Using the Genomics England National Genomic Research Library (NGRL) and UK Biobank to investigate the genetic, phenotypic and clinical landscape of thymidine kinase 2 deficiency (TK2d)

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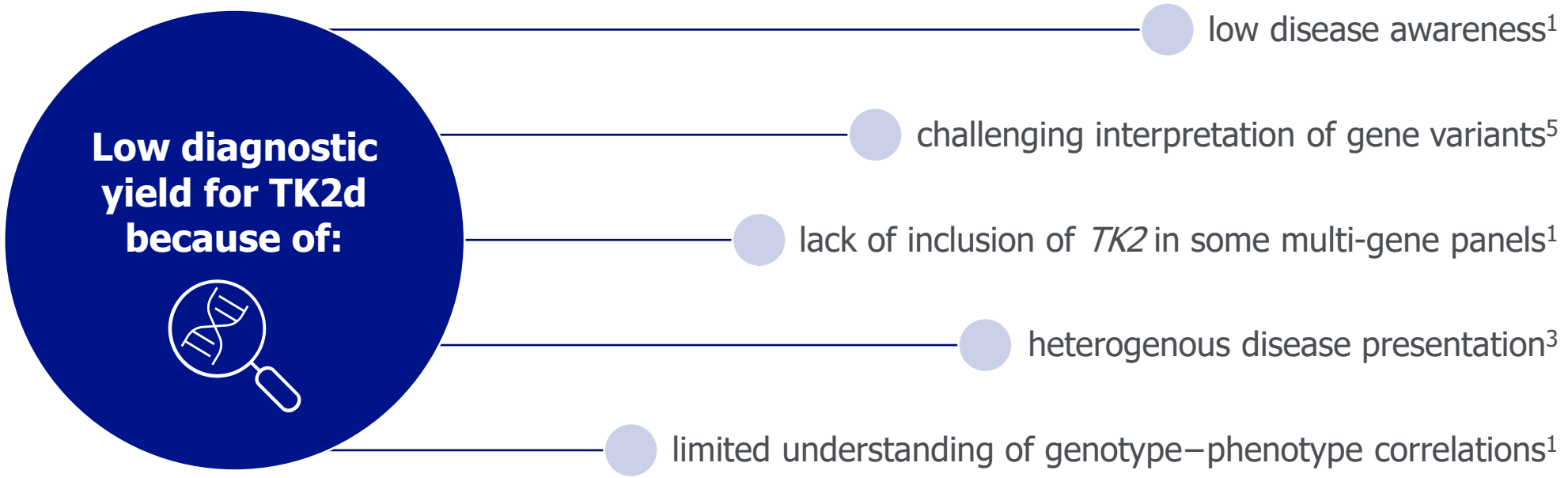
NHS, National Health Service.

TK2d is an ultra-rare, autosomal recessive, progressive and often life-threatening mitochondrial myopathy[1–3]

- Pathogenic *TK2* variants lead to impaired activity of TK2, an enzyme essential for mtDNA maintenance[1,2]
- Early diagnosis can facilitate appropriate disease management, as well as access to emerging treatments[1,4]



mtDNA, mitochondrial DNA; *TK2*, thymidine kinase 2 gene; TK2, thymidine kinase 2 enzyme; TK2d, thymidine kinase 2 deficiency.

1. Berardo A, *et al. J Neuromuscul Dis* 2022;9:225–35; 2. Wang J, *et al. Mol Genet Metabol* 2018;124:124–30; 3. Garone C, *et al. J Med Genet* 2018;55:515–21; 4. Stenton SL, Prokisch H. *EBioMedicine* 2020;56:102784; 5. Schlieben LD, Prokisch H. *Front Cell Dev Biol* 2020;8:600079.

Large cohort datasets could provide deeper insights into the genetic, phenotypic and clinical landscape of TK2d

NGRL[1]

- Managed by Genomics England
- De-identified whole-genome sequencing data, with deep phenotyping at recruitment
- Family information available for enrolled participants
- Cohorts enriched for rare diseases

100,000 Genomes Project

The UK NHS Genomic Medicine Service

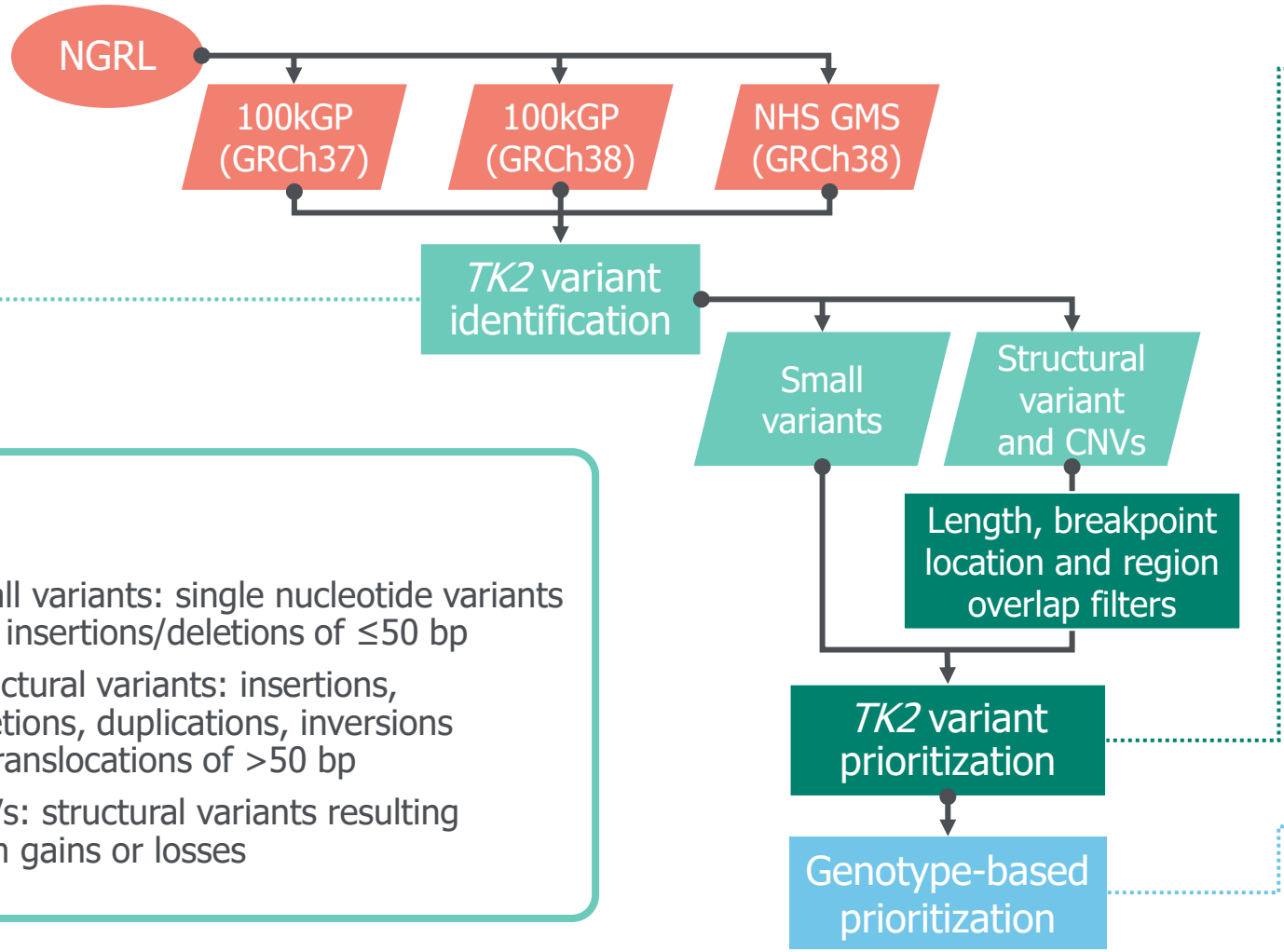
UK Biobank[2]

- Prospective cohort study of ~500,000 adults, aged 40–69 years at recruitment, from the UK[3]
- De-identified whole-exome data, with deep phenotyping at recruitment and longitudinal medical and hospital admission data[2,3]
- Nominally healthy cohort

Objectives: to use large cohort datasets to identify and characterize *TK2* variants, and to phenotypically characterize carriers of *TK2* variants

This research has been conducted using UK Biobank under Application Number 44411.
NGRL, National Genomic Research Library; NHS, National Health Service; *TK2*, thymidine kinase 2 gene; TK2d, thymidine kinase 2 deficiency.
1. The National Genomic Research Library v5.1, Genomics England. https://doi.org/10.6084/m9.figshare.4530893.v7; 2. UK Biobank [Internet]. c2025. Available from: https://www.ukbiobank.ac.uk/ (Accessed 13 June 2025);
3. Bycroft C, *et al. Nature* 2018;562:203–9.

A comprehensive variant annotation strategy was applied to prioritize *TK2* variants identified in the NGRL



- Small variants: single nucleotide variants and insertions/deletions of ≤50 bp
- Structural variants: insertions, deletions, duplications, inversions or translocations of >50 bp
- CNVs: structural variants resulting from gains or losses

Variant annotation strategy

- Allele frequencies ≤1% in both the NGRL and gnomAD
- Confirmed pathogenicity in publicly available databases (Genomics England Rare Disease Interpretation and Diagnostic Pipeline, ClinVar, VarSome)
- *In silico* predicted deleteriousness (VEP, CADD/CADD-SV, REVEL, LOFTEE, SpliceAI)
- Custom list provided by the study sponsor

Variants carried as alternate homozygous or compound heterozygous in ≥1 participant

100kGP, 100,000 Genomes Project; bp, base pairs; CADD, Combined Annotation Dependent Depletion; CADD-SV, CADD Structural Variant; CNV, copy number variant; gnomAD, Genome Aggregation Database; GRCh37, Genome Reference Consortium human build 37; GRCh38, Genome Reference Consortium human build 38; LOFTEE, Loss-Of-Function Transcript Effect Estimator; NGRL, National Genomic Research Library; NHS GMS, National Health Service Genomic Medicine Service; REVEL, Rare Exome Variant Ensemble Learner; *TK2*, thymidine kinase 2 gene; VEP, Variant Effect Predictor.

Prioritized variant carriers and family members were screened for TK2d-related phenotypes and segregation was assessed

Disease terms (recruited rare disease; HPO term; ICD-10 term) relevant to TK2d were categorized based on the following disease domains, created in collaboration with expert clinical review:

- Muscle weakness
- Impaired mobility
- Abnormal creatine kinase level
- Impaired development
- Anaemia
- Epilepsy
- Hearing loss or impairment

Group 1

- Alternate homozygous only
- TK2d-related phenotype present
- Complete penetrance

Group 2

- Alternate homozygous or heterozygous
- TK2d-related phenotype present
- Reduced penetrance

Group 3

- Alternate homozygous or heterozygous
- TK2d-related phenotype absent

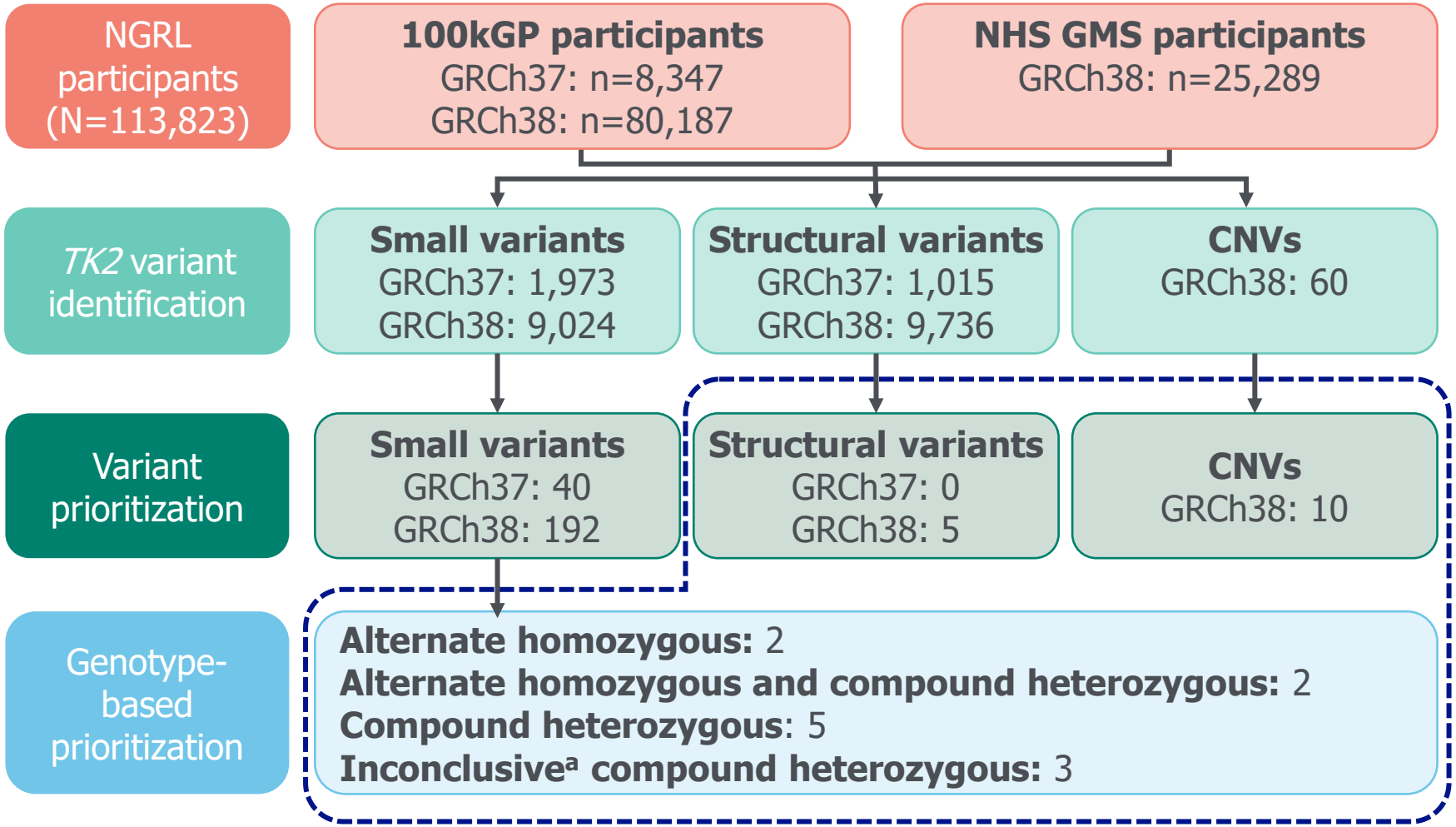
Segregation analysis

UK Biobank analysis

Prioritized alternate homozygous small *TK2* variants identified in the NGRL were screened for in UK Biobank to investigate their pathogenic significance further

HPO, Human Phenotype Ontology; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision; NGRL, National Genomic Research Library; *TK2*, thymidine kinase 2 gene; TK2d, thymidine kinase 2 deficiency.

Prioritized for further investigation were 12 small variants, 5 structural variants and 10 CNVs



Carriers of prioritized variants and their family members screened for TK2d-relevant phenotypes

[a]Inconclusive compound heterozygous variants are those that could not be confirmed through family structure or phasing information.
100kGP, 100,000 Genomes Project; CNV, copy number variant; GRCh37, Genome Reference Consortium human build 37; GRCh38, Genome Reference Consortium human build 38; NGRL, National Genomic Research Library; NHS GMS, National Health Service Genomic Medicine Service; *TK2*, thymidine kinase 2 gene; TK2d, thymidine kinase 2 deficiency.

Small missense variant chr16:66531432_G_A (p.Thr108Met in exon 5) was identified as a key contributor to TK2d

Allele frequency

- 3.74×10^{-5}

Databases

- Previously identified through the Genomics England Rare Disease Interpretation and Diagnostic Pipeline
- ClinVar/VarSome: pathogenic

In silico tools

- CADD score: ≥15
- VEP: moderate consequence

Carried as alternate homozygous by <5 participants categorized in **group 1**, with TK2d-related phenotypes including muscle weakness, impaired mobility and abnormal creatine kinase levels

Variant ID	Variant-specific counts across family structures									
	Exhibits TK2d-related phenotypes					Does not exhibit TK2d-related phenotypes				
	Singleton	Sibling-pair	Duo	Trio	Quintet	Singleton	Sibling-pair	Duo	Trio	Quintet
chr16:66531432_G_A	<5									

UK Biobank analysis

No UK Biobank participants were alternate homozygous carriers of p.Thr108Met

CADD, Combined Annotation Dependent Depletion; TK2d, thymidine kinase 2 deficiency; VEP, Variant Effect Predictor.

Variants carried by participants categorized in group 2 were considered likely to be benign in relation to TK2d

chr16:66583871_G_A (GRCh37)/ chr16:66549968_G_A (GRCh38)
(p.Arg32Trp in exon 1)

- Allele frequency: $\leq 6.01\times10^{-3}$
- ClinVar/VarSome: benign or likely benign
- CADD score: ≥15; VEP: moderate consequence
- Carried as alternate homozygous by 6 participants and family members, and heterozygous by 15 participants and family members

chr16:66543128_66546738_C_<DEL>
(feature truncating intronic variant 3,611 bp in length spanning intron 2)

- Allele frequency: 1.25×10^{-5}
- VarSome: uncertain significance
- VEP: high consequence
- Carried as heterozygous by <5 participants and family members

Variant ID	Variant-specific counts across family structures									
	Exhibits TK2d-related phenotypes					Does not exhibit TK2d-related phenotypes				
	Singleton	Sibling-pair	Duo	Trio	Quintet	Singleton	Sibling-pair	Duo	Trio	Quintet
chr16:66583871_G_A (GRCh37)/ chr16:66549968_G_A (GRCh38)		<5		<5	<5			<5	<5	
chr16:66543128_66546738_C_<DEL>	<5					<5				

UK Biobank analysis

25 participants were alternate homozygous carriers of p.Arg32Trp; <5 of these participants had HES records for anaemia and other non-TK2d-related morbidities

bp, base pairs; CADD, Combined Annotation Dependent Depletion; GRCh37, Genome Reference Consortium human build 37; GRCh38, Genome Reference Consortium human build 38; HES, Hospital Episode Statistics; TK2d, thymidine kinase 2 deficiency; VEP, Variant Effect Predictor.

Conclusions and outlook

- This study demonstrates how large sequencing datasets and deep phenotyping can be used to study ultra-rare diseases such as TK2d
- Further downstream analyses could include integrating multi-omics data and cross-referencing with TK2d-focused datasets

TK2d, thymidine kinase 2 deficiency.

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Thank you for your attention

Do you have any questions?