‘Dynamic Panelisation’ as an approach to clinical exome analysis

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Approaches to clinical exome analysis

Phenotype-agnostic approach

1. **Trios**

   Increased diagnostic yield
   De novo variant detection
   Variant inheritance
   3x cost

2. **Very low frequency, high-impact variants**

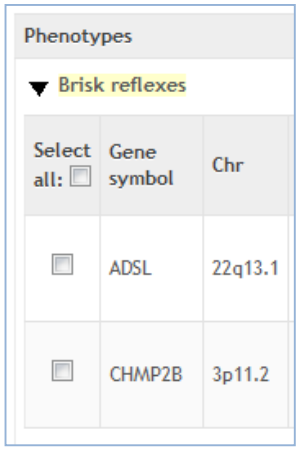
Phenotype-driven approach

1. **Filtering**

   Virtual gene panels

2. **Variant prioritisation**

   Exomizer, Phenodigm, Vaast, Phevor, PhoRank etc.



Referrals for clinical exome analysis - 1

Referrals for clinical exome analysis previously characterised by:

1. Variable method and quality of communication (emails, referral cards, phone calls).

2. Limited up-front clinical details and phenotypic information provided.

3. Long analysis and reporting times, especially for negatives.

4. Difficulties in providing a consistent approach.

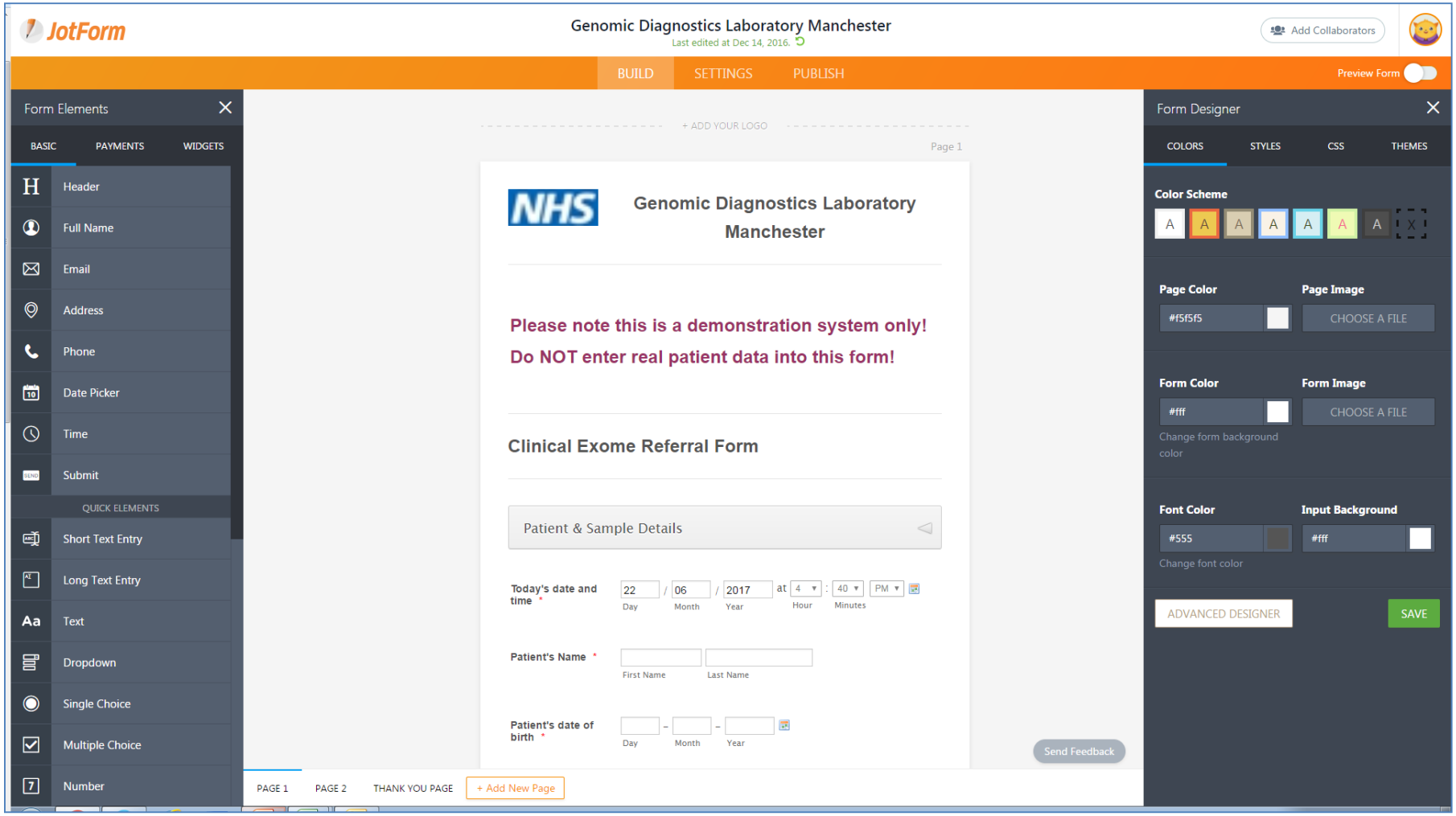
Aim was to provide a new system for referrers in our Clinical Genetics team to address the issues above.

Referrals for clinical exome analysis – 2

Design and prototyping

- A variety of tools exist to create ‘wireframes’ and mock-ups for apps (examples includes Wireframe.cc, Invision and Justinmind).
- Other tools exist for the creation of on-line forms such as JotForm, FormStack and WuFoo.
- We selected JotForm to enable us to design a prototype form to facilitate discussion with a web developer.

Referrals for clinical exome analysis – 3



Referrals for clinical exome analysis – 4

Design and prototyping continued:

- The JotForm prototype was converted into a fully functional web-based submission form by Web Developer Algy Taylor.

Features of version 1 of web referral form included:

- Secure login via an email linked to trust account
- Collection of basic referral information (demographics, referring clinician etc.)
- Capture of phenotypic codes

- Acknowledgement of submission

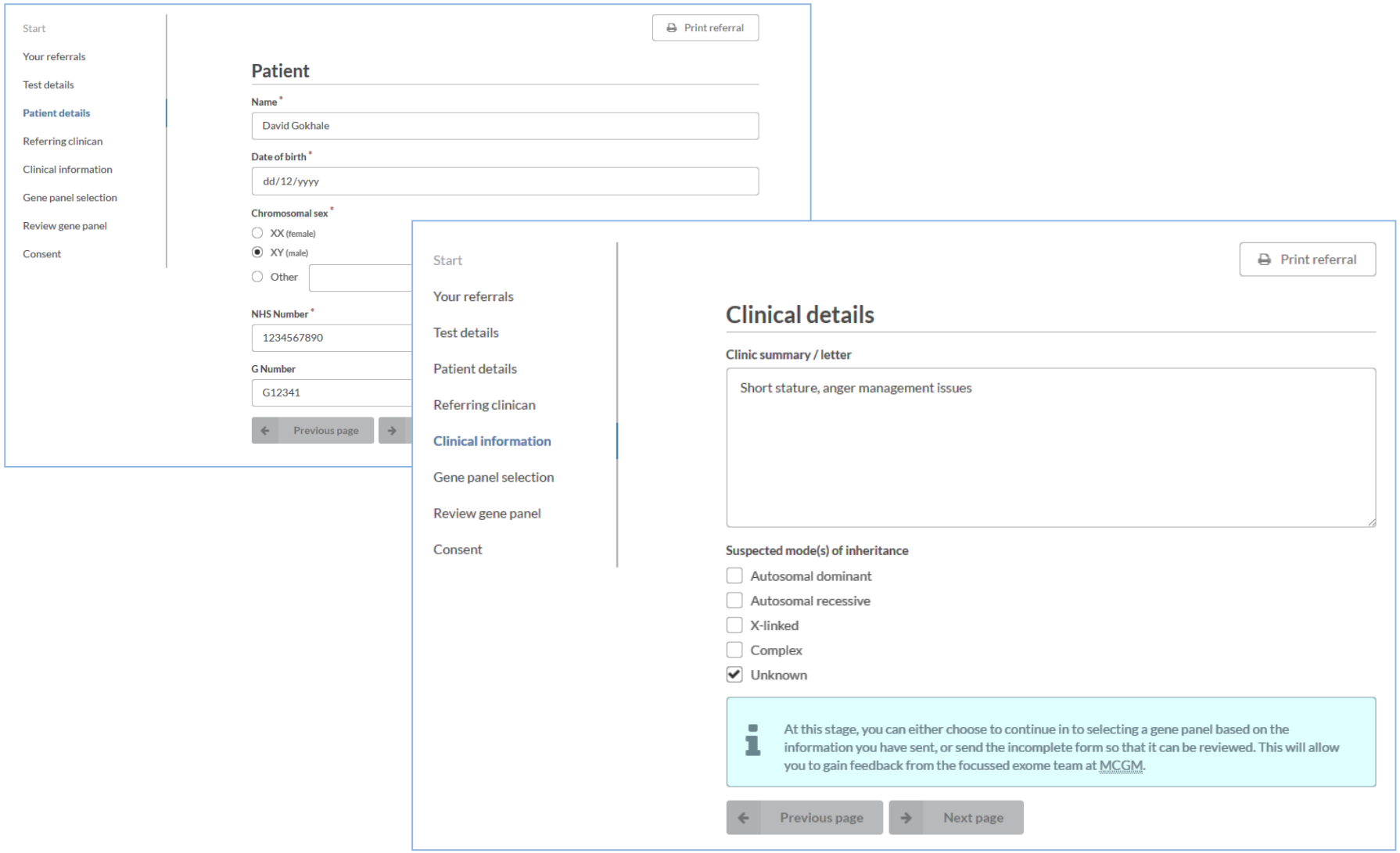
Referrals for clinical exome analysis – 5

Development of additional functionality

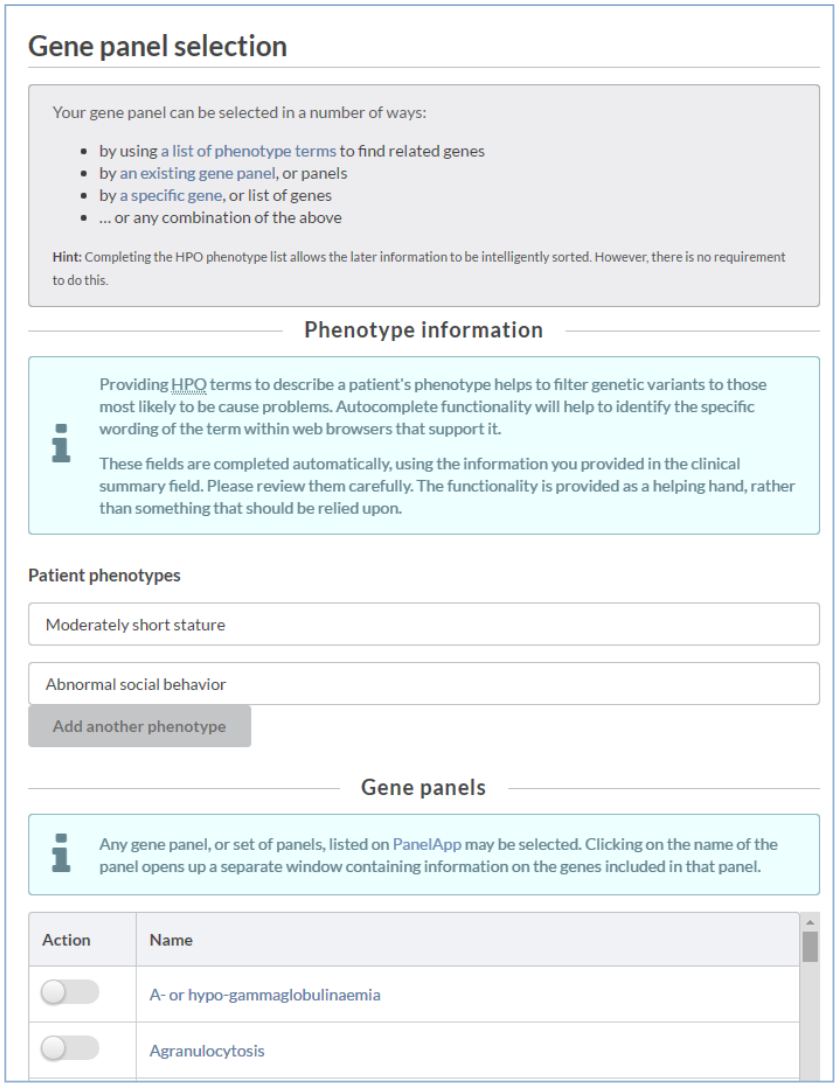
A version 2 of the system was developed further to include:

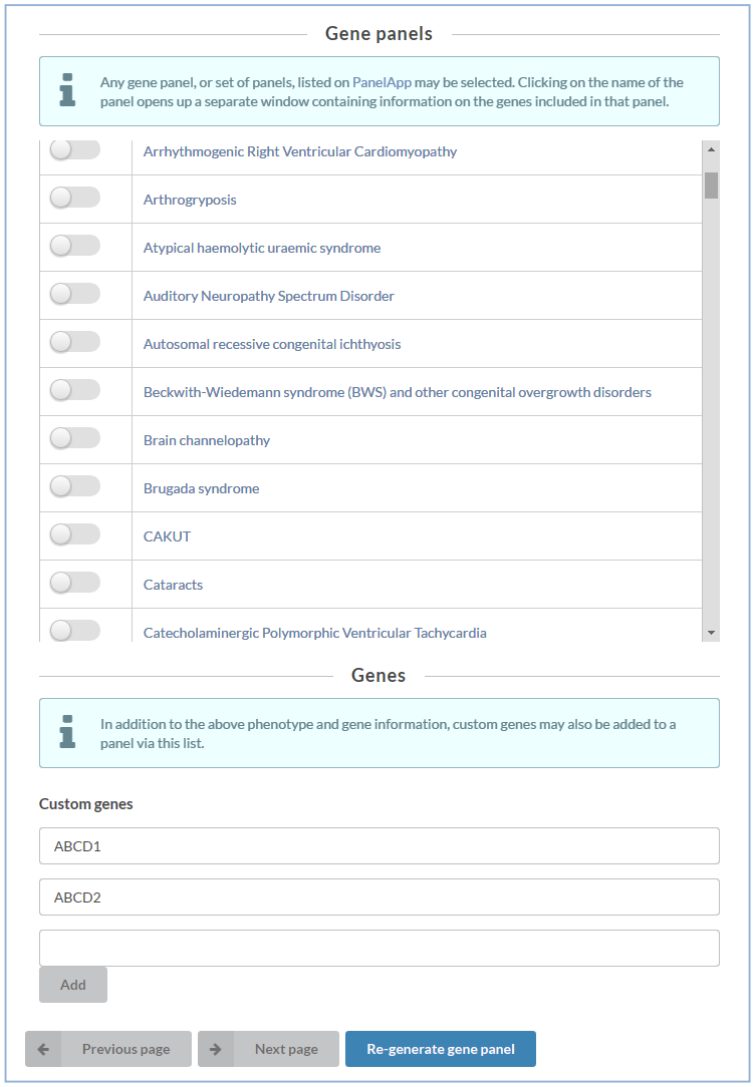
- The ability to select individual genes that are represented in the Agilent Focused Exome design
- The ability to select panels of genes from existing PanelApp panels
- The ability to select phenotypic terms and use the associated HPO code to select a gene panel
- Coverage predictions

Current Web Referral Form



Gene Panel Selection





Review of selected panel

Genes on excluded gene list

Some genes are excluded to prevent incidental findings. Ones that you have stated in your custom gene list will be automatically selected, all others will "**off**" by default.

Gene				Mean Coverage		
Include	Symbol	Reason	Disease	<18x	30x	50x
○	KCNQ1	On **Congenital hearing impairment (profound/severe)** panel	Long QT syndrome 1	0%	99.8%	97.7%

Other genes

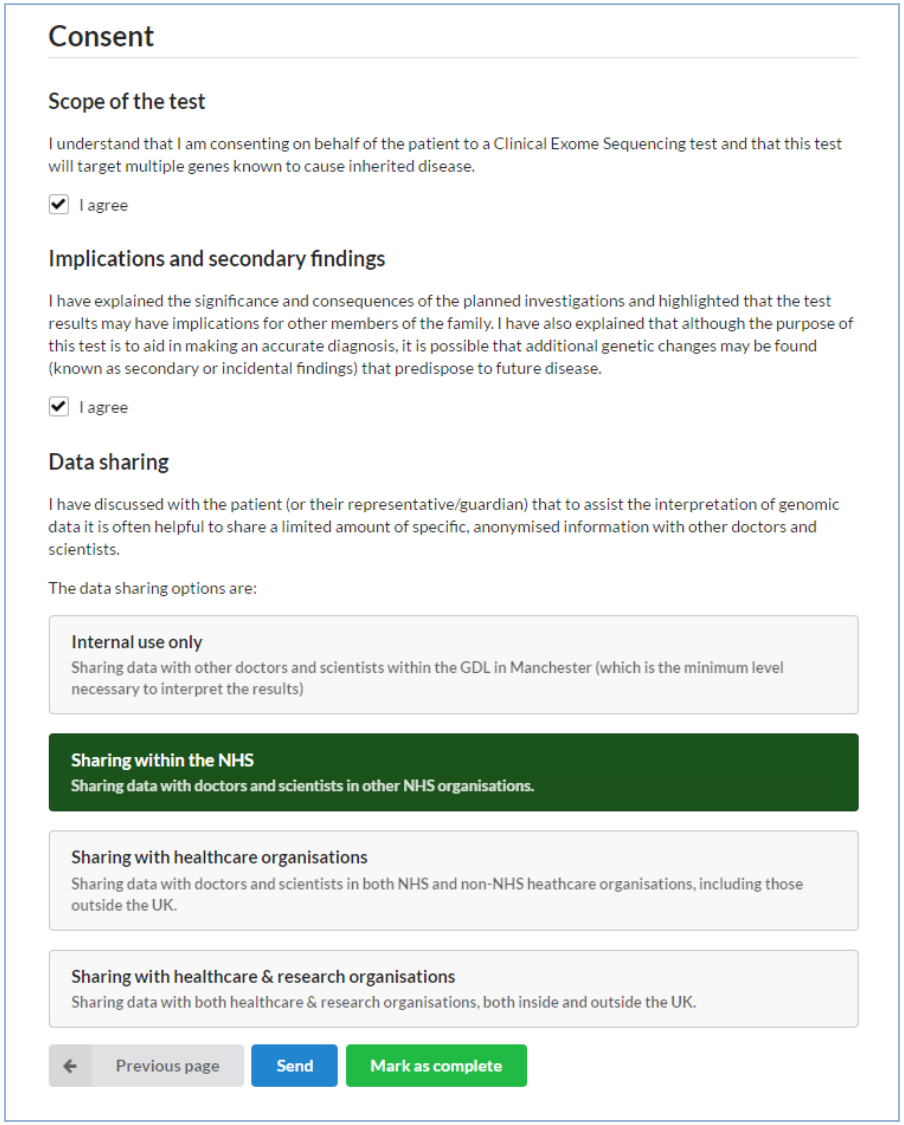
You can sort the table below by clicking on the header you wish to sort by. Clicking a second time will reverse the order.

Gene			Mean Coverage		
Include	Symbol	Reason	<18x	30x	50x
●	HOXB1	Custom gene	0%	100%	100%
●	PLXND1	Custom gene	0%	100%	98.4%
●	REV3L	Custom gene	0.9%	98%	89.1%
●	CHD7	On **Choanal atresia** panel	0%	98.9%	91.1%
●	EFTUD2	On **Choanal atresia** panel	0.3%	99.2%	95.1%
●	FAM20C	On **Choanal atresia** panel	0%	100%	100%
●	FGFR2	On **Choanal atresia** panel	0%	99.8%	93.5%
●	FGFR3	On **Choanal atresia** panel	0%	100%	100%
●	POLR1D	On **Deafness and congenital structural abnormalities** panel	0%	100%	91.3%
●	SF3B4	On **Deafness and congenital structural abnormalities** panel	21.9%	72.7%	64.1%
●	TCOF1	On **Deafness and congenital structural abnormalities** panel	0.4%	99.1%	96.6%
●	TFAP2A	On **Deafness and congenital structural abnormalities** panel	0%	99.8%	96.3%
●	AMER1	**3** linked phenotypes	0%	100%	100%
●	ANKH	**2** linked phenotypes	0%	100%	97.5%
●	DCHS1	**2** linked phenotypes	0%	99.6%	97.3%
●	FAT4	**2** linked phenotypes	0.2%	98.5%	91.5%
●	FKRP	**2** linked phenotypes	0%	100%	100%
●	GATA1	**2** linked phenotypes	0%	95.8%	89.8%
●	GJA1	**2** linked phenotypes	75.6%	14.5%	10.3%
●	LRP5	**2** linked phenotypes	0.1%	98.8%	96.5%
●	POMT1	**2** linked phenotypes	1.5%	98.2%	96.3%
●	POMT2	**2** linked phenotypes	0.3%	99.2%	95.7%
○	RPS28	**2** linked phenotypes	66.7%	27.8%	9.4%
●	SOST	**2** linked phenotypes	0%	98.3%	90.5%
●	TP63	**2** linked phenotypes	0%	99.8%	97.1%

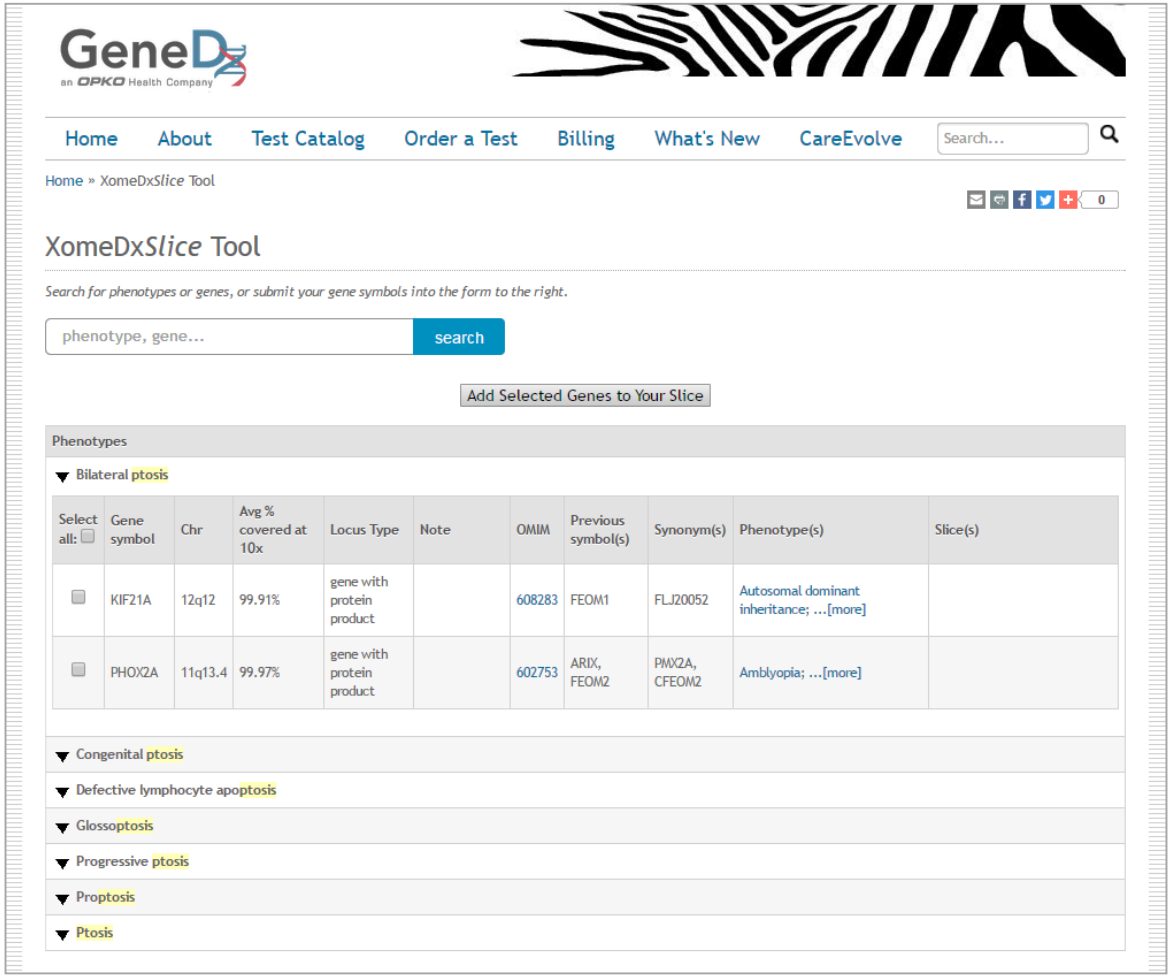
You can choose to confirm the panel you have selected, or send the form in a partially complete state. If you choose to do this, it will be reviewed by a member of staff at MCGM, with feedback sent once this has been completed.

Reject panel | Next page | Revise panel

Consent

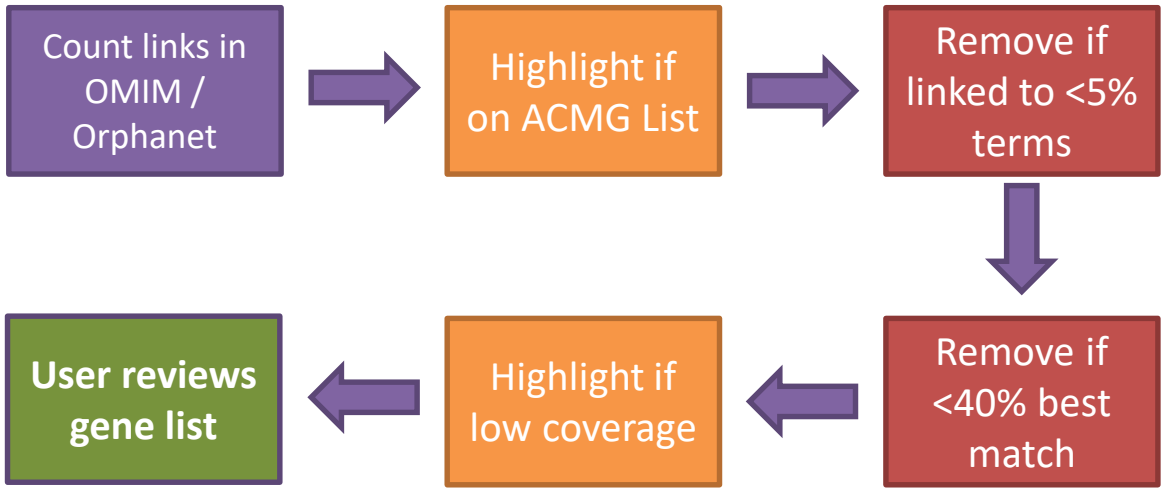

Consent
Scope of the test
I understand that I am consenting on behalf of the patient to a Clinical Exome Sequencing test and that this test will target multiple genes known to cause inherited disease.
I agree
Implications and secondary findings
I have explained the significance and consequences of the planned investigations and highlighted that the test results may have implications for other members of the family. I have also explained that although the purpose of this test is to aid in making an accurate diagnosis, it is possible that additional genetic changes may be found (known as secondary or incidental findings) that predispose to future disease.
I agree
Data sharing
I have discussed with the patient (or their representative/guardian) that to assist the interpretation of genomic data it is often helpful to share a limited amount of specific, anonymised information with other doctors and scientists.
The data sharing options are:
Internal use only
Sharing data with other doctors and scientists within the GDL in Manchester (which is the minimum level necessary to interpret the results)
Sharing within the NHS
Sharing data with doctors and scientists in other NHS organisations.
Sharing with healthcare organisations
Sharing data with doctors and scientists in both NHS and non-NHS heathcare organisations, including those outside the UK.
Sharing with healthcare & research organisations
Sharing data with both healthcare & research organisations, both inside and outside the UK.
Previous page
Send
Mark as complete

Phenotypically-driven gene panel selection



User-entered list of HPO terms

- Conductive Hearing Loss
- Microtia
- Failure to thrive
- Patent Ductus Arteriosus
- Facial Palsy

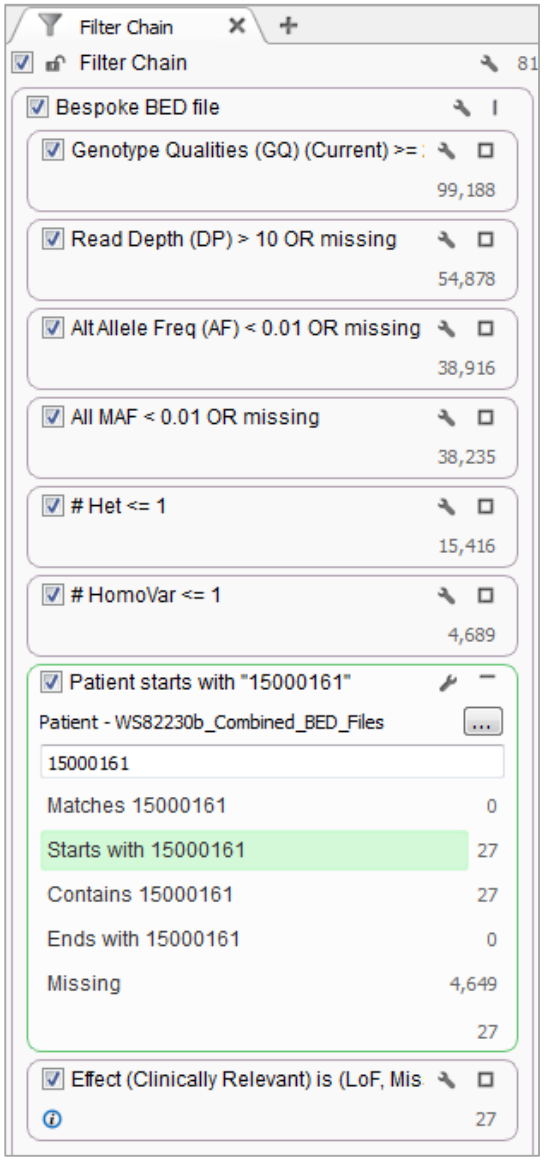


Gene	Terms Linked (OMIM/Orphanet)	ACMG	% Stated (> 5%)	% Best (> 40%)	<18x Coverage (> 9%)	30x Coverage (< 70%)
AMER1	4		80%	100%	0%	100%
SMAD4	3		60%	75%	0%	97.58%
BRAF	2		40%	50%	2.65%	87.06%
GJA1	2		40%	50%	75.64%	14.53%
OTC	1		20%	25%	0%	98.06%
ABCA4	0		0%	0%	0.09%	98.59%
BRCA1	0	X	0%	0%	4.35%	95.56%
…						

Personalised BED file generation

Status	Date of referral	Referring clinician	BED File
Submitted	26/01/2017	Elizabeth Jones, Genetics, SMH	Download
Submitted	27/01/2017	S Douzgou, MCGM	Download
Submitted	27/01/2017	S Douzgou, MCGM	Download
Submitted	27/01/2017	S Douzgou, MCGM	Download

Variant filtering



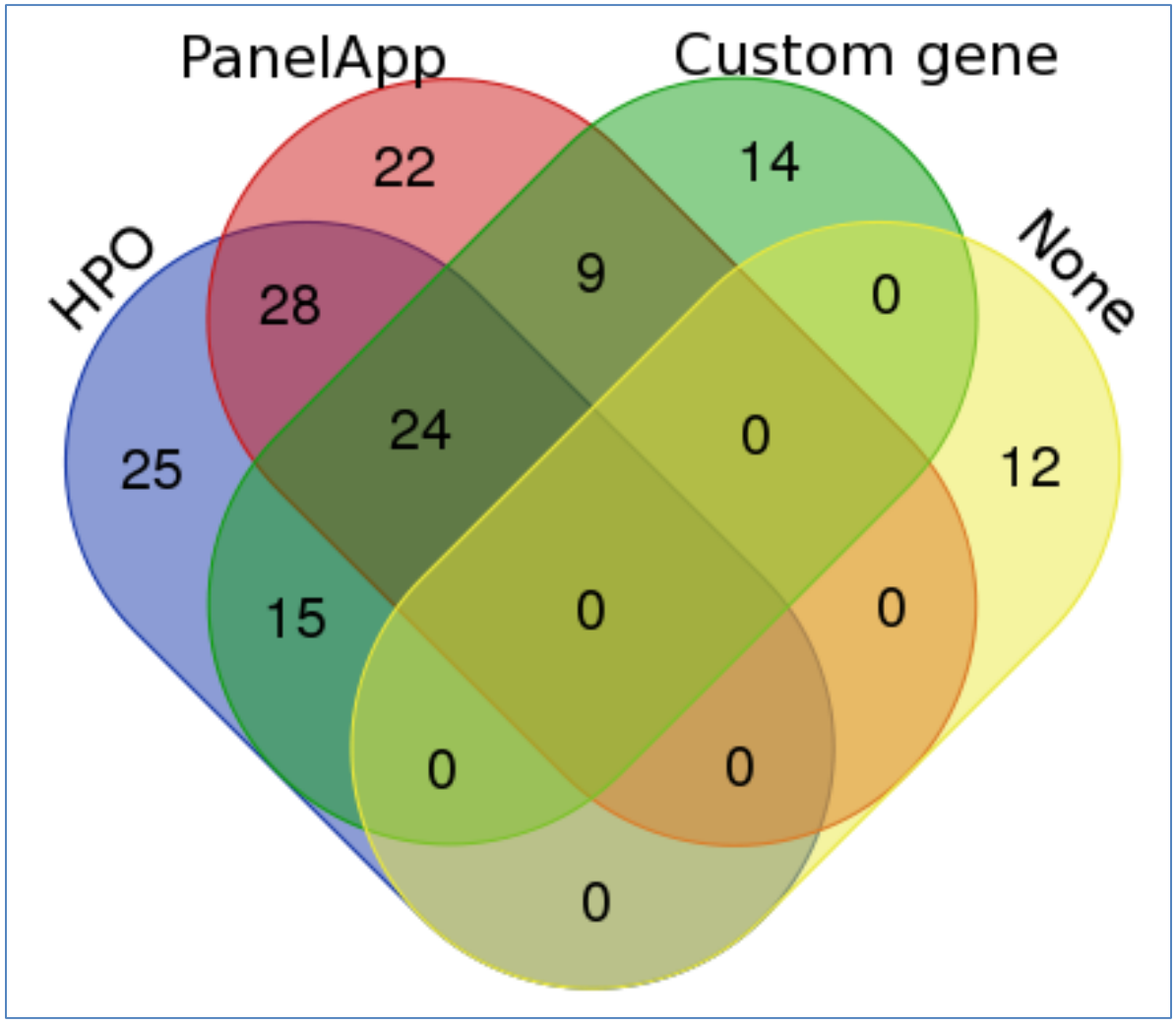
VarSeq Filter Chain

Retained:

- High quality variants
- Reasonable depth coverage
- <1% MAF
- Not a run-specific artifact present in other patients in project
- Limited to variants genes contained in the personalized .BED file

www.goldenhelix.com

Preference for method of panel creation at referral

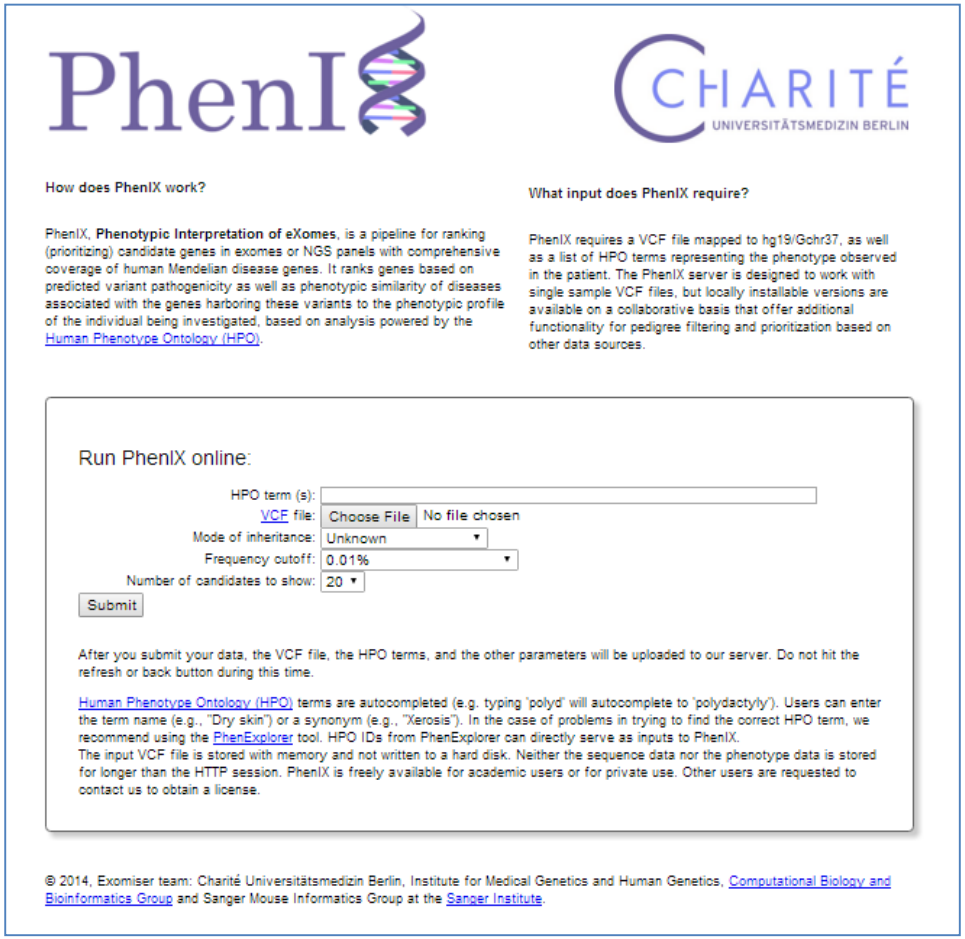


Venn diagram creator from http://bioinformatics.psb.ugent.be/webtools/Venn/

Preliminary findings

Case	Initials	HPO	PanelApp	Custom	Comments
1	AR	✓		✓	
2	ZK	✓			Consultant knew that HPO panel covered their preferences
3	MS			✓	
4	BS	✗			No specific genes suspected
5	KS	✓	✓		
6	FM	✓		✓	
7	BW	✗	✗		Likely pathogenic variant detected by PhenIX prioritisation
8	ARo			✓	
9	MA	✓			Specific gene in mind but HPO used to generate the panel
10	AC	✓		✓	
11	SM	✓	✓		

Variant Prioritisation

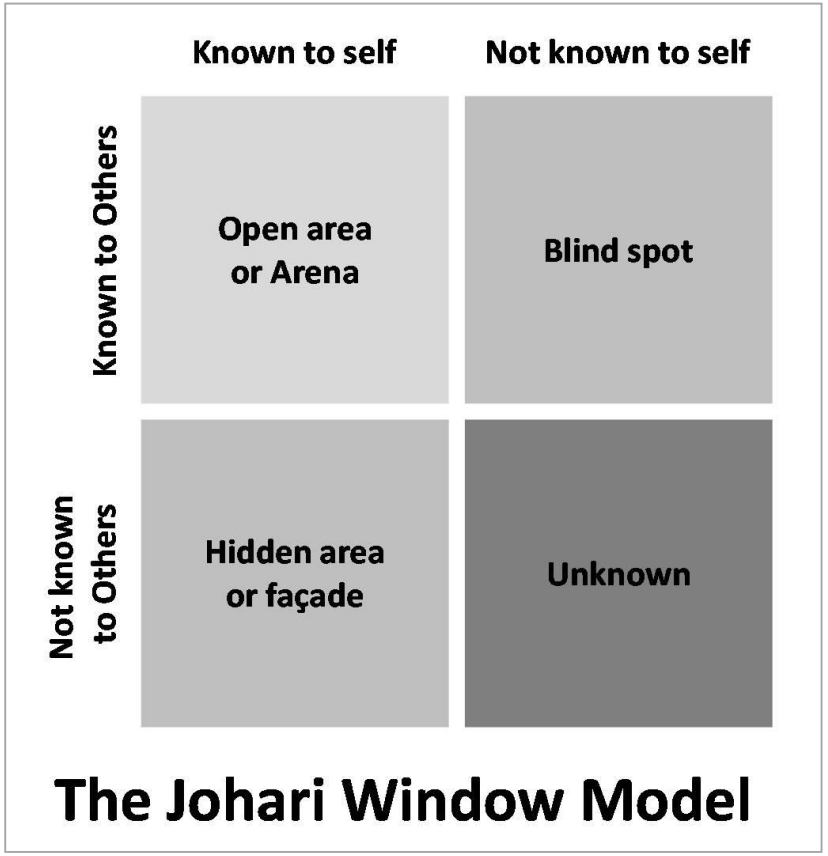


Unknown unknowns

“Reports that say that something hasn't happened are always interesting to me, because as we know, there are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns – the ones we don't know we don't know”

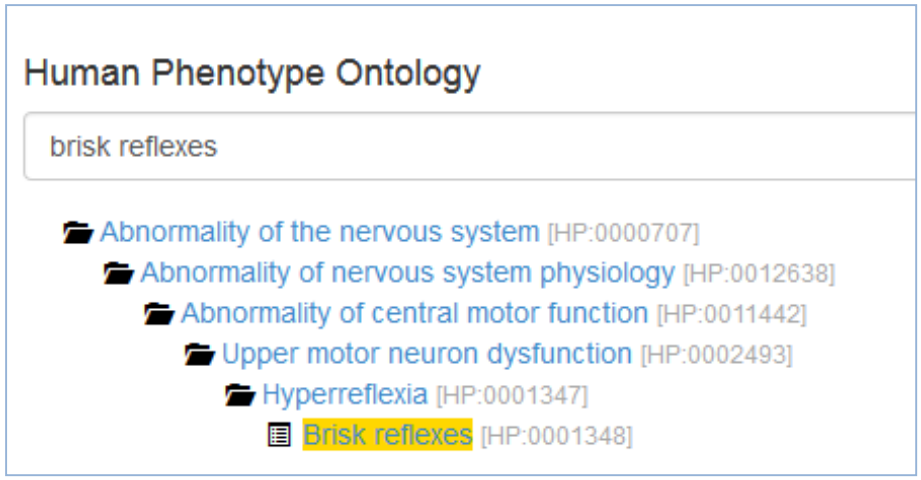
Donald Rumsfeld, United States Secretary of Defense, February 2012

Unknown unknowns



Joseph Luft and Harrington Ingham (1955)

Unknown unknowns



Phenotypes

Brisk reflexes

Select all:	Gene symbol	Chr	Avg % covered at 10x	Locus Type	Note	OMIM	Previous symbol(s)	Synonym(s)	Phenotype(s)	Slice(s)
	ADSL	22q13.1	99.97%	gene with protein product		608222			Aggressive behavior; ...[more]	
	CHMP2B	3p11.2	99.85%	gene with protein product		609512		DKFZP564O123, CHMP2.5, VPS2B	Adult onset; ...[more]	Amyotrophic Lateral Sclerosis Slice (does not include... ...[more]

Conclusions

Conclusions from preliminary cases referred using this approach:

- It is possible to rapidly select gene panels from the clinical exome using an HPO-driven ‘dynamic panelisation’ approach.
- Our preliminary results suggest that this approach should currently be used to ‘back-stop’ rather than replacing existing approaches.
- Panel selection (by humans or algorithms) needs to factor in any potential anomalies in the Human Phenotype Ontology.
- Don’t forget the acknowledgments

Acknowledgments

VarSeq optimisation
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