Integrating Red Cell Molecular Diagnostics and Research- a UK wide initiative

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OXFORD UNIVERSITY HOSPITALS NHS FOUNDATION TRUST

Conflicts of interest

- None to declare

Overview

- Rare inherited anaemias: clinical
- The patient pathway
- A novel unified approach to diagnostics
- Targeted NGS
  - Oxford panel
  - Kings panel
- Building the common pathway
- Red Cell Diagnostics- a collaborative network

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Rare inherited anaemias

	Diamond Blackfan Anaemia	Congenital Dyserythropoietic Anaemia	Sideroblastic Anaemia	Red Cell Membrane & Enzyme disorders
Age at presentation	Usually baby/young child	Usually child/young adult	Usually child/young adult	Usually child/young adult
Associated features	Bony Cardiac Cleft lip/palate	Distal limb	Ring sideroblasts on bone marrow	Hepatosplenomegaly Jaundice Gallstones
Severity	Usually severe	Usually mild to moderate	Mild to severe	Usually mild
Treatment	Steroids Transfusions & chelation BMT	Interferon Transfusions & chelation Nil	Transfusions & chelation Nil	Nil Splenectomy
Genetics	AD/de novo Ribosomal proteins	AR Multiple pathways (vesicle trafficking, chromatin assembly)	X linked AR Haem synthesis	AD AR Cytoskeleton and red cell enzymes

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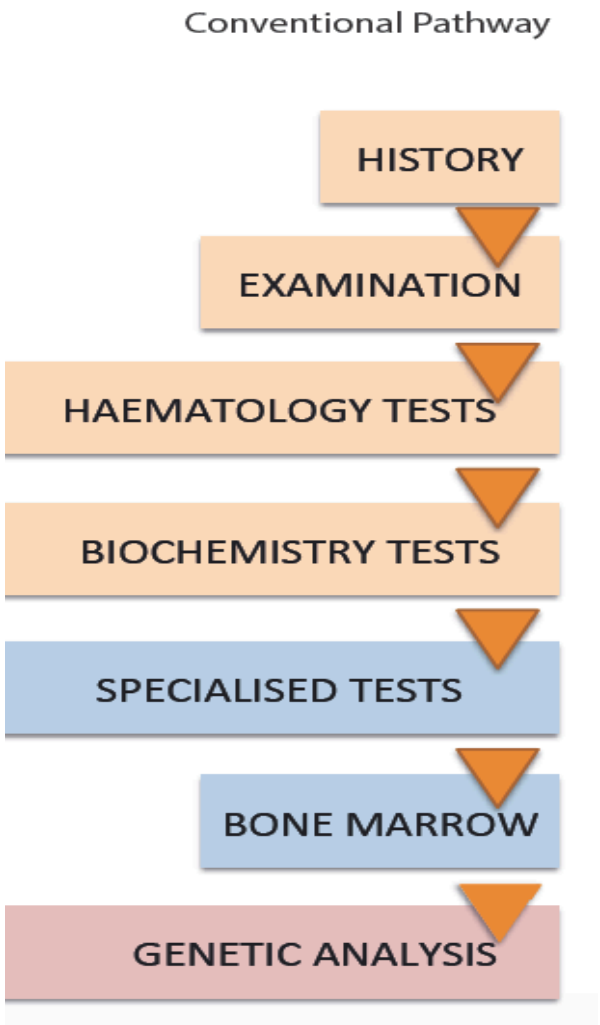
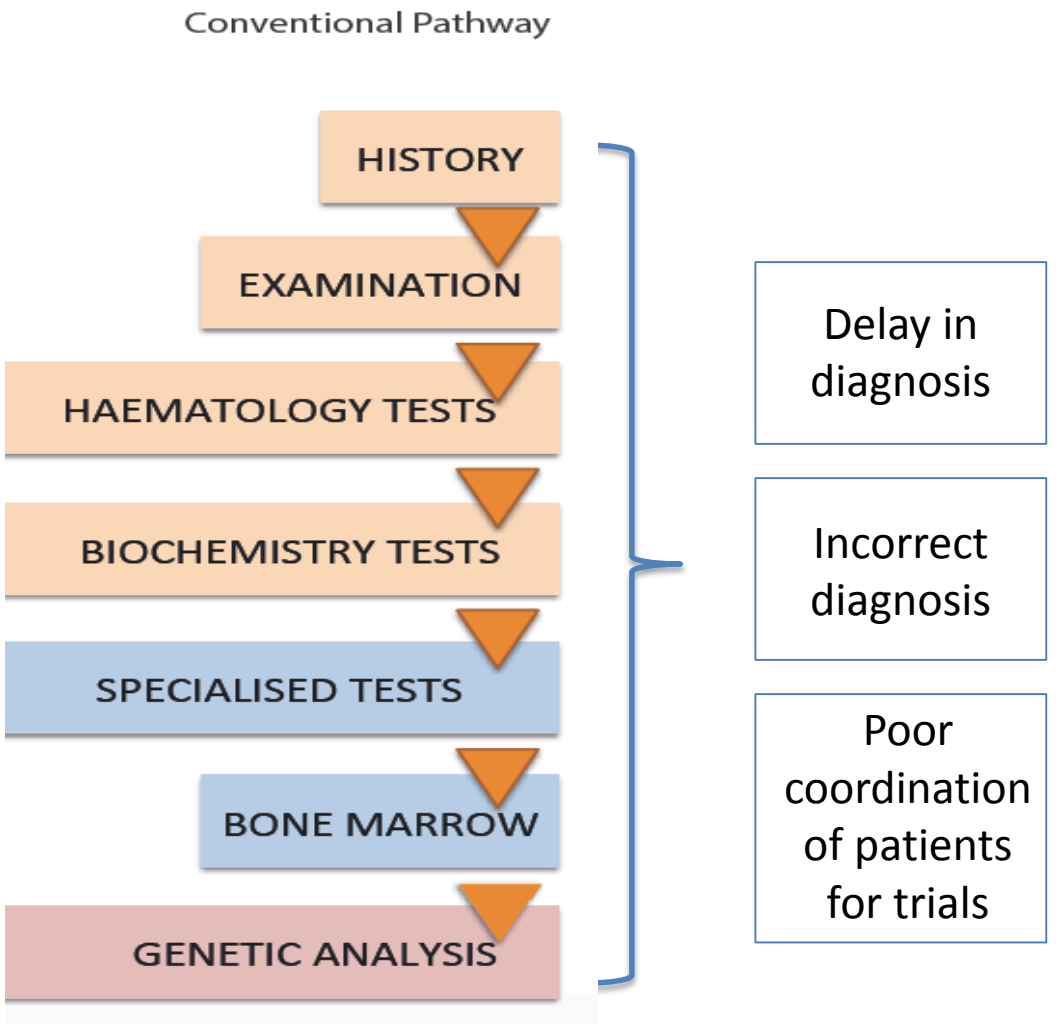
The patient pathway



The patient pathway



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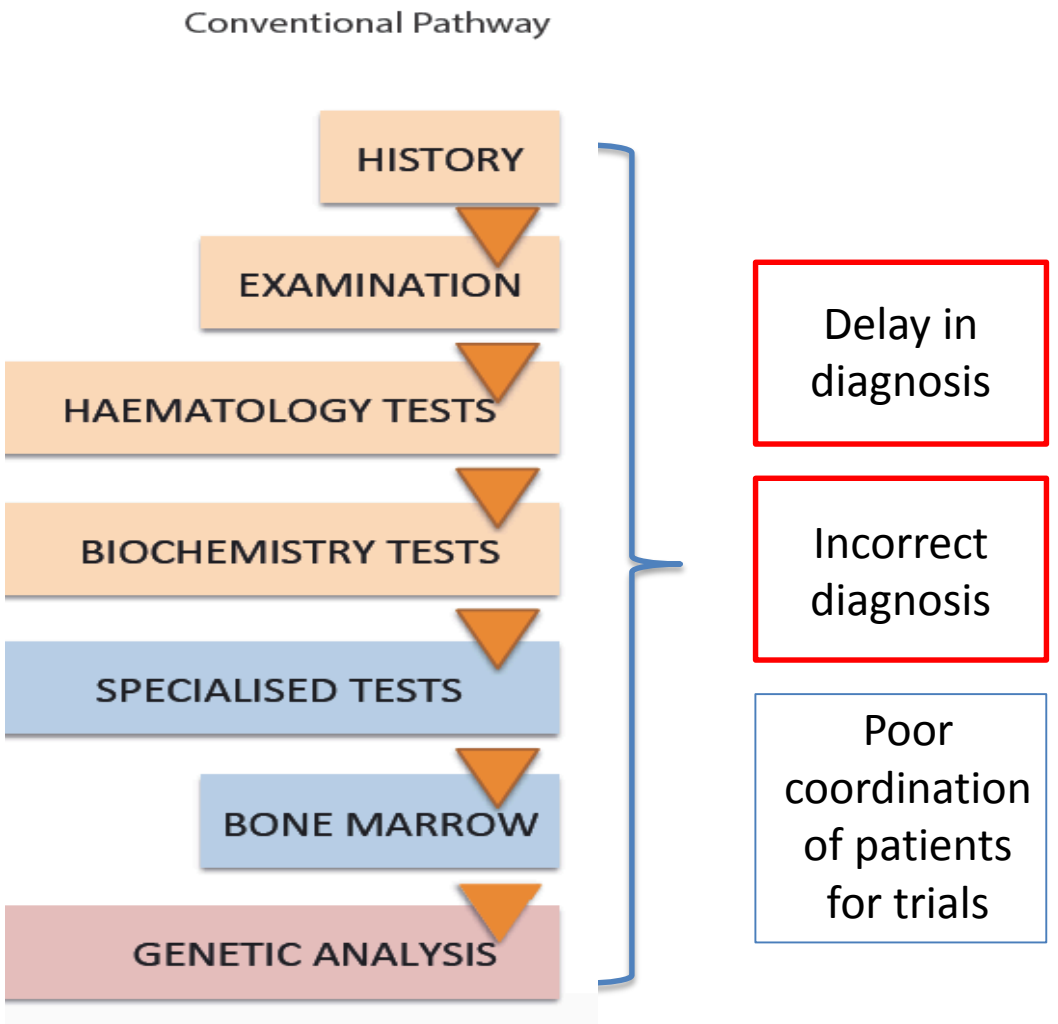

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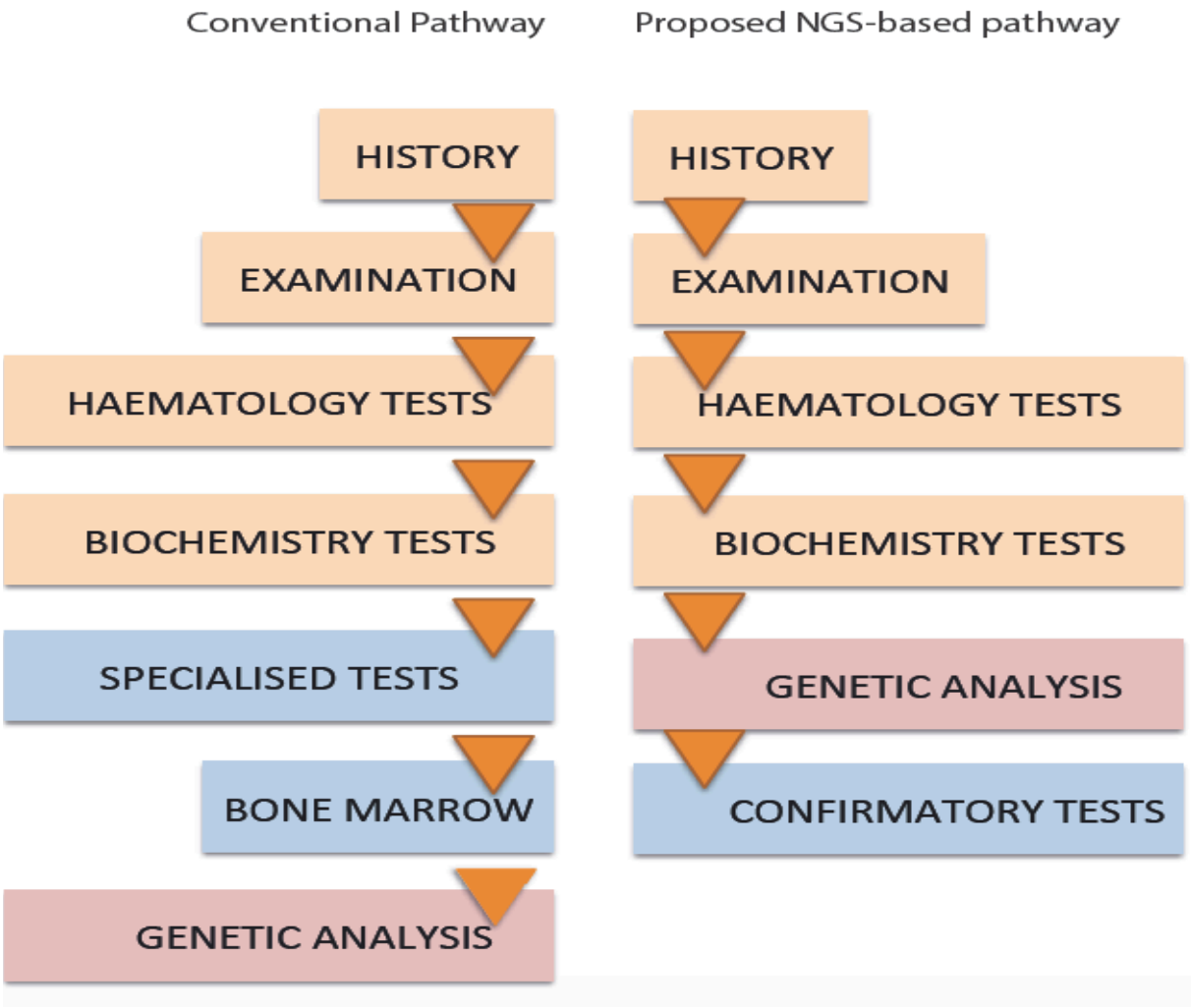

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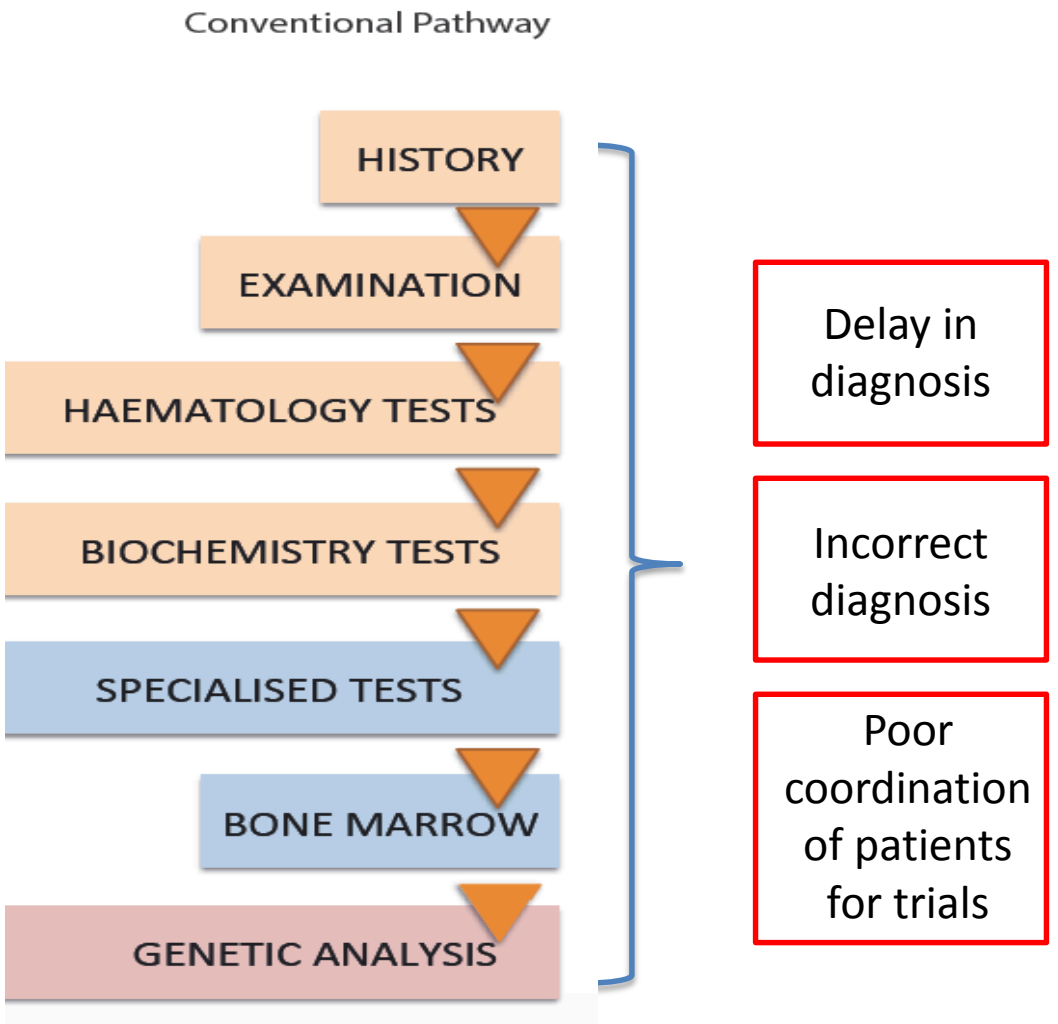

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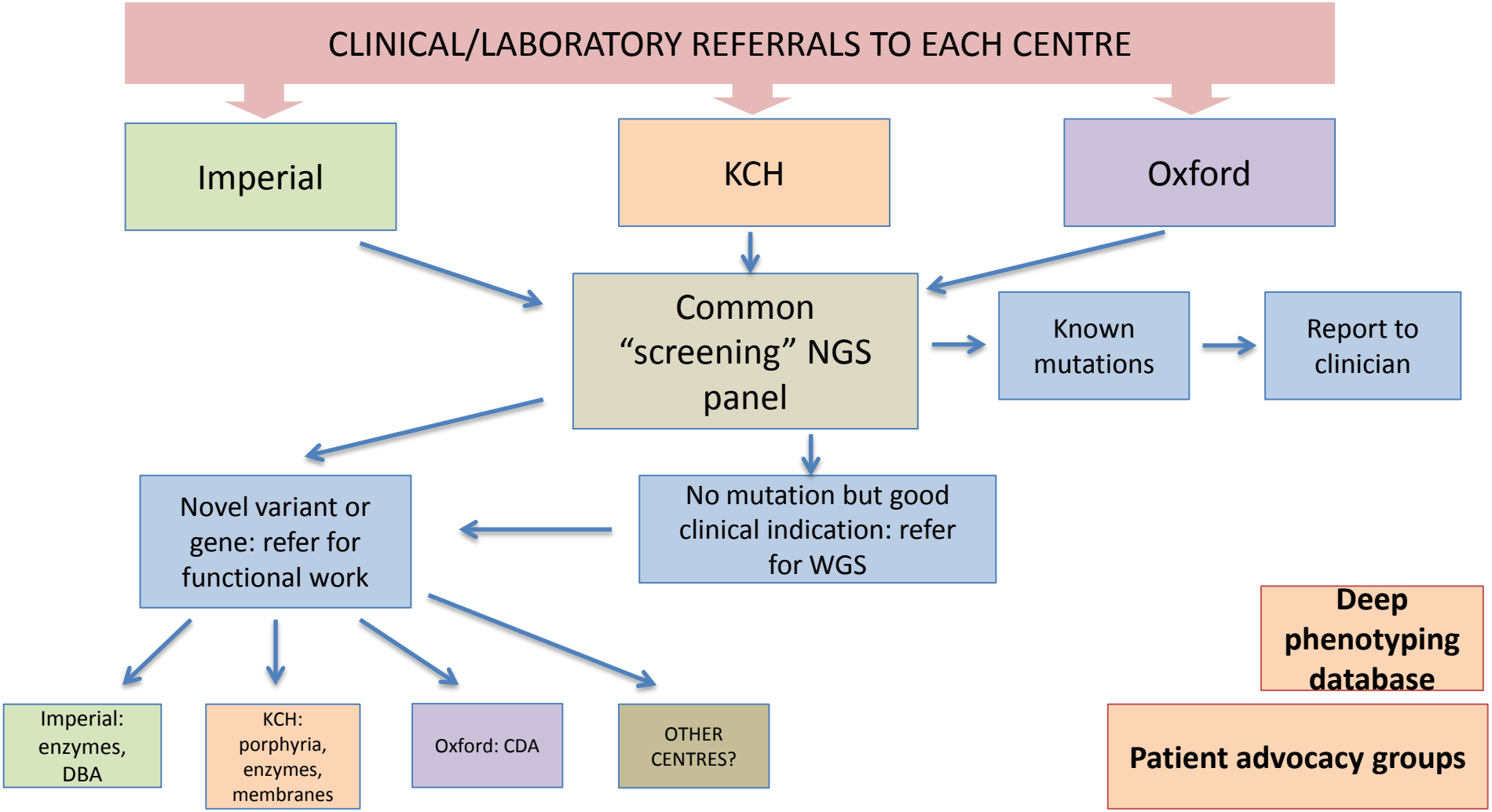
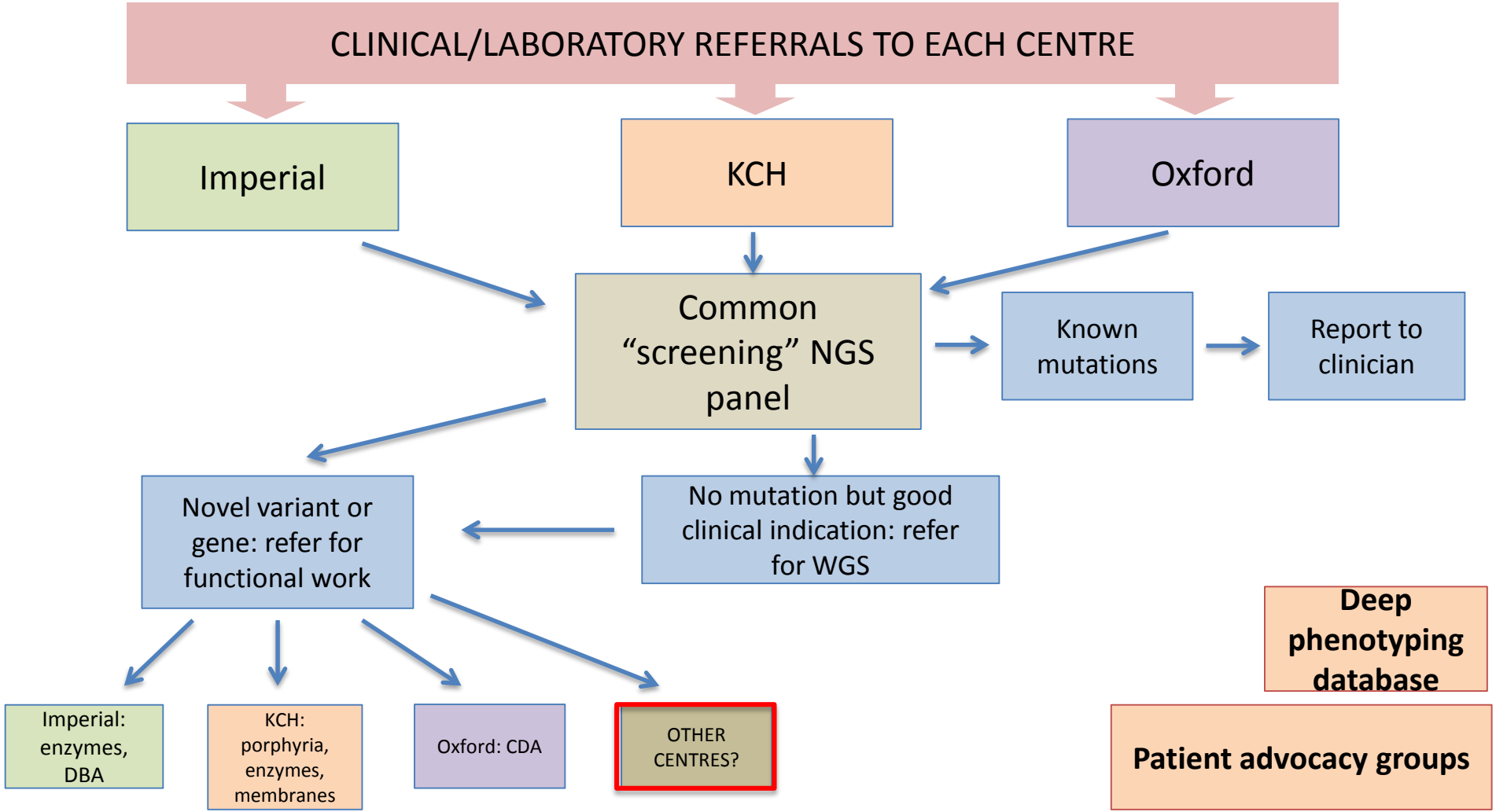

CLINICAL/LABORATORY REFERRALS TO EACH CENTRE
Imperial
KCH
Oxford
Common "screening" NGS panel
Known mutations
Report to clinician
Novel variant or gene: refer for functional work
No mutation but good clinical indication: refer for WGS
Imperial: enzymes, DBA
KCH: porphyria, enzymes, membranes
Oxford: CDA
OTHER CENTRES?
Deep phenotyping database
Patient advocacy groups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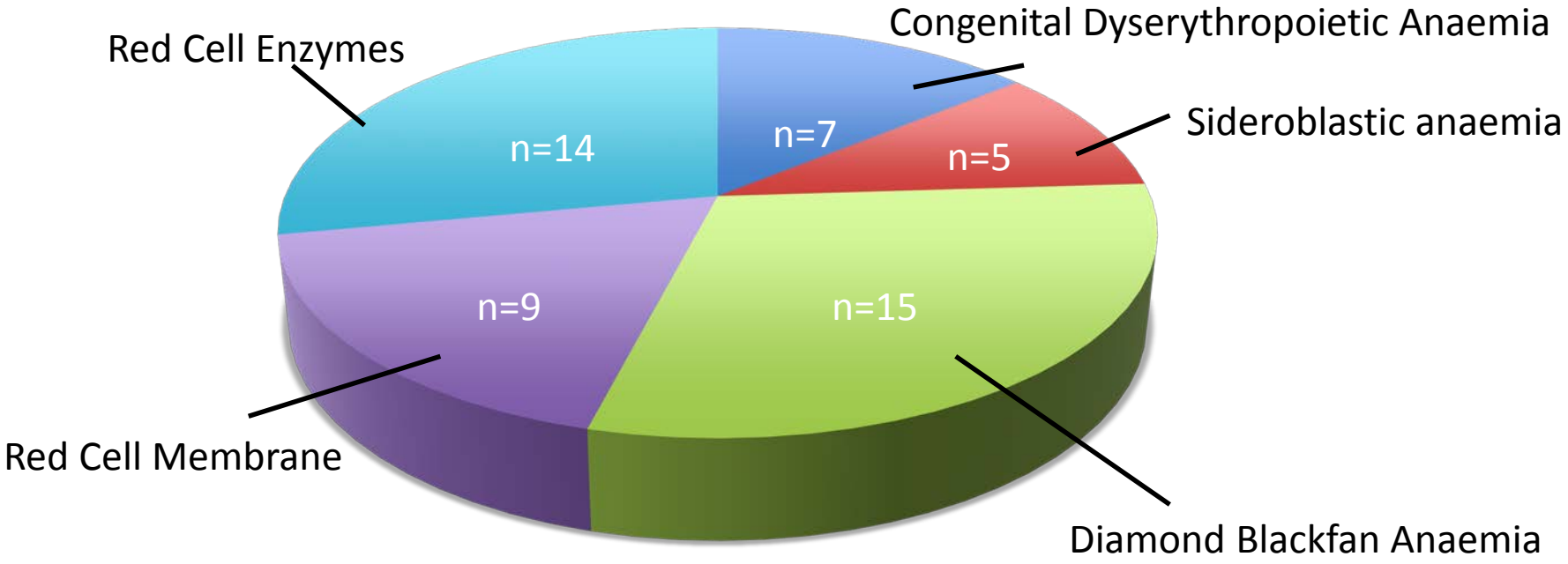
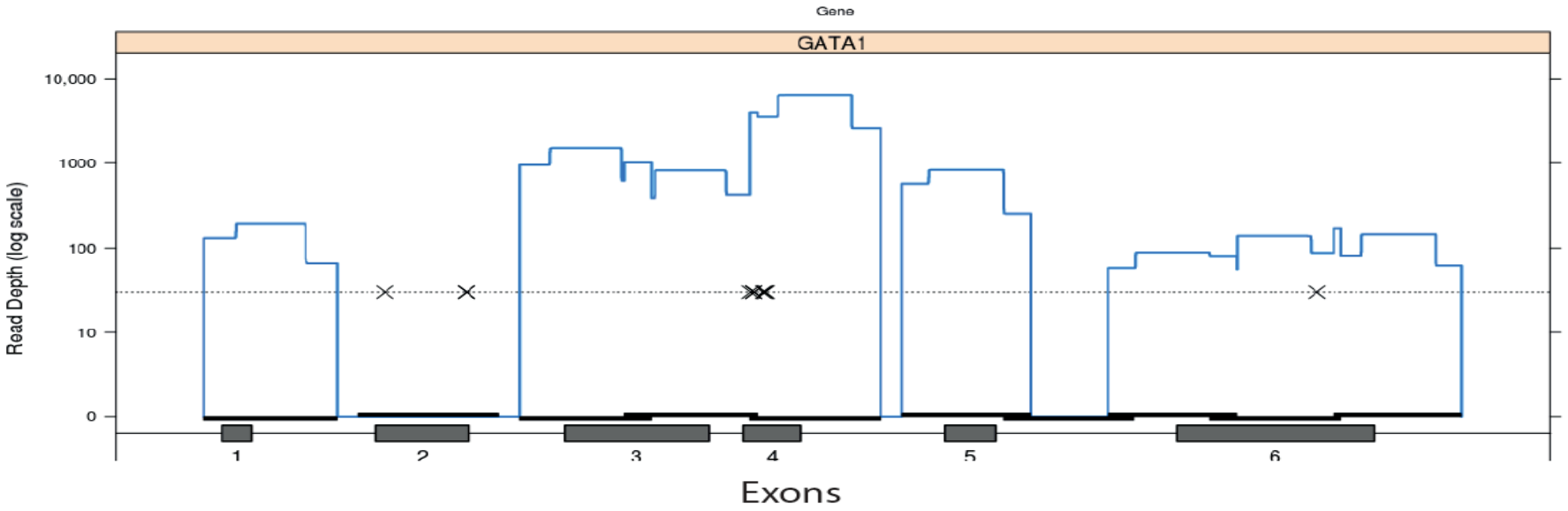
Oxford Red Cell Panel (ORCP)

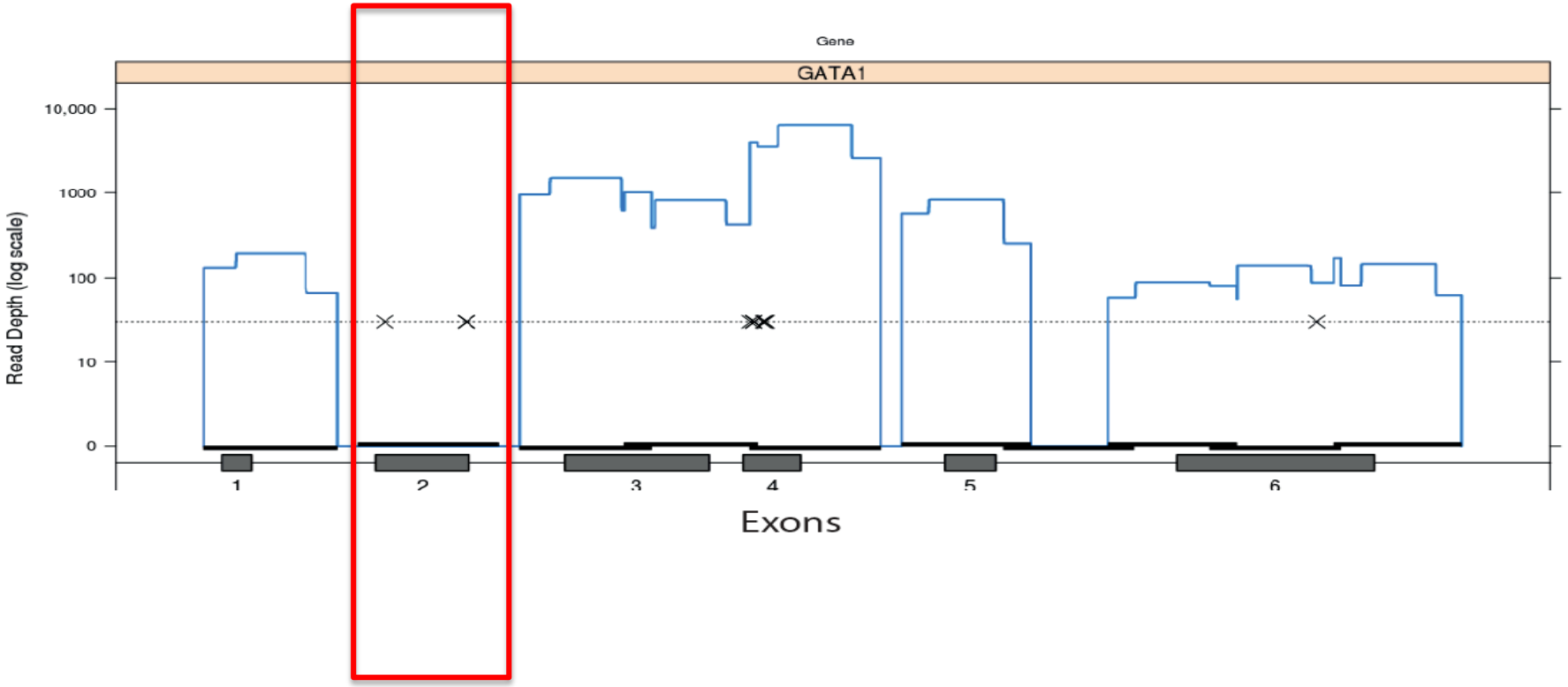


Conditions tested on ORCP

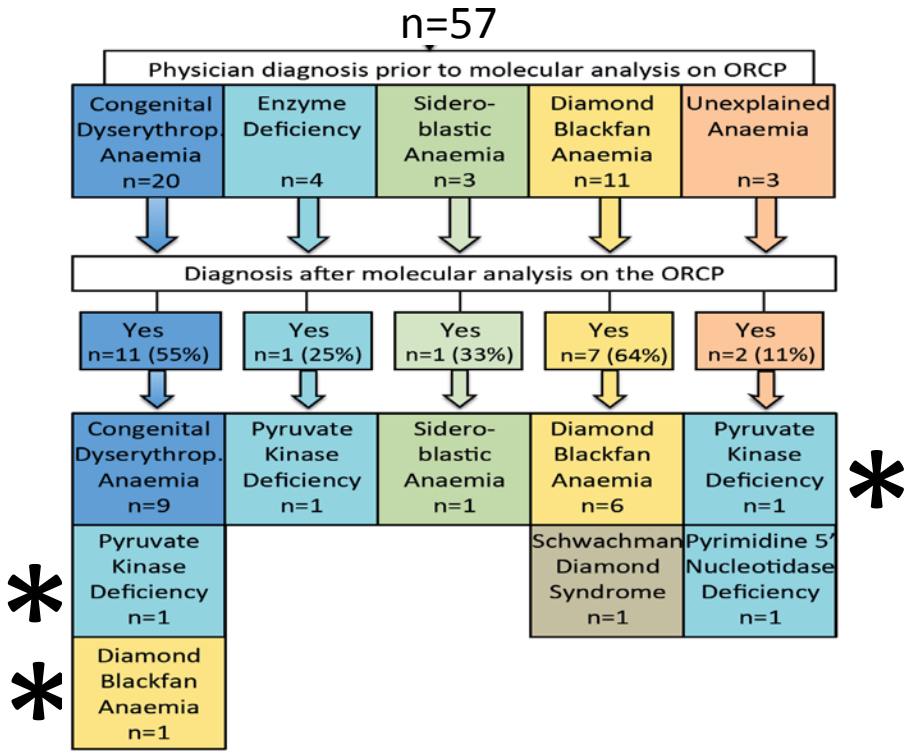
The importance of coverage analysis in making an accurate diagnosis

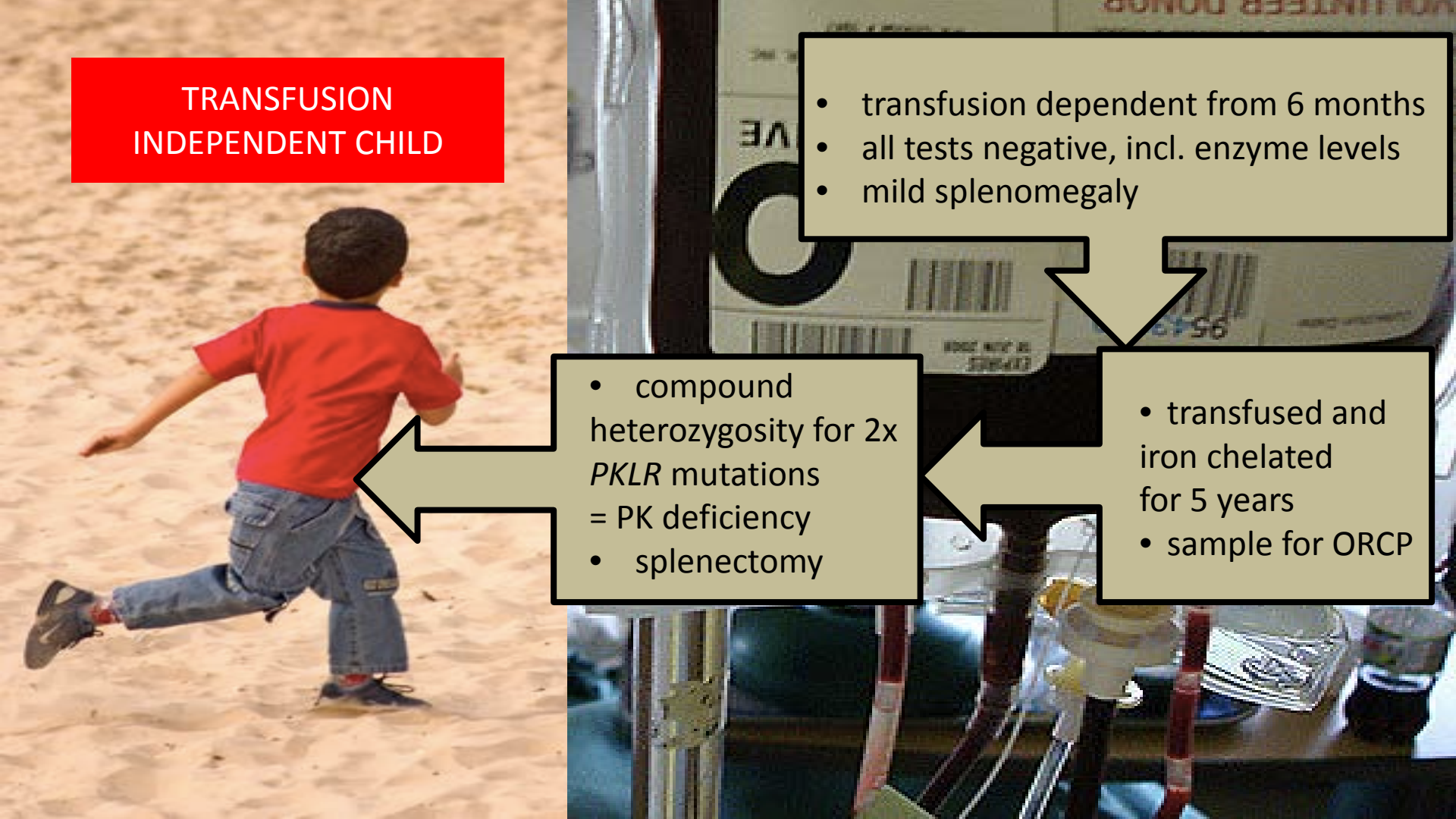


The importance of coverage analysis in making an accurate diagnosis



Targeted NGS experience with first Oxford (limited) panel




TRANSFUSION INDEPENDENT CHILD
• transfusion dependent from 6 months
• all tests negative, incl. enzyme levels
• mild splenomegaly
• compound heterozygosity for 2x *PKLR* mutations = PK deficiency
• splenectomy
• transfused and iron chelated for 5 years
• sample for ORCP

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King's : Red Cell Gene Panel

Next Generation Sequencing: Red Cell Panel V3 - Subpanels

Membranopathy	
Gene	Transcript
ABCG5	NM_022436.2
ABCG8	NM_022437.2
ADD1	NM_014189.3
ADD2	NM_001617.3
AK1	NM_000476.2
ANK1	NM_001142446.1
APOB	NM_000384.2
EPB41	NM_001166005.1
EPB42	NM_000119.2
EPB49 (DMTN)	NM_001978
GYPA	NM_002099.7
GYPB	NM_002100.5
GYPC	NM_002101.4
KCNN4	NM_002250.2
MTTP	NM_001300785.1
PIEZO1 (FAM38A)	NM_001142864.2
RhAG	NM_000324.2
SLC2A1	NM_006516.2
SLC4A1	NM_000342.3
SPTA1	NM_003126.2
SPTB	NM_001024858.2
STOM	NM_004099.5
TMOD1	NM_003275.3
TPM3	NM_153649.3
XK	NM_021083.2

Red Cell Enzymes	
Gene	Transcript
ALDOA	NM_000034.3
BPGM	NM_199186.2
CYB5A	NM_148923.3
CYB5R1	NM_016243.2
CYB5R2	NM_001302826.1
CYB5R3	NM_001129819.2
CYB5R4	NM_016230.3
CYB5RL	NM_001031672.2
ENO1	NM_001428.3
G6PD	NM_000402.4
GAPDH	NM_002046.5
GCLC	NM_001498.3
GPI	NM_001289789.1
GPX1	NM_000581.2
GSR	NM_000637.3
GSS	NM_000178.2
HK1	NM_033496.2
HK2	NM_000189.4
NT5C3A	NM_001002010.2
PFKM	NM_001166686.1
PGAM1	NM_002629.2
PGD	NM_002631.3
PGK1	NM_000291.3
PGM1	NM_001172818.1
PGM1	NM_002633.2
PKLR	NM_000298.5
TPI1	NM_001159287.1

Haemoglobinopathies	
Gene	Transcript
AHSP	NM_016633.2
Alpha globin HS40	Genomic region
ATRX	NM_000489.4
Beta globin LCR HS1-5	Genomic Region
HBA1	NM_000558.4
HBA2	NM_000517.4
HBB	NM_000518.4
HBD	NM_000519.3
HBE1	NM_005330.3
HBG1	NM_000559.2
HBG2	NM_000184.2
HBM	NM_001003938.3
HBQ1	NM_005331.4
HBZ	NM_005332.2

Congenital Dyserythropoietic Anaemia	
Gene	Transcript
CDAN1	NM_138477.2
CI5ORF41	NM_001130010.2
COX4I2	NM_032609.2
GATA1	NM_002049.3
GATA2	NM_032638.4
KIF23 (CDANIII)	NM_138555.3
KLF1	NM_006563.3
LPIN2	NM_014646.2
SEC23B	NM_032985.4
TAL1	NM_003189.5

Congenital Erythrocytosis

Gene	Transcript
BHLHE41	NM_030762.2
BPGM	NM_199186.2
EGLN1	NM_022051.2
EGLN2	NM_080732.3
EGLN3	NM_022073.3
EPAS1	NM_001430.4
EPO	NM_000799.2
EPOR	NM_000121.3
GFI1B	NM_004188.6
HBA1	NM_000558.4
HBA2	NM_000517.4
HBB	NM_000518.4
HIF1A	NM_001243084.1
HIF1AN	NM_017902.2
HIF3A	NM_152795.3
JAK2	NM_004972.3
KDM6A	NM_001291415.1
OS9	NM_006812.3
SH2B3	NM_005475.2
VHL	NM_000551.3
ZNF197	NM_006991.3

Megaloblastic Anaemia

Gene	Transcript
AMN	NM_030943.3
ATP4A	NM_000704.2
ATP4B	NM_000705.3
CBL	NM_005188.3
CBS	NM_000071.2

Megaloblastic Anaemia continued...

Gene	Transcript
CD320	NM_016579.3
CUBN	NM_001081.3
DHFR	NM_000791
DUT	NM_001025248.1
FBXO7	NM_012179.3
FTCD	NM_206965.1
GAST	NM_000805.4
GIF	NM_005142.2
HACL1	NM_012260.3
HPRT1	NM_000194.2
LMBRD1	NM_018368.3
MMAA	NM_172250.2
MMACHC	NM_015506.2
MMADHC	NM_015702.2
MT-CO1	YP_003024028.1
MTR	NM_000254.2
MTRR	NM_024010.2
MUT	NM_000255.3
PSG2	NM_031246.3
SLC19A1	NM_194255.2
SLC19A2	NM_006996.2
SLC19A3	NM_025243.3
SLC46A1	NM_080669.5
SLC5A6	NM_021095.2
TCN1	NM_001062.3
TCN2	NM_000355.3
TKT	NM_001135055.2
TPK1	NM_022445.3
UMPS	NM_000373.3

Diamond-Blackfan Anaemia

Gene	Transcript
RPL11	NM_000975.3
RPL15	NM_002948.3
RPL19	NM_000981.3
RPL26	NM_001315530.1
RPL27	NM_000988.3
RPL35A	NM_001316311.1
RPL5	NM_000969.3
RPL9	NM_000661.4
RPS10	NM_001203245.2
RPS17	NM_001021.4
RPS19	NM_001022.3
RPS24	NM_001142285.1
RPS26	NM_001029.3
RPS29	NM_001032.4
RPS7	NM_001011.3

Bone Marrow Failure

Gene	Transcript
DKC1	NM_001363.4
GATA1	NM_002049.3
GATA2	NM_032638.4
NHP2	NM_017838.3
NOP10	NM_018648.3
NT5C3A	NM_001002010.2
SBDS	NM_016038.2
TERC	NR_001566.1
TERT	NM_198253.2
TINF2	NM_001099274.1

Sideroblastic Anaemia

Gene	Transcript
ABCB6	NM_005689.2
ABCB7	NM_004299.4
ALAS1	NM_000688.5
ALAS2	NM_000032.4
GLRX5	NM_016417.2
PUS1	NM_001002020.2
SF3B1	NM_012433.3
SLC19A2	NM_006996.2
SLC25A38	NM_017875.2
YARS2	NM_001040436.2

Porphyria

Gene	Transcript
ALAD	NM_000031.5
ALAS2	NM_000032.4
CPOX	NM_000097.5
FECH	NM_001012515.2
GATA1	NM_002049.3
HMBS	NM_000190.3
PPOX	NM_001122764.1
UROD	NM_000374.4
UROS	NM_000375.2

Iron Regulation

Gene	Transcript
ACVR1	NM_001105.4
BMP6	NM_001718
BMPR1A	NM_004329
BMPR1B	NM_001256793.1
CCND3	NM_001760.4
CHRD	NM_003741.3
CP	NM_000096.3
CYBRD1	NM_024843.3
FTH1	NM_002032.2
FTL	NM_000146.3
HAMP	NM_021175
HEPH	NM_138737.4
HFE	NM_000410.3
HFE2	NM_213653.3
MCOLN1	NM_020533
NOG	NM_005450.4
PCSK7	NM_004716
SLC11A2	NM_001174125.1
SLC40A1	NM_014585
SMAD4	NM_005359
SMAD6	NM_005585.4
SMAD7	NM_005904.3
STEAP1	NM_012449.2
STEAP3	NM_182915.2
TF	NM_001063.3
TFR2	NM_003227.3
TFRC	NM_003234.2
TMPRSS6	NM_001289000.1

Haemolytic Uraemic Syndrome

Gene	Transcript
CFH	NM_000186.3
CFI	NM_000204.3

Secondary Modifiers

Gene	Transcript
HP	NM_005143.3
MTHFR	NM_005957.4
UGT1A1	NM_000463.2

Single Genes

Gene	Transcript
ATP7B	NM_000053.3
PIGA	NM_002641.3
SERPINA1	NM_000295.4

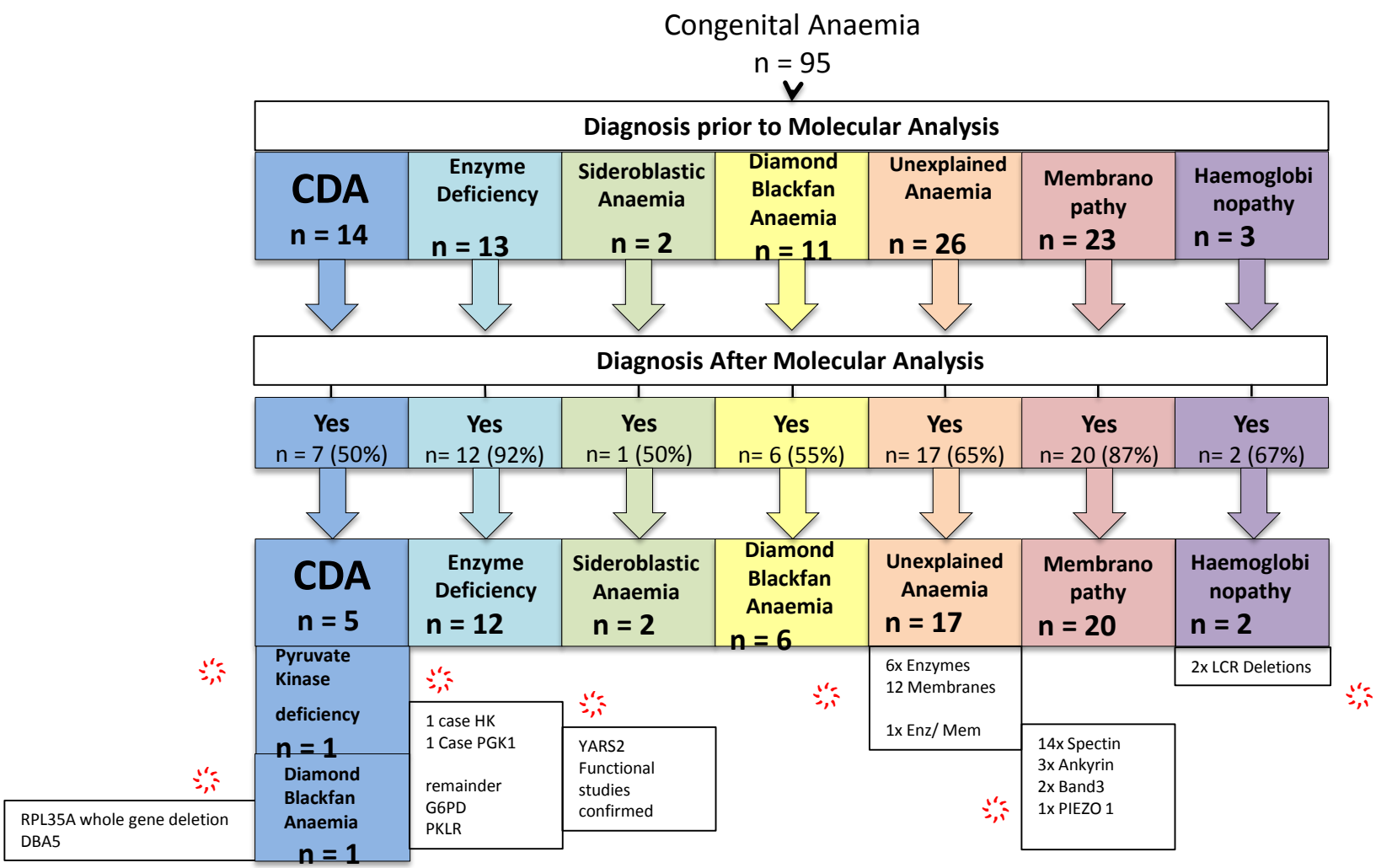
Sex Chromosome Markers

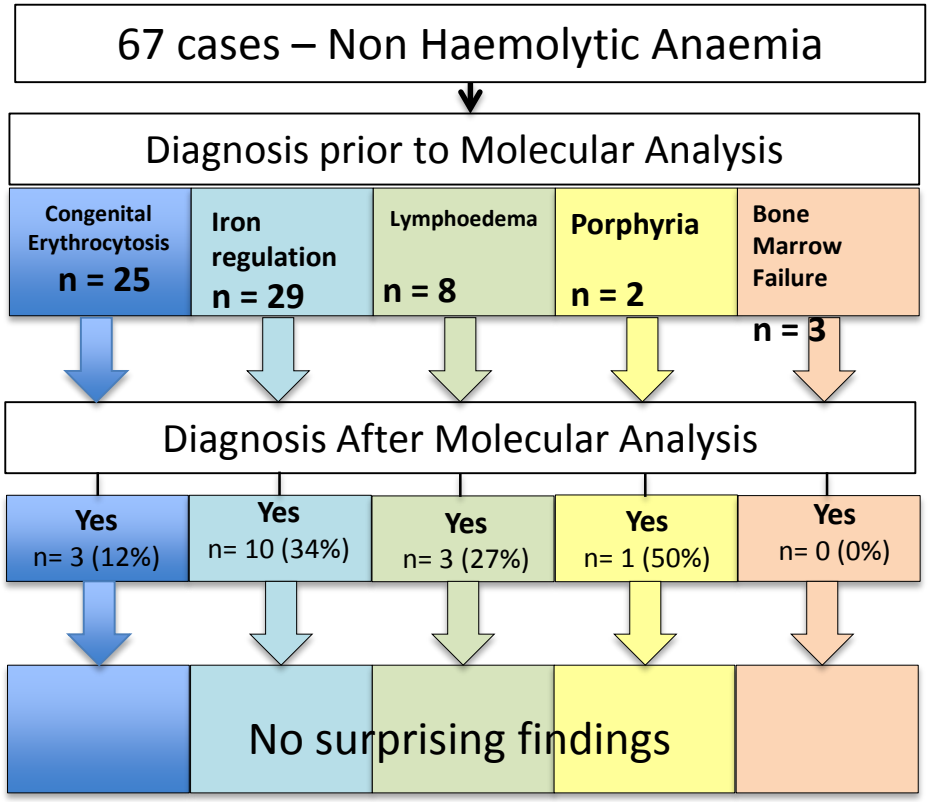
Gene	Transcript
AMELX	NM_182680.1
SRY	

King's Red Cell Gene Panel

Agilent SureSelect bait capture Targeted Gene panel

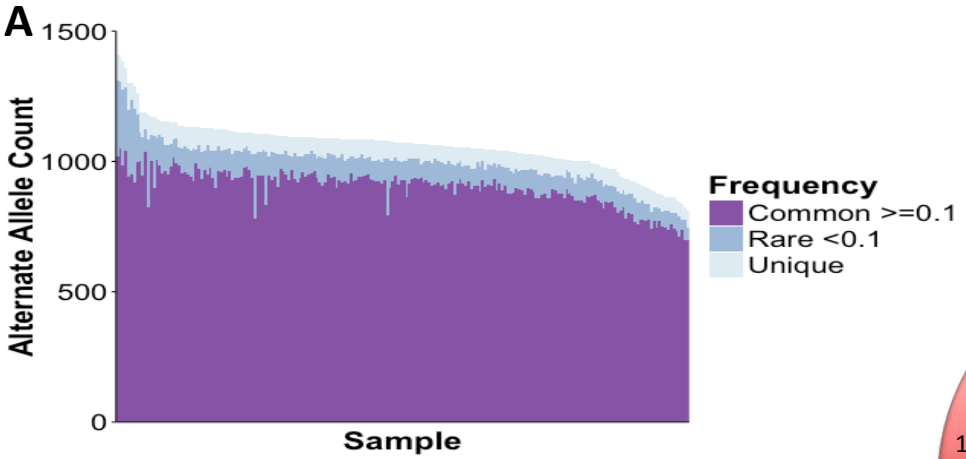
- 12 subpanels covering 197 genes – (all genes on Oxford panel included)
- Each subpanel contains all the know genes associated with the specific condition
- Searches made across Omim, Genecards and Pubmed to identify targets.
- Gene list is reviewed every time the panel is re-purchased
- Aim to get full exon coverage of each gene, ± 50bp into each intron plus UTR's
- Receiving 24 cases per month – Reported 160 cases since March 2016
- TAT 6 to 10 weeks – All reported variants confirmed by Sanger Sequencing


Congenital Anaemia
n = 95
Diagnosis prior to Molecular Analysis
CDA
n = 14
Enzyme Deficiency
n = 13
Sideroblastic Anaemia
n = 2
Diamond Blackfan Anaemia
n = 11
Unexplained Anaemia
n = 26
Membrano pathy
n = 23
Haemoglobi nopathy
n = 3
Diagnosis After Molecular Analysis
Yes
n = 7 (50%)
Yes
n= 12 (92%)
Yes
n= 1 (50%)
Yes
n= 6 (55%)
Yes
n= 17 (65%)
Yes
n= 20 (87%)
Yes
n= 2 (67%)
CDA
n = 5
Enzyme Deficiency
n = 12
Sideroblastic Anaemia
n = 2
Diamond Blackfan Anaemia
n = 6
Unexplained Anaemia
n = 17
Membrano pathy
n = 20
Haemoglobi nopathy
n = 2
Pyruvate Kinase deficiency
n = 1
1 case HK
1 Case PGK1
remainder
G6PD
PKLR
YARS2
Functional studies confirmed
6x Enzymes
12 Membranes
1x Enz/ Mem
2x LCR Deletions
Diamond Blackfan Anaemia
n = 1
RPL35A whole gene deletion
DBA5
14x Spectin
3x Ankyrin
2x Band3
1x PIEZO 1

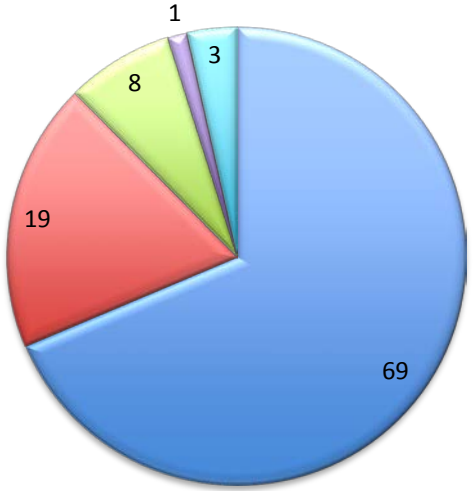

67 cases – Non Haemolytic Anaemia
Diagnosis prior to Molecular Analysis
Congenital Erythrocytosis
n = 25
Iron regulation
n = 29
Lymphoedema
n = 8
Porphyria
n = 2
Bone Marrow Failure
n = 3
Diagnosis After Molecular Analysis
Yes
n= 3 (12%)
Yes
n= 10 (34%)
Yes
n= 3 (27%)
Yes
n= 1 (50%)
Yes
n= 0 (0%)
No surprising findings

The need for functional studies

Rare variants are relatively common



Class 3 variants are a significant issue



2215 scored variants

- Class 1
- Class 2
- Class 3
- Class 4
- Class 5

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CNV Analysis

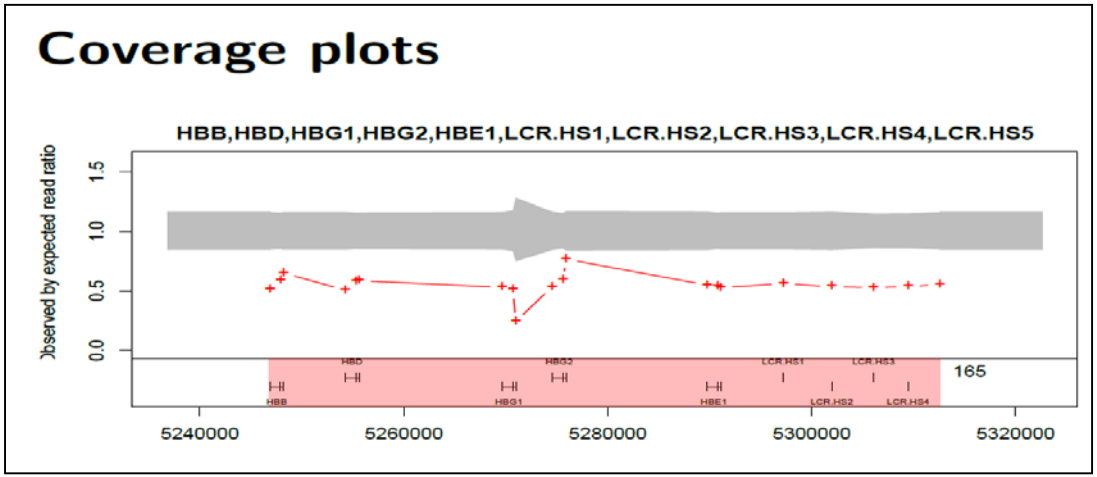
Panel: rcp3
Targets: /home/vagrant/snappy/snappy/tas/rcp3/rcp3 exomedepth.bed
Sample and references: 12B0419750, 13B1540043, 14B0257395, 16B0924419, 16B0940262, 16B0985880, 16B0996377, 16B1002232, 16B1003660, 16B1030819, 16B1035676, 16B1035765, 16B1045908, 16B1045973, G154614S, NG11-056

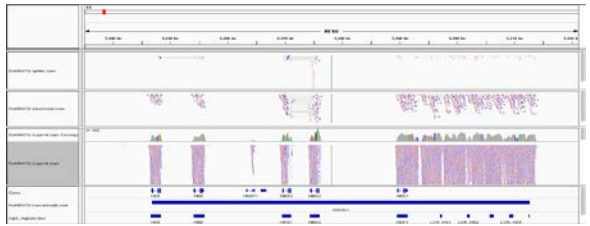
Sample G154614S

id	type	BF	reads.expected	reads.ratio	genes
chr11:5246820-5312631	deletion	165.00	10554	0.558	HBB,HBD,HBG1,HBG2,HBE1,LCR.HS1,LCR.HS2,LCR.HS3,LCR.HS4,LCR.
chr16:72091292-72093087	duplication	5.29	613	1.280	HP

Table 1/1 CNVs with negative bayes factors have been omitted.

Selected reference: 12B0419750, NG11-056, 16B1035765, 13B1540043, 16B1030819





No breakpoint spanning sequences captured so precise breakpoints unknown.

Case 1. G154614S

Oxford ORCP

↑ Or ↓

Kings panel

Haematology	
RBC (*10^{12}/l)	6.29
Hb (g/l)	102
MCV (fl)	52
MCH (pg)	16.2
HbF (%)	4.4
HbA2 (%)	2.9

Mutual learning experience

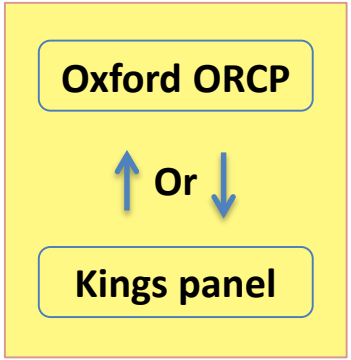
Oxford learning from Kings

- Oxford was not routinely doing haemoglobinopathy investigations on all red cell panel requests
- Beta globin MLPA was not routinely done
- Both of these have now been addressed to prevent risk of recurrence

Kings learning from Oxford

- Kings was limiting the data analysis to specific subpanels directed by the referral information.
- Defeated the object of having a single large panel
- Cases of severe PK deficiency which presented like CDA were initially missed but the sequence data was available for analysis.
- King's now reviews all the sequence variant data before issuing a negative report.

Harmonisation



- Choice of genes
- Sample exchange
- Currently 16 samples undiagnosed from Oxford being tested at Kings
- Kings to organise undiagnosed cases to send to Oxford
- Share pathogenicity scores
- Share of databases to allow electronic harmonization
- Negative cases – WGS or WES?

Deep phenotyping

Patient: EC27062010

Diagnosis

Provisional diagnosis	Isolated anaemia		
	-		
	-		
Confirmed diagnosis	-		
Classification	unclassified		

Fetal

Gestational age at delivery	40 weeks		
Fetal anaemia	N	Gestational age at onset: --	
Intrauterine transfusion	N	Units: --	Comments: --
Antenatal scans	normal		

Neonatal

Neonatal anaemia	N	Age at onset: --	
Neonatal transfusions	N	Units: --	Comments: --
Prolonged neonatal jaundice	N	Age at onset: --	Intervention required: nothing
SCBU admission	N		
Cord blood collected	--		

Clinical Information | Blood tests | Other tests | Imaging | Genetics | Marrow | **HPO** | Notes | Samples

Patient: EC27062010

HPO id	HPO name
HP:0002027	Abdominal pain
HP:0001903	Anemia
HP:0002013	Vomiting

Research studies

New patient for ongoing research studies

- DBA → Imperial
- Red cell enzymes → Kings & Imperial
- Membranes → Kings & Bristol
- Haemoglobin → Oxford & Kings
- CDA → Oxford

- Open invitation to other groups
- Need to develop and share skills

Overview

- Rare inherited anaemias: clinical
- The patient pathway
- A novel unified approach to diagnostics
- Targeted NGS
  - Oxford panel
  - Kings Panel
- Building the common pathway
- Red Cell Diagnostics- a collaborative network

Red Cell Diagnostics- a collaborative network

- Laboratory diagnostics /molecular diagnostics
- Functional studies/additional research
- Sending samples/referring cases
- We have had 3 collaborators' meetings in 2 years
  → more planned
  → let us know if you would like to be included
  – ?based on case discussions
  – ?based on research interests/functional work

Any interest? Please contact
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