HEREDITARY DISEASES AS CAUSES OF BLINDNESS IN NEWFOUNDLAND: A COHORT STUDY WITH LONG TERM FOLLOW UP

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Hereditary diseases as causes of blindness in Newfoundland: A cohort study with long term follow up.

By

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I. ABSTRACT

Background: In 1981, a cohort of 1,013 prevalent cases of blindness, registered with the CNIB, was studied. Established monogenic disease was the cause of blindness in 24% of cases and presumed in a further 6%. Since that original study, considerable new clinical information and family history data have been accumulated. In addition, incident cases with different causes of inherited blindness have been identified.

Objectives: To determine: the proportion of cases of blindness attributable to monogenic disease after long term follow up; which blindness phenotypes were observed in each geographical region of the province; whether geographic clustering of specific phenotypes occurred; and whether mutation specific disease clustered in particular geographic regions.

Methodologies: In 2007/8 all cases in the 1981 cohort were reviewed in order to determine the number in whom the clinical diagnosis had changed; the number in whom the molecular genetic diagnosis was established; and the number of families available for potential novel gene discovery. In addition, the geographic distribution of families with various monogenic causes of blindness was mapped.

Results: Long term follow up revealed that established genetic disease was the cause of blindness in 30% of the cohort and a further 12% had presumed genetic disease. Geographic clustering was observed with some inherited disorders, and random

occurrence of others around the coast of Newfoundland. Within various geographic regions there were multiple genetic causes of blindness. For example, in Conception Bay communities, nine different hereditary eye diseases were identified.

Conclusions: Newfoundland is a young founder population, consisting of multiple genetic isolates, where the peopling of these isolates has predisposed to the frequent occurrence of hereditary blindness, associated with multiple different genotypes and phenotypes. The genetic architecture of the Newfoundland population has facilitated the identification of novel genes and has resulted in families with the potential to facilitate further identification of novel genes and mutations causing blindness in Newfoundland in the future.

II. Acknowledgments

I wish to thank the following supervisors for their willing and continued participation in the development and conclusion of this project.

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V. INTRODUCTION

i. Population of Newfoundland

The Canadian province of Newfoundland and Labrador lies on the most easterly point of North America. The island portion of the province, Newfoundland, has an estimated population of 508,099 (1). The total land area of 405,212 km^2 (373,872 km^2 of Land and 31,340 km^2 of water), has a low population density of 1.35 persons /km^2. 90% of Newfoundland's population has descended from about 30,000 founders who settled in Newfoundland at the end of the 18^{th} and the start of the 19^{th} century. These founders included mainly Protestant settlers from the south-west of England and Roman Catholic settlers from the south east of Ireland (2).

The population of Newfoundland has arisen by natural increase from settlers who arrived before 1835, drawn from these highly circumscribed areas of south-western England and southern Ireland. Hundreds of small communities have grown up around Newfoundland's many natural harbours. At present, about 50% of the population of the province reside in communities of fewer than 2,500 inhabitants, and 41% reside in communities of fewer than 1,000 inhabitants (3,4). Closely consanguineous unions tend to be avoided, but matings may occur between distant relatives. The frequency of some

recessive disorders at increased frequency may be attributed to a founder effect (the chance presence of an allele in relatively few original settlers that has become common in a genetically isolated population). In addition, the large family size down the generations, and the tendency of descendants to settle near the same community, has produced large numbers of people with specific autosomal dominant disorders.

The geographic distribution of the population of Newfoundland in 1981, from information gathered from Statistics Canada, is shown in Figure 1 (1). Few people live in the interior of the island. The four cities, identified with black dots on the Trans Canada highway, are shown with their own numerical population and not included within the surrounding coastal areas. The remainder of the population is generally distributed around the coast of Newfoundland.

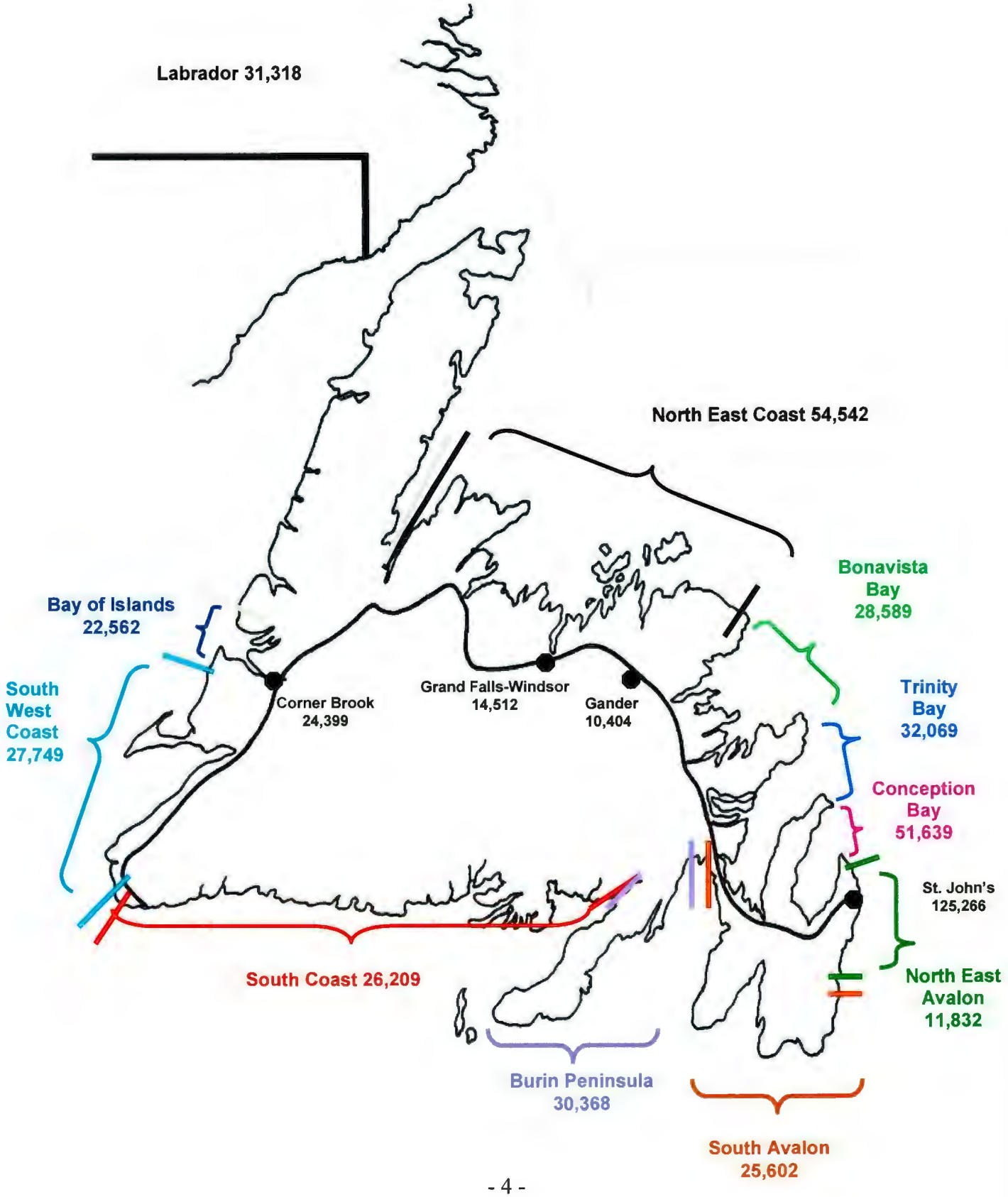
Geographic distance between settlements is a major determinant of genetic isolation (4). As about fifty percent of the population of Newfoundland resides in rural communities with 2,500 inhabitants or less, and as the average kinship between subpopulations generally decreases with the increased distance between them (4), it is not surprising that certain autosomal recessive conditions occur at increased frequency.

The geography, settlement, and socioeconomic development of Newfoundland have produced a population in which certain monogenic diseases occur frequently. As a result, important observations can be made on the clinical manifestations and outcomes of Mendelian inherited diseases, and examination of DNA for mutations in genes can provide mechanistic insights into the molecular determinants of the diseases (5). There were 27 entries in Online Mendelian Inheritance of Man (www3.ncbi.nlm.nih.gov/omim/) in 2003 whose genetic basis was elucidated using Newfoundland families (4). This

number of entries is 10-fold higher than that of the neighboring three provinces (Nova Scotia, New Brunswick and Prince Edward Island), when adjusted for population size (4).

This thesis will provide information on the clinical and genetic epidemiology of inherited eye diseases within Newfoundland through the review of pedigrees, current clinical information and previous molecular genetic studies undertaken in a cohort of patients identified nearly 30 years ago.

Figure 1: The Geographic Distribution of the Population of Newfoundland in 1981.



ii. Monogenic Disease

Monogenic causes of inherited blindness may result from autosomal dominant, autosomal recessive, X-linked, or mitochondrial inheritance. Establishing the inheritance pattern of a disease is initially through the analysis of a patient's family history, diagrammed in a pedigree (6,7,8).

Autosomal Dominant

An autosomal dominant disease results from a single abnormal gene inherited from either parent. The disease is typically observed in multiple generations (Figure 2) because affected individuals who receive a disease-causing mutation from an affected parent may pass this on to their offspring. Since these genes are located on autosomes, males and females are affected in roughly equal frequencies (6,7,8). Skipping of generations may be observed in a pedigree, when there is reduced or incomplete penetrance. A disease-causing mutation is said to have reduced or incomplete penetrance when some individuals who have the disease genotype (one copy of the mutation for an autosomal dominant disease or two copies for an autosomal recessive disease) do not display the disease phenotype (6,7,8).

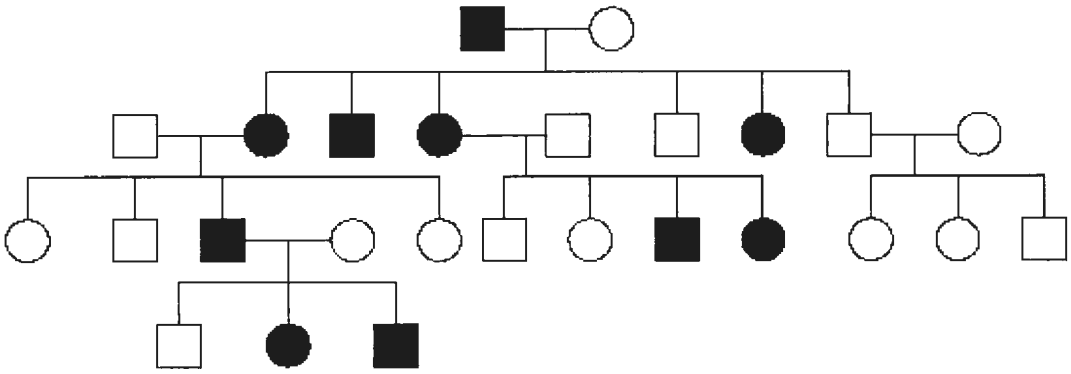


Figure 2. Autosomal Dominant Inheritance Pedigree was reproduced with consent from Dr. Tracey Weiler, Professor of Genetics, The Medical University of the Americas.

Autosomal Recessive

Autosomal recessive alleles are clinically expressed only in the homozygous state. Therefore, the offspring must inherit one copy of the disease-causing allele from each parent. Individuals with only one defective gene are considered carriers and they can pass the abnormal gene to their children. Most commonly, a homozygous or compound heterozygous affected individual results from the union of two heterozygous (carrier) parents. However, if an affected homozygote mates with a heterozygote, this could also produce an affected offspring (7,8).

In contrast to autosomal dominant diseases, autosomal recessive diseases are typically seen in only one generation of a pedigree (Figure 3). Consanguinity, the mating of related individuals (represented by the double line in the pedigree), is sometimes seen in recessive pedigrees because individuals who share common ancestors are more likely to carry the same recessive disease-causing alleles.

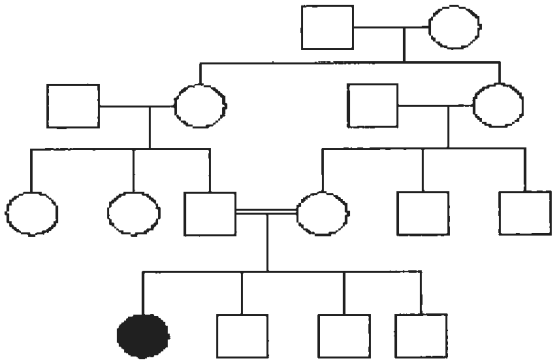


Figure 3. Autosomal Recessive Inheritance Pedigree was reproduced with consent from Dr. Tracey Weiler, Professor of Genetics, The Medical University of the Americas.

X-Linked

X-linked inherited diseases result from mutations on the X-chromosome. Since males have only one copy of the X chromosome, if a recessive disease-causing mutation occurs on the X chromosome, the male will be affected with the disease (6). Normal females have two copies of the X chromosome, so they usually require two copies of the mutation to express the disease (6,7,8). However, because X inactivation (described below), is a random process, a heterozygous female will occasionally express an X-linked recessive condition since, by random chance, most of the X chromosomes carrying the normal allele have been inactivated. Such a female is termed as a manifesting heterozygote, and since they usually have at least a small population of active X chromosomes carrying the normal allele, their disease expression is typically milder than that of a male (6,7,8).

Since males require only one copy of the mutation to express the disease, whereas females require two copies, X-linked recessive diseases are seen much more commonly in males than in females (Figure 4). Skipped generations are commonly seen because an affected male can transmit the disease-causing mutation to a heterozygous daughter, who is unaffected (carrier), but who can transmit the disease-causing allele to her sons. Male-to-male transmission is not seen in X-linked inheritance, which helps to distinguish it from autosomal inheritance (7,8).

X inactivation occurs very early in the development of female embryos. When an X chromosome is inactivated, its DNA is not transcribed into mRNA (7,8). Males inherit an X chromosome from their mother and a Y chromosome from their father, whereas females inherit an X chromosome from each parent. The X inactivation results in a more balanced functioning of the X linked genes. In a carrier female, if the chromosome with the normal gene is inactivated in relevant tissues, the female could express the disease (7).

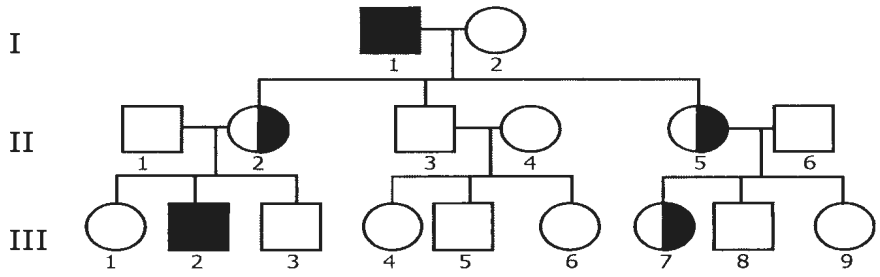


Figure 4. X-linked Recessive Inheritance Pedigree was reproduced with consent from Dr. Tracey Weiler, Professor of Genetics, The Medical University of the Americas.

There is also an inheritance pattern known as X-linked Dominant, however very few diseases meet this classification (8).

Pseudo-Dominant Inheritance

Pseudo-dominant inheritance mimics autosomal dominant inheritance, a potential pitfall when establishing autosomal dominant inheritance, therefore observation of additional family members must be completed to distinguish the inheritance (Figure 5). Pseudo-dominance can occur with mating between an affected individual of an autosomal recessive trait and an individual heterozygous for the autosomal recessive mutation. With this mating there is a 50% risk that any offspring will be affected as in AD inheritance, but this pattern will be in this generation only (7,8).

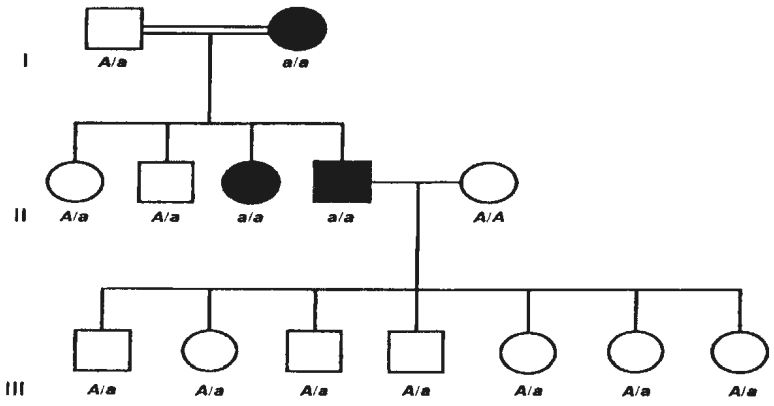


Figure 5. Pseudo-Dominant Inheritance Pedigree was reproduced with consent from Dr. Jane Green, Professor of Genetics, Memorial University of Newfoundland.

Mitochondrial

Mitochondrial inherited diseases result from mutations in the mitochondrial DNA (mtDNA) that affects mitochondria function. Mitochondria are normal structures or organelles, located within a cell's cytoplasm, outside of the nucleus, and are primarily responsible for energy production through oxidative phosphorylation (5). Each mitochondrion has a chromosome that is made of mtDNA but is otherwise quite different from the regular chromosomes within the nucleus of cells. The mitochondrial chromosome is much smaller, round in shape, and there are many copies in every cell, whereas, the regular chromosomes are much larger, rod shaped, and there is normally only one set of chromosomes in the nucleus (8).

Mitochondrial inheritance is sometimes referred to as maternal inheritance since mtDNA is strictly inherited from the mother, because sperm cells contribute no mitochondria to the egg cell during fertilization. Therefore, people affected with a mitochondrial disease may be male or female but they are always related in the maternal genetic line (Figure 6). None of the offspring of an affected male can be affected (7,8).

A typical cell contains hundreds of mitochondria in its cytoplasm. Sometimes a specific mutation is seen in only some of the mitochondria, a condition known as heteroplasmy. Variations in heteroplasmy can result in substantial variation in the severity of expression of mitochondrial diseases. Leber Hereditary Optic Neuropathy is the only mitochondrial inherited eye disease and heteroplasmy is relatively uncommon for this disease, so affected individuals tend to have similar levels of expression (8).

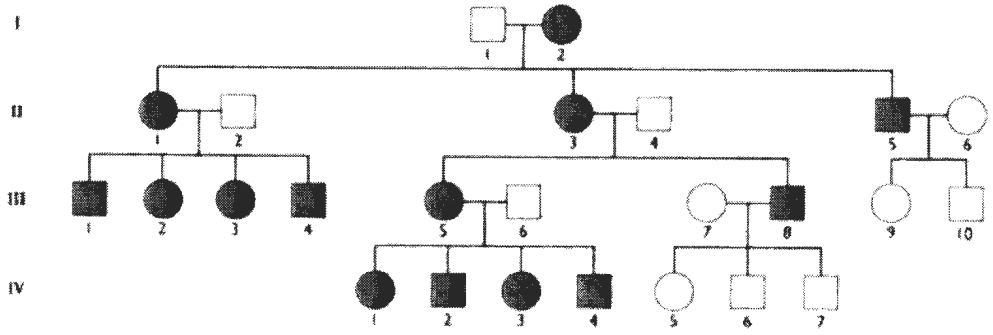


Figure 6. Mitochondrial Inheritance Pedigree was reproduced with consent from Dr. Tracey Weiler, Professor of Genetics, The Medical University of the Americas.

iii. Literature Search

A literature search was conducted through The National Library of Medicine using PubMed, Medline, and the Cochrane Collection databases. Specific search terms were used to help locate pertinent articles regarding heritable eye disease within Newfoundland and other communities, as well as the anatomy, genetics, and development of the eye including: heritable eye disease, genetic eye disease, epidemiology, ocular manifestations, genetics, and Newfoundland population. Also used were the specific eye diseases including: Retinitis Pigmentosa, Bardet-Biedl Syndrome, Cone Dystrophy, Cone-Rod Dystrophy, Newfoundland Rod-Cone Dystrophy, Achromatopsia, Leber Congenital Amaurosis, Leber Hereditary Optic Neuropathy, Optic Atrophy, Stargardt Disease, Usher Syndrome, Aniridia, Corneal Dystrophy, Cataracts, Ocular Albinism, Oculocutaneous Albinism, Microphthalmia, Retinoblastoma, Coloboma, Anophthalmia, Myopia, Glaucoma, and Peters Anomaly. Websites including: Statistics Canada (www.statcan.ca), University of Maryland Medical Centre website (www.umm.edu), and The Nuffield Council on Bioethics (www.nuffieldbioethics.org) were referenced for past and present population statistics and up to date medical definitions and guidelines. A manual search of recent articles and text books in the Health Sciences Centre Library was performed, and also a solicitation of an ocular geneticist for recent pertinent literature was done. The searches provided a tremendous amount of information regarding all facets of the eye. Several of the key articles' reference lists were checked and cross-referenced with those already located to establish if other pertinent papers could be located. Experts in the area of inherited eye disease and epidemiology were asked for input as well as abstracts from conferences for the last several years were reviewed for relevant articles.

iv. Development of the Eye

The visual system is the principal method for acquiring external sensory information in humans. The anatomy of the eye is outlined in Appendix A. The basic components of the optic system are derived from four embryonic sources: a) forebrain neuroectoderm, b) intercalating mesoderm, c) surface ectoderm, and d) neural crest. The neuroectoderm contributes cells for the formation of the retina, iris, and optic nerve; the surface ectoderm gives rise to lens and corneal epithelium; the mesoderm differentiates into the extraocular muscles and the fibrous and vascular coats of the eye; and neural crest cells become the corneal stroma, sclera and corneal endothelium (9,10).

The genes that are expressed in a cell collectively determine its primary features: its potential to grow and divide, its shape, its capacity for movement, and specific metabolic functions. Also, the expressed genes determine what signals the cell can send and receive to and from other cells (11). Genetic regulation is critical to ocular development. Two genes that are very important in ocular development are the *PAX6* gene and the *Rx* gene. Induction of *Rx* and *PAX6* genes during early embryogenesis initiates a cascade of gene activation and depression that leads to the formation of the mature eye. From the early stages of retinal development to the final mitotic divisions of the differentiating retina, *Rx* and *PAX6* are expressed in proliferating cells (12). *PAX6* has been considered a "master control gene" for ocular development. Its sequence has been conserved throughout evolution. The human protein is identical to that of mice and is almost identical to that of fish and chickens (13).

The human eye is a complex organ, with many structures working together to give us the sense of sight, allowing us to observe and learn about the surrounding world (Figure 7). It can be divided into two segments, which are anterior and posterior.

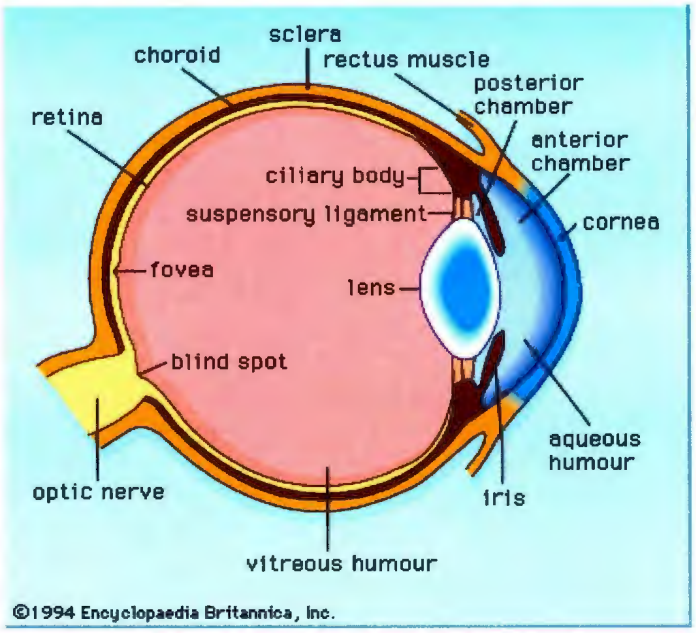


Figure7. Anatomy of the human eye, figure was reproduced from Encyclopedia Britannica.

The Globe

The eye starts to develop from neuroectoderm around the twenty-second day of fetal life when the optic primordium is first identified as the optic pits - paired structures on both sides of the midline in the ventrolateral region of the primitive forebrain (12). The optic pits extend outward to form the optic vesicles that are connected to the forebrain by the optic stalk. By the fifth week of development, the optic cup has formed from the invagination of the optic vesicle. The optic cup forms a fold known as the embryonic fissure, through which the mesenchymal and vascular tissues enter the globe. Closure of the embryonic fissure begins midway in the fissure and then extends anteriorly

and posteriorly, completing the process by the end of the seventh week of gestation (12). Incomplete closure of the fissure leads to formation of colobomas of the iris, retina, or choroid (12).

The fetal eye demonstrates rapid growth between the eighth and fourteenth weeks of life. The growth of the eye overall parallels the growth of the embryo until the thirtieth week, after which the eye growth begins to slow (12).

The Anterior Segment

The anterior segment is the front third of the eye that includes the structures in front of the vitreous humour: the cornea, aqueous humour, iris, ciliary body, lens, zonules, and pupil (Figure 8).

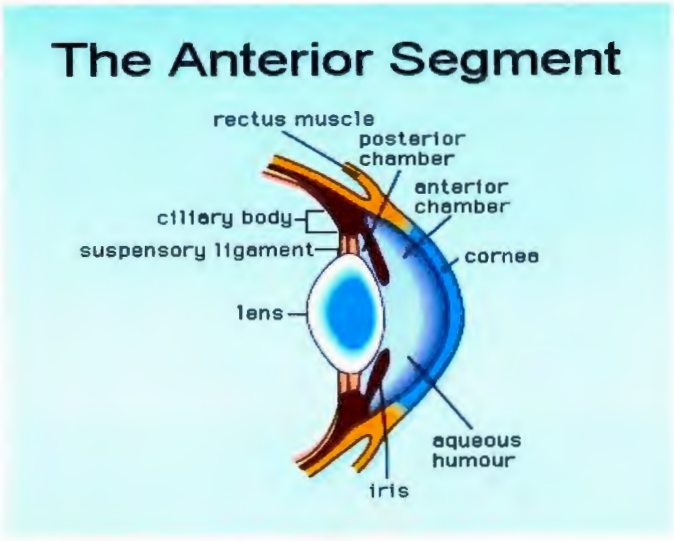


Figure 8. Anterior segment of the eye, figure was reproduced from Encyclopedia Britannica.

The Cornea

The cornea develops from three waves of neural crest-derived perilimbal mesenchymal cells that migrate between the lens and the acellular stroma at six weeks of gestation (12,14). Most of the corneal development occurs between the sixth week and fifth month of fetal life. The cornea is initially transparent and retains the uniform arrangement of parallel bundles and lamellae of collagen that allows for transparency (12). The collagen fibers are arranged with respect to one another and interact with proteoglycans in such a way as to form a mechanically strong extracellular matrix that does not scatter light, allowing transmittance of more than 99% of incident visible radiation (14).

The Iris

After six to eight months of gestation, the iris dilator and sphincter muscles develop from neuroectoderm. The iris vasculature derives from the mesoderm, and the iris stroma forms from the neural crest. At birth, the iris in many infants appears gray or blue, largely because of a lack of pigment in the iris stroma and the pigment epithelium. Iris color typically darkens during the first year of life (12).

The Lens

The cells that form the crystalline lens develop from the surface ectoderm covering the head of the embryo. After the optic vesicles and the prospective lens cells make contact they secrete an extracellular matrix that causes them to bind tightly with each other. The surface epithelial cells that adhere to the optic vesicle then elongate to

form the lens placode. Shortly after, the lens placode and adjacent cells of the optic vesicle buckle inwards resulting in the formation of the optic cup from the optic vesicle. After this lens invagination, the extracellular matrix between the optic vesicle and the lens begins to dissolve and the tissues separate. This space formed by the separation of the two tissues is the primary vitreous body, which is filled with a loose extracellular matrix (12,15).

The epithelial cells that give rise to the lens vesicle originally lie on a thin basal lamina which comes to surround the lens vesicle during the process of invagination and eventually forms the lens capsule. Soon after, the lens vesicle separates from the surface ectoderm and the cells begin to elongate into the primary fiber cells which establish the fundamental lens structure. Cells at the margin of the epithelium are stimulated to differentiate into secondary fibers cells which continue to form the final structure of the adult lens (12,15).

The Pupil

After six to eight months of gestation, the sphincter and dilator muscles that control the pupil are derived from the pigment cells of the neuroectoderm (16). The pupil can be very useful in a clinical setting due to the fact that it acts as an objective indicator of the amount of light transduction by the visual system. The amount of transient pupil contraction to a light stimulus or the steady state diameter of the pupil under constant illumination can reflect the health of the retina and the optic nerve and can be used to detect disease (17).

The Ciliary Muscle

The ciliary muscle develops from mesenchyme (embryonic connective tissue), situated at the edge of the optic cup. During the fourth month of gestation the muscle becomes organized into fibers and strands (18). Circular fibers appear on the inner anterior aspect of the meridional (middle) muscle by the seventh month and continue to increase in size but are still not completely formed at birth (18).

The Zonule of Zinn

Zonular fibers are initially seen at the end of the third fetal month. The primary cells responsible for the secretion of the zonules are the ciliary and lens epithelium (10). The zonules arise from their posterior insertion at the pars plana region of the ciliary body (19). The zonule fibers form sheets or ribbon-like structures because of the mucus associated with them (18).

The Posterior Segment

The posterior segment comprises the remaining internal structures which lie posterior to the anterior segment. This includes: the retina, macula, fovea, sclera, choroid, vitreous humour, and optic nerve (Figure 9).

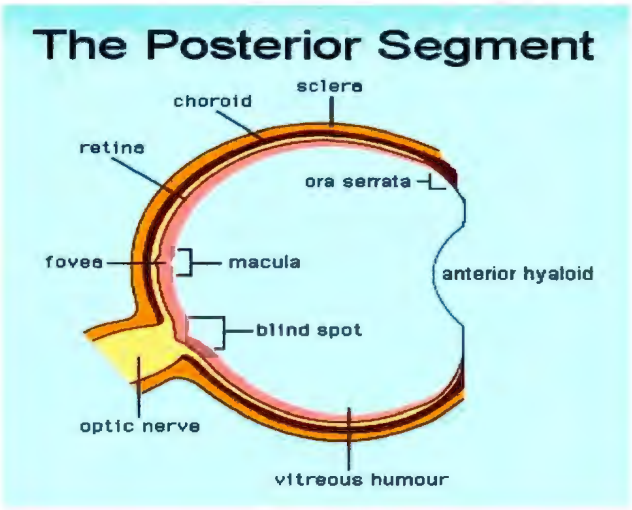


Figure 9. Posterior segment of the eye, figure was reproduced from Encyclopedia Britannica.

The Retina

The retina is one of the most interesting parts of the eye for clinicians and scientists to study due to the fact that as much as 80% of the sensory input in humans takes place through the retina, engaging about one third of the human brain for its processing. Also the retina is the simplest and best organized part of the central nervous system (CNS). It can reflect changes in the cardiovascular system, where it shows vascular changes in hypertension and diabetes. Changes in intracranial pressure can also be observed (18,20).

The retina develops from the neuroectoderm following the formation of the optic cup. The invaginating portion or inner wall of the cup gives rise to the neural retina whereas the outer wall produces the retinal pigment epithelium. The retina in its primitive state consists of 8 to 10 layers of cells with oval nuclei. The earliest cells to

differentiate in the retina are the retinal ganglion cells, and the last cells to differentiate are the rods and cones (21,22).

The initial stage of development is the differentiation of the columnar cells of the optic vesicle into a two-layered tissue known as the "neuroepithelium". This stage of development commences at the same time as the invagination of the optic vesicle in the embryo (18,23). From the sixth to the twelfth week the "neuroepithelium" gradually develops into the neuroblastic layers (23). The mature retina subsequently develops from these neuroblastic layers (23). The development follows with the differentiation of the neural elements from the inner and outer neuroblastic layers. Next is the creation of the inner nuclear layer from the inward migration of part of the outer neuroblastic layer to join the mullerian cells of the inner neuroblastic layer (23). The remaining portion of the outer neuroblastic layer will later develop into the photoreceptor cell bodies. The end of retinal maturation (except for the development of the fovea) is marked by the formation of the vascular system in the internal retina at eight months (23).

Only a few of the factors that control retinal proliferation are understood. Proliferation appears to be both stimulatory and inhibitory. Transforming growth factor-a, acidic and basic fibroblast growth factors, and epidermal growth factors stimulate the proliferation of retinal progenitor cells in cultures (20,23). The growth factors regulate the cell proliferation through intracellular signaling cascades that ultimately influence the cell-cycle control system (20). Also neurotransmitter systems may inhibit or at least regulate proliferation.

The Macula and Fovea

The development of the macula is rapid during the first three months of fetal life but then lags behind the rest of the retina until after the eighth month of gestation. The macula is not fully developed until 16 to 18 weeks after birth. The macula first develops as a localized increase in the superimposed nuclei in the ganglion cell layer, lateral to the optic disc (16). During the seventh month of gestation there is a peripheral displacement of the ganglion cells, leaving a central depression, which is known as the fovea (16).

The Vitreous Humour

The vitreous body makes up approximately 80% of the volume of the eye and is formed in three stages. The primary vitreous, a highly vascularized gel, develops in the fourth to sixth weeks of gestation (12). In the early stages as the optic cup grows, the space formed is filled by this vascularized gel which is a combination of primary fibrillar material secreted from the embryonic retina and secondary fibrillar material apparently secreted from the cell walls of the hyaloid artery after its penetration into the space (24). The primary vitreous atrophies and is replaced by the avascular secondary vitreous, which develops after the sixth week of gestation (24).

The secondary vitreous is associated with the vitreous space increasing in size and the regression of the hyaloid vascular system (12). This regression is not immediate but after some time all that is remaining is a tube of primary vitreous surrounded by secondary vitreous running from the retrolental space to the optic nerve (24).

The tertiary vitreous develops in the fourth month of gestation. This tertiary vitreous also known as the zonules is the fibrillary material that suspends the lens within the eye. During childhood the vitreous undergoes significant growth (24).

The Optic Nerve

The optic nerve forms from the optic vesicle which is originally connected to the forebrain by a short tube called the optic stalk (25). Primitive blood vessels and glial cells form a cone-shaped zone, known as the Bergmeister papilla, on the surface of the developing optic disk. These vessels and glial cells later atrophy, producing the physiologic optic cup of the disk (12).

At six weeks of gestation, axons from the retinal ganglion cells form the nerve fiber layer as they converge from all parts of the retina toward the optic disk and first enter the optic stalk. As the nerve fibers from different regions of the retina continue joining, the nerve fiber layer becomes thick near the optic disc (12). Around the ninth week of gestation these retinal ganglion cell axons first reach the dorsal nucleus of the lateral geniculate body, where they form small bundles and develop the central connections. By the eleventh week the optic chiasm is formed, which originates from these optic nerve fibers as they cross each other. Later in life, uncrossed fibers enter the optic chiasm, producing the adult configuration (12,25). Around the fifth month of gestation myelination of the optic nerve begins in the region of the geniculate bodies and reaches the globe by about the eighth month. Myelination of the optic fibers is usually complete by one month after birth (12,25).

The Sclera

The sclera first appears during the embryonic period, where it is formed by mesenchymal cells that condense around the optic cup (14). These mesenchymal cells differentiate into the tough, protective sclera after they receive inductive signals from the embryonic retinal pigment epithelium. The sclera forms first anteriorly at the limbus near the future insertion of the rectus muscles and then grows posteriorly (18).

The Choroid

The choroid develops from two embryonic tissues, the mesoderm and cranial neural crest cells (26). Inductive signals from the embryonic retinal pigment epithelium play a role in the formation of the choroid (18). Blood vessels first appear in the choroid during the 15th week of development, and arteries and veins can be recognized by the 22nd week.

Chronological Development

The eye is sophisticated organ, with a complex developmental pathway. Table 1 outlines a summary of the chronological development of the human eye, with developmental milestones and associated gestational ages.

Table 1. The following table identifies the chronological development of the human eye, with developmental milestones and associated gestational age. Table created by Deepak P. Edward, and Lawrence M. Kaufman (12).

Chronological development of the eye	
Gestational Age	**Developmental Milestone**
22 days	- Optic primordia appears
2nd month	- Hyaloid artery fills embryonic fissure - Closure of embryonic fissure begins - Lid folds appear - Neural crest cells (corneal endothelium) migrate centrally; corneal stroma follows - Choroidal vasculature starts to develop - Axons from ganglion cells migrate to optic nerve
3rd month	- Sclera condenses - Lid folds meet and fuse
4th month	- Retinal vessels grow into nerve fiber layer near optic disc - Schlemm's canal appears - Glands and cilia develop in lids
5th month	- Photoreceptors develop inner segments - Lids begin to separate
6th month	- Dilator muscle of iris forms
7th month	- Central fovea thins - Fibrous lamina cribrosa forms - Choroidal melanocytes produce pigment
8th month	- Iris sphincter develops - Chamber angle completes formation - Hyaloid vessels regress - Retinal vessels reach periphery - Myelination of optic nerve fibers is complete to lamina cribrosa - Pupillary membrane disappears

v. Hereditary Eye Disease

Hereditary eye disease can be defined as the transmission of gene defects or chromosomal aberrations/abnormalities which produce extreme variation in the structure or function of the eye. These may be evident at birth, or may become manifest later as the disorder progresses.

The pace of gene identification of the causes of inherited eye diseases has increased substantially over the past decades with the use of new technologies and the creation of the Human Genome Project databases. The contribution of ocular genetics to human gene discovery has been exceptional, beginning with a strong interest in ocular genetics by clinical ophthalmologists and ocular geneticists alike who have carefully described the patterns of inheritance of familial eye disorders (27,28). Families readily participate in research to understand the nature of their disorders with the hope that visions aids may be provided or that new treatments might be found to prevent such disorders. Patients with inherited eye disorders and their families have complex needs, which include clinical services for diagnosis and management and social and genetic counseling to help them cope with the conditions (27). A specific clinical and genetic diagnosis provides the patient and family with a framework for discussions on prognosis, treatment, and the heritability of a condition (28).

In Newfoundland and Labrador the following hereditary disorders affecting parts of the eye have been identified as a cause of genetic blindness:

Retina:

Retinitis Pigmentosa (RP), Cone Dystrophy, Cone-Rod Dystrophy, Newfoundland Rod-Cone Dystrophy (NFRCD), Achromatopsia, Leber Congenital Amaurosis (LCA), Juvenile Macular Dystrophy (Stargardt Disease), Bardet-Biedl Syndrome (BBS), Usher Syndrome.

Anterior Segment:

Aniridia, Corneal Dystrophy, Cataracts.

Optic Nerve:

Optic Atrophy, Leber Hereditary Optic Neuropathy (LHON).

Others:

Ocular Albinism, Oculocutaneous Albinism, Microphthalmia, Coloboma, Anophthalmia, Myopia, Retinoblastoma, Glaucoma.

A review of previous Newfoundland studies of inherited eye diseases is provided in Appendix B.

Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is a heterogeneous group of retinal degenerations in which abnormalities of the photoreceptors (rods and cones) or the retinal pigment epithelium (RPE) of the retina lead to progressive visual loss. Affected individuals first experience defective dark adaptation or night blindness, followed by constriction of the peripheral visual field and, eventually, loss of central vision late in the course of the disease (28,29).

The genetics of RP is complex, where the disorder can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. Autosomal dominant RP (adRP) accounts for 15%-20% of all RP cases and is characterized by significant allelic and non-allelic heterogeneity, 20%-25% are autosomal recessive (arRP), 10%-15% are X-linked (XLRP), and the remaining 40%-55% are simplex (28,30). Simplex cases are individuals with RP with no known family history so the mode of inheritance is not known. It may be arRP, adRP caused by a new mutation, and if male, may be XLRP. Many genes for RP have been identified or mapped to a particular chromosomal region including: 19 loci, with 18 identified genes for adRP, 26 loci, with 23 identified genes for arRP, and 6 loci, with 2 identified genes for XLRP (31).

New loci and genes are being identified rapidly for RP and other retinal disorders as more molecular research projects are completed. Figure 10 reveals the extent of the advances made in identifying new genes causing retinal disease over the past two decades.

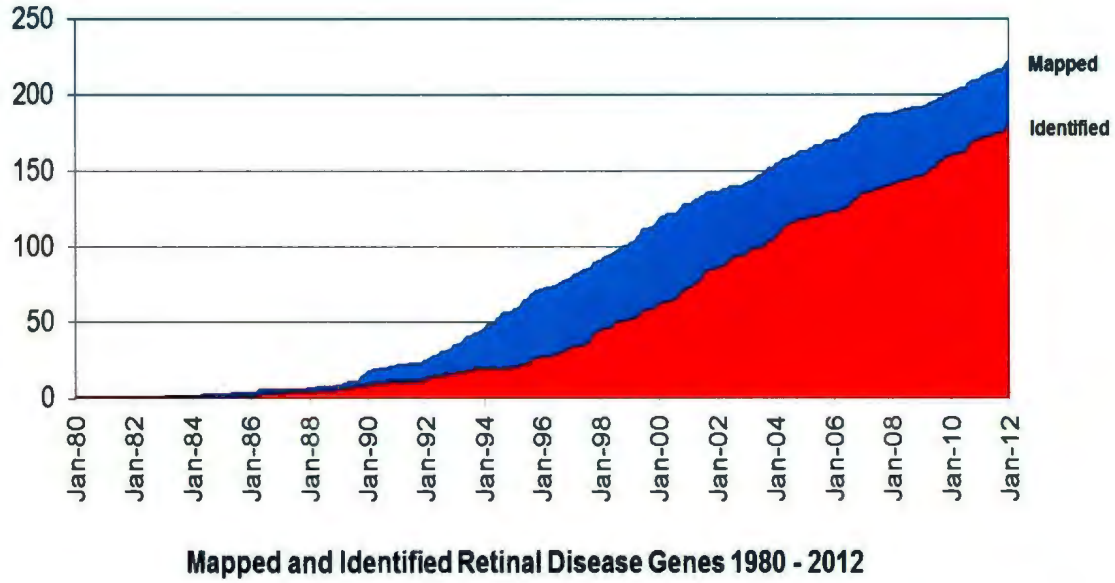


Figure 10. Mapped and Identified Retinal Disease Genes 1980-2012 was reproduced from RetNet: Summaries of Genes and Loci Causing Retinal Diseases (31).

Cone Dystrophy

Cone dystrophy is an ocular disorder characterized by the progressive dysfunction of cone cells, which are light-sensitive retinal photoreceptors responsible for both sharp central visual acuity and color vision/discrimination (32). The most common symptoms of cone dystrophy include reduced visual acuity, sensitivity to bright lights, and poor color vision (33). Individuals with cone dystrophy have normal vision initially and then develop a progressive abnormality of the cones. There is considerable clinical and genetic heterogeneity; cone dystrophies showing autosomal dominant, autosomal recessive, and X linked recessive inheritance have all been reported. X-linked cone dystrophies, COD1 and COD2 have been mapped to Xp11.4 and Xq27 respectively (34,35). COD3, involving the gene *GUCA1A,* was mapped to 6p21.1 (36).

Cone Rod Dystrophy

Cone rod dystrophies (CRDs) are retinal dystrophies that can be classified as pigmentary retinopathies. They are characterized by retinal pigment deposits visible on fundus examination, predominantly localized to the macular region. CRD is characterized by primary cone involvement or sometimes by loss of both cone and rod function that explains the predominant symptoms of the dystrophies which are: decreased central visual acuity, color vision defects, and photo sensitivity, later followed by progressive loss in peripheral vision and night blindness (37). CRDs are most frequently non syndromic, but they may also be part of other syndromes, such as Bardet-Biedl syndrome (37).

Non syndromic CRDs are genetically heterogeneous with ten cloned genes and three other loci being identified so far (37). Four major causative genes involved in the pathogenesis of CRDs are *ABCA4* (38), *CRX* (39), *GUCY2D* (40), and *RPGR* (41).

Newfoundland Rod-Cone Dystrophy

Newfoundland Rod-Cone Dystrophy (NFRCD) is a retinal dystrophy found in a particular region of Newfoundland. Initially reminiscent of retinitis punctata albescens, it is an autosomal recessive, progressive rod-cone dystrophy that is characterized by night blindness and white stippling throughout the fundus (42), but NFRCD has a substantially earlier age at onset, more-rapid progression, and a distinctive sequence of retinal changes resulting in an atrophic retina (43). Symptoms such as night blindness are evident during infancy, followed by the progressive loss of peripheral, central, and colour vision during childhood. Severe loss of vision is seen by the 2^{nd} to 4^{th} decade of life (43). Molecular

analysis showed linkage to markers on the long arm of chromosome 15, in a region encompassing *RLBP1,* a gene encoding the cellular retinaldehyde-binding protein, and confirmation as being the causative gene was established (43). *RLBP1* was initially identified as a candidate gene for the Newfoundland population because a retinal dystrophy with similar retinal appearance had been identified in an extended family in Bothnia (Northern Sweden) and homozygous mutations in *RLBP1* were identified (44,45,46).

Achromatopsia

Achromatopsia also known as rod monochromacy or total congenital color blindness is a rare autosomal recessive retinal disorder. This disorder is characterized by a total absence of the ability to discriminate colors, severely reduced visual acuity, sensitivity to light, and nystagmus observed soon after birth (33). The disorder is usually diagnosed during early infancy and does not progress after childhood (47). However, the majority of individuals with achromatopsia are registered blind.

Achromatopsia can be further divided into a complete and an incomplete form. Symptoms of complete achromatopsia are strongly reduced visual acuity, complete absence of the ability to color discriminate, extreme light sensitivity, and nystagmus. The incomplete form is characterized by a milder phenotype with residual color vision and less severely reduced visual acuity (33,47).

Three causative genes involved with achromatopsia have been identified including: *CNGA3* located at 2q11, which encodes the cone cyclic nucleotide-gated cation channel alpha subunit (48), *CNGB3* located at 8q21, which encodes the cone cyclic

nucleotide-gated cation channel beta subunit (49), and *GNAT2* located at 1p13, which encodes the cone photoreceptor-specific alpha-subunit of transducin, a G protein of the phototransduction cascade (50),

Leber Congenital Amaurosis

Leber congenital amaurosis (LCA) is a group of retinal dystrophies that are recognized at birth or very early in life. An infant presents with severely reduced vision, nystagmus, and poor pupillary reaction (32,51). Associated with LCA is a highly variable appearance of the retina, ranging from a normal appearance to a retinitis pigmentosa like picture (51). Many of the children develop a characteristic eye poking behavior. Hypermetropia and keratoconus frequently develop over the course of the disease as well (28).

The transmission of LCA is usually as an autosomal recessive trait, and rarely as an autosomal dominant trait. Ten loci and seven genes involved with LCA have been identified including: *GUCY2D* mapped to 17p13.1 (52), *CRB1* mapped to 1q31 (53), *RPE65* mapped to 1p31 (54), *RPGRIP1* mapped to 14q11 (55), *AIPL1* mapped to 17p13.1 (56), *TULP1* mapped to 6p21.3 (57), *CRX* mapped to 19q13.3 (58), and three other loci without identified genes, LCA3 on chromosome 14q24 (59), *LCA5* on chromosome 6q11-q16 (60), and *LCA9* on chromosome 1p36 (61).

Macular Dystrophy

Hereditary juvenile macular dystrophies comprise a heterogeneous group of degenerative diseases of the retinal macula characterized by central visual loss due to

atrophy of the macula and underlying retinal pigment epithelium (32,62,63). Dystrophies showing autosomal dominant, autosomal recessive, X linked recessive and mitochondrial inheritance have all been reported (32,62,63). Several genes involved with juvenile macular dystrophy have been identified including: *VMD2* mapped to 11q13 (64,65), *EFEMP1* mapped to 2p16 (66), *RDS* mapped to 6p21.2 (67,68), *MCDR1* mapped to 6q14-q16.2 (69), *TIMP3* mapped to 22q12.2-q13.2 (70) and *ABCA4* mapped to 1p22 (71,72). Stargardt disease is the most common juvenile hereditary macular degeneration.

Age-related macular degeneration (ARMD) is the most common cause of severe vision loss in people over 50 years of age in developed countries (73,74). Predisposition to ARMD includes age, environmental factors, and some genetic factors. ARMD is a degenerative disorder of the macula that results in loss of central vision (73,74).

Stargardt Disease

Stargardt disease affects the central retina (macula) and is one of the most common autosomal recessive retinal dystrophies with an estimated incidence of one in 10,000 (75). The disease is also the leading cause of juvenile macular degeneration and central vision loss. Later symptoms include loss of color vision, sensitivity to light, central scotoma and slow dark adaptation (28). Onset may occur as young as 7-12 years of age, or possibly in the teens or twenties, with the bilateral loss of central vision following over a several month to several year period (32). Depigmentation and atrophy of the macular retinal pigmentary epithelium is usually seen along with yellowish flecks surrounding the macula (76).

The gene for Stargardt disease was mapped to chromosome 1p22 in 1993 (71) and in 1997 mutations were identified in the *ABCA4* gene, encoding an ATP-binding cassette transporter (72). The *ABCA4* gene is expressed in rod and cone photoreceptors (77).

Bardet-Biedl Syndrome

Bardet-Biedl syndrome (BBS) is a rare, autosomal recessive disease characterized by retinal dystrophy, renal structural abnormalities, obesity, dysmorphic extremities, and hypogenitalism (78,79,80). BBS is genetically heterogeneous, caused by autosomal recessive inheritance, with at least 12 different genes identified: *BBS1* (11q13), *BBS2* (16q21), *BBS3* (3p12-q13), *BBS4* (15q23), *BBS5* (2q31), *BBS6* (20p12), *BBS7* (4q32), *BBS8* (14q31), *BBS9* (7p14.3), *BBS10* (12q21.2), *BBS11* (9q31-34.1), and *BBS12* (4q27) (81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93,94)

To fit the definition of having BBS a person must show at least four of the cardinal features listed above or three cardinal manifestations in a sibling of an affected person with four cardinal features (80). The current prevalence of BBS in Newfoundland is approximately 1 in 18,000 which is significantly higher than other populations around the world (79,80,84,95).

Usher Syndrome

The Usher syndromes are a genetically and clinically heterogeneous group of autosomal recessive conditions named after Charles Usher. These syndromes are characterized by congenital or early onset sensorineural hearing loss accompanied by a

retinal dystrophy indistinguishable from retinitis pigmentosa (96). The Usher syndromes are considered the most common of the syndromic retinal dystrophies (20).

The Usher syndromes are subdivided on the basis of the severity of auditory and vestibular dysfunction. Three patterns have been recognized:

1). Usher syndrome type I is characterized by congenital and severe to profound hearing impairment with vestibular dysfunction. The ability to speak rarely develops and visual symptoms are apparent by ten years of age (20,96). Six Usher syndrome type I loci have been mapped to chromosomes. USH1A maps to 14q32 (97), 1B maps to 11q13.5 (98), 1C maps to 11p15.1 (99), 1D maps to 10q21-q22 (100), 1E maps to 21q21 (101) and, 1F maps to 10q21-22 (102).

2). Usher syndrome type II is characterized by early moderate to severe hearing loss and normal vestibular function. Speech may develop and visual symptoms are apparent by the late teens (20,96). Three Usher syndrome type II loci have been mapped to chromosomes. USH 2A maps to 1q41 (99), 2B maps to 3p24.2-p23 (103), 2C maps to 5q14-q21 (104).

3). Usher syndrome type III is rare and characterized by adult onset progressive hearing loss associated with variable vestibular function (20,96). One Usher syndrome type III locus has been mapped to chromosome 3q21-q25 (105).

Aniridia

Aniridia is a rare congenital condition characterized by the underdevelopment of the iris, usually occurring in both eyes. It is associated with poor development of the retina at the back of the eye preventing normal vision development. The most

characteristic morphological feature of the iris in aniridia is a total loss of iris tissue, however partial and irregular loss of iris tissue may be seen (106).

The degree of visual impairment is quite variable from person to person (27). The iris may appear normal with only mild hypoplasia, or the iris may consist of a rudimentary stump of tissue of variable size. In some cases, this remnant can be seen on external examination, whereas in other cases it can be seen only by examining the eye.

In most cases, aniridia is inherited in an autosomal dominant fashion with almost complete penetrance but variable expressivity. One third of cases of aniridia are sporadic, most often because of a new mutation. Genetic analysis of most families with aniridia has identified a causative mutation in the *PAX6* gene, which is located on chromosome band 11p13. *PAX6* is a paired box transcription factor that is one of the master control genes involved in regulating eye development (107). This gene is still a candidate gene for the families in Newfoundland, with molecular studies being completed in the future which would identify specific mutations.

Corneal Dystrophy

Corneal dystrophies are a group of hereditary disorders, characterized by a non-inflammatory, bilateral opacity of the cornea (108,109). They commonly appear as grayish white lines, circles, or clouding of the cornea and may not significantly affect vision in the early stages. These disorders are commonly subdivided according to the layer of the cornea affected by the dystrophy. They are generally inherited in an autosomal dominant fashion with variable penetrance and expressivity (108,109). There has been several important genes discovered dealing with the corneal dystrophies.

Missense mutations seen in the *BIGH3* gene, located on chromosome 5q31, which is highly expressed in the corneal epithelium, causes mutations in the Transforming Growth Factor Beta Induced protein (TGFBI). These mutations of *BIGH3* were identified in families affected with several types of corneal dystrophies including: granular, Reis-Bucklers, lattice type I, and Avellino (110). Other genes, including, *KRT3* (chromosome 12q13) and *KRT12* (chromosome 17q12) involved with Keratin 3 and 12 proteins respectively, are specifically found in the corneal epithelium and responsible for Juvenile epithelial corneal dystrophy or Meesmann dystrophy (111,112).

Cataracts

A cataract is a clouding or any opacification that develops in the crystalline lens of the eye. Cataracts vary in degree from slight to complete opacity and may obstruct the passage of light (28,113). Cataracts can be defined by the age at onset: a congenital or infantile cataract presents within the first year of life; a juvenile cataract presents within the first decade of life; a presenile cataract presents before the age of about 45 years, and senile or age-related cataract after that (113).

Congenital cataracts are particularly serious because of the potential for inhibiting visual development, resulting in permanent blindness (28,113). Hereditary cataracts represent a major contribution to congenital and juvenile cataracts. Hereditary cataracts are most frequently inherited as autosomal dominant traits, but also can be inherited in an autosomal recessive, or X-linked fashion (113). Currently, about 39 genetic loci responsible for hereditary cataracts have been mapped and numerous genes identified. Genes involved with autosomal dominant cataracts include: *CRYBB1, CRYBB2, CRYGC,*

CRYGD, CRYAA, CRYAB, CRYBA1, MAF, BFSP2, HSF4, MIP, CX46, CX50, and *PITX3.* The genes involved with autosomal recessive cataracts are *LIM2 and CRYAA.* X-linked inherited cataracts have an association with the *CXN* gene (114,115,116,117).

Optic Atrophy

Optic atrophy is the result of atrophy of extensive amounts of optic nerve fibers (118,119, 120). Two broad types of optic atrophy exist: acquired and congenital.

The acquired (non-hereditary) type of optic atrophy may be a result of several separate causes such as: (a) blood supply changes in the eye or optic nerve, (b) inflammation or swelling within the optic nerve (optic neuritis), (c) a pressure against the optic nerve, such as from a tumour, or (d) metabolic diseases (diabetes mellitus), trauma, glaucoma, or toxicity (118,119,120).

If congenital, it is usually hereditary with deterioration starting in childhood. It may be accompanied by nystagmus. Autosomal recessive optic atrophy is a relatively rare entity, noticed occasionally in an infant and therefore termed congenital, but it is more frequently discovered around 3 to 4 years of age (118,120). Autosomal dominant optic atrophy or Kjer's optic neuropathy usually presents in early childhood (118,120). Responsible for Kjer's optic neuropathy is the *OPA1* gene which was mapped to chromosome 3q28-q29 from linkage analysis of three Danish families (121). Alternatively, non-hereditary congenital optic atrophy can be caused by a lack of oxygen during pregnancy, labour or in the early days of a child's life.

Leber Hereditary Optic Neuropathy

Leber hereditary optic neuropathy (LHON) is a mitochondrial inherited degeneration of retinal ganglion cells and their axons. Clinically, there is an acute onset of visual loss, first in one eye, and then a few weeks to months later in the other. This commonly evolves to very severe optic atrophy and permanent decrease of central visual acuity but in some individuals there is recovery of central vision. LHON is more common in males than females and often presents in the teens or twenties. LHON is due to a mutation of the mitochondrial genome and hence is passed exclusively through the mothers (28,122,123).

LHON is usually due to one of three pathogenic mitochondrial DNA (mtDNA) point mutations, occurring at nucleotide positions 11778 (G to A), 3460 (G to A) and 14484 (T to C), respectively in the *ND4*, *ND1* and *ND6* subunit genes of complex I of the oxidative phosphorylation chain in mitochondria (122,123).

Albinism

Albinism is the term used to describe a heterogeneous group of inherited disorders characterized by eye and skin hypopigmentation, and ocular abnormalities such as reduced visual acuity and nystagmus (28). Two broad types of albinism exist. Oculocutaneous albinism (OCA) involves two regions of the body: the skin and hair, and the optic system including the eye and the optic nerves. It is an autosomal recessive disease with several known genes. OCA Type I is caused by mutations in the tyrosinase gene (*TYR*) and maps to chromosome 11q14-21 (124). OCA Type II involves the human homologue, *P* gene, which maps to chromosome 15q11.2-q12 (125,126). OCA Type III

is caused by a tyrosinase-related protein (*TYRP-1*), encoded by the *B* gene, mapped to chromosome 9p23 (127). OCA Type IV is caused by a mutations in the human orthologue of underwhite that encodes a membrane associated transporter protein mapped to chromosome 5p (128).

Ocular albinism (OA) involves similar changes in the visual system with reduced pigment in the retinal pigment epithelium of the eye, however it does not affect the normal pigment of the skin or hair. OA is an X-linked disease with one gene identified, *OA1*, mapped to chromosome Xp22.3, which has been linked to defective glycosylation and thus improper intracellular transportation (129,130).

Microphthalmia

Microphthalmia is a disorder in which one or both of the eyes are abnormally small. It may be designated as simple, where it is observed without other ocular disease; or complex, where it is associated with cataracts, aniridia, retinal dysplasia, vitreous disease or other malformations (28). Microphthalmia can be further divided into colobomatous and non-colobomatous categories (28,131).

In simple microphthalmia one or both of the eyes are reduced to two-thirds of their normal volume without any other ocular abnormality except hypermetropia and occasionally aplasia of the macula. Complex microphthalmia also shows a reduction in the overall size of the eyes. It is possible that microphthalmia is secondary to associated malformations rather than merely accompanied by them (131).

Microphthalmia can be isolated, sporadic, or familial with several types of transmission such as autosomal dominant, autosomal recessive, and X-linked recessive

(131). Of monogenic causes, the genes *SOX2, PAX6, OTX2, RAX, CHX10, FOXE3,* and *CRYBA* are associated with mutations linked to microphthalmia, however only *SOX2* has to date been identified as a major causative gene for microphthalmia (132). *SOX2* has been mapped to a locus at 3q26.3 (132,133).

Coloboma

Colobomas are defects or simply "holes" in the structures of the eye, including the lens, iris, choroid or optic disc. The "hole" is present from birth and can be caused when a gap between two structures in the eye called the choroid fissure which is present early in development in the uterus, fails to close completely before a child is born. Ocular colobomas are frequently seen in association with other developmental defects such as microphthalmia. A coloboma can occur in one or both eyes (131, 134). The gene *CHD7* mapped to chromosome 8q12 has been reported to be associated with colobomas along with several other malformations (135).

Anophthalmia

In true anophthalmia, there is complete failure of primary optic vesicle outgrowth, or the optic vesicle has completely degenerated (131,136). Clinical anophthalmia is the apparent absence of the globe in an orbit that otherwise contains normal associated structures (131,136). As with microphthalmia, only the *SOX2* (mapped to 3q26.3) gene has been identified as a major causative gene for anophthalmia (132,133).

Myopia

Myopia or nearsightedness is a focusing defect in which the focal point is in front of the retina. People with myopia may see nearby objects clearly but distant objects appear blurred. With myopia, the eyeball is too long, or the cornea is too steep, so images are focused in the vitreous inside the eye rather than on the retina at the back of the eye. The common way to correct myopia is through the use of corrective lenses, such as glasses or contact lenses. It may also be corrected by refractive surgery. In high myopia, it is not possible to correct the vision defect with lenses or surgery. The majority of myopia is not caused by a single gene disorder. However there are cases of myopia, typically high myopia, which show simple Mendelian inheritance patterns of autosomal dominant, autosomal recessive, or X-linked (137). At least 17 myopia loci (*MYP 1-17*) have been mapped (28).

Retinoblastoma

Retinoblastoma (RB) is an embryonic neoplasm which originates in the primitive retinal cells. These cells undergo malignant transformation before their final differentiation. Although it is the most common intraocular malignant tumor seen in infancy and childhood, it is relatively rare with an estimated incidence between 1 in 15,000 to 1 in 20,000 live births (28,138). RB seldom arises after 3 or 4 years of age due to the disappearance of the primitive retinal cells within the first few years of life. The prevalence of RB among populations around the world is remarkably constant, where it is shown not to have a significant racial distribution and it affects both sexes equally. This

strongly implies that environmental factors play very little role in the etiology of the tumor (21,139).

In 1987, researchers found the RB1 gene, and identified its protein product, which linked the cancer to control of the cell cycle (140). *RB1* is a large tumor-suppressor gene, on chromosome 13q14, and involves 27 exons (8,135). The involved protein (known as Prb) normally binds transcription factors so that they cannot activate genes that carry out mitosis. It normally halts the cell cycle at the G1 phase. When the *RB1*gene is mutant or missing, the hold on the transcription factor is released, and cell division ensues, leading to the formation of cancers (8,28).

About half of the infants who develop RB inherit susceptibility to the disorder, where they have one germline mutant allele for the *RB1* gene, inherited in an autosomal dominant manner, in each of their cells (8,28). Cancer develops in any somatic cell where the second copy of the *RB* gene mutates or is lost. Therefore, inherited RB requires two point mutations or deletions, one germline and one somatic (8). In sporadic (non-inherited) cases, two somatic mutations occur in the *RB1* gene. The study of RB was the origin of Knudson's "two-hit" hypothesis of cancer causation, which states that two mutations (germline and somatic or two somatic) are required to cause a cancer related to tumor suppressor deletion or malfunction (141). Inheritance of the predisposition to RB is an autosomal dominant trait (138).

RB expands from its originating location to fill the eye and even extending as far as the optic nerve, orbit, sinuses, and brain (138). In developed countries the survival rate is close to 90% due to medical care being readily available, where the tumour can be detected early and removed by a surgical procedure.

Glaucoma

Glaucoma is characterized by increased intraocular pressure resulting in damage to the optic nerve and retinal nerve fibers (142). It is associated with a heterogeneous group of disorders. It is a common cause of preventable vision loss that may be treated by prescription drugs or surgery, with the majority of cases being age-related. Hereditary glaucoma can be divided into two types: primary open-angle glaucoma (POAG) and primary congenital glaucoma (PCG).

Hereditary primary open-angle glaucoma (POAG) is a severe disease that appears early in life and results in high intraocular pressure and optic nerve damage. It is often inherited in an autosomal dominant manner (28,142,143). Stone et al mapped a gene for primary congenital glaucoma, *MYOC*, to chromosome 1q21-23 (144).

Primary congenital (PCG) or infantile glaucoma occurs at birth or early childhood, usually within the first year of life, but may develop later up to three years of age (28,29). It is characterized by increased intraocular pressure, corneal edema, enlargement of the globe, and a sensitivity to light, and is mainly inherited as an autosomal recessive disorder (28,142,145). A gene for primary congenital glaucoma, *GLC3A*, was mapped to chromosome 2p21 by Sarfarazi et al in 1995 (146).

Molecular genetics is an ever expanding field of research, with many new loci and genes being identified rapidly for all inherited eye diseases in laboratories around the world. This research has the potential to revolutionize the diagnosis and treatment of inherited eye diseases.

VI. RESEARCH OBJECTIVES

In 1981 a cohort of 1,013 prevalent cases of blindness, registered with the CNIB, was studied (147,148). Established monogenic disease was the cause of blindness in 24% of cases and presumed in a further 6%. Since that original study, considerable new clinical information and family history data has been accumulated. In addition, incident cases with various causes of inherited blindness have been identified. The molecular genetic cause of several conditions including: Bardet-Biedl Syndrome, Newfoundland Rod-Cone Dystrophy, Peters Anomaly, and X-linked Ocular Albinism have been determined. The research objectives of this thesis are:

1. To determine the number of cases of eye disease attributable to monogenic disease following long term follow up and to compare this to the original results reported immediately following inception of the cohort in 1981.

2. To identify the blindness phenotypes observed in each geographical region of the province.

3. To determine whether geographic clustering of specific phenotypes occurred.

4. To determine whether mutation specific disease clustered in particular geographic regions.

VII. HYPOTHESES

1. That long term follow-up of the 1981 cohort would identify a higher proportion of eye disease being attributable to monogenic disease than originally reported.

2. That multiple different causes of blindness would be observed in each geographic region and that causes of monogenic blindness would differ from region to region.

3. That rare diseases and mutation specific diseases would cluster in specific geographic areas, consistent with the idea that Newfoundland comprises multiple genetic isolates.

VIII. SUBJECTS AND METHODS

The records of the Canadian National Institute of the Blind (CNIB) were reviewed by Dr. Jane Green in 1982/83. One thousand and thirteen patients in NL, registered as blind with The Canadian National Institute of the Blind (CNIB) on December 31, 1981, were studied. For registration in Canada a person must satisfy one of the following criteria; (a) visual acuity of 20/200 (6/60) or less in the better eye with best correction possible or (b) a total visual field of less than 10° from fixation with a white target 10mm in diameter at 1m (147). The records of each registrant were reviewed in detail in 1982/3 to determine the diagnosis and the cause of visual loss, as well as the ancestral location of the family.

Individual blind registrants were categorized by anatomical location of disease and by geographical origins. The coding used by the CNIB was adapted from the classification of the National Society for the Prevention of Blindness. There are separate codes for diagnosis (site and type of lesion) and for etiology (147). Two of the codes for etiology denote probable or possible hereditary eye disease. Where there was some ambiguity in the diagnosis or etiology, every attempt was made to arrive at the precise diagnosis, by Dr. Jane Green, through examining the person, by writing to other ophthalmologists within or outside the province or by researching the ancestral pedigree.

Many registered individuals as well as other family members were seen at regular ocular genetics clinics in St. John's or at outreach clinics at various locations around the province since 1980. Follow up appointments for further investigations such as visual field testing, colour vision testing, dark adaptation testing and electrophysiological

(electroretinogram and electrooculogram) procedures were completed. The electroretinogram (ERG) procedure tests a person's sensitivity to differing colours and intensities of light in scotopic (dark background) or photopic (light background) conditions including scotopic blue, red, and white, photopic white, and photopic flicker. The ERG is used to measure the electrical responses of the light-sensitive photoreceptor cells, the rods and cones. A dim blue light tests rod function whereas bright white light or flickering light tests cone function. The electrooculogram (EOG) procedure measures the resting potential of the retina. The EOG is used to assess the function of the pigment epithelium as well as recording eye movements. Unlike the ERG, the EOG does not represent the response to individual visual stimuli.

In 2007/8 the 1,013 cases in the 1981 cohort were reviewed again, by Dr. Jane Green and I, including clinic visit notes, and reports of visual field, color vision, dark adaption, electroretinogram, and electro-oculogram testing over the intervening years. Family histories for many individuals were updated from 1982 to 2006, and available molecular genetic results obtained. Diagnoses were revised as necessary based on new information, and ongoing studies. The cause of blindness was determined based on this updated information, and I stratified them according to anatomic location and specific diagnosis. Diagnoses made in 1982/3 (148) were compared to those now made in 2007/8.

During the period from 1982/3 to 2007/8 extended studies to identify the molecular genetic cause of certain diseases have been undertaken, in which new incident cases to the Ocular Genetics clinic, provincial ophthalmologists and Provincial Medical Genetics Program were included with the prevalent cases in the 1981 cohort. Molecular

genetic studies were performed in Bardet-Biedl Syndrome (26 families) (79), Newfoundland Rod Cone Dystrophy (six families) (43), and X- linked ocular albinism (two families) (129, 149).

Family histories were updated for all those who had a molecular genetic cause of their disease identified, and classified according to the molecular genetic diagnosis. Families with potential for novel gene discovery were identified.

A timeline representation of the Newfoundland Cohort from creation to its current day use for novel gene discovery is as follows:

1981 Cohort

↓

1983 diagnosis

↓

2007 diagnosis

↓ ↓

Monogenic Other

↓ ↓

Molecular diagnosis Potential for novel gene discovery

The database with information pertaining to all 1,013 individuals was revised using the computer program SPSS, over the first year of my project, with help from Dr. Jane Green. The information included is: name, date of birth, date of registration, age at registration, sex, CNIB #, geographical location of ancestors, maiden name (where

applicable), site type code, etiological code, hereditary classification (genetic/non-genetic), original CNIB diagnosis from 1980, revised diagnosis from 1982/3, revised diagnosis from 2007/8, extra notes pertaining to each diagnosis, and information on other identified family members. The cohort consisted of 551 male and 462 female individuals, with an average age of 52 years when the cohort was created in 1981.

The geographic location of the ancestral home of each proband and geographic distribution of each disease was mapped. Much time was spent, by Dr. Green, over several years trying to contact the families involved and reviewing family records to trace the ancestors back to their originating communities and I constructed geographic maps showing these locations. After completion, the distribution of disease in each geographic coastal area was observed to identify if any clustering of diseases had occurred or if a random occurrence was displayed.

IX. RESULTS

Monogenic causes of blindness in The 1981 Cohort

In Newfoundland in 1981 the prevalence of blindness was 1.8 per 1,000 people (1,013 registered blind in a population of 567,681). The causes of blindness among the people within the Newfoundland cohort as diagnosed initially in 1981 and following review in 2007/8 are summarized in Table 2. Of the 1,013 cases reviewed in 2007/8, 305 (30%) were found to have an established genetic monogenic disease and a further 123 (12%) have a presumed monogenic disease. This is an increase from the initial diagnosis where it was reported that 243 (24%) of cases had established and 56 (6%) had presumed monogenic genetic disease. Most changes in diagnosis occurred in people classified as congenital cataract and multiple anomalies, myopia, vascular/systemic disease, or other.

The specific causes of the established genetic diseases (N = 305) are presented in Table 3 according to anatomical location of the diseases. Fifty percent (N = 152) had Retinal disease, 17% (N = 51) Albinism, 11% (N = 35) Anterior segment disorders, 4% (N = 11) Congenital abnormalities of the whole eye, 3% (N = 10) Optic nerve disease, and 15% (N = 46) other genetic disorders.

Among the 152 patients with retinal disease 25% (N = 38) had Retinitis Pigmentosa, 20% (N = 31) had Bardet-Biedl syndrome, 13% (N = 20) had Stargardt disease, and 9% (N = 14) had Achromatopsia. Among the 51 patients with Albinism 53% (N = 27) had X-linked Ocular Albinism and 47% (N = 24) had Oculocutaneous Albinism. The groups with Anterior segment disease, Optic nerve disease, Congenital abnormalities, and other genetic disorders comprised multiple different disorders. The causes of

Table 2. Causes of blindness among the persons in Newfoundland, determined after 27 years follow-up, compared to initial diagnosis made in 1981.

Cause of Blindness	Classification (year)			
	1981		2008	
	Number of Persons	%	Number of Persons	%
Genetic Established	243	24.0	305	30.1
Genetic Presumed	56	5.5	123	12.1
Congenital: cataract and multiple anomalies	110	10.9	78	7.7
High myopia	100	9.9	61	6.0
Infectious disease	88	8.7	90	8.9
Trauma	67	6.6	68	6.7
Age related macular degeneration (ARMD)	60	5.9	63	6.2
Diabetic retinopathy	59	5.8	61	6.0
Glaucoma	48	4.7	46	4.5
Vascular or other systemic disease	41	4.0	17	1.7
Age related cataract	34	3.4	39	3.9
Tumour	22	2.2	18	1.8
Other	61	6.0	31	3.1
Undetermined	24	2.4	13	1.3
Total	**1,013**	**100**	**1,013**	**100**

The data from 1981 was reproduced from the following reference:

Green JS, Johnson GJ. Hereditary diseases as causes of blindness in Newfoundland: preliminary report. *Canadian journal of ophthalmology.* 18(6): 281-4, 1983.

Table 3. The causes of blindness in 2008 by anatomical location and specific hereditary disease among the persons in whom a genetic cause was established following long term follow-up.

Genetic Cause	Number Affected (Individuals)	Number Affected (Families)	Percentage of Total Affected (Individuals)
Established (total)	**n = 305**	**n = 192**	**100**
Retina	**n = 152**	**n = 103**	**49.9**
Retinitis Pigmentosa (RP)	38	30	12.4
Bardet-Biedl Syndrome (BBS)	31	17	10.2
Stargardt Disease	20	12	6.6
Achromatopsia	14	7	4.6
Newfoundland Rod-Cone Dystrophy (NFRCD)	8	6	2.6
Rod-Cone Dystrophy (RCD)	6	5	2
Usher Syndrome	7	5	2.3
Cone, Cone-Rod Dystrophy	2	2	0.7
Leber Congenital Amaurosis (LCA)	1	1	0.3
Retinal Dystrophy (Unspecified)	14	11	4.6
Macular Dystrophy (Unspecified)	11	7	3.6
Anterior Segment	**n = 35**	**n = 22**	**11.5**
Cataract (Hereditary, Congenital, and Juvenile)	17	10	5.6
Aniridia	7	4	2.3
Corneal Dystrophy	6	5	2
Anterior Segment (Unspecified)	5	3	1.6
Albinism	**n = 51**	**n = 17**	**16.7**
Ocular Albinism	27	2	8.8
Oculocutaneous Albinism	24	15	7.9
Optic Nerve	**n = 10**	**n = 7**	**3.3**
Optic Atrophy	8	5	2.6
Leber Hereditary Optic Neuropathy (LHON)	2	2	0.7
Congenital Abnormalities	**n = 11**	**n = 7**	**3.6**
Microphthalmia / Dwarfism	4	2	1.3
Microphthalmia	2	2	0.7
Coloboma	4	3	1.3
Anophthalmos	1	1	0.3
Other	**n = 46**	**n = 35**	**15**
Myopia	16	8	5.2
Retinoblastoma	5	5	1.6
Nystagmus	5	5	1.6
Glaucoma (Congenital)	2	2	0.7
Other Genetic	18	15	5.9

presumed genetic diseases are presented in Table 4, with the most frequent being Microphthalmia (N = 25), Myopia (N = 24), and Coloboma (N = 23). A total of 284 cases were re-diagnosed between the years 1981 and 2008. There was a substantial increase in the diagnosis of established genetic disease (N = 62), and of presumed genetic disease (N = 67).

Geographical Distribution of monogenic disorders

Figure 1 (page 4) shows the population distribution around the coastline of Newfoundland. Table 5 summarizes the geographic location of the ancestral home of families with the various inherited eye disorders identified. This includes families from the prevalent 1981 cohort and incident cases identified since that time.

Conception Bay is an area of early settlement starting in 1610. It has a relatively high population density (N = 51,639) but, despite its proximity to the urban population in and around St. John's (N = 125,266) (Figure 1), individual small communities were relatively isolated at the time of initial assessment in 1981. Within this geographic area multiple different phenotypes have been observed. The most frequent inherited causes of blindness identified include (i) Retinitis Pigmentosa, (ii) Bardet-Biedl Syndrome, (iii) Stargardt Disease, and (iv) Newfoundland Rod Cone Dystrophy. Individual cases of Usher Syndrome, Achromatopsia, Oculocutaneous Albinism, and Congenital Cataracts also occur. In addition to this diversity of disease, a branch of a family with X-linked ocular albinism had moved from the North East Coast to Conception Bay.

This diversity of inherited eye disease is observed in each geographic area, for example in Notre Dame Bay on the North East Coast (N = 54,542), 12 different

Table 4. The causes of blindness in 2008 by anatomical location and specific hereditary disease among the persons in whom a genetic cause was presumed following long term follow-up.

Genetic Cause	Number Affected	Percentage Affected
Possible (total)	**n = 123**	**100**
Retina	**n = 9**	**7.3**
Retinal Dystrophy (Unspecified)	8	6.5
Macular Dystrophy (Unspecified)	1	0.8
Anterior Segment	**n = 8**	**6.5**
Cataract	5	4.1
Anterior Segment (Unspecified)	2	1.6
Corneal Dystrophy	1	0.8
Optic Nerve	**n = 7**	**5.7**
Optic Atrophy	7	5.7
Congenital Abnormalities	**n = 53**	**43.1**
Microphthalmia	25	20.3
Coloboma	23	18.7
Anophthalmos	5	4.1
Other	**n = 46**	**37.4**
Myopia	24	19.5
Nystagmus	7	5.7
Glaucoma (Congenital and Juvenile)	5	4.1
High Hyperopia	1	0.8
Other Retina	7	5.7
Other Congenital	2	1.6

Table 5: Distribution of families* with monogenic eye diseases by geographic region of ancestral home.

	St. John's	Conception Bay	South Avalon	Burin Peninsula	South Coast	South West Coast	Bay of Islands	Northern Peninsula	North East Coast	Bonavista Bay	Trinity Bay	Total
Population (1981)	125266	51639	25602	30368	26209	27749	22562	25758	54542	28589	32069	
Retinitis Pigmentosa	2	16	9	12	2	1	2	4	6	9	4	67
Bardet Biedl Syndrome	4	6	1	1	2	3	1	1	2	1	4	26
Stargardt	2	13	1	1	-	-	1	-	2	1	1	22
Usher	1	1	4	1	-	2	-	-	3	-	3	15
Achromatopsia	-	3	-	1	-	1	-	-	1	-	-	6
Nfld Rod Cone Dystrophy	-	6	-	-	-	-	-	-	-	-	-	6
Cone Dystrophy	-	-	1	-	-	-	-	-	-	-	-	1
Ocular Albinism	-	-	-	-	1	-	-	-	1	-	-	2
Oculocutaneous Albinism	-	1	1	1	-	1	1	2	2	-	3	12
Leber's -LHON	-	-	-	-	2	-	-	-	-	-	-	2
Peters Anomaly	-	-	1	-	-	-	-	-	-	-	-	1
Congenital Cataracts	-	1	-	-	1	-	-	-	1	-	-	3
Corneal Dystrophy	-	-	-	1	-	-	-	-	4	-	-	5
Aniridia	1	-	-	1	-	-	-	-	1	1	-	4
Anterior Segment disorder	-	-	-	-	-	2	-	-	1	-	-	3
Microphthalmia Dwarfism	-	-	-	1	2	-	-	-	-	-	-	3
High Myopia retinal detachment	-	-	-	-	-	-	1	1	1	1	-	4
Retinoblastoma	2		1		1				1			5
TOTAL	12	47	19	20	11	10	6	8	26	13	15	187
TOTAL / 10,000	0.96	9.10	7.42	6.59	4.20	3.60	2.66	3.11	4.77	4.55	4.58	-

*Prevalent families identified in 1981 through review of CNIB records, and incident families identified thereafter.

Note. Five families located in Labrador were not included in the table due to information on population not being available.

monogenic diseases causing blindness were identified (Table 5). Furthermore the distribution of these conditions differs by area with some diseases occurring only in one area e.g. Leber hereditary optic neuropathy and Microphthalmia Dwarfism, both rare conditions, were observed only on the South Coast.

Mutations Detected in five disorders, with geographic distribution

During the period from 1982/3 to 2007/8 extended studies have been undertaken to identify the molecular genetic cause of certain ocular diseases including: Bardet-Biedl Syndrome, Newfoundland Rod-Cone Dystrophy, X-linked Ocular Albinism, Stargardt Disease, and Peters Anomaly. Each study included patients identified in the original cohort and also incident patients identified since 1981. Table 6 presents the identified mutations and genes pertaining to these ocular disorders within the province.

Bardet-Biedl Syndrome

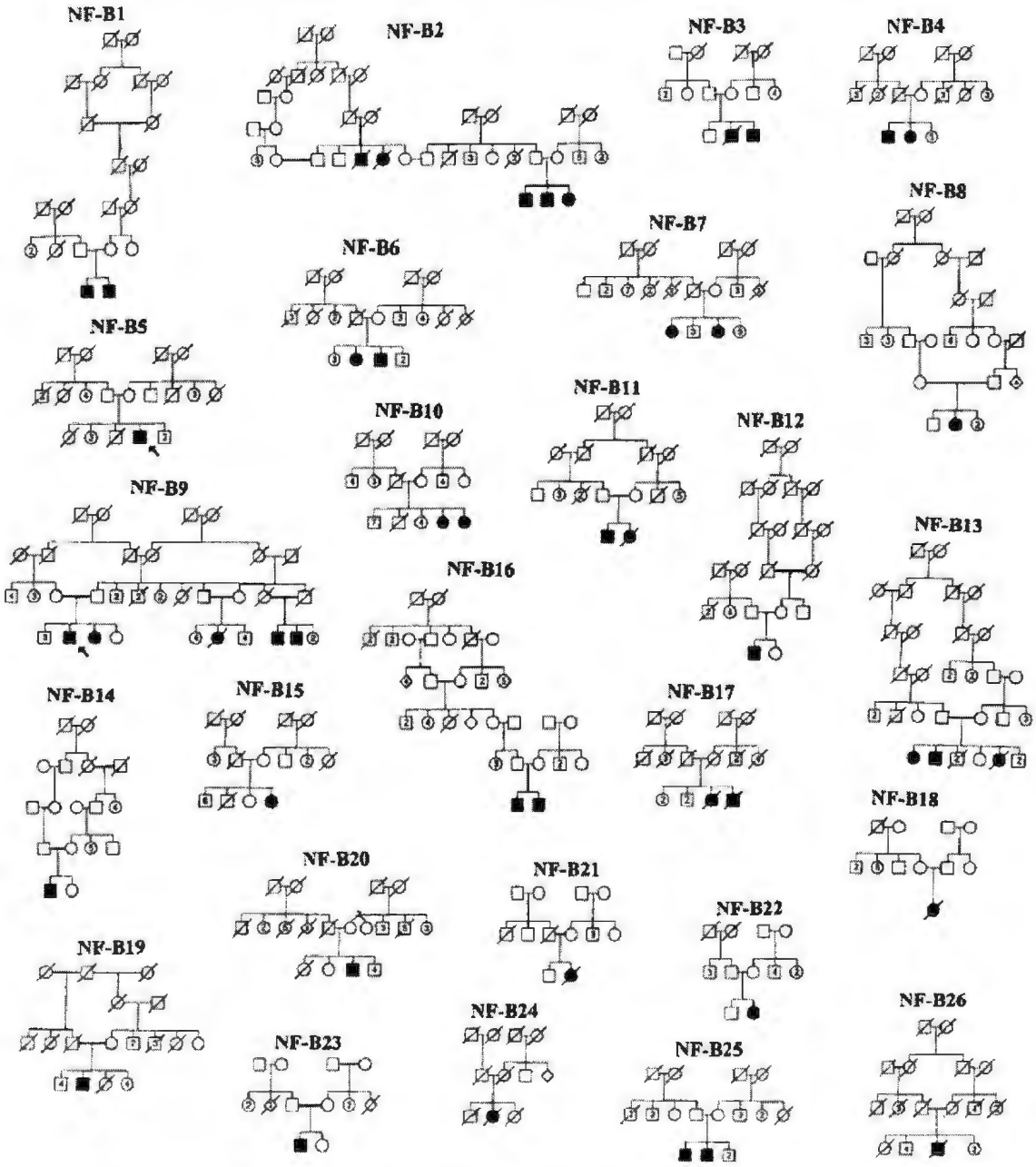
Thirty one patients (10%) of the 1981 cohort, from 17 different families, had Bardet-Biedl Syndrome (BBS). Eight of these families contained multiple individuals. By 2007 BBS was identified in 46 cases, from 26 families (Figure 11). DNA was obtained from members of 21 families, and the molecular genetic cause of the disease was identified in all of these families (Table 7). Nine mutations in six BBS genes were identified.

Some clustering of these mutations was seen. Four *BBS1* families homozygous for the M390R mutation clustered along the Southwest Coast. All families with the

Table 6. Identified genes and mutations in Bardet-Biedl Syndrome, Newfoundland Rod-Cone Dystrophy, Stargardt Disease, Peters Anomaly, and X-linked Ocular Albinism.

Genetic Ocular Condition	Cohort Study	Extended Study	Gene(s)	Map Location	References
	N	N			
Retina					
Bardet-Biedl Syndrome (BBS)	**31**	**46**			
M390R homozygous		8	*BBS 1*	11q13	79, 84
Y24X homozygous		1	*BBS 2*	16q21	79, 84
G169A homozygous		5	*BBS 3*	3p13-p12	79, 84
IVS6+3A>G homozygous		5	*BBS 5*	2q31	79, 84
D143fsX157 homozygous; F94fsX 103 homozygous; D143fsX157 / F94fsX103; F94fsX103 / L277P		15	*BBS 6*	20p12	79, 84
C91fsX95 homozygous; "F198 del, F199 del" / C91fsX95		6	*BBS 10*	12q21.2	79, 84
Unknown (No DNA)		6			
Newfoundland Rod-Cone Dystrophy (NFRCD)	**8**	**26**	*RLBP1*	15q26	43
(324G>A) homozygous					
(IVS3+2 T>C) homozygous					
(324G>A) / (IVS3+2 T>C) compound heterozygote					
Stargardt Disease	**20**	**37**	*ABCA4*	1p22	
Molecular studies in process					
Anterior Segment					
Peters Anomaly	0	11	*FOXE3*		150
c.959G>T heterozygous (AD)					
Albinism					
Ocular Albinism	**27**	**30**			
X-linked (IVS7-2 A>T)		30	*OA 1*	Xp22.3	129, 149

Figure 11. Pedigrees of Newfoundland Bardet–Biedl syndrome patients.



This figure was reproduced from the following reference:

Moore SJ, Green JS, Fan Y, Bhogal AK, Dicks E, Fernandez BA, Stefanelli M, Murphy C, Cramer BC, Dean JC, Beales PL, Katsanis N, Bassett AS, Davidson WS, Parfrey PS. Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. *American Journal of Medical Genetics.* 132(4): 352-60, 2005.

Table 7: Genotypes in Bardet-Biedl Syndrome Families in Newfoundland.

Mutation (Combinations)	BBS Families	Cases
BBS1: M390R Homozygous	B7, B8, B10, B15, B19, B23	8
BBS2: Y24X Homozygous	B14	1
BBS3: G169A Homozygous	B2	5
BBS5: IVS6+3A→G Homozygous	B9	5
BBS6: D143fsX157 Homozygous	B13, B20	4
BBS6: F94fsX103 Homozygous	B3, B4, B16, B25	8
BBS6: D143fsX157 / F94fsX103	B1	2
BBS6: F94fsX103 / L227P	B5	1
BBS10: C91fsX95 Homozygous	B12, B21	2
BBS10: F198 del, F199 del / C91fsX95	B6, B11	4
Unknown / No DNA	B17, B18, B22, B24, B26	6
Total	**26**	**46**

The data was reproduced from the following reference:

Webb M, Dicks EL, Green J, Moore S, Warden G, Gamberg J, Davidson WS, Young T, Parfrey PS. Autosomal recessive Bardet-Biedl syndrome: first-degree relatives have no predisposition to metabolic and renal disorders. *Kidney International.* 76(2): 215-23, 2009.

F94fsX103 mutation whether homozygous or compound heterozygous were originally from Conception Bay. However cases from two *BBS10* families, homozygous for the C91fsx95 mutation, were from communities geographically distant from each other (Connaigre Peninsula and Green Bay). *BBS2*, *BBS3* and *BBS5* mutations were each seen in only one nuclear or extended family in a homozygous state, each in a separate genetic isolate. Individuals who were compound heterozygotes for *BBS6* and *BBS10* mutations occurred in several communities indicating the wide spread nature of the mutations (Figure 12).

Newfoundland Rod-Cone Dystrophy

Eight patients (3%) of the 1981 cohort in 6 different families had Newfoundland Rod-Cone Dystrophy (NFRCD). At the time of an extended study during the 1990's, NFRCD had been identified in 26 patients from six families, all with ancestors from one specific region in Conception Bay (Figure 13). Fifteen of the subjects came from one extended pedigree and each of the other five families had 2-3 affected individuals (Figure 14) (43). Two splice junction mutations in the *RLBP1* gene on the long arm of chromosome 15 were identified. In the large pedigree, cases were identified who were homozygous for the 324G→A mutation, homozygous for the IVS3+2 T→C mutation, or compound heterozygous for the two mutations. Individuals in three smaller families from the same region were homozygous for IVS3+2 T→C, and affected individuals in two families were compound heterozygous with both the 324G→A and IVS3+2 T→C mutations (Table 8).

Figure 12: The Geographic Distribution (Ancestral Locations) of Bardet-Biedl Syndrome mutations in Newfoundland.

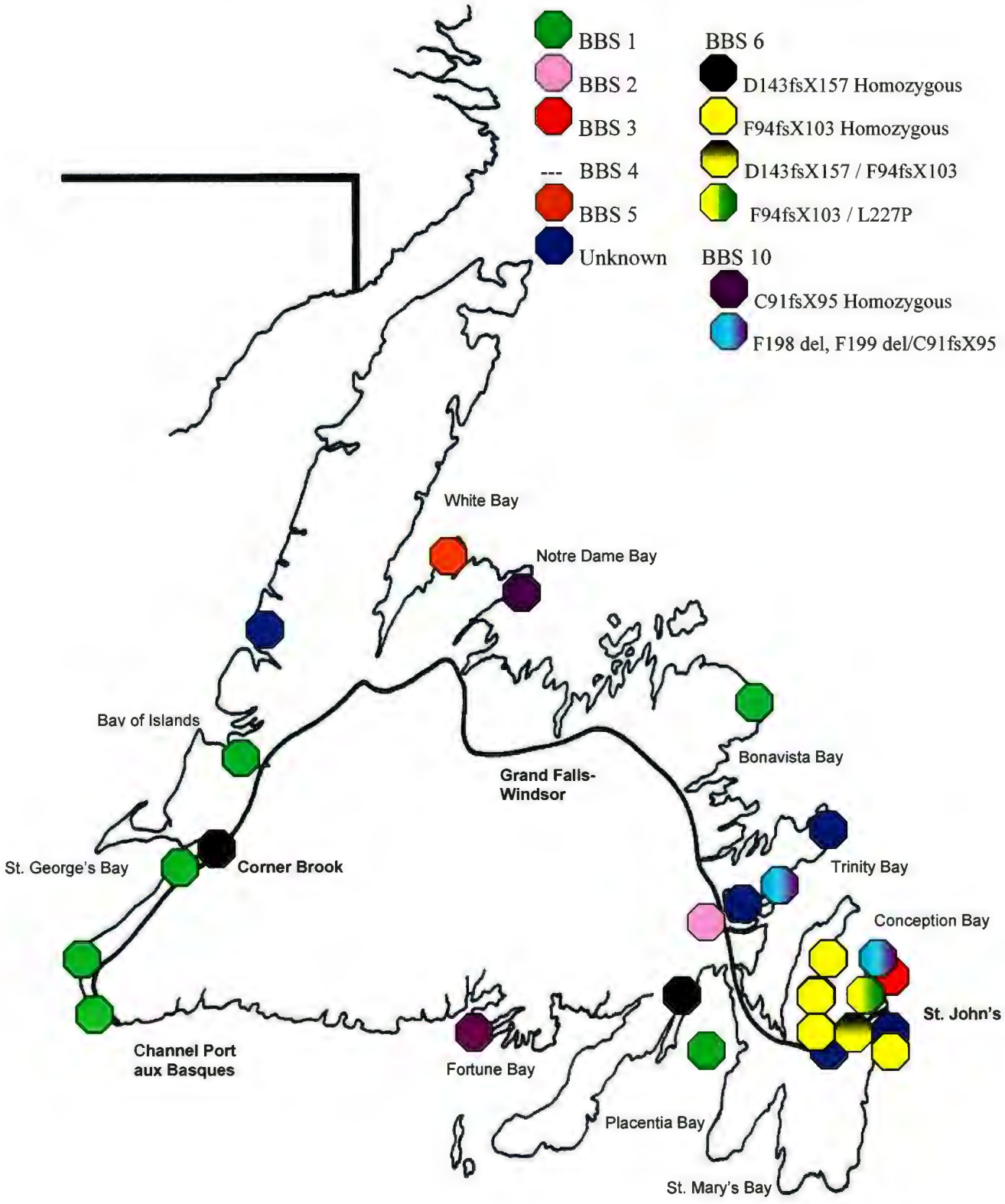


Figure 13: The Geographic Distribution (Ancestral Location) of Newfoundland Rod-Cone Dystrophy in Newfoundland.

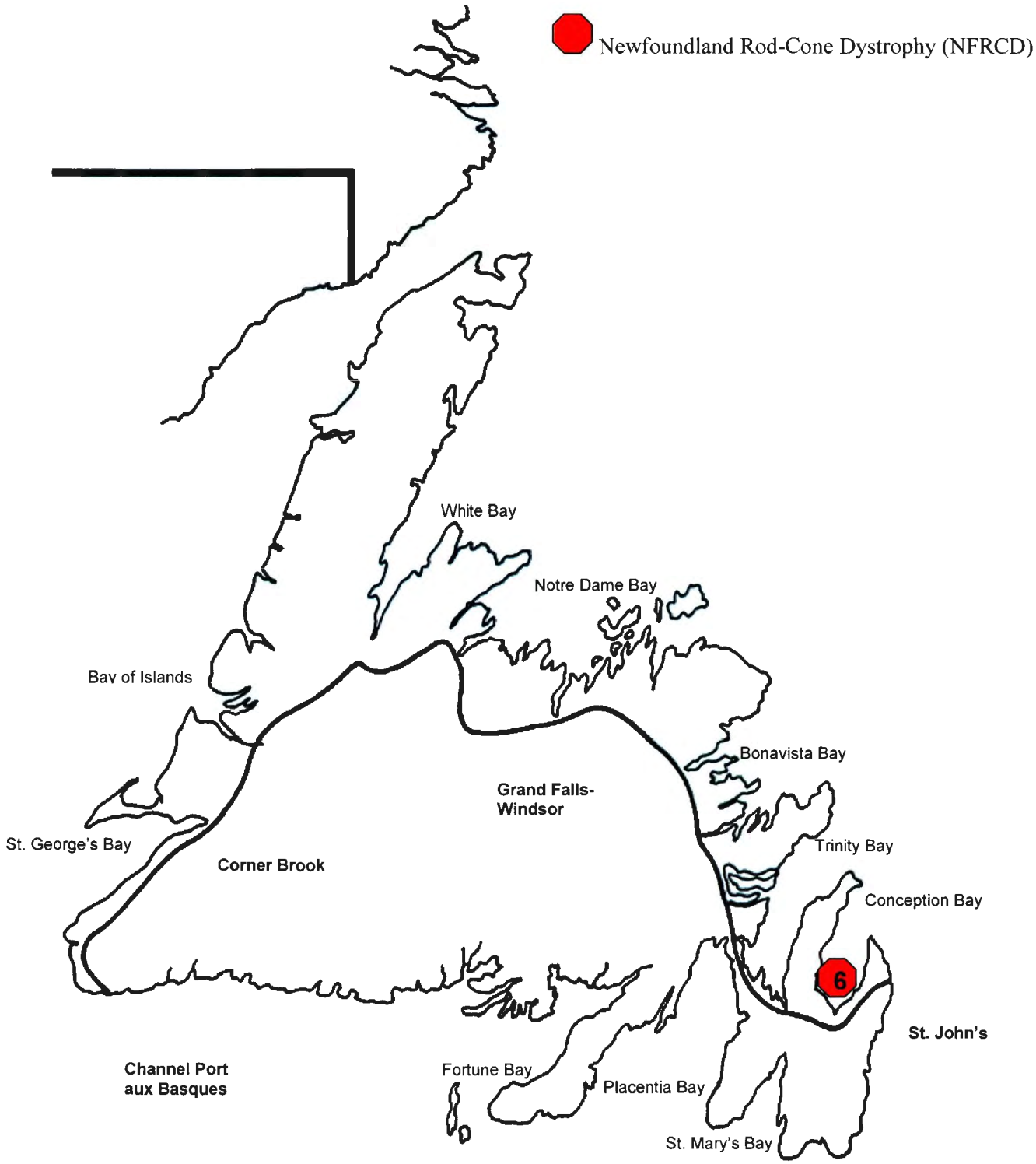
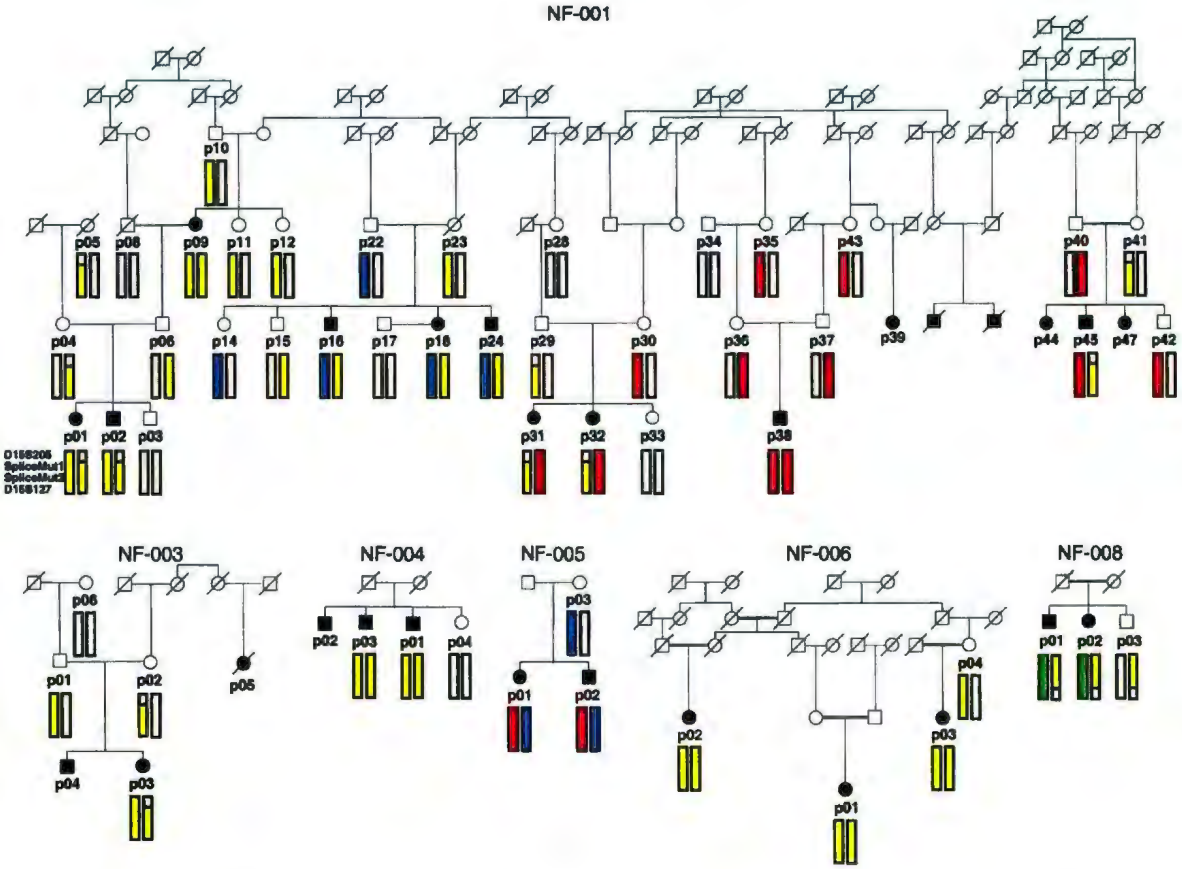


Figure 14. Pedigrees of 6 Newfoundland families diagnosed with Newfoundland Rod- Cone Dystrophy (NFRCD).



Mutation and haplotype data for six Newfoundland pedigrees diagnosed with NFRCD. All individuals were genotyped for *D15S205* and *D15S127* and were sequenced for *RLBP1*. Haplotypes were then constructed for *D15S205*-SpliceMut1-SpliceMut2-*D15S127*, with polymorphic alleles expressed in terms of base pairs. SpliceMut1 = 324G→A; SpliceMut2 = IVS3+2 T→C. Each colored bar represents a unique haplotype: yellow = 135-324G→A-wt-142; blue = 124-324G→A-wt-134; red = 159-wt-IVS3+2 T→C-136; green = 135-wt- IVS3+2 T→C-140; gray = non–disease-associated haplotypes.

The data was reproduced from the following reference:

Eichers ER, Green JS, Stockton DW, Jackman CS, Whelan J, McNamara JA, Johnson GJ, Lupski JR, Katsanis N. Newfoundland rod-cone dystrophy, an early-onset retinal dystrophy, is caused by splice-junction mutations in *RLBP1*. *American Journal of Human Genetics.* 70(4): 955-64, 2002.

Table 8: Two Splice-Junction Mutations in *RLBP1* cause Newfoundland Rod Cone Dystrophy.

Family	Mutation	Inheritance
NF-001	324G→A	Homozygous
	IVS3+2 T→C	Homozygous
	324G→A / IVS3+2 T→C	Compound heterozygote
NF-003, NF-004, NF-006	324G→A	Homozygous
NF-005, NF-008	324G→A / IVS3+2 T→C	Compound heterozygote

The data was reproduced from the following reference:

Eichers ER, Green JS, Stockton DW, Jackman CS, Whelan J, McNamara JA, Johnson GJ, Lupski JR, Katsanis N. Newfoundland rod-cone dystrophy, an early-onset retinal dystrophy, is caused by splice-junction mutations in *RLBP1*. *American Journal of Human Genetics.* 70(4): 955-64, 2002.

Albinism

Ocular Albinism (X-linked) was reported in a large Newfoundland kindred in 1971 and linkage to markers from Xp 22.3 was demonstrated in 1993 (149). The mutation in the *OA1* gene was identified as IVS7-2 A>T in 2005, through clinical studies in Ontario and was provided to the Provincial Medical Genetics Program. In the original report in 1971 (149) a pedigree of 6 generations was traced back to an individual who lived in Morton's Harbour, New World Island around 1840. The people with the disease were well known in the outports as having "dancing eyes". Twenty seven males with ocular albinism from this large family were originally examined. Descendants of these individuals have now spread out across Newfoundland from the original focus area (Figure 15). A second large kindred with ocular albinism was identified on the South Coast, and the Notre Dame Bay *OA1* mutation was excluded. This clinical work was conducted by Dr. Jane Green, and efforts to identify the specific mutation are ongoing (Figure 15).

Stargardt Disease

Thirty three families with Stargardt Disease were identified in Newfoundland, all with autosomal recessive disease. DNA was obtained from members of 22 families, including 37 affected and 25 unaffected family members. Ancestors from 13/22 (59%) families came from the Conception Bay area and ancestors of other families came from a number of different regions, particularly in the north east or south east part of the island (Figure 16). The gene for Stargardt Disease was mapped to chromosome 1p in 1993 (71) and the *ABCA4* gene was identified as the gene for autosomal recessive Stargardt Disease

Figure 15: The Geographic Distribution of Ocular Albinism (OA) in Newfoundland.

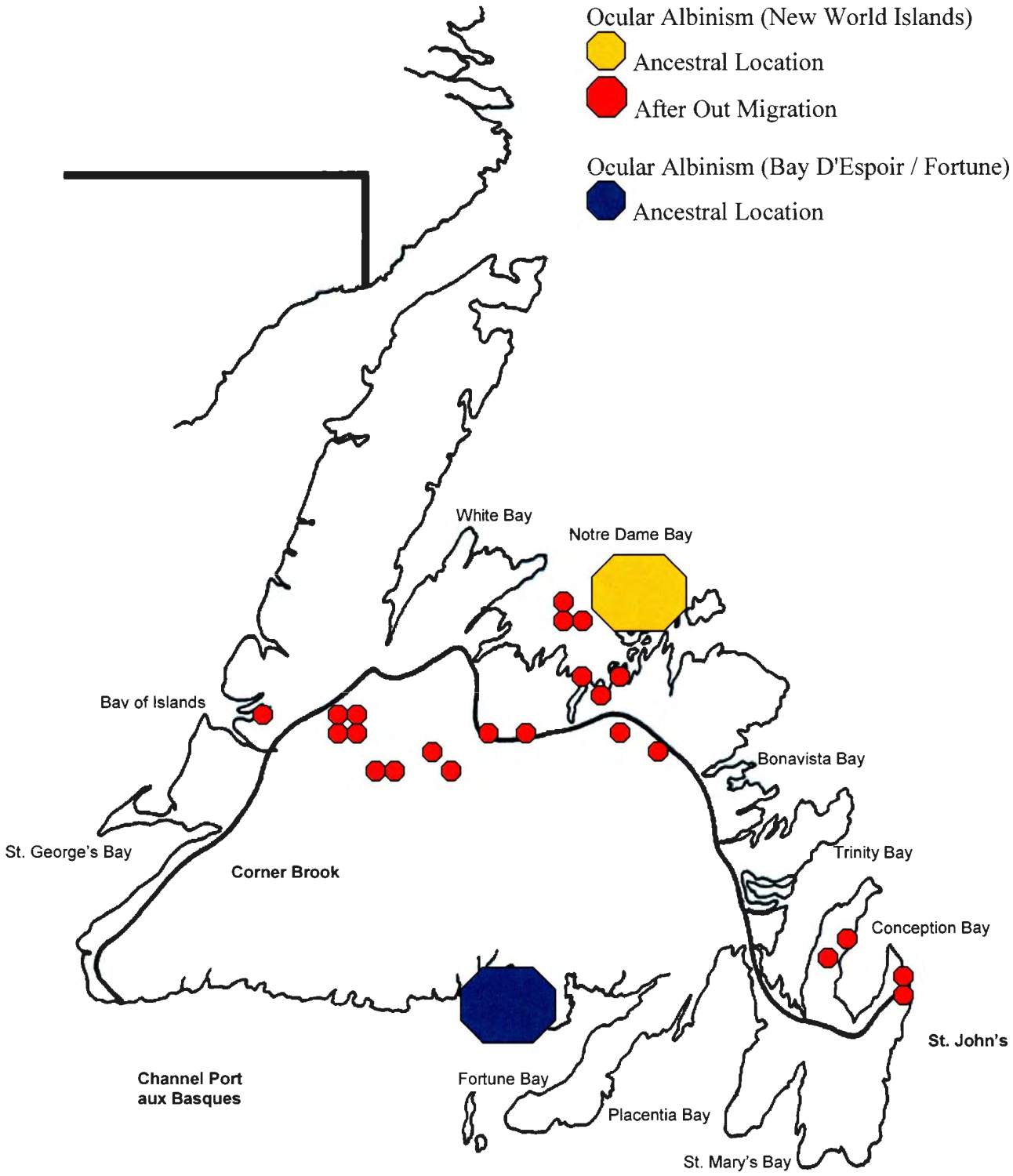
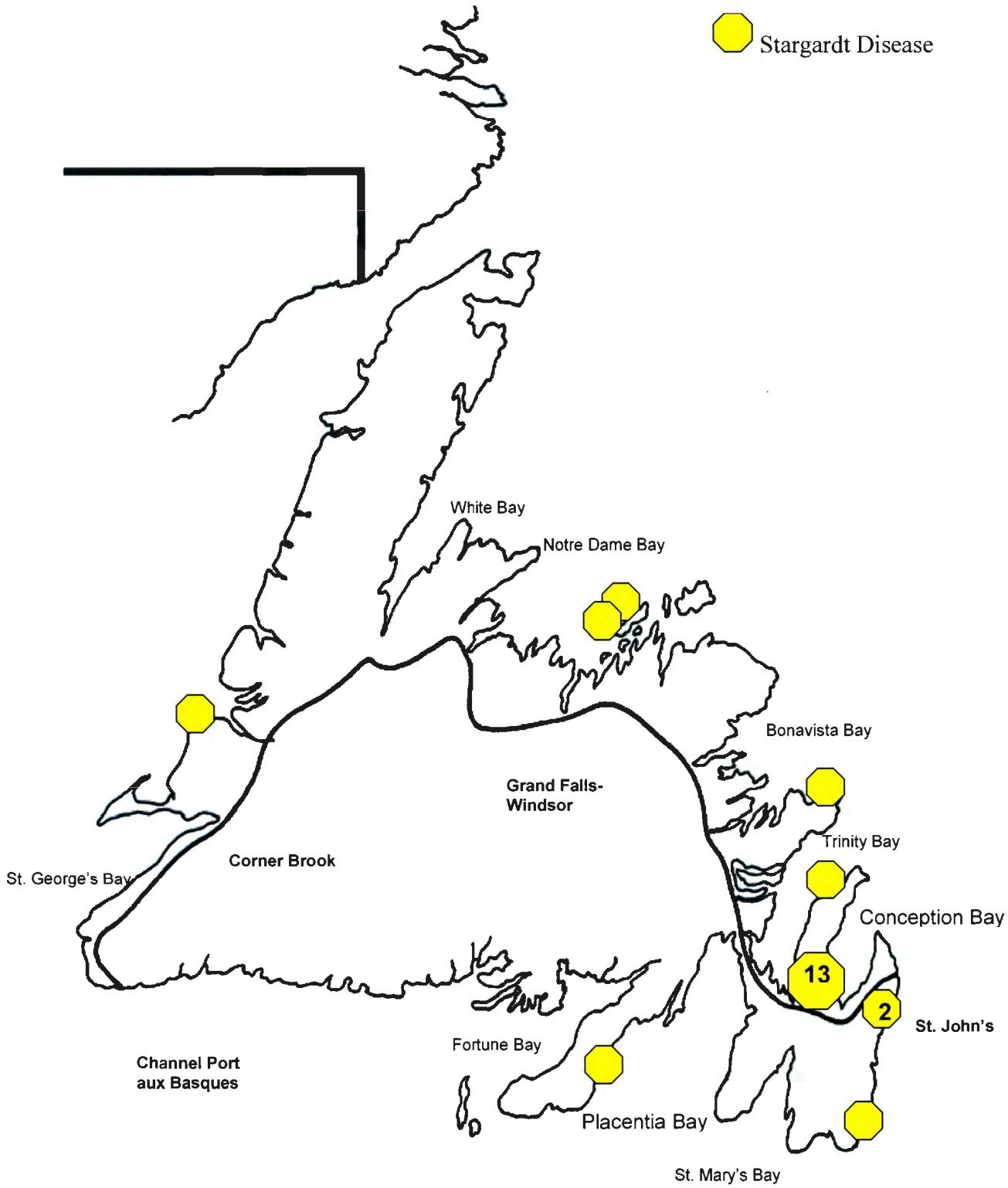


Figure 16: The Geographic Distribution (Ancestral Locations) of Stargardt Disease in 22 Newfoundland families, from whom DNA was obtained.



in a previous study in 1997 (72). *ABCA4* is a very large gene, with 50 exons. Sequencing of all 50 exons is underway to identify the specific mutations causing Stargardt Disease in the identified Newfoundland families. Since all identified families in Newfoundland have autosomal recessive disease, from pedigree analysis, the *ABCA4* gene is the only one being sequenced.

Autosomal Dominant Peters Anomaly

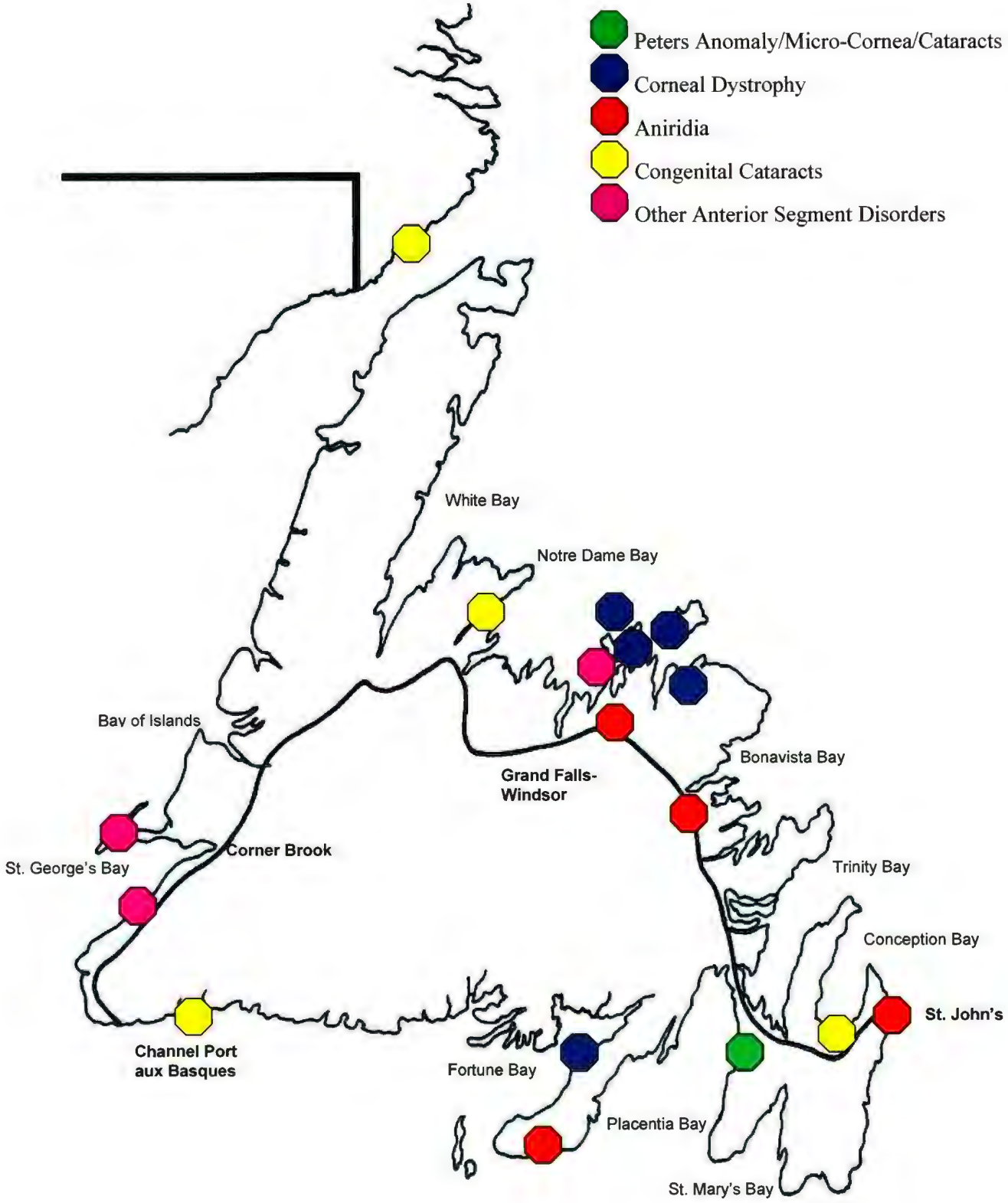
Members of a large family having a variable ocular phenotype ranging from microcornea to Peters Anomaly, segregating as an autosomal dominant trait, are from Placentia Bay (Figure 17). A molecular genetics project was recently completed to determine the molecular etiology of the variable anterior segment dysgenesis (ASD) in this family. Researchers sequenced nine candidate genes and identified 44 variants. A point mutation in *FOXE3*, which codes for a transcription factor involved in the formation of the lens and surrounding structures, co-segregated with the variable ocular phenotype. This novel mutation (c.959G>T) substitutes the stop codon for a leucine residue, predicting the addition of 72 amino acids to the C-terminus of *FOXE3* (150).

Geographic distribution of other monogenic disorders

Retinal Disorders

As the genetics of retinal dystrophies is complex, the approach taken to determine the genetic basis of retinal dystrophies has been to investigate subgroups with similar phenotypes. Consequently initial work has been undertaken in families with Bardet-Biedl

Figure 17: The Geographic Distribution (Ancestral Locations) of Anterior Segment Disorders in Newfoundland.



Syndrome, Newfoundland Rod-Cone Dystrophy, and Stargardt Disease. Retinitis Pigmentosa (RP) occurs frequently in Newfoundland, in locations all across the province (Figure 18). The inheritance pattern for RP may be autosomal dominant (AD), autosomal recessive (AR) or X-linked recessive. In Newfoundland, a total of 67 families with RP have been identified, with 37 having known inheritance patterns, including one large family with X-linked inheritance, eight families with autosomal dominant inheritance, 11 families with autosomal recessive inheritance, and 17 simplex cases (possible AR or a new mutation AD or X-linked).

Usher Syndrome has been identified in 15 families, from several areas in the province with no particular clustering (Figure 19). Specific mutations are not currently known, however a molecular genetics project is being developed to determine the specific mutation(s) involved.

Achromatopsia (with a congenital cone dysfunction causing decreased central vision and absent colour vision) is a rare autosomal recessive retinal disorder, which showed random geographic distribution of seven families. Families with affected individuals were from Conception Bay, Fortune Bay, St. George's Bay, and Notre Dame Bay (Figure 20), which is consistent with autosomal recessive disease likely caused by multiple different mutations. The genes *CNGA3, CNGB3,* and *GNAT2* have been identified as possible Achromatopsia genes, and molecular studies are ongoing to identify specific mutations.

Cone dystrophy, an autosomal recessive disease, was identified in one family from the Southern Avalon Peninsula (Figure 21).

Figure 18: The Geographic Distribution (Ancestral Locations) of Retinitis Pigmentosa in Newfoundland.

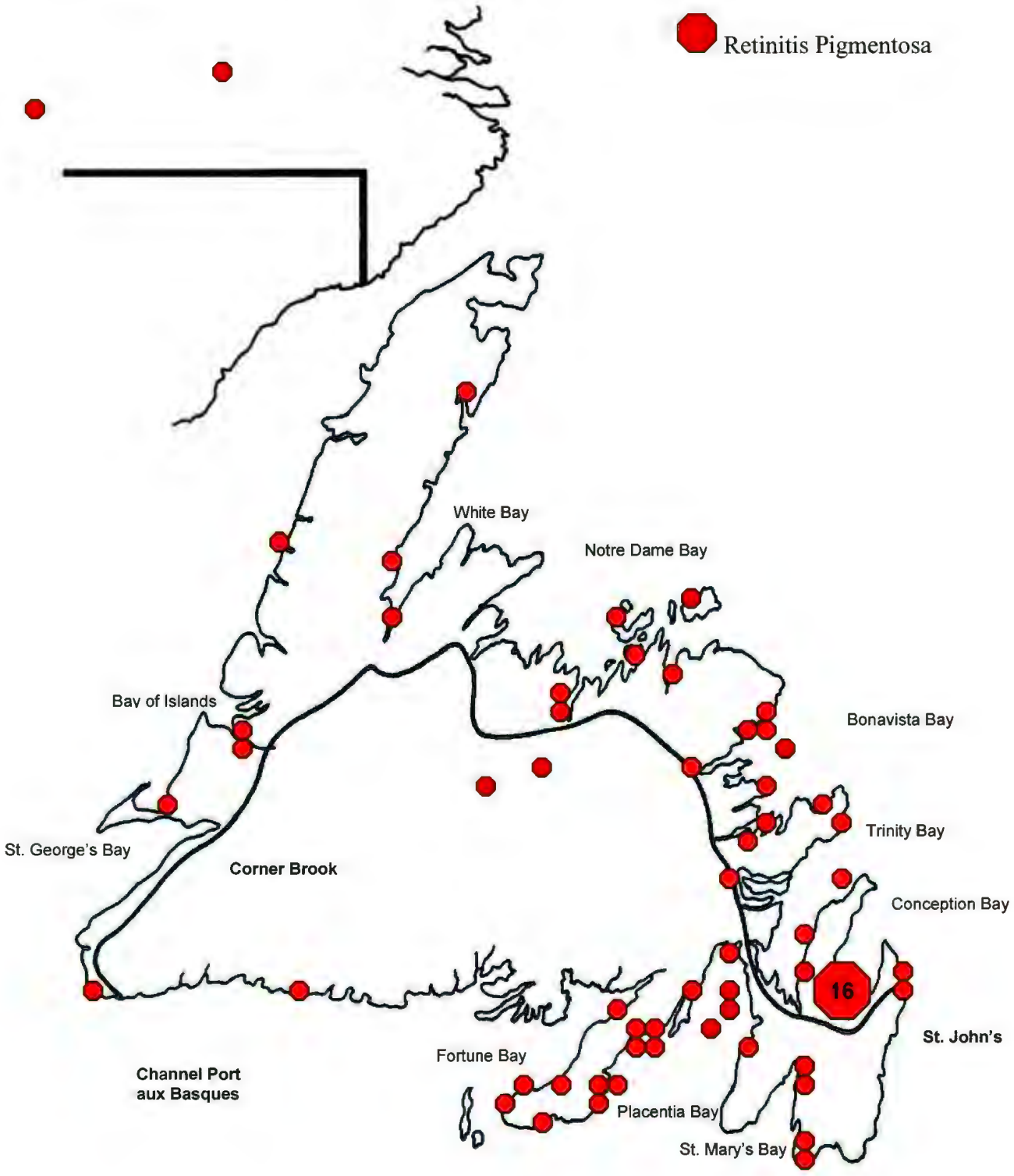


Figure 19: The Geographic Distribution (Ancestral Locations) of Usher Syndrome in Newfoundland.

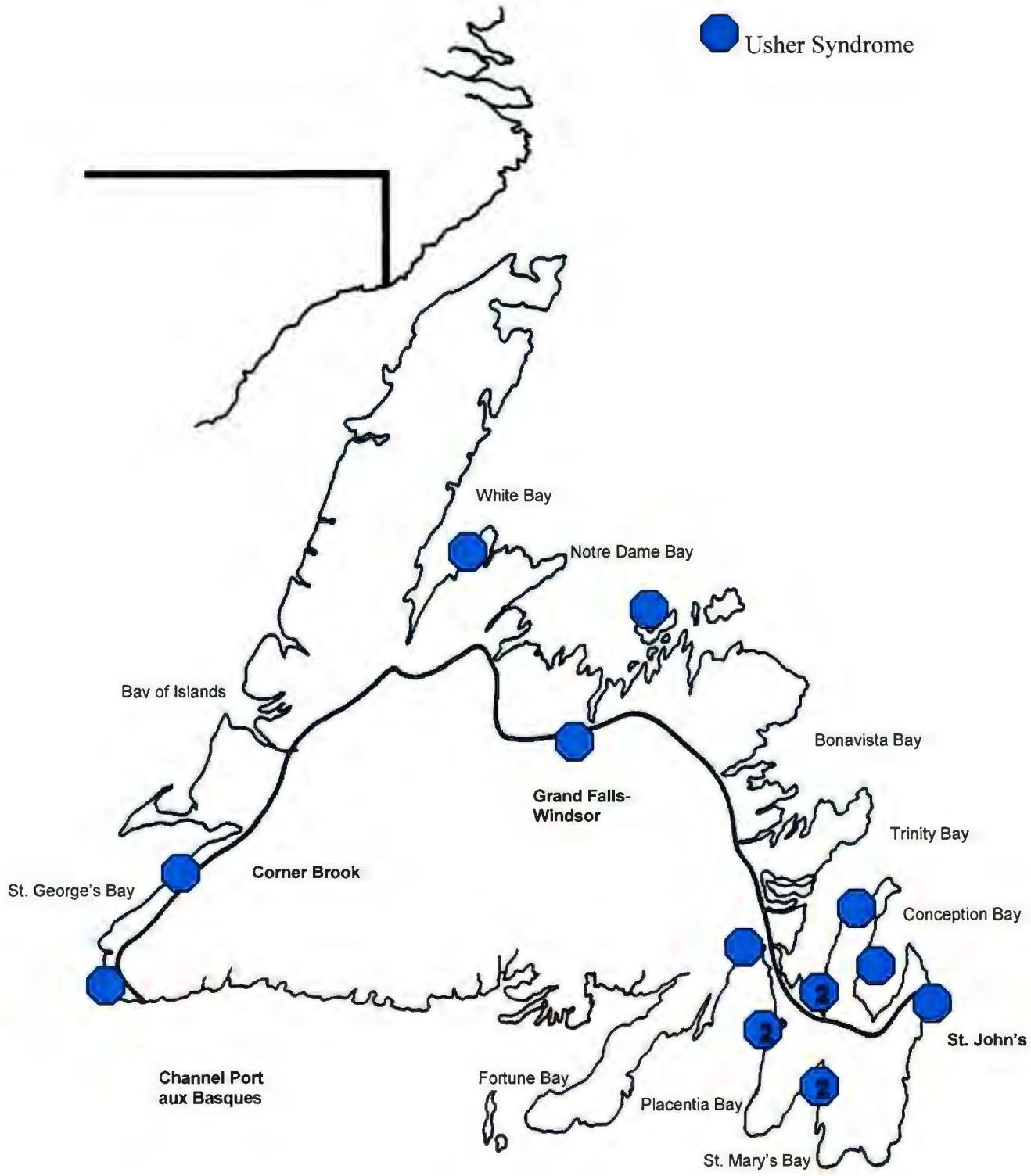


Figure 20: The Geographic Distribution (Ancestral Locations) of Achromatopsia in Newfoundland.

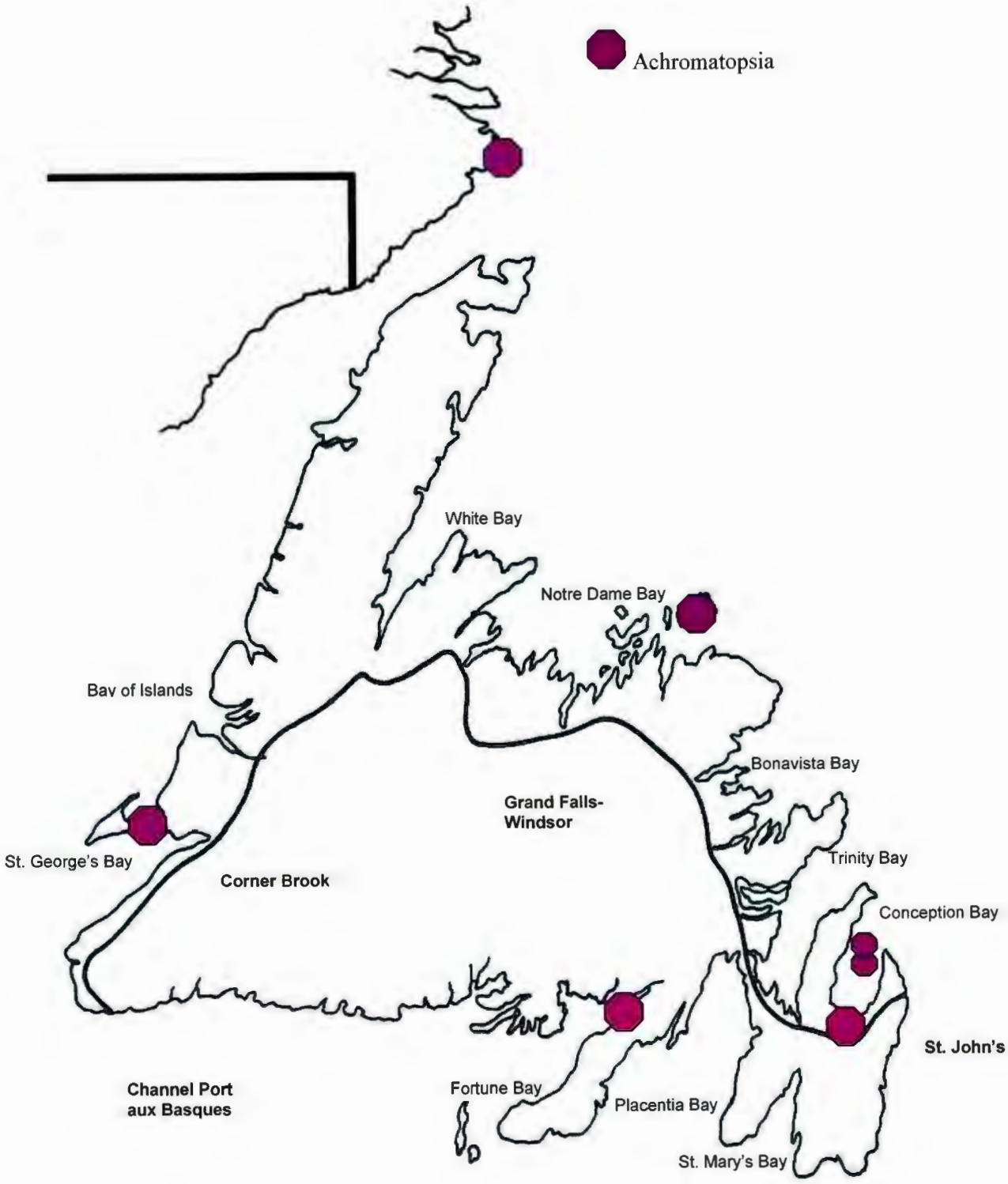
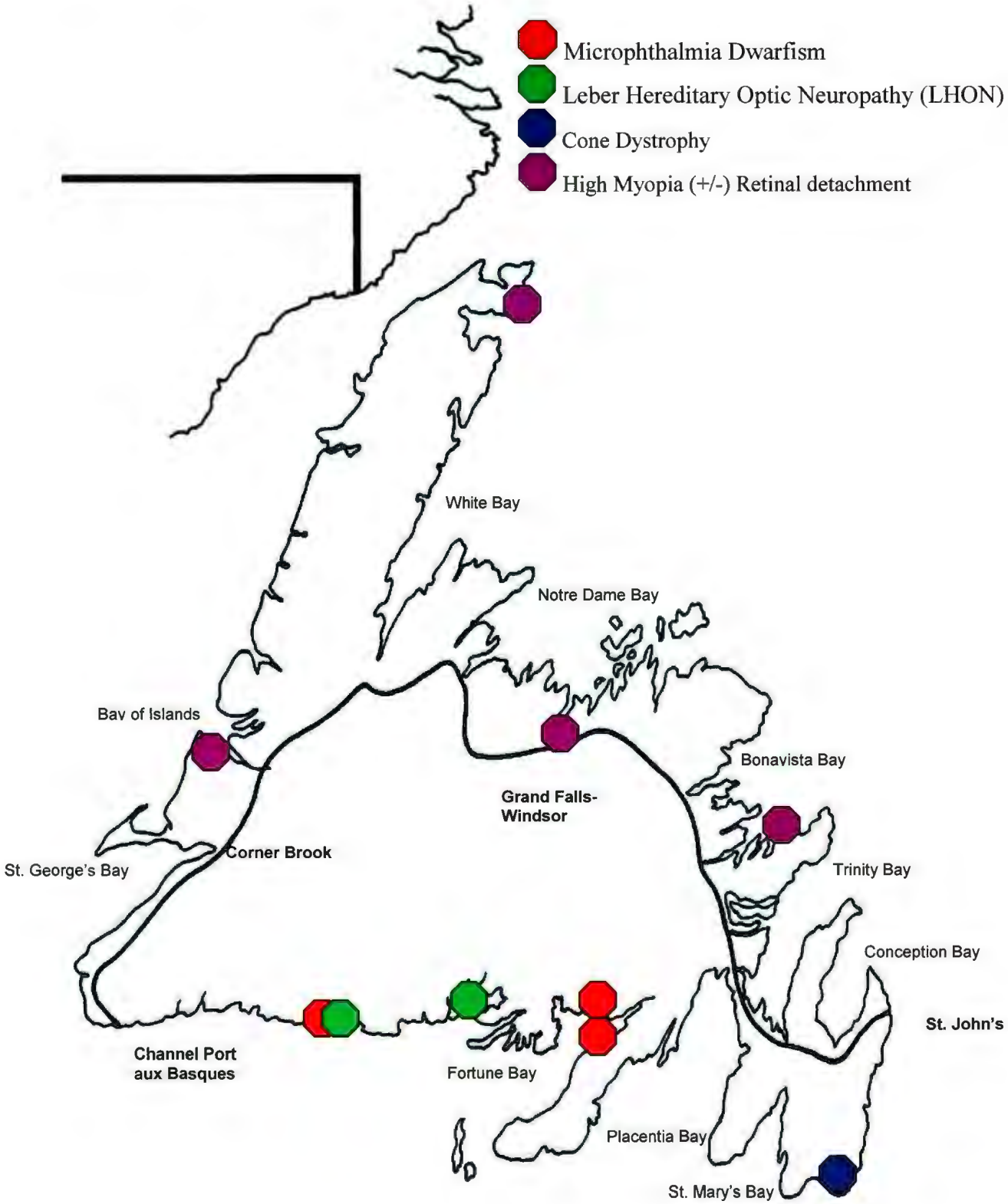


Figure 21: The Geographic Distribution (Ancestral Locations) of Rare Genetic and Ophthalmic Disorders in Newfoundland.



Anterior Segment Disorders

Several anterior segment disorders were identified including Aniridia, Congenital and Juvenile Cataracts, Corneal Dystrophy, and Peters Anomaly/Microcornea/Cataracts. Four families with Corneal Dystrophy clustered in Notre Dame Bay and a fifth in Fortune Bay were identified. No clustering was identified for any other of these rare disorders (Figure 17). Four families with Congenital Cataracts were from various locations including: the South Coast, Notre Dame Bay, Avalon Peninsula, and the South Coast of Labrador. Three additional families, unrelated to each other, with other anterior segment disorders, are located in the Notre Dame Bay and St. George's Bay area's (Figure 17).

Oculocutaneous Albinism

Oculocutaneous Albinism was identified in 15 families, with clusters of affected individuals in Trinity Bay, White Bay, and on the Northern Peninsula and several other distinct families (Figure 22).

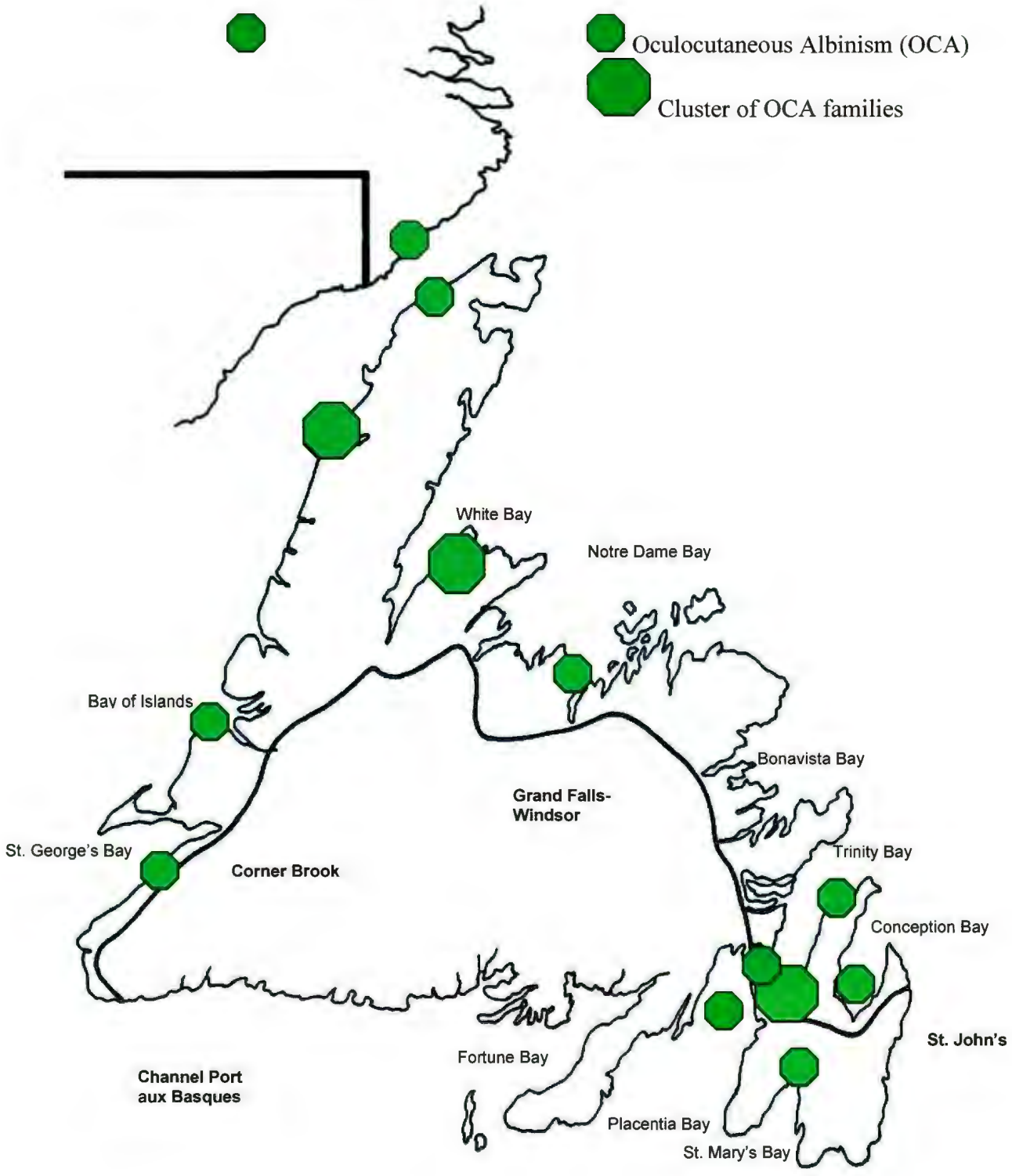
Optic Nerve

Leber hereditary optic neuropathy, a mitochondrial disease, was identified in two families located on the South Coast of the province (Figure 21).

Other Eye Disorders

Microphthalmia Dwarfism was identified in 4 individuals, from 3 families, which have been connected in an extended pedigree, are from the South Coast of Newfoundland (Figure 21). A gene discovery project is currently underway.

Figure 22: The Geographic Distribution (Ancestral Locations) of Oculocutaneous Albinism (OCA) in Newfoundland.



Families with autosomal recessive or autosomal dominant inheritance for High Myopia/Retinal Detachment, have been identified from St. George's Bay, Northern Peninsula, Notre Dame Bay, and Bonavista Bay (Figure 21).

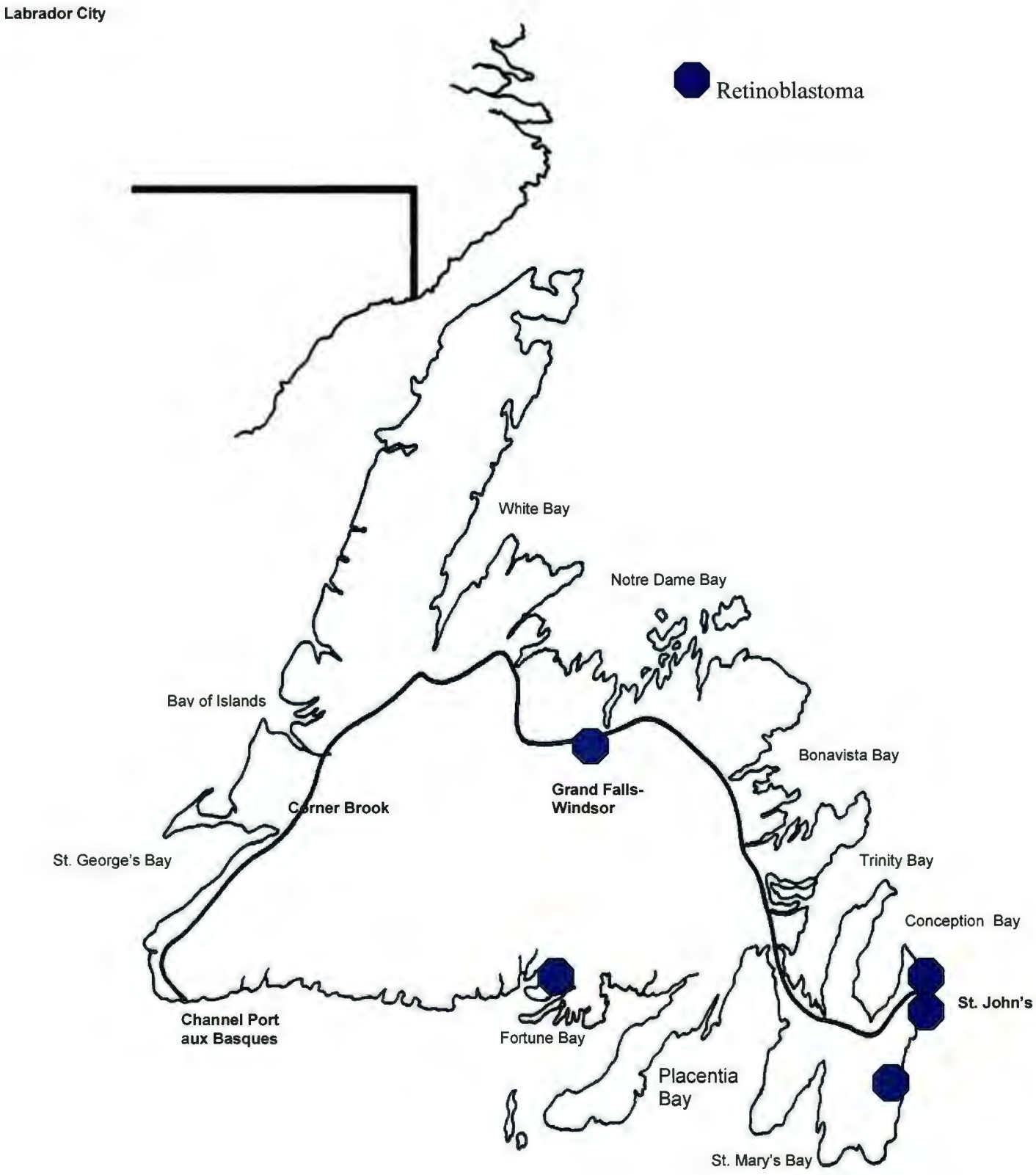
Retinoblastoma was identified in five families, three from the Avalon Peninsula, and two others in Grand Falls-Windsor and Bay D'Espoir respectively (Figure 23). A specific mutation causing Retinoblastoma has been identified in the largest family. Other mutations are yet to be identified, however linkage to the *RB1* gene has been confirmed.

Newfoundland Population in 1981 and the Present Day Clustering of Disease

In 1981 the population of the province was 567,681, of which only 174,521 (31%) lived in towns or cities of over 10,000 people (N= 4) (Figure 1, page 4). The majority lived in small communities of less than 2,000 people, distributed around the coast of Newfoundland (3). The map (Figure 1, page 4) shows the population distribution in these small communities by coastal region (N=368,217; 65%). Only 24,916 (4.4%) people lived in the interior of the province (communities of Gander and Grand Falls-Windsor).

Geographical maps indicating the locations of some inherited eye disease within Newfoundland and Labrador are provided in Figures 12, 13, and 15-23. These geographical maps indicate that inherited eye disease is widespread throughout the province, yet some specific eye diseases are concentrated in certain small regions. The study indicates the widespread distribution of some hereditary eye disorders and the occurrence of some rare conditions in specific isolated populations including: Ocular Albinism within the New World Island area, and Newfoundland Rod-Cone Dystrophy within Conception Bay.

Figure 23. The Geographic Distribution (Ancestral Locations) of Retinoblastoma in Newfoundland.



Within the Conception Bay area multiple different causes of blindness have been observed including: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by three different mutations in *BBS6* and by two mutations in *BBS10*; (iii) Newfoundland Rod-Cone Dystrophy caused by two different mutations in the *RLBP1* gene; (iv) Stargardt Disease; (v) Achromatopsia; (vi) Usher Syndrome; (vii) X-linked Ocular Albinism as a branch of the Notre Dame Bay family; (viii) Oculocutaneous Albinism; and (ix) Congenital Cataracts.

Around the coast of the Southern Avalon the causes of blindness include: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by mutations in *BBS1*; (iii) Stargardt Disease; (iv) Cone Dystrophy; (v) Autosomal dominant Peters Anomaly/Micro-Cornea/Cataracts; (vi) Aniridia; and (vii) Oculocutaneous Albinism.

On the Burin Peninsula the cause of blindness included: (i) Retinitis Pigmentosa; (ii) Stargardt Disease; (iii) Achromatopsia; (iv) Oculocutaneous Albinism; (v) Aniridia; and (vi) Corneal Dystrophy.

Along the South Coast the causes of blindness include: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by mutations in *BBS1* and *BBS10*; (iii) Ocular Albinism; (iv) Congenital Cataracts; (v) Leber Hereditary Optic Neuropathy; and (vi) Microphthalmia Dwarfism.

On the South West Coast of the province causes of blindness included: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by mutations in *BBS1* and *BBS6*; (iii) Achromatopsia; (iv) Usher Syndrome; (v) Oculocutaneous Albinism; and (vi) Anterior Segment Disease.

The causes of blindness in The Bay of Islands on the West Coast included: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by a mutation in *BBS1*; (iii) Stargardt Disease; (iv) Oculocutaneous Albinism; and (v) High Myopia/Retinal Detachment.

On the Northern Peninsula of the Island the causes of blindness included: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome; (iii) Oculocutaneous Albinism; and (iv) High Myopia/Retinal Detachment.

On the North East Coast of the Island the causes of blindness included: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by mutations in *BBS5* or *BBS10*; (iii) Stargardt Disease; (iv) Achromatopsia; (v) Usher Syndrome; (vi) X-linked Ocular Albinism; (vii) Oculocutaneous Albinism; (viii) Congenital Cataracts; (ix) Corneal Dystrophy; (x) Anterior Segment Disease; and (xi) High Myopia/Retinal Detachment.

In Bonavista Bay the causes of blindness include: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by a mutation in *BBS1*; (iii) Stargardt Disease; (iv) Aniridia; and (v) High Myopia/Retinal Detachment.

In Trinity Bay the causes of blindness include: (i) Retinitis Pigmentosa; (ii) Bardet-Biedl Syndrome caused by mutations in *BBS2* or *BBS10*; (iii) Stargardt Disease; (iv) Usher Syndrome; and (v) Oculocutaneous Albinism.

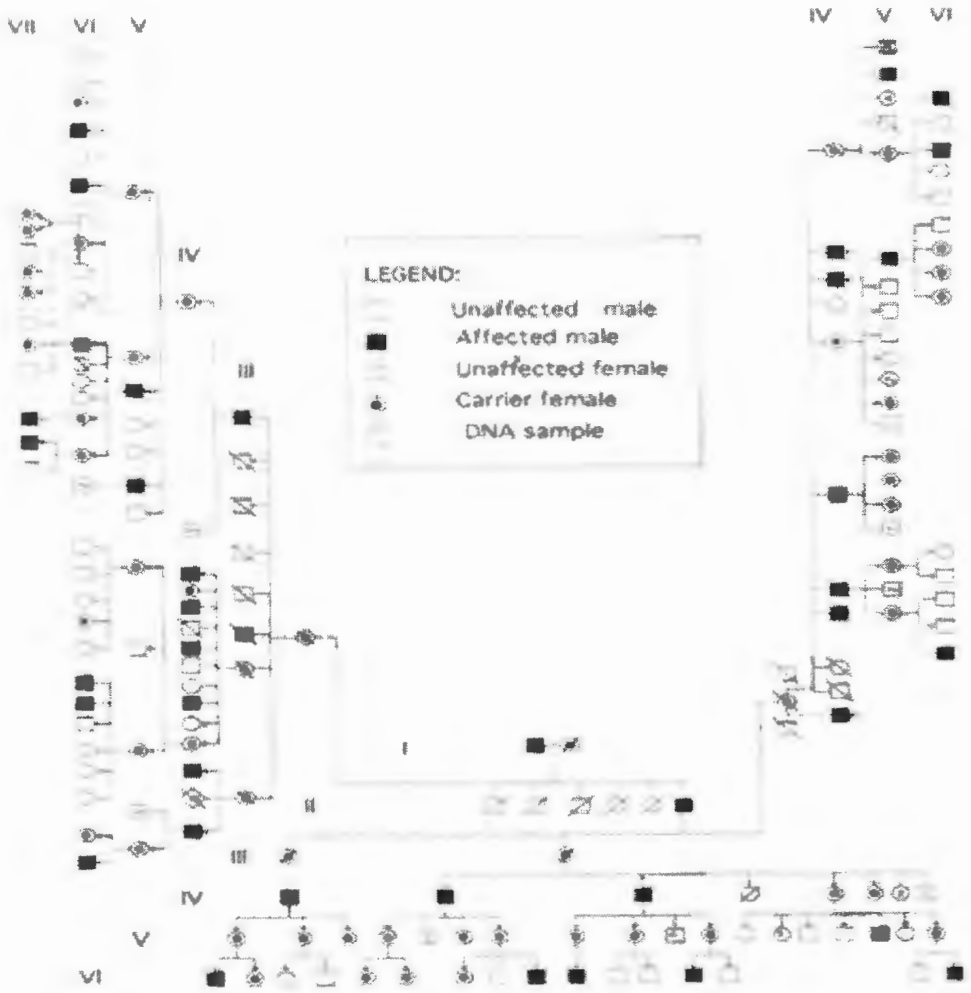
X. DISCUSSION

Newfoundland and Labrador is peopled by a young founder population, which in 1981 had a high prevalence of blindness resulting from established and presumed monogenic disease. The long follow-up (27 years) of this population-based cohort has not only revealed the multiplicity of inherited diseases causing blindness, but facilitated the examination of the genetic complexity associated with similar phenotypes. The relatively small population of the province, derived from founders from small areas of England and Ireland, consists of not just one genetic isolate but multiple isolates, each with its own genetic architecture, which has predisposed to various inherited eye diseases. These genetic isolates today represent an important source of informative families for identifying genes for inherited disorders. The important features associated with some of these families are the existence of multigenerational and extended pedigrees mostly descended from a small number of founders about 8-10 generations ago. An example is provided in Figure 24.

Cohort follow-up: Comparing 1981 to 2008

Of the 1,013 cases reviewed in 2007/8, 305 (30%) individuals were found to have an established genetic disease and a further 123 (12%) have a presumed genetic disease. This shows a 6% increase in the number of people within each of these genetic categories since the initial review was completed. The majority of changes in diagnosis occurred in people classified as congenital cataract and multiple anomalies, myopia, vascular/systemic disease, or other. This substantial increase in the number diagnosed

Figure 24. Extended multigenerational pedigree of a Newfoundland family with Ocular Albinism.



Pedigree was reproduced with consent from Dr. Jane Green, Professor of Genetics, Memorial University of Newfoundland.

with established genetic disease and with presumed genetic disease following long term follow up, is less surprising when one considers: (1) the availability of serial clinic notes and investigations over many years, (2) the enhancement of family history that occurs through multiple meetings with families, (3) the focus on making a molecular genetic diagnosis in several conditions.

The term "presumed genetic disease" was used when an individual was suspected to have a strong genetic component, on the basis of family history, to their disease. This was the case for individuals who were unable to be seen by Dr. Green, were unwilling to provide samples of DNA, or due to death. The category of established genetic disease therefore, could be, and most likely is underestimated within the Newfoundland population.

The study that was closest in scale to the current study was completed by Odland in 1981 (151), who tried to ascertain all the blind and partially sighted persons in Bergen, Norway and the surrounding county of Hordaland. Odland concluded that in terms of the isolation of communities that county did not differ from the rest of the country, and therefore the prevalence and causes of blindness in that county could be considered representative of those for the whole country. Thirty percent were judged to have an established hereditary or presumed hereditary eye disease, which shows a slightly lower proportion than this current revised study, but a very similar proportion to the initial results reported from this current study in 1981(147). One must suspect that the prevalence of genetic eye disease as a cause of blindness in Norway was underestimated, as was the prevalence in Newfoundland, and research in Norway would benefit from a long term follow up to more accurately diagnose genetic disease.

Around the world a high proportion of childhood blindness is due to hereditary disease. Genetically determined conditions accounted for 77% of childhood blindness in Lebanon (152), 79% in Cyprus (of which half were autosomal recessive disorders) (153), 84% and 89% in Saudi Arabia as reported in 2 separate studies (154,155). In 13 countries of Africa, Latin America and Asia, examined between 1990-1994, genetic disease was the cause of blindness in 39% of cases, with autosomal recessive inheritance being reported in 22-52% of cases (156).

An appropriate comparison between this Newfoundland Cohort study and other hereditary eye disease studies could not be completed due to the lack of comparable studies in the literature. This is one shortcoming of this current paper seeing that a comparison with respect to the number of people affected in each etiological category of disease within Newfoundland cannot be compared to other populations.

Newfoundland: The ideal population for identifying the genetic basis of disease.

The Newfoundland population has facilitated the elucidation of the genetic basis of many diseases. The current study of blindness arising from the 1981 cohort has facilitated the identification of several genes causing hereditary eye disease particularly: Bardet-Biedl Syndrome. The molecular genetic basis of Newfoundland Rod Cone Dystrophy, and X-linked Ocular Albinism was also determined. Studies examining the molecular genetic basis of blindness in Retinitis Pigmentosa, Achromatopsia, Stargardt Disease, Microphthalmia Dwarfism and other Anterior Segment Disorders are now underway in Newfoundland. New studies examining the molecular genetic basis of other types of hereditary blindness may follow.

Another shortcoming of this current study is the fact that there are very few molecular genetic confirmations of the clinical diagnoses of the people involved (Bardet-Biedl Syndrome, Newfoundland Rod Cone Dystrophy, X-linked Ocular Albinism and Peters Anomaly/Microcornea/Cataracts being the exceptions). However molecular genetic studies of Stargardt Disease, Achromatopsia and Retinitis Pigmentosa are underway. Even with this shortcoming, this current project has paved the way for future comparison studies within the Newfoundland population to be undertaken following the completion of current molecular genetic studies.

Newfoundland is an ideal place for identifying the genetic basis of eye disease in that it has a young founder population, consisting of multiple genetic isolates, where the original immigration of these isolates has predisposed to the frequent occurrence of blindness caused by multiple different mutations in different genes. The genetic architecture of the Newfoundland population has facilitated the identification of novel genes. Accurate phenotypic description of cases within families and determination of inheritance patterns, with geographic mapping of families, may facilitate the identification of novel genes and mutations in various diseases causing blindness in Newfoundland.

Along with the geographic isolation, the Newfoundland population demonstrates a high degree of cultural and environmental homogeneity (4). The benefit of such founder populations over heterogeneous populations to identify genes for rare monogenic disorders is unquestioned. Genetic isolates, in which founder alleles were driven to a high frequency due to restriction of gene flow in the population, still persist today in various Newfoundland outports. Although little immigration has occurred in many of

these communities during the last two centuries, improvements in transportation and significant out-migration from the Newfoundland outports, will start to change the demographics of these geographic isolates. Consequently, now is the opportune time to determine the molecular genetic basis of blindness. Our population based study has identified families with multiple genetic diseases causing blindness within multiple genetic isolates of Newfoundland to be characterized by collaboration with molecular geneticists to examine the DNA of family members.

Geographic distribution of disease

Some inherited diseases are distributed throughout the various regions of the province with some clustering observed. There are several isolated regions in which specific disorders are seen. Leber hereditary optic neuropathy and Microphthalmia Dwarfism, both rare conditions, were observed only on the South Coast of the province. Two different mutations occurred in an extended pedigree with Newfoundland Rod Cone Dystrophy living in Conception Bay, leading to homozygous inheritance of different mutations in two sibships and to compound heterozygote in a third sibship. The diversity of disease is not surprising given the complex structure of the eye, dependent on the normal function of multiple different genes. It is hypothesized that within these clusters, mutation specific diseases will be found, and that across regions, multiple different mutations will be identified as the cause of the same disorder.

This is the case for Bardet-Biedl Syndrome where there are mutations in 6 BBS genes in various communities around the province but there are specific isolates with some of these mutations in a homozygous state such as *BBS1* (on the Southwest Coast),

BBS6 (in Conception Bay), *BBS3* (in North Avalon), and *BBS5* (in White Bay). Other families with BBS caused by compound heterozygote mutations in *BBS6* and *BBS10* have been identified in diverse areas around the coast. The distribution of BBS mutations in Newfoundland demonstrates that the province is not one genetic isolate, but consists of multiple isolates.

Examination of the geographic distribution of other autosomal recessive diseases (Stargardt Disease, Usher Syndrome, Achromatopsia, and Oculocutaneous Albinism) reveals similarities to the distribution of Bardet-Biedl Syndrome, some clustering but also random distribution around the coast of the island. This suggests that multiple different mutations will be identified as causes for these diseases. It also suggests that for novel gene discovery investigation of families grouped by geographic area may be helpful.

The elucidation of the genetic basis of Retinitis Pigmentosa may be more complex, particularly as the disease may result from autosomal dominant, autosomal recessive or X-linked inheritance. However in Newfoundland clustering of Retinitis Pigmentosa occurs, particularly in Conception Bay, on the Burin Peninsula, and in Bonavista Bay. Nonetheless families with the disorder occur in every geographic area around the coast, as expected based on the substantial genetic heterogeneity of this disorder with over 50 genetic loci for RP having been identified (31).

Although new mutations occur frequently, there is no need to assume an increased mutation rate to explain the finding of multiple mutations causing specific diseases in Newfoundland. The high prevalence of blindness and the high frequency of genetic disease as a cause of blindness is likely the result of founder mutations in multiple genetic isolates.

Within many communities the genetic causes of blindness were multiple. For example, in the geographic region of Conception Bay, nine different hereditary eye diseases were identified, and some were associated with at least two different mutations (Bardet-Biedl syndrome and Newfoundland Rod Cone Dystrophy). Some of the diversity of inherited eye diseases being observed in each geographic area is the background carrier rate for multiple different disorders, but also members of families from specific regions may have moved within the province for economic reasons. This can be observed with the large New World Island family with ocular albinism, which has now spread out across Newfoundland into several communities away from the original focus area (Figure 15). This has been documented through provision of clinical genetic services to OA1 family members in different parts of the province.

Visual Aids, Counseling, and Genetic Testing: Planning for the future

Visual aids which improve support for education, employment, and transportation for people with reduced vision are of the utmost importance. In children especially even the slightest amount of vision allows for the sense of sight to develop and enter into their learning processes. The earlier a patient is introduced to visual aids in the course of their disease, when their remaining vision is still good, the easier it will be to merely adjust magnification in the future as vision decreases. A visual defect may affect an individual seriously, becoming an impediment to their social and scholastic progress, and it is vital that every attempt to help these individuals be made.

It is important to provide education about the features of these inherited eye conditions, the risk of recurrence to all affected people and their family members, and

whether genetic testing is available and when it is appropriate. Genetic counseling is an extremely valuable resource for families or individuals who are affected by these conditions and are seeking guidance or assistance to help them fit into the mainstream of society. Instruction and support from genetic counselors or physicians can be offered to all parents of affected children, or to any affected individual. Information can be made available for a child affected with a disorder at the appropriate time. Therefore, if a child is too young to be informed about their illness, or if the parents would like to defer providing information to them at such an early time, then it would be withheld until the appropriate time has come. These health care professionals help individuals and families understand the cause of the specific disease, as well as addressing the medical, ethical, sociological, and psychological issues that are associated with each specific disorder.

There is an increasing range of genetic tests for an increasing range of heritable disorders, which is all made possible by human genome research being completed around the world. If a mutation is known then screening/prenatal testing should be offered. Especially, in the case of autosomal recessive disorders, carrier screening of family members of the affected individual should be offered.

It will be feasible to produce multiple diagnostic tests for the multiple mutations causing blindness in Newfoundland. Thus it may be possible to determine which parents carry mutations, and to test pre-symptomatically in children. These scenarios have ethical issues, therefore the individuals and their families should be informed about the pros and cons of such testing, and the final decision should lie with the person involved, and they should have the ability and the right to decline any genetic testing in almost all instances.

Pre-symptomatic testing is concerned with the analysis of DNA samples taken in

early infancy in order to detect genetic diseases and facilitate appropriate interventions which can avert or ameliorate adverse outcomes or serious health problems in the future. It is of great importance if parents value diagnosis prior to the onset of visual deterioration so that this education and intervention can prepare the child and the family for a subsequent life with reduced vision. General international opinion about programs of genetic testing and screening is represented by the recommendations of the Nuffield Council on Bioethics (157). These include the requirement for informed consent and that all written information should be supplemented with counseling, individuals and parents of unborn or young children should be fully informed of the results of genetic screening and their implications for the family (157). The hope is that the general public recognizes the benefits and the potential usefulness of genetic testing and screening for individuals, for families, and for the population as a whole. However, acknowledging that there is an accompanying anxiety that genetic testing and screening arouses, all efforts to minimize any future unease should be made including: informing the public in advance, offering appropriate counseling, providing equality of access, and respecting the self-determination of those tested, making testing or screening non-compulsory.

In recent decades researchers in the province of Newfoundland and Labrador have made great strides in understanding the genetic and clinical epidemiology of inherited eye diseases. Research in Newfoundland has produced important observations on the clinical manifestations and outcomes of Mendelian inherited diseases, as well as mechanistic insights into the molecular determinants of the diseases. The molecular causes of many inherited diseases have already been identified and several others are currently being studied and will be determined in the near future.

XI. CONCLUSION

Investigation within the Newfoundland population has revealed that the prevalence of blindness in 1981 caused by monogenic disease was extremely high (0.76 per 1,000). Long term follow-up, detailed clinical investigation and analysis of extended pedigrees has facilitated accurate diagnosis, and demonstrated that multiple different causes of inherited blindness within regions were observed.

Some disorders including Bardet-Biedl Syndrome, Stargardt Disease, Usher Syndrome, Retinitis Pigmentosa, Achromatopsia, and Oculocutaneous Albinism occurred in many regions of the province, although geographic clustering of several of these conditions did occur. Consistent with the idea that Newfoundland is comprised of multiple genetic isolates, is that Bardet-Biedl Syndrome occurred in most regions and was caused by nine mutations in six different genes. Other rare disorders were observed in only one region.

Geographical mapping of families with autosomal recessive blindness of undetermined genetic etiology revealed similar patterns as for Bardet-Biedl Syndrome and Stargardt Disease suggesting that in other conditions such as Retinitis Pigmentosa, Achromatopsia, Oculocutaneous Albinism, and Usher Syndrome, multiple mutations in one or more genes will be discovered.

The peopling of Newfoundland and Labrador has predisposed to the frequent occurrence of inherited blindness, resulting from multiple mutations in different genes. The province is comprised of not just one but multiple genetic isolates. The genetic architecture of the population has facilitated the identification of novel genes and the

potential to determine the genetic basis of other causes of blindness. Geographic mapping of cases, with accurate phenotypic description and identification of inheritance patterns, may facilitate the identification of novel genes and mutations causing blindness in Newfoundland and Labrador. One goal of this genetic research in Newfoundland is the identification of novel genes and specific mutations within families, and the development of pediatric molecular diagnostic assays for future testing and screening of individuals from families with inherited disorders of the eye.

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XIII. APPENDIX

A. Anatomy of the Eye

B. Previous Newfoundland Studies: Literature Review

A. Anatomy of the Eye

The human eye is a complex organ, with many structures working together to give us the sense of sight, allowing us to observe and learn about the surrounding world. It can be divided into two segments, which are anterior and posterior (Figure 7).

The Anterior Segment

The anterior segment is the front third of the eye that includes the structures in front of the vitreous humour: the cornea, aqueous humour, iris, ciliary body, lens, zonules, and pupil (Figure 8).

The Cornea

The cornea is the transparent front part of the eye that covers the iris, pupil, and anterior chamber. It is a powerful refracting surface providing most of an eye's optical power. The cornea is extremely sensitive and has more nerve endings than anywhere else in the body. Together with the lens, the cornea refracts light and as a result helps the eye to focus (14,158).

Aqueous Humour

The aqueous is the tissue fluid that fills the space between the cornea and the iris (anterior chamber) and the space between the iris and the front face of the vitreous (posterior chamber). It is continually produced by the ciliary body. This fluid nourishes

the cornea and the lens, structures that must be transparent and therefore devoid of blood vessels. The flow of the aqueous humour provides the appropriate intraocular pressure that gives the front of the eye its form and shape (159,160).

The Iris

The iris consists of pigmented fibrovascular tissue known as a stroma. The stroma connects a sphincter muscle (sphincter pupillae), which contracts the pupil, and a set of dilator muscles (dilator pupillae) which open it. The back surface is covered by an epithelial layer two cells thick (the iris pigment epithelium), but the front surface has no epithelium. The outer edge of the iris known as the root is attached to the sclera and the anterior ciliary body (17).

The Pupil

The pupil is the opening in the center of the iris. It appears black because most of the light entering is absorbed by the tissues inside the eye. The size of the pupil determines the amount of light that enters the eye. The pupil size is controlled by the dilator and sphincter muscles of the iris (17).

The Zonule of Zinn (Suspensory Ligaments)

The zonule is a ring of fibrous strands connecting the ciliary body with the lens of the eye. The zonules hold the lens in place and function to change the focusing power of the eye by changing the tension of the zonules by contraction and relaxation of the ciliary muscle (19).

The Ciliary Muscle

The ciliary muscle is a smooth muscle that affects zonules in the eye (fibers that suspend the lens in position). During accommodation these muscles enable changes in the lens shape for light focusing (19).

The Lens

The lens or crystalline lens is a transparent, biconvex structure in the eye that along with the cornea helps to refract light to focus on the retina. The lens is flexible and its curvature is controlled by ciliary muscles through the zonules (15).

Conjunctiva

The conjunctiva is a thin, transparent tissue covering the outer surface of the eye. It begins at the outer edge of the cornea, covering the visible part of the sclera, and lining the inside of the eyelids. The conjunctiva receives nourishment from tiny blood vessels and functions to secrete oils and mucous that moisten and lubricate the eye (161).

The Posterior Segment

The posterior segment comprises the remaining internal structures which lie posterior to the anterior segment. This includes: the retina, macula, fovea, sclera, choroid, vitreous humour, and optic nerve (Figure 9).

The Retina

The retina is a thin multi-layered transparent membrane of neural cells that lines the inner surface of the large posterior segment of the eyeball (20,21). It contains millions of photoreceptors that capture light rays and convert them into electrical impulses which are sent to the brain via the optic nerve and are transformed into images. These photoreceptors can be divided into cones and rods. The cones which function best in bright light and allow us to appreciate color are concentrated in the macula, particularly within the fovea, the very center portion of the macula, the portion of the retina responsible for central vision, but are present in decreasing density in the mid-periphery and periphery. The rods which function best in dim light are responsible for peripheral and night vision, are not present in the macula but are spread throughout the peripheral retina (20).

The retina has ten distinct layers (Figure 24). Starting with the innermost layer to the outermost layer, they include (21):

1. Internal limiting membrane
2. Nerve fiber layer
3. Ganglion cell layer - Layer that contains nuclei of ganglion cells and gives rise to optic nerve fibers.
4. Inner plexiform layer
5. Inner nuclear layer
6. Outer plexiform layer
7. Outer nuclear layer

8. External limiting membrane - Layer that separates the inner segment portions of the photoreceptors from their cell nuclei.
9. Photoreceptor layer - Rod and Cone cells
10. Retinal pigment epithelium

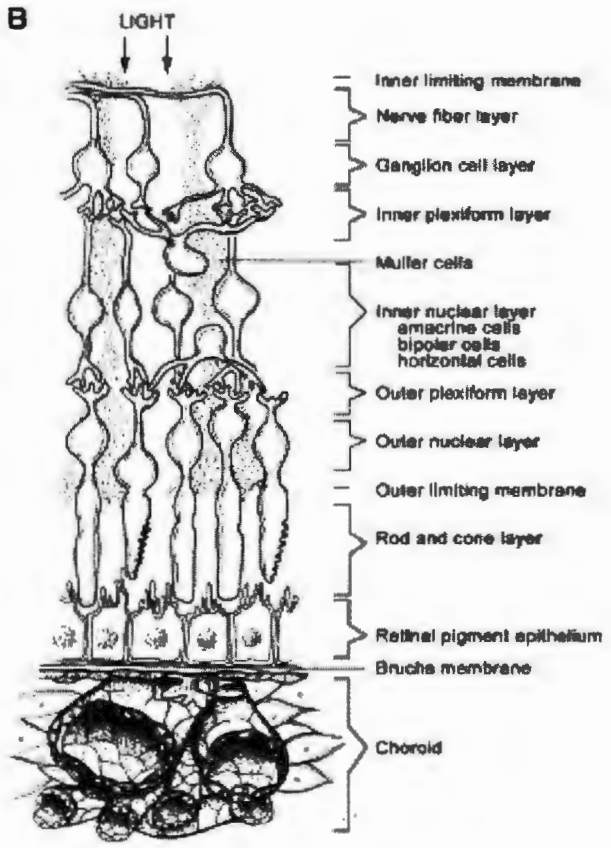

Figure 25. A schematic illustration of the ten anatomical layers of the retina and choroid. (Illustration by Adrienne J. Boutwell and Lisa J. Birmingham © University of Illinois Board of Trustees 2002.)

The Macula

The macula is located roughly in the center of the retina, temporal to the optic nerve. It is a small and highly sensitive part of the retina responsible for detailed central

vision. The fovea is the very center of the macula. The macula allows us to appreciate detail and perform tasks that require central vision such as reading (20,21).

The Sclera

The sclera is commonly known as the white of the eye. It is made of tough fibrin connective tissue that serves as the eye's protective outer coat (14).

The Choroid

The choroid lies between the retina and sclera. It is the vascular layer of the eye that provides oxygen and nourishment to the outer layers of the retina (162).

The Vitreous Humour

The vitreous humour is the clear aqueous solution that fills the space between the lens and the retina of the eyeball. The solution is 99% water and comprises about 2/3 of the eye's volume, giving it form and shape (21,24).

The Optic Nerve

In each eye the optic nerve connects the retina to target areas of the brain. These nerves transmit visual information from the retina to the brain. The optic nerve is not a peripheral nerve, but rather it is a white matter tract of the central nervous system (CNS), projecting outside the confines of the cranium (25).

B. Previous Newfoundland studies: Literature review

This section will provide an overview of the relevant study findings and the clinical work that has been published on several inherited eye diseases in Newfoundland such as, Bardet-Biedl Syndrome (BBS), Newfoundland Rod-Cone Dystrophy (NFRCD), and Ocular Albinism. The following papers are included:

1.) Hereditary diseases as causes of blindness in Newfoundland: preliminary report. Green JS, Johnson GJ. *Canadian Journal of Ophthalmology.* 1983 Oct; 18(6): 281-4, 1983. **(147)**

2.) The burden of genetically determined eye disease. Green JS, Bear JC, Johnson GJ. *The British Journal of Ophthalmology.* 1986 Sep; 70(9): 696-9. **(148)**

3.) Newfoundland rod-cone dystrophy, an early-onset retinal dystrophy, is caused by splice-junction mutations in RLBP1. Eichers ER, Green JS, Stockton DW, Jackman CS, Whelan J, McNamara JA, Johnson GJ, Lupski JR, Katsanis N. *American Journal of Human Genetics.* 2002 Apr; 70(4): 955-64. **(43)**

4.) Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. Moore SJ, Green JS, Fan Y, Bhogal AK, Dicks E, Fernandez BA, Stefanelli M, Murphy C, Cramer BC, Dean JC, Beales PL,

Katsanis N, Bassett AS, Davidson WS, Parfrey PS. *American Journal of Medical Genetics.* 2005 Feb 1; 132(4): 352-60. **(79)**

5.) Autosomal recessive Bardet-Biedl syndrome: first-degree relatives have no predisposition to metabolic and renal disorders. Webb M, Dicks EL, Green J, Moore S, Warden G, Gamberg J, Davidson WS, Young T, Parfrey PS. *Kidney International.* 76(2): 215-23, 2009. **(84)**

6.) Ocular albinism in Newfoundland. Johnson GJ, Gillan JG, Pearce WG. *Canadian Journal of Ophthalmology.* 1971 Oct; 6(4): 237-48. **(163)**

7.) Genetic mapping of X-linked ocular albinism: linkage analysis in a large Newfoundland kindred. Charles SJ, Green JS, Moore AT, Barton DE, Yates JR. *Genomics.* 1993 Apr; 16(1): 259-61. **(149)**

"Hereditary diseases as causes of blindness in Newfoundland: preliminary report":

This paper reports the underlying etiology of blindness for the registered blind population in the province of Newfoundland and Labrador in 1981. The authors state that accurate statistics on the causes of blindness and the number of people affected is needed to direct preventive efforts and to monitor trends in incidence.

The records of all 1,013 people registered with the Canadian National Institute of the Blind (CNIB) in Newfoundland were reviewed by Dr. Jane Green before December 31, 1981. There was an effort made by Dr. Green to establish the underlying pathological process or clinical entity rather than the site or secondary effects of the lesion. If it was observed that the diagnosis differed in the two eyes, the diagnosis for the second eye was used. If multiple diagnoses were given in the reviewed reports with no order specified, and the person could not be examined for other reasons, then the cause was listed as undetermined . The coding used by the CNIB was adapted from the classification of the National Society for the Prevention of Blindness. There are separate codes for diagnosis (site and type of lesion) and for etiology. Two of the codes for etiology denote probable or possible hereditary eye disease. Where there was some ambiguity in the diagnosis or etiology, every attempt was made to arrive at the precise diagnosis by examining the person, by writing to other ophthalmologists within or outside the province or by researching the ancestral pedigree . At the time of this publication, 193 of the 1,013 patients had been examined, with only 41 showing some ambiguity. Also reported was that 778 of the 1,013 cases of blindness within the province were unambiguous as to the cause of that blindness and agreed with the CNIB codes.

One major advantage reported in this study was the ability to access local information and to examine some of the patients who were in the undetermined category. This meant the proportion of cases in the undetermined category was greatly reduced compared to other previous studies.

Finally this paper stresses the importance of providing education about these conditions and their prevention to all affected people and their family members. Genetic

counseling should be more widely available for the people dealing with these conditions as well as visual aids and other educational assistance to help the individuals fit into the mainstream of the community.

"The burden of genetically determined eye disease":

This paper reports the underlying etiology of blindness for the registered blind population in the province of Newfoundland Labrador in 1984. This is a follow up and concluding report to the previous "Hereditary diseases as causes of blindness in Newfoundland: preliminary report" paper from 1981. Here the authors show analyses comparing the numbers of registrants in 1981 and 1984. It also reports an interest in the relative burden to the population of different causes of blindness, and therefore the 'person-years of blindness' was determined for the different categories as an index of the personal and population impact.

Researchers obtained the ages of death of all registrants dying in the years 1981-1984, along with their ages at registration. From these numbers they calculated the mean ages of registration for each major etiological category, the duration of blindness to the nearest whole year of each deceased registrant, and the mean duration of blindness for each major etiological category. For comparison the mean ages of registration for all registrants and for those first registered in the last five years were calculated. For each etiological category they multiplied the mean duration of blindness for deceased registrants (1981-1984) by the overall number of patients in the category as at December

31st 1984 to arrive at "person years of blindness", an indicator of the relative burden to the population of each major cause of blindness.

The overall individual and population impact of monogenic blindness is overwhelmingly greater than that for all other causes because of the much younger age of registration (25 years on average) and long survival with blindness (21 years on average of "person years of blindness"). The researchers stress that while it remains of great importance to provide support through low-vision services, itinerant teachers for the visually impaired, and employment counseling, much more attention should be paid to elucidating the genetic component of blindness.

"Newfoundland rod-cone dystrophy, an early-onset retinal dystrophy, is caused by splice-junction mutations in RLBP1":

This paper reports on a specific retinal dystrophy in Newfoundland consistent with retinitis punctata albescens but with a substantially lower age at onset and more-rapid and distinctive progression, a disorder termed "Newfoundland Rod-Cone Dystrophy" (NFRCD). Symptoms such as night blindness were evident during infancy; followed by the progressive loss of peripheral, central, and colour vision during childhood. Severe loss of vision is seen by the 2nd to 4th decade of life.

Twenty-six patients (ages 5 years to 56 years) from six families were ascertained through 12 probands referred to the Memorial University of Newfoundland Ocular Genetics Clinic. It is reported that 19 of these patients had lived within a 10-mile radius

of each other in a Newfoundland area that, until recently, had remained isolated, being settled during the mid-18th century by immigrants from southwestern England. In an attempt to identify any connection between families a detailed history of each of the families was obtained from each proband. Older relatives within the families were interviewed, as well as archives being searched. However, a single common ancestor was not identified.

Most of the 26 subjects had been followed for more than 22 years, with ophthalmological examination, visual-field testing, color-vision testing, dark-adaptation testing, electroretinography (ERG), retinal photography, and fluorescein angiography. Blood samples for DNA extraction were also collected.

The size of one of the families was sufficiently large (15 persons) to allow the researchers to perform a genomewide screen to map the NFRCD locus. Significant linkage to markers on the long arm of chromosome 15 (15q26 section), in a region encompassing *RLBP1* was detected. This gene is involved with encoding the cellular retinaldehyde–binding protein. As stated in previous reports cited throughout this paper , mutations in *RLBP1* have been associated with other retinal dystrophies such as Bothnia dystrophy, retinitis punctata albescens, retinitis pigmentosa, and fundus albipunctatus, which led to the researchers hypothesizing that *RLBP1* mutations might also cause NFRCD.

To test their hypothesis researchers sequenced the seven coding exons and splice junctions of *RLBP1*. They detected two sequence alterations, both likely to be pathogenic, since each segregated with the disease and was predicted to interfere with mRNA splicing. They stated that in contrast to some previously reported *RLBP1*

mutations, which yielded a protein that may retain some residual activity, each NFRCD mutation was likely to give rise to a null allele. This difference may account for the severe phenotype in these families and exemplifies the molecular continuum that underlies clinically distinct but genetically related entities .

The two alterations found were: 1) a G→A transition (324G→A) in the last base of exon 3, and 2) a T→C transition in the second base of intron 3 (IVS3+2 T→C). Comparison of the haplotypes of the disease-carrying chromosomes in the six pedigrees also reveals two distinct haplotypes associated with the 324G→A mutation and two haplotypes associated with the IVS3+2 T→C mutation. This suggests either that both mutations were introduced into Newfoundland through two independent mutation events on two different chromosomes or that each mutation is relatively old and has undergone haplotype divergence.

"Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study":

This paper is a prospective, population-based study, with comprehensive ascertainment, and follow-up for 22 years. The objectives of the study were: (a) to determine if Bardet-Biedl syndrome (BBS) and Laurence-Moon syndrome (LMS) are a single disorder, (b) to describe the epidemiology of BBS, (c) to extend the phenotype, and (d) to determine if there are genotype-phenotype correlations in BBS.

This study's cohort involved 46 patients, 26 being male and the remaining 20 female, from 26 families throughout the province. The reported range of age was 1.5 years to 67.9 years, with a median age of 44 years. To be enrolled in this study the patients must have had a retinal dystrophy and other features suggestive of BBS, such as renal structural abnormalities, obesity, dysmorphic extremities, and hypogenitalism.

The identification of affected patients started in 1979, with the initial and most significant tool being the registry of the CNIB. These patients gained registry as a result of their attendance at an Ocular Genetics Clinic, or through subsequent family studies completed. Hospitals were also canvassed for patients having any mention in their medical records as having BBS or LMS, however no new patients were located.

The prevalence of BBS in Newfoundland at the time of this study was approximately 1 in 18,000. This is significantly higher than other populations around the world as determined by a study from 1970 on BBS in Switzerland which reported a prevalence of 1 in 160,000. It is reported that 31 percent of the people in the 26 families involved in the Newfoundland study were affected. Founder effects, consanguinity, and large sibship size likely increased the prevalence of BBS in Newfoundland.

The results of the study show the following known BBS loci and the number of patients involved with each: eight patients have mutations in *BBS1*, one has a mutation in *BBS2*, five have mutations in *BBS3*, five have mutations in *BBS5*, and fifteen have mutations in *BBS6*. There were six patients excluded from all eight known BBS loci because the molecular investigations were inconclusive due to family structure. Six patients were excluded from the analysis due to no DNA being available.

This study gives evidence that BBS and LMS should be considered the same disorder. Through comprehensive ascertainment, the researchers involved attempted to identify all the cases of both disorders within Newfoundland. Two of the 46 patients met the criteria for LMS; however, they had mutations in BBS genes. This implies that the underlying molecular basis of both BBS and LMS is the same.

The clinical manifestations observed in the 46 patients included: blindness, dystrophy of extremities, obesity, diabetes mellitus, hypertension, renal abnormalities, genital and reproductive abnormalities, neurological, speech, and psychiatric abnormalities, craniofacial dysmorphology, gall stone disease, colonic disorders, asthma, congenital heart disease, early death, and other miscellaneous disorders. The abnormalities in most organs of the human body are consistent with defects in the terminal maturation of organs.

"Metabolic and Renal Events in Autosomal Recessive Bardet-Biedl (BBS) Syndrome and in First Degree Relatives With and Without a BBS Mutation: A population and family based study":

Researchers involved with this report state they have now identified the molecular genetic cause of BBS in all cases in Newfoundland from whom DNA has been collected. In addition they have determined which first or second degree relatives of cases are and are not carriers of a BBS mutation. They have performed the fourth assessment of these

cases over 28 years of prospective follow-up, and investigated first degree relatives for the first time.

Forty-six cases from members of 26 families were identified. Of 136 siblings, 35% had BBS. DNA was obtained from 21 families, and the molecular genetic cause of the disease was identified in all cases, amounting to nine mutations in six BBS genes being identified. The researchers conclude that the high prevalence of BBS in Newfoundland is likely the result of the high coefficient of kinship in multiple genetic isolates and the frequency with which different pathogenic BBS mutations may occur.

"Ocular albinism in Newfoundland":

This publication reports the initial discovery of ocular albinism within Newfoundland. A large family (200 persons) located in the New World Islands area, in Notre Dame Bay, just off the north east coast of the province, was identified and a pedigree of six generations was traced back to a woman who lived in Moreton's Harbour, approximately 130 years ago. This family stemmed from early settlers who came from England. During examination of ten affected males it was found that all had features described for ocular albinism, which included: impaired visual acuity, photophobia, nystagmus and a generalized deficiency of pigment in the retinal pigment epithelium. From previous studies, this condition was known to be due to an abnormal gene on the X chromosome. Males, with only one X chromosome show the full effect of the mutant gene, while females known to be carrying the abnormal gene on one of their two X

chromosomes (heterozygote carrier) have no functional disability but may show a patchy alteration in retinal pigmentation. Usually the term "carrier" is defined on the basis of genetic and not phenotypic status, however in this study it was stated this way and one goal of the study was to establish that the patchy depigmentation seen in the "carriers" was a reliable indicator of the heterozygote state. Unaffected individuals have neither the functional disability nor show any alteration in retinal pigmentation.

Results reported indicate that a total of 29 affected males along with 50 carrier females were examined. The progeny of affected males were reported as male children not showing any ocular impairment, with results stating there were 40 unaffected sons and no affected sons, while all female children showed signs of a patchy alteration in retinal pigment, with results reporting 26 carrier daughters and no unaffected daughters. The progeny of carrier females were reported showing many children with the presence and absence of an ocular impairment or a patchy alteration in retinal pigment, with results stating there were 40 affected sons and 28 unaffected sons, as well as 20 carrier daughters and 28 unaffected daughters.

The majority of affected individuals had a visual acuity of 20/200 or less and were registered as blind. It was reported that reading vision was in general not so severely affected as distance vision, which causes considerable problems for children, having to hold books very close to their face and having trouble reading a blackboard in school. Although their vision was on the borderline of legal blindness, some affected individuals were able to work satisfactorily in the fishing or forestry industries as the requirements for excellent acuity were not as great as newly introduced trades. The investigators

stressed that unless the next generation of individuals decides to limit its reproduction, the number of people have ocular albinism in the future will increase considerably.

"Genetic mapping of X-linked ocular albinism: linkage analysis in a large Newfoundland kindred":

This short communication reports on a genetic linkage study in a large Newfoundland family affected by X-linked ocular albinism (OA1). The family as reported in a previous publication "Ocular albinism in Newfoundland" is from the New World Island area, in Notre Dame Bay, just off the north east coast of the province. X-linked ocular albinism is stated to cause reduction is visual acuity and nystagmus in males, associated with iris translucency and foveal hypoplasia. Carrier females have normal vision, but ophthalmic examination may show a characteristic "mud-splattered" fundus appearance. Venous blood samples were obtained from 91 individuals (including 18 affected males, and 36 obligate carriers females) for DNA extraction and Xg blood grouping. The Xg blood group system, a classification of human blood based on the presence of proteins called Xg antigens on the surfaces of red blood cells, was used since this is the only blood group in which the antigen-encoding genes are located on the X chromosome. Discovery of the Xg system in 1962 greatly aided mapping of the X chromosome (164).

The researchers used DNA markers DXS237, DXS143, and DXS85 during the study. Results reported from this analysis showed linkage to markers from chromosome

Xp22.3. Also one recombinant mapped the disease proximal to DXS143 and two recombinants mapped the disease distal to DXS85. These results are confirmed with other studies involving 16 British families also showing close linkage between OA1 and DXS143.