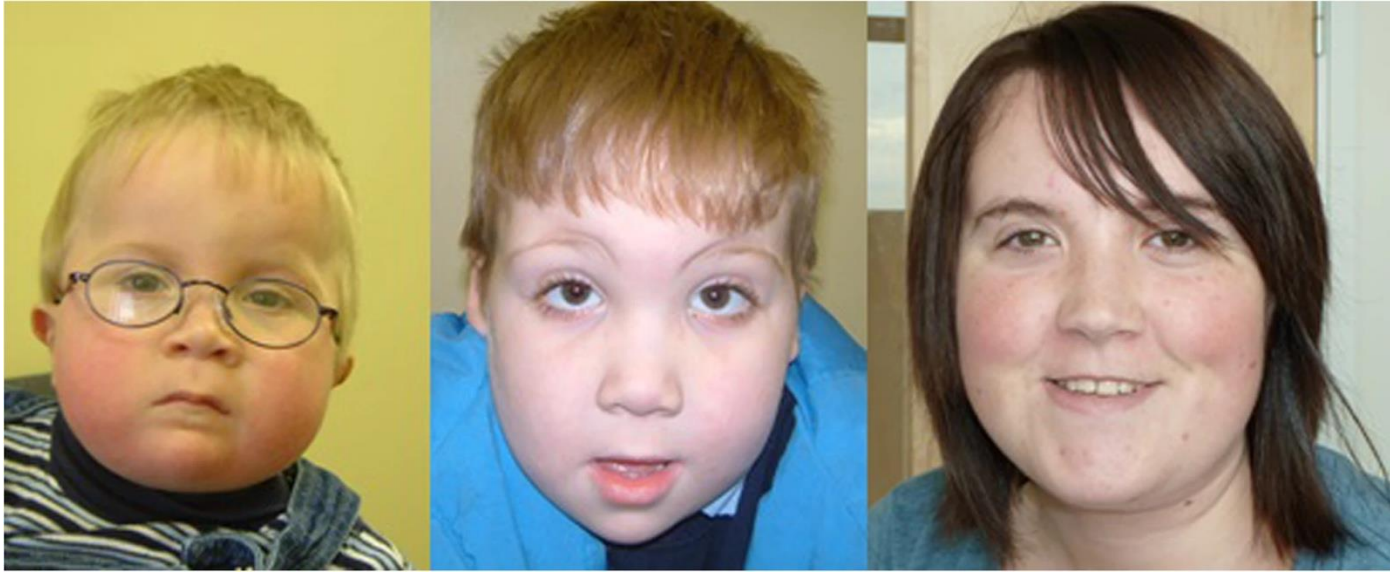


# Blood DNAm as a biomarker to predict disease onset



DNAm arrays can  
also be used to  
identify  
Epivariants

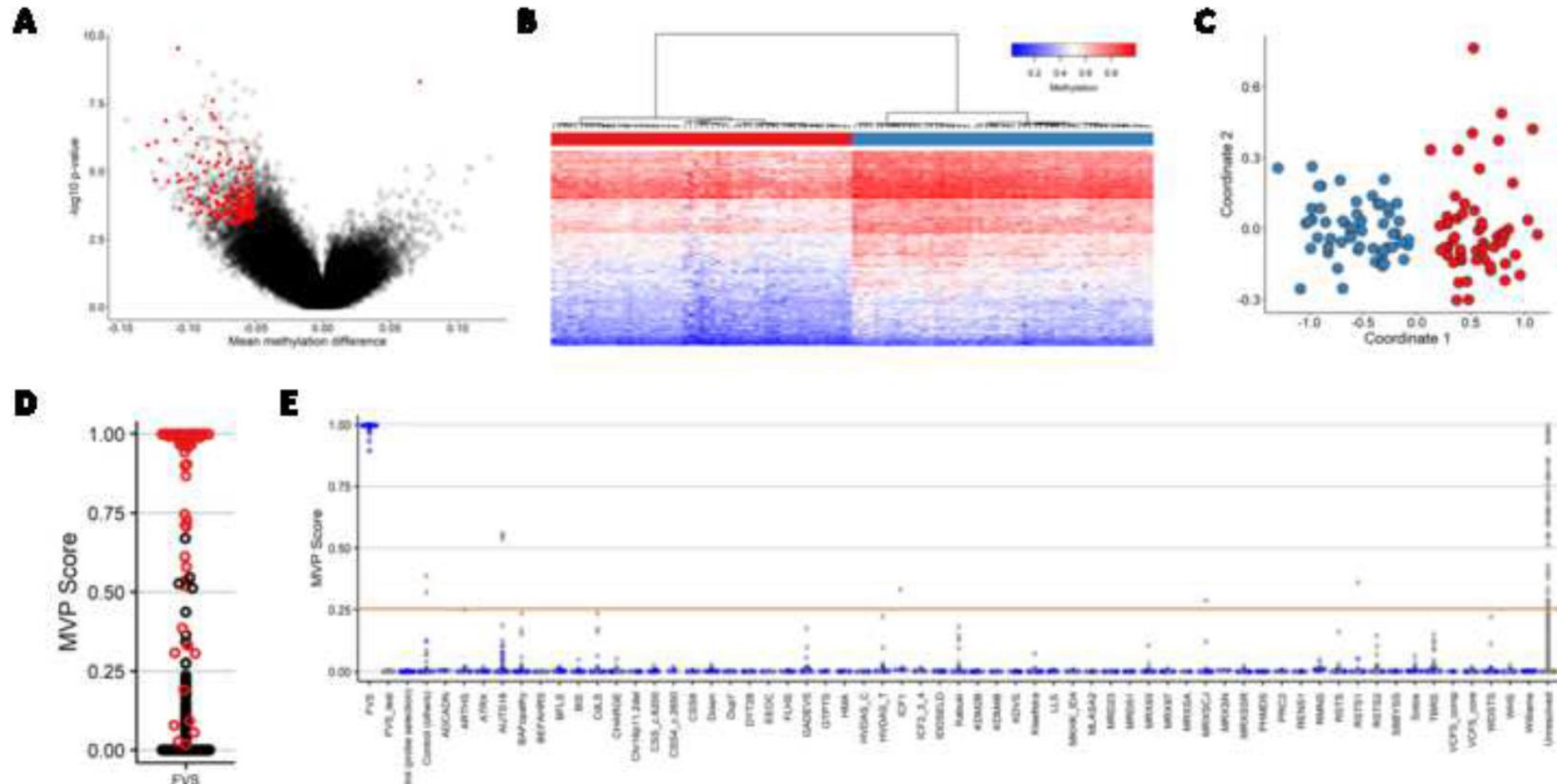




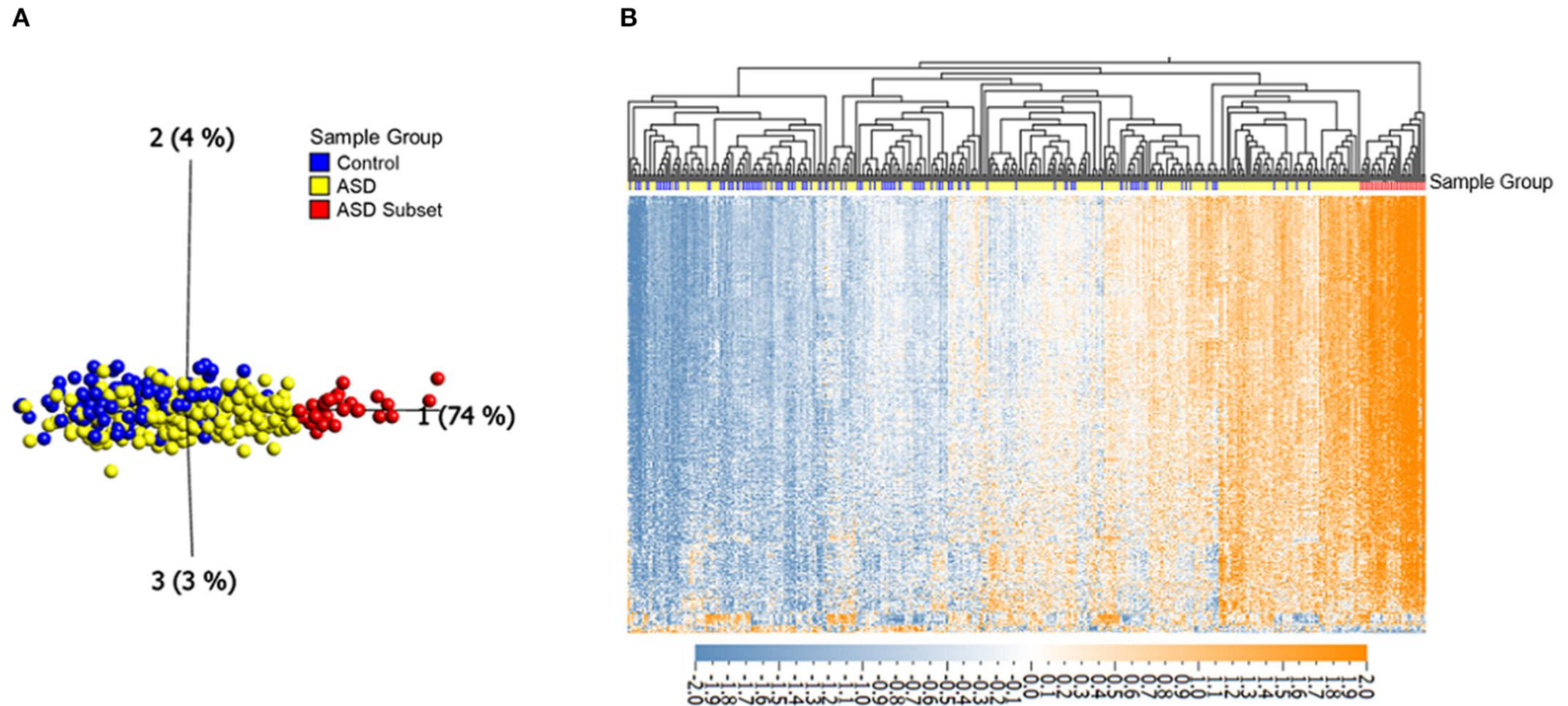
- Flat philtrum, thin upper lip, full, everted lower lip, short anteverted nose, small mouth, epicanthic folds, neat arched eyebrows, broad nasal root
- Cognitive difficulties, social communication difficulties, autism spectrum disorder
- Spina bifida
- Congenital cardiac defect
- Cleft palate
- Metopic suture synostosis
- Radial ray defect
- Genitourinary malformations
- Laryngomalacia/stridor
- Joint laxity
- Talipes requiring surgery
- Digital anomalies
- Ophthalmological anomalies
- Enuresis/poor bladder control



# Episignatures for environmental exposures

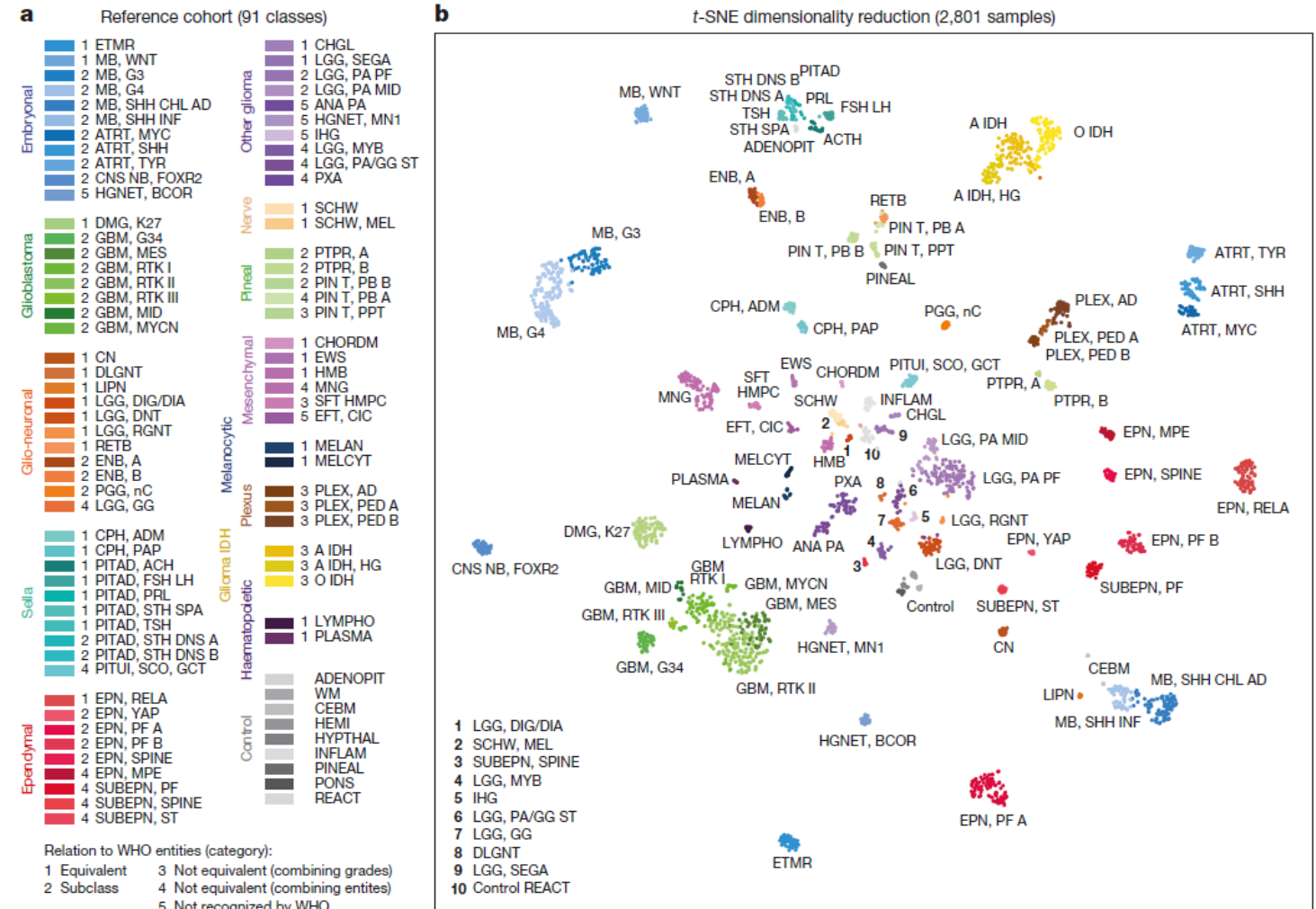


# DNA methylation profiles for stratifying multi-factorial diseases

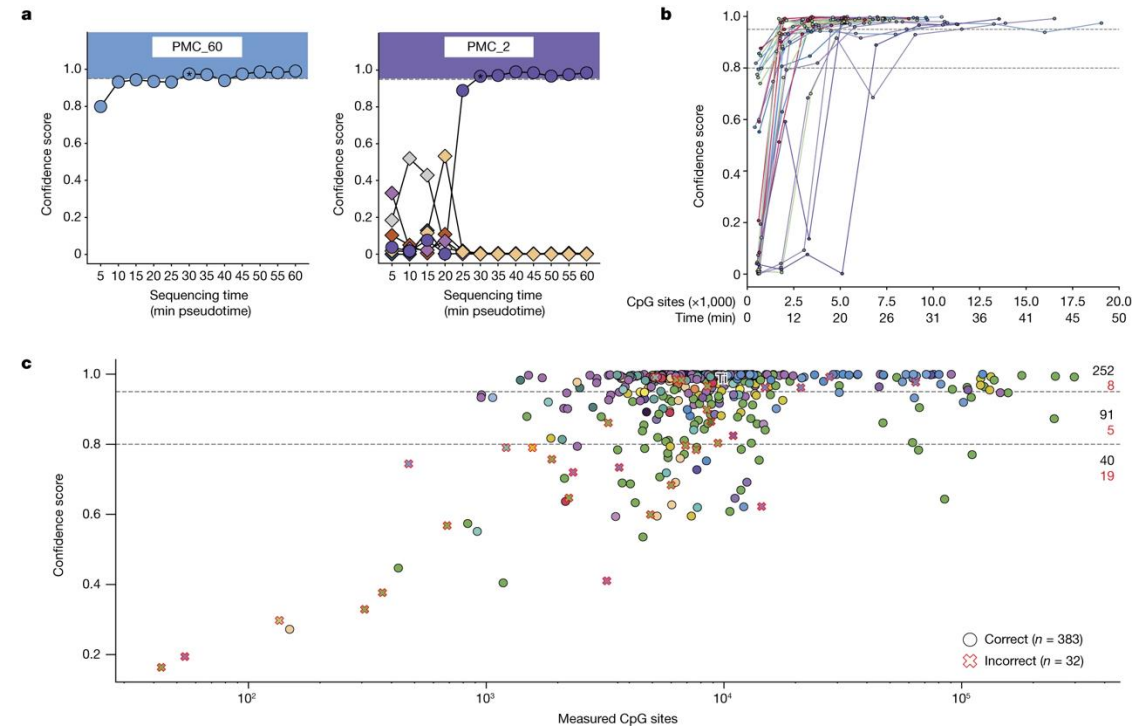
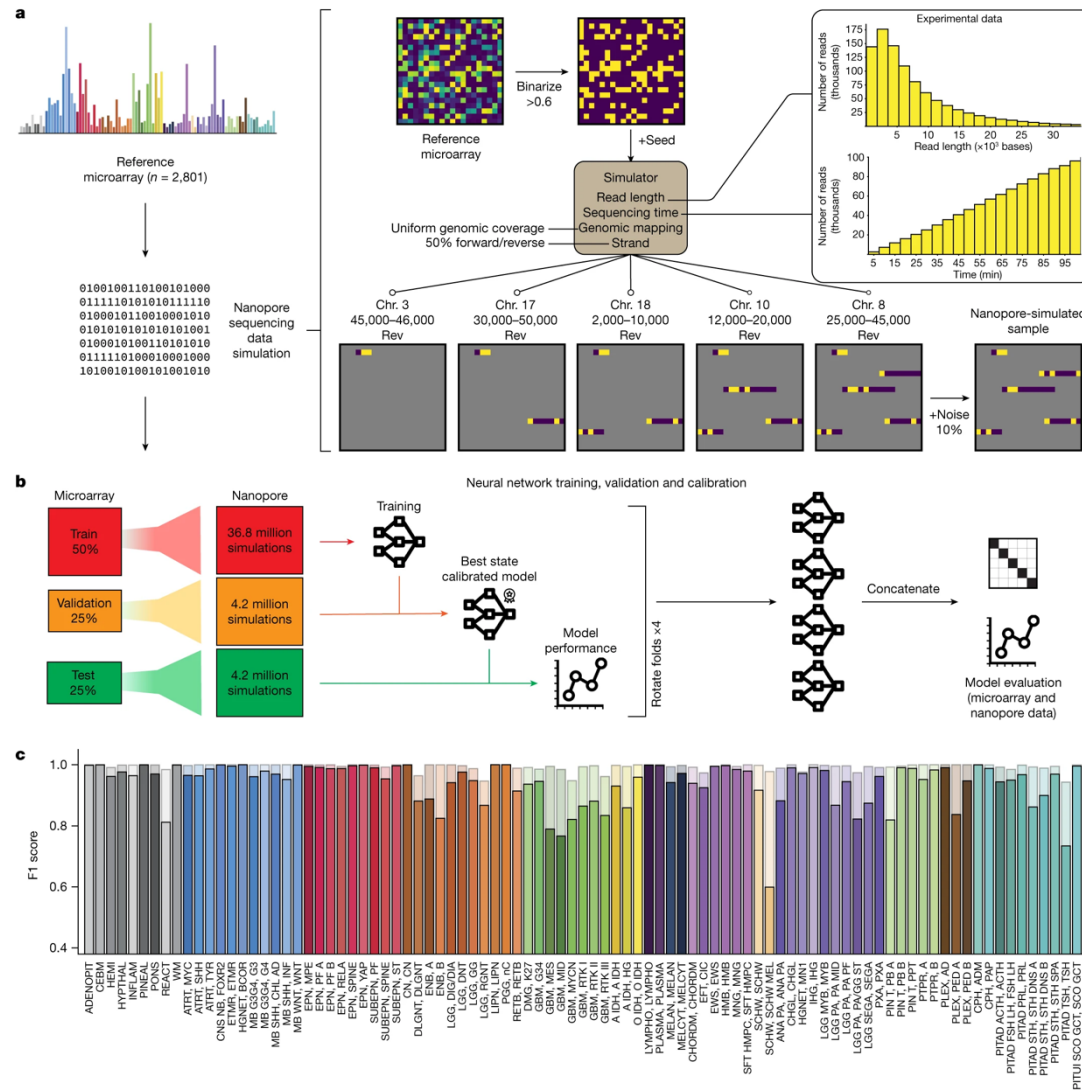


# DNAm based classification of tumors

- Represents known histopathological types
- Enables sub-classification of tumor types
- Potentially novel tumour categories identified



# Ultra-fast deep-learned CNS tumour classification during surgery using Nanopore DNAm data



# Take home messages

- DNAm profiling (episignature) is a biomarker that can improve diagnosis for rare disease patients with or without VUS.
- Additional rare disease clinical applications such as biomarkers for environmental exposure, disease severity, penetrance, therapy response, stratification may develop further in future.
- Interpretation with caution...