Nottingham University Hospitals NHS
NHS Trust

Clinical exome sequencing in patients with rare genetic disease found to have loss of heterozygosity by microarray

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Introduction

- Approximately **8% of children will have a genetic disorder**
- In up to **50% of cases a genetic diagnosis will not be made**
- There may be a lengthy **diagnostic odyssey** involving a large number of expensive and unnecessary tests before a diagnosis can be confirmed
- Rare disorders can be **hard to identify** based on the patient's phenotype alone

Benefits of a genetic diagnosis:

- Reduced anxiety for the family
- Improved chance of finding support groups
- Prenatal diagnosis (PND), pre-implantation genetic diagnosis (PIGD) and reproduction counselling available
- Guide treatment and future monitoring
- Prevent unnecessary, expensive investigations and ineffective treatments

Single nucleotide polymorphism (SNP) microarray is now a first line test for many patients with a suspected genetic disorder and can detect regions with loss of heterozygosity (LOH). LOH can indicate:

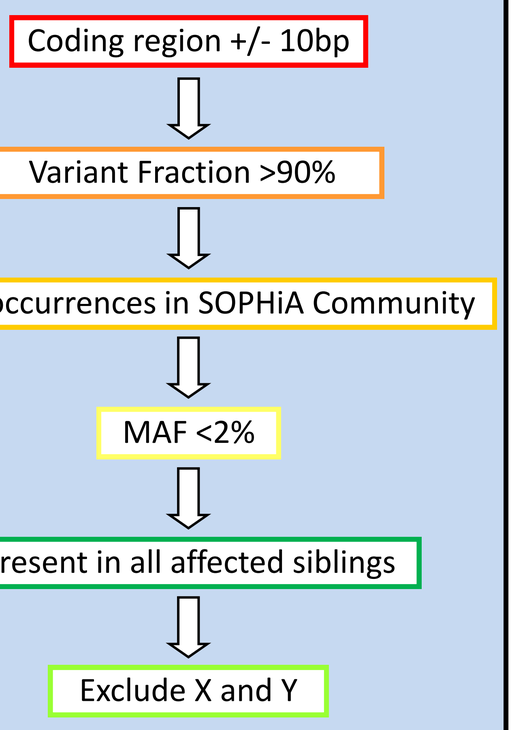
- Consanguinity or endogamy in the family history
- Increased risk of rare, autosomal recessive disorder
- Homozygous pathogenic variant(s)

Project aim

Assess the clinical utility in following up an LOH result with a phenotype agnostic Clinical Exome test in the proband and filtering the results assuming the presence of a homozygous pathogenic variant.

Method

- 11 probands recruited by Nottingham Clinical Genetics
- Suspected rare genetic disorder and LOH on SNP microarray
- Sequenced proband and any affected siblings if DNA was available
- Clinical Exome Solution from SOPHiA GENETICS
- Illumina NextSeq
- Approximately 4500 genes associated with genetic disease
- SOPHiA DDM software for bioinformatics
- Variants assessed for pathogenicity using ACMG guidelines
- MDT with Clinical Genetics

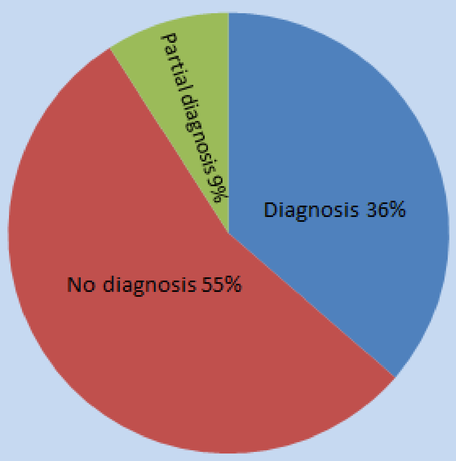


Results

Homozygous pathogenic or likely pathogenic variants were found in 4 of the 11 families that explained the phenotype. A 36% diagnostic yield. In addition a variant was found in another family that explained the patient's biochemical phenotype, but not the complete syndrome as it was later found to only be heterozygous in two affected siblings.

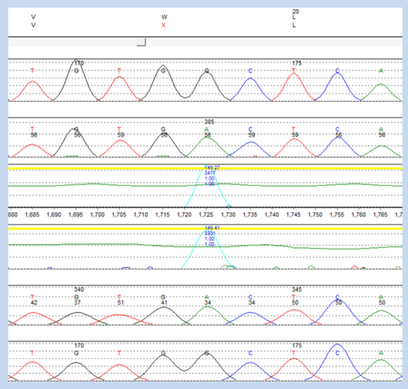
At least 98.3% of the target region was covered at >50x in all samples with the majority being >99%.

The library preparation method was simple, required no specialist equipment and could be completed in two working days. SOPHiA DDM allowed for fast, user friendly variant analysis and filtering.



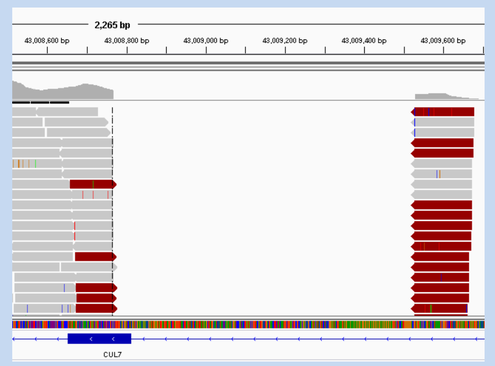
Family 1:

- Male, age 23
- Developmental delay, learning difficulties, retinal dystrophy, nystagmus, seizures, nocturnal parasomnia, obesity, large hands with tapering fingers and resting tremors
- Parents are first cousins
- Homozygous *SRD5A3* c.57G>A p.(Trp19*) – class 5
- Congenital disorder of glycosylation (CDG) type Iq



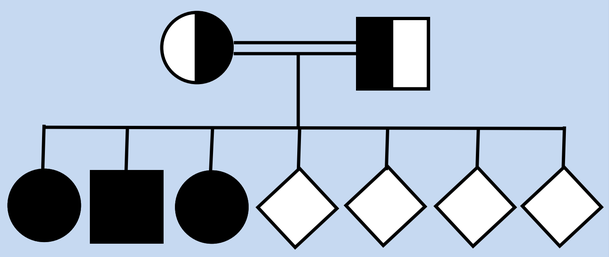
Family 2:

- Male, age 3
- Short stature, short fingers and toes with hyper flexibility, Hirshsprung's disease, motor developmental delayed, multiple capillary angiodysplasia of the colon, normal head circumference, deep palmar creases
- Suspected 3-M syndrome, gene panel testing negative in another laboratory
- Parents are first cousins
- SOPHiA DDM called *CUL7* c.3944_3945ins93
- Deletion suspected using IGV and confirmed to be 761bp deletion by Sanger sequencing
- Homozygous c.3898-715_3943del - class 5
- 3-M syndrome confirmed
- Maternal carrier status confirmed
- Variant not detected by previous gene panel



Family 3:

- Three siblings, aged 20, 9 and 7
- Global developmental impairment, white matter changes on MRI, leukodystrophy, thin corpus callosum, microcephaly
- Parent are first cousins
- Homozygous *TRAPPC9* c.1708C>T p.(Arg570*) - class 5
- Autosomal Recessive mental retardation type 13
- White matter changes match those reported in other patients
- Parental carrier status confirmed



Family 4:

- Male, age 8
- Severe learning disability, hypotonia, scoliosis, convergent squint, posterior urethral valves, undescended left testis, weight 0.4th centile, OFC 2nd centile, myopathic face with tented upper lip, broad-tipped nose, deep set eyes with prominent infra-orbital creases, upturned ear lobes
- Parents are first cousins
- Homozygous *UNC80* c.409C>T p.(Arg137*) - class 4
- Infantile hypotonia with psychomotor retardation and characteristic facies 2

Conclusions

- Using an LOH result as an indication for a singleton clinical exome is a time and cost-effective diagnostic testing method with a good diagnostic yield.
- The technique used was a fast, high quality library preparation method with simple, user friendly data analysis without the need for a specialist bioinformatician.
- Introduction of a clinical exome early in the diagnostic pathway based on SNP microarray results could **prevent a long diagnostic odyssey**, **direct future treatment** and provide the families with the options for **PND and PIGD** while potentially **saving money** for the health service by preventing further unnecessary tests and ineffective treatments.