Supplemental Information

Table 1. Accrual of eyeGENE® samples by disease category and comparison to estimated U.S. prevalence

eyeGENE® Disease Diagnosis Category	Prevalence	Number of Individuals Accrued in eyeGENE®	High-end Estimate of Affected in the U.S.	Percentage of Estimate Captured in eyeGENE® to date
Retinitis Pigmentosa and other Retinal Degenerations	1 in 5,000 to 10,000**	1,356	62,800	2.16%
Stargardt Disease	1 in 8,000 to 10,000*	937	39,250	2.39%
Cone-Rod Dystrophy	1 in 100,000**	300	3,140	9.55%
Choroideremia	1 in 50,000 to 100,000*	186	6,280	2.96%
Pattern Dystrophy	unknown**	177	unknown	n.d.
X-linked Juvenile Retinoschisis	1 in 5,000 to 25,000 males*	134	62,800	0.21%
Best Vitelliform Macular Dystrophy	unknown*	124	unknown	n.d.
Retinoblastoma	1 in 100,000**	109	3,140	3.47%
Optic Atrophy Type 1	1 in 50,000*	90	6,280	1.43%
Familial Exudative Vitreoretinopathy	unknown*	79	unknown	n.d.
Usher Syndrome	1 in 100,000**	75	3,140	2.39%
Doyne Honeycomb Dystrophy	unknown**	56	unknown	n.d.
Congenital Stationary Night Blindness	unknown*	46	unknown	n.d.
Aniridia	1 in 50,000 to 100,000*	36	6,280	0.57%
Corneal Dystrophy	unknown*	34	unknown	n.d.
Sorsby Fundus Dystrophy	unknown**	25	unknown	n.d.

Albinism	1 in 20,000 (OCA)/1 in 60,000 males (OA)*	25	15,700/5,233	0.12%
Bietti's Crystalline Corneo-Retinal Dystrophy	1 in 67,000*	25	4,686	0.53%
Leber Hereditary Optic Neuropathy (LHON)	1 in 100,000**	19	3,140	0.61%
Microphthalmia (MO) and Anophthalmia (AO)	1 in 10,000 (MO)/1 in 250,000 (AO)*	19	31,400/1,256	0.06%
Mitochondrial Conditions with Ophthalmic Manifestations	unknown*	16	unknown	n.d.
Glaucoma	1 in 10,000 (primary congenital) to 50,000 (juvenile open-angle)*	13	31,400/6,280	0.04%
Pantothenate Kinase-Associated Neurodegeneration (PKAN)	1 in 1,000,000**	13	314	4.14%
Axenfeld - Rieger Syndrome	1 in 200,000*	8	1,570	0.51%
Stickler Syndrome	1 in 7,500 to 9,000*	8	41,866	0.02%
Congenital Cranial Dysinnervation Diseases (CCDD)	1 in 230,000 (CFEOM)* 1 in 5,000 to 10,000 (Duane)**	7	1,365/62,800	0.01%
Infantile Neuroaxonal Dystrophy	<1 in 1,000,000**	4	314	1.27%
Occult Macular Dystrophy	unknown**	3	unknown	n.d.
Fundus Albipunctatus	unknown**	2	unknown	n.d.
Hermansky-Pudlak Syndrome	1 in 500,000 to 1,000,000 (1 in 1,800 in Puerto Rico)*	1	628	0.16%
Achromatopsia	1 in 30,000*	0	10,466	0.00%

Lowe Syndrome	1 in 500,000*	0	628	0.00%

Based on US population of 314,000,000 from census.gov on July 4, 2012.
n.d. – not determined
*Retrieved from Genetic Home Reference http://ghr.nlm.nih.gov/ 4-30-13
**Retrieved from Orphanet http://www.orpha.net/consor/cgi-bin/index.php 5-1-13

Table 2. Diagnoses eligible for inclusion and some of the genes tested through eyeGENE®

Achromatopsia	CNGA3, CNGB3
Albinism	**Recessive** TYR, OCA2, TYRP1, SLC45A2 **X-linked** GPR143 (OA1)
Aniridia and other developmental eye anomalies	PAX6, WT1#, DCDC1#, ELP4# (# del/dup testing only)
Axenfeld - Rieger Syndrome	PITX2, FOXC1
Best Disease	BEST1
Bietti's Crystalline Corneo-Retinal Dystrophy	CYP4V2
Choroideremia	CHM
Chronic Progressive External Ophthalmoplegia (CPEO)	POLG
Cone Rod Dystrophy	ABCA4, RPGR, CRX, GUCY2D *(codon R838)*
Congenital Cranial Dysinnervation Diseases (CCDD)	KIF21A, CHN1, SALL4, TUBB3, HOXA1, PHO2A, ROBO3, HOXB1
Familial/Congenital Nystagmus (X-linked cases only)	FRMD7
Congenital Stationary Night Blindness/Oguchi Disease	GPR179, RHO, NYX, TRPM1, SAG
Corneal Dystrophy	TGFBI, KRT3, KRT12
Doyne Honeycomb Dystrophy	EFEMP1
Familial Exudative Vitreal Retinopathy	FZD4, LRP5, NDP, TSPAN12
Fundus Albipunctatus/Bothnia Retinal Dystrophy	RDH5, RLBP1
Glaucoma (juvenile open angle and congenital only)	CYP1B1, OPTN, MYOC
Hermansky-Pudlak Syndrome	HPS1 and HPS3
Juvenile X-linked Retinoschisis	RS1
Kearns-Sayre Syndrome (KSS), Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS), Myoclonis Epilepsy associated with Ragged Red Fibers (MERRF), Neuropathy, Ataxia, and Retinitis Pigmentosa (NARP)	Mitochondrial gene panel
Leber Hereditary Optic Neuropathy (LHON)	LHON panel (MT-ND4, MT-ND1, MT-ND6/mutations 11778G>A, 3460G>A, 14484T>C, and 14459G>A)
Lowe Syndrome	OCRL
Microphthalmia and Anophthalmia	RAX, SOX2, OTX2, VSX2, STRA6 and SIX6del/dup analysis
Neurodegeneration with Brain Iron Accumulation (NBIA)	FA2H, MMIN, PANK2, PLA2G6
Occult Macular Dystrophy	RP1L1 (R45W)
Optic Atrophy, Dominant	OPA1, OPA3
Papillo-renal Syndrome	PAX2
Pattern Dystrophy	PRPH2
Retinitis Pigmentosa (RP) and Retinal Degenerations	**Dominant** (panel including RHO, PRPH2, RP1, IMPDH1, PRPF8, NR2E3, PRPF3, TOPORS, PRPF31, RP1, KLHL7, SNRPN200), CA4, CRB1, C1QTNF **X-linked** RPGR, RP2 **Recessive:** single genes available on as needed basis - please inquire with CC
Retinoblastoma^ (12/50 enrolled for 2013)	RB1
Sorsby Fundus Dystrophy	TIMP3
Stargardt Disease	ABCA4, ELOVL4, PRPH2
Stickler Syndrome^ (6/50 enrolled for 2013)	COL2A1
Usher Syndrome^ (Limit exceeded for 2013)	Usher panel (CDH23, CLRN1, DFNB31, GPR98, MYO7A, PCDH15, USH1C, USH1G, USH2A)

The eyeGENE® enrollment process

Eligible individuals can participate in eyeGENE® through a certified eye health care provider or genetics professional. To qualify for enrollment, the participant must exhibit symptoms of an inherited eye disease for which eyeGENE® has a diagnostic molecular test (Table 1), be able to submit a blood sample, participate in an eye exam, and agree to have a portion of their DNA used for research. Ideally, the patient, the provider, and a genetic counselor work together to discuss the expectations of the program and the implications of genetic testing, complete the required paperwork, enter phenotypic data (eye exam and family history) on the eyeGENE® Database, and submit a blood sample to the eyeGENE® Coordinating Center.

There are several inherited conditions for which eyeGENE® allows patients to be enrolled, but does not currently offer testing because of the lack of cost efficient testing methods. However, these DNA samples may be tested on a research basis through a Stage 2 research study. If a disease-causing mutation is found to be the likely cause of the individual's eye condition, eyeGENE® will confirm the research finding in a Network CLIA lab so the results may be reported back to the referring clinician. If research testing does not yield a disease-causing mutation, the sample will continue to be held in the eyeGENE® Biorepository and will continue to be available to researchers.

Referring providers must register online to become an eyeGENE® Registered Clinical Organization (RCO) before enrolling a patient in the program. Once approved by the eyeGENE® Coordinating Center, the referring provider agrees to arrange for genetic counseling of patients before and after results are generated. An RCO can be any size; i.e. a sole practitioner in a private practice, or a medium to large entity with multiple practitioners. Online instructions for registering in the eyeGENE® Network are at http://www.nei.nih.gov/resources/eyegene/professionals.asp.

Since most of the diseases included for testing in eyeGENE® are rare, some providers will not likely enroll more than a few individuals each year. Individuals referred by an RCO enrolling fewer

than 10 subjects per year may be enrolled as offsite participants of the NIH and are not required to have their own IRB approval. However, a referring provider should always check with their local IRB, if available, for their local requirements and regulations. For sites enrolling more than10 patients per calendar year, the eyeGENE® protocol requires local or commercial IRB approval.

Enrolling an individual into eyeGENE® requires the RCO to enter the patient's data into a disease specific assessment form on the secure online eyeGENE® database. This includes family history and all relevant clinical findings. Additionally, eyeGENE® often requests additional files to be uploaded (e.g. fundus photographs, OCT pictures, ERGs and other measurements of visual function). The clinical assessment form includes a series of questions related to the suspected clinical diagnosis. This information is crucial to the research efforts of eyeGENE®, and is reviewed and curated by a team of eyeGENE® ophthalmologists to ensure accuracy and significance for research. It is also used to determine which genetic test(s) should be performed and in what order. The eyeGENE® Coordinating Center may request additional patient information if it is not submitted at the time of enrollment.

Once a patient sample is accepted into the Network, the patient's DNA is extracted from the submitted blood sample in the eyeGENE® CLIA Laboratory. A majority of the de-identified portion of each patient's DNA is stored in the eyeGENE® Biorepository, while another smaller portion of the DNA may be sent to a Network CLIA-certified laboratory for molecular diagnostic testing. The Network CLIA diagnostic lab performs the necessary procedures and produces a genetic test result with interpretation, which they return to the eyeGENE® Coordinating Center in a report coded in the same way as the DNA that they received. eyeGENE® staff link the code back to the patient, reviews the report and the corresponding information entered into the database, and sends the results back to the referring provider. Finally, the referring provider shares the results with the participant.

Storage of patient DNA in the eyeGENE® Repository and genetic testing of patient sample are free of charge to the referring clinician and the patient, regardless of the number of genes tested, but

eyeGENE® does not cover any costs associated with phlebotomy or blood shipment, ophthalmic evaluation and any testing or photography performed, or fees associated with genetic counseling.

Turnaround time (TAT) for the receipt of molecular diagnostic results varies, but ranges between six months and two years from the receipt of blood for conditions for which eyeGENE® has a test available. Turnaround time is mostly dependent on the time to completion of study requirements, which include signed consent forms, receipt of correct blood volume, receipt of clinical data and changes in eyeGENE® procedures due to quality assurance testing or changes to federal contract regulations which govern diagnostic testing services. While a downstream benefit of eyeGENE® is that it increases the accessibility of clinical molecular diagnostic testing for individuals with inherited eye disease, irrespective of insurance coverage, this benefit is not the principal goal of the program, which remains eye disease research.

Limitations of eyeGENE®

eyeGENE® has evolved substantially since its inception in response to an ever-changing environment, but within set budget parameters. From a clinical perspective, the main limitation is that testing has a long turnaround time (TAT). But it is important to remember that facilitating vision research is the ultimate goal of the initiative. As such, the success of the program requires certain conditions be met that are absent from fee-for-service diagnostic testing entities. eyeGENE® requires more blood, paperwork, and clinical information than a traditional testing service. Also, all the clinical information received is curated. Extracted DNA and original blood samples are subjected to quality assurance testing, which includes gender and short tandem repeat (STR) marker analysis. As these extra measures have been added to the process, the TAT has been impacted. Another contributor to TAT is the receipt of sufficient clinical data. The Network continues to work with RCOs to stress the importance of receiving quality phenotypic information for each patient early in the process to avoid delays in testing. Many of the eyeGENE® processes have been streamlined over the last year, in efforts to positively impact the TAT. While eyeGENE® continues to improve TAT through streamlining

processes, resource levels may also impact TAT if program demands continue to grow. It is important to note that fee-based clinical diagnostic testing for ocular diseases has become much more available, and is a viable option for those wishing a faster TAT. An individual who has opted to pursue diagnostic testing through commercial testing may still enroll in eyeGENE® to participate in the database and bioreposbenefits that eyeGENE® offers. Information on genetic testing laboratories, clinical resources and a guide for locating genetics professionals can be found through the Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/).

Another limitation of the program is that it does not offer testing for all known genetic causes of ocular disease. This is in part a function of the historical circumstances involved in the genesis of the program. The ten awarded NEI administrative supplements to CLIA-laboratories and CLIA-associated research laboratories defined the original diseases and genes tested through the Network. There was some overlap of disease categories chosen to enable quality assurance testing among Network laboratories. The original disease categories considered for eyeGENE® included retinal diseases, corneal disease, glaucoma, cataract, strabismus and retinoblastoma. The eyeGENE® Network CLIA laboratories are no longer supported through administrative grants supplements. Instead, there was an open competition which afforded all interested CLIA diagnostic laboratories the opportunity to compete for contracts to handle the diagnostic testing aspects of the Network. This allows for greater flexibility in both testing and partnering. The disease categories tested through eyeGENE® represent those testing categories offered by the contracting CLIA laboratories. The eyeGENE® framework has been honed over seven years of application and currently focuses on a specific subset of inherited eye conditions. As eyeGENE® grew, so did the expertise of the laboratories and additional genetic tests were added to disease categories and sub-categories. At the same time, some laboratories were unable to maintain operations or complete CLIA accreditation requirements leading to changes in the availability of disease categories. Disease accrual categories have mostly consisted of existing labs

broadening their offerings. eyeGENE® continues to expand by adding new tests and CLIA diagnostic laboratories, following the guidance of the eyeGENE® Steering Committee and given budget constraints. For instance, eyeGENE® is looking to enhance offering of anterior segment genes for rare conditions. This will allow the existing framework to extend to additional participants, clinicians, and vision researchers and engage a wider audience. eyeGENE® is also supporting the development of next generation sequencing technologies to cover more ocular disease categories.

Figure Legend for Figure A.

Figure A. Schematic illustration of the centralized infrastructure components of eyeGENE®.