



‘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.

Phenotypes		
▼ Brisk reflexes		
Select all: ☐	Gene symbol	Chr
☐	ADSL	22q13.1
☐	CHMP2B	3p11.2

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.

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



Genomic Diagnostics Laboratory Manchester
Last edited at Dec 14, 2016.

Add Collaborators

BUILDSETTINGSPUBLISHPreview Form

Form Elements

BASICPAYMENTSWIDGETS

H

Header

Full Name

Email

Address

Phone

Date Picker

Time

Submit

QUICK ELEMENTS

Short Text Entry

Long Text Entry

Aa

Text

Dropdown

Single Choice

Multiple Choice

7

Number

+ ADD YOUR LOGO

Page 1

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Manchester

Please note this is a demonstration system only!
Do NOT enter real patient data into this form!

Clinical Exome Referral Form

Patient & Sample Details

Today's date and time *
22 / 06 / 2017 at 4 : 40 PM

Patient's Name *
First Name Last Name

Patient's date of birth *
Day Month Year

Send Feedback

Page 1PAGE 2THANK YOU PAGE+ Add New Page

Form Designer

COLORSSTYLESCSSTHEMES

Color Scheme

A

A

A

A

A

A

A

X

Page ColorPage Image

#f5f5f5

CHOOSE A FILE

Form ColorForm Image

#fff

CHOOSE A FILE

Font ColorInput Background

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ADVANCED DESIGNERSAVE

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

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

Start

Your referrals

Test details

Patient details

Referring clinician

Clinical information

Gene panel selection

Review gene panel

Consent

Print referral

Patient

Name *

David Gokhale

Date of birth *

dd/12/yyyy

Chromosomal sex *

☐ XX (female)

☒ XY (male)

☐ Other

NHS Number *

1234567890

G Number

G12341

Previous page

Next page

Start

Your referrals

Test details

Patient details

Referring clinician

Clinical information

Gene panel selection

Review gene panel

Consent

Print referral

Clinical details

Clinic summary / letter

Short stature, anger management issues

Suspected mode(s) of inheritance

☐ Autosomal dominant

☐ Autosomal recessive

☐ X-linked

☐ Complex

☒ Unknown

 At this stage, you can either choose to continue in to selecting a gene panel based on the information you have sent, or send the incomplete form so that it can be reviewed. This will allow you to gain feedback from the focussed exome team at [MCGM](#).

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Gene Panel Selection

Gene panel selection

Your gene panel can be selected in a number of ways:

- by using a list of [phenotype terms](#) to find related genes
- by an existing gene panel, or panels
- by a specific gene, or list of genes
- ... or any combination of the above

Hint: Completing the HPO phenotype list allows the later information to be intelligently sorted. However, there is no requirement to do this.

Phenotype information



Providing [HPO](#) terms to describe a patient's phenotype helps to filter genetic variants to those most likely to be cause problems. Autocomplete functionality will help to identify the specific wording of the term within web browsers that support it.

These fields are completed automatically, using the information you provided in the clinical summary field. Please review them carefully. The functionality is provided as a helping hand, rather than something that should be relied upon.

Patient phenotypes

Moderately short stature

Abnormal social behavior

Add another phenotype

Gene panels



Any gene panel, or set of panels, listed on [PanelApp](#) may be selected. Clicking on the name of the panel opens up a separate window containing information on the genes included in that panel.

Action	Name
☐	A- or hypo-gammaglobulinaemia
☐	Agranulocytosis

Gene panels



Any gene panel, or set of panels, listed on [PanelApp](#) may be selected. Clicking on the name of the panel opens up a separate window containing information on the genes included in that panel.

☐	Arrhythmogenic Right Ventricular Cardiomyopathy
☐	Arthrogryposis
☐	Atypical haemolytic uraemic syndrome
☐	Auditory Neuropathy Spectrum Disorder
☐	Autosomal recessive congenital ichthyosis
☐	Beckwith-Wiedemann syndrome (BWS) and other congenital overgrowth disorders
☐	Brain channelopathy
☐	Brugada syndrome
☐	CAKUT
☐	Cataracts
☐	Catecholaminergic Polymorphic Ventricular Tachycardia

Genes



In addition to the above phenotype and gene information, custom genes may also be added to a panel via this list.

Custom genes

ABCD1

ABCD2

Add

← Previous page

→ Next page

Re-generate gene panel

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%
☒	DCSH1	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 healthcare 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



[Home](#) [About](#) [Test Catalog](#) [Order a Test](#) [Billing](#) [What's New](#) [CareEvolve](#)

Home » XomeDxSlice Tool

XomeDxSlice Tool

Search for phenotypes or genes, or submit your gene symbols into the form to the right.

Phenotypes

▼ Bilateral **ptosis**

Select all: ☐	Gene symbol	Chr	Avg % covered at 10x	Locus Type	Note	OMIM	Previous symbol(s)	Synonym(s)	Phenotype(s)	Slice(s)
☐	KIF21A	12q12	99.91%	gene with protein product		608283	FEOM1	FLJ20052	Autosomal dominant inheritance; ...[more]	
☐	PHOX2A	11q13.4	99.97%	gene with protein product		602753	ARIX, FEOM2	PHOX2A, CFEOM2	Amblyopia; ...[more]	

▼ Congenital **ptosis**

▼ Defective lymphocyte apo**ptosis**

▼ Glosso**ptosis**

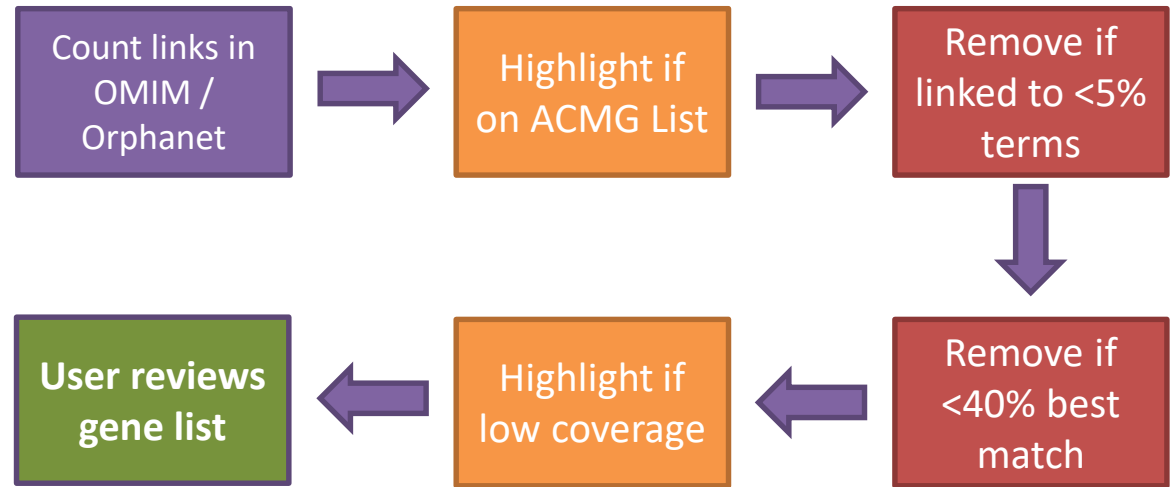
▼ Progressive **ptosis**

▼ Pro**ptosis**

▼ **Ptosis**

User-entered list of HPO terms

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

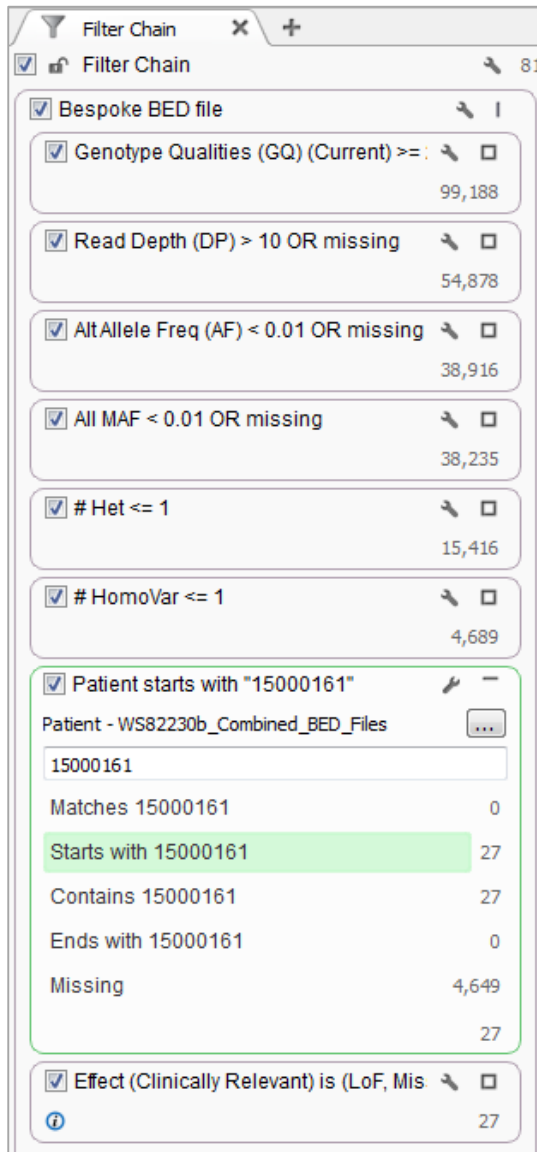


Gene	Terms Linked (OMIM/Orphanet)	ACMG	% Stated (> 5%)	% Best (> 40%)	<18x Coverage (> 9%)	30x Coverage (< 70%)
AMER1	4		80%	100%	0%	100%
SMAD4	3		60%	75%	0%	97.58%
BRAF	2		40%	50%	2.65%	87.06%
GJA1	2		40%	50%	75.64%	14.53%
OTC	1		20%	25%	0%	98.06%
ABCA4	0		0%	0%	0.09%	98.59%
BRCA1	0	X	0%	0%	4.35%	95.56%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

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



The screenshot shows the VarSeq Filter Chain interface. It displays a list of filters applied to a dataset, with the total count of variants remaining after each filter. The filters are:

- ☒ Bespoke BED file (81)
- ☒ Genotype Qualities (GQ) (Current) ≥ 10 (99,188)
- ☒ Read Depth (DP) > 10 OR missing (54,878)
- ☒ Alt Allele Freq (AF) < 0.01 OR missing (38,916)
- ☒ All MAF < 0.01 OR missing (38,235)
- ☒ # Het ≤ 1 (15,416)
- ☒ # HomoVar ≤ 1 (4,689)
- ☒ Patient starts with "15000161" (27)
 - Patient - WS82230b_Combined_BED_Files
 - 15000161
 - Matches 15000161: 0
 - Starts with 15000161: 27
 - Contains 15000161: 27
 - Ends with 15000161: 0
 - Missing: 4,649
- ☒ Effect (Clinically Relevant) is (LoF, Mis) (27)

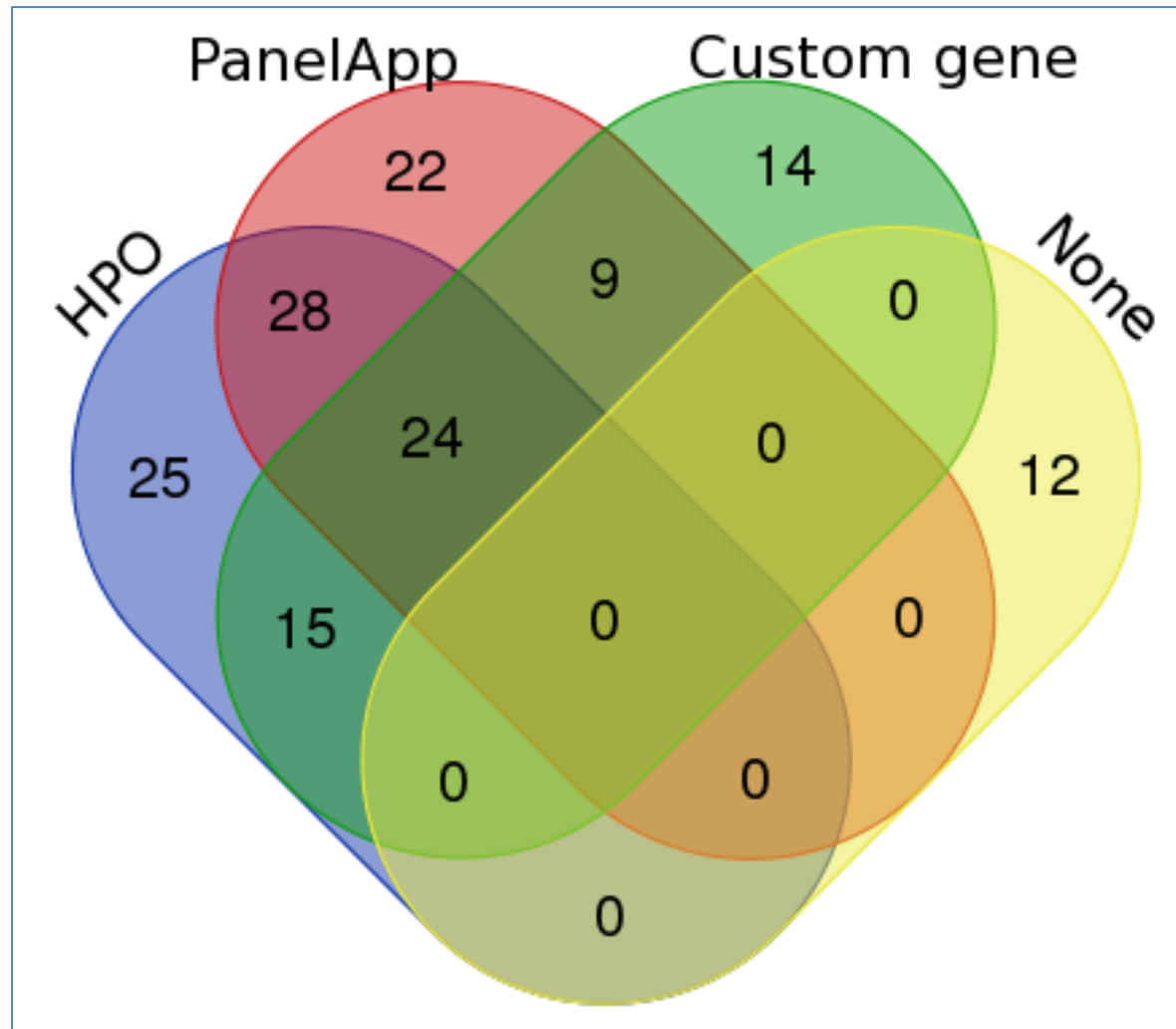
VarSeq Filter Chain

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



How does PhenIX work?

PhenIX, **Phenotypic Interpretation of eXomes**, is a pipeline for ranking (prioritizing) candidate genes in exomes or NGS panels with comprehensive coverage of human Mendelian disease genes. It ranks genes based on predicted variant pathogenicity as well as phenotypic similarity of diseases associated with the genes harboring these variants to the phenotypic profile of the individual being investigated, based on analysis powered by the [Human Phenotype Ontology \(HPO\)](#).

What input does PhenIX require?

PhenIX requires a VCF file mapped to hg19/Gchr37, as well as a list of HPO terms representing the phenotype observed in the patient. The PhenIX server is designed to work with single sample VCF files, but locally installable versions are available on a collaborative basis that offer additional functionality for pedigree filtering and prioritization based on other data sources.

Run PhenIX online:

HPO term (s):

VCF file: No file chosen

Mode of inheritance:

Frequency cutoff:

Number of candidates to show:

After you submit your data, the VCF file, the HPO terms, and the other parameters will be uploaded to our server. Do not hit the refresh or back button during this time.

[Human Phenotype Ontology \(HPO\)](#) terms are autocompleted (e.g. typing 'polyd' will autocomplete to 'polydactyly'). Users can enter the term name (e.g., "Dry skin") or a synonym (e.g., "Xerosis"). In the case of problems in trying to find the correct HPO term, we recommend using the [PhenExplorer](#) tool. HPO IDs from PhenExplorer can directly serve as inputs to PhenIX. The input VCF file is stored with memory and not written to a hard disk. Neither the sequence data nor the phenotype data is stored for longer than the HTTP session. PhenIX is freely available for academic users or for private use. Other users are requested to contact us to obtain a license.

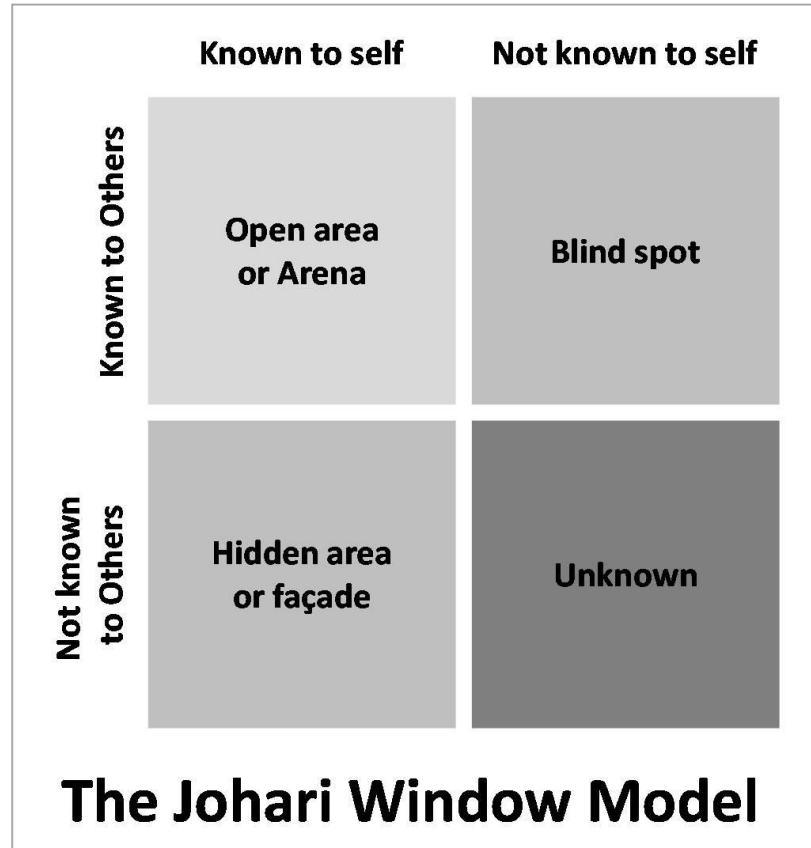
Unknown unknowns



“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

Human Phenotype Ontology

brisk reflexes

- 📁 [Abnormality of the nervous system](#) [HP:0000707]
 - 📁 [Abnormality of nervous system physiology](#) [HP:0012638]
 - 📁 [Abnormality of central motor function](#) [HP:0011442]
 - 📁 [Upper motor neuron dysfunction](#) [HP:0002493]
 - 📁 [Hyperreflexia](#) [HP:0001347]
 - 📄 **Brisk reflexes** [HP:0001348]

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 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

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