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Panel-Based Clinical Genetic Testing in 85 Children with Inherited Retinal Disease

Rachel L. Taylor, PhD,[1,2] Neil R.A. Parry, PhD,[3,4] Stephanie J. Barton, BSc,[2] Christopher Campbell, BSc,[2] Claire M. Delaney, MSc,[3,4] Jamie M. Ellingford, MRes,[1,2] Georgina Hall, MSc,[1,2] Claire Hardcastle, BSc,[2] Jiten Morarji, MSc, BMBS,[3] Elisabeth J. Nichol, DBO,[3] Lindsi C. Williams, DBO,[3] Sofia Douzgou, PhD, MD,[1,2] Jill Clayton-Smith, MD(Res), FRCP,[1,2] Simon C. Ramsden, PhD, FRCPath,[1,2] Vinod Sharma, FRCOphth,[3] Susmito Biswas, FRCOphth,[3] I. Chris Lloyd, FRCOphth,[3,5] Jane L. Ashworth, PhD, FRCOphth,[1,3] Graeme C. Black, DPhil, FRCOphth,[1,2] Panagiotis I. Sergouniotis, PhD, FEBO[1,2,3]

Purpose: To assess the clinical usefulness of genetic testing in a pediatric population with inherited retinal disease (IRD).

Design: Single-center retrospective case series.

Participants: Eighty-five unrelated children with a diagnosis of isolated or syndromic IRD who were referred for clinical genetic testing between January 2014 and July 2016.

Methods: Participants underwent a detailed ophthalmic examination, accompanied by electrodiagnostic testing (EDT) and dysmorphologic assessment where appropriate. Ocular and extraocular features were recorded using Human Phenotype Ontology terms. Subsequently, multigene panel testing (105 or 177 IRD-associated genes) was performed in an accredited diagnostic laboratory, followed by clinical variant interpretation.

Main Outcome Measures: Diagnostic yield and clinical usefulness of genetic testing.

Results: Overall, 78.8% of patients (n = 67) received a probable molecular diagnosis; 7.5% (n = 5) of these had autosomal dominant disease, 25.4% (n = 17) had X-linked disease, and 67.2% (n = 45) had autosomal recessive disease. In a further 5.9% of patients (n = 5), a single heterozygous *ABCA4* variant was identified; all these participants had a spectrum of clinical features consistent with *ABCA4* retinopathy. Most participants (84.7%; n = 72) had undergone EDT and 81.9% (n = 59) of these patients received a probable molecular diagnosis. The genes most frequently mutated in the present cohort were *CACNA1F* and *ABCA4*, accounting for 14.9% (n = 10) and 11.9% (n = 8) of diagnoses respectively. Notably, in many cases, genetic testing helped to distinguish stationary from progressive IRD subtypes and to establish a precise diagnosis in a timely fashion.

Conclusions: Multigene panel testing pointed to a molecular diagnosis in 84.7% of children with IRD. The diagnostic yield in the study population was significantly higher compared with that in previously reported unselected IRD cohorts. Approaches similar to the one described herein are expected to become a standard component of care in pediatric ophthalmology. We propose the introduction of genetic testing early in the diagnostic pathway in children with clinical and/or electrophysiologic findings, suggestive of IRD. *Ophthalmology 2017;■:1–7 © 2017 by the American Academy of Ophthalmology*

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Inherited retinal disease (IRD) denotes a heterogeneous group of predominantly monogenic disorders that feature loss or dysfunction of photoreceptor cells as a primary or secondary event.[1] These conditions are clinically and genetically diverse and have been linked to more than 250 genes (Retinal Information Network, available at https://sph.uth.edu/Retnet/). The retinal appearance and pattern of visual loss are typically related to the degree to which cone and rod photoreceptor cells are affected. Notably, distinguishing progressive (e.g., macular, cone/cone-rod, and rod–cone dystrophies) from relatively nonprogressive (e.g., cone dysfunction syndromes or congenital stationary night blindness) IRD subtypes may be a formidable challenge, especially in pediatric patients.

Collectively, IRD is a major cause of visual impairment in children.[2] It can also sometimes be one of the first presenting features of a syndromic condition such as a ciliopathy or a neurometabolic disorder.[3] Timely recognition and appropriate multidisciplinary management of these disorders can have important implications for the child's health and development.[4] Over the past decade, the advent of high-throughput DNA sequencing technologies has revolutionized genetic testing for IRD, accelerating diagnosis and facilitating a precision medicine approach.[5,6]

The aim of this study was to assess the current clinical validity and usefulness of genetic testing in children with IRD. The potential to improve diagnosis and management

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and to allow the definition of specific care pathways is highlighted.

Methods

Recruitment and Phenotypic Data Collection

Unrelated participants, 16 years of age or younger, with a diagnosis of IRD were ascertained retrospectively through the database of the Manchester Regional Genetic Laboratory Service, Manchester, United Kingdom. Only individuals for whom a referral was received between January 2014 and July 2016 (30 months total) were included. All study participants were diagnosed through tertiary ophthalmic genetic clinics at Central Manchester University Hospitals, Manchester, United Kingdom. The care pathway in these clinics includes offering genetic testing to all families with children that have clinical history and examination findings suggestive of IRD.

A 3-generation pedigree and a full ocular, developmental, and medical history were obtained for each patient. Clinical assessment included visual acuity testing using age-appropriate optotypes, dilated fundus examination, and in selected cases, cycloplegic refraction, orthoptic assessment, color fundus photography, fundus autofluorescence imaging, and optical coherence tomography. Electrodiagnostic testing (EDT) was performed in most cases; the protocols used incorporated the standards of the International Society of Electrophysiology of Vision for full-field and pattern electroretinography.[7] At the initial electrodiagnostic assessment, Ganzfeld electroretinograms were obtained from 50.6% of patients (n = 43; age range, 3−14 years). A reduced pediatric protocol was used in 29 very young or uncooperative children (34.1%; age range, 4 months−4 years), using a handheld photic stimulator (Grass PS33, Grass Instruments, Quincy, MA). Both dark-adapted and light-adapted handheld electroretinograms were recorded at the first visit, except for 1 uncooperative patient who underwent only light-adapted tests, but subsequently underwent full Ganzfeld electroretinography at 6 years of age. Twelve of the reduced-protocol patients underwent further EDT, half of whom consented to undergo Ganzfeld electroretinography at the second visit. Thirteen patients (15.3%) did not undergo EDT. Where extraocular features were present or suspected, a full systemic and dysmorphic assessment was undertaken by a clinical geneticist.

After obtaining informed consent from the affected individual or family, multigene panel testing for IRD was requested. Key clinical information was included in the referral request to facilitate variant interpretation. These phenotypic data subsequently were converted into Human Phenotype Ontology terms (Human Phenotype Ontology build no. 1701; accessed October 10, 2016); the Human Phenotype Ontology project is an international initiative aiming to provide both a standardized vocabulary and a computer-interpretable representation of phenotypic abnormalities encountered in human disease.[8] Ethics committee approval for the study was obtained from the North West Research Ethics Committee (identifiers, 11/NW/0421 and 15/YH/0365), and all investigations were conducted in accordance to the tenets of the Declaration of Helsinki.

Clinical Genetic Testing and Bioinformatics Analysis

Multigene panel testing and analysis were performed at the Manchester Genomic Diagnostic Laboratory, a United Kingdom Accreditation Service Clinical Pathology Accredited medical laboratory (Clinical Pathology Accredited identifier, no. 4015). DNA samples were processed using Agilent SureSelect (Agilent Technologies, Santa, Clara, CA) target enrichment kits designed to capture all exons and 50 base pairs of flanking intronic sequence of either 105 genes (samples tested between January and June 2014)[9] or 177 genes (samples tested between July 2014 and July 2016).[10] All tested genes have been previously associated with IRD in humans and were selected after interrogating publically available databases (Retinal Information Network; https://sph.uth.edu/Retnet/) and the literature. A list of all tested transcripts and genes can be found in Tables S1 and S2 (both available at www.aaojournal.org). Notably, the following disease-associated intronic mutations also were included in the 177-gene panel: *CEP290* c.2991+1665A→G (this variant was also covered by the 105-gene panel), *USH2A* c.7595−2144A→G, *OFD1* c.935+706A→G, *ABCA4* c.5196+1056A→G, *ABCA4* c.5196+1137G→A, *ABCA4* c.5196+1216C→A, *ABCA4* c.4539+2001G→A, *ABCA4* c.4539+2028C→T, and *ABCA4* c.5461−10T→C.

Sequencing and bioinformatics analysis were performed as described previously.[9,10] Briefly, after enrichment, the samples were sequenced on an Illumina HiSeq 2000/2500 system (Illumina, Inc, San Diego, CA) according to the manufacturer's protocols. Sequence reads subsequently were demultiplexed using CASAVA software version 1.8.2 (Illumina, Inc, San Diego, CA) and aligned to the hg19 reference genome using the Burrows Wheeler Aligner (BWA-short version 0.62).[11] Duplicate reads were removed using Samtools before base quality score recalibration and insertion-deletion realignment using the Genome Analysis Tool Kit (GATK-lite version 2.0.39).[12] The UnifiedGenotyper within the Genome Analysis Tool Kit was used for single nucleotide variant and insertion-deletion discovery.[13] To reduce the number of false-positive variants, we primarily limited the clinical analysis to changes with sequencing quality metrics above specific criteria: single nucleotide variants with ≥50× independent sequencing reads and ≥45 mean quality value, and insertions-deletions with support from >25% of the aligned and independent sequencing reads were considered.[10] Copy number variants were detected from high-throughput sequencing read data using ExomeDepth version 1.1.6.[14] Copy number variants then were confirmed by dosage polymerase chain reaction; reactions were analyzed against a GS500 ROX standard (Applied Biosystems, Waltham, MA) and run on an ABI 3130XL Genetic Analyzer according to the manufacturer's instructions, using a generic multiplex ligation-dependent probe amplification analysis setting.

Variant Filtering and Classification

Clinical interpretation of variants was performed using a previously described strategy[10] and criteria consistent with the 2015 American College of Medical Genetics and Genomics best practice guidelines.[15] In short, variants with a minor allele frequency of more than 1% in large publically available datasets (National Heart, Lung, and Blood Institute Exome Sequencing Project Exome Variant Server ESP6500 and dbSNP version 135) were considered benign and were not analyzed further. The Ensembl Variant Effect Predictor[16] was used to predict functional consequences of the remaining genetic changes, and a pathogenicity classification score[15] was assigned after extensive appraisal of the scientific literature, the patient's clinical referral, and in silico modeling. A clinical report was then generated and variants that possibly or probably accounted for the tested individual's clinical presentation were highlighted. For the purpose of this study, participants were split in to 3 groups:

1. Probable molecular diagnosis group: patients with clearly or likely disease-associated variant(s) in an apparently