# Optical Genome Mapping facilitates rapid characterisation of structural variants in families with developmental eye anomalies

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## Introduction

- Developmental eye anomalies, including Anophthalmia (absent eye), Microphthalmia (small eye) and Coloboma (optic fissure closure defects) (AMC), affect 11.9 per 100,000 live births[1]
  - ~50% remain undiagnosed using standard whole exome/genome sequencing (WES/WGS)
- Structural variants (SVs) represent an under investigated subset of variants implicated in AMC since most detection methods focus on single nucleotide variants and small indels
  - Conventional methods to detect SVs, including array CGH and WGS, provide challenges due to limited resolution and difficulty in detecting and interpreting complex rearrangements
- Optical Genome Mapping (OGM) (Bionano Genomics, USA) analyses long DNA fragments and detects balanced and unbalanced SVs at a high resolution, including complex rearrangements

## Methods

**Cohort**

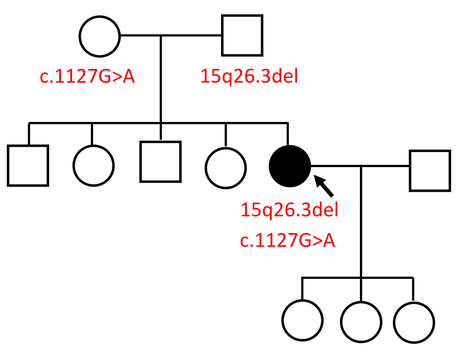
- We screened for SVs in 57 AMC families recruited via our 'National Genetics of Eye and Brain anomalies Study' UK (REC (04/Q0104/129) using OGM
- We prioritised cases with severe bilateral eye anomalies or unilateral eye anomalies plus systemic features and no genetic diagnosis at the time of the study
- Consent was obtained from all participants according to the tenets of the Declaration of Helsinki

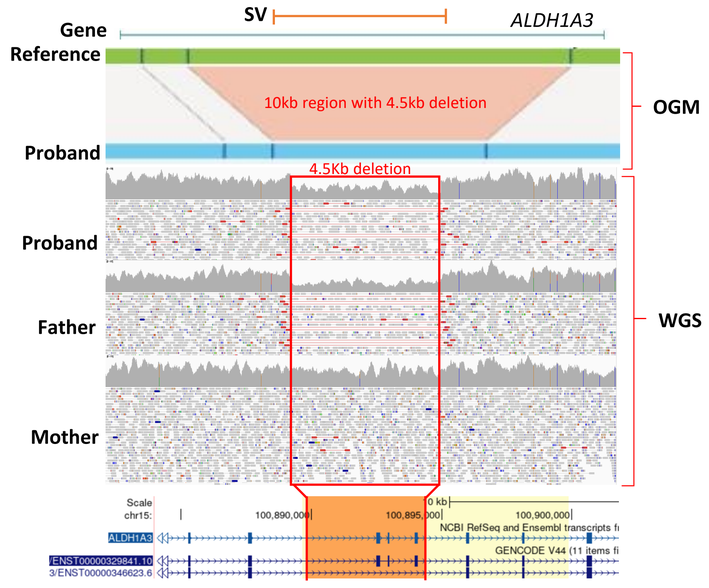
**OGM protocol** (Bionano Genomics, USA)

- High molecular weight (HMW) DNA was extracted from whole blood, fluorescently labelled at specific genomic markers, scanned using the Bionano Saphyr system, and compared to reference genomes
- SVs were filtered to retain rare variants (<5% in the Bionano control cohort) affecting 488 genes associated with structural eye disease (PanelApp 2.3[2])

## Two AMC families with compound heterozygous variants including intragenic deletions detected by OGM

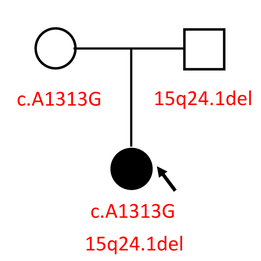
### Family 1: *ALDH1A3* deletion of exons 4-6 and p.(Gly269Glu)

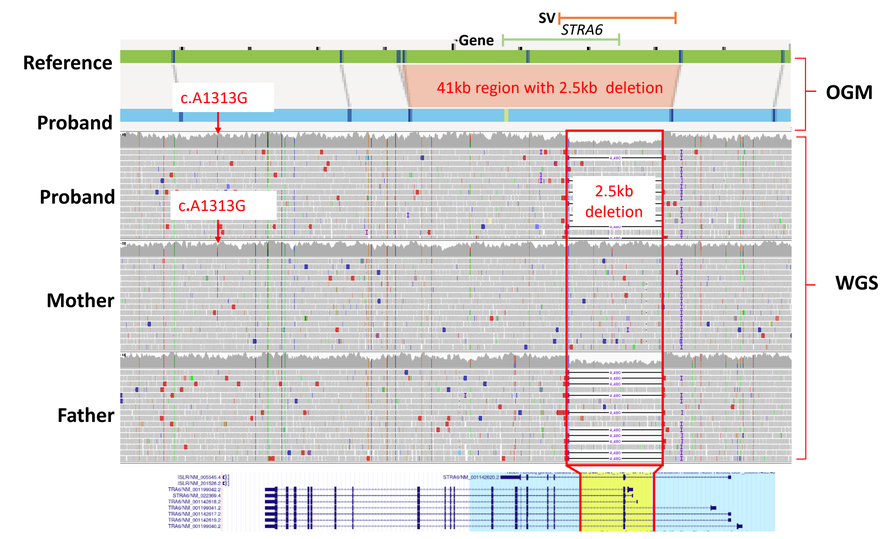



- **Phenotype:** Bilateral anophthalmia
- **OGM:** 4.5kb deletion overlapping *ALDH1A3* (orange) in a 10kb region (yellow)
  - WGS: refined breakpoints to *ALDH1A3* exons 4-6 (NM_000693.4); Paternally inherited
- **WGS:** *ALDH1A3* c.806G>A p.(Gly269Glu); MAF = 0.000001859 (gnomAD 4.1.0)
  - Maternally inherited
- *ALDH1A3* is a diagnostic gene for autosomal recessive AMC +/- other features[3]

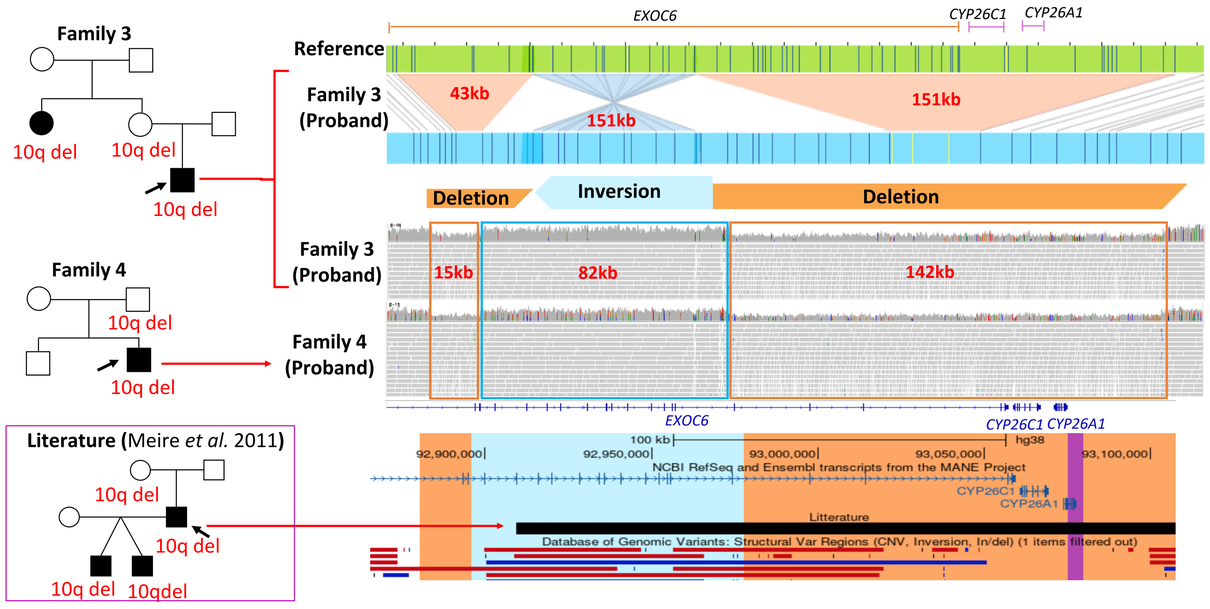
### Family 2: *STRA6* deletion of exons 1 - 2 and p.(Gln438Arg)




- **Phenotype:** Bilateral anophthalmia, learning disability, autism, urinary reflux
- **OGM:** 2.5kb deletion overlapping *STRA6* (blue) in a 41kb region (blue)
  - WGS: refines breakpoints to *STRA6* exons 1 and 2 (NM_022369.4); Paternally inherited
- **WGS:** *STRA6* c.806G>A p.(Gln438Arg); MAF = 0.000002485 (gnomAD 4.1.0)
  - Maternally inherited
  - Previously reported pathogenic[4]
- *STRA6* is a diagnostic gene for autosomal recessive AMC plus systemic anomalies[5]

## Families 3 and 4: Complex rearrangement on Chr10 in two unrelated families




**Family 3:**

- Phenotype: Bilateral anophthalmia, developmental delay
- aCGH: 4.5kb loss at 10q23 (violet) in proband, unaffected mother and affected aunt
- OGM: Complex SV at 10q23: 2 deletions (43kb and 152kb; orange) flanking a 50.1kb inversion (blue)
- WGS: Validated complex SV, refined breakpoints: 2 deletions (15kb and 142kb) flanking 82kb inversion]
- Segregation: SV found in asymptomatic mother, symptomatic aunt (anophthalmia)

**Family 4:**

- Phenotype: Bilateral microphthalmia and learning difficulties
- WGS: The same complex SV as seen in family 3

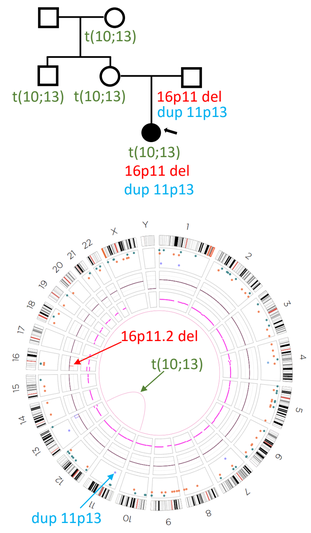
**The 10q complex SV:**

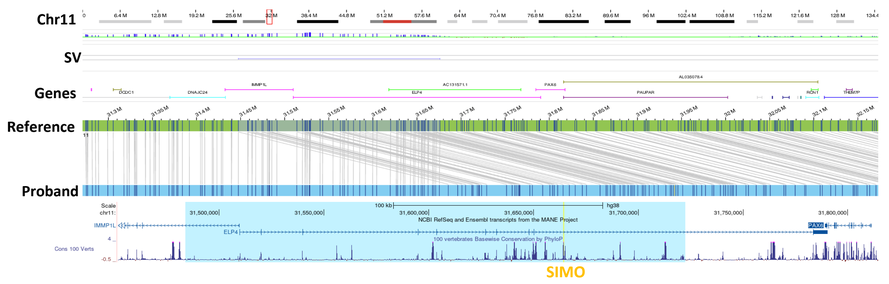
- Frequency: Absent in GnomAD SV, DGV, ClinVar
- Literature: Meire et al. report a 10q deletion (black line in bottom panel) associated with optic nerve hypoplasia and incomplete penetrance of microphthalmia[6]
- Genes: *EXOC6, CYP26A1*, and *CYP26C1*

**Conclusion:**

- CYP26A1/CYP26C1 part of vitamin A pathway essential for ocular development
- Classified as variant of unknown significance (VUS), possible incomplete penetrance

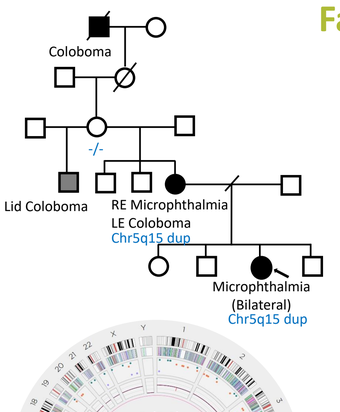
## Family 5: Three SVs of unknown significance

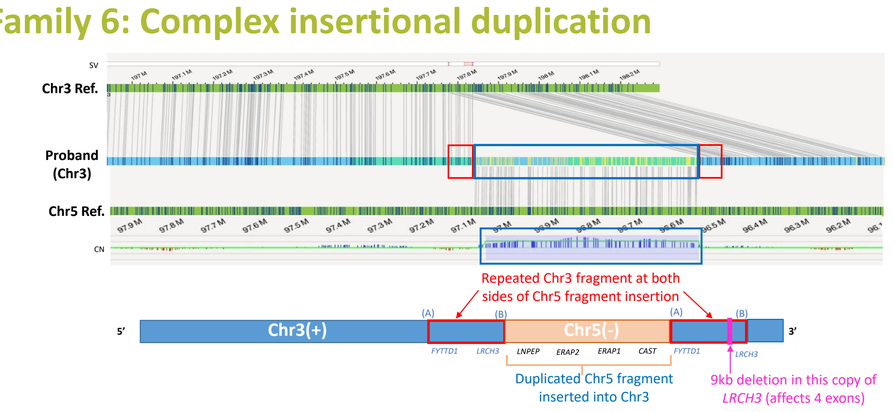



- **Phenotype:** Proband (Severe right microphthalmia, learning difficulties, epilepsy); Mother (Epilepsy); Maternal Uncle (Learning difficulty)
- **OGM**: Unbalanced translocation t(10:13) (15Mb DNA gain in Chr13); 16p11.2 loss (649kb); 11p13 duplication (220kb)
- Duplication 11p13 (blue): Paternally inherited; absent in (gnomAD SV, DGV, ClinVar).
  - Overlaps genomic regulatory region of *PAX6* (3'), containing the SIMO regulatory element (yellow) which, when deleted, is associated with aniridia[7]
- VUS under further investigation

## Family 6: Complex insertional duplication




- **aCGH:** 5q15 duplication (Proband and Mother)
- **OGM:** 5q15 duplication (520kb); translocation t(5;3); 3q29 deletion (9kb); All absent in maternal grandmother
- **Interpretation:** Duplicated Chr5q15 fragment inserted into Chr3 with repeated Chr3 segment
- **Conclusion:** 3q29 microduplications previously associated with AMC[8]
  - VUS under further investigation

## Discussion

- Rapid identification of 2 intragenic deletions in *ALDH1A3* and *STRA6* inherited *in trans* with previously identified SNVs (Families 1 and 2) resulting in genetic diagnoses

**OGM versus existing SV/CNV detection methods**

- OGM gives high resolution SV identification (500bp) compared to CGH/SNP array
- OGM improved characterisation of SVs/CNVs
- All SVs of interest identified were visible on WGS. However, OGM aids interpretation of complex rearrangements

## References

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