Application of the Genomics England (GEL) tiering system to NGS variant analysis

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Salisbury NHS Foundation Trust

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Outline of talk

process to stratify sequence variants and speed up analysis

experiences of variant analysis and reporting

need to streamline analysis and reporting

evaluation to look at effect on analysis time and diagnostic yield

considerations for development and implementation

Variant interpretation and reporting BPG

acgs
Association for Clinical Genetic Science
Part of the British Society for Genetic Medicine

VKGL

Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics.

Original guidelines ratified by the UK Clinical Molecular Genetics Society (11th January, 2008) and the Dutch Society of Clinical Genetic Laboratory Specialists (Vereniging Klinisch Genetische Laboratoriumspecialisten; VKGL) (22nd October, 2007).

Guidelines updated by the Association for Clinical Genetic Science (formally Clinical Molecular Genetics Society and Association of Clinical Cytogenetics) and the Dutch Society of Clinical Genetic Laboratory Specialists (approved September 2013).

5 class system for variant classification

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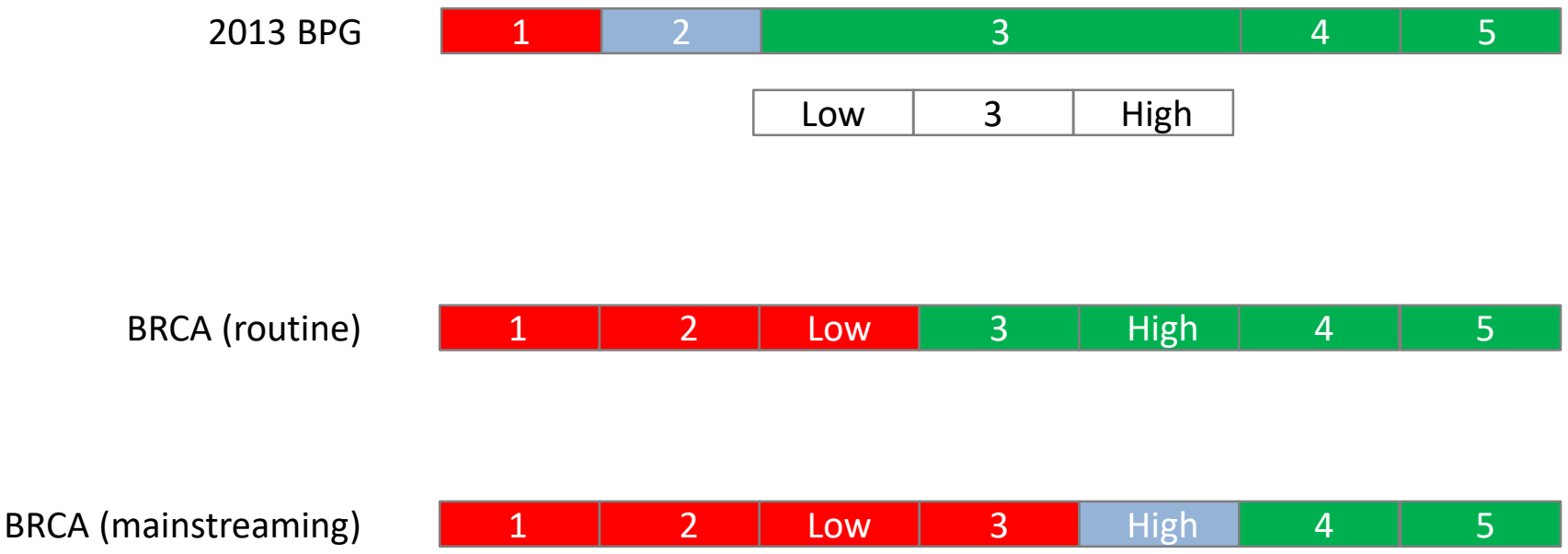
Variant interpretation and reporting BPG



Key: Don't report ? Report

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Clinical exome development

Illumina Trusight One Clinical exome (4813 genes)
analysis pipeline filters variants with MAF > 2%

140 samples tested in parallel by an NGS panel test

- Exported May 2015 - Feb 2016
- 36 different UKGTN panels
- total of 460 variants
- average 3.3 per sample

114 historic patients without a previous genetic diagnosis

- single genes, fixed and bespoke panels
- total 1051 variant
- average 9.4 per sample

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GEL 100,000 genomes project

maximise diagnostic efficiency
balance sensitivity and specificity
accept that some diagnostic variants will not be automatically prioritised

Variants within virtual gene panel Green list are divided into tiers:

Tier 1 variants
known pathogenic
protein truncating

Tier 2 variants
protein altering
intronic (near splice site)

Wessex GMC 100,000 genomes results

51 Tier 1 and Tier 2 variants returned

Tier 1	6	12 %
Tier 2	45	88 %

Diagnostic yield

Tier 1	all 6 pathogenic or likely pathogenic
Tier 2	37 no further follow up 7 awaiting decision 1 taken to MDT (class 3-4)

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Modified tiering approach

Tier 1: known pathogenic
protein truncating
HGMDPro - DM and ? DM
ClinVar - Pathogenic or likely pathogenic with ≥ 2* rating

Tier 2: all other variants

Evaluate analysing Tier 1 only vs Tier 1 and 2

take clinical exome data
apply gene selection for each patient
standard bio-informatic analysis with filter variant > 2%

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Impact on number of variants to be analysed

100KGP results	532 genes / trio	6 / 51 Tier 1	12%
140 parallel tests UKGTN panels	51 genes / patient	92 / 460 Tier 1	20%
114 bespoke tests mixed panels	109 genes / patient	108 / 1051 Tier 1	10% ##

abstract was 102 samples and 16 % Tier 1 variants

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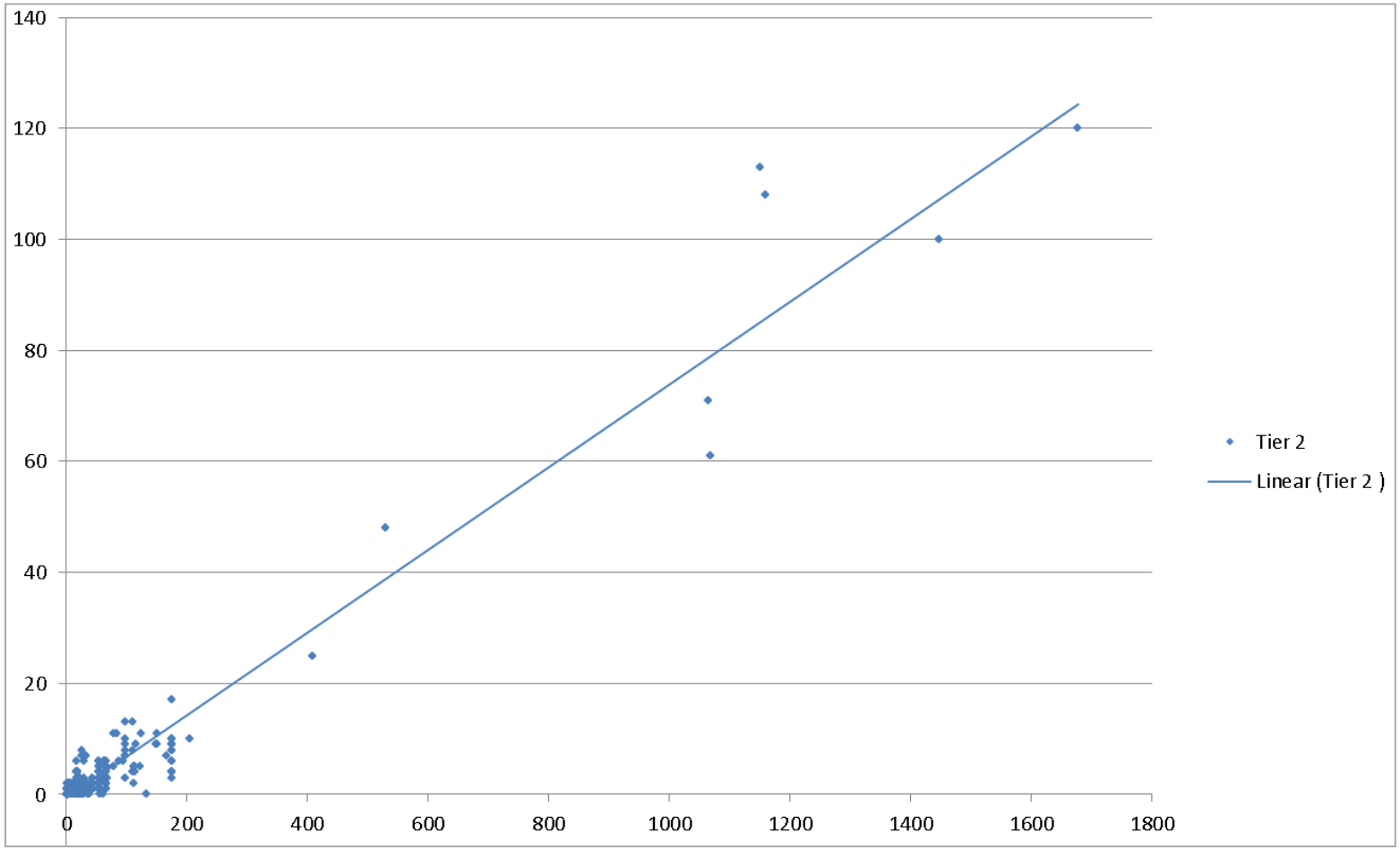
Effect of panel size on number of variants



Combined data from all 140 and 114 samples

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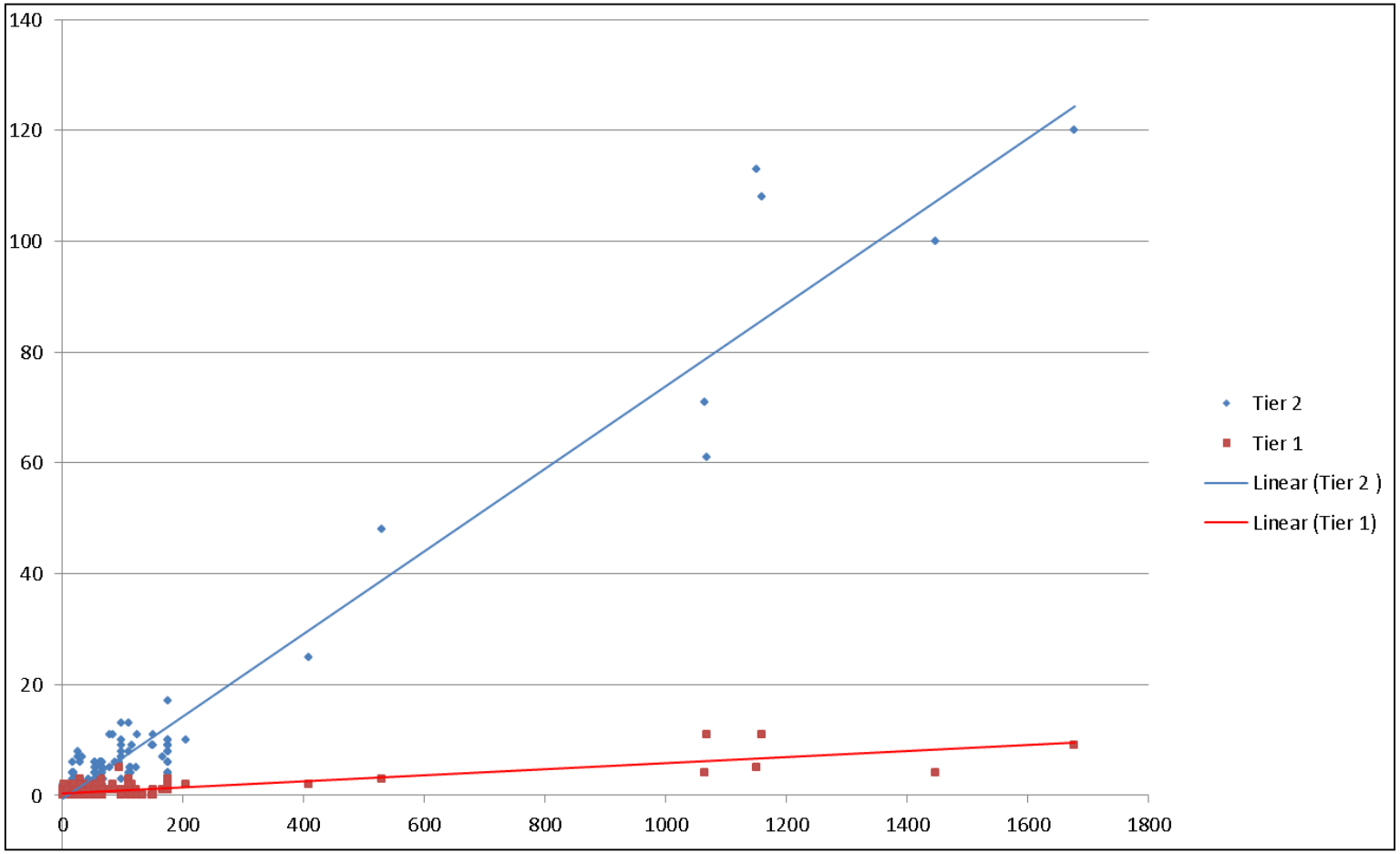
Effect of panel size on number of variants



Combined data from all 140 and 114 samples

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Impact on number of variants to be analysed

Looking only at Tier 1 variants reduces the number of variants by 80-90%

Reduction depends upon size of region of interest

Likely to take less per variant for Tier 1 compared to Tier 2

Effect of tiering on diagnostic yield

140 samples tested in parallel: UKGTN panel vs clinical exome sequence
external laboratory's report taken as "gold standard" for comparison

57 variants reported as pathogenic or likely pathogenic
55 in genes covered by the clinical exome

52 were Tier 1	yield 52 / 92	56.5 %
3 were Tier 2	yield 3 / 368	0.8 %

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Effect of tiering on diagnostic yield

Inheritance	Sample No.	Variant No.	Tier 1	Tier 2
Dominant or XL	24	24	22	2
AR (comp het)	6	12	11	1
AR (hom)	9	9	9	0
AR (carrier)	10	10	10	0
TOTAL	48	55	52	3

39 Molecular diagnosis made by external laboratory

Tier 1 only:

36 identical outcome
2 diagnosis missed
1 one variant missed = carrier

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further development

Review inclusion criteria:

DM or ?DM on HGMDPro

Pathogenic or likely pathogenic on ClinVar with ≥ ** rating

Automate the selection process

More sensitive - *can pathogenic Tier 2 be identified?*

- *look at Tier 2 in AR condition with pathogenic Tier 1*

More specific - *which Tier 1 were not pathogenic and how were identified?*

More evidence for effect on diagnostic yield in 114 patient cohort

Gain of function, very rare or poorly characterised conditions

Can this be used in conjunction with phenotype-based prioritisation tools?

Implementation

Envisage two stage process:

1) analyse and report Tier 1
2) sample passes to research or full analysis requested

Standardisation of testing across RGLs
National ACGS approach

More development before using for UKGTN tests
Use for specific applications within region

Discussion with both Clinical Genetics and other specialities
Acceptance of principle of faster, simpler reports with small chance of missing variant

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