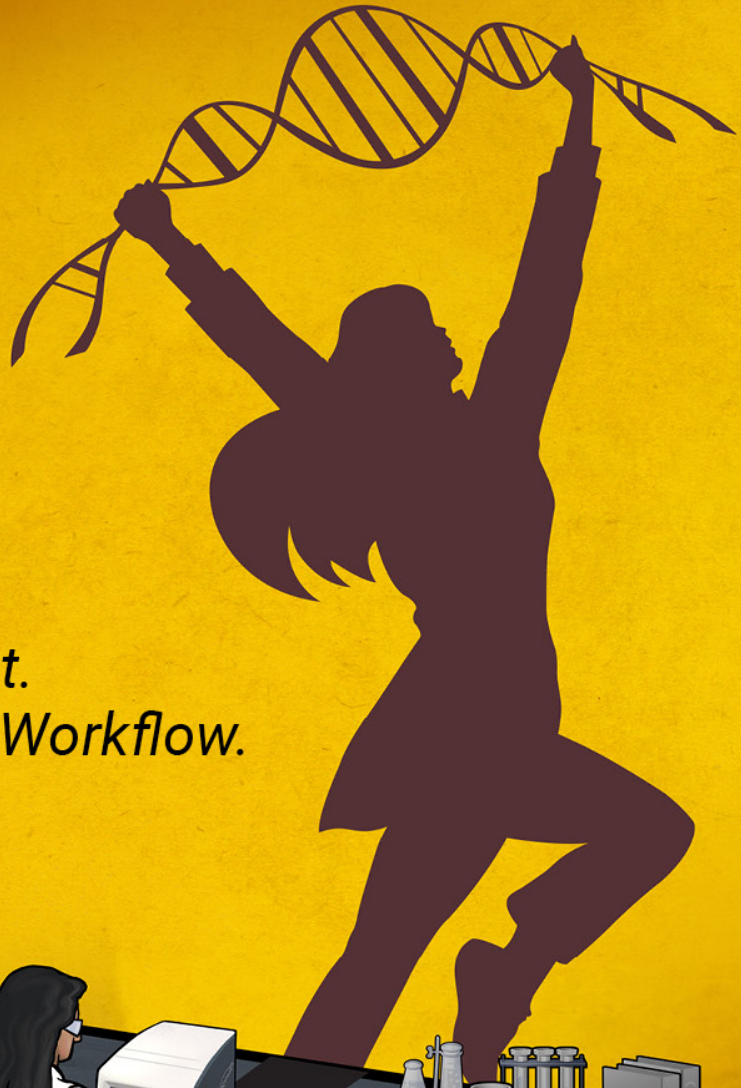




Small but Mighty

*One Small Instrument.
Take Charge of Your Workflow.*






The Spectrum Compact CE System offers Sanger sequencing and 6-dye fragment analysis. With an easy-to-use integrated touch screen, remote access software, plug-and-play prefilled consumables and unparalleled support, you can take charge of your workflow.

Discover the possibilities:

promega.com/SpectrumCompactCE



New variants and in silico analyses in GRK1 associated Oguchi disease

James A. Poulter¹  | Molly S. C. Gravett² | Rachel L. Taylor³ |
Kaoru Fujinami^{4,5,6,7}  | Julie De Zaeytijd⁸ | James Bellingham⁶ |
Atta Ur Rehman⁹ | Takaaki Hayashi¹⁰ | Mineo Kondo¹¹ | Abdur Rehman¹² |
Muhammad Ansar¹³ | Dan Donnelly¹⁴ | Carmel Toomes¹ | Manir Ali¹ |
UK Inherited Retinal Disease Consortium, Genomics England Research Consortium |
Elfride De Baere⁸ | Bart P. Leroy^{8,15} | Nigel P. Davies¹⁶ |
Robert H. Henderson¹⁷ | Andrew R. Webster^{5,6} | Carlo Rivolta^{18,19,20}  |
Christina Zeitz²¹ | Omar A. Mahroo^{5,6} | Gavin Arno^{4,5,6} |
Graeme C. M. Black^{3,22} | Martin McKibbin^{1,23} | Sarah A. Harris²⁴ |
Kamron N. Khan^{1,22} | Chris F. Inglehearn¹

¹Division of Molecular Medicine, Leeds Institute of Medical Research, University of Leeds, Leeds, UK

²School of Molecular and Cellular Biology, University of Leeds, Leeds, UK

³Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicines and Health, University of Manchester, Manchester, UK

⁴National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Centre, Tokyo, Japan

⁵Moorfields Eye Hospital, London, UK

⁶UCL Institute of Ophthalmology, London, UK

⁷Keio University School of Medicine, Tokyo, Japan

⁸Ghent University, Ghent, Belgium

⁹Division of Genetic Medicine, Centre Hospitalier Universitaire Vaudois (CHUV), University of Lausanne, Lausanne, Switzerland

¹⁰The Jikei University School of Medicine, Tokyo, Japan

¹¹Mie University Graduate School of Medicine, Mie, Japan

¹²Department of Genetics, Faculty of Science, Hazara University Mansehra, Dhodial, Pakistan

¹³Clinical Research Center, Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland

¹⁴School of Biomedical Sciences, University of Leeds, Leeds, UK

¹⁵Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

¹⁶St Thomas's Hospital, London, UK

¹⁷Department of Ophthalmology, Great Ormond Street Hospital, London, UK

¹⁸Department of Genetics and Genome Biology, University of Leicester, Leicester, UK

¹⁹Clinical Research Center, Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland

²⁰Department of Ophthalmology, University Hospital Basel, Basel, Switzerland

²¹Sorbonne Université, INSERM, CNRS, Institut de la Vision, Paris, France

James A. Poulter and Molly S. C. Gravett should be considered joint first author.

Sarah A. Harris, Kamron N. Khan, and Chris F. Inglehearn should be considered joint senior author.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Human Mutation* published by Wiley Periodicals LLC

²²Manchester Centre for Genomic Medicine, Saint Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, UK

²³Leeds Teaching Hospitals NHS Trust, St James' University Hospital, Leeds, UK

²⁴School of Physics and Astronomy, University of Leeds, Leeds, UK

Correspondence

James A. Poulter, Division of Molecular Medicine, Leeds Institute of Medical Research, University of Leeds, St James' University Hospital, Beckett St, Leeds LS9 7TF, UK.
Email: J.A.Poulter@leeds.ac.uk

Funding information

Fight for Sight UK, Grant/Award Numbers: Early Career Investigator Award, RP Genome Project GR586; RP Fighting Blindness, Grant/Award Number: RP Genome Project GR586; Engineering and Physical Sciences Research Council, Grant/Award Number: EP/S0306971/1

Abstract

Biallelic mutations in G-Protein coupled receptor kinase 1 (GRK1) cause Oguchi disease, a rare subtype of congenital stationary night blindness (CSNB). The purpose of this study was to identify disease causing GRK1 variants and use in-depth bioinformatic analyses to evaluate how their impact on protein structure could lead to pathogenicity. Patients' genomic DNA was sequenced by whole genome, whole exome or focused exome sequencing. Disease associated variants, published and novel, were compared to nondisease associated missense variants. The impact of *GRK1* missense variants at the protein level were then predicted using a series of computational tools. We identified twelve previously unpublished cases with biallelic disease associated GRK1 variants, including eight novel variants, and reviewed all *GRK1* disease associated variants. Further structure-based scoring revealed a hotspot for missense variants in the kinase domain. In addition, to aid future clinical interpretation, we identified the bioinformatics tools best able to differentiate disease associated from nondisease associated variants. We identified *GRK1* variants in Oguchi disease patients and investigated how disease-causing variants may impede protein function in-silico.

KEYWORDS

CSNB, GRK1, Oguchi disease, rhodopsin

1 | INTRODUCTION

The first member of the G protein-coupled receptor kinase (GRK) family was discovered when enzymatic activity was observed in rod membranes that phosphorylated rhodopsin in a light-dependent manner (Kuhn & Dreyer, 1972). The enzyme, now known as GRK1 (MIM# 180381), was found to be essential for quenching and recycling light-activated rhodopsin. To be recycled, light-activated rhodopsin is phosphorylated multiple times by GRK1, followed by binding of Arrestin-1 to activated-phosphorylated rhodopsin to block further transducin activation by steric exclusion (Krupnick et al., 1997; Wilden et al., 1986). Failure of this process results in a build-up of activated rhodopsin, which continues to activate the photo-transduction cascade, shutting off circulating current in the rod photoreceptors, rendering them unable to respond electrically to light. Importantly, a build-up of activated rhodopsin appears to be well tolerated by photoreceptors, with no obvious cell death or structural consequences in the retina, meaning any future therapies leading to the deactivation of rhodopsin could restore visual acuity. Activated cone opsin is likely to be more reliant on GRK7 than GRK1 phosphorylation, as although both enzymes are expressed in cones, loss of GRK1 function has minimal impact on human photopic vision.

GRK1 is a serine/threonine protein kinase with a central catalytic AGC protein kinase (PK) domain that sits within a regulator of G

protein signaling homology (RH) domain (Figures 1 and 3). The PK domain binds ATP and the polypeptide substrate which is subsequently phosphorylated (Arencibia et al., 2013). The RH domain is thought to have a key role in receptor binding, with loss of this domain preventing binding and phosphorylation of rhodopsin (He et al., 2017). While the N-terminus encodes a short alpha-helical domain, the C-terminus encodes a lipid-binding region, which is crucial for prenylation-dependent docking of GRK1 into the outer segment membranes of rod photoreceptors (Komolov & Benovic, 2018).

Oguchi disease (MIM# 613411), a rare form of congenital stationary night blindness (CSNB), results from biallelic variants in either *SAG* (encoding Arrestin-1) or *GRK1*. *SAG*-mediated disease is most prevalent in Japanese patients, whilst pathogenic variants in *GRK1* are more common in South East Asians. Typically, Oguchi disease is characterized clinically by the Mizuo-Nakamura phenomenon—the presence of a golden-yellow colouration to the retina that disappears in the dark-adapted state and reappears shortly after light exposure (Miyake et al., 1996). Electro-physiologically there is normal cone function, delayed rod dark adaptation and marked rod desensitization to a bright flash. Most reported cases have this distinctive phenotype and no significant variation in disease expression has been reported, with the exception of a few rare cases (Hayashi et al., 2007; Nishiguchi et al., 2019).

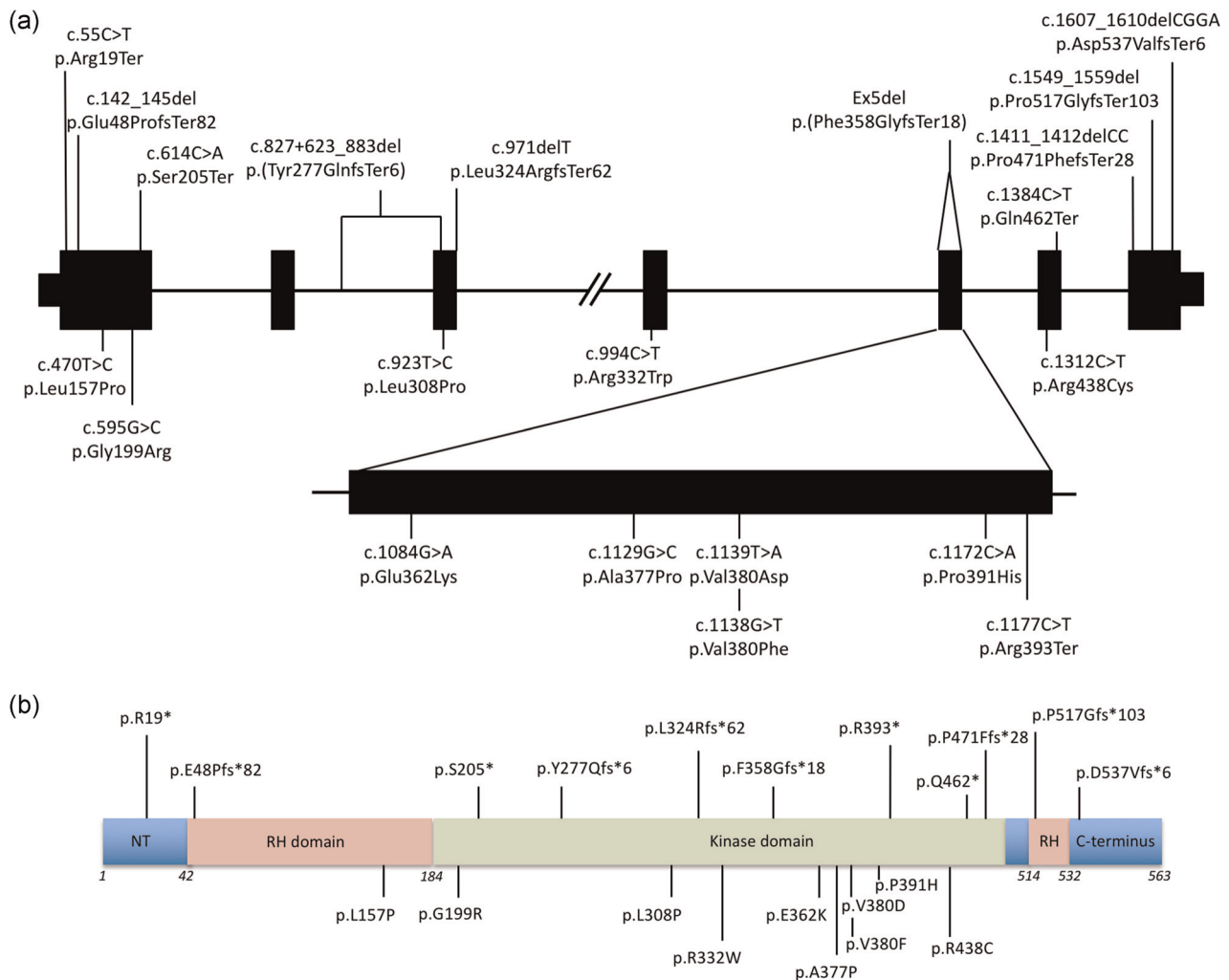


FIGURE 1 Distribution of disease associated variants within GRK1. All variants shown are annotated according to human genome GRCh37/hg19, using GRK1 gene and protein accession numbers NM_002929 and NP_002920, respectively. Loss of function variants are shown above the gene/protein and missense variants are given below. (a) Genomic organization of GRK1 showing the location of all novel and published Oguchi disease causing variants. (b) Domain structure of GRK1 showing the location of all GRK1 variants. The start and end amino-acid positions of each domain are based on Lodowski et al. (2006). NT, N-terminus; RH, regulator of G-protein signaling homology

Distinguishing between Oguchi disease and other forms of CSNB is important as they may have different prognoses—some patients with Oguchi disease report very slowly progressive visual dysfunction, whereas those with classical CSNB do not. Minimally progressive retinal degeneration has been observed in SAG-associated Oguchi disease, and for one patient, this resulted in their disease being reclassified as retinitis pigmentosa 26 years after being diagnosed with a stationary rod dysfunction syndrome (Oguchi disease; Nishiguchi et al., 2019). As most of our knowledge of Oguchi disease stems from our understanding of loss of SAG function further research is required to better understand GRK1-related Oguchi disease.

To date, thirteen pathogenic variants in GRK1 have been implicated in Oguchi disease, eight of which are predicted to be null variants (Azam et al., 2009; Cideciyan et al., 1998; Godara et al., 2012; Hayashi et al., 2007; Jespersgaard et al., 2019; Li et al., 2017; Mucciolo et al., 2018; Oishi et al., 2007; Skoczyk-Werner et al., 2015; Teke et al., 2016; Yamamoto et al., 1997; Q. Zhang et al., 2005).

The molecular mechanisms by which these contribute to disease are poorly understood. Here, we present eight further disease associated, likely pathogenic variants in GRK1 and review new and known variants causing Oguchi disease. Using in-silico techniques we compare likely pathogenic disease-associated variants with nonpathogenic GRK1 missense variants to identify features that could be contributing to the phenotype. Together this will help define disease mechanisms and assist in predicting likelihood of pathogenicity for variants identified in GRK1.

2 | METHODS

2.1 | Editorial policies and ethical considerations

Ethical approval for this study was obtained from the Yorkshire and The Humber – Leeds East Research Ethics Committee (REC

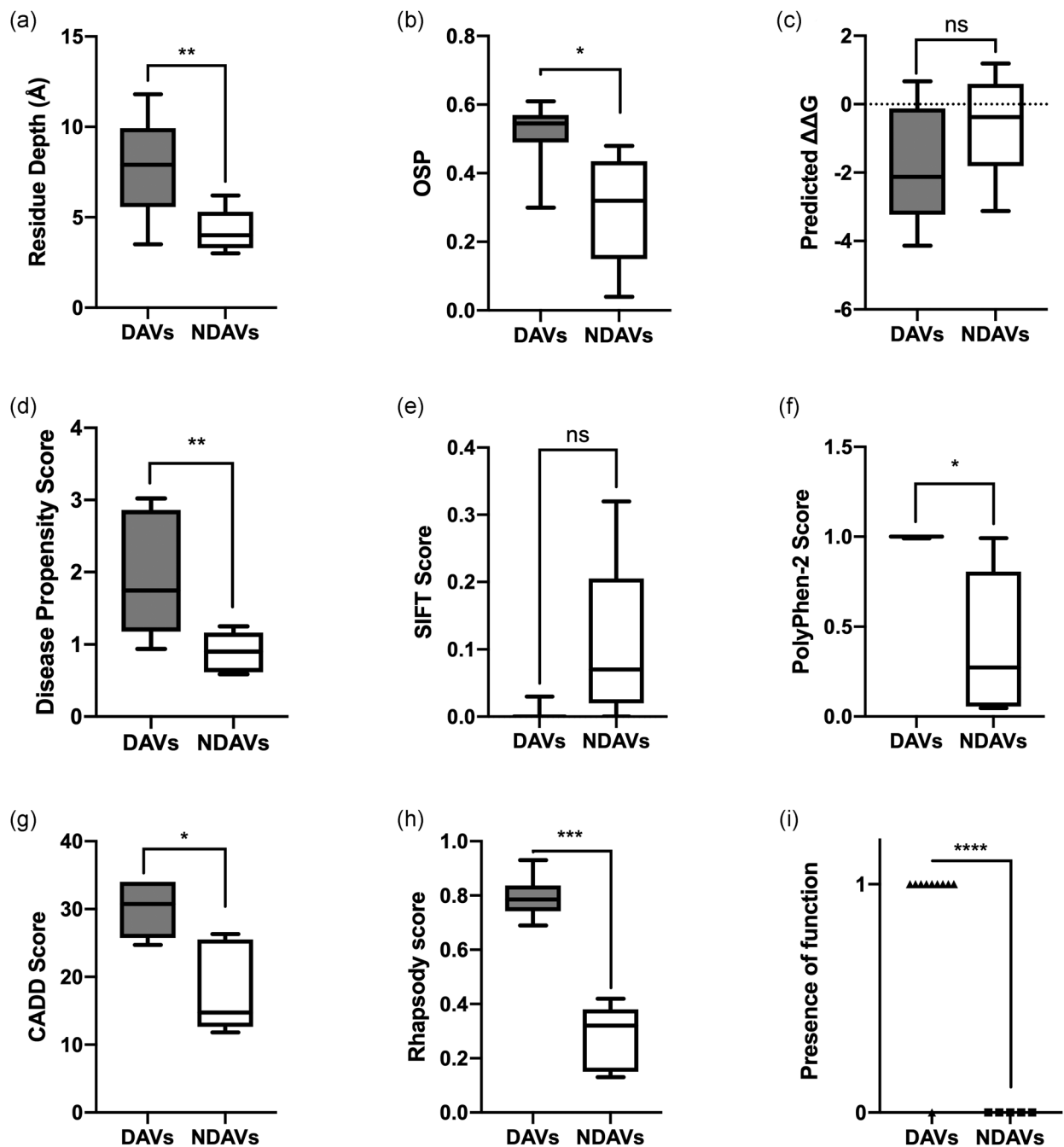


FIGURE 2 Bioinformatic prediction scores of disease associated and nondisease associated GRK1 variants. Comparison of predicted pathogenicity scores for all novel or published disease associated missense GRK1 variants, with likely nonpathogenic missense variants identified in gnomAD. The whiskers in the box plots show the minimum and maximum values. Statistical significance was calculated using Welch's *t*-test in GraphPad prism 8.0.2. *ns* = $p \geq .05$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$, **** = $p < .0001$. (a) Wildtype (WT) residue depth (Å) from site-directed mutator (SDM), (b) WT occluded surface packing (OSP) from SDM, (c) thermostability score SDM, (d) disease propensity scores as predicted in VarSite, (e) SIFT scores, (f) PolyPhen-2 scores, (g) CADD (v1.3) scores, (h) Rhapsody scores and (i) ConSurf predicted roles (1 = structural/functional role predicted, 0 = no role predicted). DAVs, disease associated variants; NDAVs, nondisease associated variants

reference: 17/YH/0032). The study adhered to the tenets of the Declaration of Helsinki. Blood samples were taken with informed consent from each participant, or with parental informed consent on behalf of children.

2.2 | Clinical assessment

Study participants were ascertained from St James' University Hospital, Leeds, England; Manchester Centre for Genomic

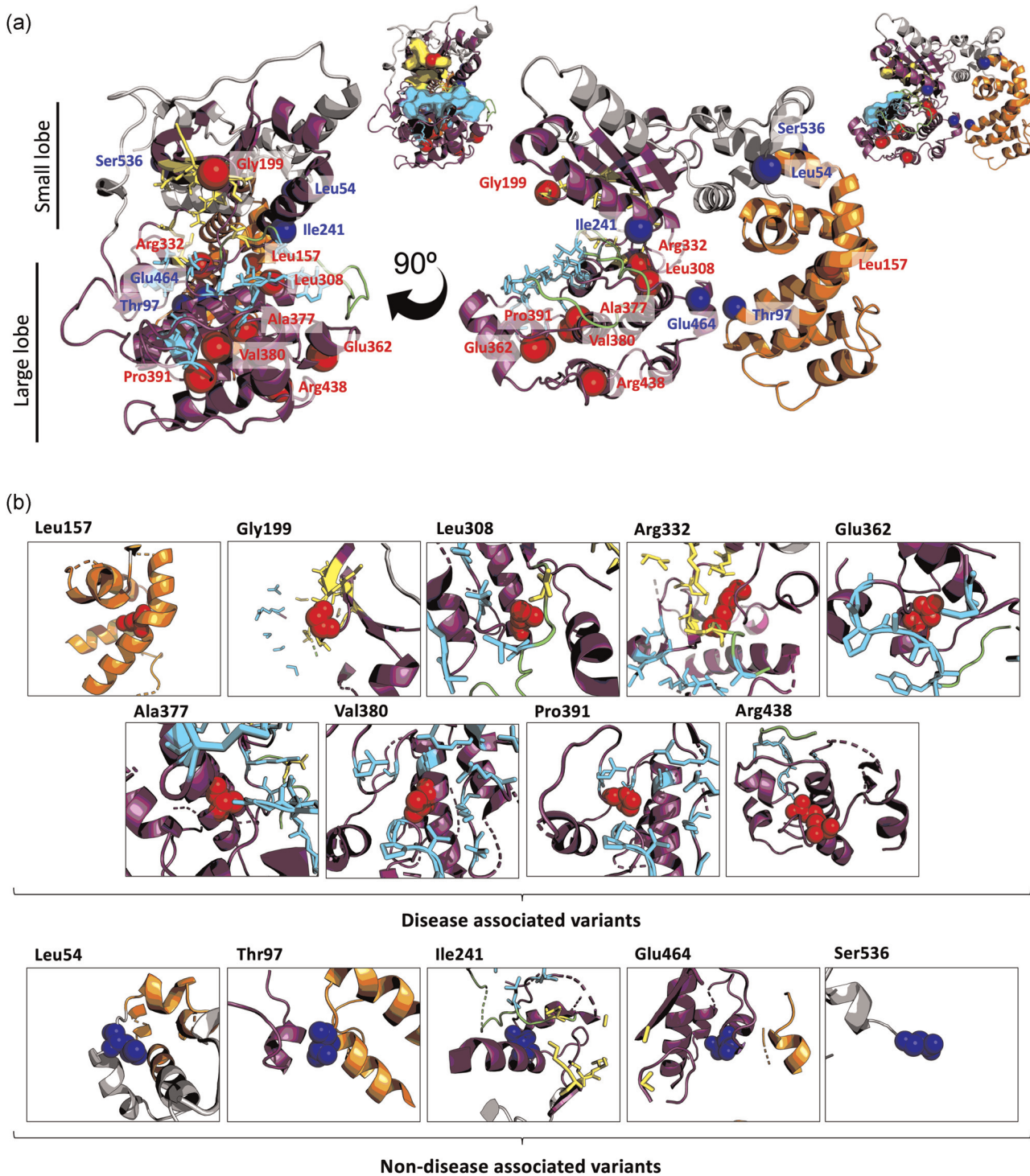


FIGURE 3 – Location of disease associated and nondisease associated variants in a homology model of GRK1. Homology model of human GRK1 based upon bovine GRK1 structure (PDB: 4PNI). Wildtype residues of disease associated variants are labeled in red (Leu157, Gly199, Leu308, Arg332, Glu362, Ala377, Val380, Pro391, and Arg438) and nondisease associated variants in blue (Leu54, Thr97, Ile241, Glu464, and Ser536). The protein kinase domain is colored in purple, RH domain in orange, activation loop in green, ATP binding residues are represented as yellow sticks and polypeptide substrate binding residues as cyan sticks. (a) A visualization of variant location in the overall structure. Variants are highlighted as beads. The smaller representations to the right, with the ATP and polypeptide binding surface displayed demonstrate how intramolecular residue changes may deform the binding site surface. (b) A focus on residue location and local interactions, displaying only features within 15 Å of the residue of interest. Variant residue atoms are represented as spheres

Medicine, Manchester, England; Moorfields Eye Hospital, London, England; Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium; The Jikei University School of Medicine, Tokyo, Japan; Mie University Graduate School of Medicine, Mie, Japan; and National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Centre, Tokyo Japan; individuals were recruited following clinical examination by an experienced ophthalmologist, with the exception of Families 10 and 11 who were ascertained by local doctors in Pakistan.

2.3 | Sequencing

Exome and clinical exome sequencing was performed on 3 µg of genomic DNA using the Agilent SureSelectXT Human All Exon (V6) and Focussed Exome respectively (Agilent Technologies), according to the manufacturer's instructions. Captured libraries were pooled and sequenced on an Illumina HiSeq3000 sequencer (Illumina). The resulting fastq files were aligned to the Human genome assembly GRCh37 using Burrows-Wheeler Aligner and reads processed using samtools, Picard, and the Genome Analysis Toolkit. Variants within the captured regions were called and annotated using variant effect predictor (NCBI) and further filtered for those within genes known to cause retinal disease. This analysis pipeline is described in more detail elsewhere (Panagiotou et al., 2017). Copy number variation analysis was performed using ExomeDepth, comparing patient BAM files with BAM files from unrelated individuals in the same sequencing run. All novel variants were confirmed by Sanger sequencing using standard methods.

The affected individual and unaffected parents of Family 1 underwent Genome sequencing as part of the 100,000 Genome Project, as previously detailed by Taylor et al. (2017).

2.4 | Identifying nonpathogenic variants

To identify protein-level characteristics influencing a disease phenotype we compared the nine disease associated missense variants with a group of five assumed nondisease associated missense variants. For the nonpathogenic variants, we identified all homozygous *GRK1* missense variants in gnomAD and only included those with a MAF > 0.00035, the level above which variants are calculated to be too common to cause Oguchi disease (Whiffin et al., 2017). The five missense variants in gnomAD with frequencies above this level, p.Leu54Phe, p.Thr97Met, p.Ile241Thr, p.Glu464Gln, and p.Ser536Leu, also all occur at least once as homozygotes in gnomAD. These are therefore assumed to be non-disease associated polymorphisms (further details in Table S1).

2.5 | Structural analysis

A homology model of human wild-type *GRK1* based on the bovine structure (PDB: 4PNI) was produced using SwissModel (Waterhouse et al., 2018). The locations of the RH and PK

domains, ATP binding site, polypeptide substrate binding site and activation loop were identified using InterPro (Mitchell et al., 2019). The structure was visualized, and regions of interest labeled using PyMOL (Schrodinger, 2015). Residue depth and connectivity within the structure was determined using Site-Directed Mutator (SDM; Pandurangan et al., 2017). To further explain variant impact, changes in residue physical characteristics; molecular weight, isoelectric point, and hydrophobicity (Laskowski et al., 2020) were reviewed.

2.6 | Scoring missense variants

A number of bioinformatics tools were reviewed to identify which approach best classified the variants. Scoring methods included: SDM (Pandurangan et al., 2017), VarSite (Laskowski et al., 2020), SIFT v6.2.1 (Sim et al., 2012), Polyphen-2 v2.2.2r394 (Adzhubei et al., 2010), CADD v1.3 (Kircher et al., 2014), Rhapsody (Ponzoni et al., 2019), and ConSurf (Ashkenazy et al., 2016). All statistical analyses were performed in GraphPad Prism 8.0.2 (for macOS, GraphPad Software, www.graphpad.com) using Welch's *t*-test.

3 | RESULTS

3.1 | Identification of novel *GRK1* variants associated with Oguchi disease

Screening of inherited retinal disease patients by an international consortium of retinal genetics laboratories identified 12 cases with Oguchi disease harboring nine different disease associated variants in *GRK1*, of which eight are previously unreported (Table 1). The majority of cases in this study were identified in families of South Asian descent, except for Family 2 who are East Asian (Japanese) and Family 5 who are Eastern European (Polish). All variants were confirmed and segregated in available family members (summarized in Figure S1). Each patient had a history of non/minimally progressive night blindness, and retinal examination revealed the Mizuo-Nakamura phenomenon, consistent with a clinical diagnosis of Oguchi disease.

A literature search revealed a further thirteen published Oguchi disease causing *GRK1* variants from 12 studies (Azam et al., 2009; Cideciyan et al., 1998; Godara et al., 2012; Hayashi et al., 2007; Jespersgaard et al., 2019; Li et al., 2017; Mucciolo et al., 2018; Oishi et al., 2007; Skoczyk-Werner et al., 2015; Teke et al., 2016; Yamamoto et al., 1997; Q. Zhang et al., 2005). The nomenclature for these variants was updated based on the current HGVS nomenclature guidelines and the GRCh37 version of the Human Genome, and together with the new variants reported here, these have been included in a Leiden Open Variation Database (LOVD) of *GRK1* variants (http://dna2.leeds.ac.uk/GRK1_LOVD/genes/GRK1).

TABLE 1 Summary of all novel and published mutations in GRK1

DNA variant	Exon	Protein variant	rs number	gnomAD Frequency (1 homozygous)	Domain	PolyPhen-2	CADD (v1.3)	References
c.55C>T	1	p.Arg19Ter	rs370713047	17/239,700	N-Terminus	N/A	37.0	Li et al. (2017)
c.142_145del	1	p.Glu48ProfsTer82	rs748680704	6/248,384	RH domain	N/A	28.5	This study (F1, F8, F11)
c.470T>C	1	p.Leu157Pro	N/A	0	RH domain	1.00 (Prob. D)	26.0	Mucciolo et al. (2018)
c.595G>C	1	p.Gly199Arg	N/A	0	Kinase	1.00 (Prob. D)	24.7	This study (F2)
c.614C>A	1	p.Ser205Ter	N/A	0	Kinase	N/A	36	Azam et al. (2009)
c.827+623_883del	3	p.(Tyr277GlnfsTer6)	N/A	0	Kinase	N/A	27.8	Zhang et al. (2005)
c.923T>C	3	p.Leu308Pro	N/A	0	Kinase	0.996 (Prob. D)	29.2	Teke et al. (2016)
c.971del	3	p.Leu324ArgfsTer62	N/A	0	Kinase	N/A	35	Oishi et al. (2007)
c.994C>T	4	p.Arg332Trp	rs372114657	0	Kinase	1.000 (Prob. D)	29.5	This study (F12)
Ex5 del	5	p.(Phe358GlyfsTer18)	N/A	0	Kinase	N/A	25.1 ^a	Yamamoto et al. (1997); Cideciyan et al. (1998)
c.1084G>A	5	p.Glu362Lys	N/A	0	Kinase	1.00 (Prob. D)	34	This study (F3)
c.1129G>C [*]	5	p.Ala377Pro	N/A	0	Kinase	0.991 (Prob. D)	25	Godara et al. (2012) ¹
c.1138G>T	5	p.Val380Phe	N/A	2/173,226	Kinase	0.998 (Prob. D)	32	This study (F4)
c.1139T>A [*]	5	p.Val380Asp	rs777094000	5/173,124	Kinase	0.999 (Prob. D)	34	Yamamoto et al. (1997) ² ; Godara et al. (2012) ¹
c.1172C>A	5	p.Pro391His	rs570621429	1/141,802	Kinase	1.00 (Prob. D)	34	Hayashi et al. (2007)
c.1177C>T	5	p.Arg393Ter	rs137877289	6/141,764	Kinase	N/A	46	This study (F5) ³
c.1312C>T	6	p.Arg438Cys	rs765070399	2/141,922	Kinase	1.00 (Prob. D)	34	This study (F9)
c.1384C>T	6	p.Gln462Ter	N/A	0	Kinase	N/A	42	Jespersgaard et al. (2019)
c.1411_1412del	7	p.Pro471PhefsTer28	N/A	0	Kinase	N/A	35	Oishi et al. (2007)
c.1549_1559del	7	p.Pro517GlyfsTer130	N/A	0	RH domain	N/A	35	This study (F6)
c.1607_1610del	7	p.Asp537ValfsTer7	rs756235051	89/172,958	C-Terminus	N/A	26.7	Yamamoto et al. (1997) ² ; Skorzcyk-Werner et al. (2015); This study (F5 ³ , F7, F10)

Note: Lodowski et al. (2006). All variants identified are all homozygous except for ^{1,2,3} which are compound heterozygous.

^{*}Predicted – DNA mutation not provided in publication, Prob. D = Probably Damaging as scored by PolyPhen2.

^aBased on deletion of whole of exon 5 and surrounding splice sites as exact coordinates are unknown.

3.2 | Disease associated variant distribution and domain structure

The 21 Oguchi disease associated variants (DAVs) identified to date include missense ($n = 10$), frameshift ($n = 6$), and nonsense ($n = 4$) variants (Table 1) as well as a large deletion encompassing exon 5 (Figure 1a). Each variant has been identified in only a single family with the exception of p.Val380Asp (two families), p.Glu48ProfsTer82 (two families), the deletion of exon 5 (p.(Phe358GlyfsTer18); three families), and the C-terminal frameshift variant p.(Asp537ValfsTer6; six families), with the latter variant having the highest allele frequency in gnomAD (89/172,958). Of the 89 p.Asp537ValfsTer7 alleles in gnomAD, 42 are in European (non-Finnish) and 35 in South Asian populations, which is consistent with this mutation being identified in cases from Eastern Europe and South Asia in this study.

Sixteen of the variants lie within the protein kinase domain, three within the RH domain and one at each of the N- and C-termini (Figure 1b). While the frameshift and nonsense variants are present throughout the protein, all except one of the missense variants ($n = 8$ out of 9) are present within the protein kinase domain (Figure 2a), and five cluster within a 30 amino-acid region between Glu362 and Pro391 (Figure 2a). These are likely to impair phosphorylation of light-activated rhodopsin.

Three variants located after this cluster introduce frameshifts in the final exon, giving rise to transcripts which would not be expected to undergo nonsense-mediated decay (Thermann et al., 1998). The extreme C-terminus of GRK1, which these three variants would disrupt, is prenylated to facilitate anchoring of GRK1 in the membranes of the rod photoreceptor outer segments (Pitcher et al., 1998), which maximizes the likelihood of interaction with activated rhodopsin. Lack of this domain is therefore likely to reduce the level of anchored GRK1, which will in turn reduce the quantity of rhodopsin being bound and phosphorylated.

3.3 | Disease associated missense variants affect key regions in GRK1 active site

To further understand the mechanism by which the missense variants affected GRK1 function, we performed structure-based scoring on the SDM server using our homology model (Figure 2a–c). Although SDM's primary function is predicting thermal stability, the most interesting results came from the scoring of wildtype (WT) residue depth and occluded surface packing (OSP), with both scores showing a significant difference between DAVs and likely nonpathogenic, nondisease associated variants (NDAVs) ($p = .0021$ and $p = .0390$ respectively). This demonstrated that DAVs were located deeper within the three-dimensional (3D) structure of GRK1 than NDAVs (residue depth = 3.5–11.8 and 3–6.2, respectively; Figure 2a, Table S2) and were more connected/densely packed (OSP = 0.3–0.61 and 0.04–0.48, respectively; Figure 2b, Table S2). Exceptions to these rules were seen in DAV p.Gly199Arg, which is located at the surface of the protein (residue depth = 3.5 Å and OSP = 0.3), and

NDAV p.Ile241Thr, which is located deeper in GRK1 (residue depth = 6.2 and OSP = 0.48). Gly199 is positioned within the conserved glycine rich loop of the PK domain and is part of the ATP binding site (Figure 3). Mutation to a bulky positively charged arginine (Table S2) is likely to disrupt both the shape and charge of the ATP binding pocket (Figure 3). Although Ile241 is buried in the binding pocket of the PK domain (Figure 3), there is little change in size and charge (Table S2) which could explain why this variant is nonpathogenic.

Further investigation of mutation location in the protein structure demonstrated DAVs were located proximal to key sites for protein function, including the substrate and ATP binding domains, while NDAVs were not (Figure 3a). DAVs were predicted to be likely to disrupt the shape and charge of the active site either directly as in the case of p.Gly199Arg and p.Arg332Trp, or allosterically through introduction of proline kinks into alpha helices (e.g., p.Leu308Pro and p.Ala377Pro), intramolecular charged groups (classically exposed; e.g., p.Val380Asp and p.Pro391His), and bulky side groups within densely packed regions (e.g., p.Val380Phe) close to key residues (Figure 3b and Table S2). In the case of p.Arg438Cys and p.Glu362Lys, the equivalent arginine and glutamic acid residues are conserved and form a salt bridge in the bovine protein (Figure S2). It is therefore likely that substitution of Glu362 and Arg438 changes the charge of these residues in the human protein and is disrupting this salt bridge interaction (Figure S2). Meanwhile NDAVs were often subtler changes, and not local to key residues (Figure 3b and Table S2). We hypothesize DAVs within the protein kinase domain are inhibiting protein function through disruption of the shape and dynamics of this key region, while NDAVs prove less disruptive to shape as they are not internal and/or have subtler changes in residue characteristics. This is supported by Consurf's prediction that most of the DAVs occur at loci important for protein structure or function (Figure 2i and Table S2).

Only one DAV, p.Leu157Pro, lies outside of the kinase domain, instead being present within the RH domain of GRK1, the domain primarily responsible for rhodopsin binding (He et al., 2017). The introduction of a proline residue within an α -helix is likely to disrupt the secondary structure and introduce a characteristic "kink" due to its rigidity and constrained phi angle. This could potentially impact on the ability of Rhodopsin to correctly bind GRK1 in order for phosphorylation to occur.

To determine which bioinformatic tools were most successful in differentiating between disease associated and nondisease associated variants, we scored missense variants using: VarSite, SDM, PolyPhen-2, SIFT, CADD, Rhapsody, and Consurf. Predictions were compared between DAV and NDAV sets, with significance assessed by Welch's *t*-test (Figure 2, Tables 1, and S2). VarSite disease propensity, WT OSP, WT residue depth, PolyPhen-2, CADD, Rhapsody, and Consurf, were able to distinguish between DAVs and NDAVs with statistical significance. Consurf's structure/function prediction and Rhapsody, which both take the variant's location in the protein

(e.g., the formation of novel H-bond or salt bridge interactions, or the disruption of interactions within non-conserved residues). However, it is possible to draw general conclusions about the surrounding residue location and impact on secondary and tertiary structure. Therefore, loss of kinase activity through disruption of its shape and dynamics is a second mechanism by which Oguchi disease can occur.

Three variants have been identified in the last exon of *GRK1*, all of which result in frameshift transcripts that potentially escape nonsense mediated decay, leading to a *GRK1* protein with a disrupted C-terminus. This region plays a crucial role in embedding *GRK1* within the photoreceptor outer segment membrane; *GRK1* localizes directly to the outer segment membranes due to a short prenylation sequence within the C-terminus that interacts with the prenyl-binding protein, delta (*PrBP/δ*), which facilitates attachment to the membranes (Huang et al., 2011; Roosing et al., 2014). Deletion of *PrBP/δ* has also been shown to result in impaired trafficking of prenylated-*GRK1* to photoreceptor outer segments, highlighting the importance of this interaction in correct localization of *GRK1* (H. Zhang et al., 2007). This cellular localization is essential for *GRK1* function as it brings active rhodopsin into contact with docked *GRK1* protein through a two-dimensional search rather than 3D diffusion, which is essential for ultrarapid signal termination (Sato et al., 2015). Lack of this sequence is likely to cause failure of prenylation, leading to abolished binding of *PrBP/δ*, incorrect localization of *GRK1* and a reduction in Rhodopsin phosphorylation, a third mechanism by which disease can arise. This is supported by studies showing a marked reduction in Rhodopsin phosphorylation in cells transfected with the p.Asp537ValfsTer7 variant (Khani et al., 1998).

One missense variant, p.Leu157Pro, was identified in the N-terminal RH domain, which of the two RH domains present in *GRK1*, contains most of the Rhodopsin binding capacity (~70%). While complete loss of the N-terminal RH domain has been shown to cause a significant reduction in Rhodopsin binding (He et al., 2017), no studies have yet determined the effect of single amino acid substitutions on this activity. The importance of Leu157, however, is highlighted by it being conserved across all known human GRKs, suggesting it is crucial for the function of the protein. Binding of Rhodopsin has also been shown to require the protein kinase domain to be in an active conformation (Homan & Tesmer, 2014), therefore missense substitutions in the kinase domain may also have an impact on Rhodopsin binding. Further studies are required to determine the full extent of missense substitutions on *GRK1* function.

Our study therefore highlights three potential molecular mechanisms by which *GRK1* variants lead to disease, each of which ultimately reduce or abolish the phosphorylation of light-activated rhodopsin. These are (a) lack of *GRK1* protein due to frame shift or nonsense variants in all exons but the last, resulting in NMD; (b) missense variants leading to inability of *GRK1* to phosphorylate rhodopsin due to a dysfunctional kinase domain or failure to bind rhodopsin, ATP or Mg^{2+} ; or (c) a frameshift in the final exon, which would be expected to escape nonsense mediated decay and produce a protein retaining the ability to bind and phosphorylate light-activated rhodopsin, but which cannot localize to the outer segment

membranes. We would therefore predict that, while variants in the first category lead to no protein being produced, those in the second produce proteins that correctly localize within the outer segments but have inhibited kinase activity, and those in the third produce a *GRK1* protein likely to maintain kinase activity but which fails to localize and so rarely comes into contact with activated rhodopsin. Interestingly, the kinase activity of the p.(Asp537ValfsTer6) variant was found to be significantly reduced in transfected cos7 cells, suggesting C-terminal mutations may affect kinase activity as well as protein localization. However, this lack of activity may be explained by difficulties in expressing the mutation containing *GRK1* protein rather than on protein function (Khani et al., 1998).

Our comparison of *GRK1* DAVs to NDAVs identified from gnomAD facilitated an assessment of pathogenicity scores, which could inform future variant interpretation. We found that VarSite disease propensity, Polyphen2, CADD, Rhapsody and ConSurf were able to significantly differentiate between DAVs and NDAVs, as were biophysical scores of residue depth and occluded surface packing. Rhapsody was the best of all the tools at differentiating between DAVs and NDAVs. Rhapsody incorporates dynamics information from elastic network models of protein structure, as well as PolyPhen-2 and EVMutation (conservation) scores using a machine learning approach. This broad range of sequence-, structure-, and dynamics-based information could account for its success since the impact of a variant can arise from a multitude of different physiochemical effects. Despite producing the best separation (Rhapsody) and describing features (SDM), structure-based tools are limited by the availability of the protein's structure or a homologous structure. Although homology models are not always accurate for atomic level information, coarser detail such as whether residues are buried is likely to be reliable. Similarly, although ConSurf can be used without a pdb structure it is dependent on homologous proteins having a structure. In this study we found using ConSurf without a pdb structure was sufficient to predict pathogenicity based on whether a residue is likely to be buried or exposed, relating to its location in homologous proteins with known structures. Ultimately, these scores can only be used as a guide, and variant pathogenicity should be confirmed by segregation analysis and functional assays wherever possible, as rare nonpathogenic variants can also score highly.

Interrogation of gnomAD revealed a further three rare variants that were homozygous in at least one individual and could not be excluded as a cause of Oguchi disease based on allele frequency alone (Table S3 and Figure S3). We therefore calculated the same set of pathogenic prediction scores to assess the likely pathogenicity of these variants of unknown significance. The p.Met185Val variant, although rare in gnomAD, produced scores that were comparable with the NDAVs for each tool, suggesting it does not contribute to disease. However, both p.Ala353Ser and p.Ala387Val, produced scores that were compatible with DAVs, suggesting that the two individuals in gnomAD that are homozygous for these variants may have Oguchi disease. gnomAD contains exome and genome sequence data from over 130,000 unrelated individuals, with only those individuals affected by a severe pediatric disorder being removed. It is

therefore possible that individuals with CSNB or Oguchi disease are present in the gnomAD database which could explain the presence of two homozygous, likely DAVs.

In summary, we present eight novel variants in *GRK1* as a cause of Oguchi disease and perform an in-depth analysis of all *GRK1* variants to understand their contribution to disease. We describe three mechanisms likely to account for all *GRK1*-related disease, each of which ultimately result in a failure to phosphorylate light-activated rhodopsin. We assessed the ability of different in silico pathogenicity scores to differentiate DAVs and NDAVs and found that Rhapsody out-performed all tools tested for this data set. Finally, we have created an LOVD database into which all the known DAVs and several common NDAVs described herein have been entered, to guide future variant interpretation in *GRK1*-associated disease.

ACKNOWLEDGMENTS

The authors would like to thank all the families for agreeing to participate in this study, and Roman A. Laskowski and Arun Prasad Pandurangan for assistance with VarSite and SDM respectively, and Ivet Bahar, Luca Ponzoni and the rest of the Bahar group for their help with Rhapsody. This study was supported by the European Retinal Disease Consortium (ERDC), the European Reference Network for Rare Eye Disease (ERN-EYE) and the Japan Eye Genetics Consortium (<https://www.jegc.org/>).

This study was funded by RP Fighting Blindness and Fight for Sight as part of the UK Inherited Retinal Disease Consortium (RP Genome Project GR586) and the UK National Health Service (NHS) Foundation Trust, the National Institute for Health Research Biomedical Research Centre (NIHR-BRC) at Moorfields Eye Hospital and the NIHR-BRC at Great Ormond Street Institute of Child Health, Moorfields Eye Charity and the Great Britain Sasakawa Foundation. JAP is supported by a UKRI Future Leaders Fellowship (MR/T02044X/1). MScG is on the Wellcome Trust 4-year PhD studentship in The Astbury Centre funded by The University of Leeds. GA is supported by a Fight For Sight (UK) Early Career Investigator Award. SAH acknowledges the EPSRC for their support (EP/S0306971/1). OM is funded by Wellcome Grant 206619_Z_17_Z. This study was made possible through access to the data and findings generated by the 100,000 Genomes Project managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council have also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support.

Members of the UK Inherited Retinal Disease Consortium include: Graeme C. Black, DPhil, FRCOphth (study chair); Georgina Hall, MSc; Stuart Ingram, BSc; Rachel L. Taylor, PhD; Panagiotis Sergouniotis, PhD; Andrew R. Webster, MD(Res), FRCOphth; Alison J. Hardcastle, PhD; Michel Michaelides, MD(Res), FRCOphth; Nikolas Pontikos, PhD; Michael Cheetham, PhD; Gavin Arno, PhD; Alessia Fiorentino, PhD;

Chris F. Inglehearn, PhD; Carmel Toomes, PhD; Manir Ali, PhD; Martin McKibbin, FRCOphth; James A. Poulter, PhD; Kamron N. Khan, PhD, FRCOphth; Claire E.L. Smith, PhD; Susan Downes, MD, FRCOphth; Jing Yu, PhD, and Veronica van Heyningen, PhD.

Members of the Genomics England Research Consortium include: Ambrose J. C., Arumugam P., Baple E. L., Bleda M., Boardman-Pretty F., Boissiere J. M., Boustred C. R., Brittain H., Caulfield M. J., Chan G. C., Craig C. E. H., Daugherty L. C., de Burca A., Devereau, A., Elgar G., Foulger R. E., Fowler T., Furió-Tarí P., Hackett J. M., Halai D., Hamblin A., Henderson S., Holman J. E., Hubbard T. J. P., Ibáñez K., Jackson R., Jones L. J., Kasperaviciute D., Kayicki M., Lahnstein L., Lawson K., Leigh S. E. A., Leong I. U. S., Lopez F. J., Maleady-Crowe F., Mason J., McDonagh E. M., Moutsianas L., Mueller M., Murugaesu N., Need A. C., Odhams C. A., Patch C., Perez-Gil D., Polychronopoulos D., Pullinger J., Rahim T., Rendon A., Riesgo-Ferreiro P., Rogers T., Ryten M., Savage K., Sawant K., Scott R. H., Siddiq A., Sieghart A., Smedley D., Smith K. R., Sosinsky A., Spooner W., Stevens H. E., Stuckey A., Sultana R., Thomas E. R. A., Thompson S. R., Tregidgo C., Tucci A., Walsh E., Watters, S. A., Welland M. J., Williams E., Witkowska K., Wood S. M., and Zarowiecki M.

CONFLICTS OF INTEREST

All the authors declare that there are no conflicts of interests.

DATA AVAILABILITY STATEMENT

All disease associated *GRK1* variants described to date have been collated into a Leiden Open Variation Database (http://dna2.leeds.ac.uk/GRK1_LOVD/genes/GRK1). The variants reported in this paper have been deposited into the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) at the National Center for Biotechnology Information under accession numbers SCV001245346 - SCV001245364. Supplemental Data includes three figures and three tables. The 3D homology model of Human *GRK1* based upon the bovine *GRK1* crystal structure (PDB: 4PNI) is available on request from the corresponding author. The full data that supports the findings of this study are available in the supplementary material of this article.

ORCID

James A. Poulter  <https://orcid.org/0000-0003-2048-5693>

Kaoru Fujinami  <http://orcid.org/0000-0003-4248-0033>

Carlo Rivolta  <http://orcid.org/0000-0002-0733-9950>

REFERENCES

- Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., Kondrashov, A. S., & Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. *Nature Methods*, 7(4), 248–249. <https://doi.org/10.1038/nmeth0410-248>
- Arencibia, J. M., Pastor-Flores, D., Bauer, A. F., Schulze, J. O., & Biondi, R. M. (2013). AGC protein kinases: from structural mechanism of regulation to allosteric drug development for the treatment of human diseases. *Biochimica et Biophysica Acta/General Subjects*, 1834(7), 1302–1321. <https://doi.org/10.1016/j.bbapap.2013.03.010>

- Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., & Ben-Tal, N. (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Research*, 44(W1), W344–W350. <https://doi.org/10.1093/nar/gkw408>
- Azam, M., Collin, R. W., Khan, M. I., Shah, S. T., Qureshi, N., Ajmal, M., & Cremers, F. P. (2009). A novel mutation in GRK1 causes Oguchi disease in a consanguineous Pakistani family. *Molecular Vision*, 15, 1788–1793. <https://www.ncbi.nlm.nih.gov/pubmed/19753316>
- Cideciyan, A. V., Zhao, X., Nielsen, L., Khani, S. C., Jacobson, S. G., & Palczewski, K. (1998). Null mutation in the rhodopsin kinase gene slows recovery kinetics of rod and cone phototransduction in man. *Proceedings of the National Academy of Sciences of the United States of America*, 95(1), 328–333. <https://www.ncbi.nlm.nih.gov/pubmed/9419375>
- Godara, P., Cooper, R. F., Sergouniotis, P. I., Diederichs, M. A., Streb, M. R., Genead, M. A., McAnany, J. J., Webster, A. R., Moore, A. T., Dubis, A. M., Neitz, M., Dubra, A., Stone, E. M., Fishman, G. A., Han, D. P., Michaelides, M., & Carroll, J. (2012). Assessing retinal structure in complete congenital stationary night blindness and Oguchi disease. *American Journal of Ophthalmology*, 154(6), 987–1001. <https://doi.org/10.1016/j.ajo.2012.06.003>
- Hayashi, T., Gekka, T., Takeuchi, T., Goto-Omoto, S., & Kitahara, K. (2007). A novel homozygous GRK1 mutation (P391H) in 2 siblings with Oguchi disease with markedly reduced cone responses. *Ophthalmology*, 114(1), 134–141. <https://doi.org/10.1016/j.ophtha.2006.05.069>
- He, Y., Gao, X., Goswami, D., Hou, L., Pal, K., Yin, Y., Zhao, G., Ernst, O. P., Griffin, P., Melcher, K., & Xu, H. E. (2017). Molecular assembly of rhodopsin with G protein-coupled receptor kinases. *Cell Research*, 27(6), 728–747. <https://doi.org/10.1038/cr.2017.72>
- Homan, K. T., & Tesmer, J. J. (2014). Structural insights into G protein-coupled receptor kinase function. *Current Opinion in Cell Biology*, 27, 25–31. <https://doi.org/10.1016/j.ccb.2013.10.009>
- Huang, C. C., Orban, T., Jastrzebska, B., Palczewski, K., & Tesmer, J. J. (2011). Activation of G protein-coupled receptor kinase 1 involves interactions between its N-terminal region and its kinase domain. *Biochemistry*, 50(11), 1940–1949. <https://doi.org/10.1021/bi101606e>
- Jespersgaard, C., Fang, M., Bertelsen, M., Dang, X., Jensen, H., Chen, Y., Bech, N., Dai, L., Rosenberg, T., Zhang, J., Møller, L. B., Tümer, Z., Brøndum-Nielsen, K., & Grønskov, K. (2019). Molecular genetic analysis using targeted NGS analysis of 677 individuals with retinal dystrophy. *Scientific Reports*, 9(1), 1219. <https://doi.org/10.1038/s41598-018-38007-2>
- Khani, S. C., Nielsen, L., & Vogt, T. M. (1998). Biochemical evidence for pathogenicity of rhodopsin kinase mutations correlated with the oguchi form of congenital stationary night blindness. *Proceedings of the National Academy of Sciences of the United States of America*, 95(6), 2824–2827. <https://doi.org/10.1073/pnas.95.6.2824>
- Kircher, M., Witten, D. M., Jain, P., O’Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, 46(3), 310–315. <https://doi.org/10.1038/ng.2892>
- Komolov, K. E., & Benovic, J. L. (2018). G protein-coupled receptor kinases: Past, present and future. *Cellular Signalling*, 41, 17–24. <https://doi.org/10.1016/j.cellsig.2017.07.004>
- Krupnick, J. G., Gurevich, V. V., & Benovic, J. L. (1997). Mechanism of quenching of phototransduction. Binding competition between arrestin and transducin for phosphorhodopsin. *Journal of Biological Chemistry*, 272(29), 18125–18131. <https://www.ncbi.nlm.nih.gov/pubmed/9218446>
- Kuhn, H., & Dreyer, W. J. (1972). Light dependent phosphorylation of rhodopsin by ATP. *FEBS Letters*, 20(1), 1–6. <https://www.ncbi.nlm.nih.gov/pubmed/11946367>
- Laskowski, R. A., Stephenson, J. D., Sillitoe, I., Orengo, C. A., & Thornton, J. M. (2020). VarSite: Disease variants and protein structure. *Protein Science*, 29(1), 111–119. <https://doi.org/10.1002/pro.3746>
- Li, L., Chen, Y., Jiao, X., Jin, C., Jiang, D., Tanwar, M., Ma, Z., Huang, L., Ma, X., Sun, W., Chen, J., Ma, Y., M’hamdi, O., Govindarajan, G., Cabrera, P. E., Li, J., Gupta, N., Naeem, M. A., Khan, S. N., ... Hejtmancik, J. F. (2017). Homozygosity mapping and genetic analysis of autosomal recessive retinal dystrophies in 144 consanguineous Pakistani families. *Investigative Ophthalmology and Visual Science*, 58(4), 2218–2238. <https://doi.org/10.1167/iov.17-21424>
- Lodowski, D. T., Tesmer, V. M., Benovic, J. L., & Tesmer, J. J. (2006). The structure of G protein-coupled receptor kinase (GRK)-6 defines a second lineage of GRKs. *Journal of Biological Chemistry*, 281(24), 16785–16793. <https://doi.org/10.1074/jbc.M601327200>
- Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., Brown, S. D., Chang, H. Y., El-Gebali, S., Fraser, M. I., Gough, J., Haft, D. R., Huang, H., Letunic, I., Lopez, R., Luciani, A., Madeira, F., Marchler-Bauer, A., Mi, H., ... Finn, R. D. (2019). InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Research*, 47(D1), D351–D360. <https://doi.org/10.1093/nar/gky1100>
- Miyake, Y., Horiguchi, M., Suzuki, S., Kondo, M., & Tanikawa, A. (1996). Electrophysiological findings in patients with Oguchi’s disease. *Japanese Journal of Ophthalmology*, 40(4), 511–519. <https://www.ncbi.nlm.nih.gov/pubmed/9130055>
- Mucciolo, D. P., Sodi, A., Murro, V., Passerini, I., Palchetti, S., Pelo, E., Virgili, G., & Rizzo, S. (2018). A novel GRK1 mutation in an Italian patient with Oguchi disease. *Ophthalmic Genetics*, 39(1), 137–138. <https://doi.org/10.1080/13816810.2017.1323341>
- Nishiguchi, K. M., Ikeda, Y., Fujita, K., Kunikata, H., Akiho, M., Hashimoto, K., Hosono, K., Kurata, K., Koyanagi, Y., Akiyama, M., Suzuki, T., Kawasaki, R., Wada, Y., Hotta, Y., Sonoda, K. H., Murakami, A., Nakazawa, M., Nakazawa, T., & Abe, T. (2019). Phenotypic features of oguchi disease and retinitis pigmentosa in patients with S-antigen mutations: A long-term follow-up study. *Ophthalmology*, 126(11), 1557–1566. <https://doi.org/10.1016/j.ophtha.2019.05.027>
- Oishi, A., Akimoto, M., Kawagoe, N., Mandai, M., Takahashi, M., & Yoshimura, N. (2007). Novel mutations in the GRK1 gene in Japanese patients with Oguchi disease. *American Journal of Ophthalmology*, 144(3), 475–477. <https://doi.org/10.1016/j.ajo.2007.03.025>
- Panagiotou, E. S., Sanjurjo Soriano, C., Poulter, J. A., Lord, E. C., Dzulova, D., Kondo, H., A., Hiyoshi, C., Chung, B. H. Y., Chu, Y. W. Y., Lai, C. H. Y., Tafoya, M. E., Karjosukarso, D., Collin, R. W. J., Topping, J., Downey, L. M., Ali, M., Inglehearn, C. F., & Toomes, C. (2017). Defects in the cell signaling mediator beta-catenin cause the retinal vascular condition FEVR. *American Journal of Human Genetics*, 100(6), 960–968. <https://doi.org/10.1016/j.ajhg.2017.05.001>
- Pandurangan, A. P., Ochoa-Montano, B., Ascher, D. B., & Blundell, T. L. (2017). SDM: a server for predicting effects of mutations on protein stability. *Nucleic Acids Research*, 45(W1), W229–W235. <https://doi.org/10.1093/nar/gkx439>
- Pitcher, J. A., Freedman, N. J., & Lefkowitz, R. J. (1998). G protein-coupled receptor kinases. *Annual Review of Biochemistry*, 67, 653–692. <https://doi.org/10.1146/annurev.biochem.67.1.653>
- Ponzoni, L., Oltvai, Z. N., & Bahar, I. (2019). Rhapsody: Pathogenicity prediction of human missense variants based on protein sequence, structure and dynamics. *bioRxiv*. <https://doi.org/10.1101/737429>
- Roosing, S., Collin, R. W., den Hollander, A. I., Cremers, F. P., & Siemiakowska, A. M. (2014). Prenylation defects in inherited retinal diseases. *Journal of Medical Genetics*, 51(3), 143–151. <https://doi.org/10.1136/jmedgenet-2013-102138>
- Sato, P. Y., Chuprun, J. K., Schwartz, M., & Koch, W. J. (2015). The evolving impact of g protein-coupled receptor kinases in cardiac health and

- disease. *Physiological Reviews*, 95(2), 377–404. <https://doi.org/10.1152/physrev.00015.2014>
- Schrodinger, L. L. C. (2015). The PyMOL molecular graphics system. *Version*, 1, 8.
- Sim, N. L., Kumar, P., Hu, J., Henikoff, S., Schneider, G., & Ng, P. C. (2012). SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Research*, 40(Web Server issue), W452–W457. <https://doi.org/10.1093/nar/gks539>
- Skorczyk-Werner, A., Kociecki, J., Wawrocka, A., Wicher, K., & Krawczynski, M. R. (2015). The first case of Oguchi disease, type 2 in a Polish patient with confirmed GRK1 gene mutation. *Klinika Oczna*, 117(1), 27–30. <https://www.ncbi.nlm.nih.gov/pubmed/26349155>
- Taylor, R. L., Arno, G., Poulter, J. A., Khan, K. N., Morarji, J., Hull, S., & The, G. P. (2017). Association of steroid 5alpha-reductase type 3 congenital disorder of glycosylation with early-onset retinal dystrophy. *JAMA Ophthalmol*, 135(4), 339–347. <https://doi.org/10.1001/jamaophthalmol.2017.0046>
- Teke, M. Y., Citirik, M., Kabacam, S., Demircan, S., & Alikasifoglu, M. (2016). A novel missense mutation of the GRK1 gene in Oguchi disease. *Molecular Medicine Reports*, 14(4), 3129–3133. <https://doi.org/10.3892/mmr.2016.5620>
- Thermann, R. (1998). Binary specification of nonsense codons by splicing and cytoplasmic translation. *EMBO Journal*, 17(12), 3484–3494. <https://doi.org/10.1093/emboj/17.12.3484>
- Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., de Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research*, 46(W1), W296–W303. <https://doi.org/10.1093/nar/gky427>
- Whiffin, N., Minikel, E., Walsh, R., O'donnell-Luria, A. H., Karczewski, K., Ing, A. Y., Barton, P. J. R., Funke, B., Cook, S. A., MacArthur, D., & Ware, J. S. (2017). Using high-resolution variant frequencies to empower clinical genome interpretation. *Genetics in Medicine*, 19(10), 1151–1158. <https://doi.org/10.1038/gim.2017.26>
- Wilden, U., Hall, S. W., & Kuhn, H. (1986). Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. *Proceedings of the National Academy of Sciences of the United States of America*, 83(5), 1174–1178. <https://www.ncbi.nlm.nih.gov/pubmed/3006038>
- Yamamoto, S., Sippel, K. C., Berson, E. L., & Dryja, T. P. (1997). Defects in the rhodopsin kinase gene in the Oguchi form of stationary night blindness. *Nature Genetics*, 15(2), 175–178. <https://doi.org/10.1038/ng0297-175>
- Zhang, H., Li, S., Doan, T., Rieke, F., Detwiler, P. B., Frederick, J. M., & Baehr, W. (2007). Deletion of PrBP/delta impedes transport of GRK1 and PDE6 catalytic subunits to photoreceptor outer segments. *Proceedings of the National Academy of Sciences of the United States of America*, 104(21), 8857–8862. <https://doi.org/10.1073/pnas.0701681104>
- Zhang, Q., Zulfiqar, F., Riazuddin, S. A., Xiao, X., Yasmeen, A., Rogan, P. K., & Hejtmancik, J. F. (2005). A variant form of Oguchi disease mapped to 13q34 associated with partial deletion of GRK1 gene. *Molecular Vision*, 11, 977–985. <https://www.ncbi.nlm.nih.gov/pubmed/16319817>

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Poulter JA, Gravett MSC, Taylor RL, et al. New variants and in silico analyses in GRK1 associated Oguchi disease. *Human Mutation*. 2021;42:164–176. <https://doi.org/10.1002/humu.24140>