Making (non)sense of Variant Interpretation for patients tested on a 30 gene Next Generation Sequencing(NGS) panel for Disorders of Sex Development

By Nick Drinkall
Trainee Clinical Scientist

Birmingham Women's NHS
NHS Foundation Trust

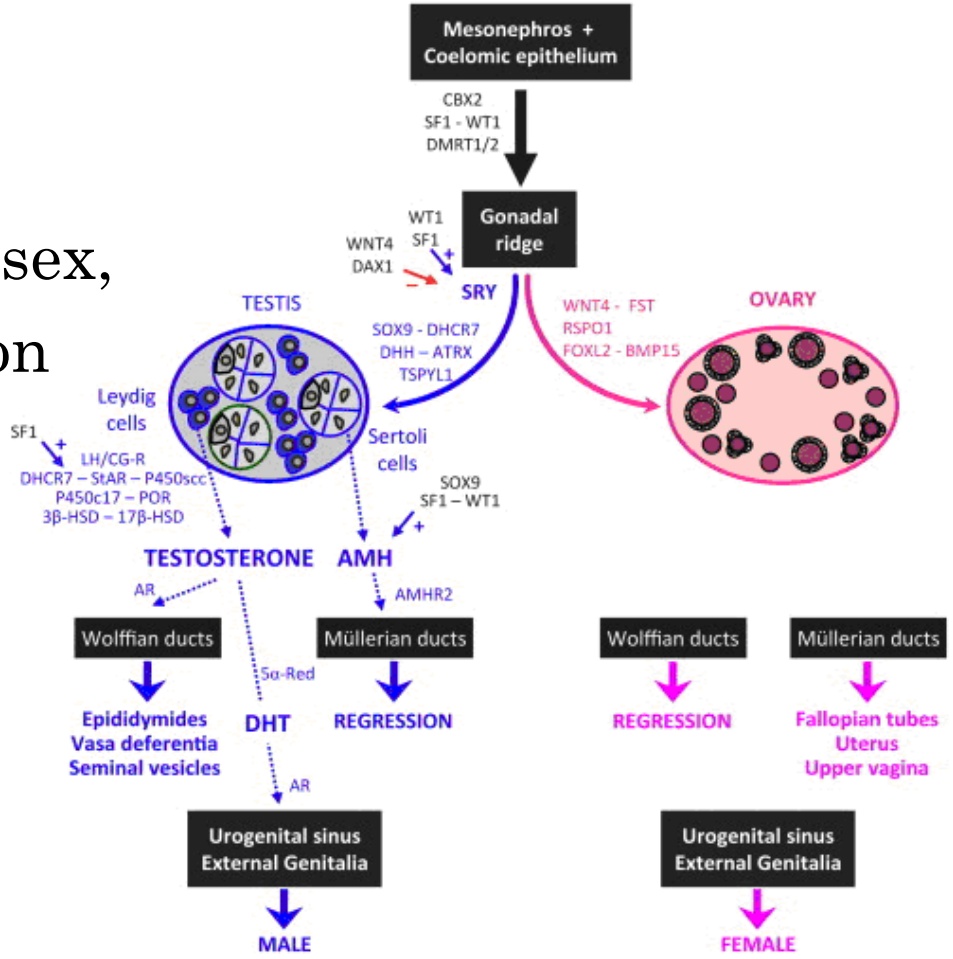
local care:
global impact

Disorders of Sex Development

- Replacing the term Intersex, along with sub-classification nomenclature changes
- Sexual differentiation

Has many genetic factors

- Phenotypic Spectrum



Male ⟵ ⟶ Female

46,XX
46,XY

46,XX
46,XY

Birmingham Women's NHS
NHS Foundation Trust

local care: global impact

DSD Gene panel

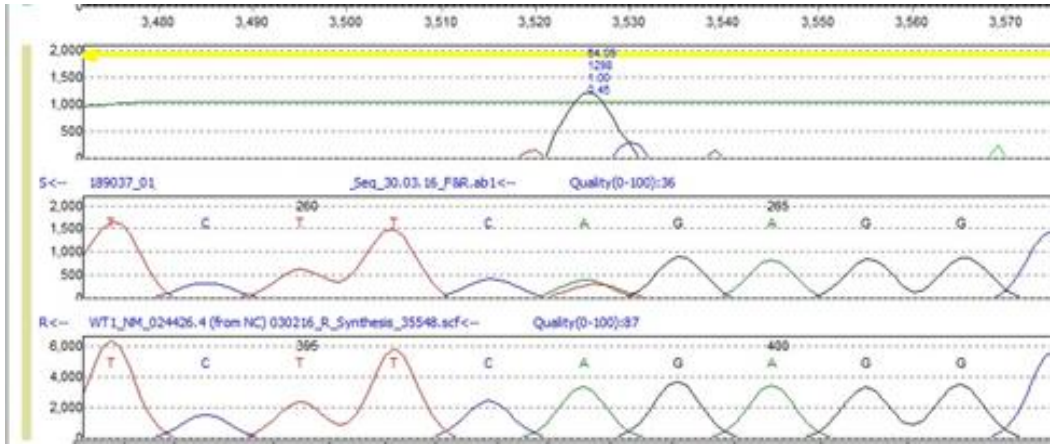
- 30 gene NGS panel on HiSeq, using TSO. Offered as a clinical service to Clinical Genetics, Endocrinology and Adolescent Gynaecology
- Collaborating with Clinical Genetics – gene list by Trevor Cole and Nils Krone
- Genes involved with; Testicular development, hormone synthesis and action, ovarian development, androgen excess.

Total	Negative	Pathogenic Positives	VOUS
38	22	6	10

- Positive results in WT1, HSD3B2, DHCR7, HSD17B3
- VOUS – in WT1, POR, NR5A, RSPO1, HSD3B2, CBX2, CYP11A1
- Challenging in terms of counselling, prenatal VOUS and incidental findings

Case 1 - Counselling

- Baby boy (46,XY) - ambiguous genitalia, no palpable gonads, small phallus, hypospadias (defect in urethral opening), fused labioscrotal folds, U/S - no uterus or gonads observed
- Biochemistry – hyponatraemia (low NaCl), responded to testosterone test
- Results - WT1 N/c.1087A>T p.(Arg363*)



Birmingham Women's NHS
NHS Foundation Trust

local care:
global impact

Case 1 – Counselling Results

- Mutations linked to; Wilms Tumour, WAGR Syndrome, Denys-Drash Syndrome and Frasier Syndrome
- Predicted protein truncation
- Not previously reported
- Downstream nonsense reported with Wilms tumour – class 4 likely pathogenic

Issues

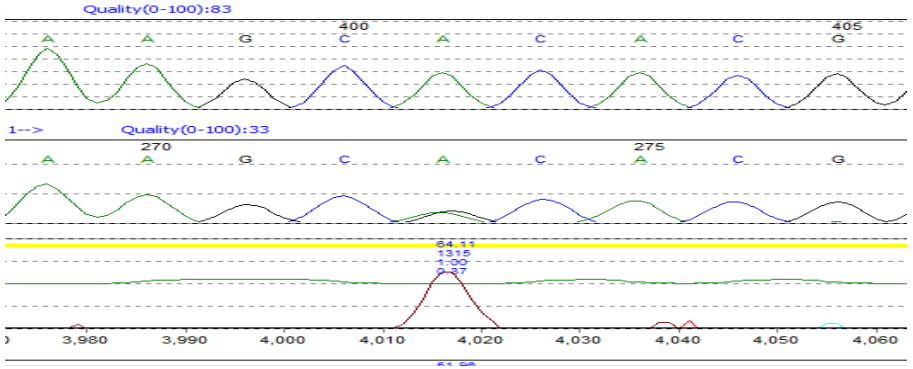
- What do you counsel? – Tumour risk?
- What do the parents understand/remember?
- Unnecessary worry
- Parents primary concern – sexing?

Case 2 – VOUS and Prenatal

- Previous child (46,XY) with hypospadias with paternal pathogenic c.307C>T p.(Arg103*) in SRD5A2 (2p23.1) – 5-alpha-reductase deficiency – lack conversion T to DHT
- Also maternal VOUS in SRD5A2 – c.107A>G, p.(His36Arg)
- AR inheritance
- Pregnant– will we do prenatal testing?
- ffDNA at GOSH predicted male fetus
- Unknown if second mutation is pathogenic
- 1 in 4 chance of inheriting both mutations
- Decided to report only on presence of mutations/variant

Case 2 – VOUS and Prenatal - Results

- Prenatal – compound heterozygote for paternal nonsense c.307C>T, p.(Arg103*) and maternal VOUS c.107A>G, p.(His36Arg)



- "Given the uncertainty regarding the pathogenicity of the p.His36Arg variant we are unable to comment further on the likely phenotypic consequences for the fetus."
- VOUS in prenatal–not normally reported (i.e. microarray)
- ?if only VOUS present – report and counsel?
- Clinical impact – considering TOP
- Patients well informed

Birmingham Women's NHS NHS Foundation Trust

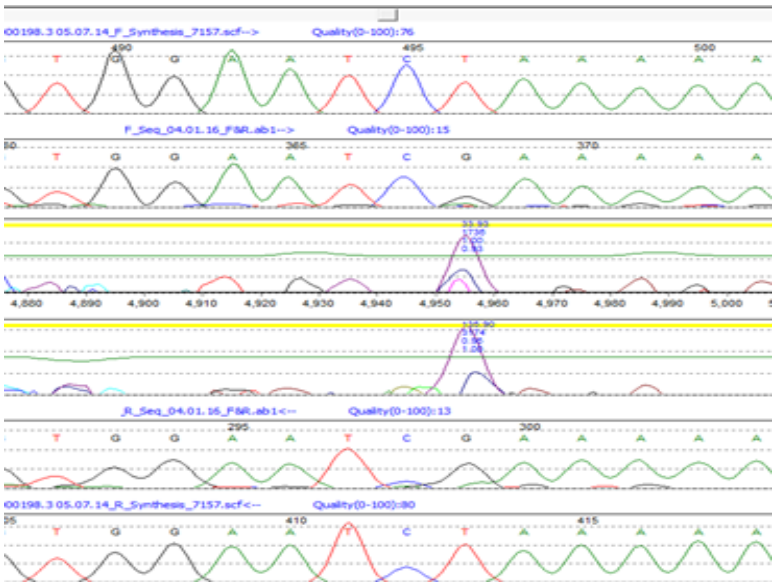
local care: global impact

Case 3 – Incidental Findings

- Baby with 46,XY DSD - Bilateral labioscrotal folds with testes, ASD, Creases on earlobe
- FISH SRY +ve, normal testosterone, cortisol, aldosterone
- ?5-alpha Reductase deficiency
- c.518T>G, p.(Leu173Arg)
- (HOM) in HSD3B2 (1p12)
- Pathogenic missense

associated with (CAH)

Congenital Adrenal Hyperplasia

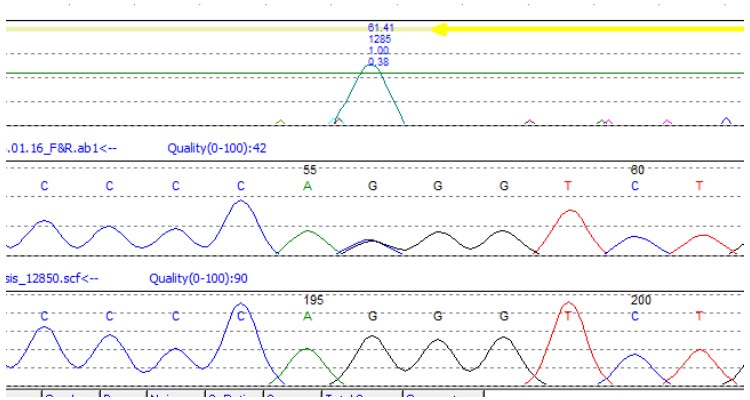


Birmingham Women's NHS
NHS Foundation Trust

local care: global impact

Case 3 – Incidental finding - Results

- Also N/c.964-1G>C splice site mutation in DHCR7 (11q13.4)
- Pathogenic, therefore at least carrier of Smith-Lemli-Opitz Syndrome



- CAH fits phenotype but also incidental SLOS carrier
- Gene Panels don't eliminate incidental findings – rather reduce findings to those more 'relevant'
- SLOS carrier – counsel
- Potential missed mutation

Summary

- Technology – discover but not always interpret
- Patient Counselling – used to more binary results
- VOUS – don't known until more information – hard to explain to patients
- Data sharing essential
- VOUS can be difficult to interpret for a number of reasons – ethics, lack of known information, uncertain phenotype
- Over time hopefully less VOUS – until next technological milestone