How genetics works? An illustrative case report

Vikas Khetan[1], Mario Zanolli[1], Jenina Capasso[1], Nanette Zale Refice[2], Kimberley Neeley[3], Alex V Levin[1,4]

In this communication, we report the case of a four year old boy who presented with reduced vision in the right eye. He had visual acuity of light perception right eye and 6/12 in the left eye and anterior segment examination was normal. Fundus examination of the right eye showed a falciform retinal fold extending from the optic nerve temporally involving the entire retina with exudates within the falciform fold and dense pigmentation peripherally. The left eye showed mild macular temporal dragging of the vessels and 360° of peripheral laser scars. In addition he also had some characteristic systemic features such as developmental delay, obesity, dysmorphic facies and tapered fingers. Using this case as an example, we present a systematic, logical approach to a patient with a possible genetic disorder. The growing field of ocular genetics now allows for improved diagnosis using step-wise cost efficient testing as demonstrated herein.

Key words: Familial exudative vitreoretinopathy, genetics, microarray, mutations, retina

Ocular genetics is a relatively new field in India with very few practicing professionals. Specialists in the field provide a unique perspective by attention paid to systemic findings, inclusion of detailed family history, experience with rare disorders which they by definition see more commonly, and knowledge of the rapidly emerging biotechnology related to diagnosis and future treatment. Herein, we use a case to illustrate the deductive process by which the ocular geneticist solves an unusual clinical dilemma and in doing so, assists other ophthalmologists in providing a diagnosis for their patient, which allows for better understanding also by the family.

Illustrative Case

A 4-year-old boy was referred for poor vision, strabismus, and a history of laser treatment to his right eye. He weighed -3.2 kg at birth and was delivered at 41 weeks.

Access this article online

Quick Response Code:

Website: www.ijo.in

DOI: 10.4103/0301-4738.185629

[1]Department of Ocular Genetics, Wills Eye Hospital, Philadelphia, [2]Geisinger Health System, Sranton, [3]Penn State Hershey Eye Center, [4]Thomas Jefferson University, PA, USA

Correspondence to: Dr. Alex V Levin, Pediatrics Ophthalmology and Ocular Genetics, Wills Eye Hospital, Suite 1201, 840 Walnut Street, Philadelphia, PA 19107-5109, USA. E-mail: alevin@willseye.org

Manuscript received: 07.12.15; Revision accepted: 21.02.16

He had surgery for double aortic arch (tracheal ring) with secondary pulmonary concerns. He also had speech and developmental delay with moderate obesity and dysmorphic facies. He had tapered fingers [Fig. 1]. Family history was unremarkable. He was the only affected person in the family.

Ophthalmic examination revealed best-corrected visual acuity of light perception right eye and −6/12 in the left eye using Snellen's visual acuity chart. Anterior segment examination was normal. He had right pseudoexotropia with full ocular motility. Cycloplegic refraction was −4.25 right eye and −3.50 left eye. Right, fundus examination showed a falciform retinal fold extending from the optic nerve temporally involving the entire retina [Fig. 2a]. There was exudate within the falciform fold and peripherally with dense pigmentation peripherally. The left eye showed mild macular temporal dragging of the vessels and 360° of peripheral laser scars [Fig. 2b]. Fluorescein angiography revealed areas of capillary nonperfusion, staining, and new vessels in the left eye posterior to and within the laser treated areas and in the right eye peripheral leakage [Fig. 2c and d]. Further laser treatment was performed to ablate these areas.

Step 1: Is this a known disease?

When approaching a complex presentation with multiple features, the first step is to use pattern recognition or other resources to see if the co-occurrence of findings has been reported previously.[1,2] Our patient had retinopathy, double aortic arch, developmental delay, obesity, abnormal facies, and abnormal finger shape. Pattern recognition did not allow us to suggest a unifying diagnosis. A search of the medical literature and online resources such as the Online Mendelian Inheritance in Man (OMIM: http://www.ncbi.nlm.nih.gov/omim) did not show any previously described link between all of the features.

Step 2: One disease or two?

The question then becomes whether the multiple features are due to an abnormality in one gene, multiple contiguous genes, or a chance occurrence. Multiple abnormalities in the same patient may be due to a single chromosomal abnormality that is affecting multiple genes simultaneously, for example, a contiguous deletion or duplication of a chromosome segment involving more than one gene. Karyotyping is a test which examines whole chromosomes extracted from the nuclei of cells, usually blood lymphocytes to identify aberrations of chromosome number or structure.[3] As humans have approximately 25,000 genes distributed on 23 chromosome pairs, there are on average hundreds or thousands of genes per chromosome. Any microscopically visible aberration in chromosome number or structure, even if involving only a small section of the chromosome, will by definition involve multiple genes thus resulting in multiple manifestations in the patient.[4]

Cite this article as: Khetan V, Zanolli M, Capasso J, Refice NZ, Neeley K, Levin AV. How genetics works? An illustrative case report. Indian J Ophthalmol 2016;64:399-402.

Figure 1: Tapered fingers in the patient

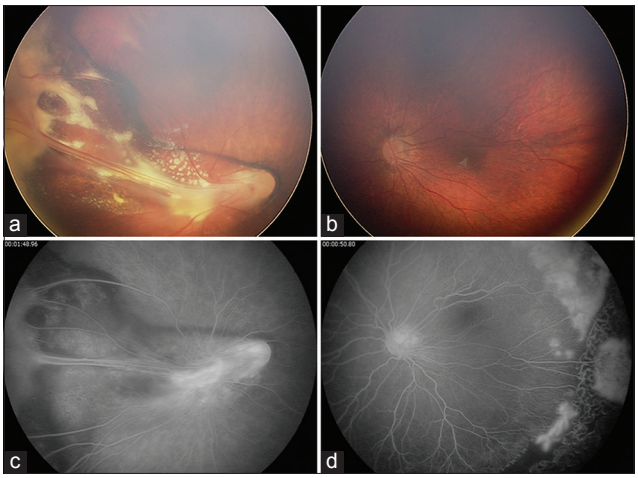

Figure 2: (a). Right fundus shows a falciform retinal fold extending from the optic nerve temporally, involving the entire retina. There is exudation within the falciform fold and peripherally. (b) The left eye shows mild macular temporal dragging of the vessels and an incidental prefoveal gliotic tuft. (c) Fluorescein angiography shows peripheral and posterior leakage in the right eye within the falciform fold. (d) Note the areas of capillary nonperfusion, staining, and new vessels in the left eye posterior to and within the laser treated areas

A karyotype is therefore indicated when a patient has more than two malformations that are not otherwise recognizably related.

Deletions or duplications may be too small to be visualized by karyotyping yet still be clinically significant involving multiple genes. Chromosome microarray is useful for detecting small copy number variations (CNVs).[5] A single nucleotide polymorphism (SNP) array is a type of DNA microarray which is used to detect polymorphisms within a population. These variations are the most frequent type of variation in the genome. There are approximately 50 million SNPs that have been identified in the human genome.[1] SNPs, therefore, serve as an excellent genotypic marker. The patient's blood is compared to the microarray panel to create labeling of the DNA which can then be quantified to identify the presence of deleted or duplicated regions which appear as abnormally low or high dosing of a particular area of the genome. SNP microarray technology can also detect regions of homozygosity which may help to suggest a higher likelihood of an autosomal recessive disorder involving a single gene in that region.[6] Microarray cannot detect balanced structural rearrangements since the complement of DNA is neither duplicated nor deleted – just rearranged. Mutations in a single gene cannot be detected. Even the best microarray chips available today have small gaps in coverage based on the number of SNP probes used. A normal microarray does not rule out a deletion or duplication smaller than the distance between probes, a structural rearrangement that could cause disruption of a gene, or genes, at a breakpoint, or a single gene abnormality.

Our patient's karyotype was normal. Microarray revealed a duplication of 3p25.3-26.1 and a deletion in 4q33-34.1.

Step 3: Interpretation of the microarray

CNV (deletions or duplications) can be nonpathogenic. Therefore, one must determine if the results on microarray are related to the patient's phenotype. The first step in this process is to consult the literature and available databases (e.g., http://www.genome.ucsc.edu/cgi-bin/hgGateway) to see if there have been prior reports of similar array abnormalities that are known to be associated with a phenotype. Testing the parents can also be useful. If an unaffected parent has the same CNV as the affected child, then it can be concluded the CNV is likely not causative. In the absence of a known phenotype and when normal parents do not have the CNV, one must consider the possibility that the CNV is a newly recognized cause of the patient's phenotype. To explore this possibility one can use available databases (e.g., http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606andbuild=previous) to identify candidate genes within the CNV that are either known to produce manifestations as seen in the patient or are expressed in organs affected in the patient.

Our patient's deletion on 4q has not been previously reported as pathogenic and contains no genes that seemed relevant to his phenotype. His mother's microarray was normal. His father was found to harbor the del4q thus confirming its likely nonpathogenicity as he does not have any clinical abnormalities. The patient's 3p duplication has been previously reported with a nonocular phenotype consistent with that seen in our patient.[7] This is likely due to deletion of the GHRL and *OXTR* gene and manifests with obesity, developmental delay, cardiac malformations and a facies very similar to our patient. This explains some but not all of our patient's findings. Could his ocular abnormalities be due to the dup3p? A search for other genes in the duplicated region failed to reveal any that are known to be associated with ocular disease and none that are expressed in the eye.

Step 4: Consider the possibility of a second disorder

If all of the findings cannot be explained by a unifying genetic diagnosis or test, then one must consider the possibility of a coincidental occurrence of two unrelated disorders.

The eye findings suggest familial exudative vitreoretinopathy. This disorder demonstrates genetic heterogeneity and may be due to mutations in *NDP*, *FZD4*, *LRP5*, or *TSPN12*.[8] Sequencing of the latter three genes was unremarkable, but the patient was found to show a hemizygous nonsense mutation in *NDP* exon 3, 388G>T (Glu130X). His mother was heterozygous for the same mutation. Although her clinical eye exam was normal,

IVFA revealed areas of peripheral nonperfusion commonly seen in the female carriers of this X-linked recessive form of the disorder. The child has his mother's *NDP* mutation leading to his ocular phenotype. We have now explained all of the clinical features in this patient. The genetic testing is summarized in Table 1.

Discussion

Ophthalmologists are frequently faced with ocular disorders that occur in the setting of systemic abnormalities which may or may not be related. The presence of such systemic abnormalities may help guide the ophthalmologist to a recognized unifying diagnosis. One may then order genetic testing to confirm the diagnosis if such a test is available. If the systemic associations are not previously described in the literature, then this may represent a contiguous gene deletion/duplication syndrome due to a chromosomal aberration, the presence of two different and genetically unrelated disorders or a new syndrome due to a single gene. A karyotype can identify larger chromosomal aberrations, but the microarray now allows us to identify submicroscopic contiguous gene deletion/duplication disorders. If there is no CNV or a CNV, which does not adequately explain all of the findings, then the physician may try to reclassify the findings into separate disorders and engage in confirmatory testing.

This process of deductive and inductive reasoning requires a fundamental yet broad knowledge of ocular genetic disorders and systemic genetic disorders associated with ocular findings. Genetic testing is becoming readily available to clinicians worldwide, and tests are being ordered with increasing frequency. The difficulty lies in developing a cost-effective strategy for testing, interpreting the sometimes complicated results, exploring the veracity, and relevance of the results both through database mining and family member evaluation and testing, and counseling the patient and family. Ocular genetics is a new ophthalmic fellowship-trained subspecialty, especially suited to engage in such evaluations particularly in light of the multitude of new and emerging technologies. Concentrating these rare disorders in the practice of an ocular geneticist allows for the development of experience and expertise while also freeing other ophthalmology subspecialists from the almost insurmountable task of keeping up-to-date with recent genetic developments, including research trials and diagnostic testing.

Table 1: Results of genetic testing

Genetic test result	Results
Proband	
Karyotype	Normal 46, XY
Oligo-SNP array	Duplication 3p25.3-p26.1 Deletion 4q33-q34.1
LRP5 sequencing	No mutation
TSPAN12 sequencing	No mutation
NDP sequencing	Nucleotide change 388G >T, protein change Glu130X (stop codon)
Mom	
Oligo-SNP array	Normal array
NDP sequencing	388G >T
Maternal Grandmother	
NDP sequencing	Normal sequencing
Father	
Karyotype	Normal 46, XY
FISH for dup3p and del4q	No duplication 3p26.1 Positive for deletion 4q33-q34.1

SNP: Single nucleotide polymorphism

Finding a genetic diagnosis is often difficult and time-consuming, yet there are great benefits to the patient and family. In our experience, many of these patients have seen multiple physicians only to be frustrated by incorrect diagnosis or a lack of diagnosis. The power of a diagnosis is apparent in the family's ability to understand the causation of the disease, associated findings for which screening may be indicated, and prognosis. Genetic counseling must be a part of test interpretation and result disclosure.[9] Families may be offered information relevant to family planning options, recurrence risk, and identification of at-risk family members or carriers. Having a definitive diagnosis also allows families to find support networks and participate in research trials, or when available, receive appropriate disease specific treatment.

Every ophthalmologist has a contact in some way with patients affected by genetic disorders. The growing field of ocular genetics now allows for improved diagnosis using stepwise, cost-efficient testing as demonstrated herein. New technologies will make testing even more accurate, available, and cheaper. With the success of gene therapy for ocular disorders, it becomes even more critical that proper diagnoses through appropriate genetic testing is conducted, hopefully in the setting of a knowledgeable ocular geneticist and supportive genetic counselor.[10-13]

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Neel JV, Julius S, Weder A, Yamada M, Kardia SL, Haviland MB. Syndrome X: Is it for real? Genet Epidemiol 1998;15:19-32.
2. Cohen MM Jr. Syndromology: An updated conceptual overview. X. References. Int J Oral Maxillofac Surg 1990;19:89-96.
3. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, *et al.* dbSNP: The NCBI database of genetic variation. Nucleic Acids Res 2001;29:308-11.
4. Rafiee L, Mohsenzadeh S, Saadat M. Nonrandom gene distribution on human chromosomes. EXCLI J 2008;7:151-3.
5. Manning M, Hudgins L; Professional Practice and Guidelines Committee. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. Genet Med 2010;12:742-5.
6. Grote L, Myers M, Lovell A, Saal H, Sund KL. Variable approaches to genetic counseling for microarray regions of homozygosity associated with parental relatedness. Am J Med Genet A 2014;164A:87-98.
7. Bittel DC, Kibiryeva N, Dasouki M, Knoll JH, Butler MG. A 9-year-old male with a duplication of chromosome 3p25.3p26.2: Clinical report and gene expression analysis. Am J Med Genet A 2006;140:573-9.
8. Nikopoulos K, Venselaar H, Collin RW, Riveiro-Alvarez R, Boonstra FN, Hooymans JM, *et al.* Overview of the mutation

spectrum in familial exudative vitreoretinopathy and Norrie disease with identification of 21 novel variants in FZD4, LRP5, and NDP. Hum Mutat 2010;31:656-66.

9. Wong N, Lasko D, Rabelo R, Pinsky L, Gordon PH, Foulkes W. Genetic counseling and interpretation of genetic tests in familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. Dis Colon Rectum 2001;44:271-9.
10. Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr., Mingozzi F, Bennicelli J, *et al.* Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 2008;358:2240-8.
11. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, *et al.* Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med 2008;358:2231-9.
12. Jacobson SG, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, *et al.* Gene therapy for leber congenital amaurosis caused by RPE65 mutations: Safety and efficacy in 15 children and adults followed up to 3 years. Arch Ophthalmol 2012;130:9-24.
13. Xia CH, Liu H, Cheung D, Wang M, Cheng C, Du X, *et al.* A model for familial exudative vitreoretinopathy caused by LPR5 mutations. Hum Mol Genet 2008;17:1605-12.