Phenotyping and Genotyping of Idiopathic Infantile Nystagmus

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Background: Nystagmus can be a manifestation of ocular or systemic disorders. However, it may represent a separate disease entity by itself as in idiopathic infantile nystagmus (IIN). In 2004, Kerrison et al. localised the gene causing X-linked IIN to Xq26-27 (NYS1); however, the gene/genes causing IIN had not been identified.

Aims and Objectives: The aims of this study were threefold.

1. To ascertain families and singletons (sporadic subjects) with IIN.
2. To further refine the locus NYS1 and to identify the gene causing X-linked IIN
3. To describe and compare the phenotype of subjects with IIN

Methods: 39 families and 78 singletons with nystagmus were recruited and phenotyped. Genotyping with microsatellite markers were performed in the X-linked families to refine the genetic interval at Xq26-27. Gene sequencing was carried out by our collaborators (not by the author) at the Sanger Institute. The clinical features and eye movement recordings of 90 subjects with mutations in the *FRMD7* gene were compared to 48 subjects with IIN not associated with mutations in this gene (non-*FRMD7* group).

Results:

I: 149 familial subjects and 78 sporadic subjects with IIN were phenotyped. 121 subjects from 30 families were diagnosed to have X-linked IIN while 28 subjects from 9 families had other diagnosis such as albinism and aniridia.

II: Genetic mapping in 16 families with X-linked IIN, refined the critical interval at Locus NYS1 (Xq26-17) to a 9mB region between markers DXS8072 and DXS8094 which contained about 80 genes. High throughput DNA sequencing was carried out at the Sanger Institute which led to the discovery of *FRMD7*, mutations in which is associated with X-linked IIN.

III: The median visual acuity in subjects with a *FRMD7* mutation was log MAR 0.301. The number of subjects with good stereopsis (Lang positive) was higher in the *FRMD7* group (93.4%) compared to subjects in the non- *FRMD7* group (78.4%). None of the subjects in the *FRMD7* group had severe (>15˚) anomalous head posture (AHP) while 27% of subjects in the non-*FRMD7* group had AHP more than 15˚.
52.17% of obligate female carriers of a *FRMD7* mutation were clinically affected.

Discussion: This study identified the first gene causing idiopathic infantile nystagmus. The phenotypic characteristics of these subjects will help in clinically identifying subjects with IIN due to mutations in *FRMD7*. In addition it has generated a stage for further research into the mechanisms behind ocular motor control.

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1 Introduction

Nystagmus consists of repetitive to and fro movements of the eye/eyes. The term nystagmus arise from the Greek word 'νυσταγμός'(*nystagmos*) which is used to describe the head movements of a person in a drowsy state; typically a slow downward drift followed by a corrective quick upward movement. Nystagmus is described as *pendular* when it consists of sinusoidal slow-phase oscillations. If the slow phases are followed by corrective quick phases, then it is called *jerk nystagmus*. Nystagmus can be a manifestation of various ocular and systemic disorders. However, it may represent a separate disease entity by itself as in idiopathic infantile nystagmus.

1.1 Terminology

In 1967 Cogan classified congenital nystagmus into four predominant types: sensory-defect nystagmus, motor-defect nystagmus, latent nystagmus and periodic alternating nystagmus.[1] Nystagmus was considered 'sensory' if it is associated with a defect in the afferent visual system, and 'motor' if due to a deficit in the efferent mechanism. Cogan described sensory nystagmus as predominantly pendular and motor nystagmus as jerky.[1] Although there is a large overlap of nystagmus waveforms between groups, it has been confirmed that waveforms in certain types of nystagmus associated with afferent pathway disorders such as achromatopsia, congenital stationary night blindness or low vision are different from idiopathic nystagmus.[2-4]

Infantile nystagmus has its onset within up to six months after birth. It was previously called congenital nystagmus, however, the term 'infantile nystagmus' is preferred

because in many patients, abnormal eye movements do not occur immediately at birth.

Nystagmus originating in the first months of life has been termed as 'infantile nystagmus syndrome' by the committee for the Classification of Eye Movement Abnormalities and Strabismus Workshop sponsored by the National Eye Institute of the United States of America.[5] This classification is mainly based on eye movement recordings and does not consider the differences between various underlying disorders such as ocular albinism and rod monochromacy, which would be helpful for the clinician.

1.2 Classification of Nystagmus

Physiological nystagmus includes optokinetic nystagmus, end point nystagmus and vestibular nystagmus. Pathological nystagmus can be classified based on the time of onset into *early onset*, i.e. infantile nystagmus (onset before 6 months of age) and *late onset*, i.e. acquired nystagmus (onset after 6 months).

1.2.1 Infantile Nystagmus

Infantile nystagmus can be idiopathic or it may be associated with a number of ocular or systemic conditions ranging from common co-morbid conditions such as albinism and strabismus, to rare syndromes and metabolic disorders.

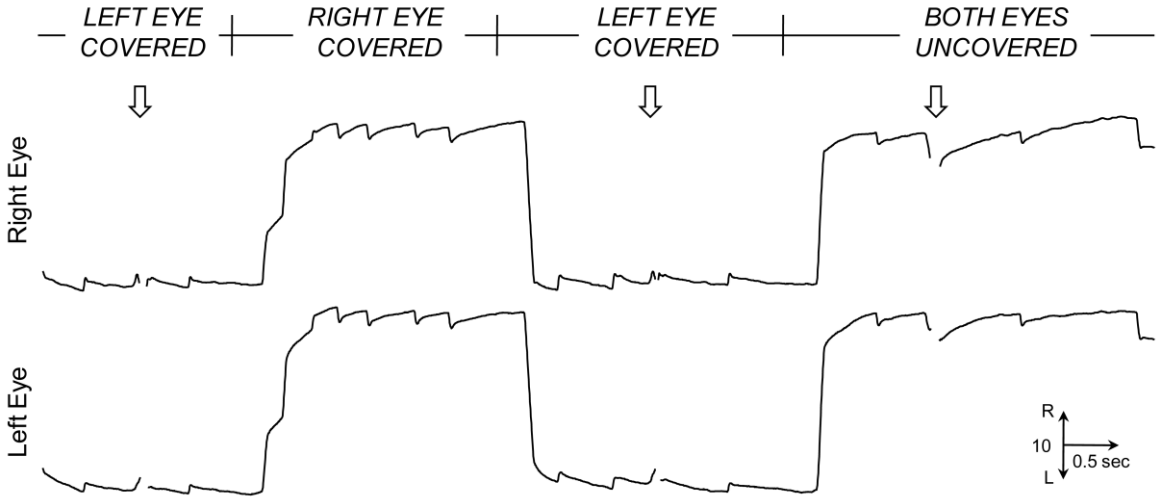
1.2.1.1 Idiopathic infantile nystagmus

Idiopathic infantile nystagmus (IIN) has onset at birth or within 6 months of life. It can be sporadic or familial; of the familial forms, X-linked inheritance is the commonest.[6, 7] IIN is described in detail later in this chapter.

1.2.1.2 Latent and manifest latent nystagmus

Latent and manifest latent nystagmus are most commonly associated with strabismus.[8] Manifest latent nystagmus (MLN) is a horizontal jerk nystagmus that increases on covering one eye (Figure 1.1). The fast phase of the nystagmus beats towards the fixing eye and it has decelerating or linear slow phase velocity.[9] Latent nystagmus is similar to MLN; however the nystagmus is present only on monocular viewing. True latent nystagmus (no nystagmus when both eyes are open) is very rare as most of these patients, when examined with eye movement recordings, have minimal or subclinical nystagmus when both eyes are open.[9, 10]

Figure 1.1: *Eye movement recording in a subject with manifest latent nystagmus. The fast phase of the nystagmus is towards the fixing eye. When both eyes are open, the nystagmus is left-beating.*



1.2.1.3 Spasmus nutans

This disorder consists of a triad of nystagmus, head nodding and anomalous head posture. Unlike other types of infantile nystagmus, spasmus nutans has a later onset, usually before the first birthday or even later.[11] The nystagmus is often disconjugate (occasionally monocular) and is of high frequency and low amplitude. Unlike other forms of nystagmus, in spasmus nutans head nodding suppresses nystagmus.[12] Signs and symptoms improve with time. It usually subsides by the age of 2-4 years, even though subclinical nystagmus may persist longer.[13] Intracranial tumors such as gliomas and other abnormalities of the anterior visual pathways can present with nystagmus mimicking spasmus nutans and therefore a thorough clinical work up and neuro-imaging is required to exclude these conditions.[14-16]

1.2.1.4 Nystagmus Associated with ocular diseases

1.2.1.4.1 Albinism

Albinism comprises a group of genetic disorders in which there is congenital hypopigmentation of hair, skin and eyes or the eyes alone. It may be associated with systemic disorders.

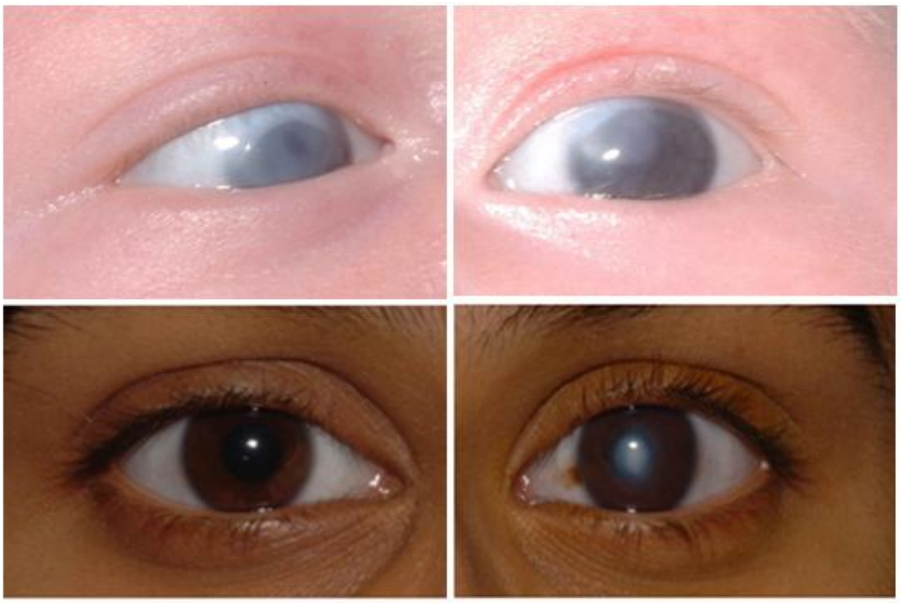
X-linked and autosomal recessive forms of ocular albinism are known. Ocular albinism type 1 (MIM300500) is caused by mutations in the *OA1* (Xq22.32) and it affects pigment production in the eye.[17, 18] Autosomal recessive ocular albinism (AROA) is genetically heterogeneous. Most of the patients with AROA represent clinically mild oculocutaneous albinism.[19]

Oculocutaneous albinism (OCA) is genetically heterogeneous and is characterised by congenital hypopigmentation of the eyes, skin and hair. OCA1A and OCA1B are caused by mutations in the *TYR* gene (11q14-q21). Oculocutaneous albinism type II is caused by mutations in the OCA2 gene (15q11.2-q12). OCA3 and OCA4 are caused due to mutations in *TYRP1* and *SLC45A2* genes.

1.2.1.4.2 Anterior segment abnormalities and cataract

Peters anomaly (MIM 604229) is a rare developmental anomaly which is characterized by corneal opacity (Figure 1.2), absence of Descemet membrane and the presence of irido-corneal and kerato-lenticular adhesions. Most cases are sporadic. In familial forms, autosomal recessive and dominant inheritance have been reported. Mutation in *PAX6* (11p13) was found in a family with Peters anomaly of autosomal dominant inheritance and variable expression.[20] Mutations in *CYP1B1* (2p22.2)[21], *PITX2* (4q25)[22], and *FOXC1* (6p25)[23] have been found to be associated with Peters anomaly.

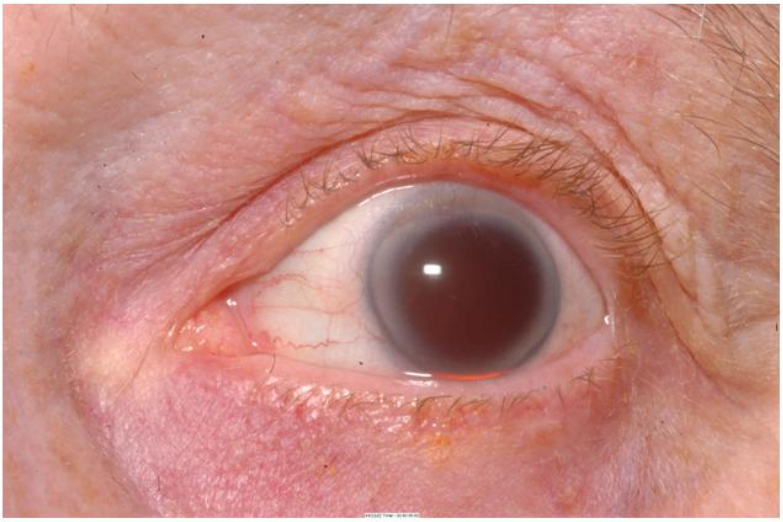
Figure 1.2: *Peters anomaly is bilateral in about 80% of cases. The top panel shows bilateral corneal opacities in an infant with Peters anomaly. The bottom panel shows a child with unilateral disease.*



Aniridia (MIM106210) (Figure 1.3) is a congenital bilateral ocular condition of variable expressivity and is characterized by incomplete development of the iris. Most commonly aniridia is associated with mutations in *PAX6* (11p13).[24,25] Other associated ocular manifestations include foveal hypoplasia, optic nerve hypoplasia, glaucoma, cataracts, ectopia lentis, strabismus and nystagmus.[26] A third of cases of aniridia are sporadic. In familial cases, the inheritance is autosomal dominant. **Gillespie syndrome** (MIM 206700) consists of aniridia, mental retardation and cerebellar ataxia. Autosomal recessive inheritance is most common. A mutation in *PAX6* has been reported in one possible case.[27] **WAGR syndrome** (MIM194072) consists of increased susceptibility to Wilms' tumour, aniridia, genitourinary abnormalities, and mental retardation. It is a

contiguous gene deletion disorder involving the *WT1* and *PAX6* genes at chromosome 11p13.[28-30]

Figure 1.3: *Near total absence of iris in a subject with aniridia.*



Congenital Cataracts of various aetiologies can be associated with nystagmus. Mutations in *CRYAA* (21q22.3) are associated with autosomal dominant congenital cataract (MIM123580).[31] Congenital cataract associated with nystagmus may occur in mutations of the gamma-D-crystallin gene (CRYGD) (2q33-q35).[32] A syndrome of microphthalmia, congenital cataract and nystagmus (MIM212550) caused by mutation in *SIX6* (14q23) was reported by Gallardo et al.[33] Mutations in *PAX6* has also been reported in cases with foveal hypoplasia, congenital nystagmus and presenile cataracts.[34]

Furthermore, there are a number of syndromes associated with congenital cataract.

Nance-Horan Syndrome (cataract-dental syndrome)(MIM302350) is an X-linked disorder characterized by congenital cataracts, dental abnormalities, and facial features such as anteverted pinnae. Other associated findings include developmental delay, microcornea, microphthalmos and nystagmus.[35] It is caused by mutations in the

NHS gene.[36] **Marinesco-Sjögren syndrome** (MIM248800) is associated with bilateral congenital/infantile cataracts. It is inherited as an autosomal recessive condition and is characterized by sensorymotor neuropathy, cerebellar ataxia, nystagmus and progressive myopathy. Other associated features include mental retardation and hypergonadotropic hypogonadism.[37] Mutations in the *SIL1* gene (5q31) which encodes a nucleotide exchange factor for the heat-shock protein HSPA5, has been found to be causative.[37]

Sengers Syndrome (MIM212350) is characterized by congenital cataracts and cardiomyopathy. The inheritance is of autosomal recessive type and the 12 patients reported by Cruysberg et al, developed total bilateral cataracts in the first week of life.[38] **Oculocerebrorenal syndrome of Lowe** (MIM309000) is caused by mutations in the *OCRL* gene (Xq26.1) and is characterized by congenital cataracts, renal tubular dysfunction and mental retardation.[24, 39] **Hallermann-Streiff syndrome** (MIM234100) is also called François Dyscephalic Syndrome and oculo-mandibulo-facial syndrome. It is characterized by bird-like facies with hypoplastic mandibles, dental anomalies, proportionate dwarfism, microphthalmos and congenital cataracts.[40, 41] **Familial remitting chorea** (MIM601372) described by Wheeler et al. in 1993, is characterized by monocular nystagmus, cataract and self-remitting chorea. The nystagmus improved by the age of six in one of the affected subjects[42]. This association has not been reported since then. **Karsch-Neugebauer syndrome** (Nystagmus-Split Hand Syndrome) (MIM183800) as the name suggests, comprises of congenital nystagmus, spilt hand and split foot.[43] Pendular nystagmus associated with malformation of the limbs in two sisters with Karsch-Neugebauer syndrome was reported by Wong et al.[44] The

inheritance is considered to be autosomal dominant. This syndrome can be associated with congenital cataracts.

1.2.1.4.3 Retinal Disorders and associated syndromes

Early onset **Cone-Rod Dystrophies** can cause nystagmus. X-linked cone-rod dystrophy (CORDX1) (MIM304020) is caused by mutations in exon 15 of the Retinitis Pigmentosa GTPase Regulator (RPGR) gene (Xp21.1). The symptoms include reduced vision, photophobia and colour blindness.

Congenital stationary night blindness (CSNB) (MIM 310500 and 300071) is clinically and genetically heterogeneous. X-linked and autosomal recessive forms present with non-progressive visual loss and nyctalopia, whilst the autosomal dominant form has normal visual acuity, but presents with nyctalopia. X-linked CSNB can be complete (non-recordable rod-specific ERG) or incomplete (detectable rod-specific ERG). Mutations in *NYX* (Xp11.4)[45] causes complete CSNB (CSNB1) while *CACNA1F* (Chromosome Xp11.23) is implicated in incomplete CSNB (CSNB2).[46] Mutations in *GRM6* (Chromosome 5q35) and *CABP4* (Chromosome 11q13.1) causes autosomal recessive CSNB (type 1B and type 2b respectively).[47] Autosomal dominant CSNB is caused by mutations in *RHO* gene (3q21-q24), *PDEB* (4p16.3) and *GNAT1 gene* (3p21).

Achromatopsia is characterized by poor vision, defective colour vision, photophobia and nystagmus. It is autosomal recessive and may occur in the complete (typical) or incomplete (atypical) forms. The typical form is characterized by complete absence of colour vision and is due to mutations in genes which encode components for cone photo-transduction ((*CNGA3* (2q11.2), *CNGB3* (8q21.3) and *GNAT2* (1p13)).[48-50] Incomplete achromatopsia characterized by variable loss of cone function and normal

rod function could also be caused by mutations in *CNGA3* gene. **Blue cone monochromacy** (MIM303700) is an X-linked recessive disorder characterized by absence of L and M cone function. The L (red) and M (green) pigment genes consist of a tandem array of two or more repeat units and is located at chromosome Xq28 whilst the S (blue) pigment gene is located on chromosome 7. Mutations in the L and M gene array encoding the red and green cone pigments form the basis of blue cone monochromatism.[51, 52]

Macular disorders such as foveal hypoplasia and macular coloboma can be isolated or syndromic and are associated with nystagmus. Foveal hypoplasia could be caused due to mutations in *PAX6* gene, in addition to conditions such as aniridia and albinism. A family with bilateral macular coloboma with autosomal dominant inheritance has been reported in literature.[53] Bilateral macular colobomas could be associated with nystagmus and apical dystrophy of hand and feet (**Sorsby syndrome**)(MIM120400)[54] or Leber's congenital amaurosis.[55]

Norrie disease (MIM310600) is a neuro-developmental disorder causing blindness in infancy, characterized by incomplete retinal vascularisation leading to vitreous haemorrhage and retinal detachment.[56, 57] Patients usually present with poor vision and leukocoria. Systemic associations include sensory-neural deafness and other neurological defects. Mutations in the *NDP* gene (Xp11.4) which encodes *norrin*, a cysteine-rich protein is causative. The mode of inheritance is considered to be X-linked recessive, however, a case of manifesting heterozygote whose diagnosis was made on clinical grounds has been reported in literature.[58]

Bardet-Biedl syndrome (BBS) (MIM209900) is a genetically heterogeneous disorder and is characterized by retinal dystrophy (Figure 1.4), polydactyly, mental retardation and obesity. These patients can have nystagmus.[59]

Leber's Congenital Amaurosis [60] comprises a group of autosomal recessive retinal dystrophies. These patients have severe or moderately severe visual loss at or soon after birth. They have nystagmus, sluggish pupillary light reflex and absent or poor ERG responses. LCA is genetically heterogeneous. In the genetic subtype caused by mutations in *RPE65* (1p31), genetic replacement therapy has been found to be promising.[61,62] Patients can have a normal appearing fundus at birth; however, most develop features of retinitis pigmentosa later on in life (Figure 1.5).

***Figure 1.4**: Fundus picture of a subject with Bardet Biedl syndrome. Note the choroidal atrophy, narrow blood vessels, and pigmentation in the periphery.*

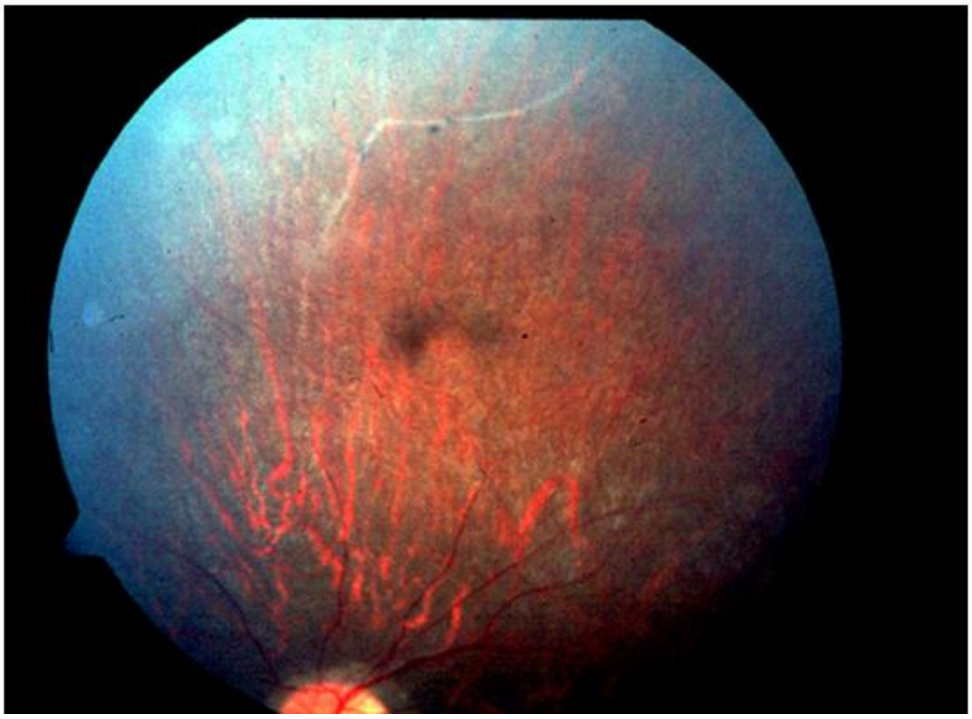
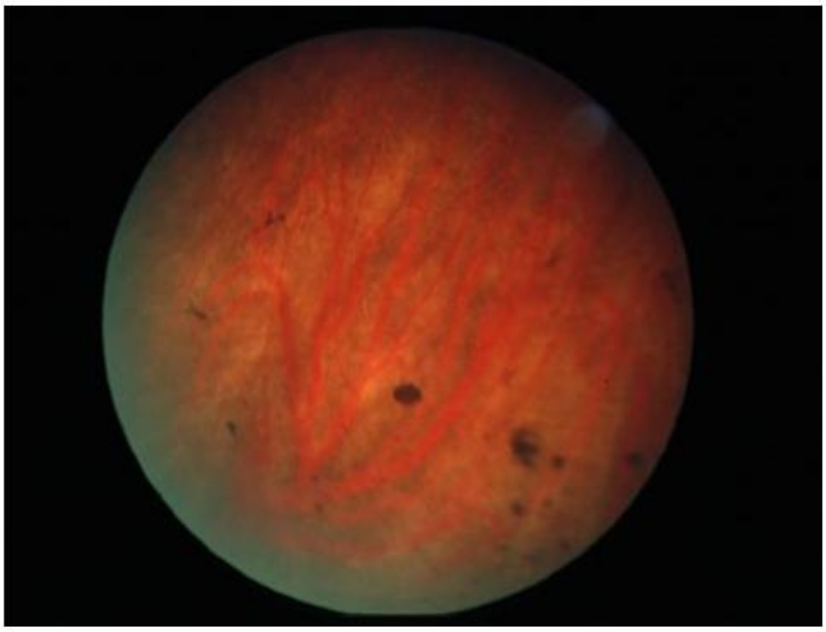


***Figure 1.5:** Fundus picture of a 19-year-old patient with Leber's congenital amaurosis. They may have a normal fundus appearance in early childhood. However, optic disc pallor and other features of retinitis pigmentosa develop later in life as shown in the figure.*

Senior-Loken syndrome is an autosomal recessive disorder and is genetically heterogeneous. It consists of familial juvenile nehronophthisis and retinal dystrophy. The retinal pathology can vary from pigmentary retinopathy[63] (Figure 1.6) , exudative retinopathy[63] and congenital retinal amaurosis.[64] Mutations in the *NPHP1* (2q13), *NPHP4* (1p36) and *NPHP5* (3q21.1) are causative. This phenotype has also been mapped to a locus on 3q22.

Figure 1.6: *Shows pigmentary retinopathy in a patient with Senior-Loken syndrome.*



Alström syndrome (MIM203800) is an autosomal recessive disorder characterized by childhood obesity, diabetes mellitus & hyperinsulinemia, neural deafness, cone-rod dystrophy and dilated cardiomyopathy.[65-67] The gene involved in Alström syndrome *ALMS1,* is localised to 2p13.[68] Patients develop nystagmus and photophobia during the first year of life. Phenotypic variability is the norm. They usually have profound visual loss in the first/second decade of life. This disorder is similar to Bardet Biedl

syndrome. However, in Alström syndrome, digital abnormalities are less common, and the progression of the disease is faster.[66]

Åland island eye disease (MIM300600) is an X-linked disorder, the first reported family being from Åland Island in the sea of Bothnia.[69] It is due to mutations in the *CACNA1F* gene characterized by hypopigmentation of the fundus, foveal hypoplasia, nystagmus, protan type of colour blindness, defective dark adaptation with reduced scotopic b-wave amplitudes on ERG and progressive myopia. Patients can have iris transillumination defects and the phenotype is similar to ocular albinism, however, this disorder is not associated with misrouting of the optic nerve fibers. Congenital stationary night blindness type 2 (MIM300071) is allelic.

Jeune syndrome which is also termed asphyxiating thoracic dystrophy (MIM 208500) is a rare form of chondrodysplasia, characterized by small thorax leading to respiratory insufficiency in infancy. [70, 71] It is associated with polydactyly, renal, hepatic and ocular abnormalities which include retinal dystrophy leading to night blindness, reduced visual acuity and subnormal ERG.[70, 72]

Joubert Syndrome (MIM 213300) is clinically and genetically heterogeneous. Familial agenesis/hypoplasia of cerebellar vermis (molar tooth sign on axial MR imaging), episodic hyperpnoea, abnormal eye movements, retinal dystrophy, ataxia and psychomotor retardation were delineated in four siblings by Joubert et. al in 1969.[73] This disorder and can also be associated with renal abnormalities (MIM609583).[74] Mutations in *AHI1* (6q23), *TMEM67* (8q21) and RPGRIP1L (16q12.2) are causative. Joubert syndrome is also caused by mutations in the NPHP1 and CEP290 genes (allelic to Senior-Loken syndrome).

Recently many of these conditions such as the Bardet-Biedl syndromes, Leber congenital amaurosis, Senior-Loken syndrome, Joubert syndrome and some of the cone-rod dystrophies are grouped together as **retinal ciliopathies**.[75] This term refers to a group of disorders associated with abnormal ciliary function of the photoreceptors. It was noted that photoreceptors axonemes and sperms were abnormal in Usher syndrome[76] and this led to the hypothesis that abnormal ciliary function could be the basis for the retinal degeneration.[75] Nystagmus can be a symptom in these syndromes.

1.2.1.4.4 Optic nerve disorders and associated syndromes

Optic nerve hypoplasia is characterized by reduced number of optic nerve fibers and this disorder can be unilateral or bilateral. *In-utero* exposure to drugs such as opiates and benzodiazepines can cause optic nerve hypoplasia[77] and it is a feature of foetal alcohol syndrome.[78] Bilateral optic nerve hypoplasia can be caused due to mutations in *PAX6* gene.[79]

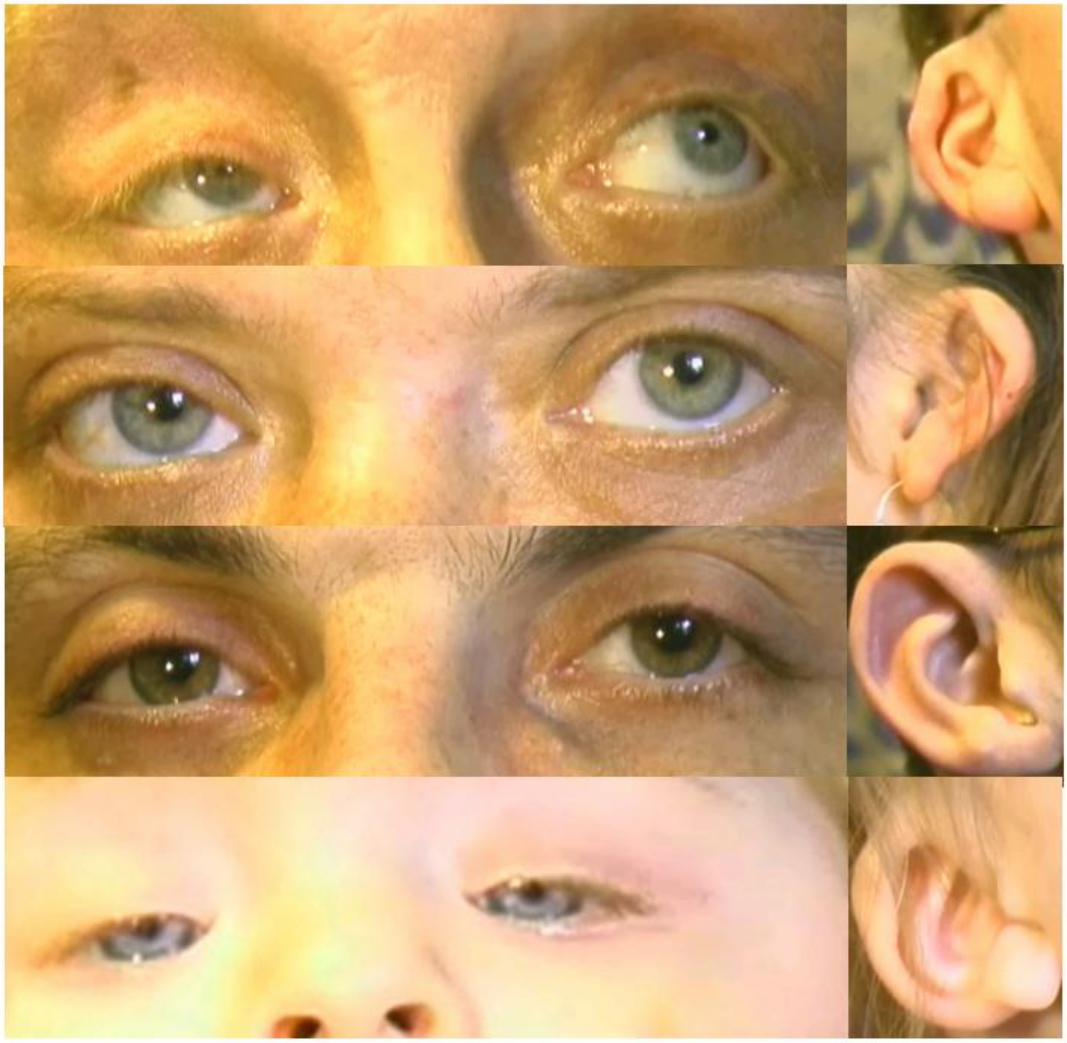
De Morsier syndrome (Septo-optic dysplasia) comprises of optic nerve hypoplasia, pituitary hypoplasia and midline brain abnormalities such as absent septum pellucidum and corpus callosum.[80, 81] Mutations in the homeobox gene *HESX1* (3p21.2-p21.1) has been implicated in causing the phenotype.[82]

Other causes of infantile nystagmus include optic nerve head coloboma associated with renal disease (papillo-renal syndrome) and early onset bilateral optic atrophy of the Behr type[83].

1.2.1.5 Craniosynostosis and Structural Anomalies

Craniosynostoses are a group of disorders in which there is premature closure of the cranial suture/sutures leading to distortion of the skull. Many of these patients can have nystagmus. **Apert syndrome** (MIM101200) is caused by mutation in the *FGFR2* gene (Chromosome 10q26) and is characterized by bicoronal craniosynostosis and symmetrical syndactyly of the hands and feet. It is associated with midface hypoplasia, cleft palate and number of ocular manifestations which include nystagmus.[84] Most reported cases are sporadic, but autosomal dominant inheritance is also reported.[85] **Crouzon syndrome** is one of the common craniosynostosis (MIM123500) and is an autosomal dominant condition. It is characterized by premature closure of the coronal and sagittal sutures resulting in shallow orbits, proptosis and hypertelorism and can be associated with amblyopia in 21% of cases and optic atrophy in 7% of cases.[86] Unlike Apert syndrome, abnormalities of the limbs are absent. **Baller-Gerold syndrome** (MIM218600) is characterized by craniosynostosis and radial abnormalities. Growth retardation and poikiloderma[87] are associated features. Nystagmus is also reported in the phenotypic spectrum.[88] **Saethre-Chotzen Syndrome** (MIM101400) is an autosomal dominant condition and is caused by mutations in the *TWIST* gene[60, 89] is associated with nystagmus in 30% of the cases.[84] It is characterized by coronal craniosynostosis, prominent forehead, low frontal hairline, facial asymmetry, maxillary hypoplasia and partial cutaneous syndactyly. Ocular features include blepharoptosis, strabismus and nystagmus (Figure1.7).

Figure 1.7: *Shows the eyes and ears of four subjects of a family with Saethre-Chotzen syndrome. Patients can have unilateral or bilateral blepharoptosis. Abnormal shaped ears (e.g. long pointed crus as seen in the figure) is an associated feature.*



Muenke Syndrome (MIM602849) is characterized by unilateral or bilateral coronal craniosynostosis and mutations in the fibroblast growth factor receptor 3 gene (*FGFR3*)[90] is associated with nystagmus in 18% of cases.[84]

1.2.1.6 Inborn Errors of Metabolism

Most of the inborn errors of metabolism are autosomal recessive.

Lysosomal storage disorders such as Type 1 Generalised **GM_1 gangliosidosis** (MIM230500) and **GM_2 gangliosidosis** (Tay-Sachs disease and Sandhoff disease) may be associated with nystagmus.[91-93] **Sialidosis** (cherry-red spot-myoclonus syndrome) (MIM256550) caused by the deficiency of the enzyme neuraminidase presents with progressive neurological deterioration and can develop nystagmus.[94]

Disorders of Lipid Metabolism such as **Metachromatic leukodystrophy** (MIM250100) and **Refsum disease**[95] can cause nystagmus. Metachromatic leukodystrophy is a disorder of lipid metabolism caused by the deficiency of the enzyme, arylsuphatase A, and it can present with nystagmus.[96,97]

Adrenoleukodystrophy[98], an X-linked disorder of peroxisomes, can cause nystagmus.

Disorders of amino-acid metabolism can cause nystagmus. **Maple syrup urine disease** (MIM248600), an autosomal recessive disorder of branch chain amino acids can cause optic atrophy and nystagmus if left untreated.[99] **Hartnup disease** (MIM234500) is associated with pellagra like skin lesions and aminoaciduria. Patients can develop cerebellar ataxia and nystagmus.[100]

Mitochondrial disease such as **Leigh's disease** (subacute necrotizing encephalopathy of infancy or childhood), (MIM256000) and **Cytochrome C oxidase deficiency** (CCOD) are

associated with nystagmus.[101, 102] Neonatal **hypothyroidism** is reported to cause nystagmus in up to 10% of cases.[103]

1.2.1.7 Teratogens

In utero exposure to alcohol[104] and drugs such as amiodarone[105], opiates[77] and benzodiazepines[77] can cause nystagmus secondary to optic nerve damage.

1.2.1.8 Chromosomal abnormalities

Trisomy 21 (Down syndrome) is one of the most common chromosomal anomalies in live born children. Its phenotype is variable. The prevalence of nystagmus in Down syndrome was found to be variable between 12.7%[106] and 23%.[107, 108] Latent/manifest latent nystagmus is the most common type and was found in 23% of adults with Down syndrome in a study conducted in 1999.[108] Waveforms compatible with IIN can also be present in Down syndrome.[108]

Turner Syndrome is a chromosomal disorder that affects females, and is characterized by partial or complete absence of one
X-chromosome. Ocular features include ptosis, hypertelorism, epicanthus, and red green colour-blindness. It can be associated with periodic alternating nystagmus.[109] Nystagmus is also reported in **18q deletion** and partial deletion of the short arm of chromosome 3.

1.2.1.9 Other syndromes and diseases associated with nystagmus

Aicardi syndrome (MIM 304050) is characterized by the presence of clustered chorioretinal lacunae around the optic disc, infantile spasms and agenesis of the corpus callosum. It is an X linked dominant disorder which is fatal in males.[110]

Cornelia de Lange syndrome (CDLS) (MIM122470) is characterized by low anterior hair line, maxillary prognathism, growth retardation and mental retardation.[111] Ocular manifestations include ptosis, myopia and nystagmus. The three genes identified in this syndrome (*NIPBL* (5p13.1), *SMC1A* (Xp11) and *SMC3* (10q25)) code for components of the cohesion complex.

Dandy-Walker malformation (MIM220200) is comprised of cerebellar hypoplasia, upward rotation of cerebellar vermis, and posterior fossa cyst contiguous with the fourth ventricle.[112] Affected individuals present with delayed motor development, ataxia, hypotonia and nystagmus. Grinberg et al. localised the genetic interval to the region which encompasses the genes *ZIC1* and *ZIC4* on chromosome 3q2 and the authors concluded that heterozygous mutation in the above genes cause the disease phenotype in subjects with deletion chromosome of 3q.[113]

Chiari malformation is characterised by herniation of parts of cerebellum and/or brain stem through the foramen magnum. Downbeat nystagmus can be a presenting symptom of **Chiari malformation** (MIM 118420 and 207950). [114] [115]

Klippel-Trenaunay Weber syndrome (MIM149000) is a rare phakomatosis and is characterized by cutaneous haemangiomas and hypertrophy of the related tissues and underlying bones. It is associated with varicosities and abnormal limb length and bulk.[99, 116, 117] Congenital nystagmus and strabismus can be present.[99]

Lowry-Wood syndrome (MIM226960) is associated with short stature, microcephaly and congenital nystagmus. Reported in 1975 by Lowry and Wood[118], this condition is associated with small irregularly shaped epiphyses and square iliac bones were detected on radiography.[118, 119]

Schizencephaly (MIM269160) is characterized by infolding of the gray matter along a cleft formed at the primary cerebral fissures.[120, 121] Mutations in the *EMX2* gene (10q26.1) have been found to be associated with some cases of this disease.[120] The clinical features depend on the severity of the malformation, and include mental retardation, seizures, hypotony and spasticity.

Noonan syndrome (MIM163950) is characterized by dysmorphic features, short stature, it has features similar to Turners' syndrome. It is associated with mental retardation, webbed neck, cardiac abnormalities such as pulmonary stenosis and hypertrophic cardiomyopathy. Mutations in *PTPN11* (12q24.1), a gene encoding intracellular protein-tyrosine phosphatase has been found to be causative.[122] Ocular manifestations include hypertelorism, downward slanting eyes, ptosis, strabismus, nystagmus, prominent corneal nerves, and cataracts.[123]

Pierson Syndrome (MIM609049) consists of congenital nephritic syndrome and microcornea.[124] First reported by Pierson et al in 1963, it is caused by mutations in the *LAMB2* gene (3p21).[125] Other ocular features include nystagmus, strabismus, myopia and hypopigmented fundus.[126]

Arima syndrome (Cerebro-oculo-hepato-renal syndrome) (MIM243910) is characterized by congenital amaurosis of the Leber type, agenesis of the cerebellar vermis, infantile polycystic kidneys and hepatic changes such as fatty liver and hepatic fibrosis.[127]

Sotos syndrome (cerebral gigantism)(MIM117550) is characterized by rapid and excessive growth during childhood, acromegaly and mental retardation. Mutations in the *NSD1* gene (5q35) has been found to be the major cause for this disorder[128]. Ocular manifestations include strabismus, refractive errors and nystagmus.[129]

Pelizaeus-Merzbacher disease(MIM312080) is a dysmyelinating leukodystrophy of the central nervous system and is caused by mutations in the proteolipid protein 1 (PLP1) gene (Xq22) which encodes a major component of CNS myelin proteins. Spatic paraplegia is allelic and nystagmus is a presenting symptom.[130, 131] Other cerebral degenerative disorders such as **orthochromatic leukodystrophy**[132] can also cause nystagmus.

1.2.2 Acquired Nystagmus

In addition to those mentioned above, there are many other causes for nystagmus that occurs later in life. In contrast to infantile nystagmus patients with acquired nystagmus often experience oscillopsia, the illusion that the visual environment moving.

Acquired nystagmus can be caused by afferent visual disorders, intoxication and most commonly by central nervous disease. It can be associated with visual loss (e.g. cone dystrophy).[133] Intoxication with alcohol or other drugs such as lithium[134], barbiturates[135], phenytoin[136], lamotrigine[137], benzodiazepines, carbamazepine and ibuprofen[138] can cause nystagmus. Thiamine deficiency (Wernicke's encephalopathy) is often associated with nystagmus.

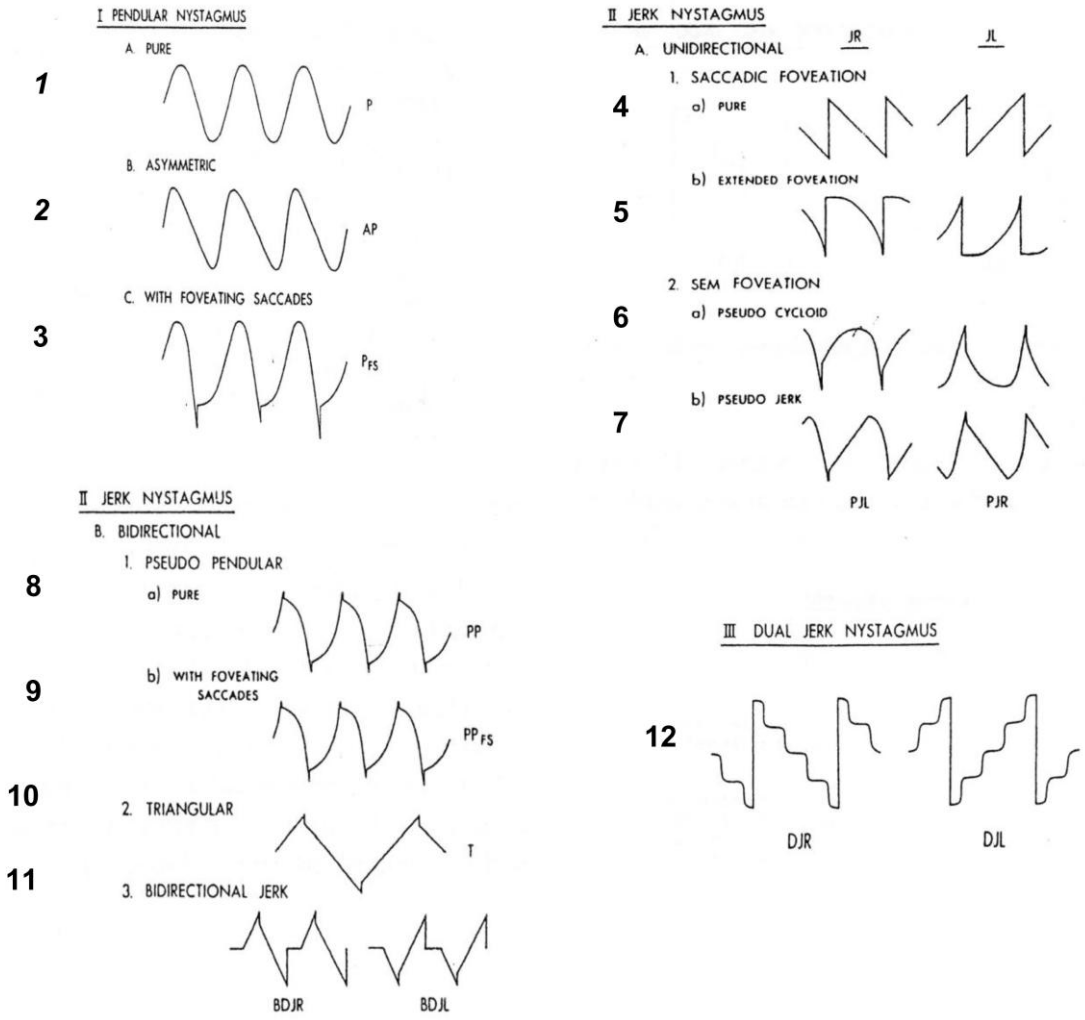
Multiple sclerosis is the most common cause of acquired nystagmus.[139] In addition central nervous diseases such as space occupying lesions, cerebrovascular incidents and various types of spinocerebellar ataxia can cause nystagmus.

1.3 Nystagmus waveforms

After the advent of accurate eye movement recording instruments there were attempts in literature to group nystagmus of various etiology based on their waveform. Dell'Osso and Daroff in 1975 classified the waveforms of congenital nystagmus (CN) into twelve types. Their classification includes four main categories: pendular, jerk, bidirectional and dual jerk types. These waveforms are illustrated in Figure 1.8.

Figure 1.8: *The 12 waveforms described by Dell'Osso & Daroff (1975): The waveforms can be categorised into pendular (1-3), unidirectional jerk (4-7), bidirectional jerk (8-10) and dual jerk nystagmus (12).*

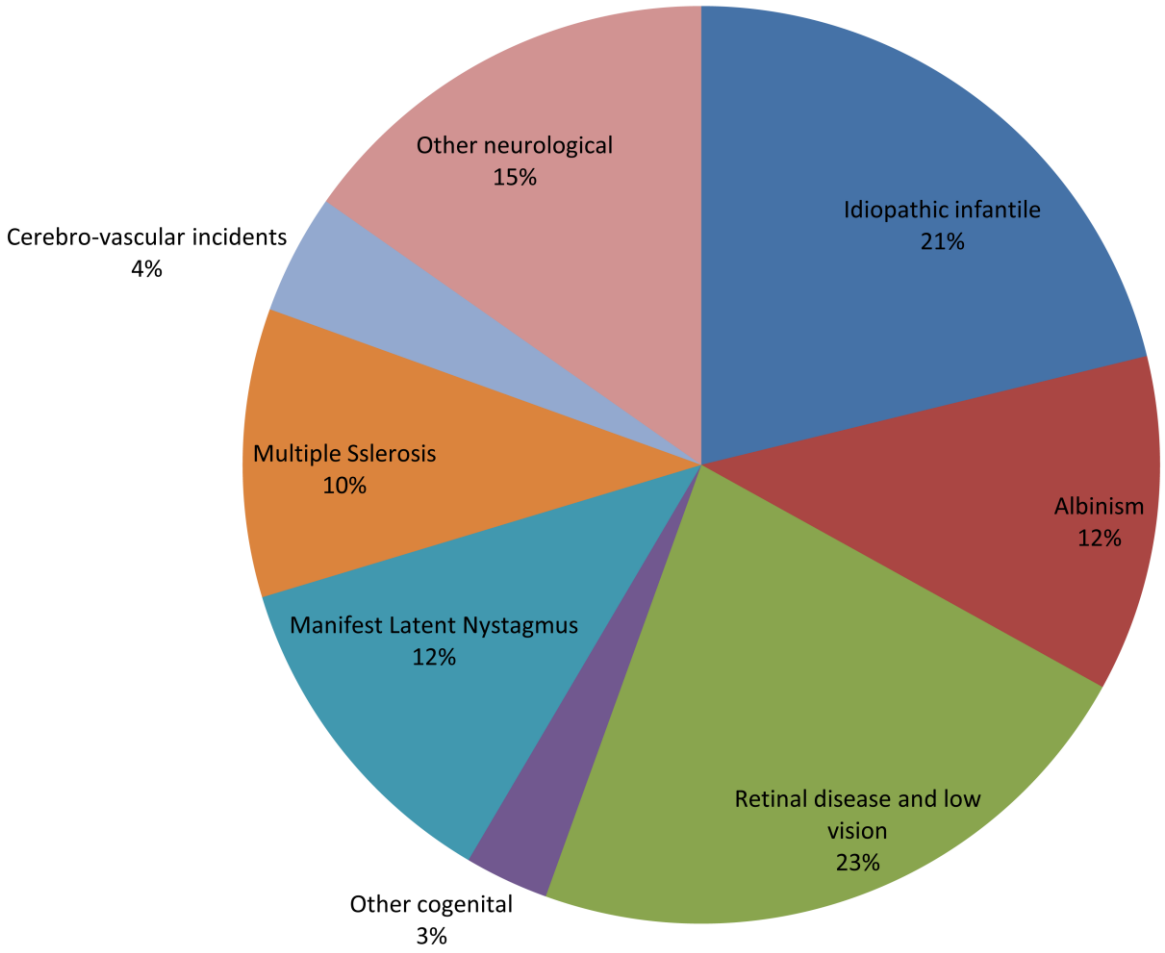
(Reproduced with permission from Springer Science +Business Media:< Doc Ophthalmol 1975; 39:155-182; Figures 16-19.)



1.4 Prevalence of Nystagmus

The prevalence of nystagmus in the general population has previously been estimated to be 1/20000[140], 1/6500[141], 1/1500[142] and 1/1000[143] in three studies. However, these studies were not primarily aimed at investigating the prevalence of nystagmus and included only children and young adults. A recent study conducted in Leicestershire to estimate nystagmus prevalence used data from three sources, (i) a hospital based database, (ii) all patients registered partially sighted and blind and (iii) children registered as visually impaired by teachers in Leicestershire. The prevalence of nystagmus among general population was estimated to be 24 per 10,000.[139] Of the 237 patients who presented in clinics in Leicestershire with nystagmus from the hospital based database, approximately 70% had infantile nystagmus forms and 30% acquired nystagmus. A breakdown of nystagmus to various aetiologies in 237 children seen in Leicestershire is shown in Figure 1.9.

Figure 1.9: *A breakdown of different types of nystagmus: From a survey of 237 patients attending clinics in Leicester Royal Infirmary, UK between February 2002 and October 2007. (Data from Leicestershire Nystagmus Survey, Invest Ophthalmol Vis Sci 2009)*



1.5 Idiopathic Infantile Nystagmus- Clinical Aspects and Work up

Idiopathic infantile nystagmus is a heterogeneous disorder and the clinical features vary. The onset is usually within six months after birth. In most patients with IIN, a history of 'wobbly eyes' starting between birth and the first 6 to 8 weeks of age is present. It has been documented that a subject with normal eye movements at 5 weeks of age developed pendular nystagmus by the age of 8 weeks.[144]

In order to classify nystagmus, it is essential to establish whether the presentation corresponds to the typical pattern of idiopathic infantile nystagmus or not. Clinical examination and investigations should be aimed at detecting any associated pathology.

Relevant points to look for in taking the medical history include:

- Family history of nystagmus
- Parental consanguinity
- Exposure to teratogens during pregnancy
- Birth and perinatal history
- Time of onset of nystagmus
- Type and description of nystagmus
- Changes of nystagmus over time
- Visual development
- Oscillopsia
- Night blindness
- Photophobia
- Presence of squint

Usually parents of infants with idiopathic nystagmus report improvement of visual development in their children some months after onset of nystagmus. Although oscillopsia often indicates acquired nystagmus some patients with idiopathic nystagmus can have mild oscillopsia especially if they are looking in directions of gaze away from their null point.[145, 146] Photophobia/light sensitivity is pronounced in patients with achromatopsia and to a lesser degree in albinism whilst nyctalopia may indicate congenital stationary night blindness or other diseases affecting rods.

Examination of patients should start with documenting their visual acuity and it might give clue towards diagnosis. Colour vision could be checked using the Ishihara pseudo-isochromatic chart and/or the D15 test.

Orthoptic assessment should follow to document the degree of binocular vision, and to detect any manifest/latent strabismus.

Slit-lamp examination is performed to look for anterior segment and posterior segment abnormalities, paying particular attention to detect iris transillumination defects that could be a sign of albinism. The degree of iris transillumination in albinism varies and this is best detected with a bright thin beam of light directed into the posterior segment through the pupil whilst focusing on the iris. This examination needs to be performed in a dark room. Examination of parents and older siblings may be helpful in detecting iris transillumination, which might give a clue to the diagnosis in an infant where the examination is difficult as carriers of ocular albinism may have iris transillumination defects.[147, 148]

Electrophysiology including visual evoked potential (VEPs) and electroretinograms (ERGs) are useful tools in arriving at a diagnosis in patients with nystagmus. ISCEV

standards (International Society of Electrophysiology of Vision)[33, 149, 150] should be adhered to whilst doing electrophysiological tests.

Electroretinograms (ERGs) should be performed in all patients with infantile nystagmus which may be abnormal in retinal diseases. If the patients report nyctalopia, dark adaptometry can be performed to confirm and quantify the defect.

Chiasmal 'miswiring' as seen in albinism results in asymmetry in VEPs where the findings obtained on stimulation of one eye show an asymmetrical distribution that is reversed when the other eye is stimulated.

Optical coherence tomography (OCT) is helpful in the diagnosis of certain retinal pathologies where it shows specific abnormalities, e.g. to detect fovea hypoplasia in albinism.[151]

Neuro-imaging should be considered in patients with infantile nystagmus who present with atypical waveforms. For example, when both eyes have dissociated movements, or when the nystagmus is vertical.

1.5.1 Treatment of IIN

Most of the management options of nystagmus are empirical and do not solve the problem completely. A cure cannot be expected until we comprehend the aetiology and pathogenesis of nystagmus in a better way. Current management options of IIN can be classified into

a. Optical

b. Pharmacological

c. Surgical

d. Others

Prismatic spectacles may be used to orient the eyes towards the null point to eliminate an abnormal head posture. Bilateral base out prisms in conjunction with concave (minus) lens correction can also be used to induce artificial divergence (stimulate fusional convergence), which can dampen IIN. However, these prisms can be thick and heavy. Fresnel prisms can interfere with vision, thus limiting their use. Contact lenses have also been tried with variable results in the management of nystagmus.[152, 153] It is possible that they give patients a better optical quality than glasses because they move with the eye.

There are several drugs used in the treatment of nystagmus on an empirical basis. Gabapentin and baclofen are the most commonly used drugs to treat acquired nystagmus in the UK.[154] Anticholinergic drugs, sodium or potassium channel blockers, alcohol, clonazepam, and other antiepileptic drugs have also been administered for acquired nystagmus.[155, 156] Gabapentin has been used successfully to treat congenital nystagmus.[157] NMDA receptor antagonists such as memantine have been found useful in reducing the intensity of acquired nystagmus. In a recent prospective randomised placebo controlled study[158], it was found that gabapentin and memantine were effective in improving the visual acuity and reducing the intensity of congenital nystagmus. Aminopyridines has been found to be useful in controlling vertical nystagmus, irrespective of its aetiology.[159, 160]

Anomalous head posture associated with eccentric null position can be improved by changing the position of the eyes through surgical methods. The principle of this surgery was first advocated by Anderson in 1953, who recommended bilateral recession of muscles responsible for the slow phase of the nystagmus.[161] In 1954 Kestenbaum introduced combined recession and resection and later Parks suggested

increased amounts of the same surgery for better results.[162, 163] In addition to the effect on head posture (beneficial in preventing and treating torticollis), these surgeries can help in reducing the intensity of nystagmus, improving the vision and broadening the null region.[164] The downside of extensive surgery is that some of these patients will be left with restricted gaze. However, many prefer to be able to keep their head straight at the expense of a restricted gaze which can be overcome by moving their head. Recently it has been reported that extraocular muscle tenotomy has helped in improving the visual acuity and nystagmus in subjects with IIN.[87] The effectiveness and usefulness of this last procedure remains to be proven.

Many children with IIN have relatively good vision, however, some would benefit from extra help at school. They might need to be seated in a specific position near the front of the classroom to match their anomalous head posture to achieve the best possible visual acuity.

1.6 Genetic Mapping

1.6.1 Overview of Genetics

Genetics is the study of heredity and inheritance. The modern science of genetics began with the work of Gregor Mendel in the latter half of 19th century. His work on pea plants (Pisum sativum) became the basis of the Mendel's laws of inheritance. Mendel's first law is about the segregation of characteristics in organisms. For each trait, an organism carries two alleles, one from each parent and they segregate during the production of gametes. Mendel's second law (law of independent assortment) states that the emergence of one trait is not affected by the presence of another and that different traits are inherited independently of each other. However, this law is valid only if two alleles are not linked to each other.

When the loci of two genes are on the same chromosome, and are close enough together that they do not segregate independently, they are said to be linked. X-linked inheritance was first documented by Thomas H Morgan in 1910 during his studies of the white eye mutation in Drosophila.[165] Morgan suggested that two genes located close to each other on a chromosome are less likely to have a chiasma between them. He proposed the term 'crossing over' to describe the physical exchange leading to recombination. It was Morgan's student, Alfred H Sturtevant who used Morgan's theory to map the X-chromosome of Drosophila.[166]

1.6.2 Recombination Fraction

If two loci are on different chromosomes, they will segregate independent of each other. This means that for two loci on different chromosomes, 50% of the gametes will

be recombinant and 50% will be non-recombinant. Here the recombination fraction is 0.5. If the loci are syntenic, they might segregate together unless they are separated by a *crossing over* during meiosis I. The closer the two loci are to each other, the lesser the chance of recombination. If the loci are far apart on a chromosome, the chance of them separated by a recombination is higher and so the recombination fraction is a measure of the distance between two loci. Recombination fraction is a measure of the genetic distance and not the physical distance between the loci. A single recombination event produces two recombinants and two non-recombinant chromatids. When the two loci are far apart on a chromosome or are on 2 different chromosomes, the overall effect is to give 50% recombinants and so the recombination fraction never exceeds 0.5. Two loci which have 1% chance of being separated by a recombination is defined as being one centimorgan (cM) apart on a genetic map.

1.6.3 Physical and Genetic Maps

Physical map literally means the order of the features of the chromosome and its distance can be expressed in kilo or mega bases. Genetic map is based on the amount of recombination occurring between adjacent loci rather than the actual number of base pairs separating them. They are constructed statistically characterizing the number of crossovers observed in parental meioses leading to the transmission of alleles to their offspring. Each individual vary in the number of crossovers per meiosis and so can vary in their genetic map lengths. Recombination is more common towards the telomeric region of chromosomes in males unlike in females where the

centromeric region has more crossovers.[167] Recently Jeffreys et al. found that recombination is not random at the DNA sequence level. Human chromosome seems to contain conserved regions (20-50 kB length) separated by recombination hot spots. Ninety-five percent of the recombination occurs in these 1-2 kb.[168, 169]

1.6.4 Mapping Function

The mathematical relationship between recombination fraction and genetic map distance is described by the mapping function.

Haldane, in 1919 postulated a map function assuming that the crossovers occurred at random along a bivalent without any influence on each other.

Haldane function is

$$w = -\tfrac{1}{2}\ln(1-2\theta)$$

where

w=map distance, θ=recombination fraction and ln=logarithm to the base e

The presence of one chiasma inhibits the formation of another one in the vicinity (interference) and therefore, applying Haldane function to observed data showed variable results depending on the distance between markers.[170] There are other mathematical equations to predict the map function and one of the widely used one is Kosambi's function which accommodates for the phenomenon of interference. The equation is:

$$w = \tfrac{1}{4}\ln[(1+2\theta)/(1-2\theta)]$$

1.6.5 DNA polymorphism

DNA polymorphism literally means the presence of more than one form of DNA in a population. The DNA of homologous chromosomes may vary in their nucleotide sequence or the number of repeated nucleotide units at the same locus. Two or more alleles may be present at a chromosomal locus in a given population. The chromosomal sites at which there are many alternate forms of DNA sequence are ideal sites for genetic markers as these DNA segments could be tracked down over generations. In the human genome the polymorphic sites which has variation in the 'repetition of the same short DNA sequence a variable number of times' (tandem repeat sequence) can be detected using Southern blotting or polymerase chain reaction.

1.6.6 Genetic Markers

Genetic polymorphisms are used as markers to follow a chromosomal segment through a pedigree. Informative meiosis is essential for linkage analysis and the chances of getting informative meioses are greater if the heterozygosity of the marker is high.

Restriction Fragment length polymorphisms (RFLPs) typed using either Southern blot or PCR were used as genetic markers. RFLPs have only two alleles with a maximum hetrozygosity of 0.5 and hence its low informativeness.

VNTRs (variable number tandem repeats) are polymorphisms arising from instability of tandem array of repeat DNA. Markers based on VNTR has high heterozygosity and are

more informative than diallelic markers such as RFLPs.[171, 172] Minisatellite regions which are highly polymorphic within the genome was described in 1980's.[171, 173] The repeat units are more than 9 base pairs long and are typed using Southern blots. Microsatellite markers are di-, tri- and tetra nucleotide repeats that are distributed throughout the genome. They are highly informative and are typed using PCR.[174, 175] Single nucleotide polymorphism (SNP) is a DNA sequence variation where a single nucleotide in the genome differ from other individuals of the same species. They form the most common DNA sequence variation in human genome.[176, 177] and they can be used as genetic markers. They can be used for high-throughput genotyping. However they are bialleic with low heterozygosity.

1.6.7 Linkage Analysis

Linkage analysis is the technique typically used to determine the genetic location of a disease gene and is useful especially when there are no other indicators for the position of the gene such as co-inherited disorders, cytogenetic abnormality or good candidate genes.

The goal of linkage analysis is to identify a DNA fragment of known location that is co-segregating with all the family members affected by the disorder being studied, and is not inherited by any of the unaffected family members. Genetic linkage is a function of recombination. If a marker co-segregates with the disorder, a statistical program is used to calculate the odds that you are in the correct location. This is expressed as log of the odds (LOD score).[178]

For a simple pedigree where the number of recombinant meiosis is known, it can be calculated with the formula

$$\text{Log}_{10}\left(\frac{(1-\emptyset)^{NR} \times (\emptyset)^{R}}{(1/2)^{NR+R}}\right)$$

Where Ø is the recombination fraction; NR, the number of non-recombinant meiosis; and R, the number of recombinant meiosis.

In simple pedigrees, if the markers are not fully informative and the phase of inheritance of some of the markers are not known, lod score can be calculated using a modification of the above formula

$$\text{Log}_{10}\left(\frac{(1-\emptyset)^{NR} \times (\emptyset)^{R}}{(1/2)^{NR+R}} + \frac{(1-\emptyset)^{R} \times (\emptyset)^{NR}}{(1/2)^{NR+R}}\right)$$

For complex pedigrees and multiple markers, manual calculation of lod score is quite tedious and error prone. There are a number of software program s available freely on the net to calculate lod scores.

The threshold for accepting linkage is Z= 3.0 with a 5% chance of error. Linkage can be rejected if the lod score is below Z < -2.0. Values between -2 and +3 are inconclusive. For X-linked characters the threshold for accepting linkage is lower and the accepted value is 2.3 or above.[179]

1.6.8 Parametric and nonparametric linkage analysis

In parametric linkage analysis, a specific disease model is used to describe the segregation of the trait with the marker. The mode of inheritance, allele frequency and the estimated penetrance of the disease should be known in order to carry out parametric linkage analysis.

Non parametric linkage analysis does not require a specific inheritance model and it involves testing whether the inheritance pattern deviates from the expected pattern under independent assortment.[180]

When individual markers, one at a time, are checked for co-segregation with the trait to establish the evidence of linkage, it is called two-point linkage analysis. Either a sib-pair analysis after breaking the pedigree into nuclear families or the extended relative pair analysis[181] that computes IBD-sharing (identity by descent) probabilities for all pairs of affected individuals in a pedigree can be used to do two-point linkage analysis. Software programs such as *LINKAGE*[182], *MLINK* and *ILINK* (*http://linkage.rockefeller.edu/soft/linkage/sec3.3.html*) can be used to carry out two-point linkage analysis. Two point linkage analysis has its own inherent deficiencies as it does not use the full inheritance information from the pedigree. Multipoint linkage analysis is superior and it uses haplotype information from multiple markers to infer the IBD. The program '*genehunter*' was made available in 1996 which computes multipoint linkage involving many markers in a family.[180] However this program is conservative when the descent information is incomplete.[183] To adjust for this, a modified version of the program, '*genehunter-plus*' was introduced.[183] Newer programs such as

MERLIN [184] and *Allegro*[185] which are faster and more user friendly are available now. Compared to the older programs, these newer programs can handle larger pedigrees and more markers.

There are many other programs available freely and a detailed list of available programs are given of the Rockefeller university website (http://linkage.rockefeller.edu/soft/list3.html#m)

1.7 How to identify a gene

1.7.1 Positional cloning

Disease genes can be identified through positional cloning methods or through position independent strategies. In positional cloning the approximate position of the gene (candidate region) is known but not the function of the gene. The gene for X-linked chronic granulomatous disease was the first gene identified based on the information of its map position, without prior reference to a specific protein.[186]

The first step in positional cloning is to confirm the locus of the genetic defect. This can be done through linkage analysis or using chromosomal abnormalities such as translocations, inversions or deletions.

Once the position of the gene of interest is mapped, the next step is to define the candidate interval using the haplotype data and this depends on the number of meiosis available for the study. Single recombinants define the limits of the candidate region. They are more reliable if identified in an affected individual rather than an unaffected individual who could be a non-penetrant carrier of the genetic defect.

A catalogue of all genes in the candidate region can be obtained from one of the genome browsers (e.g. http://www.ensembl.org/biomart). However this data may be incomplete and may need to be supplemented with additional experiment and computer work.[187]

Genes from the candidate region must be selected and prioritised for mutation screening based on its expression profile, function or homology to other known genes with appropriate expression or function.

1.7.2 Position independent strategies

Here the candidate genes are identified without prior knowledge of its approximate position. Identification of β globin gene was based on the knowledge of its gene product rather than the map position.[188]

If the protein product is known, oligonucleotide probes could be generated and used to screen libraries to identify the cDNA.

Another strategy is to use antibodies which are specific to a protein to immuno-precipitate the mRNA. This method was used in the identification of phenylalanine hydroxylase.[189]

Animal models of human disease are used successfully in identifying human disease genes, for e.g., the identification of *SOX10*.[190]

1.7.3 Confirming the disease gene

Candidate genes must be tested individually to prove that mutations in it cause the disease phenotype. Mutation screening in the affected individuals is one of the methods for confirming the candidate gene. If a sequence change is identified in an affected individual it is imperative to find out if it is a non-pathogenic polymorphism or a pathogenic mutation. Screening unaffected members of the family and other normal controls will help detect non-pathogenic polymorphisms. Collins and Schwartz in their paper in 2002 have examined the number and type of controls required to detect polymorphisms. Normal controls should ideally be selected from the same ethnic background, and they should be past the age of presentation of the phenotype. For X-linked diseases, the controls should be males so that unaffected carrier females are

not included. To detect a 5% polymorphism with 95% power, one needs to examine 65 chromosomes and to detect 1% polymorphism with 95% power, a total of 346 chromosomes need to be examined.[191]

1.7.4 Pathogenic Mutations

Mutations are changes to base pair sequence of the genetic material of an organism. Allelic sequence variations with a frequency of more than 0.01 are considered and described as DNA polymorphisms rather than mutations. Pathogenic mutations are changes which results in altered gene expression either though a change in the coding sequence or by an alteration of intragenic or extragenic sequences which are important for gene expression.

There are a number of mechanisms through which the expression of a gene can be altered by a change in sequence. A single nucleotide change in the coding sequence can result in a stop codon; replace an essential amino acid or induce a frameshift in translation resulting in abnormal product. A similar change affecting the promoter region of a gene also can lead to abnormal gene expression. Mutations can also prevent normal splicing by inactivating the donor or acceptor splice site or by activating a cryptic splice site. Deletion of a gene or a chromosomal segment containing a gene can cause disease as in WAGR syndrome.[30] Other pathogenic changes include disruption of the gene structure by translocations or inversions.

1.8 Clinical and Molecular Genetics of Idiopathic Infantile Nystagmus

IIN may be sporadic or inherited. In the inherited type, autosomal dominant, autosomal recessive and X-linked forms have been described.

1.8.1 Autosomal Dominant and Recessive IIN

Several families with nystagmus of autosomal dominant inheritance have been described in the literature. Allen in 1942 described three pedigrees of primary hereditary nystagmus of possible autosomal dominant inheritance.[192] In 1964 Dichgans and Kornhuber described another family with dominant inheritance.[193] Kerrison et al. gave the localization of the NYS2 gene as 6p12 based on study of a single large African-American family with autosomal dominant congenital nystagmus.[194, 195]

Patton et al. observed a balanced reciprocal 7;15 translocation (p11.2;q11.2) in a mother and son with congenital nystagmus.[196]

Klein et al.[197] reported a family in which three individuals had congenital nystagmus inherited in an autosomal dominant pattern. It was found that all three affected individuals shared a common haplotype at chromosome 7, suggesting a locus responsible for the phenotype at 7p11.2 (NYS3) (MIM 608345).

Locus 'NYS4' (MIM193003) was described by Ragge et al. in 2003.[198] They carried out a phenotypic study of a four generation family with nystagmus. Affected family members developed vestibulocerebellar type nystagmus in the first two years of life. A higher incidence of strabismus (64%) was noted in affected members. Haplotype analysis linked the disorder to chromosome 13q31-q33.

Nystagmus of autosomal recessive inheritance (MIM257400) was described by Waardenburg in 1963.[199]

1.8.2 X-linked Idiopathic Infantile Nystagmus

X-linked IIN is genetically heterogeneous. In a single four generation French family with 12 individuals with idiopathic congenital nystagmus over, Cabot et al mapped the gene to the short arm of the X chromosome by showing close linkage of the locus to polymorphic markers on Xp11.4-p11.3 (maximum lod = 3.20 with DXS993).[200] This region contains several genes associated with forms of congenital nystagmus related to retinal diseases such as congenital stationary night blindness, retinitis pigmentosa, cone dystrophy, Norrie disease, exudative vitreoretinopathy, Åland Island eye disease and X-linked optic atrophy, raising the possibility that congenital nystagmus is allelic with one of these disorders.

In 1999, Kerrison et al studied three families with idiopathic congenital nystagmus inherited in an X-linked pattern.[7] They found that the penetrance of the disease among obligate female carriers was 54%. Linkage analysis showed linkage to Xq26-q27. Evaluation of markers in the region of the genes for ocular albinism (OA1- Xp22.3), congenital stationary night blindness (CSNB1- Xp11.4), and blue cone monochromatism (BCM-Xq28) revealed no evidence of linkage, supporting the hypothesis that X-linked congenital idiopathic nystagmus represents a distinct clinical entity. Assessment of haplotypes and multipoint linkage analysis placed the gene in a region between two specific markers GATA172D05 and DXS1192. Evaluation of

candidate genes CDR1 (cerebellar degeneration-related autoantigen) (MIM302650), which maps to Xq27.1- q27.2, and SOX3 (MIM313430) which causes suppression of neural differentiation and maps to Xq26- q27, revealed no mutations in affected male subjects.

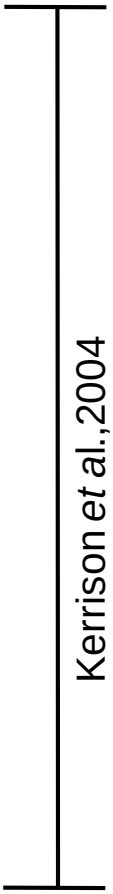
In 2001 Kerrison et al. refined the NYS1 locus to a 5-cM interval between DXS9909 and DXS1211 on Xq26-q27.[6] Genetic analysis excluded mutations in the SLC25A14 gene (MIM 300242). Based on examination of an extended pedigree, the estimated penetrance among obligate female carriers was 29%.

In 2004, Kerrison et al. managed to narrow the NYS1 locus (Xq26-q27) to a 12mB region between marker DXS8078 and DXS1211 based on a study on six families with congenital motor nystagmus.[201]

A diagrammatic representation of the genetic interval at locus NYS1 refined by Kerrison et.al is shown in Figure 1.10. They sequenced fifteen genes (out of 37 candidate genes expressed in the brain and retina) to identify one DNA sequence alteration, which was present in 4.4% of normal human controls. They concluded that identification of candidate genes for X–linked congenital nystagmus is hampered by the large genetic interval and the lack of knowledge as to where the primary biologic defect is located.

Figure 1.10: *A description of the genetic locus of X linked nystagmus (NYS1) at Xq26-27 based on the study by Kerrison et al in 2004. They localised the genetic interval to a 12 mega base region between markers DXS8078 and DXS1211.*

Marker	Position (mB)
DXS8078	126.3
DXS8044	126.3
DXS1047	127.1
DXS8072	130.2
DXS8071	131.2
DXS6748	132.1
DXS1114	133
DXS8041	133.4
DXS8033	133.8
DXS691	135.1
GDB:204469	135.5
DXS8094	136
DXS1041	136.3
DXS8050	136.7
DXS1062	137
DXS1211	138

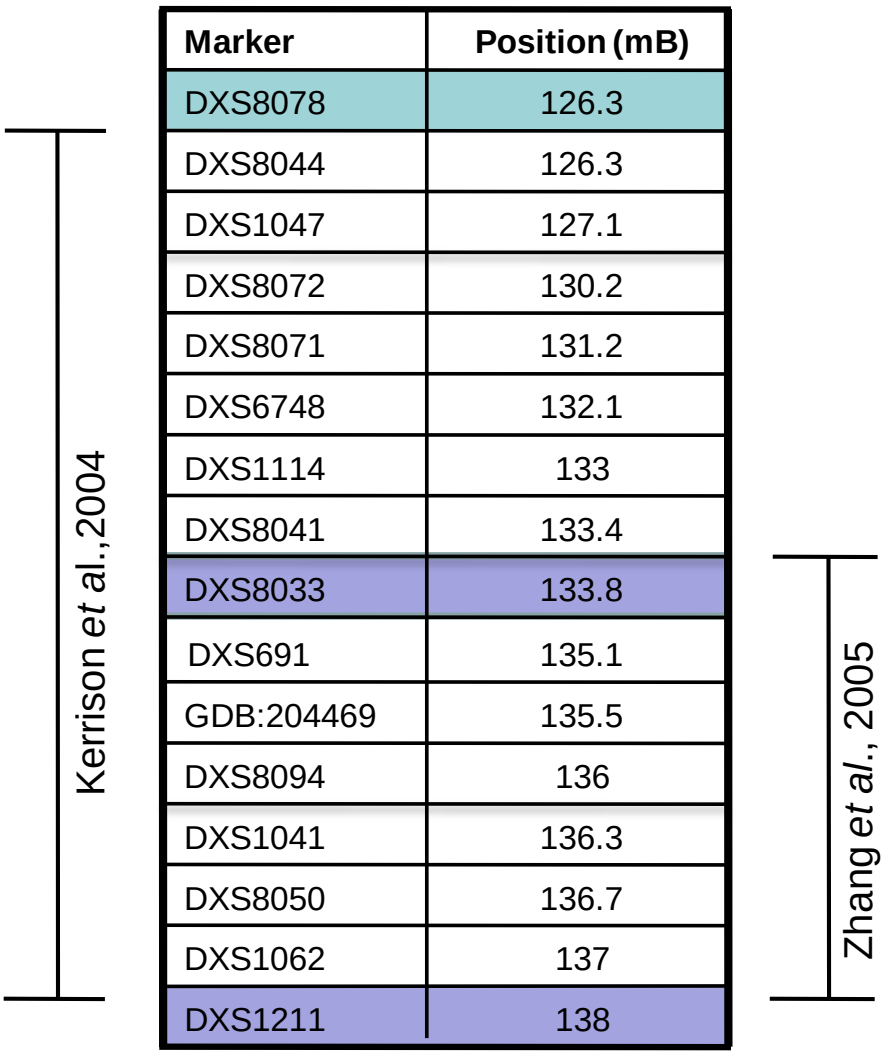


In 2005 Zhang et al tried to refine the genetic interval at locus NYS1 further.[202] They identified a large Chinese family with congenital motor nystagmus. Genotyping and

linkage analysis was performed on 22 individuals from this family. The genetic locus was refined to a 4.4 cM region at Xq26.3 - Xq27.1 between markers DXS8033 and DXS1211 (Figure1.11).

However, this study has its own pitfalls. Zhang et al presumed that the disease is fully penetrant in this family and they used X-linked dominant model for the linkage analysis with presumed penetrances of 1 and 0.95 in males and females respectively. However previous studies have shown that the penetrance of X-linked IIN in females is much lower than 100% (54% and 29% in two studies by Kerrison et al). [6, 7] They used recombinant events in two unaffected females to refine the genetic interval and this can give spurious results if those two unaffected females are nonpenetrant carriers of a mutation.

Figure 1.11: *A description of the genetic locus of X linked nystagmus (NYS1) at Xq26-27 based on the study by Zhang et al in 2005. They refined the region to a ~4mB region between markers DXS 8033 and DXS 1211, however this data was based on a recombinant event in a clinically unaffected female in a family with presumed 95% penetrance. (The region refined by Kerrison et.al in 2004 is also shown in the figure)*



Marker	Position (mB)
DXS8078	126.3
DXS8044	126.3
DXS1047	127.1
DXS8072	130.2
DXS8071	131.2
DXS6748	132.1
DXS1114	133
DXS8041	133.4
DXS8033	133.8
DXS691	135.1
GDB:204469	135.5
DXS8094	136
DXS1041	136.3
DXS8050	136.7
DXS1062	137
DXS1211	138

1.8.3 Summary- Current Knowledge about locus NYS1

Kerrison et al. identified several families with IIN linked to locus NYS1 at Xq26-q27 which seems to be the major locus for X-linked IIN. They analysed the haplotype in six families which placed the causative gene between markers DXS8078 and DXS1211, an interval spanning 12 mega bases.

Zhang et al further refined the region to a 4.4 cM interval; however this data may not be fully reliable because of the reasons mentioned in section 1.8.2.

1.9 Aims/objectives of the Study

The aims of this study were threefold:

1) To ascertain subjects with familial and sporadic IIN

This involved recruiting subjects with possible IIN into the study. History, clinical examination, eye movement recording and investigations such as electro diagnostic tests were performed to diagnose IIN. Particular attention was paid to taking family history in order to identify inherited forms of the disease.

2) To further refine the NYS1 locus (Xq26-q27) and to identify the gene causing X-linked idiopathic infantile nystagmus

Upon completing the first objective, i.e., ascertaining subjects with sporadic and familial IIN, it was evident that the most common form of inheritance of IIN in this series was X-linked.

Having recruited many families with X-linked IIN, our next aim was to check if these families link to this locus (NYS1 at Xq26-27). Genotyping with microsatellite markers was carried out in order to achieve this objective. Analysis of the haplotype was carried out to reduce the potential genetic interval.

Sequencing of genes in the region was then carried out by our collaborators at Sanger institute.

3) To describe and compare the phenotype of patients with idiopathic infantile nystagmus (IIN)

Sequencing of genes in this locus by Tarpey *et al*. at Sanger institute led to the discovery of the first gene causing IIN (*FRMD7*). Having identified the gene causing X-linked IIN, our next aim was to describe the phenotype of IIN caused by mutations in *FRMD7* and compare it to IIN not associated with this gene.

2 Subjects and Methods

The outcome of this project was dependent on the number and size of the families recruited. Very careful phenotyping was essential to avoid contamination of the cohort with other conditions associated with nystagmus such as ocular albinism.

The fact that the Ophthalmology group at the University of Leicester is a tertiary referral centre for nystagmus and other eye movement disorders in the UK, helped us recruit a large number of subjects in a relatively short span of time. Professor Irene Gottlob who has special interest in eye movement disorders has a number of international collaborators which meant that the net could be widened to include families recruited from centres in Austria, Germany, and USA.

Phenotyping of the subjects required slit-lamp examination, eye movement recording and fundus examination.

Electro diagnostic tests (EDT) were performed on at least one subject from each family. This meant that even if I was able to examine many families on field trips, some of the family members needed to visit the base centre in Leicester for electro-diagnostics.

This work was done in collaboration with a number of researchers in Leicester and at other institutes.

Initials of the people who helped with project are:

Irene Gottlob -	IG
Frank A Proudlock -	FP
Nagini Sarvananathan -	NS
Chris Degg -	CD
Eryl Roberts -	ER
Musarat Awan -	MA
Rebecca McLean -	RM
Shegufta Farooq -	SF
Mylvaganam Surendran -	MS
Laura Baumber -	LB
Chris Talbot -	CT
Colin Veal -	CV
Patrick Tarpey-	PT
Lucy Raymond-	LR

2.1 Ethics Committee Approval

This project has the full approval of the Leicestershire research ethics committee. Documents such as the patient information sheet (adult and child), and the consent form were designed in accordance with the guidelines of the local ethics committee. We also recruited subjects from overseas centers where the work was based on the ethics approval of the local authorities.

2.2 Recruitment of Subjects

Most of the subjects were recruited from referrals to the eye clinic at the Leicester Royal infirmary. In addition many patients and their relatives contacted us through the Nystagmus Network, UK (http://www.nystagmusnet.org). The group also have international collaborators and have recruited families from oversees.

A patient information sheet was given to every subject. Informed consent was taken from the subjects before enrolling subjects into the study. Parents/ guardians were requested to consent for their children to be enrolled in the study.

2.3 Field Trips

The author organised a number of field trips in the UK and overseas to recruit subjects for the study. Subjects were seen in the local GP surgery or community centres depending on the availability of facilities. Some of the families were recruited through overseas collaborators of Professor Gottlob.

The largest family recruited was from the north-east of England. Field trips to many locations locally in Leicester and away in Dorset, Glasgow, Hereford, Isle of Man,

Leeds, Leyland, Manchester, Nottingham, Preston, Stockport and Swindon helped to recruit more families and subjects into the study.

Four families were enrolled through field trips to Graz (Austria) and were phenotyped with the help of clinical collaborators of Professor Irene Gottlob at the University hospitals of Graz. Two families from USA and one from Madagascar were also recruited through clinical collaborators.

2.4 Clinical work-up and Investigations

Clinical examination of most of the subjects were performed by the author. However, he was helped especially on field trips by IG, NS, MA and SF.

The clinical work-up consisted of:

- **History:** Patients and/or their parents/guardians were interviewed to elucidate the history, especially the time of onset of nystagmus and of any significant events during the antenatal period, postnatal period and early childhood. History about consanguinity was obtained. Speaking to more than one person of the family and interviewing subjects on more than one occasion was found to be useful in obtaining accurate family histories.
- **Visual acuity** was measured using a Snellen chart or Sheridan-Gardner test.
- **Colour vision** was tested using the Ishihara's pseudo-isochromatic chart
- **Stereo-visual acuity** was quantified using Frisby and the Lang test. In those subjects who were Lang negative, Wirt fly test and Bagolini striate glasses were used to detect and document the level of binocular vision.

- **Cover test** was performed at near and distance fixation on every subject to evaluate any manifest or latent strabismus.
- **Anomalous head posture** was determined when patients were reading visual acuity charts at distance and was classified into three groups (No AHP: i.e. <5˚of head turn; moderate AHP: i.e. 5-15˚ of head turn and large AHP: i.e. >15˚ head turn)
- **Slit lamp examination** was performed on every subject to detect or rule out any anterior segment pathology. Careful examination was done in the dark to look for subtle iris transillumination in all these subjects. Fundus examination was also done using a 78D lens.
- **Description of nystagmus.** Any subject with nystagmus was observed to characterise the waveform in various positions of gaze. The null position of nystagmus and the effect of convergence were also noted.
- **Video recording** of the eye movements were performed for confirmation of the findings and for future reference.

2.4.1 Eye Movement Recording

Eye movement recording was done by FP, RM, ER, and MS. The data was analysed by the author.

Eye movements were recorded using a high-resolution pupil tracker at a sample rate of 250Hz (EyeLink I, SensoMotoric Instruments GmbH, Berlin, Germany). The eye tracker has a resolution of 0.005° and a range of ± 30° with a noise level of <0.01°. The EyeLink I system consists of three miniature cameras mounted on a headband (Figure 2.4.). Two cameras track the pupil permitting accurate binocular tracking of horizontal and vertical eye movements. The system works by illuminating the eye with infrared light and determining the centre of the pupil from the centre of a dark ellipse on the image where reflected light is low. The third camera measures head position by monitoring four infrared lamps mounted around the monitor/screen. Consequently, EyeLink provides head compensated gaze data. In addition, a chinrest was used when possible. EyeLink data files were converted offline to Spike2 neurophysiological software system files for subsequent analysis (Cambridge Electronic Design, UK).
Visual stimuli was generated using a VisLab system (SensoMotoric Instruments GmbH, Berlin, Germany) generating stimuli either on a LCD monitor (Samsung SyncMaster 710v, viewing distance 0.4m) on field trips or on a rear projection screen (1.8x1.2m, viewing distance 1.2m) using a video projector (Hitachi CP-X958) in lab based recordings. Each eye was calibrated separately offline by selecting foveations when fixating 0° and horizontal and vertical points at ±15° eccentricity. A range of visual tests

were performed which were modified depending upon time constraints. These included:

- Measuring nystagmus when maintaining steady fixation (up to 1 minute recording at primary position and secondary positions at 15°)
- Measuring nystagmus when maintaining steady fixation at primary position under monocular viewing (left eye open and right eye open).
- Horizontal and vertical smooth pursuit (10°/s, 20°/s and 40°/s velocities, ±20° amplitude),
- Horizontal and vertical optokinetic nystagmus (10°/s, 20°/s and 40°/s velocities)
- Horizontal and vertical saccades (following targets from –20° to 20° in 10° steps moving every 1.5s)

Eye movement recoding data was analysed using the 'Spike2' (Cambridge electronic design) software to detect the amplitude, frequency and waveform of nystagmus.

2.4.2 Electrodiagnostics

Most of the electro-diagnostic tests were performed by Dr Chris Degg and his team at the department of medical physics at the Leicester Royal infirmary. However some of the ERG's and VEP's were carried out at other hospitals when subjects were seen on field trips.

Electroretinograms (ERGs) and Visual evoked potentials (VEP) were peformed on all singletons included in the study and at least from one subject from each family (more subjects when possible) to detect/rule out retinal disorders and albinism.
Multi-channel VEPs were performed when a diagnosis of albinism was suspected for detecting the asymmetrical distribution of VEP over the posterior scalp. Chiasmal 'miswiring' as seen in albinism results in 'crossed asymmetry' where the findings obtained on stimulation of one eye show an asymmetrical distribution that is reversed when the other eye is stimulated. ISCEV standards[150 203] were adhered to, whilst selecting the stimulus parameters, recording parameters and the clinical protocols.

2.5 Laboratory work

Laboratory work was started after recruiting eleven families into the study. Another five families were recruited subsequently and they were included in the laboratory work. Genotyping using microsatellite markers were carried out in subjects from these sixteen families.

2.5.1 Collection of Tissue Sample

Tissue samples were collected by the author and was helped by IG, NS and ER.

Blood samples (2 x 9ml EDTA tubes) were collected from the enrolled subjects. The samples were transported to the laboratory at 4° C. In children and in those who were not willing to be give blood, saliva samples were collected using 'The Oragene DNA self-collection Kit' (*DNA Genotek Inc. Ottawa, Canada*). Buccal swabs (BuccalAmp™ DNA extraction kits- Epicentre Biotechnologies) were used in infants and small children.

Blood samples were frozen at -80° C. Saliva samples were stored in room temperature. Buccal swabs were refrigerated at 4°C.

2.5.2 DNA Extraction

Most of the DNA extraction was done by the author and was helped by ER.

A standard protocol was used for DNA extraction from blood samples. Frozen blood was thawed by keeping in a water bath at 37°C. The defrosted blood (9 ml) was diluted

with cold sterile water to get a final volume of 50 ml. This sample was spinned at 3000 rpm for 10 minutes. This step would lyse the white blood cells. The supernatant which contained the plasma was discarded and the remaining buffy coat was suspended in 25 ml of sucrose lysis buffer. The solution was vortexed and left for 30 minutes. This would lyse the red blood cells. The cells were separated by centrifuging at 3000 rpm for 10 minutes. The pellet containing the cells was re-suspended in 3ml of 'CVS buffer' and 25µl (20mg/ml) of 'Proteinase K'. This solution was incubated at 60°C for upto 3 hours to ensure extraction of the proteins. 2 ml of sodium chloride at 5M concentration was added and DNA was precipitated using 100% ethanol. The precipitated DNA was re-suspended in 1 ml of sterile water. The concentration of DNA was checked using a spectrometer before storing it at -20°C. DNA extraction was done by the author and EOR.

2.5.3 Amplification of DNA and Genotyping

Amplification of the DNA and genotyping with microsatellite markers were done by the author under the supervision of CT, LB and CV.

The DNA was amplified through the standard technique of Polymerase Chain Reaction (PCR).

Thirteen polymorphic micro-satellite markers bordering the NYS1 region (Xq26-27) were used for genotyping. Primers were obtained from 'Invitrogen'. Gradient PCR was done to optimize the temperature for the reactions. The details of the primers are given in Table 2.1

Table 2.1: *The details of thirteen microsatellite markers used in the study are given in the table below.*

Marker	Forward primer	Reverse primer	Size	Chromosome Position	
				Start	End
DXS1047	CCGGCTACAAGTGATGTCTA	CCTAGGTAACATAGTGAGACCTTG	196-210	128902978	128903267
DXS8072	GTAAAAATTTACGGTTGTNCCAA	TCTCCCTATCCAACTCATGC	215-239	130255232	130255527
DXS8071	CACAATAACCAAGATGTGGA	CATAATGCCATCAAGTTTCA	185-201	131263387	131263697
DXS6748	TGCATTTGTCTATCACCCTA	TGCTGTGCTATCATGTTTG	211	132225222	132225573
DXS1114	TGACTACATTATAAAAGCACAATGC	ACTAAAAAAAAGAGTTTGACTACCTC	109-115	133122952	133123064
DXS8041	GCAAGACTCCGTCTCAAATAATAAC	TTCCTGCTACCTGCAATTCC	144-164	133531327	133531654
DXS8033	GAAGACAAACCCCATGAG	ATGCGTTGATAGGTGCAG	222-266	133915500	133915872
DXS691	TATGGGTAGGTTTGGGTTGA	GTTACACTCTTTCAGCCAGC	144-166	135221095	135221361
GDB:204469	AAGAAGAGAACTGACTAGCAACG	TCGGACAGTTATTCATTCTCTTT	197	135569771	135570015
DXS8094	GCCATTGTAAAATAAAATTCAG	ATGGTCTTGAGTCACTGTCT	225-239	136064421	136064780
DXS1041	GTCCTCTTGGAACATGAGAA	CGAACAATTATGGGTTGTCT	120-176	136363544	136363874
DXS8050	CAGTTCCTTGACCTACCC	CTCCAGATTTGACATAATAATACTC	194-200	136813621	136813949
DXS1062	GAGATGTGTGACCTTGAGCACT	GTTGCCTGTTAAGCACTTTGAATC	222-248	137130620	137130909

2.5.4 Agarose Gel Electrophoresis

Electrophoresis on agarose gel was performed to quantify the results of PCR.

1% agarose gel was prepared in 1 x TBE buffer. The preparation was heated for about 2 minutes in microwave to dissolve the solute and then cooled down. 2-3 µL of ethidium bromide per 100 ml of solution was added before pouring it in the tray with the combs in position.

Five µL of the PCR product was mixed with 2.5 µL of gel loading buffer. Orange G (mixed with glycerol 5:1) was used as the colour marker in these experiments (The other option was bromophenol blue mixed with glycerol 5:1).

In one of the wells, 5µL of a molecular marker such as PBR322Hae III or Lamda *Hind* III was added to facilitate estimation of band size. The electrophoresis was done at ~100v for approximately one hour. The gel was transferred to the UV transilluminator to compare the bands with the known sizes of the molecular marker to determine the approximate size of the PCR product.

2.5.5 Polymerase chain reaction

The Primers for the PCR were diluted to 10 µM working concentration. The PCR conditions for the reactions were as given in table 2.2. At the end of the reaction, 3 µL of the PCR product was run on a 2% agarose gel and was photographed for reference. The remaining PCR products were stored at

-20°C for genotyping.

Table 2.2: *PCR conditions for the NYS1 markers for a 10µL reaction. All the reactions were done at 56°C.*

10ul reaction	
Ingredient	Volume (µL)
Template 30ng/µL	1.2
Water	4.4
Q-solution	0
10XBuffer	1
dNTP	0.8
Fwd. Primer [10]	1.25
Rev. Primer [10]	1.25
Taq	0.1
Total Volume:	10

2.5.6 Preparation of Acrylamide Gel

Once the PCR products were ready, the next step was genotyping using the ABI 377 DNA sequencer (Applied Biosystems). This involved a number of steps as detailed below.

The plates for running the gel were cleaned with denatured water (dH2O) and dried. Acrylamide gel mixture was prepared in a glass beaker using 10 ml of 6% sequagel, 5ml of sequagel buffer complete and 50µL of 10% (w/v) ammonium persulphate (APS). The gel mixture was taken in a 20 ml syringe and was injected into the space between the plates, to form a thin layer. Once the gel was poured, the comb was positioned back to front between the plates to form a straight edge and was left for 60-90 minutes for polymerising. Once the gel was set, the comb and the bull-dog clips were removed carefully. The plates and its groove were cleaned with dH2O to remove all the residual acrylamide without disturbing the gel. The groove was then filled with dH2O and the comb was replaced with the teeth facing down, until the teeth sat just below the surface of the acrylamide gel.

2.5.7 Preparations of Reagents (PCR products) for Genotyping

Once the polyacylamide gel was set, the PCR products were processed for loading on to the plates. The PCR products were diluted according to the strength and pooled together in accordance with the compatible markers. Hundred micro litre (100 µL) of dH2O was aliquoted into a plate and suitable volume of each PCR product (strong products were diluted 1 in 100 and weaker products ~1 in 40) was added to pool compatible markers.

Formamide loading dye was prepared by mixing 5 ml of the de-ionised formamide, 1 ml of 25 mM EDTA and blue dextran for colour. A master mix of formamide loading dye (1.5 µL per reaction) and TAMRA 500 size standard (0.3 µL per reaction) was prepared and an aliquot of 1.8 µL of this master mix was prepared on a separate plate. 1.5 µL of the pooled and diluted PCR product was added into each well to make up the volume in each well to 3.3 µL. This reaction was denatured at 96°C for 2-3 minutes and immediately transferred on to ice to maintain the denatured state until the gel electrophoresis.

2.5.8 ABI 377 Sequencer

An ABI 377 DNA sequencer (Applied Biosystems) was used for genotyping. The acrylamide gel plates were placed in a cassette and positioned in the ABI machine. The 'genescan 2.1' programme was run in the ABI 377 collection programme. Plate check was set to "Plate check C" and GS run and GS pre-run programmes were selected (36C-1200). Plate check was done and the scan/gel image windows were checked to make sure that there was no background fluorescence. Plates were re-cleaned when

necessary before loading the reactions. The upper and lower buffer chambers were filled with 1 x TBE and the pre-run programme was started. While the machine was on the 'pre-run' the sample sheets (labelling each lane on the gel plate) were prepared. The pre-run was cancelled after approximately about 30 minutes when the gel temperature was optimum as seen on the status window. A syringe with a cannula was used to remove any air bubbles before loading the reaction for genotyping. Stagger loading method was used to load the denatured PCR products mixed with the loading dye and the size standard i.e., odd lanes were loaded first; the electrolysis was run for 1.5 to 2 minutes before loading the even lanes. The correct sample sheets were inserted and the gel was run. 0.55 µL of the reaction was used to load in a 96-well gel and 1.5 µL was used in a 48-well gel.

2.5.9 Genotyping

Once the PCR products were run on the ABI 377 DNA sequencer, genotyping was done using the *ABI* software *Genescan* and *Genotyper* 2.1. The results were checked manually and scored. Weak bands and overlapping lanes were excluded and the experiment was repeated when necessary. The data was extracted onto an Excel sheet for review.

2.5.10 Linkage analysis

The haplotype data was tabulated in *Microsoft notepad* and the data was checked for errors using *pedstats (Merlin)*. Pedigrees were drawn either using *Cyrillic* or Microsoft power point and the haplotype data was entered manually. *Merlin software for X-linked pedigrees* (MINX) was used to do multipoint linkage analysis. The author performed linkage analysis using *Merlin* and was supported by Dr Chris Talbot and Dr Gonçalo Abecasis (online support).

For the linkage analysis, X-linked model was used with a phenocopy rate (penetrance with 0 affected alleles) estimated as 2/3rd of the prevalence of IIN i.e. 0.0002. Disease allele frequency was estimated to be 0.0001. Penetrance of the disease in males without the mutation was taken as 0.0002, and 0.99 for hemizygotes, and for 0, 1 and 2 copies of the mutated allele in females penetrances were 0.0002, 0.5 and 0.99.

2.6 Phenotype-Genotype Correlation and Statistics

Phenotype-genotype correlation study was carried out by the author. He was helped by FP to create Figures 3P.1, 3P.4, 3P.5 and 3P.8 and to do the statistical analysis.

Subjects with IIN were classified into two groups (those with mutations in *FRMD7* and those without mutations in *FRMD7*). Visual acuity, stereopsis, anomalous head turn, strabismus and parameters such as amplitude, frequency and the waveform of nystagmus were compared between these two groups of subjects with IIN. Linear mixed models were used to statistically compare the effects of *FRMD7* and non-*FRMD7* inheritance including family, gender and eccentricity (for eye movements) as fixed factors excluding any non-significant interactions from the final models. Non-parametric data were either log transformed (i.e. amplitudes and frequencies) or analysed using Mann–Whitney U-tests (i.e. comparing VAs between *FRMD7* and non-*FRMD7* groups and also OKN and smooth pursuit eye movements of *FRMD7* carriers to age-matched controls). The Pearson chi-squared test and the γ-statistic were used to compare relative proportions between groups.

3 Results

This chapter is subdivided into clinical and laboratory results. The first section comprises of the findings on clinical examination of all the subjects.

The second section relates to the lab work done by the author and comprises the results of genotyping using microsatellite markers and linkage analysis.

3.1 Clinical Results

The author recruited 39 families and 78 singletons with nystagmus. The clinical details of each recruited subject is detailed in this section.

The families recruited initially in whom the data from linkage analysis was available prior to gene sequencing is numbered with Prefix 'N' .

A number of smaller families with IIN were included in the study even after the first batch of haplotype analysis. DNA from these families were submitted for gene sequencing prior to haplotype analysis. Families in whom the haplotype data was unavailable prior to submitting for gene sequencing are denoted with a Prefix 'F'.

Four small families (prefixed with 'G') with possible X-linked inheritance were recruited later. Haplotype analysis was not carried out on these samples. However the DNA was submitted for gene sequencing.

Families with nystagmus associated with disorders other than X-linked IIN were not included in the genetic analysis and are denoted with a prefix 'E'.

In each section the families are listed in the chronological order of recruitment.

3.1.1 Families in which Linkage Analysis was Performed (Families N1-N16)

Family N1

Two subjects from this family were referred to the neuro-ophthalmology clinic at Leicester Royal Infirmary and the rest of the subjects were recruited with the help of the proband's parents.

This large four-generation family (Figure 3N.01) is from the Preston-Leyland region (Lancashire) with a branch of the family living in Swindon (Wiltshire) and a few members in Australia.

Phenotyping of these subjects required multiple trips to Preston and Leyland. Subject 21238 and her children lives in Swindon and they were examined on a house visit by the author. On a later date, 21238 and 21239 travelled to Leicester for detailed examination and eye movement recording.

Subjects 21281 and 21282 live in Australia.

Fourteen affected members were recruited from this family. Eight of the twelve male subjects had predominantly horizontal pendular nystagmus while three had jerk waveform. The waveform of subject 21282 was not determined. The only female subject with clinically detectable nystagmus (21239) had horizontal jerk waveform. Eye movement data of subject 20905 and 21239 are shown in Figure 3N.01a. Subject 21020 was initially classified as unaffected, however, was found to have subclinical nystagmus on eye movement recording (Figure 3N.01b)

Most of the subjects with nystagmus in this family had Snellen visual acuity between 0.5 and 1.0. Two subjects had anomalous head posture (AHP) of ~10° and another two

had and AHP of ~5°. The level of stereopsis was variable. None of the subjects with nystagmus had manifest strabismus. Subject 20828 was blind in one eye due to injury in childhood and had left exotropia, but not nystagmus. The clinical details of the subjects are given in Table 3N.01. ERG and VEP of the subject 20905 is shown in Figures 3N.01c and 3N.01d

Figure 3N.01: *shows Family N1*

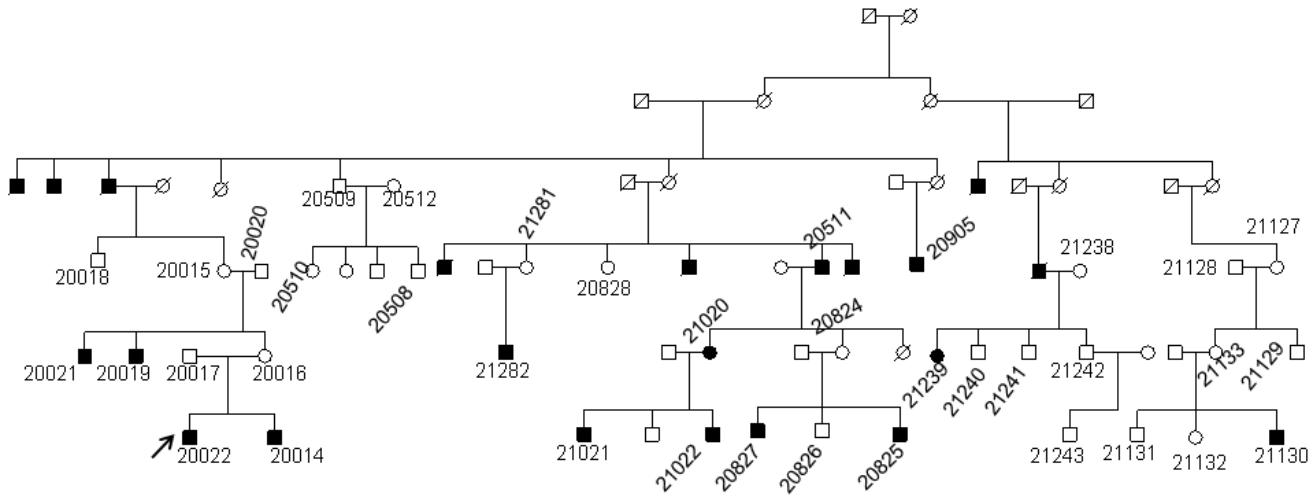


Table 3N.01: *Clinical details of subjects from family N1. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
20014	13	M	0.50	0.50	0.50	Horizontal jerk	10° to Left	150	Nil	Normal EDT
20022	2	M	0.50	0.50	0.50	Horizontal jerk	Nil	1200	Nil	
20019	36	M	0.50	0.67	0.67	Horizontal jerk	10° to Left	170	Nil	
20021	35	M	1.00	0.67	1.00	Horizontal conjugate	5° to Left	85	Nil	Normal EDT
20511	62	M	0.67	0.67	0.67	Horizontal conjugate	5° to Left	100	Nil	
20825	21	M	0.67	0.50	0.67	Horizontal conjugate	Nil	550	Nil	
20827	17	M	0.50	0.67	0.67	Horizontal conjugate	Nil	Bagolini +ve	Nil	
20905	48	M	0.50	0.50	0.50	Horizontal jerk	Nil	150	Nil	Normal EDT
21022	21	M	0.50	0.67	0.67	Horizontal conjugate	Nil	150	Nil	Normal EDT
21130	11	M	0.33	0.50	0.50	Horizontal conjugate	Nil	170	Nil	
21239	37	F	1.00	1.00	1.00	Horizontal jerk	Nil	300	Nil	
21021	13	M	1.00	1.00	1.00	Horizontal conjugate	Nil	150	Nil	
21282	39	M	U	U	U	Present	U	U	U	
21020	40	F	1.00	1.00	1.00	Subclinical nystagmus	Nil	150	Nil	
20017	40	M	1.00	1.20	1.20	Nil	Nil	55	Nil	
20016	40	F	1.00	1.50	1.50	Nil	Nil	150	Nil	
20018	70	M	1.00	1.00	1.00	Nil	Nil	40	Nil	
20015	61	F	1.00	1.00	1.00	Nil	Nil	240	Nil	
20020	64	M	1.00	1.00	1.00	Nil	Nil	75	Nil	
20508	54	M	1.00	0.67	1.00	Nil	Nil	U	Nil	
20510	55	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
20512	77	F	1.00	1.00	1.00	NIl	Nil	U	Nil	
20509	80	M	0.67	0.67	0.67	Nil	Nil	U	Nil	
20824	39	F	1.20	1.20	1.20	NIl	Nil	60	Nil	
20826	20	M	1.20	1.20	1.20	Nil	Nil	120	Nil	
20828	61	F	1.00	NPL	1.00	Nil	Nil	Nil	L exotropia	L traumatic cataract
21127	70	F	1.00	0.67	1.00	Nil	Nil	85	Nil	
21128	75	M	0.67	1.00	1.00	NIl	Nil	85	Nil	
21129	45	M	1.00	1.00	1.00	Nil	Nil	110	Nil	
21131	13	F	1.00	1.00	1.00	Nil	Nil	40	Nil	
21133	41	F	0.50	1.00	1.00	Nil	Nil	85	Nil	
21131	8	F	1.00	1.00	1.00	Nil	Nil	U	U	
21238	63	F	1.00	1.00	1.00	Nil	Nil	U	U	
21240	34	M	1.20	1.20	1.20	Nil	Nil	150	Nil	
21241	27	M	1.00	1.00	1.00	Nil	Nil	150	Nil	
21243	6	M	1.00	1.00	1.00	Nil	Nil	340	Nil	
21242	24	M	1.00	1.00	1.00	Nil	Nil	U	Nil	
21281	57	F	U	U	U	Nil	U	U	U	

Figure 3N.01a: *Eye movement recording of two subjects from family N.01. The top panel shows horizontal jerk waveform in subject 20905 and the bottom panel shows horizontal jerk nystagmus in subject 21239. Both subjects do not have any vertical nystagmus.*

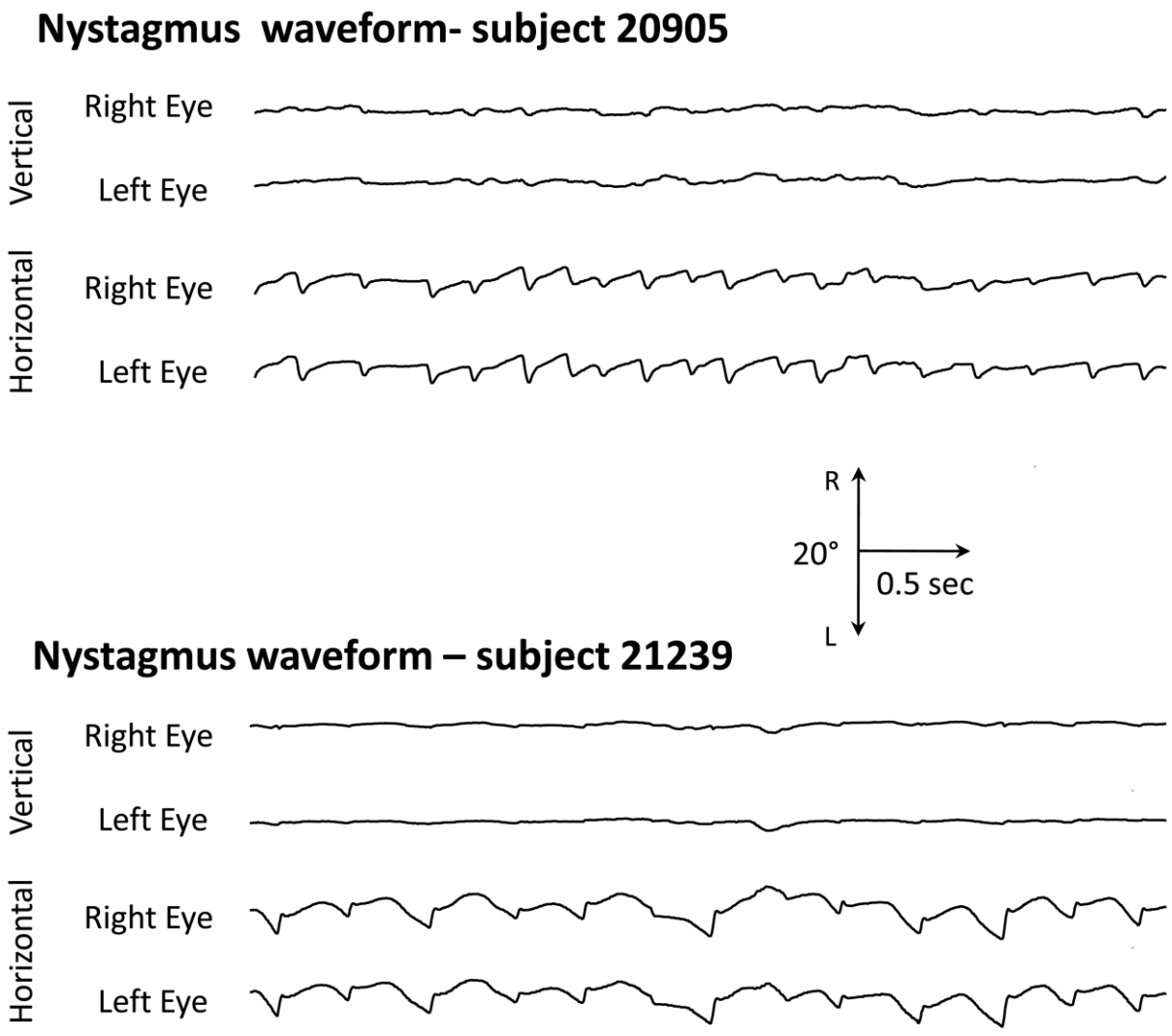


Figure 3N.01b: *Eye movement recording of the subject (no.21020). Clinical examination was normal with no evidence of nystagmus. However, eye movement recording showed fine nystagmus with slow rightward drifts and fast beats to the left (~0.5°).*

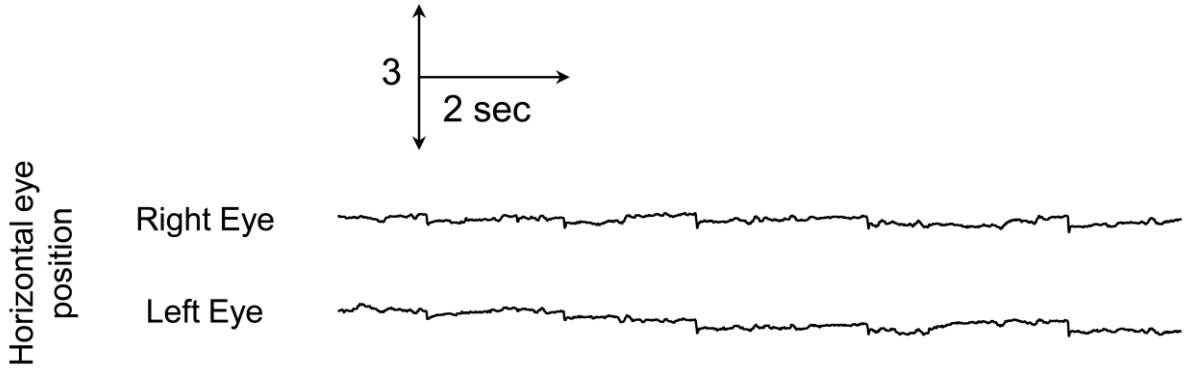


Figure 3N.01c: Normal Electroretinogram of subject 20905. *Photopic (top panel) and scotopic ERG (bottom panel) shows normal 'a' and 'b' waves. 'ISCEV 2004 standard flash' (SF) which was reduced at -0.25 log units per flash was used for photopic ERG. A low intensity flash which was increased stepwise at 0.25 log units to reach a maximum intensity of 'ISCEV 2004 standard flash' was used for the scotopic ERG.*

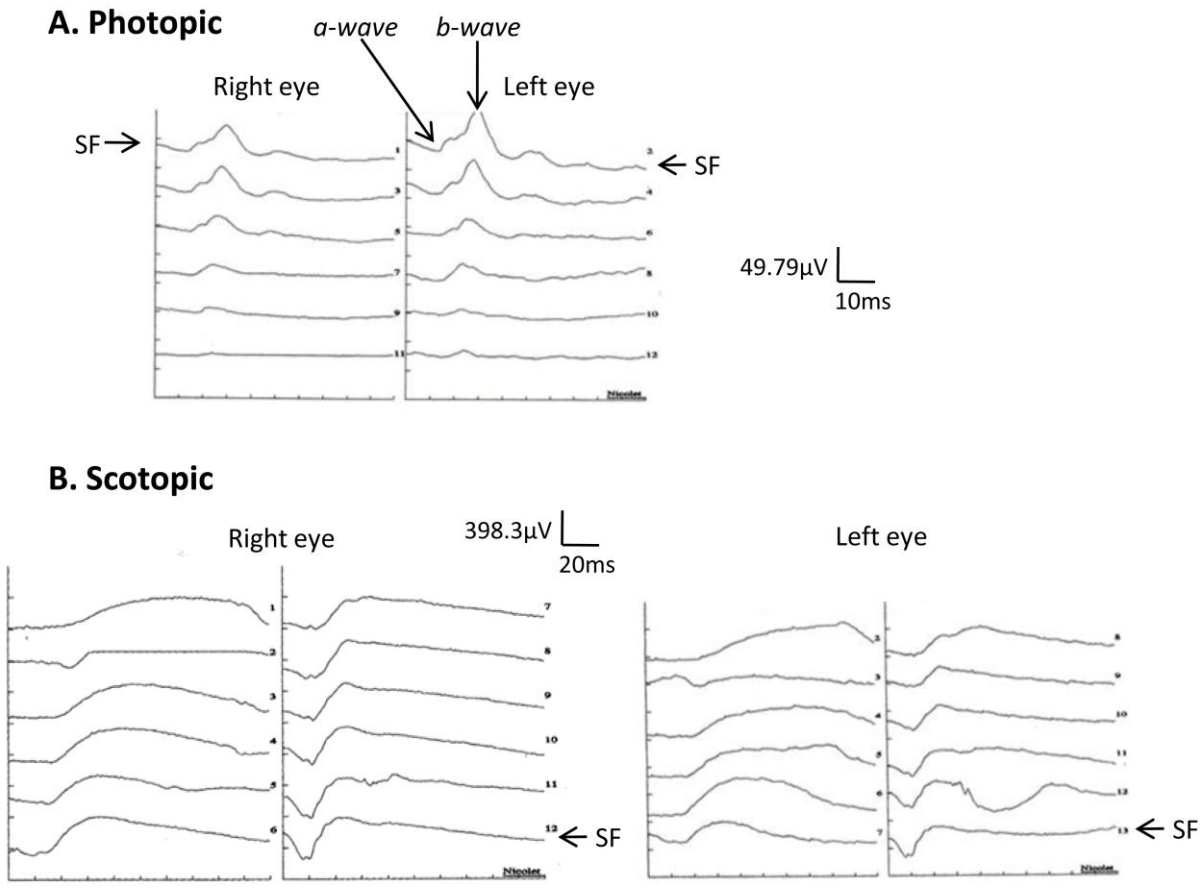
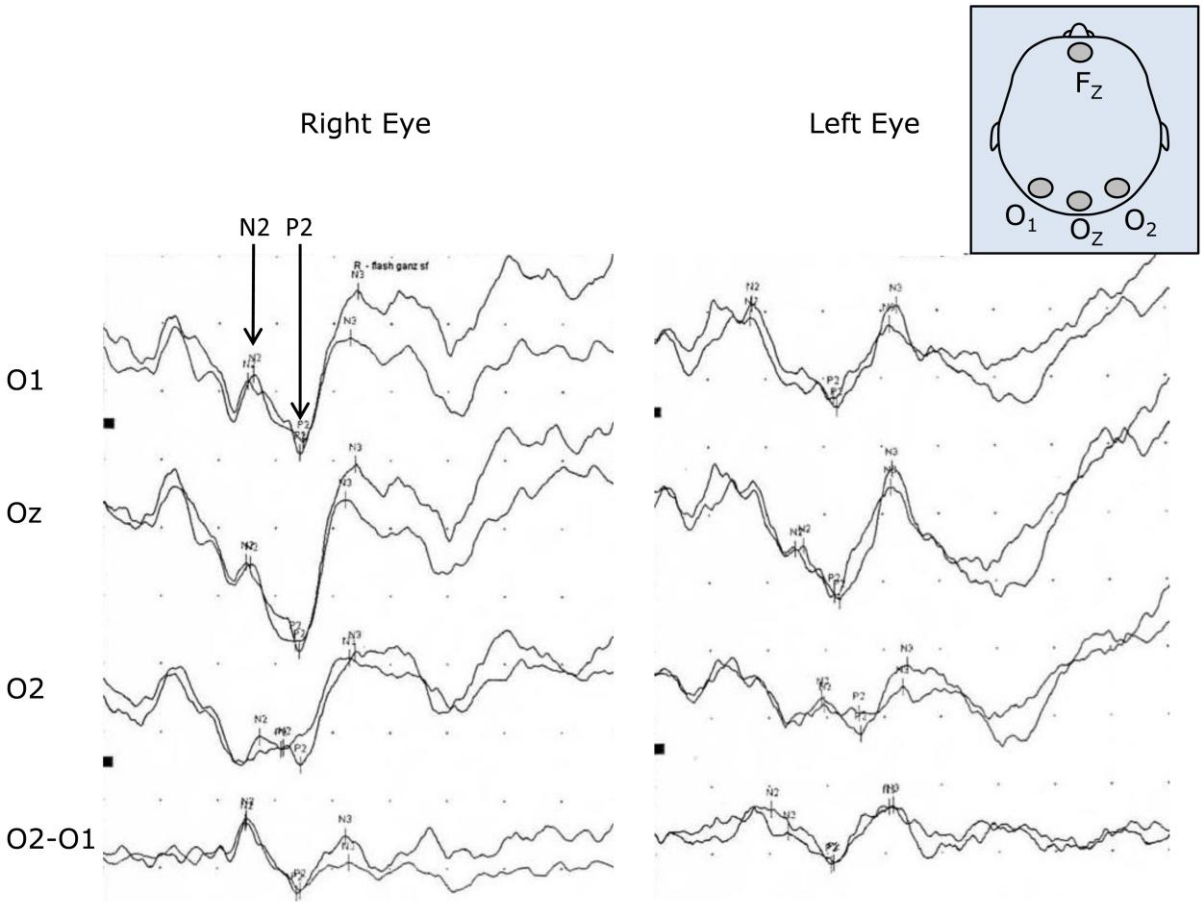


Figure 3N.01d: *Normal VEP of subject 20905. The panel on the left shows the response on stimulating of the right eye whilst the panel on the right shows the response on stimulating the left eye. The position of electrodes O1, O2 and Oz are at the occipital region on the left side, right side and in the midline respectively as shown in the inset.*



Family N2

Family N2 and N3 (Figure 3N.2 and 3N.3) was recruited on a field trip by IG, FP and MA. The author's involvement included liaising with the subjects and collaborators in USA, obtaining permission from HM customs and 'the inspector of anatomy' to import the tissue samples to UK, tabulating and analysing the data.

Seven subjects with IIN were recruited from this family of which five were males and two were females. Horizontal pendular and jerk waveforms were present (Figure 3N.02a). Snellen visual acuities ranged between 0.5 and 1.0 except for subject 21080 who had poorer visual acuity due to senile cataracts. None of the subjects had significant AHP.

One of the members of the family underwent electro-diagnostic tests (EDT's) which showed normal results. ERG is shown in Figure 3N.02b and the clinical details are given in Table 3N.02.

Figure 3N.02: *Shows the pedigree N2*

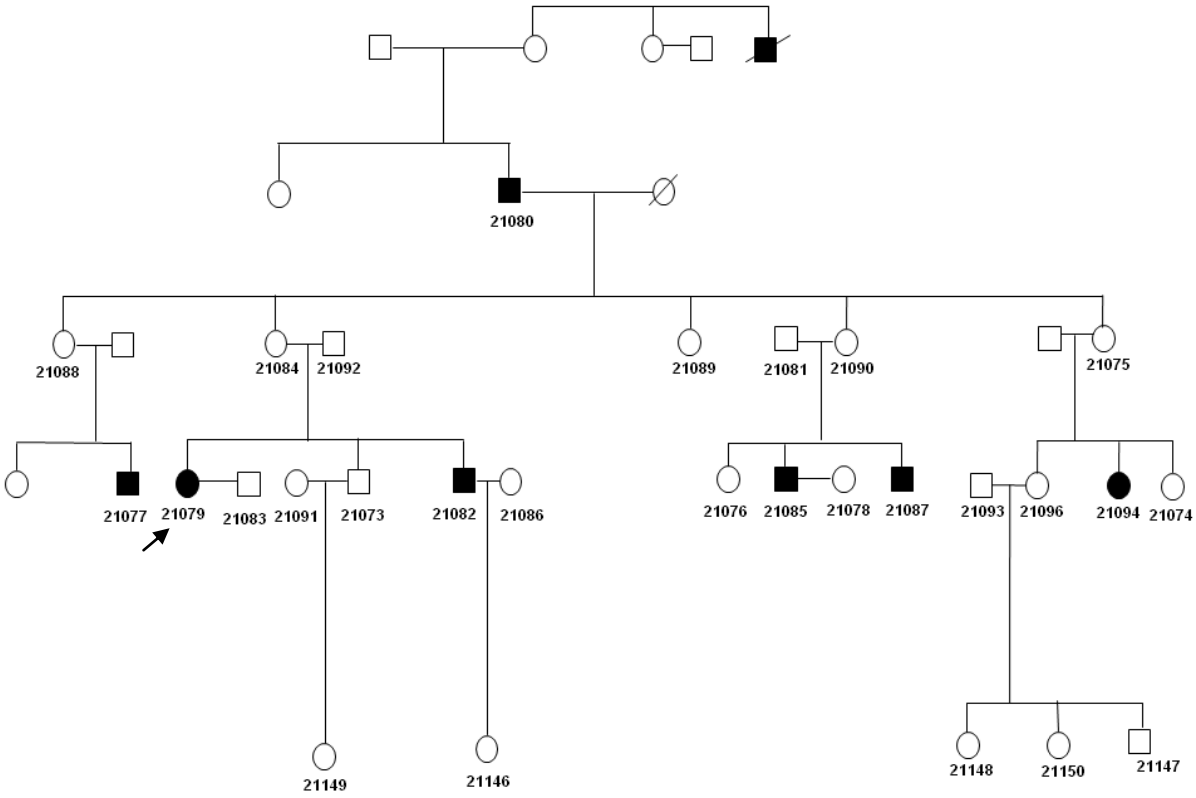


Figure 3N.02a: *Eye movement recording of two subjects from family N.02. The top panel shows horizontal pendular waveform in subject 21079 and the bottom panel shows horizontal jerk nystagmus in subject 21077. Both subjects do not have any vertical nystagmus.*

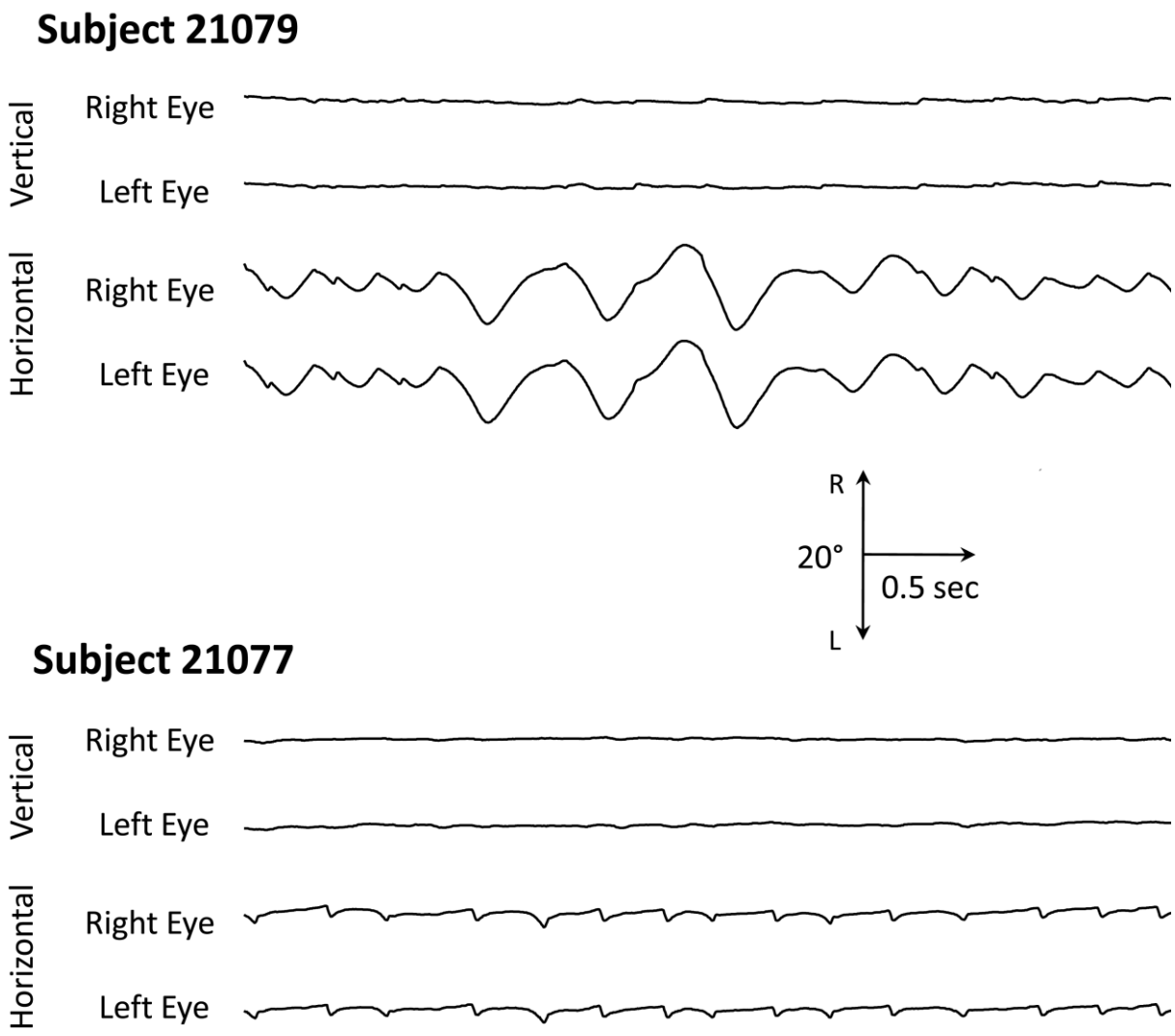


Figure 3N.02b: *Normal ERG of subject 21087 of family N.02. The figure on the left-hand side is photopic ERG and the one on right-hand side is scotopic ERG. In each figure, the top trace is of right eye and the bottom trace is of left eye. (This was performed at Wills Eye Hospital, Philadelphia, USA). Normal 'a' wave (denoted 1) and 'b' wave (denoted 2) are seen in both photopic and scotopic traces. The scale of the ERG- time (5mS/division) on the X-axis and the amplitude (20µV/division) on the Y axis is also shown.*

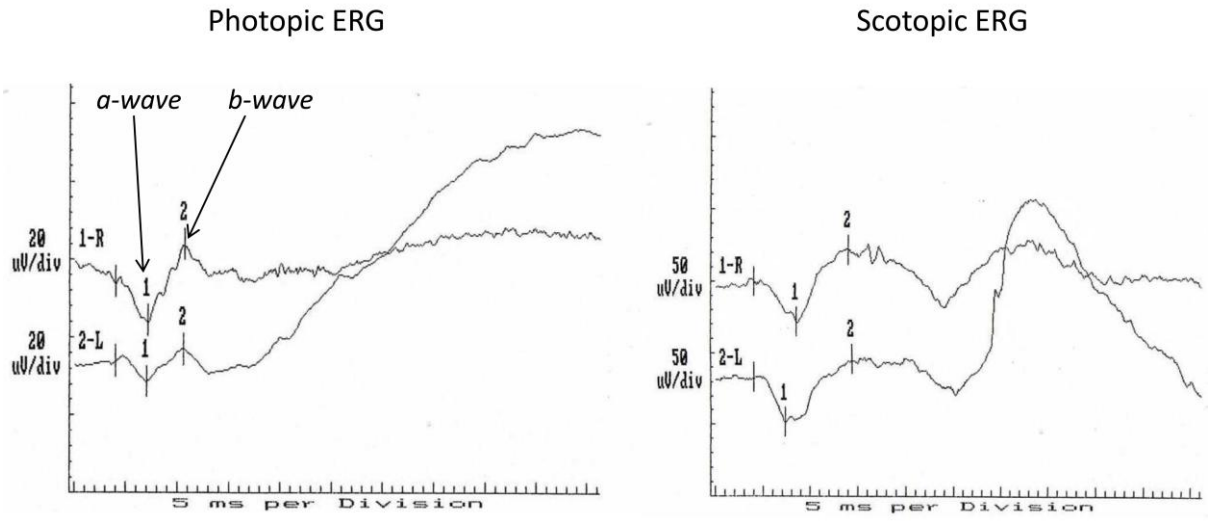


Table 3N.02: *Clinical details of subjects from family N2. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21077	24	M	1.00	1.00	1.00	Horizontal pendular	Nil	30	Nil	
21079	27	F	0.80	0.80	0.80	Horizontal pendular	Nil	60	Nil	
21080	86	M	0.17	0.17	0.17	Horizontal pendular	Nil	170	Nil	Cataract
21082	34	M	0.50	0.33	0.50	Horizontal jerk	Minimal face turn to right	85	Nil	
21085	33	F	0.80	0.50	0.63	Horizontal jerk	Minimal face turn to left	240	Nil	
21087	35	M	0.32	0.83	0.83	Horizontal pendular	Minimal face turn to left	75	Nil	EDT: Normal
21092	61	F	0.50	U	0.50	Latent nystagmus	Nil	85	R exotropia	
21094	39	F	0.67	0.50	0.67	Horizontal jerk	Nil	40	Nil	
21073	36	M	1.18	1.18	1.18	Nil	Nil	60	Nil	
21074	34	F	0.80	0.50	0.80	Nil	Nil	300	Nil	
21075	62	F	1.00	1.18	1.18	Nil	Nil	30	Nil	
21076	24	F	0.80	1.00	1.00	Nil	Nil	60	Nil	
21078	29	F	1.18	1.18	1.18	Nil	Nil	30	Nil	
21081	60	M	1.18	1.18	1.18	Nil	Nil	240	Nil	
21083	31	M	0.50	0.80	U	Nil	Nil	30	Nil	
21084	55	F	0.50	0.50	0.50	Nil	Nil	120	Nil	
21086	31	F	1.20	1.20	1.20	Nil	Nil	155	Nil	
21088	53	F	1.18	1.00	1.00	Nil	Nil	240	Nil	
21089	58	F	0.80	0.80	0.80	Nil	Nil	600	Nil	
21090	59	F	0.80	0.80	0.80	Nil	Nil	300	Nil	
21091	38	F	0.67	0.17	0.67	Nil	Nil	Nil	L exotropia	
21093	42	M	1.20	1.50	1.20	Nil	Nil	60	Nil	
21096	37	F	1.50	1.50	1.50	Nil	Nil	40	Nil	
21146	3	F	U	U	U	Nil	Nil	550	Nil	
21147	8	M	1.18	1.18	1.18	Nil	Nil	30	Nil	
21148	1	F	U	U	U	Nil	Nil	U	Nil	Fixes & follows objects
21149	1	F	U	U	U	Nil	Nil	U	Nil	Fixes & follows objects
21150	6	F	1.00	1.18	1.00	Nil	Nil	60	Nil	

Family N3

Nine affected subjects (eight males and one female) were recruited from this four generation family (Figure 3N.03). Subjects had either horizontal pendular or jerk waveforms. All the subjects with nystagmus had Snellen visual acuity better than 0.3. None of the affected subjects had manifest strabismus. Two of these subjects had minimal AHP. EDT's were done on two subjects from the family and was reported normal.

Figure 3N.03*: The pedigree of family N3.*

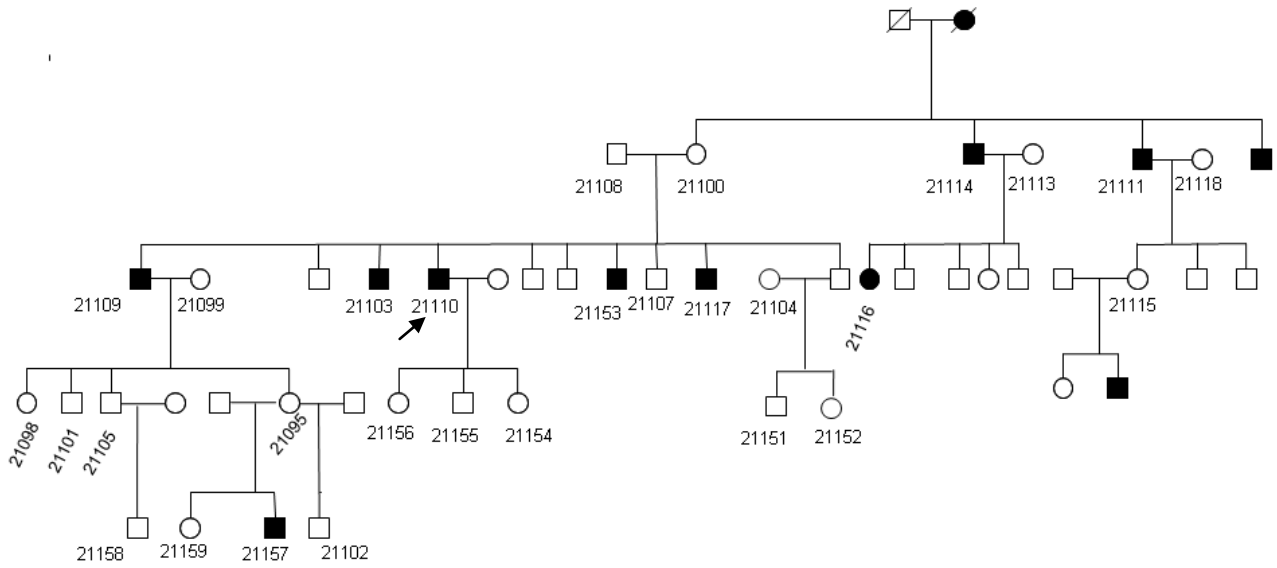


Table 3N.03: *Clinical details of subjects from family N03. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21103	48	M	0.50	0.50	0.50	Horizontal pendular	Minimal face turn to left	240	Nil	EDT: Normal; Head nodding present
21109	50	M	0.50	0.40	0.50	Horizontal pendular	Nil	40	Nil	EDT: Normal
21110	40	M	0.50	0.63	0.63	Horizontal pendular	Nil	240	Nil	
21111	75	M	U	U	U	Horizontal pendular	Nil	U	U	Parkinsons disease
21114	74	M	0.50	0.50	0.50	Horizontal jerk	Nil	350	Nil	
21116	42	F	0.80	1.00	1.00	Horizontal jerk	Nil	85	Nil	
21117	42	M	0.32	0.40	0.40	Horizontal pendular	Nil	120	Nil	
21153	46	M	0.40	0.63	0.63	Horizontal pendular	Minimal chin depression	240	Nil	
21157	1	M	U	U	U	Horizontal pendular	Nil	U	Nil	Fixes & follows objects
21095	30	F	1.00	1.00	1.00	Nil	Nil	550	Nil	
21098	25	F	1.18	1.50	1.50	Nil	Nil	30	Nil	
21099	51	F	1.18	1.00	1.00	Nil	Nil	150	Nil	
21100	72	F	1.00	0.63	1.00	Nil	Nil	85	Nil	
21101	24	M	0.80	1.00	1.00	Nil	Nil	60	Nil	
21102	12	F	1.18	1.18	1.18	Nil	Nil	30	Nil	
21104	42	F	1.20	1.20	1.20	Nil	Nil	60	Nil	
21105	28	M	0.80	1.00	1.00	Nil	Nil	240	Nil	
21106	35	M	U	U	U	Nil	Nil	U	U	
21107	35	M	1.60	1.60	1.60	Nil	Nil	30	Nil	
21108	74	M	0.63	0.63	0.63	Nil	Nil	U	Nil	
21113	73	F	1.18	1.18	1.18	Nil	Nil	85	Nil	
21115	43	F	0.63	1.18	1.18	Nil	Nil	85	Nil	
21118	73	F	U	U	U	Nil	Nil	85	Nil	
21151	11	M	1.00	1.00	1.00	Nil	Nil	60	Nil	
21152	13	F	1.18	1.18	1.18	Nil	Nil	30	Nil	
21154	7	F	1.18	1.18	1.18	Nil	Nil	60	Nil	
21155	12	M	1.18	1.54	1.54	Nl	Nil	30	Nil	
21156	13	F	1.00	1.00	1.00	Nil	Nil	60	NIl	
21158	4	M	0.63	0.63	0.63	Nil	Nil	U	Nil	
21159	3	F	U	U	U	Nil	Nil	550	Nil	Fixes & follows objects

Family N4

Five affected subjects and twenty unaffected relatives were recruited from this family (Figure 3N.04). Three affected subjects of this family were seen at the ophthalmology clinic at Leicester and the rest of the subjects were recruited on a field trip by IG, FP and ER in the Isle of Man.

All except one child with nystagmus had a Snellen visual acuity better than 0.5. A three year old child with nystagmus was found to have alternating esotropia. Two subjects had minimal AHP. Electro diagnostic tests were done on two affected subjects and were found to be normal. The clinical details of these subjects are given in Table 3N.04.

Figure 3N.04*: The pedigree of Family N4*

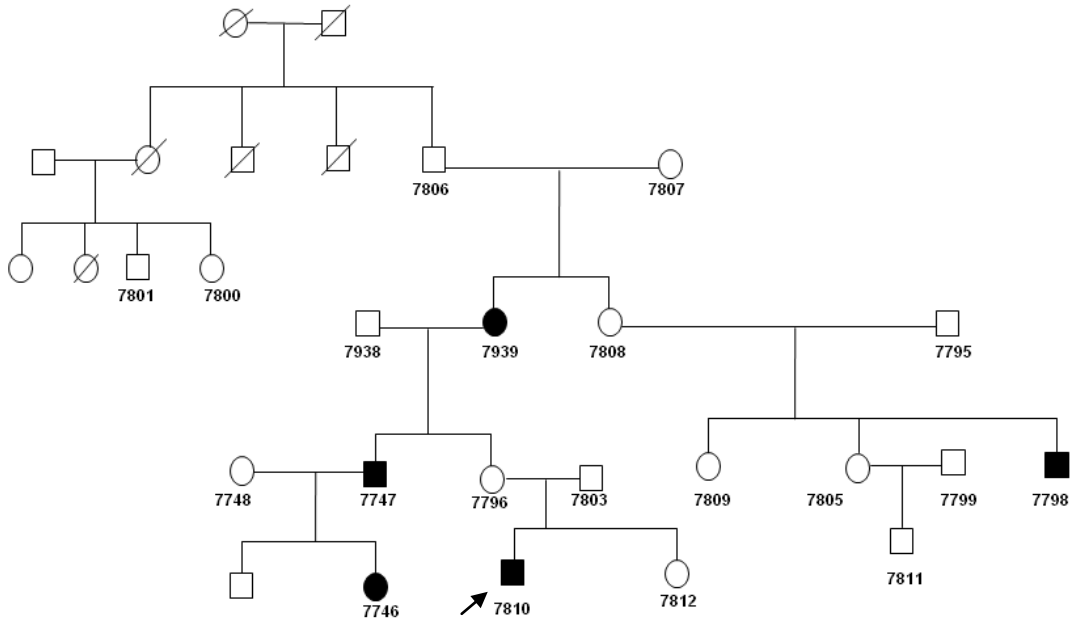


Table 3N.04: *Clinical details of subjects from family N4. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
7746	3	F	U	U	U	Horizontal pendular	Nil	U	Alternating esotropia	
7747	39	M	0.67	0.50	0.67	Horizontal jerk	Slight face turn to R	U	Nil	EDT: Normal
7798	21	M	0.67	0.50	0.67	Horizontal pendular/torsional	Nil	150	Nil	
7810	4	M	0.17	0.17	0.17	Horizontal pendular	Nil	550	Nil	
7939	65	F	0.67	0.50	0.67	Horizontal pendular	Slight face turn to L	40	Nil	EDT: Normal
7811	3	M	U	U	U	Nil	Nil	U	Nil	
7800	71	F	0.50	1.00	1.00	Nil	Nil	550	Nil	
7799	33	M	1.50	1.20	1.50	Nil	Nil	40	Nil	
7938	63	M	1.20	1.00	1.20	Nil	Nil	30	Nil	
7796	36	F	1.00	1.00	1.00	Nil	Nil	550	Nil	
7748	34	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
7809	30	F	1.50	1.20	1.50	Nil	Nil	550	Nil	
7805	29	M	1.20	1.20	1.20	Nil	Nil	550	Nil	
7795	59	M	1.00	1.00	1.00	Nil	Nil	550	Nil	
7808	54	F	1.00	1.20	1.20	Nil	Nil	550	Nil	
7801	72	M	1.20	0.67	1.40	Nil	Nil	300	Nil	
7807	81	F	1.00	1.00	1.00	Nil	Nil	550	Nil	
7806	86	M	1.00	1.00	1.00	Nil	Nil	550	Nil	
7812	6	F	1.00	1.00	1.00	Nil	Nil	550	Nil	
7803	34	M	2.00	2.00	2.00	Nil	Nil	U	Nil	

Family N5 - N7

These are smaller families with IIN with possible X-linked transmission.

Subject 20912 was phenotyped in the ophthalmology clinic whilst the rest of the subjects of Family N5 were recruited on a house visit by the author.

The affected subjects with nystagmus from this family had visual acuity better than 0.5. Both had horizontal pendular nystagmus.

The subjects of family N6 were seen at the ophthalmology clinic. All the affected subjects had good Snellen visual acuity (better than 0.5), minimal AHP and none of them had manifest strabismus.

Subjects 7920 and 7921 of family N7 was seen in the eye clinic at Leicester. The rest of the family members were recruited through home visits by the author. All affected subjects had vision comparable to subjects from other families. None of the subjects had manifest strabismus, however stereopsis in one subject (469) was poor (Bagolini positive)

The family trees are shown in Figure 3N5- 3N7. EDT's were performed on subjects 20912 and 7921 and were normal. Figure 3N.07a shows the horizontal pendular waveform in subject 7921.

Figure 3N.05- 3N.07*: shows the pedigrees of family N5-N7*

Figure 3N.05

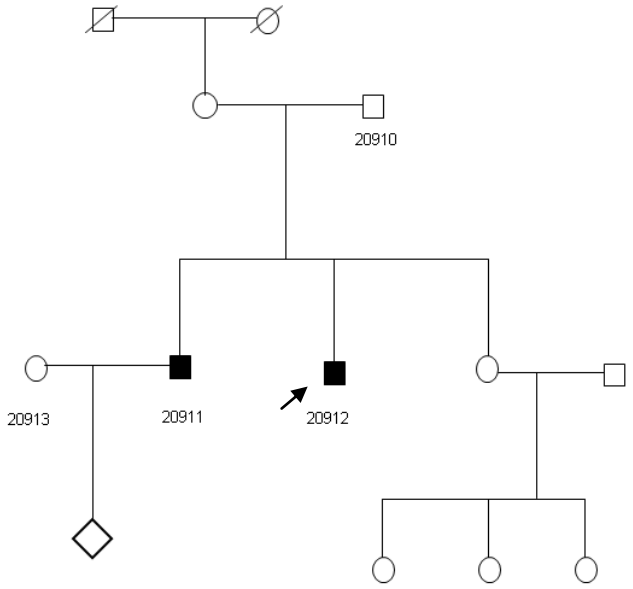


Figure 3N.06

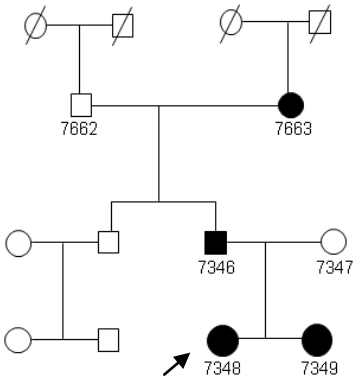


Figure 3N.07

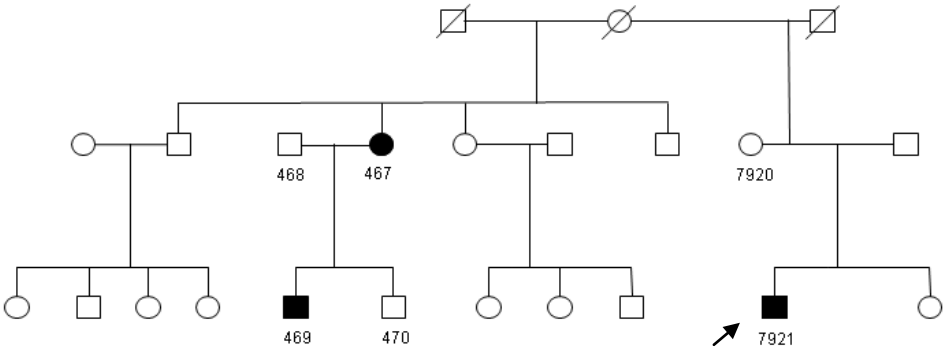


Figure 3N.07a: *shows the eye movement recording of the subject '7921'showing horizontal pendular nystagmus.*

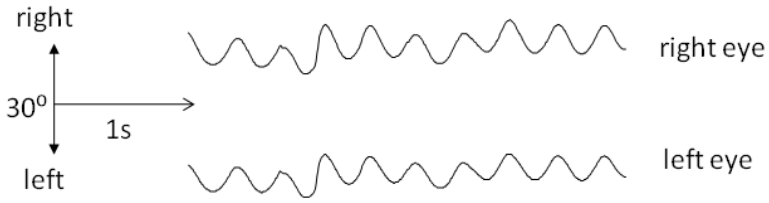


Table 3N.05-3N.07: *Clinical details of subjects from family N5 (top panel), N6 (middle panel) and N7 (lower panel). Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, binocular vision (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
20911	34	M	0.67	0.67	0.67	Horizontal pendular	Face turn 5° to right	150	Nil	EDT: Normal
20912	37	M	0.50	0.50	0.50	Horizontal pendular	Nil	110	Nil	EDT: Normal
20913	39	F	U	U	U	Nil	NIl	40	Nil	
Nil	U	F	1.00	1.00	1.00	Nil	Nil	40	Nil	
20910	64	M	1.20	1.20	1.20	Nil	Nil	110	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
7663	68	F	0.50	0.50	0.50	Horizontal pendular	Nil	550	Nil	
7348	9	F	1.00	1.00	1.00	Horizontal pendular	5° chin down	60	Nil	
7349	6	F	0.67	0.67	0.67	Horizontal pendular	Nil	60	Nil	
7346	43	M	0.67	0.67	0.67	Horizontal pendular	Face turn 5° to right	150	Nil	EDT: Normal
7662	75	M	1.00	1.00	1.00	Nil	Nil	300	Nil	
7347	39	F	U	U	U	Nil	U	U	U	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
7921	32	M	0.67	0.67	0.67	Horizontal pendular	Mininal left head tilt	110	Nil	EDT: Normal
467	40	F	0.67	0.67	0.67	Horizontal jerk	Nil	550	Nil	
469	14	M	0.67	0.67	0.67	Horizontal pendular	Nil	Bagolini +ve	Nil	
7920	53	F	U	U	U	Nil	U	U	Nil	
468	41	M	0.80	0.67	0.67	Nil	Nil	U	Nil	
470	12	M	1.50	1.50	1.50	Nil	Nil	550	Nil	Endpoint nystagmus

Family N8-N10

Families N8 and N9 were recruited by clinical collaborators of Professor Irene Gottlob. This clinical details and saliva samples were given to the author by Dr A Zubcov and Dr C Pieh. The visual acuity and other clinical characteristics of subjects from these families are comparable to the subjects from the previously described families.

Family N10 lives in Lincolnshire and was recruited after a member was referred to the neuro-ophthalmology clinic at Leicester. Visual acuity is comparable to subjects from other families and subjects had either horizontal pendular or jerk waveform. One subject from the family has manifest strabismus. Subject 8165 had normal EDT.

Figure 3N.08 – 3N.10: *The top and middle picture (Figure 3N.08 and 3N.09) shows the pedigree of two families of German origin (I would like to acknowledge Dr A Zubcov and Dr C Pieh for providing the clinical details and blood samples). The bottom panel (Figure 3N10) shows family N10.*

Figure 3N.08

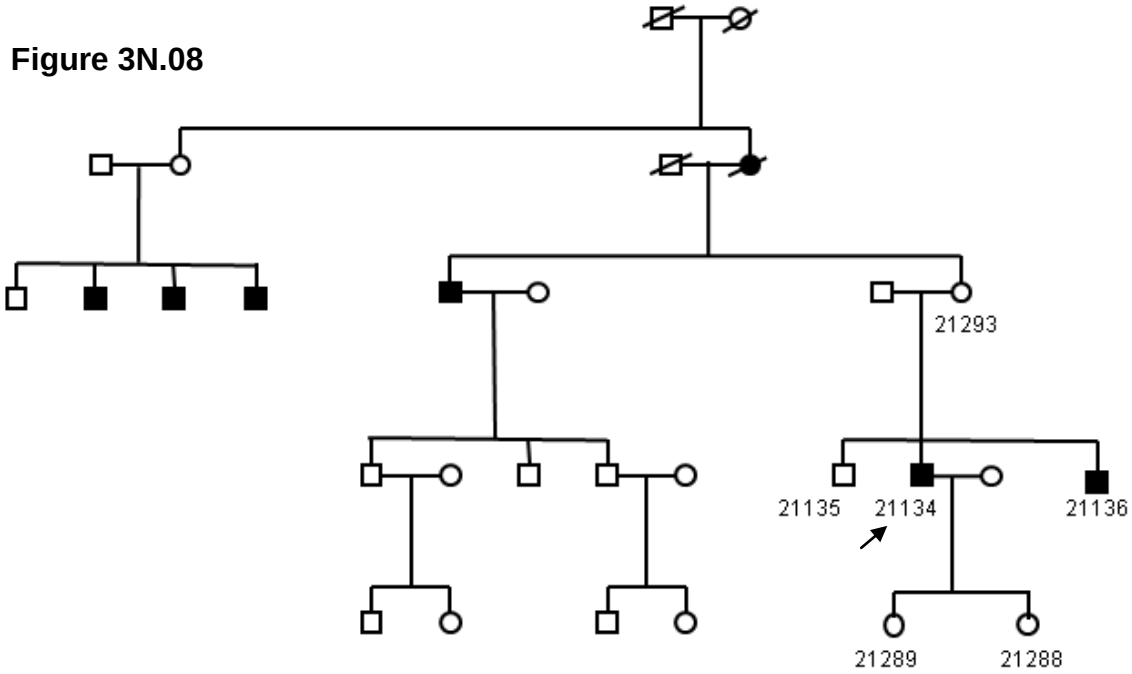


Figure 3N.09

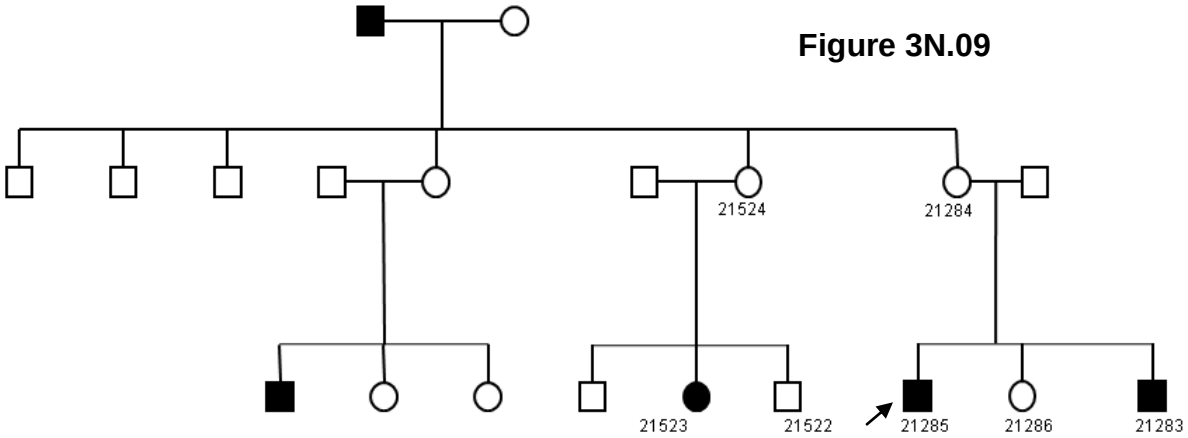


Figure 3N.10

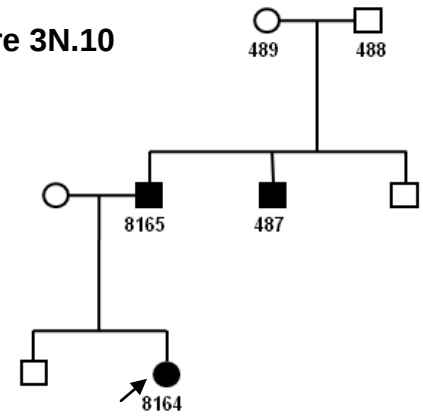


Table 3N.08 – 3N.10: *Clinical details of subjects from family N8 (top panel), N9 (middle panel) and N10 (bottom panel). Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21136	28	F	0.67	0.67	U	Horizontal pendular	Nil	U	Nil	
21134	37	M	0.67	0.67	U	Horizontal pendular	Nil	U	Nil	EDT: normal
21135	41	M	0.33	0.25	0.33	Nil	Nil	U	Nil	Unaided vision
21288	11	F	U	U	U	Nil	Nil	U	Nil	
21289	2	F	U	U	U	Nil	Nil	U	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21283	8	M	0.50	0.50	0.50	Horizontal pendular	Nil	U	Nil	
21285	16	M	0.50	0.50	0.50	Horizontal pendular	Nil	U	Nil	EDT: normal
21523	10	F	U	U	U	Present	U	U	Nil	
21524	35	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
21284	37	F	1.00	1.00	1.00	NIl	Nil	U	Nil	
21286	12	F	1.00	1.00	1.00	NIl	NIl	U	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
8165	37	M	0.67	0.67	0.67	Horizontal pendular	Nil	150	Nil	EDT: Normal
487	40	M	0.50	0.50	0.50	Horizontal jerk	Nil	150	R esotropia	
8164	2	F	U	U	U	Horizontal pendular	NIl	U	NIl	
489	69	F	1.00	1.00	1.00	Nil	Nil	U	NIl	Endpoint nystagmus
488	71	M	1.00	1.00	1.00	Nil	Nil	U	Nil	

Family N11

This family of Scottish ethnicity was recruited after the proband was referred to the eye clinic at Leicester. Sixteen affected subjects were phenotyped, however, four were excluded due to oculo-cutaneous albinism. Of the twelve subjects with a diagnosis of IIN, 6 were males. Most of the subjects from this family lives in Glasgow and were recruited on a field trip by the author, IG, FP, RM and SF.

Two subjects with nystagmus had manifest strabismus and another two subjects had high myopia. Subjects had either pendular or jerk waveform and the incidence of AHP was low.

A branch of the family (21352 and 21353) lives in Hereford and was seen on a house visit by the author. Subject 21353 who was married in to the family has ocular albinism. 21354 (daughter of 21353) also had a diagnosis of ocular albinism and her daughter (21355) was found to have iris transillumination; hence this branch of the family was excluded from the study.

Another branch of the family (21325 and children) lives in Nottingham and was seen on a house visit by the author. Subject 21328 was found to have horizontal pendular nystagmus and had stereopsis of 85'' of arc. We could not organise electro-diagnostic tests and due to the uncertainty in the diagnosis, this subject was excluded from the genetic analysis.

The details of the family are shown in Figure 3N.11 and Table 3N.11. Subject 8185 had normal EDT.

Figure 3N.11*: Shows the pedigree of family 11. Note that subject no. 21352 has IIN while 21353 has ocular albinism (green colour). The children of this couple with nystagmus were not included in the study.*

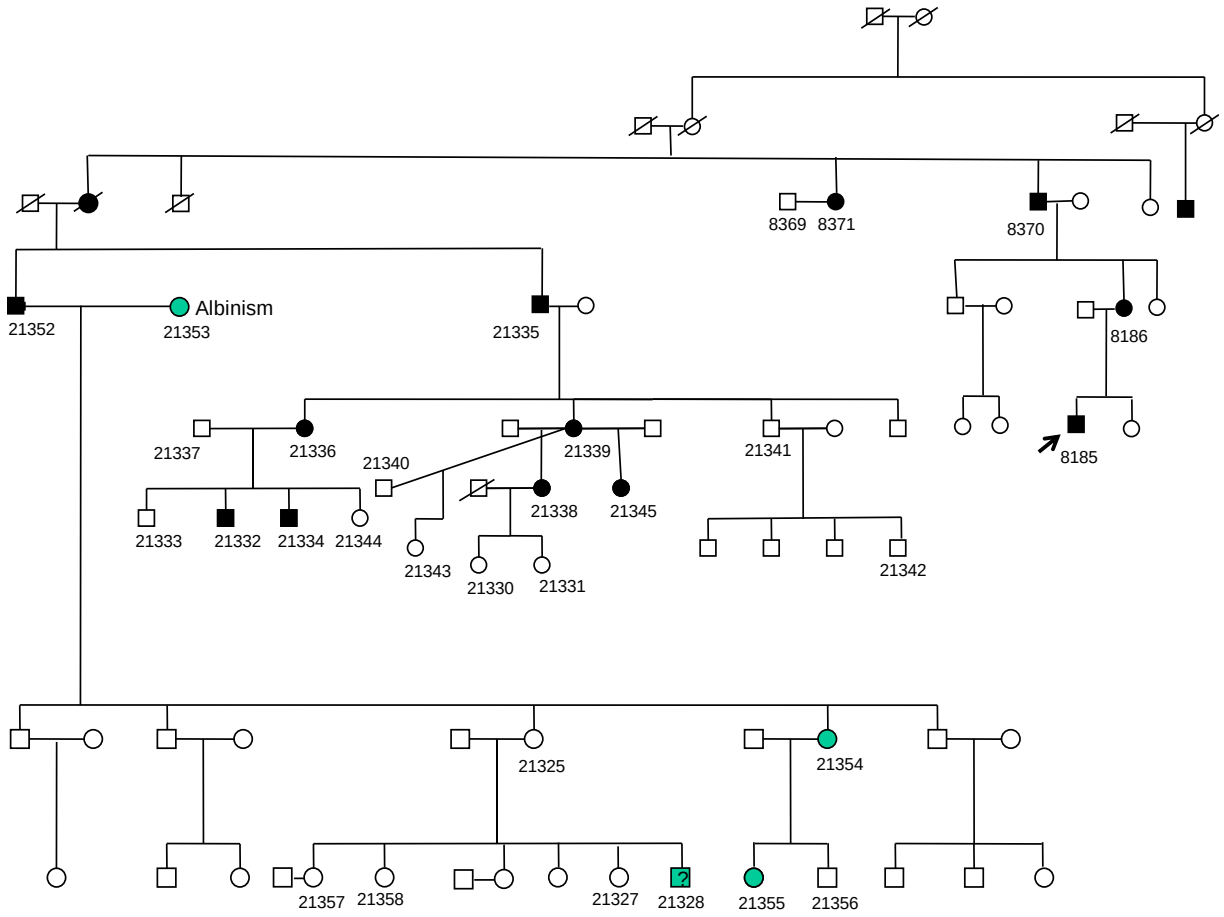


Table 3N.11: *Clinical details of subjects from family N11. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
8185	20	M	1.00	0.80	1.00	Horizontal pendular	Nil	85	Nil	EDT: Normal
8370	71	M	1.00	1.00	1.00	Horizontal jerk	5° to R	40	Nil	
8371	75	F	0.67	0.67	0.67	Horizontal pendular	5° to R	60	Nil	
21332	15	M	0.63	0.67	0.67	Horizontal pendular	5°	85	Nil	
21334	14	M	0.20	0.25	0.25	Horizontal pendular	5°	3000	Alt. exotropia	
21335	65	M	0.40	0.33	0.40	Horizontal pendular	U	150	Nil	
21336	45	F	0.80	0.80	0.80	Horizontal pendular	Nil	40	Nil	
21338	27	F	0.33	0.40	0.40	Horizontal pendular	Nil	150	Nil	
21339	44	F	0.33	0.40	0.40	Horizontal jerk	Nil	300	Nil	High myopia; EDT: Normal
21345	23	F	0.17	0.25	0.25	Horizontal jerk	5°	300	Nil	High myopia; EDT: Normal
21352	67	M	0.67	0.67	0.67	Horizontal pendular	Nil	40	Nil	
8186	54	F	0.67	0.67	0.67	Horizontal pendular	U	U	Nil	
21328	9	M	U	U	0.63	Horizontal pendular	Nil	85	Nil	Grandmother: Albinism
21353	66	F	0.17	0.17	0.17	Horizontal pendular	Nil	U	U	Albinism
21354	37	F	U	U	U	Horizontal pendular	U	U	Nil	Albinism
21355	15	F	0.17	0.17	0.17	Horizontal pendular	Nil	U	L esotropia	Iris transillumination
21325	39	F	0.80	0.80	0.80	Nil	Nil	85	Nil	
8369	76	M	U	U	U	Nil	Nil	U	Nil	
21337	47	M	1.20	1.00	1.20	Nil	Nil	60	NII	
21333	26	M	1.50	1.20	1.50	Nil	Nil	40	Nil	
21344	12	F	0.50	0.80	0.80	Nil	Nil	150	Nil	
21341	43	M	0.80	HM	0.80	Nil	Nil	Nil	L exotropia	
21342	13	M	1.20	1.20	1.20	NII	Nil	170	Nil	
21343	11	F	0.80	0.80	0.80	Nil	Nil	150	Nil	
21340	44	M	0.80	0.63	0.80	Nil	Nil	85	Nil	
21330	7	F	0.80	1.00	1.00	Nil	Nil	480	NII	
21331	6	F	1.00	0.63	1.00	NII	Nil	550	Nil	
21327	9	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
21356	9	M	U	U	U	Nil	U	U	U	
21357	22	F	0.33	0.33	0.33	Nil	Nil	U	Nil	Unaided vision
21358	21	F	U	U	U	Nil	U	U	U	

Family N12

This family with five affected subjects (three males and two females) was referred by Dr O Backhouse and were recruited on a trip to Bradford by the author who was accompanied by ER. All the affected subjects had visual acuity between 0.5 and 1. Two subjects in this family had strabismus (hypertropia and esotropia). One of the subjects (383) underwent EDT's at the referring hospital and was reported as normal. The clinical details of these subjects are shown in Table 3N.12

***Figure 3N.12**: Shows the pedigree of family N12.*

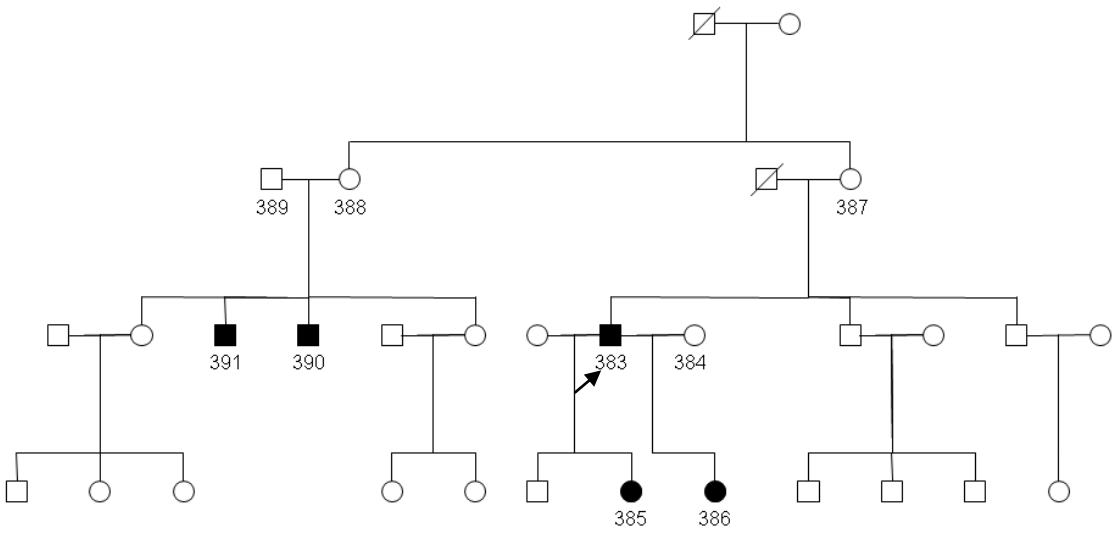


Table 3N.12*: Clinical details of subjects from family N12. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
391	39	M	0.10	0.33	0.33	Horizontal pendular	Nil	Bagolini -ve	R esotropia	
390	32	M	0.80	0.50	0.80	Horizontal jerk	Nil	3000	L hypertropia	
383	38	M	0.40	0.63	0.40	Horizontal pendular	Nil	150	Nil	EDT: Normal
386	4	F	0.50	0.63	0.63	Horizontal pendular	Nil	80	Nil	
385	14	F	0.80	0.80	0.80	Horizontal pendular	Nil	170	Nil	
384	36	F	1.20	1.20	1.20	Nil	Nil	150	Nil	
387	70	F	0.80	0.80	0.80	Nil	Nil	150	Nil	
388	64	F	1.00	0.80	0.80	Nil	Nil	150	Nil	
389	70	M	0.80	1.00	1.00	Nil	Nil	300	Nil	

Family N13 and N14

Family N13 was recruited by clinical collaborators (Mr O Backhouse and Dr R Gale) from Madagascar. The clinical characteristics of the subjects are similar except that a higher proportion of the subjects have significant AHP in this family.

Family 14 was recruited by the author, IG and FP on a field trip to Graz (Austria). Snellen visual acuities ranged between 0.5 and 1.0 and of the four affected subjects, two had significant AHP. The clinical details of these families are given in table 3N.13 and 3N.14.

Figure 3N.13- 3N.14: *The figure on top (Figure 3N.13) shows the pedigree of family N13 and the bottom figure (Figure 3N.14) shows family N14.*

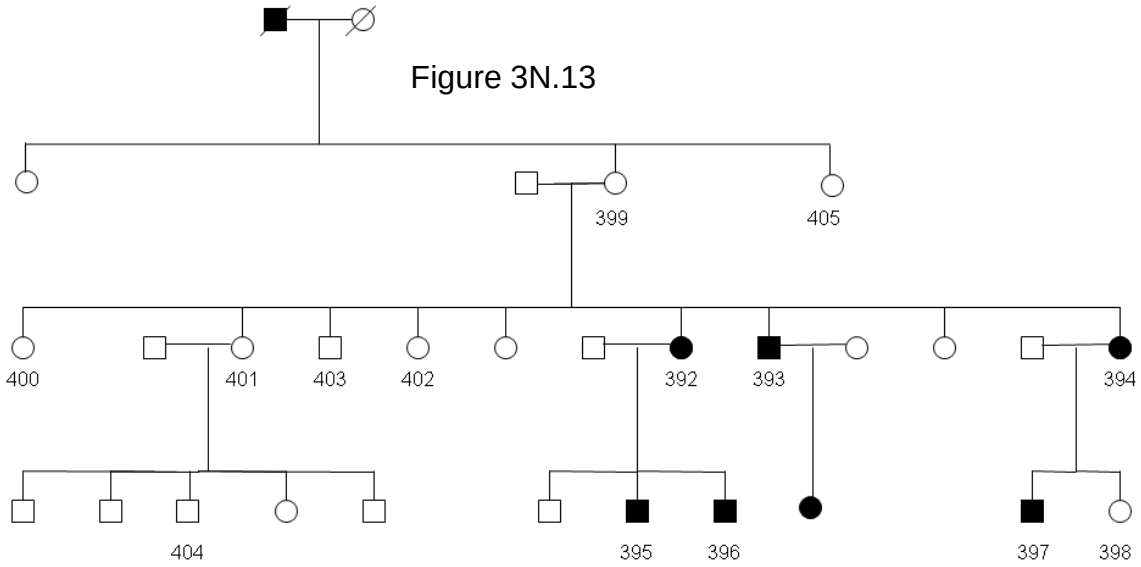


Figure 3N.13

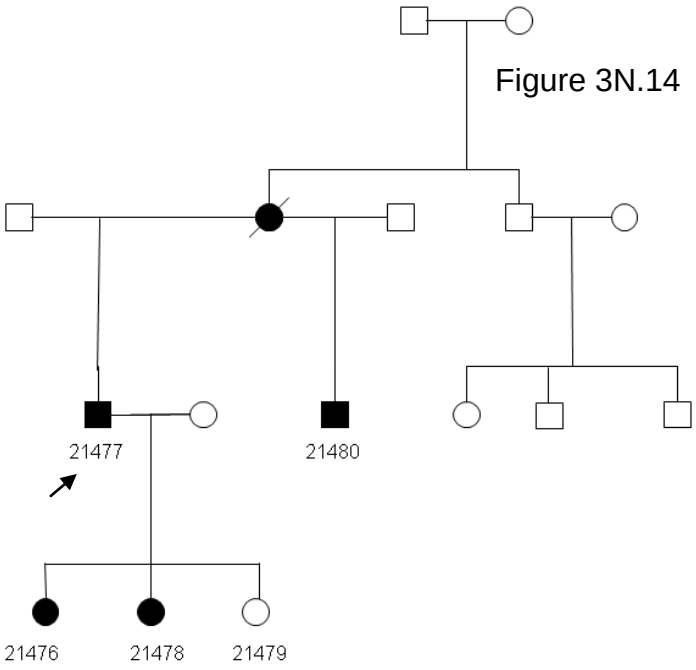


Figure 3N.14

Table 3N.13*: Clinical details of subjects from family N13. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, binocular vision (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
392	30	F	0.90	0.90	0.90	Horizontal pendular	10° to L	200	L exotropia	EDT: normal
393	33	M	0.50	0.50	0.90	Horizontal pendular	10° to R	600	Nil	
394	24	F	0.80	0.80	1.00	Horizontal pendular	10° to L	200	Exotropia	
395	4	M	0.17	0.22	0.22	Horizontal pendular	10° to L	600	Nil	
396	1	M	U	U	U	Horizontal pendular	20° to R	U	Nil	Fixes and follows objects
397	6	M	0.20	0.20	0.28	Horizontal pendular	10° to L	200	Nil	
398	1	M	U	U	U	Nil	Nil	U	Nil	
399	67	F	1	0.9	0.9	Nil	Nil	600	Nil	
400	46	F	1	1		Nil	Nil	600	Nil	
401	42	F	1	1	1	Nil	Nil	200	Nil	
402	41	F	1	1	1	Nil	Nil	200	Nil	
403	31	M	1	1	1	Nil	Nil	200	Nil	
404	15	M	0.9	1	0.9	Nil	Nil	150	Nil	
405	65	F	0.5	1	0.7	Nil	Nil	200	Nil	

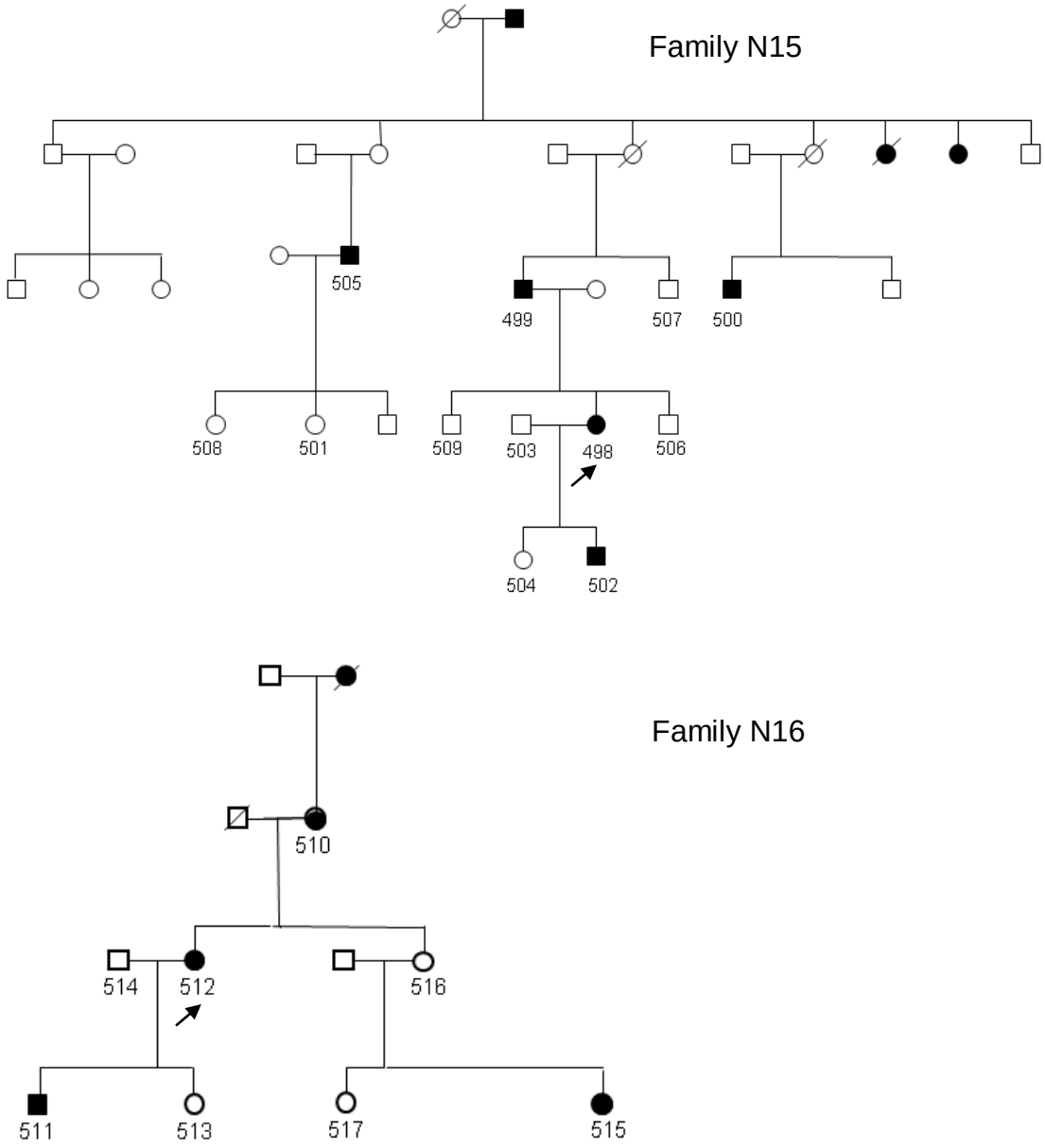
Table 3N.14: *Clinical details of subjects from family N14. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, binocular vision (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21476	12	F	0.80	0.80	0.80	Horizontal pendular	10°	550	Nil	
21477	38	M	0.50	0.50	0.50	Horizontal pendular	10°	U	Nil	EDT: normal
21478	9	F	0.90	0.90	0.90	Horizontal pendular	Nil	550	Nil	
21480	36	M	1.00	1.00	1.00	Horizontal pendular	5°	550	Nil	
21479	1	F	U	U	U	Nil	Nil	U	Nil	

Family N15 and N16

These two families were recruited with the help of clinical collaborators of Professor Irene Gottlob. They were diagnosed with IIN by Dr R Hertle and Dr D Hunter in USA and the saliva samples were sent to the author by subjects 498 and 512. The family trees are shown in Figure N15 and Figure N16. All the subjects had visual acuities better than 6/12. Except for the presence of nystagmus, clinical examination was reported to be normal. EDT's were reported to be normal in the probands.

Figure 3N.15 and 3N.16*: shows the pedigree of Family N15 and N16. The numbers on the figures are the designated lab numbers.*



3.1.2 Families in which haplotype data was unavailable prior to sequencing

Ten relatively smaller families with IIN with possible X-linked inheritance were included for haplotype analysis at a second phase. However the results of genotyping performed in Leicester were not available before the submission of DNA for sequencing at Sanger Institute. These families are prefixed 'F' and the details are given in this section.

Family F1, **F2** and **F3**

F1 is a family from Leeds with two affected members. Both these subjects were examined at the Leicester Royal Infirmary. They both had visual acuities of 6/6 and are professionals by occupation. One of the affected subjects had an AHP of 10°.

F2 is a three generation family from Leicestershire with three affected members. One subject was seen in the hospital and the other subjects were seen on a house visit by the author.

F3 was recruited on the field trip to Austria. There were two affected subjects in this family. These subjects had Snellen visual acuities better than 0.5 and both had significant AHP's. One of the subjects from family F3 (21483) had only minimal nystagmus in extremes of gaze which was quite difficult to differentiate form physiological end-point nystagmus and is shown in green colour in the figure. Clinical details of these subjects are shown in Table 3F.01 to 3F.03.

Figures 3F.01, 3F.02 and 3F.03: *shows the pedigree of F1, F2 and F3.*

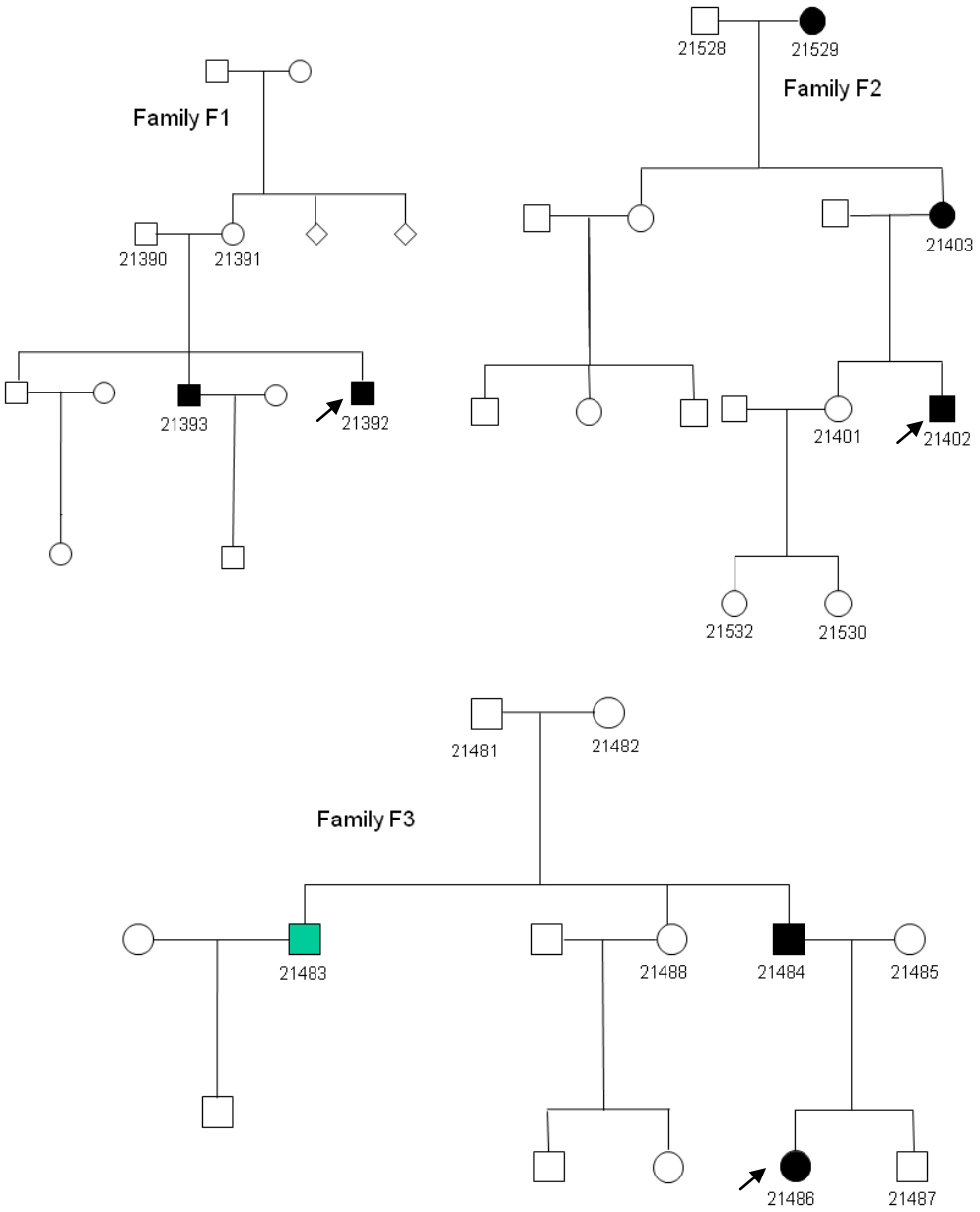


Table 3F.01, 3F.02 and 3F.03*: Clinical details of subjects from family F1, F2 and F3 are shown in the tables below. Tables are arranged in numerical order with table 3F.01 on top. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, binocular vision (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21392	31	M	1.00	1.00	1.00	Horizontal pendular	10 degree to R	40	Nil	EDT: reported normal
21393	34	M	1.00	1.00	1.00	Horizontal pendular	NIl	U	Nil	
21390	70	M	1.00	1.00	1.00	Nil	Nil	U	Nil	
21391	66	F	1.00	1.00	1.00	Nil	Nil	U	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21402	31	M	0.67	0.67	0.67	Horizontal pendular	5 degree to R	150	Nil	EDT: Normal
21529	70	F	U	U	U	Horizontal pendular	U	U	Nil	
21403	55	F	U	U	U	Horizontal pendular	Nil	U	Nil	
21401	33	F	1.00	0.67	1.00	Nil	Nil	U	Nil	
21528	78	M	U	U	U	Nil	U	U	Nil	
21530	3	F	U	U	U	Nil	Nil	550	Nil	Left congenital Horner syndrome
21532	6	F	1.00	1.00	1.00	Nil	Nil	U	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21484	31	M	1.00	0.67	1.00	Horizontal pendular	20 degree to R	550	Nil	EDT: Normal
21486	4	F	0.50	0.50	0.50	Horizontal jerk	21 degree to R	Bagolini +ve	Nil	
21481	58	M	1.20	1.20	1.20	Nil	Nil	550	Nil	
21482	56	F	1.20	1.20	1.20	Nil	Nil	550	NIl	
21483	37	M	1.00	1.20	1.20	U	Nil	550	Nil	Endpoint nystagmus
21485	26	F	1.20	1.20	1.20	Nil	Nil	U	Nil	
21487	1	M	U	U	U	Nil	Nil	U	Nil	
21488	36	F	1.20	1.20	1.20	Nil	Nil	550	Nil	

Family F4 and F5

Family F4 is of Romanian origin and was phenotyped by the author on the field trip in Austria. One affected subject was examined and was found to have good vision, binocularity and no AHP. A saliva sample from another subject from this family (462) was received through post, however, the author does not have the details of clinical examination of this patient.

Family F5 is of Austrian ethnicity. Two affected subjects were phenotyped from this family. The clinical details of these subjects are shown in table 3F.04-3F.05. Subjects 21489 and 21504 had normal findings on EDT's.

Figures 3F.04 and 3F.05: *shows the pedigrees of Families F4 and F5.*

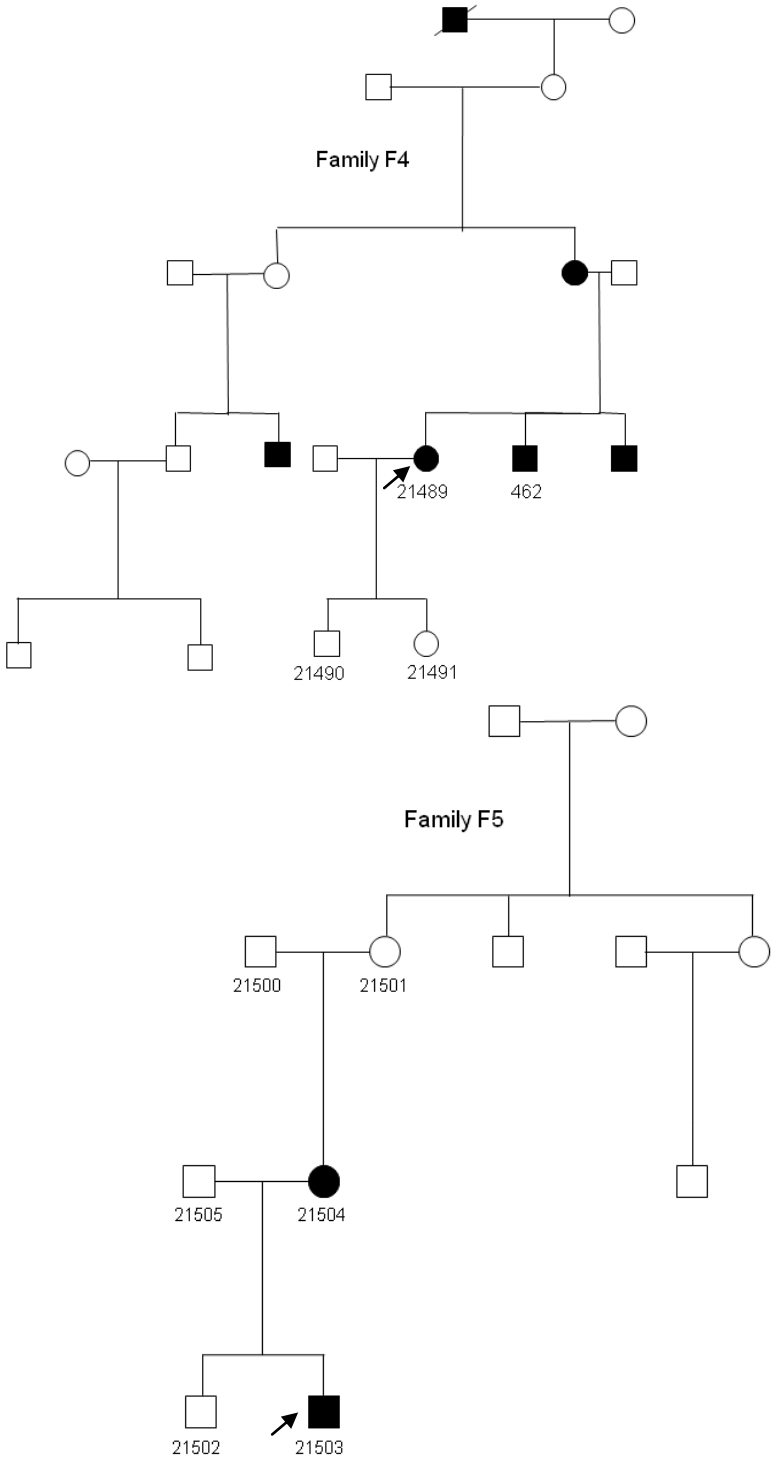


Table 3F.04 and 3F.05*: Clinical details of subjects from family F4 (top table) and F5 (bottom table) are shown in the tables. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21489	33	F	0.80	0.80	0.80	Horizontal Pendular	Nil	550	Nil	EDT-Normal
462	40	M	U	U	U	Present	U	U	U	
21490	5	M	1.00	1.00	1.00	Nil	Nil	550	Nil	
21491	4	F	0.67	0.67	0.67	Nil	Nil	550	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21504	37	F	1.25	1.25	1.25	Horizontal Pendular	5 degree to R	550	Nil	EDT-Normal
21503	10	M	0.63	0.63	0.63	Horizontal Pendular	20 degree to L	600	Nil	
21500	56	M	1.00	1.00	1.00	Nil	Nil	150	Nil	
21501	56	F	1.20	1.20	1.20	Nil	Nil	U	Nil	
21502	12	M	1.200	1.20	1.20	NIl	Nil	150	Nil	
21505	38	M	0.80	1.20	1.20	Nil	Nil	150	Nil	

Families F6, F7 and F8

Family F6 lives in Leicester and one of the subjects were seen in the eye clinic. The other members of the family were seen on house visits by the author.

Family F7 is from Lancashire. One affected subject from this family was seen at the neuro-ophthalmology clinic in Leicester. Saliva sample from subject 21544 was received by post and the author does not have clinical details.

Family F8 lives in Dorset. Subject 416 along with his mother was seen during a 'Nystagmus Network' workshop in Birmingham. The proband's grandmother travelled to Leicester for clinical examination. The rest of the family members were phenotyped by the author on a house visit to Dorset. The clinical details of these families are given in Table 3F.06-3F.08.

Figure 3F.06, 3F.07 and 3F.08: *Shows the pedigrees of Families F6, F7 and F8.*

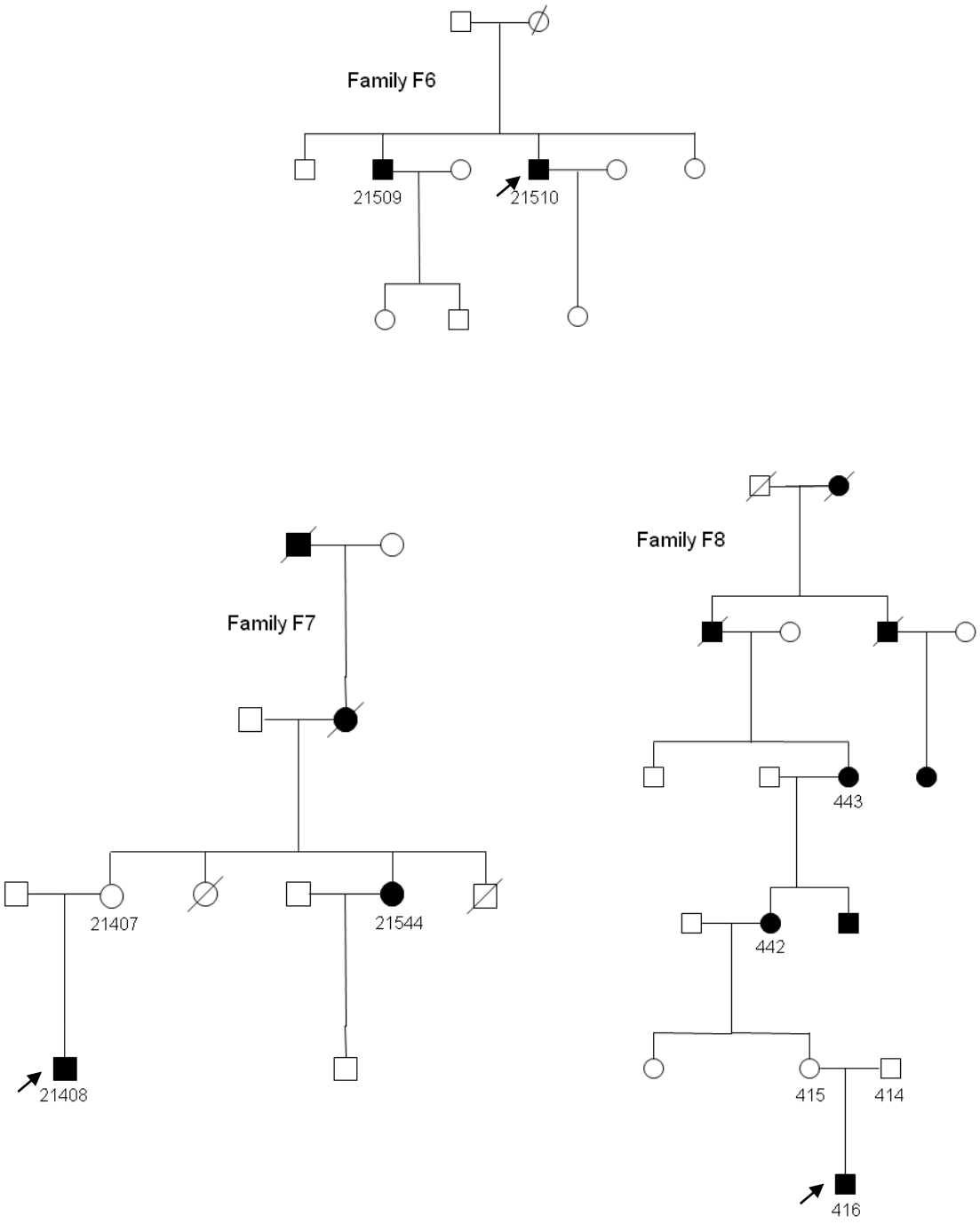


Table 3F.06, 3F.07 and 3F.08*: Clinical details of subjects from family F6 (top panel), F7 (middle panel) and F8 (bottom panel) are shown in the tables. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21509	53	M	0.67	0.67	0.67	Horizontal pendular	Nil	U	Nil	
21510	50	M	0.67	0.67	0.67	Horizontal pendular	Nil	U	Nil	EDT: Normal

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21408	28	M	0.50	0.50	0.50	Horizontal jerk	Nil	150	Nil	EDT: Normal
21544	62	F	U	U	U	Present	U	U	U	
21407	66	F	U	U	U	Nil	U	U	U	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
416	1	M	U	U	U	Horizontal pendular	Slight chin down	U	Nil	
442	55	F	0.67	0.67	0.67	Horizontaljerk	Nil	40	Nil	EDT: Normal
443	79	F	0.67	0.67	0.80	Horizontal jerk	Nil	150	Nil	
415	28	F	U	U	U	Nil	Nil	U	Nil	

Family F9 and F10

The proband from family F9 (7672) was seen in the eye clinic. He is hypermetropic and has a visual acuity of 6/12. This branch of the family lives in Luxembourg.

Proband's maternal cousin sister (472) who lives with her parents in Manchester was affected and that branch of the family was phenotyped on a house visit by the author.

Interestingly subject 474 (father of 472) was found to be unaffected and this was confirmed on eye movement recordings. If nystagmus is segregating as an X-linked disorder, then 474 would be the first recruited unaffected male who has the disease genotype.

Another branch of the family (447 and children) lives in Dorset was phenotyped on house visits by the author, however, none of them was found to be affected.

All together sixteen subjects were phenotyped from Family F9 which had two affected subjects.

Subject 7672 had normal EDT's. The clinical details of this family is shown in Table 3F.09.

Members of Family F10 were seen in the eye clinic at Leicester. This family is especially interesting due to the fact that all the affected subjects were females. Visual acuity was variable, there was significant AHP in some of the subjects and one of the affected subjects had manifest strabismus. In addition it was found that subject 8072 had inferior choroidal coloboma.

The clinical details of these subjects are given in Table 3F.10. Subject 7312 had normal EDT's.

Figure 3F.09 and 3F.10: *Shows the pedigree for Families F9 and F10. Subject 8072 has choroidal coloboma (shown in green colour)*

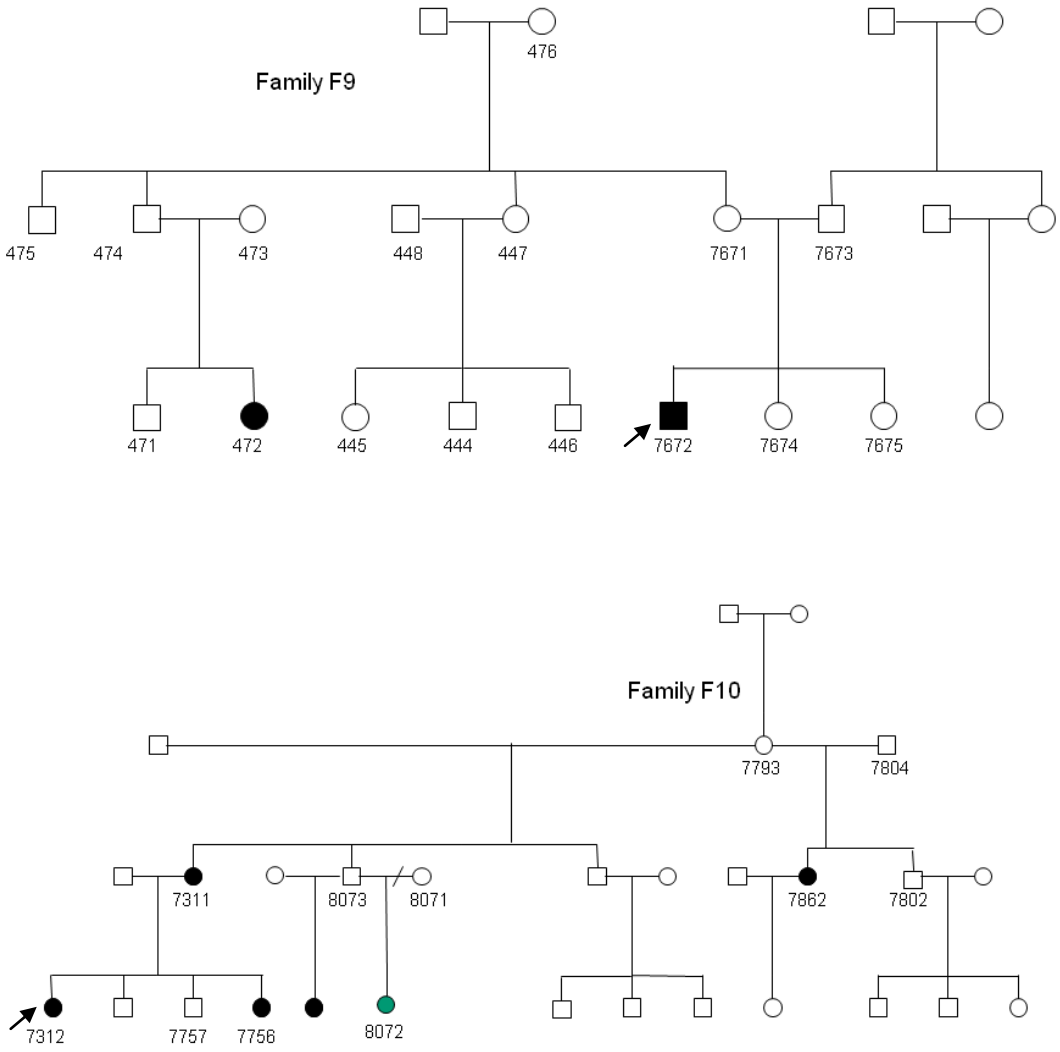


Table 3F.09*: Clinical details of subjects from family F9 is shown in the table below. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
7672	7	M	0.50	0.50	0.50	Horizontal jerk	5 degree to L at near	120	Nil	Hypermetropia 7D; EDT:normal
472	4	F	0.33	0.33	0.33	Horizontal jerk	10 degree to R	550	Nil	
474	36	M	1.60	1.60	1.60	Nil	Nil	550	Nil	
7673	45	M	1.20	1.00	1.00	Nil	Nil	30	Nil	
7671	37	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
471	2	M	U	U	U	Nil	Nil	U	Nil	
448	40	M	U	U	U	Nil	U	U	Nil	
447	38	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
445	12	F	1.00	1.00	1.00	Nil	Nil	50	Nil	
446	10	M	1.00	1.00	1.00	Nil	Nil	50	Nil	
444	4	M	U	U	U	Nil	Nil	Nil	Accommodative esotropia	
473	33	F	1.50	1.20	1.50	Nil	Nil	550	Nil	
475	32	M	1.50	1.20	1.20	Nil	Nil	550	Nil	
476	67	F	1.00	1.00	1.00	Nil	Nil	550	Nil	Endpoint nystagmus
7674	3	M	U	U	U	Nil	Nil	U	Nil	
7675	5	F	U	U	U	Nil	Nil	U	Nil	

Table 3F.10*: Clinical details of subjects from family F10 is shown in this table. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
7311	42	F	0.67	0.67	0.67	Horizontal pendular	5° chin up	550	Nil	
7312	24	F	0.25	0.33	0.33	Horizontal pendular	10° to R	U	Nil	EDT: Normal
7756	12	F	1.50	1.50	1.50	Horizontal pendular	20° to L	50	Nil	
7862	33	F	0.33	0.33	0.50	Horizontal pendular/torsional	20° to L	600	L esotropia	
7757	16	M	1.50	1.50	1.50	Nil	Nil	40	Nil	
7793	60	F	1.00	0.80	1.00	Nil	Nil	550	Nil	
7804	31	M	1.00	1.00	1.00	NIl	Nil	U	Nil	
8071	37	F	U	U	U	Nil	U	U	U	
8072	8	F	HM	0.80	0.80	Nil	Nil	Nil	Nil	R choroidal coloboma
7802	32	M	U	U	U	Nil	U	U	U	
8073	38	M	U	U	U	Nil	U	U	U	

3.1.3 Families in whom genotyping was not carried out

There were four small families which were recruited later on in the study in which genotyping was not carried out. However a sample of DNA was send for sequencing to the Sanger Institute. The details of these families are given in this section.

Families G1 and G2

Family G1 comprised of an affected mother and daughter (shown in Figure 3G.01). They were seen in the Ophthalmology clinic in Leicester. The child with nystagmus had significant AHP.

Family 3G.02 is from Birmingham and has two affected subjects and interestingly, there is consanguinity in the family. One of the subjects was seen in Leicester and the rest of the members were phenotyped on a house visit by the author and MA.

The clinical details are given in Table 3G.01 and 3G.02.

Figure 3G.01 and 3G.02: *Shows the pedigree for Families G1 and G2.*

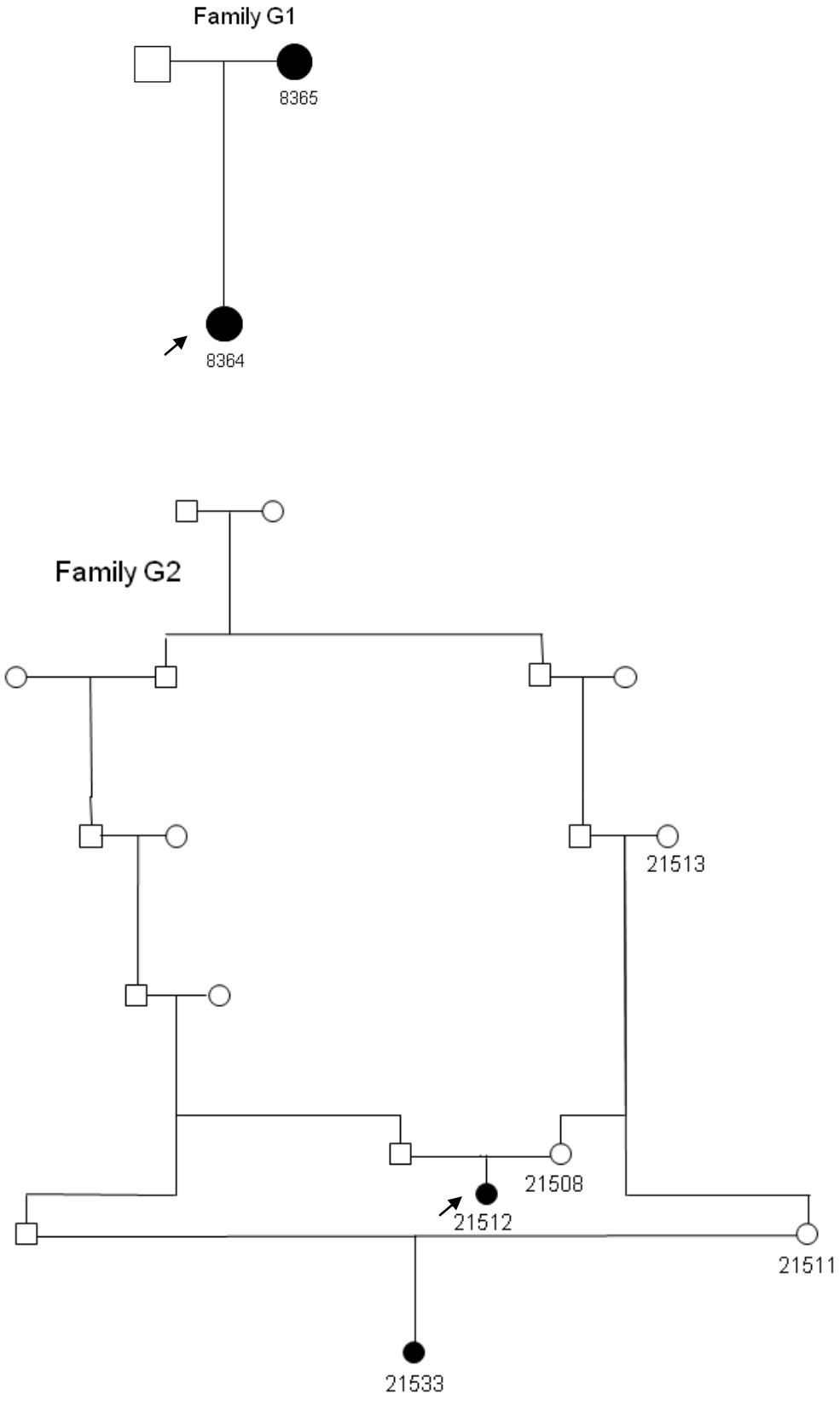


***Table 3G.01 and 3G.02**: Clinical details of subjects from family G1 (top table) and G2 (bottom table) are given below. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
8364	4	F	0.67	0.67	0.67	Horizontal jerk	10° to L	U	Nil	
8365	34	F	0.67	0.67	0.67	Horizontal pendular	Nil	40	Nil	EDT: Normal

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21512	14	F	0.50	0.50	0.50	Horizontal pendular	Nil	Frisby neg	Nil	EDT: Normal
21533	18	F	0.17	0.25	0.25	Horizontal pendular	Nil	Frisby neg	Nil	
21508	37	F	1.00	1.00	1.00	Nil	Nil	150	Nil	
21511	38	F	U	U	U	Nil	U	U	U	
21513	62	F	1.00	0.67	0.67	Nil	Nil	150	Nil	

Family G3 and G4

Subjects from family G3 and G4 were phenotyped in the eye clinic at Leicester. Two affected subjects were recruited from family G3. Both affected subjects had horizontal pendular nystagmus.

All the three subjects with nystagmus from family G4 had vertical conjugate nystagmus. Their visual acuity was poor compared to other families. Two affected subjects had AHP more than 10°. Subject 480 had manifest strabismus. These pedigrees are shown in Figures 3G.03 and 3G.04. Eye movement recording of subject 479 is shown in Figure 3G.03a. Clinical details are shown in Tables 3G.03 and 3G.04

Figure 3G.03 and 3G.04: *Shows the pedigrees of Family G3 and G4.*

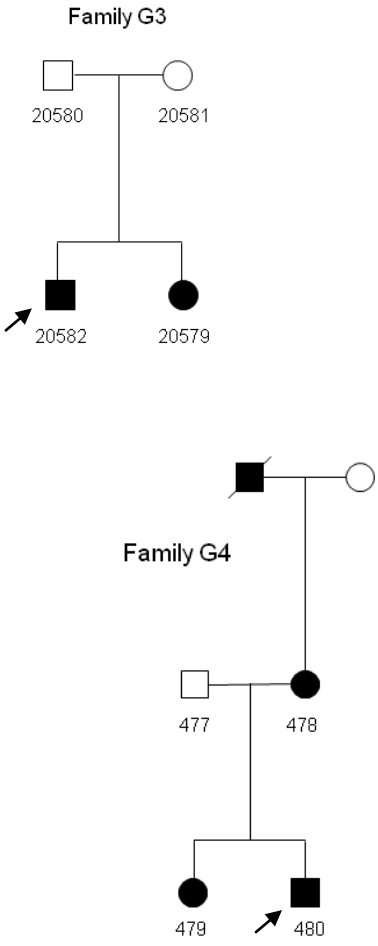


Figure 3G.03a: *Eye movement recoding of subject 479. The upper two traces show the nystagmus in the vertical meridian. The lower two traces shows absence of nystagmus in the horizontal meridian.*

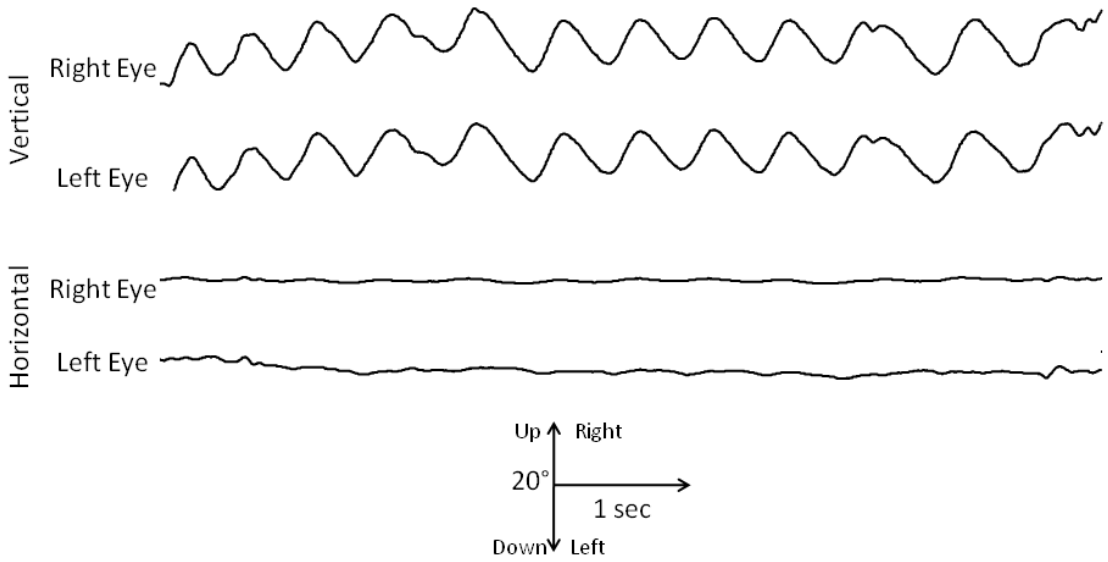


Table 3G.03 and 3G.04*. Clinical details of subjects from family G3 (top table) and G4 (bottom table) are given below. Snellen visual acuities (right eye, left eye & both eyes); head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
20579	2	F	U	U	U	Horizontal pendular	5° turn L	U	Nil	
20582	5	M	0.50	0.50	0.50	Horizontal pendular	5° chin down	550	Nil	EDT: Normal

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
478	36	F	0.33	0.33	0.40	Vertical jerk (down beat)	Nil	300	Nil	EDT: Normal
479	7	F	0.17	0.17	0.17	Vertical jerk	20° to L	Nil	Nil	
480	6	M	0.17	0.17	0.17	Vertical jerk	10° Chin up, 10° L	Nil	L esotropia	
477	36	M	1.00	1.00	1.00	Nil	Nil	85	Nil	

3.1.4 Families excluded after initial work up

A number of subjects with familial forms of infantile nystagmus were excluded after initial examination. The families are denoted with a prefix 'E'. The clinical details of these subjects are given below.

Family E1, E2 and E3

This family of Austrian ethnicity (Family E1) was recruited with an initial diagnosis of IIN. However, slit lamp examination showed significant iris transillumination defects (Figure 3E.01a). The clinical details are given in Table 3E.01.

The proband in family E2 (8366) was diagnosed with IIN after clinical examination and electro diagnostic tests. However his mother was found to have complete aniridia and therefore the family was excluded from the study. Clinical details are given in Table 3E.02. Subject 8366 had normal EDT's.

Family E3 has two affected subjects (Figure 3E.03). The proband has an unaffected twin brother. However this family was excluded because of family history of albinism. Table 3E.03 shows the clinical details of these subjects.

Figure 3E.01-3E.03: *Shows the pedigrees of families E1, E2 and E3. Subject 304 of Family E2 (showed in blue colour) has complete aniridia.*

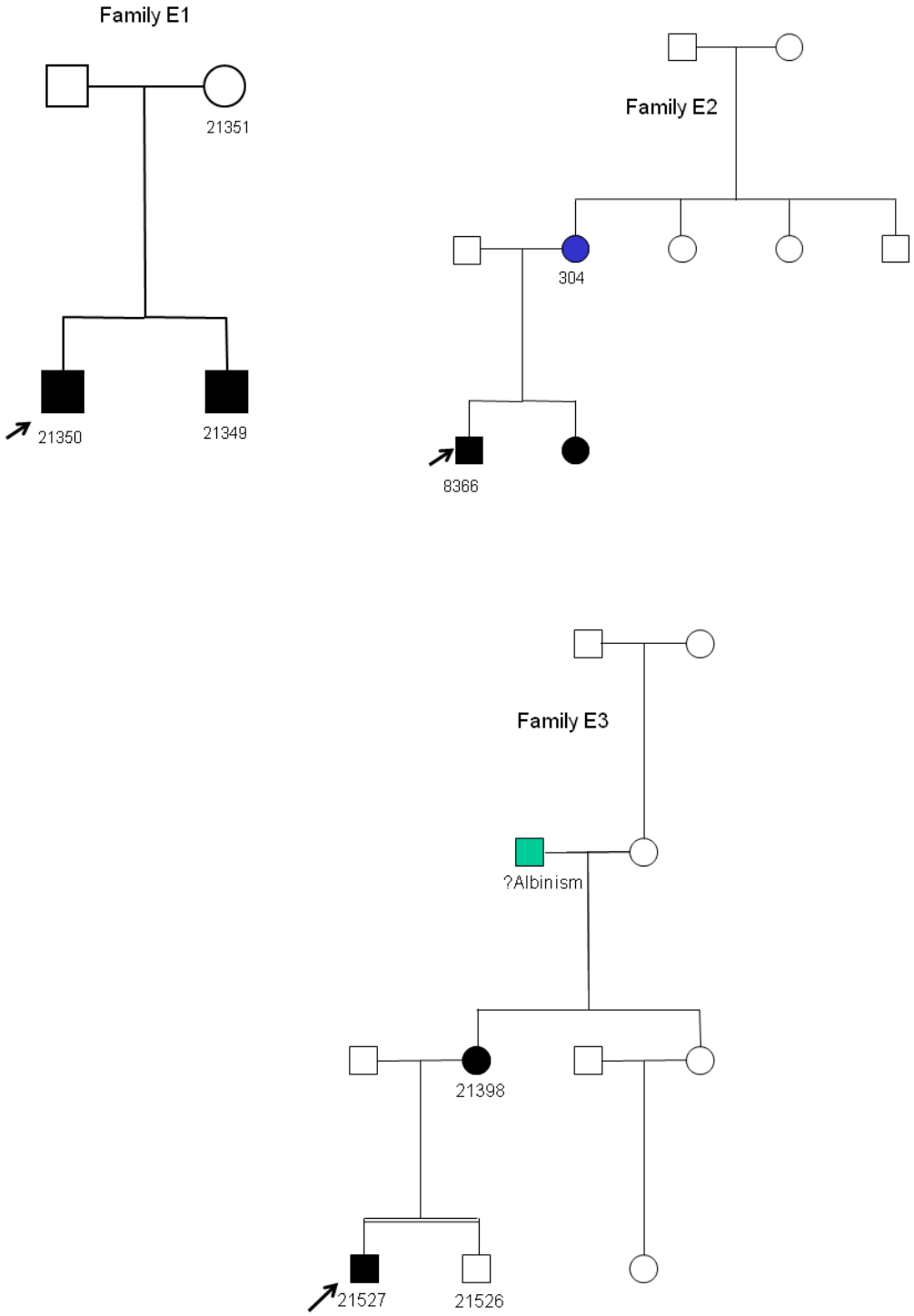


Figure 3E.01a: *Shows iris transillumination in subject 21350.*

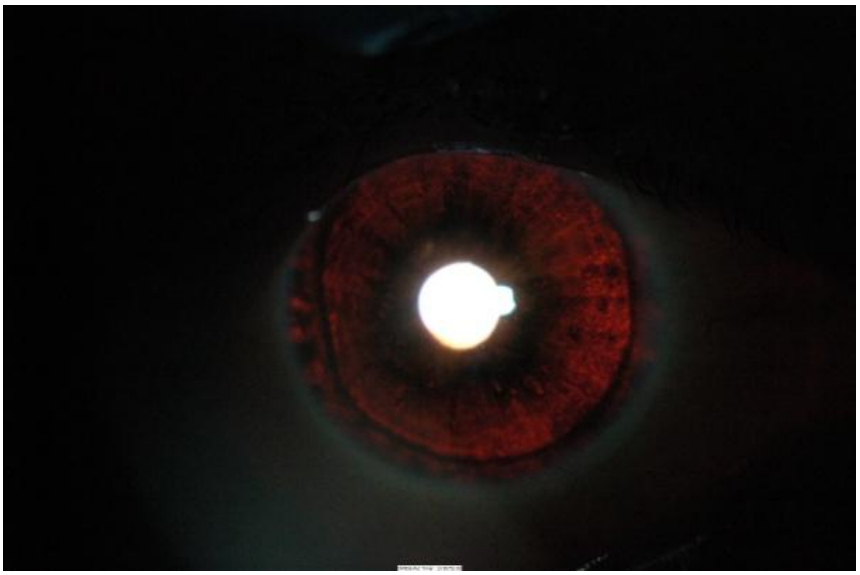


Table 3E.01- 3E.03*: Clinical details of subjects from family E1 (top panel), E2 (middle panel) and E3 (bottom panel). Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21349	5	M	U	U	U	Horizontal pendular	Nil	U	Nil	
21350	11	M	0.50	0.50	0.50	Horizontal pendular	Nil	550	Nil	EDT: Normal
21351	45	F	1.00	1.00	1.00	Nil	Nil	150	Nil	
8366	34	M	0.67	0.67	0.67	Horizontal jerk	Slight turn to R	120	Nil	EDT: Normal
304	55	F	CF	0.17	0.17	Horizontal pendular	Nil	Nil	R exotropia	Aniridia

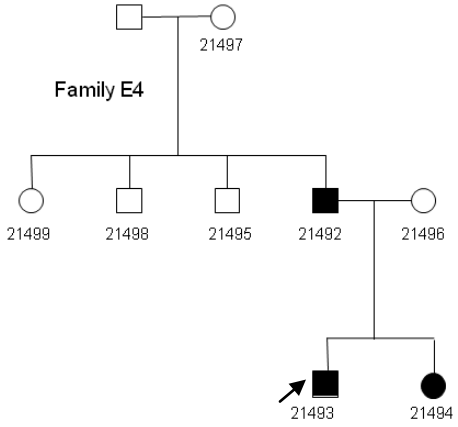
Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21398	27	F	0.800	0.800	0.800	Horizontal jerk	Nil	55	Nil	Nil
21527	1	M	U	U	U	Horizontal pendular	Nil	U	Nil	
21526	1	M	U	U	U	Nil	Nil	U	Nil	

Family E4 and E5

E4 is a family (Figure 3E.04) recruited on the field trip to Graz. There were three subjects with nystagmus in this family. All the affected subjects had Snellen visual acuity better than 0.5 and the clinical characteristics were similar to those of previously recruited families. However there was male to male transmission in this family and therefore was excluded from the study.

Members of family E5 was seen in the eye clinic at Leicester. Two subjects with nystagmus were examined. In subject number 7676, rod-specific ERG showed a negative waveform and the cone-specific ERG was normal (Figure 3E.05a). A diagnosis of congenital stationary night blindness was made and the family was excluded. Clinical details are given in Table 3E.04-3E.05.

Figure 3E.04 and 3E.05*: Pedigrees of families E4 and E5.*



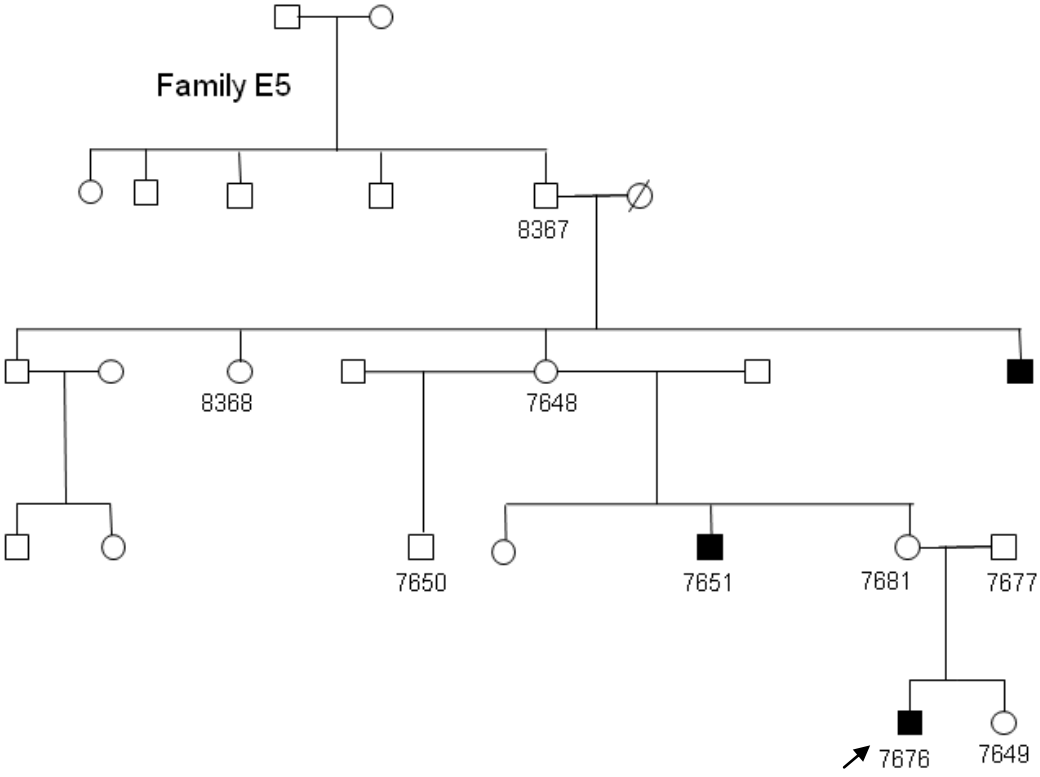
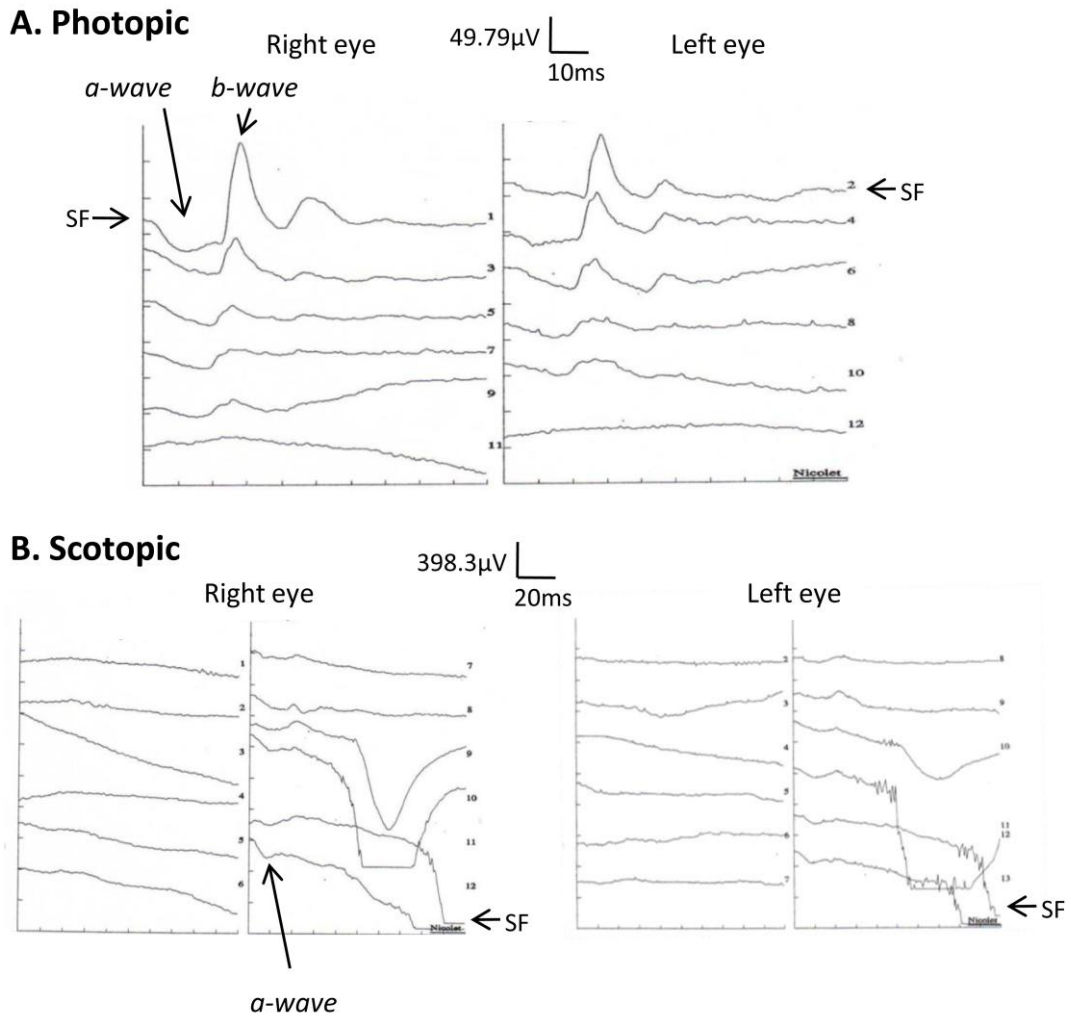


Table 3E.04- 3E.05*: Clinical details of subjects from family E4 (top panel), and E5 (bottom panel). Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown. U=unknown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
21492	32	M	0.80	0.80	0.80	Horizontal pendular	Nil	550	Nil	EDT: Normal
21493	8	M	0.67	0.80	0.80	Horizontal pendular	Nil	550	Nil	
21494	4	F	0.50	0.50	0.50	Horizontal jerk	Nil	550	Nil	
21495	36	M	1.00	1.50	1.50	Nil	Nil	550	Nil	
21496	30	F	1.50	1.50	1.50	Nil	Nil	550	Nil	
21497	62	F	1.00	1.00	1.00	Nil	Nil	550	Nil	
21498	38	M	1.20	1.20	1.20	Nil	Nil	550	Nil	Red-Green color blind
21499	40	F	1.25	1.25	1.25	Nil	Nil	550	Nil	

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
7676	8	M	0.50	0.33	0.50	Horizontal jerk	5°to left	Nil	L exotropia	Myopic astigmatism (-0.5/-1.0x20^ and -0.75/-1.75x160^ ERG:CSNB
7651	28	M	U	U	U	Present	U	U	U	
8367	85	M	0.67	0.50	0.67	Nil	Nil	U	Nil	
8368	62	F	1.00	1.00	1.00	Nil	Nil	U	Nil	
7648	55	F	U	U	U	U	U	U	U	
7649	3	F	U	U	U	Nil	Nil	U	Nil	
7650	9	M	U	U	U	U	U	U	U	
7677	32	M	U	U	U	U	Nil	U	U	
7681	30	F	U	U	U	Nil	U	U	U	

***Figure 3E.05a**: **Shows the ERG of subject number 7676 of family E5**. Photopic ERG (top panel) appears normal except for the broad 'a'wave. Scotopic response (bottom panel) shows negative ERG with reduced b: a wave ratio. 'ISCEV 2004 standard flash' (SF) which was reduced at -0.25 log units per flash was used for photopic ERG. A low intensity flash which was increased stepwise at 0.25 log units to reach a maximum intensity of 'SF' was used for the scotopic ERG.*



Family E6

One of the affected siblings of this family and his mother was phenotyped. Subject number 461 had the characteristics of IIN. However his mother (subject 463) had poor vision and iris transillumination. ERG was normal. However VEP showed asymmetry which reversed polarity on monocular stimulation (Figure 3E.06a).

Figure 3E.06*: Pedigree of Family E6.*

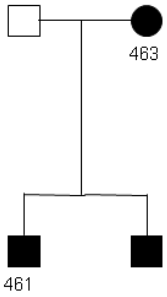


Figure 3E.06a: *Visual evoked response of subject 463 showing reversal of polarity on monocular stimulation. The upper panel shows the response on stimulating of the right eye whilst the lower panel shows the response on stimulating the left eye. The position of electrodes O1, O2 and Oz are at the occipital region on the left side, right side and in the midline respectively as shown in the inset. O2-O1 trace in the upper panel (stimulating the right eye) is a mirror image (indicated by blue arrow) of the O2-O1 trace in the lower panel (stimulating the left eye). Asymmetric VEP response which reverses polarity on monocular stimulation is a sign of chiamal miswiring.*

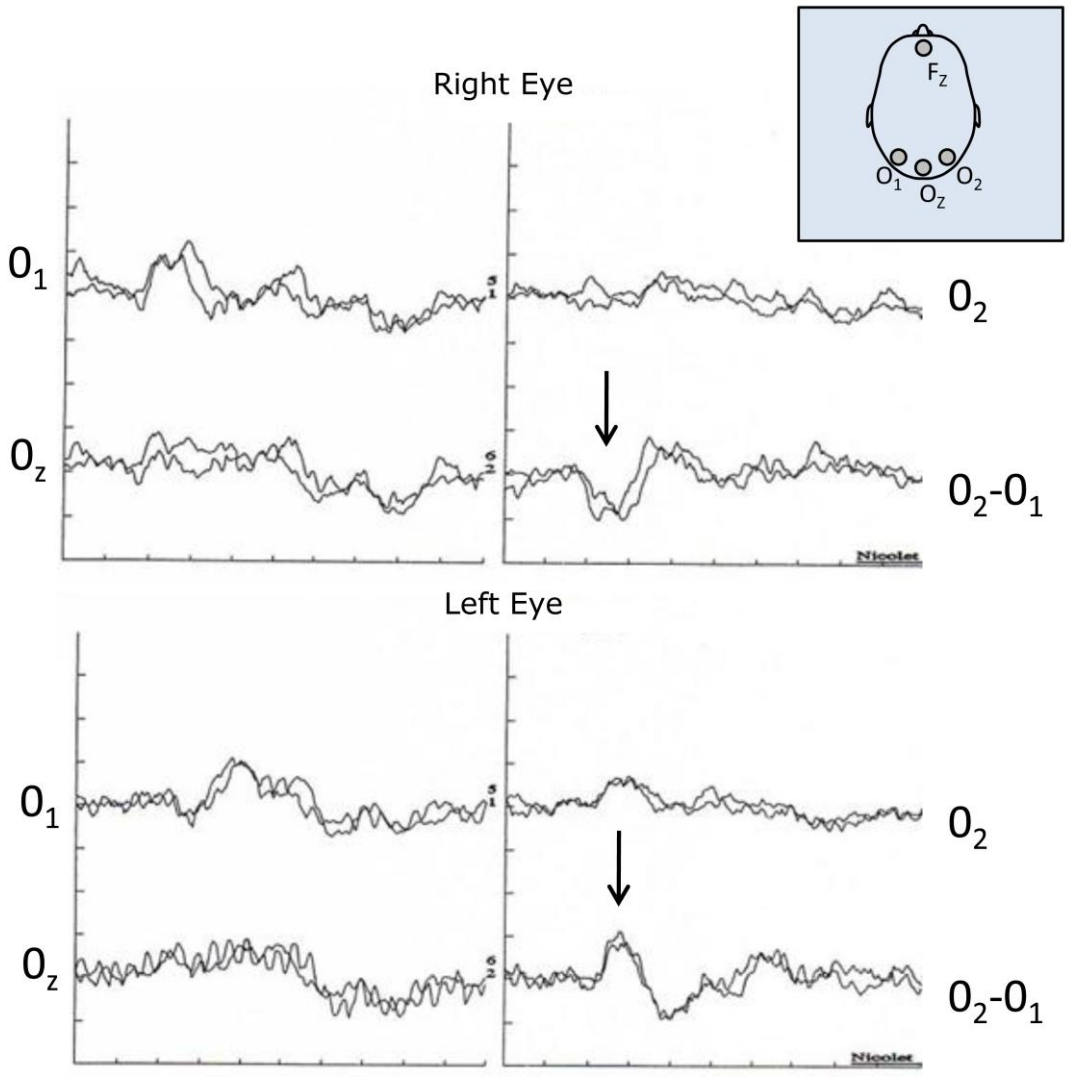


Table 3E.06. *Clinical details of subjects from family E6. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown.*

461	35	M	0.67	0.67	0.67	Horizontal pendular	Nil	550	Nil	ERG: Normal, VEP Normal
463	61	F	0.25	CF	0.25	Horizontal pendular	Nil	Nil	Nil	Iris Transillumination, Ocular albinism

Family E7

This is a large 5-generation family (Figure 3E.07) with history of congenital nystagmus, pre-senile cataracts. Few of the subjects had blepharoptosis. Subjects with poor vision had foveal hypoplasia which was confirmed with OCT (Figure 3E.07a)

Figure 3E.07: *Pedigree of Family E7.*

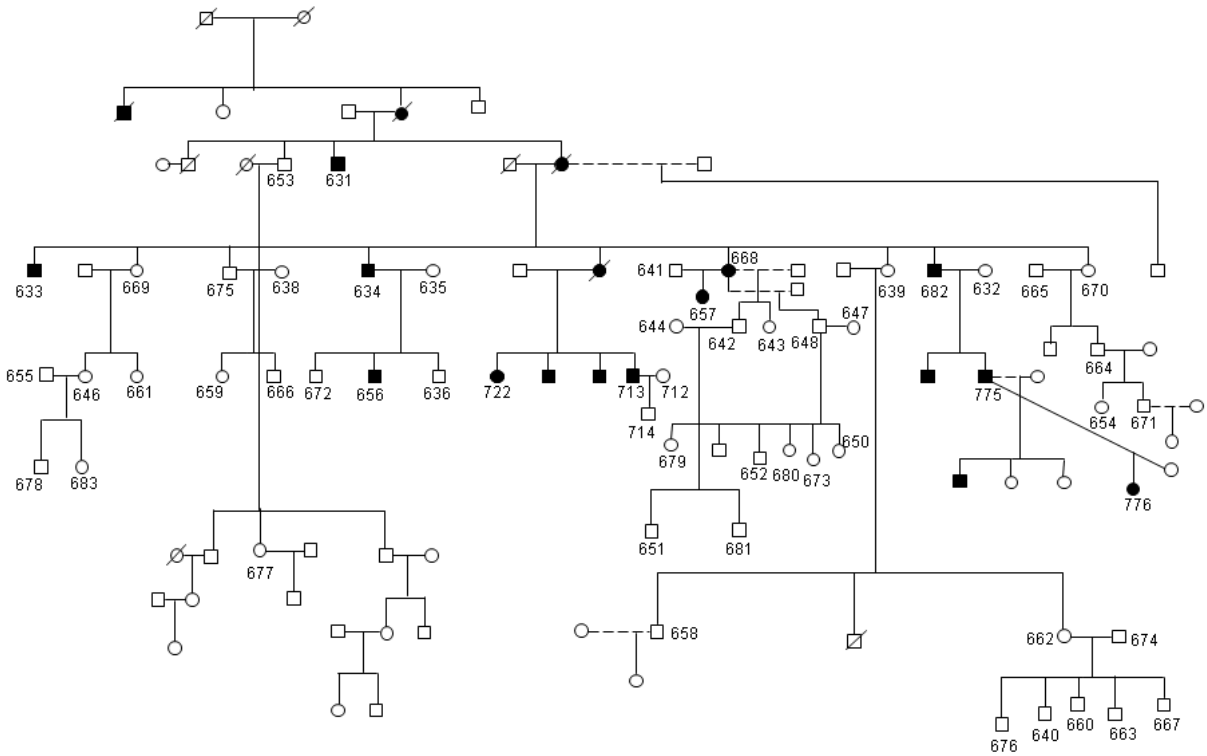


Figure 3E.07a: *OCT of subject 668 showing the ill-developed fovea in both eyes.*

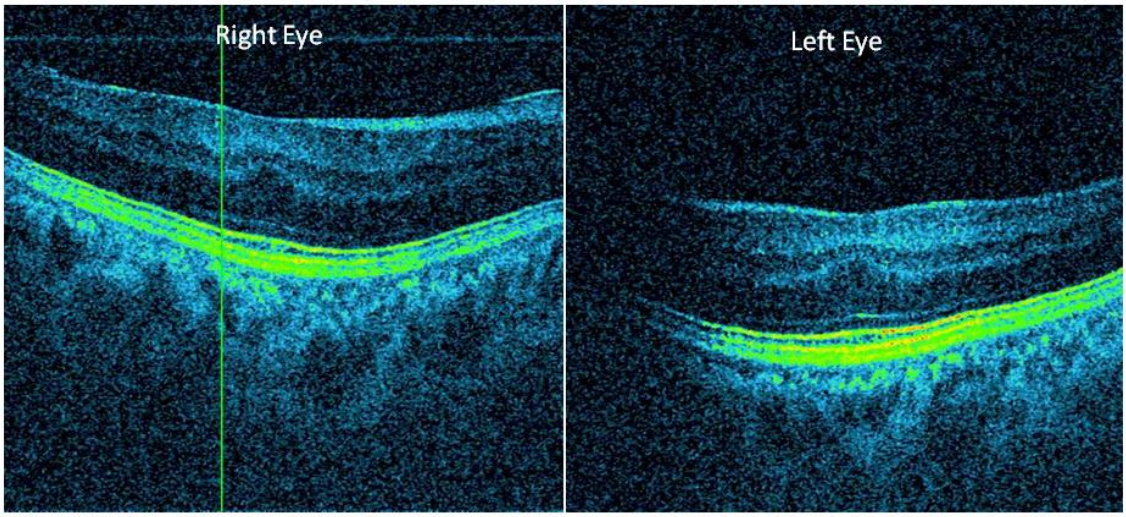


Figure 3E.07b: *Right eye posterior subcapsular cataract in subject 713. He is pseudo-phakic in the left eye.*

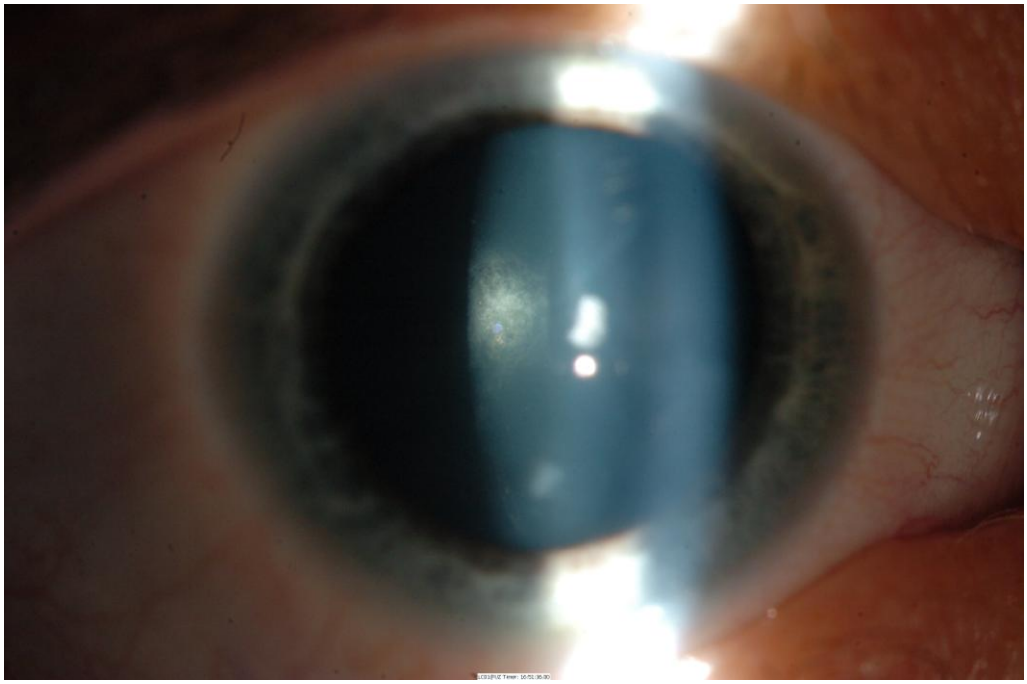


Table 3E.07: *Clinical details of subjects from family E7. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown.*

Number	Age	RVA	L VA	BE VA	Nystagmus	Cataract	Strabismus	Stereopsis	Other
631	77	0.50	Phthysis	0.50	Horizontal pendular in 1ry postn. vertical upbeat in upgaze.	R aphakia x 30 years; left phthysis	Nil	Nil	R eye peaked pupil
632	54	U	U	U	Nil	Nil	Nil	U	Hypermetrope(+6-BE)
633	50	CF		CF	Pendular in 1ry postn. jerk on versions	R aphakia, L phthysis- following RD surgery at age 15.	L exotropia	Nil	
634	47	0.17	0.07	0.07	Horizontal pendular, vertical on upgaze	PCIOL-BE- Surg at age 17 and 18	Alt exotropia	Bagolini +ve	
636	15	U	U	U	Nil	Nil	Nil	U	
637	51	U	U	U	Horizontal pendular/jerk	Bilateral cataract surgery at age 26	U	U	Renal stones
638	41	0.80	0.80	0.80	Nil	Nil	Nil	U	Brother has ? Duanes syndrome
639	50	0.80	1.00	1.00	Nil	Minimal nuclear sclerosis	Nil	55	
640	12	1.00	1.00	U	Nil	Nil	Nil	55	
642	28	U	U	U	Nil	Nil	Nil	U	
643	29	U	U	U	Nil	Nil	Nil	U	
644	25	U	U	U	Nil	Nil	Nil	U	
646	24	1.50	1.50	1.50	Nil	Nil	Nil	55	
647	34	0.80	0.80	U	Nil	Cortical opacity in right eye, left lens clear	R exotropia	55	
648	33	0.80	1.00	U	Nil	Nil	Nil	U	
650	11	0.80	0.80	U	Endpoint nystagmus	Nil	Nil	55	
651	5			U	Nil	Nil	Nil	U	
652	10	0.80	0.50	U	Nil	Nil	Nil	150	
653	74	0.67	0.50	U	Nil	Nuclear sclerosis	Nil	U	
655	24	U	U	U	Nil	Nil	Nil	U	
656	13	U	U	U	Horizontal pendular	Early PSC lens opacity	Nil	Bagolini +ve	Bilateral ptosis
657	8	0.10	0.17	U	Horizontal and vertical (elliptical)	Nil	Nil	U	

Number	Age	RVA	L VA	BE VA	Nystagmus	Cataract	Strabismus	Stereopsis	Other
658	29	1.00	1.00	1.00	Nil	Nil	Nil	55	
660	4	1.00	1.00	U	Nil	Nil	Nil	U	
661	10.3.	1.20	1.20	1.20	Nil	Nil	Nil	55	
662	32	1.00	0.80	U	Nil	Nil	Nil	55	
663	6	1.00	1.00	U	Nil	Nil	Nil	55	
665	62	U	U	1.20	Nil	Nil	Alt exotropia	U	Hypermetrope
666	15	U	U	U	Present	Nil	L exotropia	U	Disc pallor (L>R), congenital hydrocephalus
667	9	1.00	1.00	U	Nil	Nil	Nil	55	
668	48	0.67	0.17	0.25	Horizontal jerk	BES- PCIOL in 1990	L exotropia	Bagolini +ve	R ptosis, Left ptosis surg
669	40	1.00	1.00	1.00	Nil	Nil	Exophoria	55	
670	54	U	U	1.00	Nil	Nil	Nil	U	
672	5	0.80	0.80	U	Nil	Nil	Nil	170	
673	8	0.50	0.50	U	Endpoint nystagmus	Nil	Nil	120	
674	34	U	U	U	Nil	Nil	Nil	U	
675	45	1.00	1.00	U	Nil	Nil	Nil	55	
676	14	0.80	0.80	U	Nil	Nil	Nil	55	
677	49	1.00	1.00	U	Nil	Nil	Nil	550	
678	6 wks	U	U	U	Nil	Nil	Nil	U	
679	1	U	U	U	Nil	Nil	Alt exotropia	U	
680	5	0.80	0.80	U	Nil	Nil	Nil	150	
683	1	U	U	1.00	Nil	Nil	Nil	U	
713	20	0.17	0.25	0.25	Horizontal jerk	R PSCLO; L PCIOL	R exotropia	Bagolini-neg	
714	1	U	U	U	Nil	Nil	Alt esotropia	U	Fixes and folllows objects

Family E8

A mother and her child were seen from this family. (Figure 3E.08). Both the mother (736) and her child (735) had infantile nystagmus and macular coloboma (Figure E.08a). The best corrected visual acuities were 6/36 in both the subjects. ERG was subnormal an VEP showed delayed latency. The clinical details are given in table 3E.08.

Figure 3E.08*: Pedigree of Family E8*

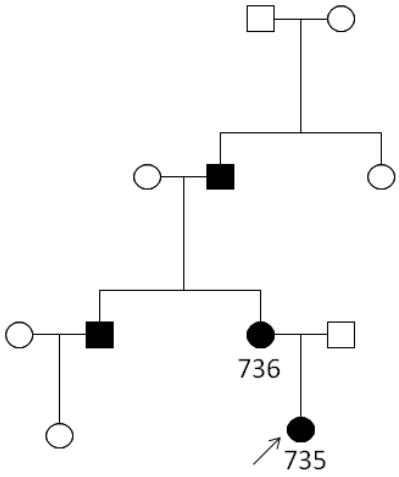


Figure 3E.08a*: Shows the fundus picture of subject 736 showing left eye macular coloboma.*

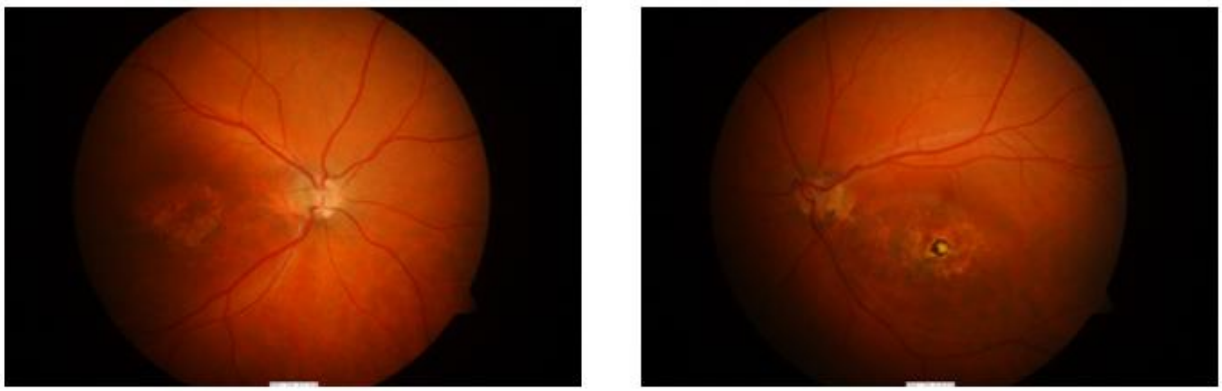
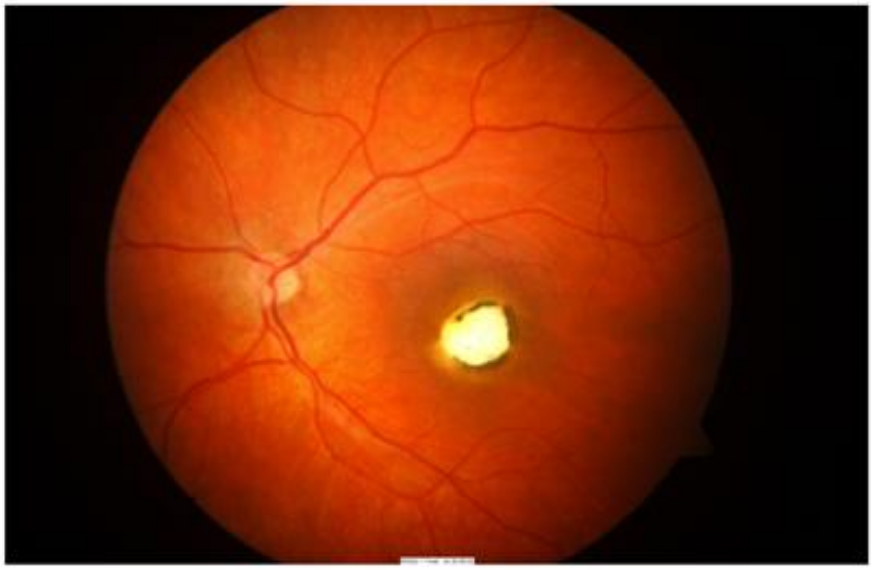


***Figure 3E.08b**: Left eye macular coloboma in subject 735.*



Family E9

Two members of this family (Figure 3E.09) were seen in the ophthalmology clinic at Leicester. The proband had infantile nystagmus. Her mother (809) had similar nystagmus and the best corrected visual acuity was 6/36. Subject 809 had bilateral cortical cataracts (Figure 3E.09a) and mild peripheral corneal vascularisation. The disorder seems to be of autosomal dominant inheritance and was excluded from the study.

Figure 3E.09: *Pedigree of Family E9.*

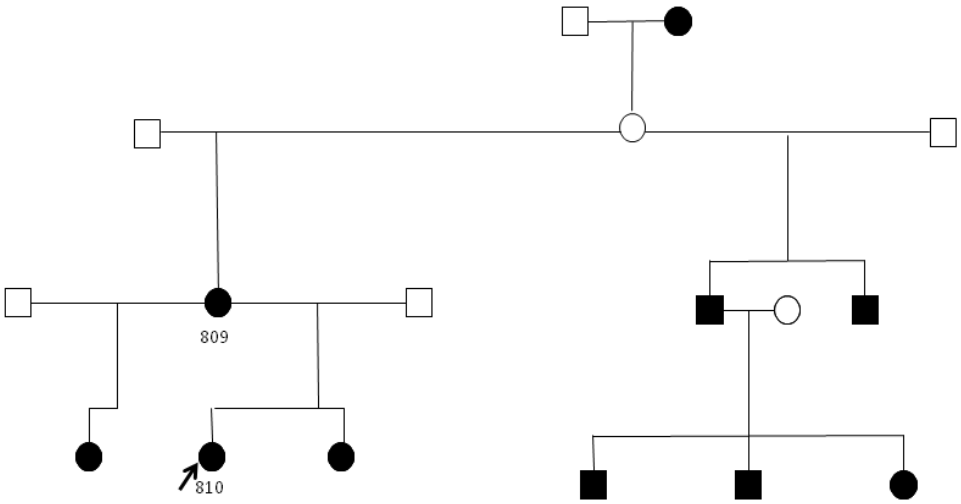


Figure 3E.09a*: Shows bilateral cortical cataracts in subject 809.*

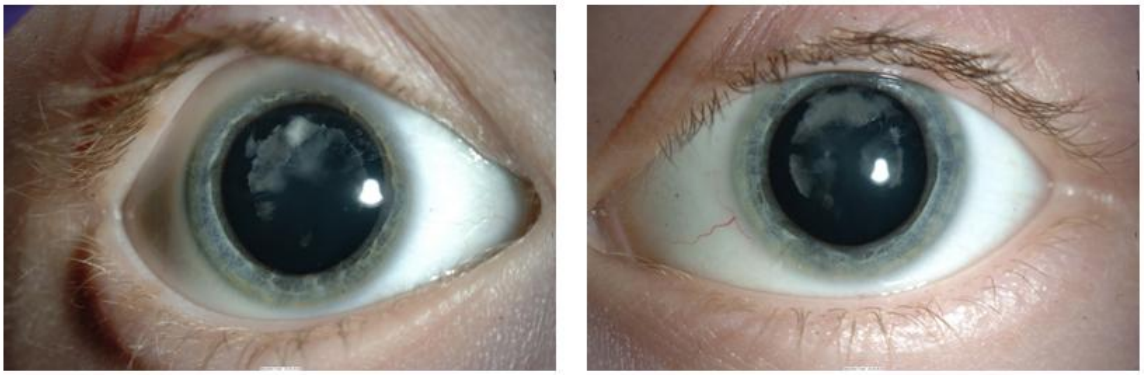


Table 3E.08 and 3E.09: *Clinical details of subjects from family E9. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
735	5	F	0.17	0.10	0.17	Horizontal pendular	Nil	Nil	Nil	
736	29	F	0.10	0.17	0.17	Horizontal pendular	Nil	Nil	Nil	ERG-Normal; VEP-abnormal

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings
809	24	F	0.10	0.17	0.17	Horizontal pendular	Nil	Nil	Nil	EDT-Normal
810	5	F	0.10	0.10	0.10	Horizontal pendular	Nil	Nil	Nil	

3.1.5 Singletons (Sporadic Patients) with Nystagmus

In addition to the families, seventy-eight individual subjects (singletons) without family history of nystagmus were also phenotyped.

Forty-two of these subjects were diagnosed with IIN and were included in the genetic study. The diagnosis of subjects excluded from the study include ocular and oculo-cutaneous albinism, achromatopsia, congenital stationary night blindness, spasmus nutans, congenital squint syndrome, dissociated vertical deviation, Browns syndrome, congenital cataract, congenital fibrosis of extra ocular muscles (CFEOM) and optic disc anomalies.

There were many subjects and families with ocular and oculo-cutaneous albinism referred and seen in the eye clinic at Leicester. Most of these subjects were not enrolled into this study as the diagnosis was made before the recruitment. However in seven subjects (1001, 1003, 1005, 1006, 1013, 1018 and 1034), a diagnosis of IIN was made on initial examination. Two of these subjects (1006 and 1034) had normal visual acuity (6/6). There were no ocular features of albinism except for subtle iris transillumination defects (Figure 3S.01). However VEP showed asymmetry which reverses polarity on monocular stimulation.

Four subjects (1001, 1005, 1013 and 1018) had poor vision, manifest strabismus and pale fundus and a clinical diagnosis of ocular albinism was made. A sixth subject (1003) had poor visual acuity, minimal iris transillumination defects and had abnormal crossing at the chiasm detected on VEP.

Figure 3S.01: *Shows subtle iris transillumination defect in subject 1006.*

Fourteen subjects were diagnosed with latent and/or manifest latent nystagmus. Eleven of these subjects had manifest strabismus. Two of them had exophoria and one was diagnosed with dissociated vertical deviation. Three of these subjects with MLN had possible birth injury. Subject 1027 was seen in clinic with history of nystagmus from birth. His birth history was significant in that the labour was delayed and had possible perinatal hypoxia. The visual acuities were 6/36 and 6/60 in the right and left eye respectively. Optic disc pallor was evident on fundus examination (Figure 3S.02). Subject 1032 was diagnosed with MLN and had a history of birth injury details of which were not known. An MRI of his brain showed bilateral occipital infarcts (Figure 3S.03). Subject 1033 with infantile nystagmus was found to have pale optic discs and bilateral

occipital infarcts on MRI (Figure 3S.04). He had history of delayed birth and neonatal convulsions.

Figure 3S.02: *Bilateral optic disc pallor in subject 1027.*

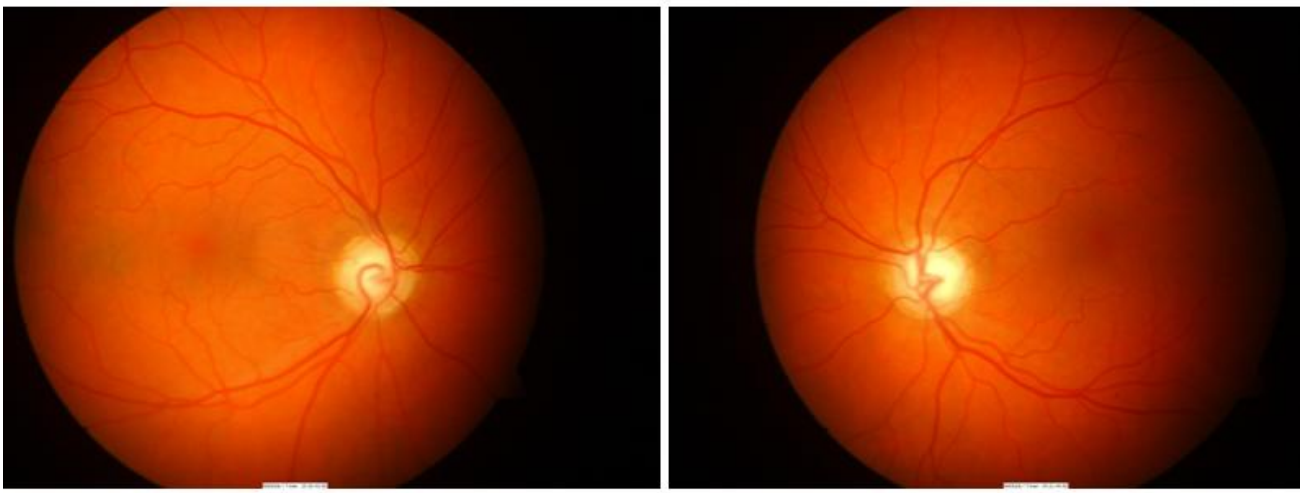


Figure 3S.03: *Cranial MR scan of subject 1032. Axial T2 weighted scan shows hyperintense region at the medial occipital region bilaterally.*

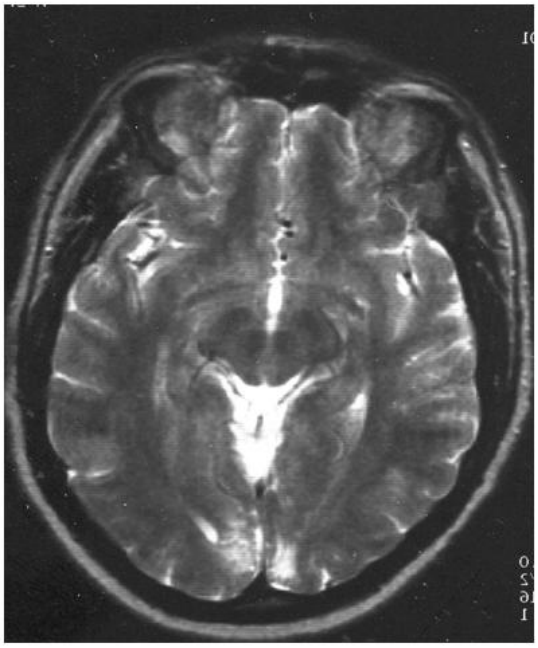
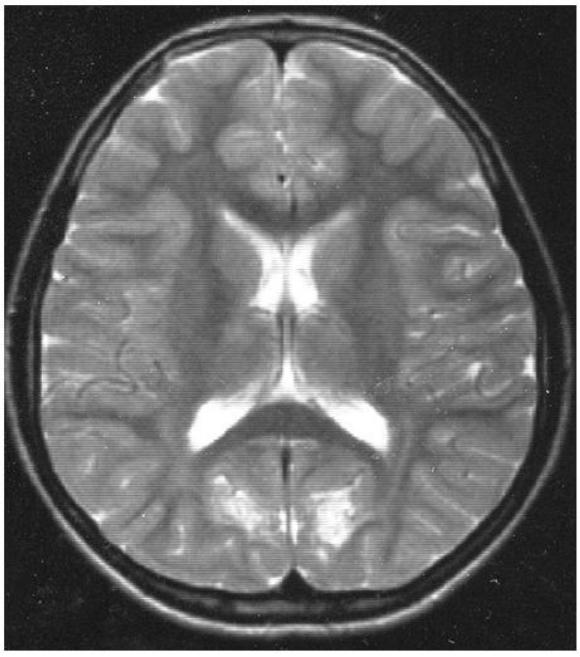


Figure 3S.04: *Cranial MR scan of subject 1033 showing bilateral occipital infarcts. (Axial T2 weighted image).*



Two subjects with infantile nystagmus were found to have bilateral cataracts and manifest strabismus. Congenital fibrosis of extra ocular muscles (CFEOM) was the diagnosis in two subjects.

Two adults who were referred to clinic with infantile nystagmus were found to have optic nerve hypoplasia. In addition a child with infantile nystagmus was diagnoses with septo-optic dysplasia.

Three subjects were diagnosed with achromatopsia and two subjects with congenital stationary night blindness.

Another subject with infantile nystagmus had a diagnosis of Noonan syndrome.

Clinical features of these sporadic subjects with nystagmus are shown in Table 3S.01

***Table 3S.01**: Clinical details of all the phenotyped singletons. Snellen visual acuities (right eye, left eye & both eyes; head posture in degrees, stereopsis (seconds of arc) of recruited subjects is shown.*

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings & Inv.
7293	12	M	0.33	0.50	0.50	Horizontal pendular	>15° turn to left	Nil	R esotropia	Kestenbaum procedure
7309	30	F	0.10	0.33	0.33	Horizontal jerk	Slight tilt to right & slight chin elevation	150	R esotropia	
7667	20	F	U	U	U	Present			Nil	
7669	34	F	0.50	0.67	0.67	Horizontal jerk	Nil	Nil	Left esotropia	
7670	3	M	U	U	U	Horizontal pendular	Nil	U	Nil	
7686	32	M	0.50	0.50	0.50	Horizontal jerk	20° to left	30	Nil	
7689	43	M	1.00	0.67	1.00	Horizontal jerk	20° chin up	55	Nil	
7749	34	M	0.50	0.50	0.50	Horizontal jerk	Nil	100	Nil	
7814	10	F	1.00	0.67	1.00	Horizontal jerk	20° chin up	40	Nil	
7925	30	M	0.67	0.67	0.67	Horizontal jerk	Nil	250	Nil	
7940	12	M	0.50	0.50	0.50	Horizontal pendular	20° tilt to left and slight chin up	30	Nil	
7949	16	M	0.50	0.50	0.50	Horizontal pendular	Nil	150	Nil	
7950	43	M	0.67	0.67	0.67	Horizontal pendular	Nil	150	Nil	
7954	43	M	0.50	0.50	0.67	Horizontal jerk	Nil	215	Nil	
7957	75	M	0.33	0.33	0.50	Horizontal pendular	5° to left	240	Nil	
8162	40	M	1.20	1.20	1.20	Horizontal pendular	Nil		Nil	
8170	30	F	1.00	1.00	1.00	Horizontal jerk	10° to right	30	Alt esotropia	
8175	11	M	0.67	0.67	1.20	Horizontal pendular	5° to right	150	Nil	
8176	5	M	0.67	0.67	0.67	Horizontal jerk	20° to left	50	Nil	

Table 3S.01-continued---

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings & Inv.
8481	13	M	0.67	0.67	0.67	Horizontal pendular	5° to left	85	Nil	
20652	27	F	0.67	0.67	0.67	Horizontal pendular	Nil	60	Nil	
20903	72	M	U	U	U	Horizontal pendular	Nil	U	Nil	
21019	51	M	0.67	0.67	0.67	Horizontal jerk	Nil	111	Nil	
21262	43	F	0.50	0.50	0.50	Horizontal pendular	10° to right	Nil		
21326	60	M	0.67	0.67	0.67	Horizontal pendular	Nil	U	Nil	
21372	10	M	0.67	0.67	0.67	Horizontal pendular	Nil	U	Nil	
21386	40	F	0.50	0.50	0.50	Horizontal pendular	Nil	U	Nil	
21521	34	F	0.33	0.33	0.33	Horizontal pendular	Nil	U	NIl	
21527	1	M	U	U	U	Horizontal pendular	Nil	U	Nil	
21535	8	M	1.00	1.00	1.00	Horizontal pendular	Nil	U	Nil	
406	2	F	0.25	0.25		Horizontal pendular	20° to left	Nil	Nil	
429	1	M	0.33	0.33	0.33	Horizontal pendular, latent component	20° to left	Nil	L esotropia	
436	40	M	0.50	0.50	0.50	Horizontal pendular	Nil	300	Nil	
437	72	F	0.10	0.50	0.50	Horizontal pendular	Nil	Nil	Nil	
438	45	M	0.67	0.67	0.67	Horizontal pendular	20° to left	150	Nil	
439	60	M	0.67	0.67	0.67	Horizontal pendular	5° chin up	85	Nil	
440	36	F	0.67	0.67	0.67	Horizontal pendular	Nil	85	Nil	
450	1	M	U	U	U	Horizontal pendular	U	U	U	

Table 3S.01 continued---

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings & Inv.
537	5	M	0.50	0.50	0.50	Horizontal pendular	20° to left	U	Nil	
549	4	M	0.50	0.50	0.50	Horizontal pendular	20° to right	240	Nil	
7310	20	F	1.00	1.00	1.00	Horizontal jerk	5° to left	550	Nil	
426	6	F	0.67	0.33	0.67	Horizontal pendular	5° to left	3000	Nil	
1001	9	M	0.25	0.17	0.25	Horizontal jerk, Latent component	20° to left	Nil	Alt esotropia	Ocular albinism
1003	17	M	0.50	0.33	0.50	Horizontal pendular	Nil	Nil	Nil	Ocular albinism
1005	41	M	0.17	0.17	0.17	Horizontal pendular	Nil	Nil	Alt esotropia	Ocular albinism
1006	9	F	1.00	1.00	1.00	Horizontal pendular	Nil	85	Nil	Subtile iris transillumionation, but clear evidence of asymmetry on VEP
1013	11	M	0.1	0.1	0.1	Horizontal pendular	5-10° to left	Nil	Alt esotropia	Ocular abinism
1034	30	M	1.00	1.00	1.00	Horizontal jerk	Nil	85	Nil	Subtile iris transillumionation, but evidence of asymmetry on VEP
1018		M	0.7	0.7	0.7	Horizontal pendular	5° to right	Nil	L exotropia	Oculo-cutaneous albinism
1002	12	F	0.02	0.67	0.67	Horizontal pendular	Nil	Nil	R esotropia	R congenital cataract
1023	63	F	0.1	0.1	0.1	Horizontal pendular	Nil	Nil	R esotropia	Congenital cataract
1004	3	F	1.00	1.00	1.00	Nil	Nil	550	Nil	R Brown's syndrome
1007	2	F	U	U	U	Rotary nystagmus	Head Nodding	U	R esotropia	Spasmus nutans
1008	5	M	0.67	0.67	0.67	Rotary nystagmus	Slight chin up	U	Nil	CFEOM
1011	25	F	1.00	0.67	1.00	Horizontal Jerk	Slight chin up	U	Nil	CFEOM
1009	35	F	1.20	0.67	1.20	Latent nystagmus	Nil	Bagolini +ve	Nil	Dissociated vertical deviation
1010	29	M	0.05	1.20	1.20	Latent nystagmus	Nil	Nil	R exotropia (consecutive)	History of squint surgery
1014	9	M	0.7	1.0	1.0	Manifest latent nystagmus	15° to right	55	Nil	Exophoria

Table 3S.01 continued---

Number	Age	Sex	R VA	L VA	VA BE	Nystagmus	Head posture	Stereopsis	Strabismus	Other findings & Inv.
1015	59	M	1.2	0.2	1.2	Manifest latent nystagmus	Nil	Nil	L exotropia	Manifest latent nystagmus
1016	40	F	0.7	1.2	1.2	Manifest latent nystagmus	Nil	Nil	R esotropia	Manifest latent nystagmus
1017		F	0.1	0.1	0.1	Manifest latent nystagmus	Nil	Nil	R esotropia	Manifest latent nystagmus
1024	41	M	0.3	0.3	0.5	Manifest latent nystagmus	Nil	Nil	Alt esotropia	Latent nystagmus
1027	41	M	0.2	0.1	0.2	Manifest latent nystagmus	Nil	Nil	Left esotropia	Optic disc pallor, birth injury
1029	33	M	0.0	0.1	0.1	Manifest latent nystagmus	Nil	Nil	R esotropia	
1032	40	M	0.2	0.1	0.2	Manifest latent nystagmus	Nil	Nil	left esotropia	Birth injury- occipital infarcts
1033	28	M	0.3	0.1	0.3	Manifest latent nystagmus	Nil	Nil	left esotropia	Deayed birth, recurrent convulsions at age 2
1026	44	M	0.3	0.3	0.3	Manifest latent nystagmus	Slight chin down	Nil	R exotropia	Red green blind
1020		M	0.0	0.7	0.7	Manifest latent nystagmus	Nil	Nil	R esotropia	Latent nystagmus
1021	24	F	0.7	0.3	0.7	Manifest latent nystagmus	5° chin up	55	Nil	Exophoria
1028	52	M	0.3	0.3	0.3	Horizontal pendular/rotary	Nil	150	Nil	ON hypoplasia
1022	60	F	0.0	0.3	0.3	Horizontal pendular	Nil	Nil	Nil	ON hypoplasia
1035	1	M	U	U	u	Horizontal and vertical pendular	Nil	U	Nil	Septo-optic dysplasia
1019	48	M	0.1	0.1	0.2	Horizontal jerk	Nil	Nil	Right esotropia	Achromatopsia
1025	44	M	0.1	0.2	0.2	Horizontal jerk	Nil	Nil	left esotropia	Achromatopsia
1030	39	F	0.3	0.3	0.3	Horizontal jerk	Slight turn to right	300	Nil	Achromatopsia
1012	14	F	0.3	0.5	0.5	Horizontal jerk	Slight turn to left	550	L hypertropia	Congenital stationary night blindness
1031	4	M	0.2	0.2	0.2	Horizontal jerk	20° chin up	600	Nil	Congenital stationary night blindness
1034	7	F	0.5	0.5	0.5	Horizontal jerk	Nil	U	Nil	Noonan syndrome

3.1.6 Interim conclusion- Summary of clinical results

During this study we phenotyped 149 subjects with infantile nystagmus and 218 unaffected relatives from 39 families. In addition, we phenotyped 78 sporadic subjects with infantile nystagmus.

Thirty families were diagnosed to have X-linked IIN and one family with autosomal dominant IIN. Three families were diagnosed with albinism. Subjects with an obvious diagnosis of albinism were not enrolled into this study and hence the number of families diagnosed with albinism (three) does not represent its actual burden as a cause of congenital nystagmus. These three families had an initial diagnosis of IIN, but were found to have features of albinism on detailed examination and hence excluded from the study. Subjects from two families were found to have congenital nystagmus associated with cataract and/or foveal hypoplasia. One family each were diagnosed with aniridia, congenital stationary night blindness and cone-rod dystrophy.

Of the 149 subjects with infantile nystagmus, 121 subjects (71 males and 50 females) were found to have X-linked IIN. A breakdown of families with their diagnosis, the number of affected males and females in the families, their mode of inheritance are given in Table 3S.02.

Seventy-eight sporadic cases of congenital nystagmus were recruited into the study of which forty-two (28 males and 14 females) were confirmed as having IIN. Thirty-six subjects with other diagnosis were excluded from the genetic study. The details of these subjects are shown in Table 3S.03

***Table 3S.02**: Shows a breakdown of all familial subjects recruited into the study. The family number, diagnosis, number of affected males and females are shown in the table.*

Family Number	Diagnosis	Affected Males	Affected Females	Total number
N1	X linked IIN	12	2	14
N2	X linked IIN	5	2	7
N3	X linked IIN	8	1	9
N4	X linked IIN	3	2	5
N5	X linked IIN	2	0	2
N6	X linked IIN	1	3	4
N7	X linked IIN	2	1	3
N8	X linked IIN	2	0	2
N9	X linked IIN	2	1	3
N10	X linked IIN	2	1	3
N11	X linked IIN	6	6	12
N12	X linked IIN	3	2	5
N13	X linked IIN	4	2	6
N14	X linked IIN	2	2	4
N15	X linked IIN	4	1	5
N16	X linked IIN	1	3	4
F1	X linked IIN	2	0	2
F2	X linked IIN	1	2	3
F3	X linked IIN	1	1	2
F4	X linked IIN	1	1	2
F5	X linked IIN	1	1	2
F6	X linked IIN	1	1	2
F7	X linked IIN	1	1	2
F8	X linked IIN	1	2	3
F9	X linked IIN	1	1	2
F10	X linked IIN	0	4	4
G1	X linked IIN	0	2	2
G2	X linked IIN	0	2	2
G3	X linked IIN	1	1	2
G4	X linked IIN	1	2	3
E1	Albinism	2	0	2
E2	Aniridia	1	1	2
E3	Albinism	1	1	2
E4	Autosomal dominant IIN	2	1	3
E5	CSNB	2	0	2
E6	Albinism	1	1	2
E7	Cataract/Nystagmus/Foveal hypoplasia	7	4	11
E8	Cone-Rod dystrophy	0	2	2
E9	Nystagmus/Cataract	0	2	2

Table 3S.03: *Details of the sporadic subjects recruited into the study. The diagnosis, number of males, females and the total number of subjects in each group are shown in the table. (CFEOM= congenital fibrosis of extra ocular muscles; MLN= manifest latent nystagmus; DVD= dissociated vertical deviation; CSNB= congenital stationary night blindness)*

Diagnosis	Affected Males	Affected Females	Total Number
IIN	28	14	42
Ocular Albinism	5	1	6
Oculocutaneous albinism	1	0	1
Congenital cataracts	0	2	2
Brown's syndrome	0	1	1
Spasmus nutans	0	1	1
CFEOM	1	1	2
DVD	0	1	1
Strabismus and MLN	10	3	13
Optic nerve hypoplasia	1	1	2
Septo-optic dysplasia	1	0	1
Achromatopsia	2	1	3
CSNB	1	1	2
Noonan syndrome	0	1	1

3.2 Laboratory Results

3.2.1 Linkage Analysis

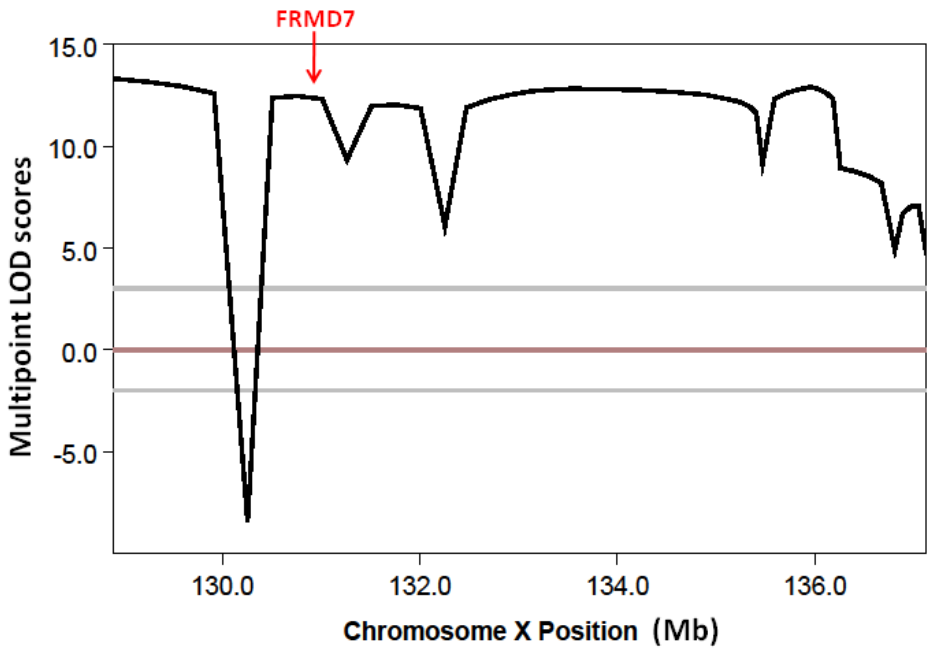
Genotyping of these families were performed in three steps.

In the first step, thirteen microsatellite markers from across the previously identified minimal region of X-chromosome were genotyped for a total of 154 individuals from eleven families. In the second step, nine microsatellite markers from the genetic interval were genotyped for 38 individuals from 5 families. In the third step, 63 individuals from ten smaller families were genotyped using nine microsatellite markers. The details of the microsatellite markers are given in Table 2.1.

The microsatellite data were analysed using the *MERLIN* Software. Haplotype was checked for errors using 'pedstats'.[204] Parametric linkage analysis was done and the LOD scores were calculated using '*MERLIN* for X-linked pedigrees' as explained in chapter 2.

The results of the parametric linkage analysis in the initial eleven families are shown in Figure 3L.01.

Figure 3L.01: *Multipoint LOD scores for marker between dxs1047 and dxs1062 . The position of FRMD7, which was identified later, is indicated.*



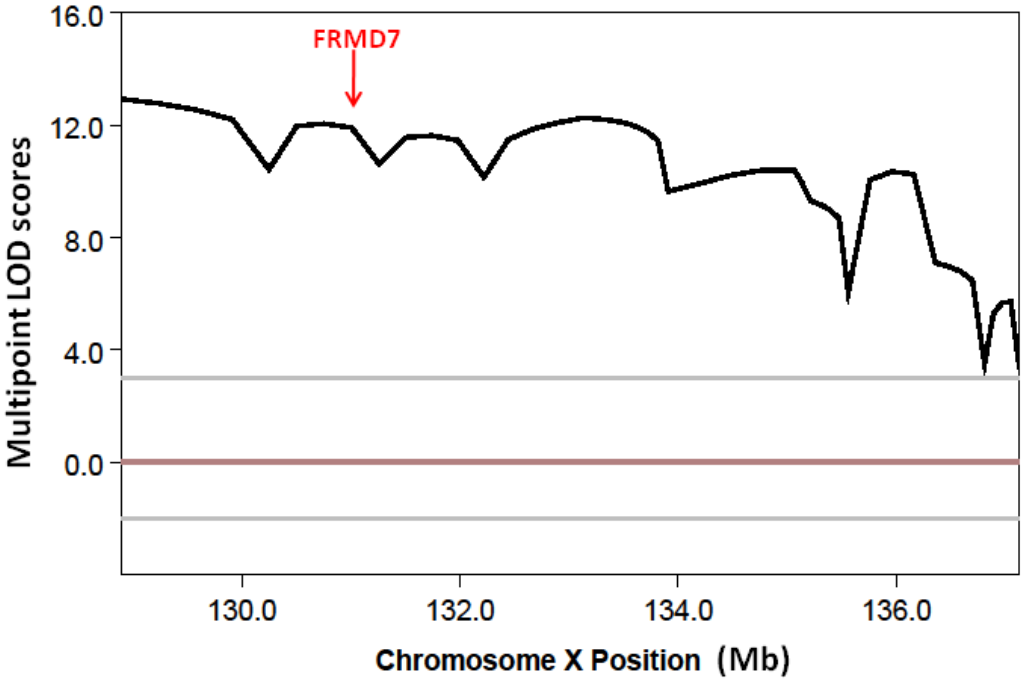
Five more families were included in the study and the results of the linkage analysis in these families (sixteen families) is shown in Figure 3L.02.

Linkage analysis using *'MERLIN'* gave high LOD scores across the region for all markers which confirmed linkage. This is in agreement with the work by Kerrison et.al.[201]

LOD score at all the selected markers scored above 4.0; however the highest LOD score of 12.449 was obtained for marker DXS8072 at chromosome position 130.225.

Haplotype analysis refined the genetic interval as discussed in section 3.2.2.

***Figure 3L.02**: Multipoint LOD scores for marker between dxs1047 and dxs1062. The position of FRMD7, which was identified later, is indicated.*



3.2.2 Haplotype Analysis

After confirming the locus through linkage analysis as explained in section 3.1.1, we used the haplotype data to refine the genetic interval further.

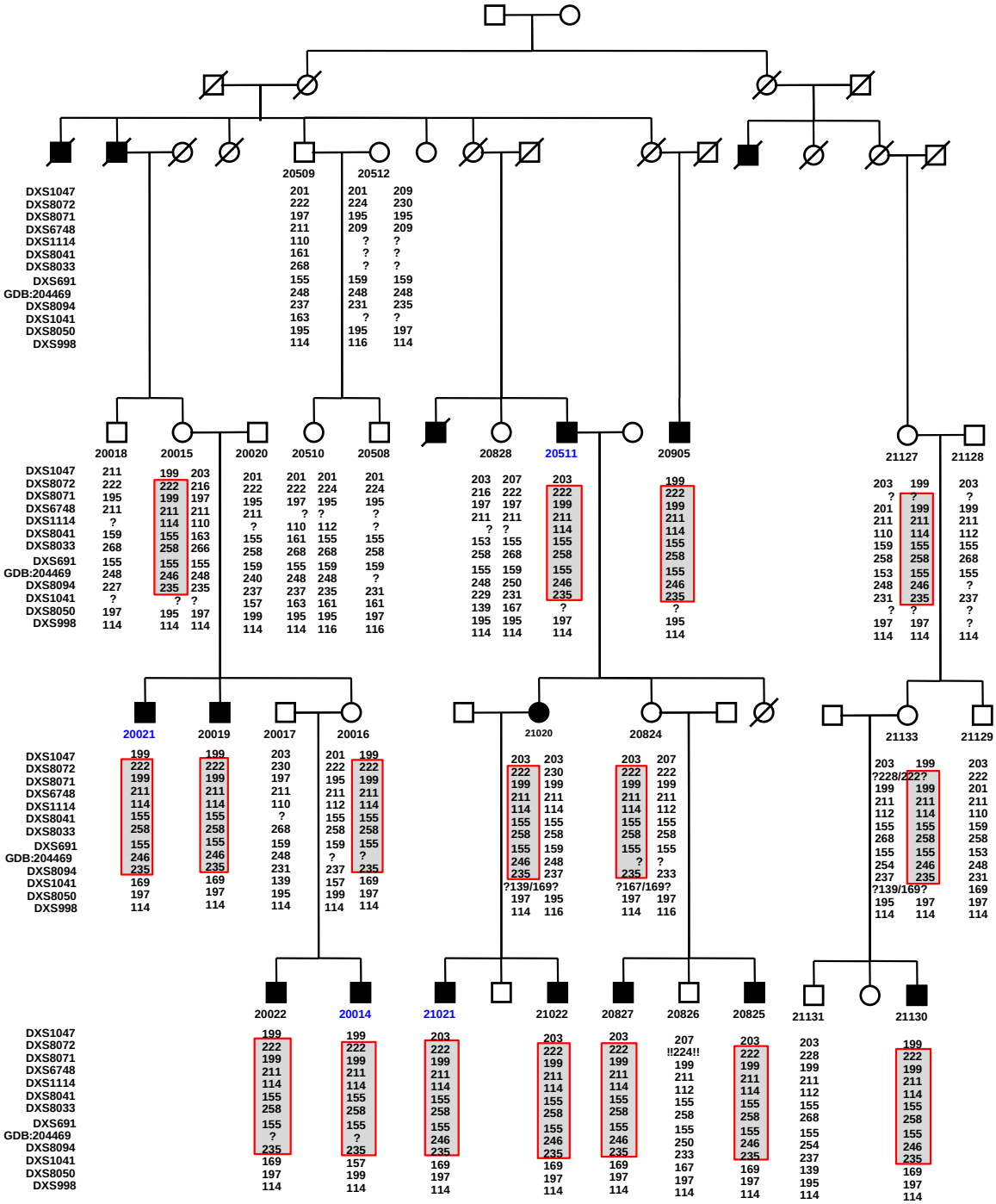
Kerrison et.al in 2004, had mapped the gene at locus NYS1 to a 12 mB region between markers DXS8078 and DXS1211.[201]

We analysed the haplotype data in 26 families and found that the data from family N1 has recombination events which refined the centromeric and telomeric boundaries of the shared haplotype at locus NYS1. The new genetic interval (9 mB region) was defined by markers DXS8072 and DXS8094. Haplotype data in Family N1 is shown in Figure 3H-N1. The shared haplotype between the markers DXS8072 and DXS8094 is highlighted in the red box. The centromeric boundary of the shared interval is defined by a recombination event between markers DXS8072 and DXS1047 in individual 20511. (Compare with other affected males 20019 and 20014 in Figure 3H-N1). The telomeric boundary of the interval is defined by a recombinant between markers DXS8094 and DXS1041 in subject number 20014 (e.g. discordant with subject number 20021).

The new genetic interval at locus NYS1 is shown in Figure 3H-N17.

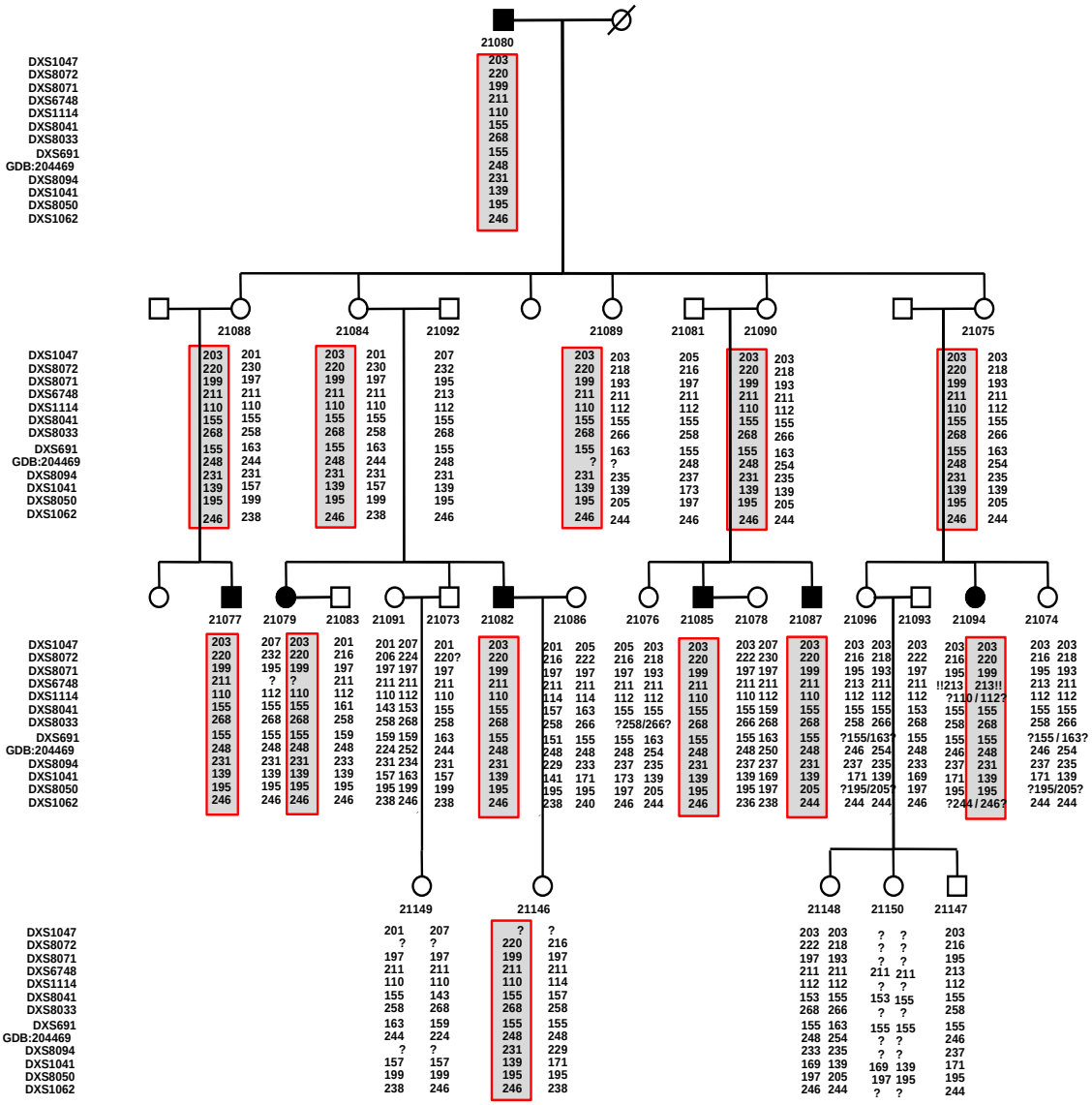
The author would like to draw attention to the work done by Zhang et.al (ref) which is misleading. Zhang et.al in 2005 refined the genetic interval to a 4mB region between markers DXS8033 and DXS1211 as explained in section 1.8.2. However this data is inaccurate because they presumed that the disease is fully penetrant in the family and based their assumptions on two recombination events in unaffected females, without considering the fact that they could be unaffected obligate carriers.

***Figure 3H-N1**: The haplotype data from family N1. The shared haplotype is highlighted in red coloured boxes.*



Haplotype data from family N2 is shown in Figure 3H-N2.

***Figure 3H-N2**: Haplotype data form family N2. Shared haplotype is highlighted.*



Haplotype data from Families N3 & N4 is shown below in Figures 3H-N3 and 3H-N4

Figure 3H-N3: *Haplotype data from family N3 is shown. Shared haplotype is highlighted.*

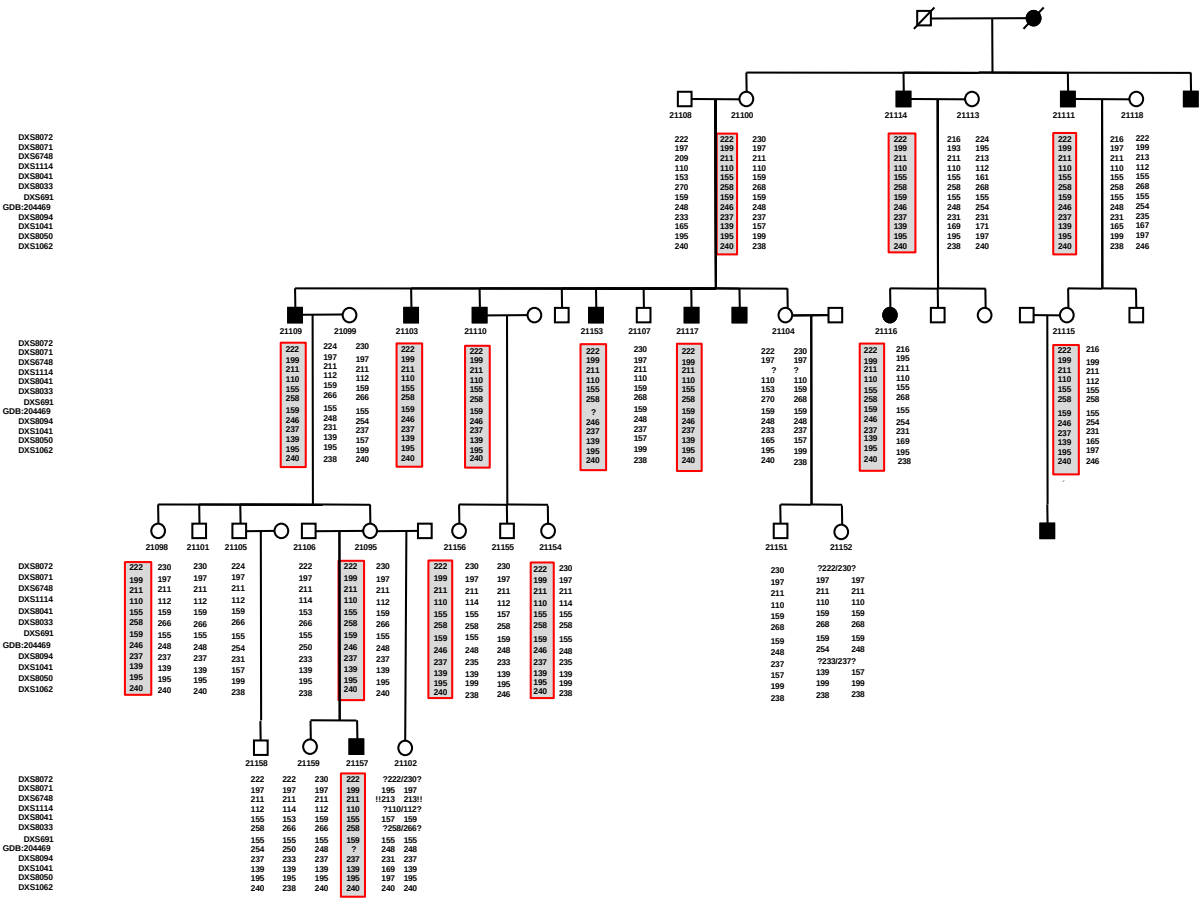


Figure 3H-N4: *Haplotype data from family N4 is shown. Shared haplotype is highlighted.*

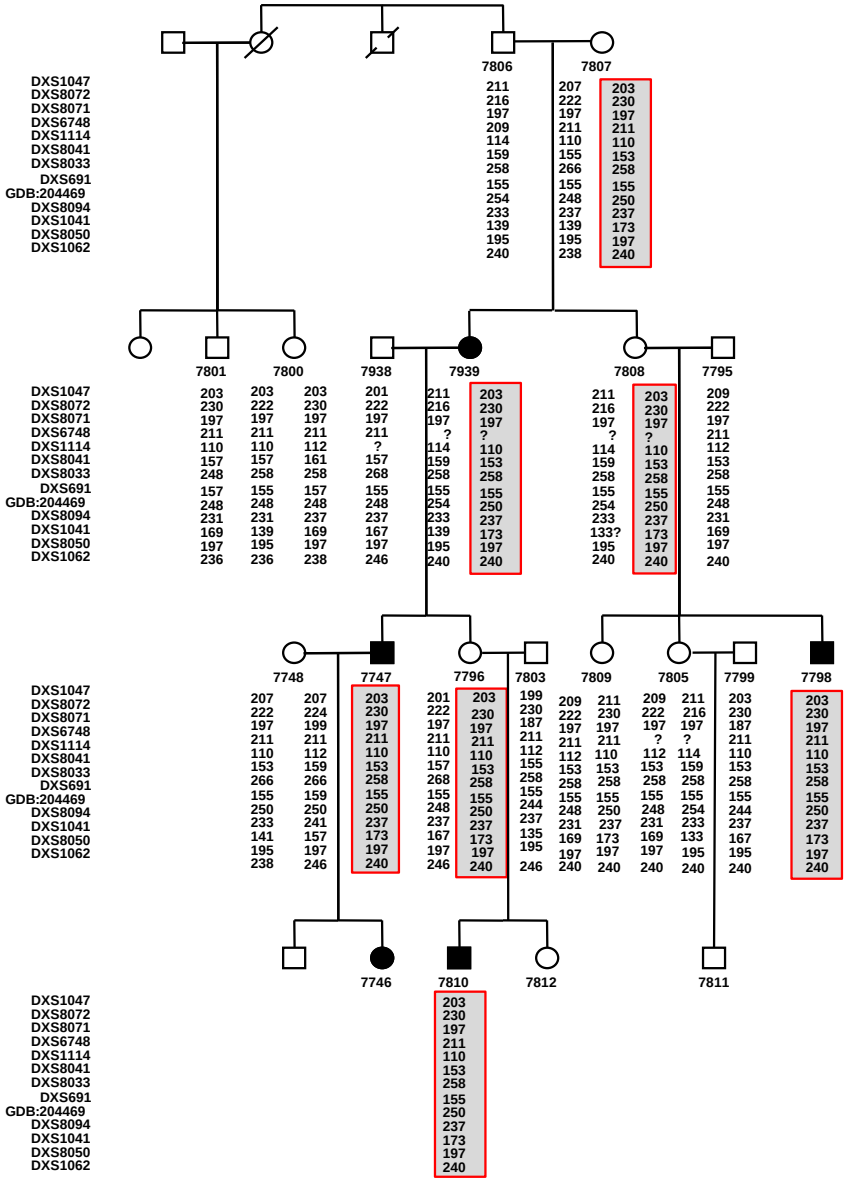


Figure 3H-N5: *Haplotype data from family N5 is shown. Shared haplotype is highlighted.*

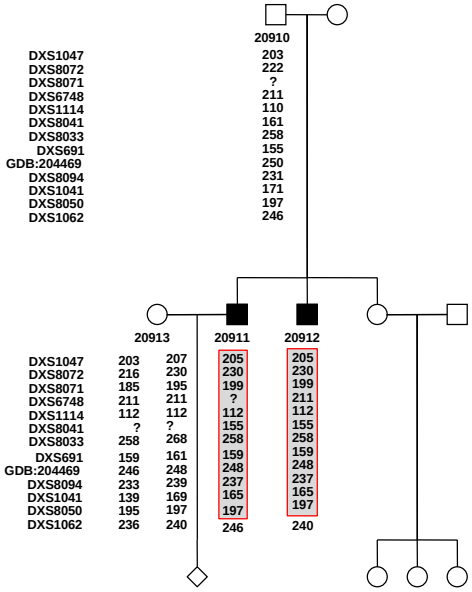


Figure 3H-N6: *Haplotype data from family N6 is shown. Shared haplotype is highlighted.*

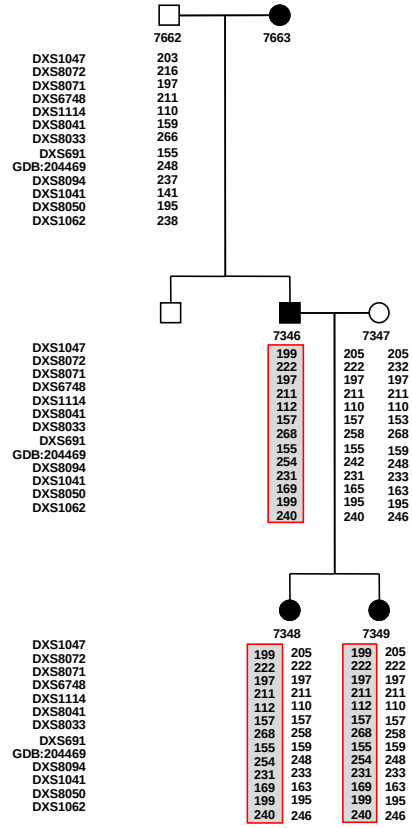


Figure 3H-N7: *Haplotype data from family N7 is shown. Shared haplotype is highlighted.*

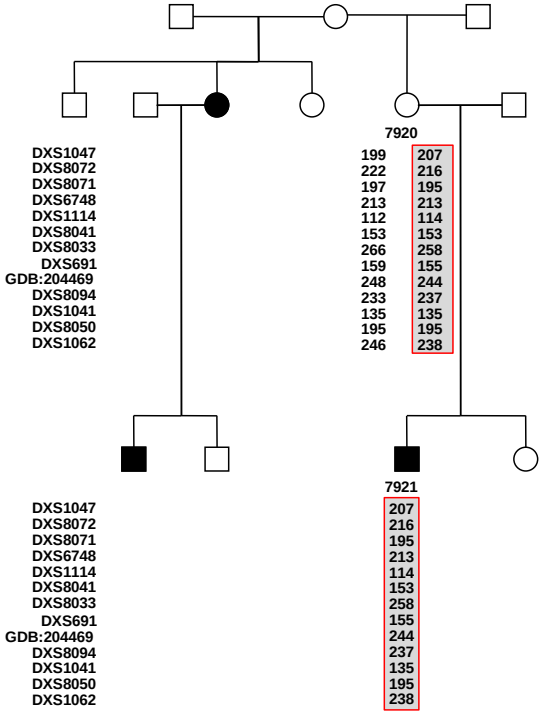


Figure 3H-N8: *Haplotype data from family N8 is shown. Shared haplotype is highlighted.*

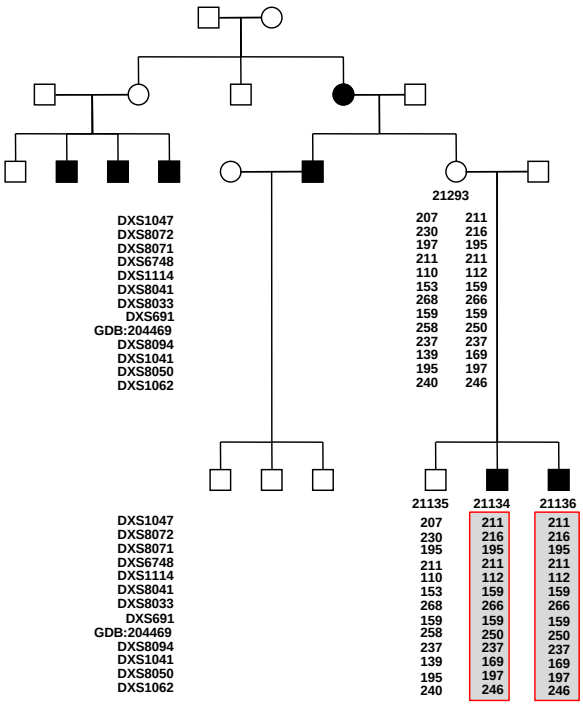


Figure 3H-N9: *Haplotype data from family N9 is shown. Shared haplotype is highlighted.*

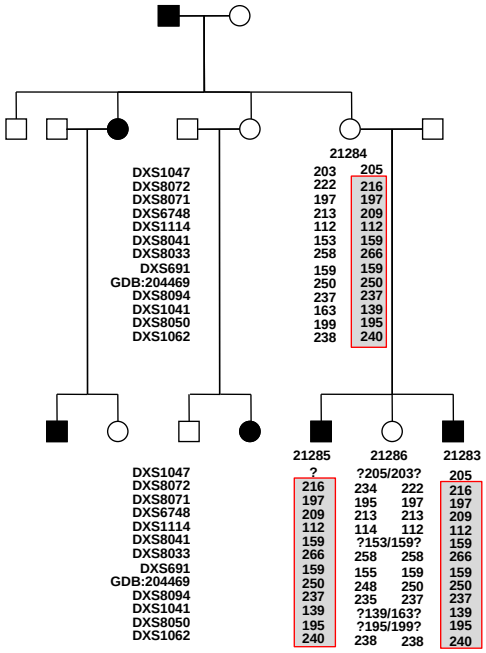


Figure 3H-N10: *Haplotype data from family N10 is shown. Shared haplotype is highlighted.*

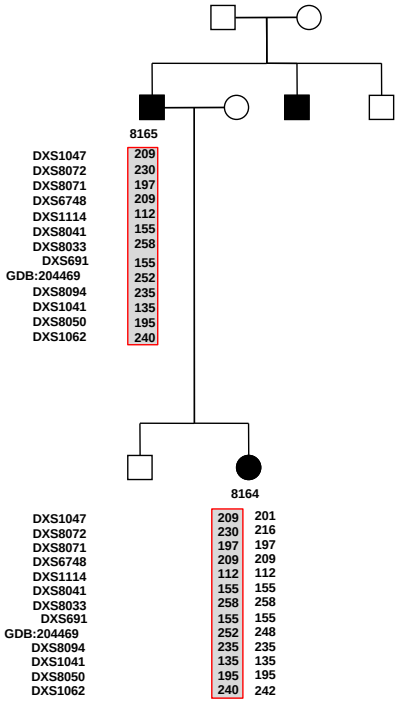


Figure 3H-N11: *Haplotype data from family N11 is shown. Shared haplotype is highlighted.*

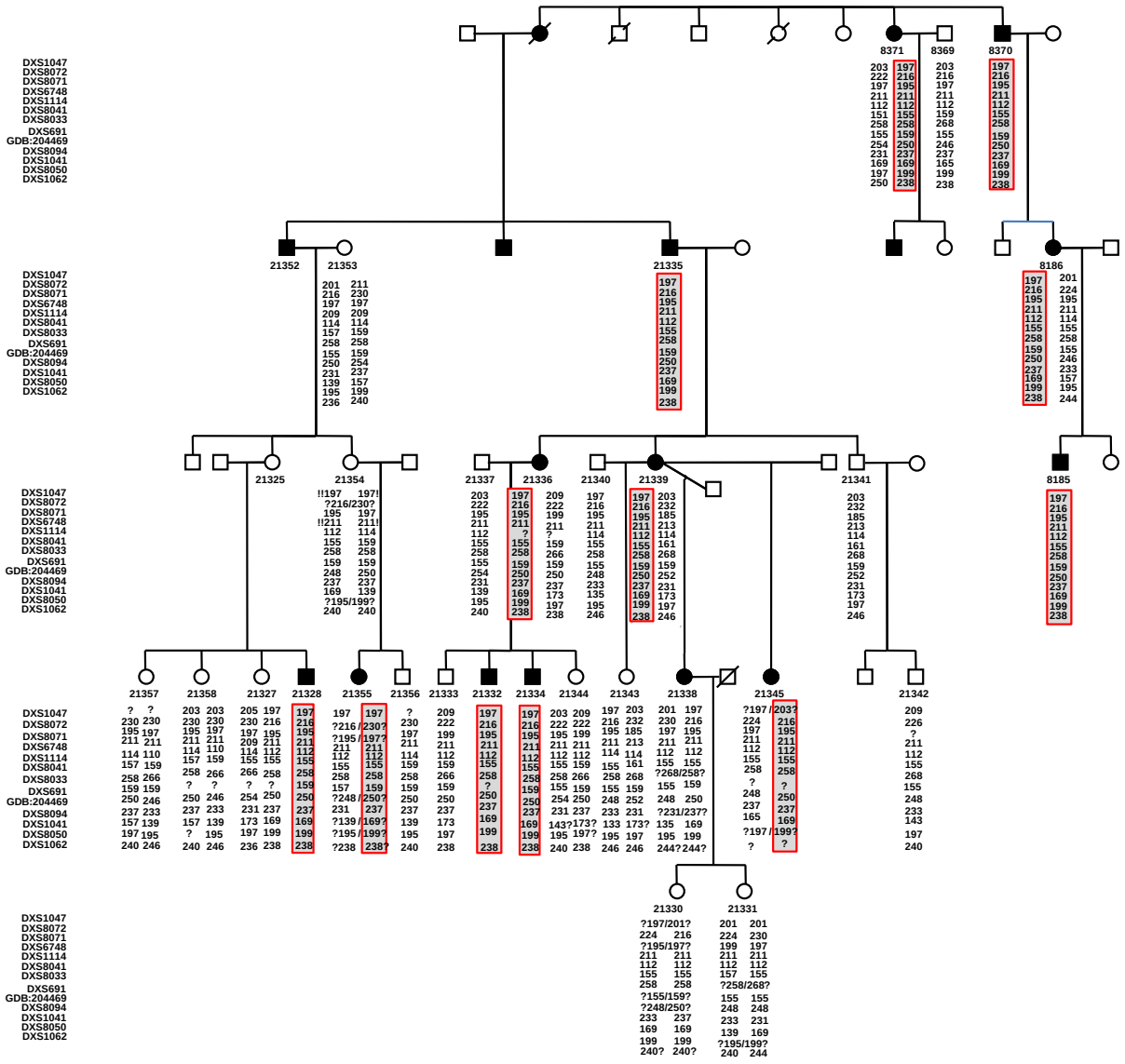


Figure 3H-N12: *Haplotype data from family N12 is shown. Shared haplotype is highlighted.*

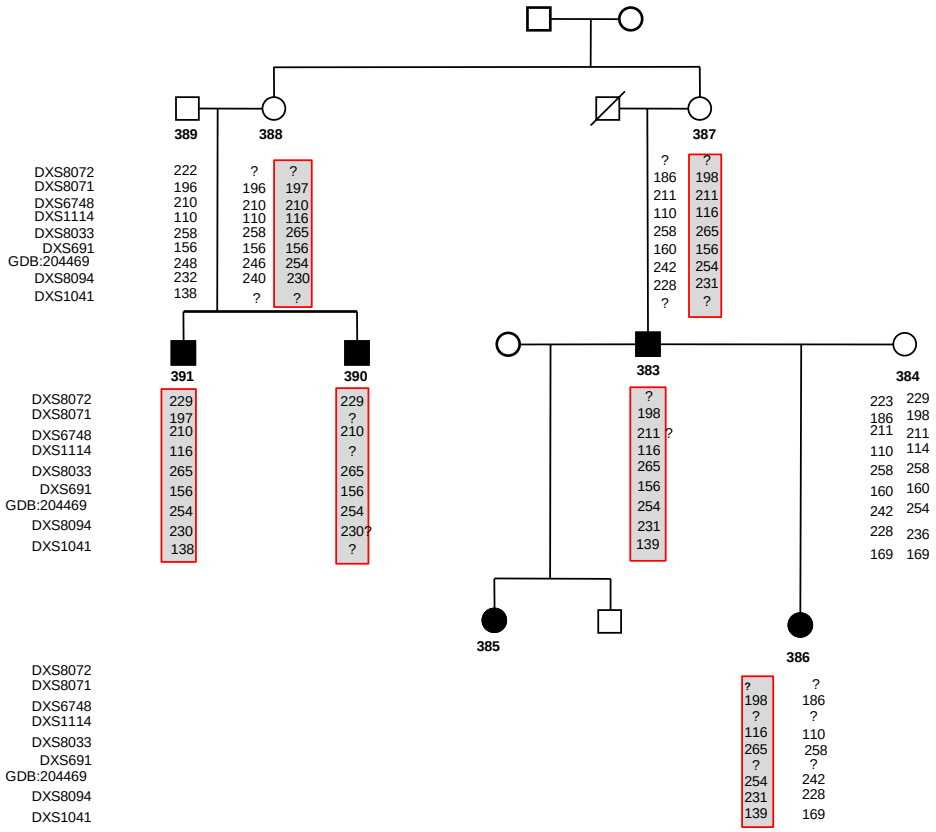


Figure 3H-N13: *Haplotype data from family N13 is shown. Shared haplotype is highlighted.*

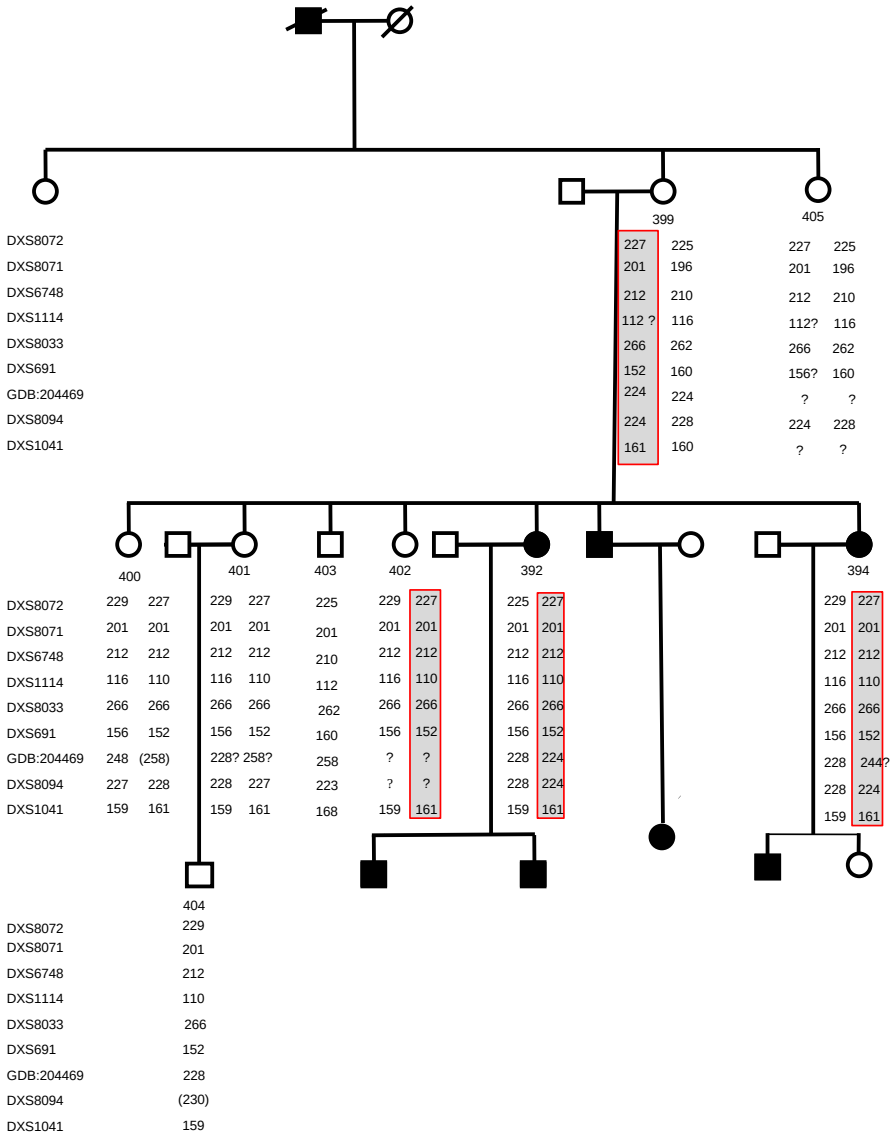


Figure 3H-N14: *Haplotype data from family N14 is shown. Shared haplotype is highlighted.*

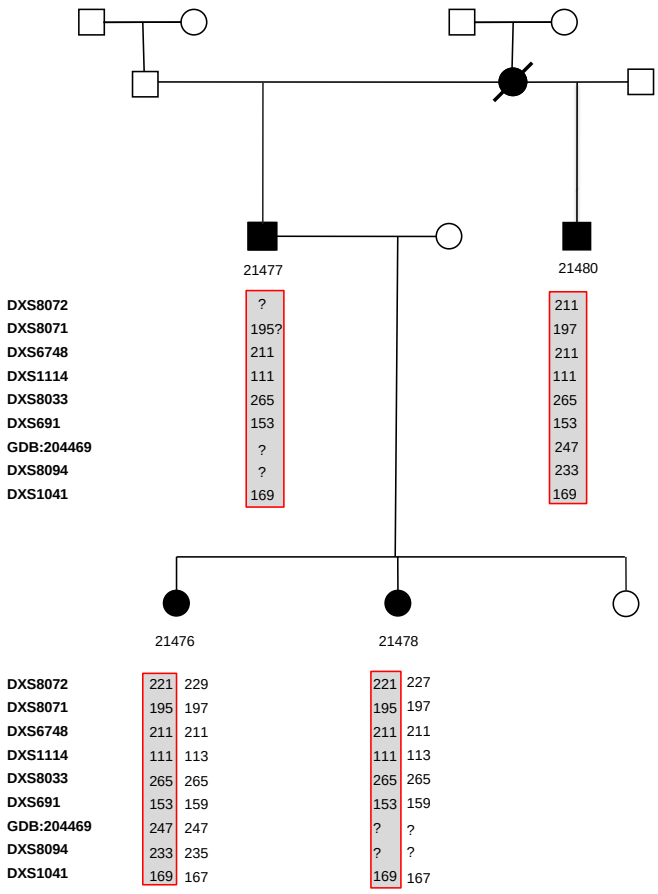


Figure 3H-N15: *Haplotype data from family N15 is shown. Shared haplotype is highlighted.*

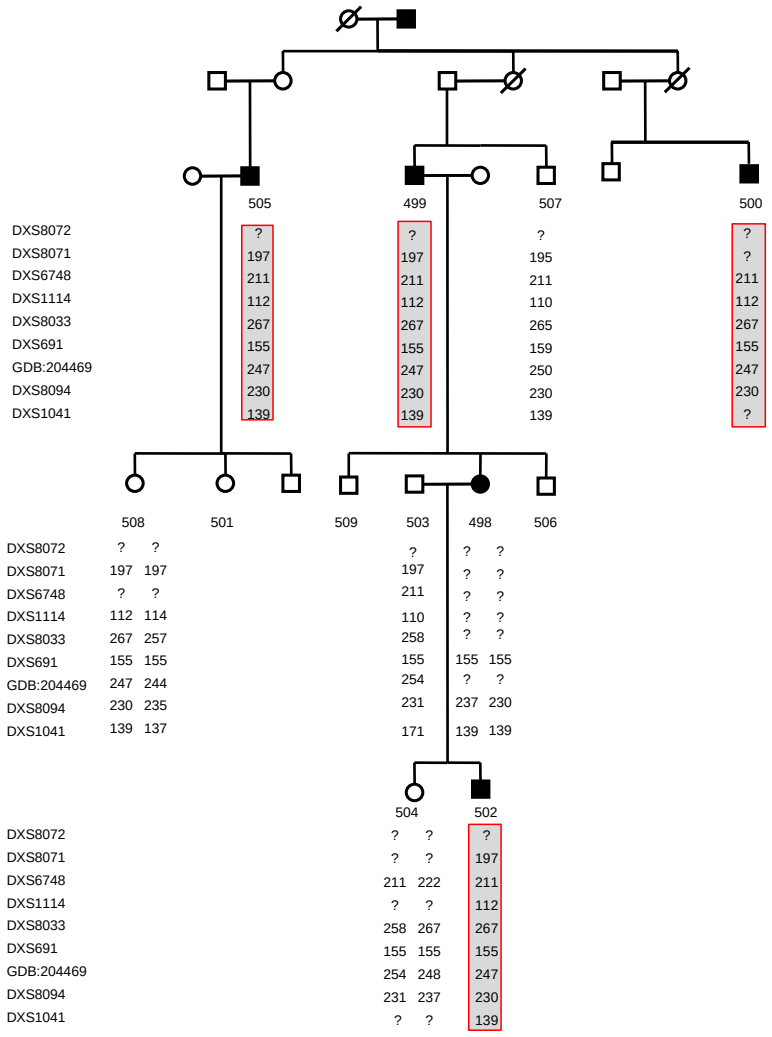


Figure 3H-N16: *Haplotype data from family N16 is shown. Shared haplotype is highlighted.*

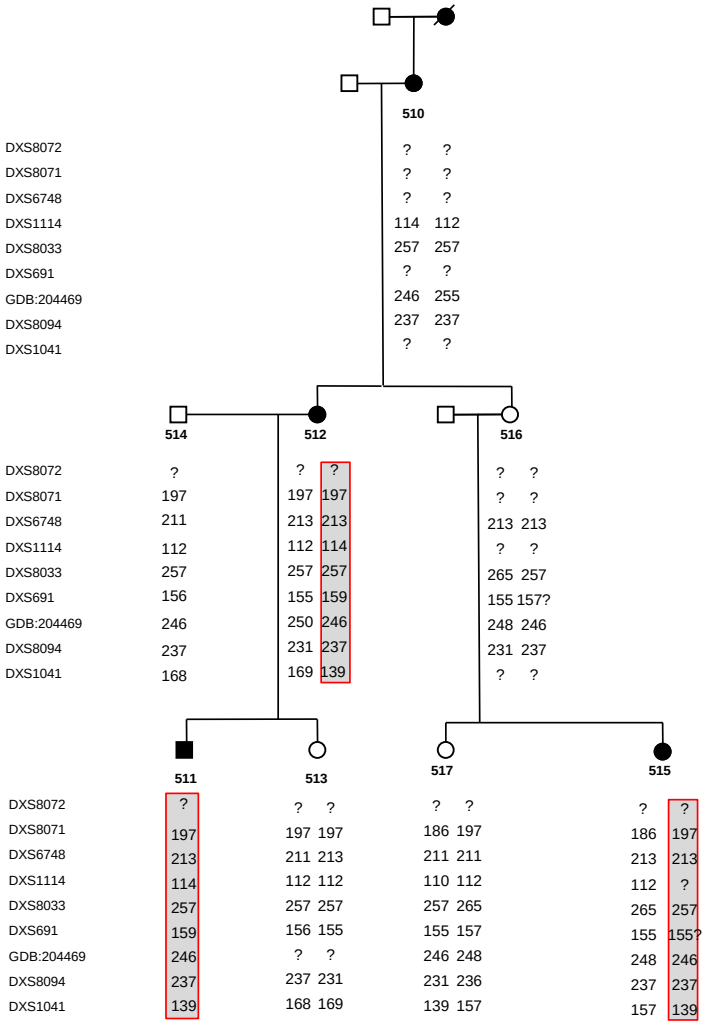
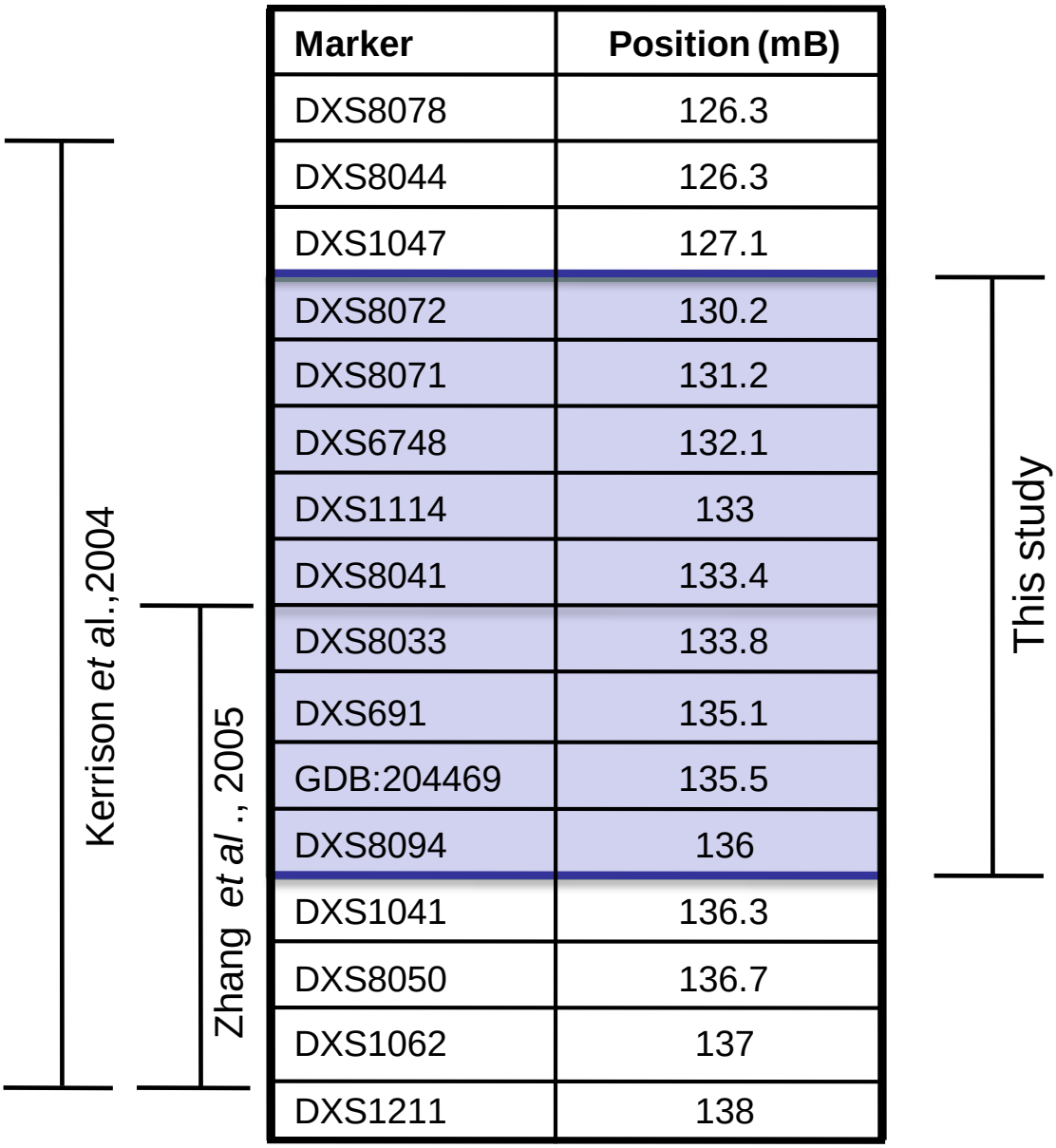


Figure 3H-N17: *A description of the localization of the genetic locus of X linked nystagmus (NYS1) at Xq26-27 based on this study (~9mB the region defined by the markers DXS1047 and DXS1041). The genetic interval refined by Kerrison et.al (2004) and Zhang et.al (2005) are also shown in the figure.*



Marker	Position (mB)
DXS8078	126.3
DXS8044	126.3
DXS1047	127.1
DXS8072	130.2
DXS8071	131.2
DXS6748	132.1
DXS1114	133
DXS8041	133.4
DXS8033	133.8
DXS691	135.1
GDB:204469	135.5
DXS8094	136
DXS1041	136.3
DXS8050	136.7
DXS1062	137
DXS1211	138

Families with prefix 'F'

Haplotype analysis on the smaller families and those recruited later were done at a second step. The results of analysis of these families are as follows.

Figure 3H-F1 (Family F1): *Haplotype data from family F1 is shown. Shared haplotype is highlighted.*

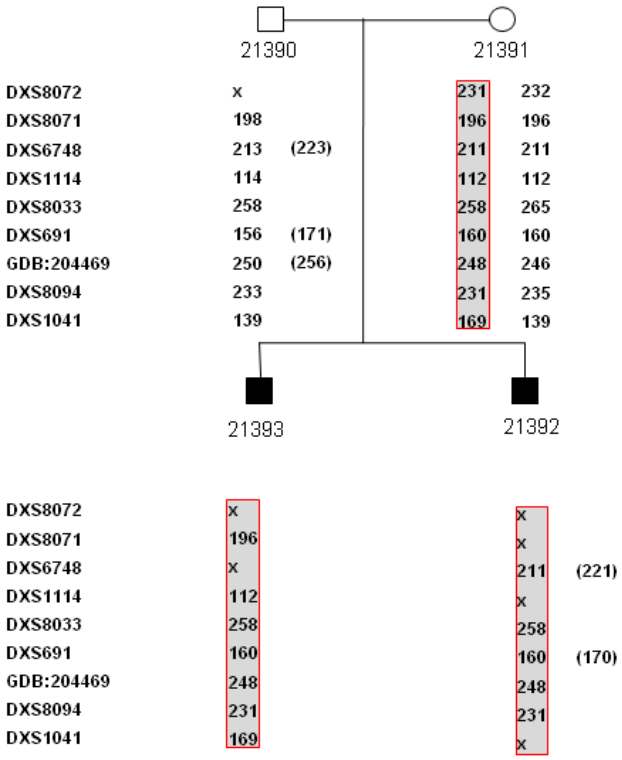


Figure 3H-F2 (Family F2): *Haplotype data from family F2 is shown. Shared haplotype is highlighted.*

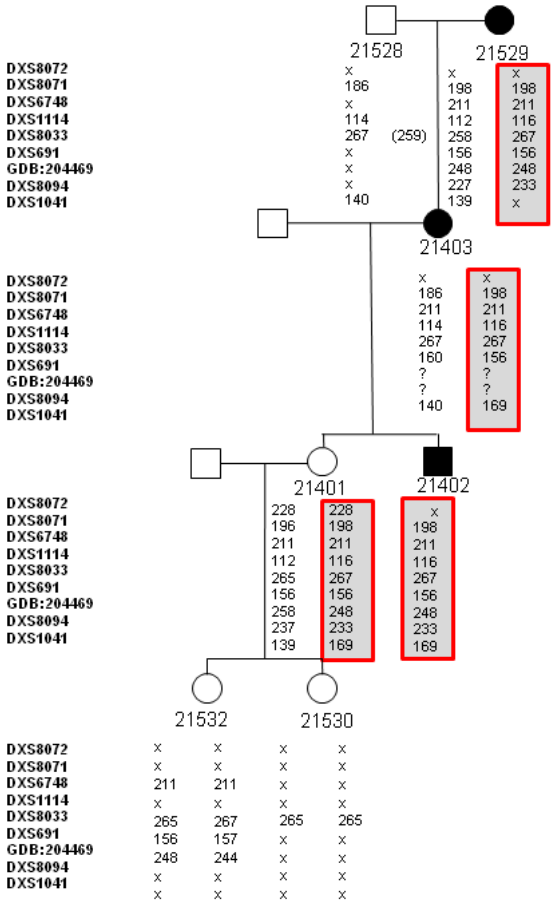


***Figure 3H-F3 (Family F3)**: Haplotype data from family F3 is shown. Shared haplotype is highlighted.*

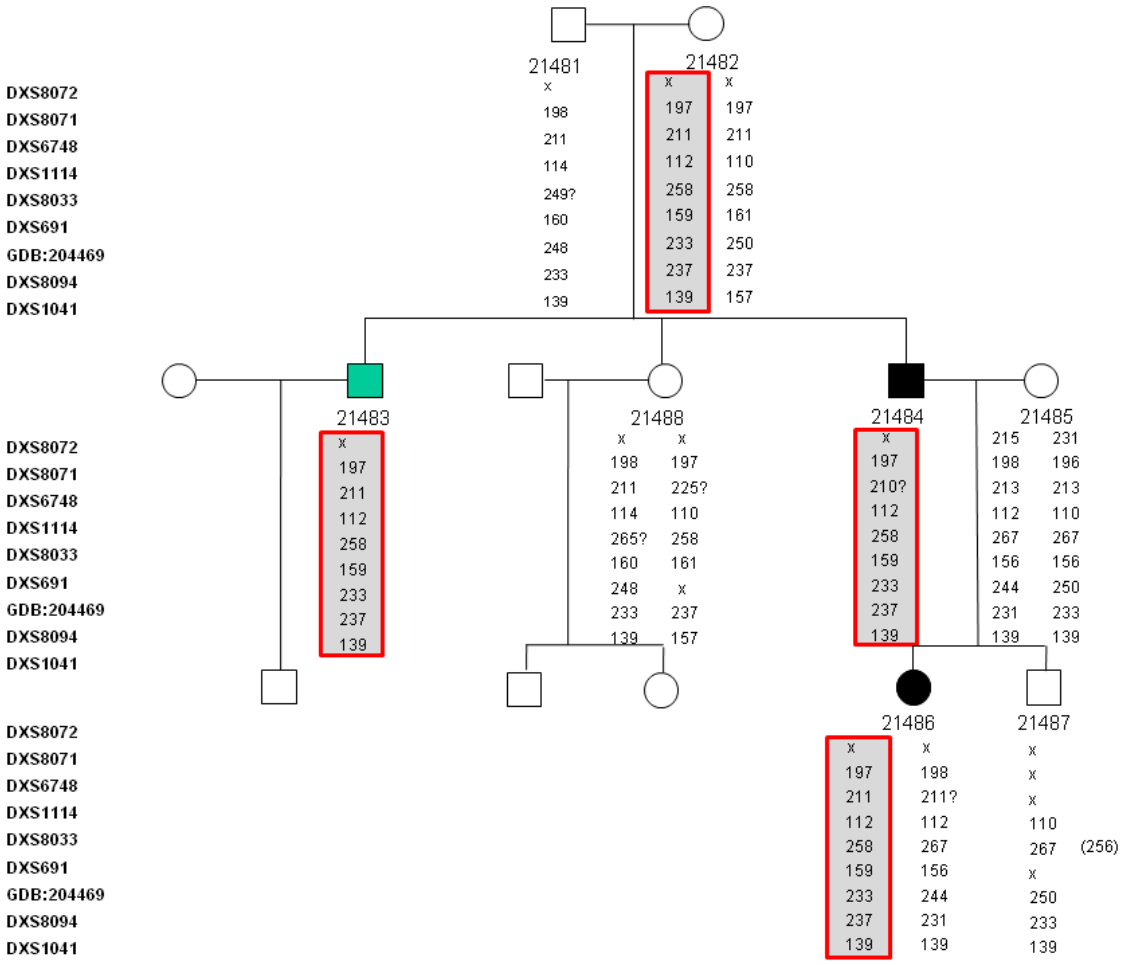


Figure 3H-F4 (Family F4): *Haplotype data from family F4 is shown. Shared haplotype is highlighted.*

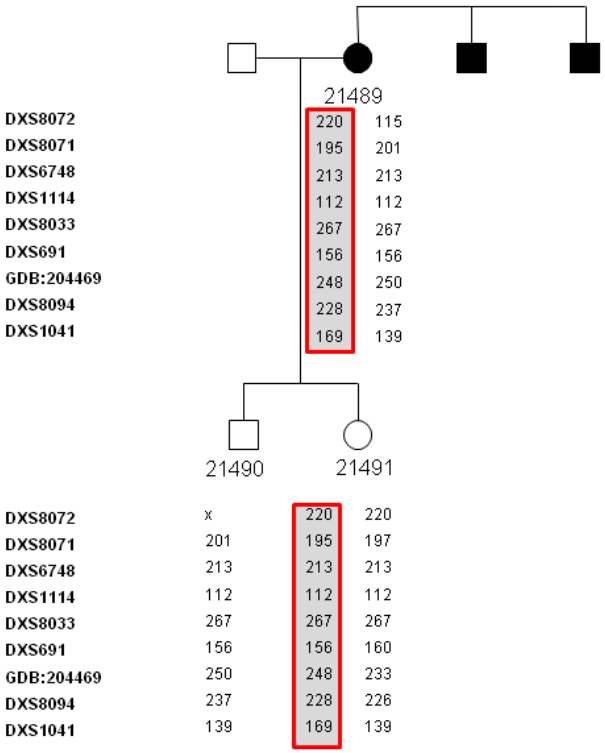


***Figure 3H-F5 (Family F5)**: Haplotype data from family F5 is shown. Shared haplotype is highlighted.*

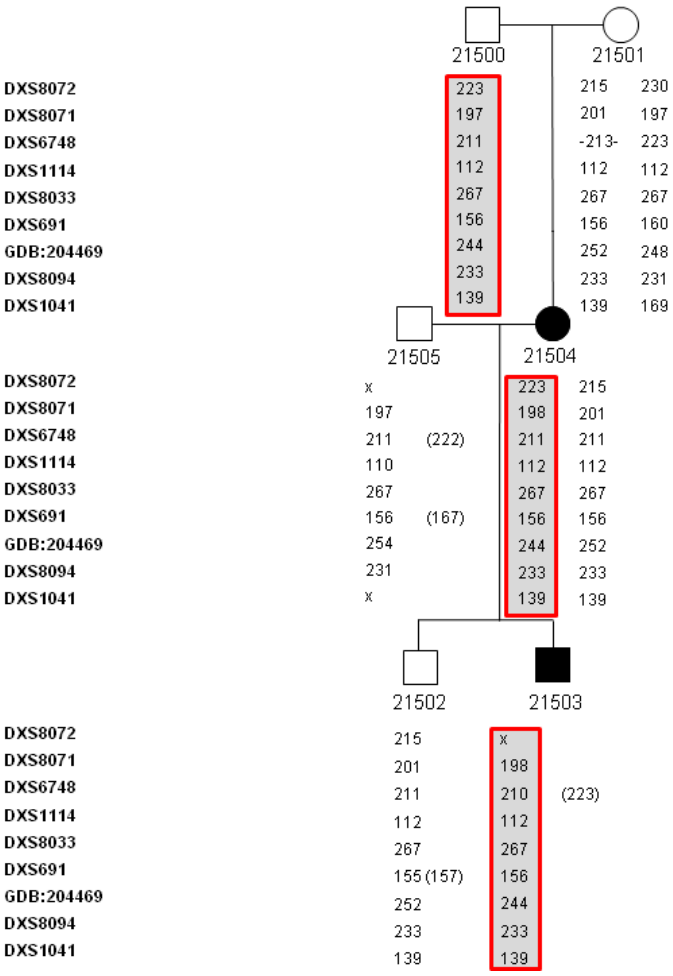


***Figure 3H-F6 (Family F6)**: Haplotype data from family F6 is shown. Shared haplotype is highlighted.*

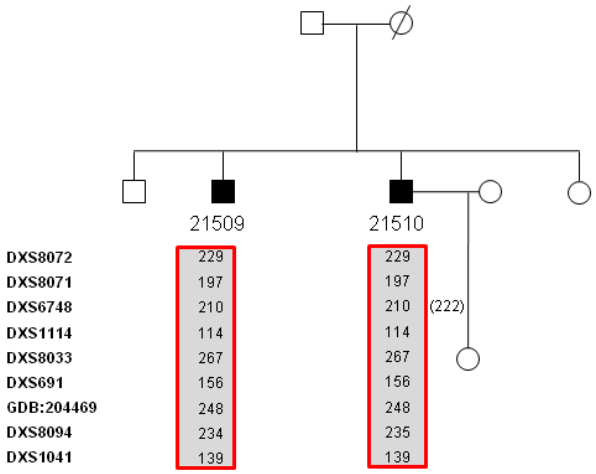


***Figure 3H-F7 (Family F7):** Haplotype data from family F7 is shown. Shared haplotype is highlighted.*

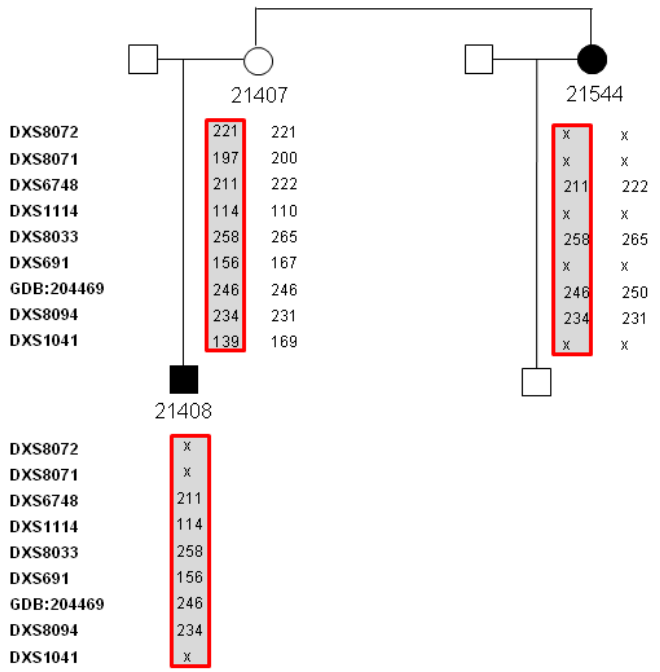


Figure 3H-F8 (Family F8): *Haplotype data from family F8 is shown. Shared haplotype is highlighted.*

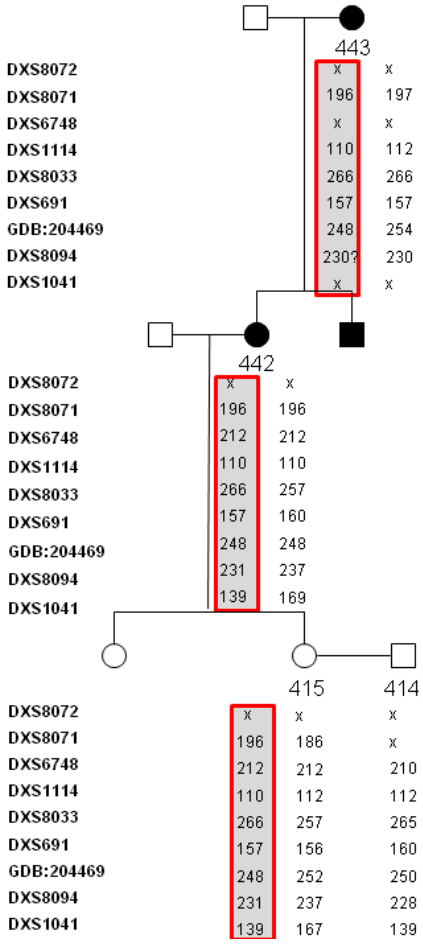


Figure 3H-F9 (Family F9): *Haplotype data from family F1 is shown. Shared haplotype is highlighted.*

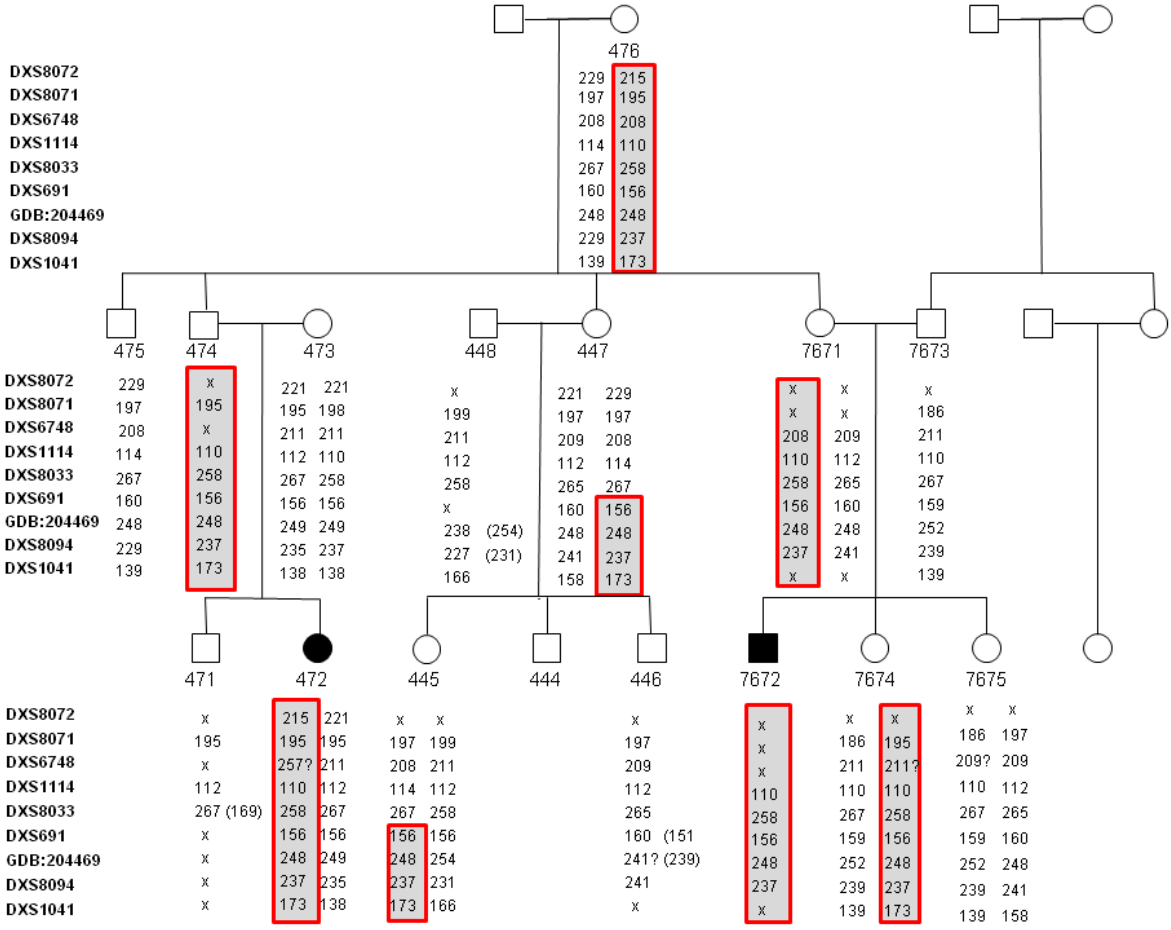
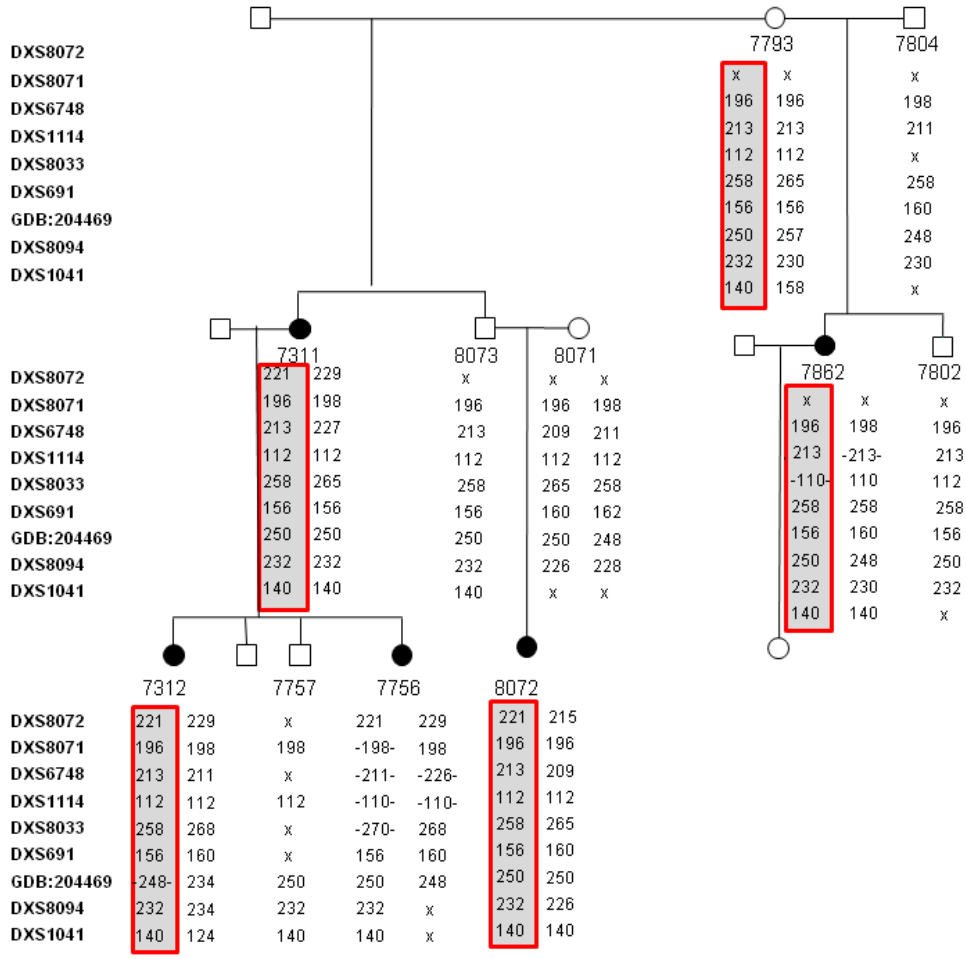


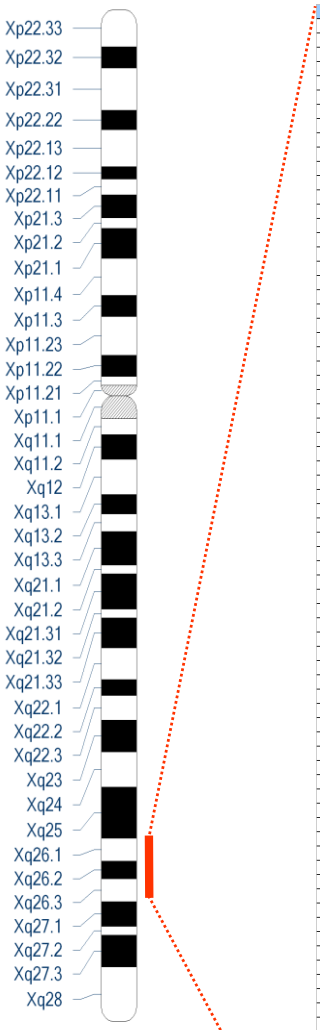
Figure 3H-F10 (Family F10): *Haplotype data from family F1 is shown. Shared haplotype is highlighted.*



3.2.3 Locus NYS1 refined in this study

We refined the critical genetic interval at locus NYS1 to a ~ 9mB region between markers dxs8072 and dxs8094. This region had about 80 gens as shown in Figure 3H-F11.

Figure 3H-F11: *A list and description of genes at locus Xq26-27 (indicated by the thick red line) in Homo sapiens. (I would like to acknowledge P Tarpey for help with this figure)*



start	stop	Symbol	Cyto	Description
128842204	128917136	BCORL1	Xq25-q26.1	BCL6 co-repressor-like 1
128924440	128970007	ELF4	Xq26	E74-like factor 4 (ets domain transcription factor)
128988872	129025343	PDCD8	Xq25-q26	programmed cell death 8 (apoptosis-inducing factor)
129031308	129044379	RAB33A	Xq25	RAB33A, member RAS oncogene family
129062224	129128408	SUHW3	Xq25	suppressor of hairy wing homolog 3 (Drosophila)
129199582	129232870	SLC25A14	Xq24	solute carrier family 25 (mitochondrial carrier, brain), member 14
129243949	129244956	GPR119	Xq25	G protein-coupled receptor 119
129261542	129273163	RBMX2	Xq25	RNA binding motif protein, X-linked 2
129354450	129356267	FAM45B	Xq25	family with sequence similarity 45, member B
129482894	129762743	COVA1	Xq25-q26.2	cytosolic ovarian carcinoma antigen 1
129917751	129949388	FLJ30058	Xq26.1	hypothetical protein FLJ30058
130063604	130064552	OR11Q1P	Xq26.1	olfactory receptor, family 11, subfamily Q, member 1 pseudogene
130133020	130146790	IGSF1	Xq25	immunoglobulin superfamily, member 1
130163843	130184822	OR11N1P	Xq26.1	olfactory receptor, family 11, subfamily N, member 1 pseudogene
130201038	130201961	OR5AW1P	Xq26.2	olfactory receptor, family 5, subfamily AW, member 1 pseudogene
130257558	130258466	OR5BH1P	Xq26.2	olfactory receptor, family 5, subfamily BH, member 1 pseudogene
130283810	130284750	OR1AA1P	Xq26.2	olfactory receptor, family 1, subfamily AA, member 1 pseudogene
130351788	130352402	LOC392542	Xq26.2	similar to NACHT-, LRR- and PYD-containing protein 7 (PYRIN-containing A
130403683	130404509	OR13H1	Xq26.2	olfactory receptor, family 13, subfamily H, member 1
130422238	130423321	OR13K1P	Xq26.2	olfactory receptor, family 13, subfamily K, member 1 pseudogene
130456630	130456712	OR7L1P	Xq26.2	olfactory receptor, family 7, subfamily L, member 1 pseudogene
130478583	130479472	OR2AF1P	Xq26.2	olfactory receptor, family 2, subfamily AF, member 1 pseudogene
130562215	130690206	LOC286467	Xq26.2	hypothetical protein LOC286467
130882862	130935506	MST4	Xq26.2	Mst3 and SOK1-related kinase
130936558	130987585	LOC90167	Xq26.2	hypothetical protein LOC90167
130989649	130990915	LOC392543	Xq26.2	similar to hypothetical protein FLJ10842
131062588	131077724	RAP2C	Xq25	RAP2C, member of RAS oncogene family
131237714	131299253	MBNL3	Xq26.2	muscleblind-like 3 (Drosophila)
131485579	131820947	HS6ST2	Xq26.2	heparan sulfate 6-O-sulfotransferase 2
131885027	131887820	USP26	Xq26.2	ubiquitin specific protease 26
132076217	132077896	TFDP3	Xq26.2	transcription factor Dp family, member 3
132160584	132274725	GPC4	Xq26.1	glypican 4
132395298	132845186	GPC3	Xq26.1	glypican 3
133103779	133105465	LOC347475	Xq26.2	hypothetical LOC347475
133131837	133233116	LOC402425	Xq26.2	similar to 5-nucleotidase, cytosolic II-like 1 protein; chromosome 6 open rea
133232862	133288339	PHF6	Xq26	PHD finger protein 6
133319777	133360216	HPRT1	Xq26.1	hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)
133403979	133406173	MGC16121	Xq26.3	hypothetical protein MGC16121
133425393	133518029	PLAC1	Xq26	placenta-specific 1
133629116	133656232	LOC159090	Xq26.3	similar to hypothetical protein MGC17347
133666744	133714136	LOC159091	Xq26.3	hypothetical protein BC017868
133747182	133774817	MOSPD1	Xq26.3	motile sperm domain containing 1
133809099	133812932	LOC340581	Xq26.3	hypothetical protein LOC340581
133821230	133821858	LOC392545	Xq26.3	similar to High mobility group protein 4 (HMG-4) (High mobility group protein
133880932	133882052	LOC441518	Xq26.3	similar to DKFZP564B147 protein
133891917	133893096	CXX1	Xq26	CAAX box 1
133910488	133911677	DKFZP564B147	Xq26.3	DKFZP564B147 protein
134015983	134031271	FLJ20527	Xq26.3	hypothetical protein FLJ20527
134039699	134053740	LOC392546	Xq26.3	similar to hypothetical protein FLJ20527
134061168	134075459	LOC389888	Xq26.3	similar to FLJ20527 protein
134145240	134203477	ZNF75	Xq26.3	zinc finger protein 75 (D8C6)
134204241	134222858	FLJ23614	Xq26.3	hypothetical protein FLJ23614
134281391	134285191	MGC39606	Xq26.3	hypothetical protein MGC39606
134282463	134298260	LOC392547	Xq26.3	similar to KIAA0445 protein
134380104	134441954	LOC203522	Xq26.3	hypothetical protein LOC203522
134443096	134444130	LOC392548	Xq26.3	similar to C-ETS-2 protein
134458249	134493088	LOC402426	Xq26.3	similar to sarcoma antigen 1; putative tumor antigen
134497487	134537132	LOC139587	Xq26.3	similar to sarcoma antigen 1; putative tumor antigen
134537456	134547730	LOC442465	Xq26.3	similar to sarcoma antigen 1; putative tumor antigen
134591734	134599770	MGC27005	Xq26.3	hypothetical protein MGC27005
134609008	134618913	LOC441519	Xq26.3	similar to Hypothetical protein MGC27005
134653851	134662255	LOC441520	Xq26.3	similar to hypothetical protein MGC27005
134671118	134679514	LOC441521	Xq26.3	similar to hypothetical protein MGC27005
134701305	134720740	SAGE1	Xq26	sarcoma antigen 1
134769751	134781654	TMEM32	Xq26.3	transmembrane protein 32
134793147	134854812	SLC9A6	Xq26.3	solute carrier family 9 (sodium/hydrogen exchanger), isoform 6
134865507	134886310	LOC392549	Xq26.3	similar to Glyceraldehyde 3-phosphate dehydrogenase, liver (GAPDH)
134905179	134906027	LOC139542	Xq26.3	similar to E2F transcription factor 6 isoform 1
134955200	135019030	FHL1	Xq26	four and a half LIM domains 1
135020901	135104363	FLJ12649	Xq26.3	hypothetical protein FLJ12649
135130576	135244735	GPR112	Xq26.3	G protein-coupled receptor 112
135295645	135300118	BRS3	Xq26-q28	bombesin-like receptor 3
135305191	135320010	HTATSF1	Xq26.1-q27.2	HIV TAT specific factor 1
135339831	135364483	VGLL1	Xq26.3	vestigial like 1 (Drosophila)
135455889	135468069	TNFSF5	Xq26	tumor necrosis factor (ligand) superfamily, member 5 (hyper-IgM syndrome)
135473234	135589023	ARHGEF6	Xq26	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6
135596291	135599459	LOC392550	Xq26.3	similar to 60S ribosomal protein L7
135681140	135687114	RBMX	Xq26	RNA binding motif protein, X-linked
135717074	135829332	LOC441522	Xq26.3	hypothetical gene supported by AK055694
135837827	135839353	GPR101	Xq25-27.1	G protein-coupled receptor 101
136248729	136249716	RAC4	Xq26.3	ras-related C3 botulinum toxin substrate family member 4
136373866	136379779	ZIC3	Xq26.2	Zic family member 3 heterotaxy 1 (odd-paired homolog, Drosophila)
136469241	136475314	LOC389892	Xq26.3	similar to Mothers against decapentaplegic homolog interacti

More than a quarter of these genes encoded hypothetical proteins. In addition, since the pathogenesis and the molecular basis of nystagmus are unknown, it was difficult to select candidate genes to investigate. A collaboration was set up with Dr F Lucy Raymond at CIMR, Cambridge, who leads a study of genes associated with X-linked mental retardation (http://goldstudy.cimr.cam.ac.uk). Since this involve high throughput sequencing of genes on the X chromosome, accessing their resources made identification of the gene mutated in X-linked nystagmus from such a large critical region a realistic proposition.

3.2.4 DNA Sequencing and identification of FRMD7

Automated high throughput DNA sequencing was carried out at the Sanger Institute. All coding exons and the splice junctions of fourty genes in the centromeric half of this 9mB region were sequenced in one affected male individual from 16 families (N1-N16). One gene stood out from the rest in terms of the number and type of mutations present in this cohort and the absence of mutations in a larger cohort of controls. This gene was annotated as a hypothetical protein LOC90167 and was since renamed *FRMD7*.

Once the gene was identified, all the affected individuals with IIN (both familial and sporadic cases) were sequenced to check for co-segregation. These results were published and I have attached a copy of the paper for reference (Appendix 1).

This work at Sanger institute does not form part of this thesis; however the paper is included in order to have a continuation with the next chapter where we have correlated the phenotype with the genotype.

3.2.5 Mutations in FRMD7

Fifteen of the sixteen linked families (prefixed by 'N') were found to have mutations in *FRMD7*. Of the ten smaller families in whom haplotype analysis was done (prefixed by 'F'), eight families were found to have mutations in the gene. Four small families (prefixed by 'G') in whom haplotype analysis was not carried out were also screened for mutations in the gene; however, none of these subjects were found to have mutations in *FRMD7*. Families and the corresponding mutations are shown in Table 3L.01.

All mutations identified in *FRMD7* co-segregated with the disease in the linked family members and were absent in 300 male control chromosomes.

The nonsense mutations (C601T and C1003T) were predicted to cause truncated protein. Four splice-site mutations were at conserved residues (at position +1 and +2) and are predicted to cause classical exon skipping and nonsense mediated decay. In family N7, pathogenicity of the mutation (IVS2+5G>A) was confirmed through RT-PCR, were only negligible amounts of transcript was amplified compared to normal controls.

A silent mutation (G252A) was detected in family N5. This change created a cryptic spice acceptor site within exon 4 resulting in loss of transcript containing the sequence of exons 1-5 which was demonstrated using RT-PCR. In family N5, a transcript in which exon 4 was skipped was also identified.

The missense mutations involved highly conserved residues that are invariant in *Rattus norvegicus, Mus musculus, Gallus gallus* and *Xenopus tropicalis* suggesting that they are critical to the normal function of the protein. Furthermore at CIMR, they modelled the effects of these mutations on the three dimensional structure of the protein by mapping

them onto the closest ortholog of known structure: the core domain of the protein 4.1R. The crystal structure extends from residues 1-279 and this is the region were most of the missense mutations are present. It was found that the helical domain of the wild-type structure is disrupted with these mutations.

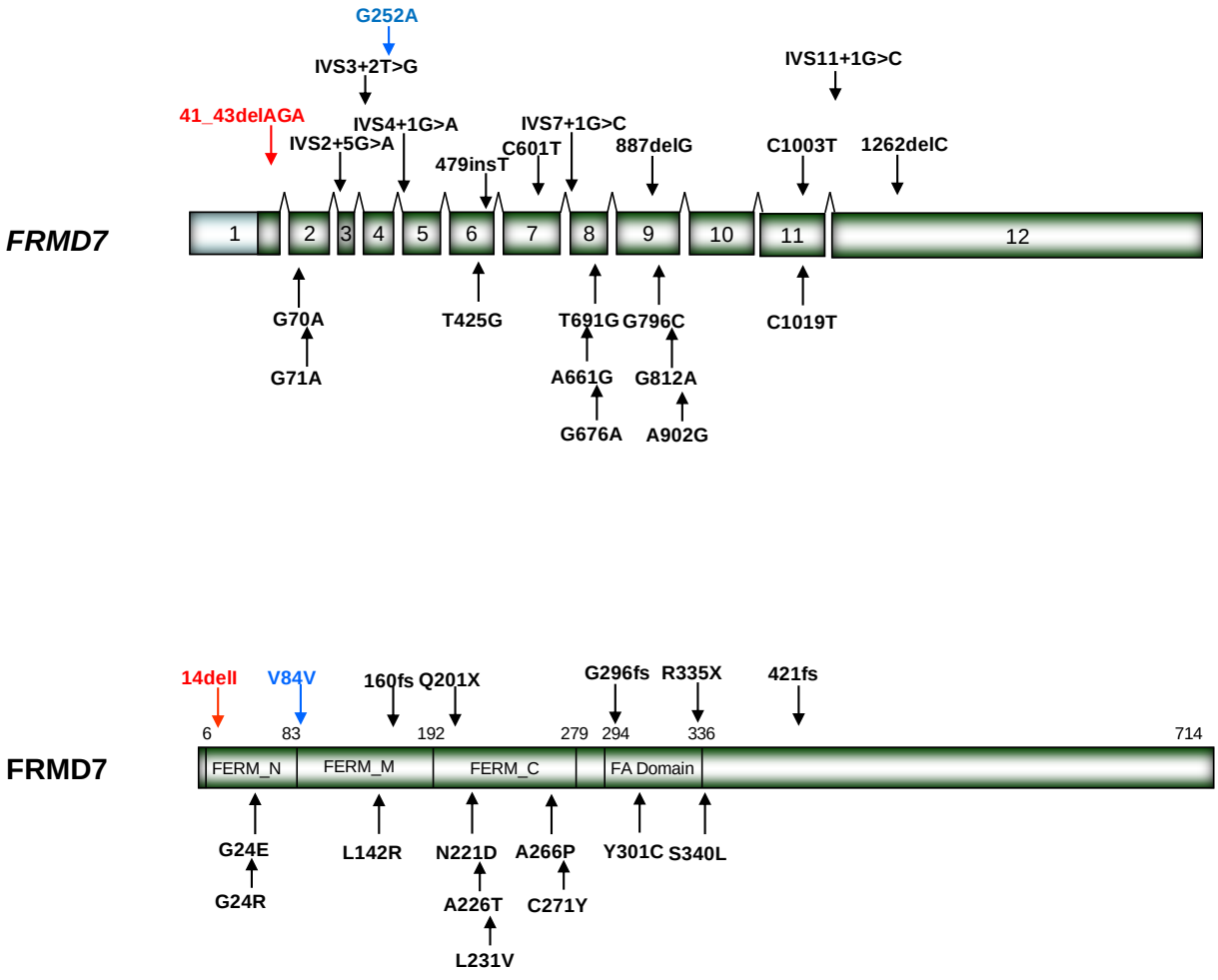
A schematic representation of the gene, protein and the mutations detected in this study are shown in Figure 3L.03

Table 3L.01: Shows the mutations detected in FRMD7.

The family number, sequence change, amino acid change, the class of mutation and the ethnicity of the family are shown. Family N1 and F7 have the same mutation. Also Family N10 and F6 share the same mutation.

Family	Sequence change	Amino acid change	Class	Ethnicity
N1	IVS4+1G>A		Truncating	England
N2	C601T	Q201X	Truncating	Italy-Germany
N3	T691G	L231V	Missense	Italy-Germany
N4	IVS3+2 T>G		Truncating	England
N5	G252A	V84V	Silent	England
N6	G796C	A266P	Missense	England
N7	IVS2+5G>A		Truncating	England
N9	IVS11+1G>C		Truncating	Germany
N10	C1003T	R335X	Truncating	England
N11	G812A	C271Y	Missense	Scotland
N12	A902G	Y301C	Missense	England
N13	IVS7+1G>C		Truncating	Madagascar
N14	887delG	G296fs	Truncating	Austria
N15	G70A	G24R	Missense	Ireland
N16	T425G	L142R	Missense	Ireland
F1	A661G	N221D	Missense	England
F2	G676A	A226T	Missense	England
F4	C1019T	S340L	Missense	Romania
F5	G71A	G24E	Missense	Austria
F6	C1003T	R335X	Truncating	India
F7	IVS4+1G>A		Truncating	England
F8	479insT	160fs	Truncating	England
F9	41_43delAGA	14delI	Inframe deletion	England
Singleton	1262delC	421fs	Truncatiing	England
Singleton	10003C>T	R335X	Truncating	England
Singleton	G796C	A266P	Missense	England

***Figure 3L.03**: **A schematic representation of the mutations in FRMD7**. **Upper panel:** The 12 exons and the introns are shown. Non-coding region of exon1 is lightly shaded. **Lower panel:** Diagram of the protein* FRMD7*, with its functional domains. Missense mutations are shown below the diagram of the gene/protein. Mutations predicted to cause a truncated protein is shown in black above the diagram of the gene/protein. In-frame deletion and a silence mutation in two families are shown in red and blue colour respectively.*



3.2.6 FERM domain containing 7 (*FRMD7*)

The FRMD7 gene is approximately 51 kb in length and consists of 12 exons. It encodes a 714-residue protein which has a FERM domain at the N terminal end.

In-situ hybridization experiments in human embryonic brain have shown that FRMD7 is expressed in the developing neuro-retina and in the areas of the brain involved in ocular motor control.[205] It is also expressed in low levels in various human adult tissues such as liver, kidney and pancreas.

FRMD7 has close homology with FARP1 and FARP2 which is concentrated at the N terminal end. FARP2 modulates the branching and growth of neuritis in rat embryonic cortical neurons.[205, 206] Whether the function of FRMD7 is similar to that of FARP2 is not known.

FARP1 (Chr13) has been implicated in the regulation of microfilament organization.[207] Interestingly fine mapping of the family described in locus NYS4 restrict the locus to a region between makers D13S1300 (start 91409148; end 91409485) and D13S158 (start 102774398; end 102774526) and this locus falls in the vicinity of FARP1 (at chromosome 13:97593435-97695559).

3.2.7 Summary of Molecular Genetic Work

DNA extraction, genotyping with microsatellite markers and linkage analysis were done by the author at Leicester. Sequencing of the genes, confirmation of the pathogenicity of the mutations through RT-PCR and in-silico analysis were done by Dr LR and Dr PT at CIMR and the Sanger Institute.

Molecular genetic work began with extraction of DNA from 149 subjects and 218 unaffected relatives from 39 families, and another 79 sporadic subjects with IIN. Thirteen microsatellite markers from across the previously identified minimal region of X-chromosome (locus NYS1) were genotyped for 255 individuals from 26 families and this was done in three steps.

Linkage analysis in 16 families using *'MERLIN'* gave high LOD scores across the region for all markers which confirmed linkage. This is in agreement with the work done by Kerrison et.al in 2004. [201] LOD score at all the selected markers scored above 4.0; however the highest LOD score of 12.449 was obtained for marker DXS8072 at chromosome position 130.225.

After confirming the locus through linkage analysis, the haplotype data was used to refine the genetic interval further. Data from family N1 has recombination events which refined the centromeric and telomeric boundaries of the shared haplotype at locus NYS1. The new genetic interval (9 mB region) was defined by markers DXS8072 and DXS8094.

This region contained about 80 genes. A collaboration was set up with Dr F Lucy Raymond, who leads a study of genes associated with X-linked mental retardation at the Cambridge

Institute of Medical Research to carry out high throughput sequencing of the genes in the candidate region.

All coding exons and the splice junctions of fourty genes in the centromeric half of this 9mB region were sequenced in one affected male individual from 16 families and this led to identification of mutations in a gene annotated as a hypothetical protein LOC90612. This gene was since renamed *FRMD7*.

The *FRMD7* gene is approximately 51 kb in length and consists of 12 exons. It encodes a 714-residue protein which has a FERM domain at the N terminal end.

In-situ hybridization experiments in human embryonic brain have shown that FRMD7 is expressed in the developing neuro-retina and in the areas of the brain involved in ocular motor control.

The function of *FRMD7* is not known; however, *FARP2* (which has close homology to *FRMD7*), a guanine nucleotide exchange factor for Rho family of GTPase, modulates the branching and growth of neuritis in rat embryonic cortical neurons.

3.3 Phenotype-Genotype Correlation

Phenotype-genotype correlation was done by the author.

The main objective of this chapter is to compare the phenotype of subjects with IIN associated with mutations in *FRMD7* to those subjects with IIN not associated with *FRMD7*. In addition, the author compares the clinical features and the penetrance of *FRMD7* related IIN, in families with truncating and missense mutations in *FRMD7.*

3.3.1 IIN- *FRMD7* vs. non-*FRMD7*

We identified 90 subjects with IIN due to mutations in *FRMD7* (mean age 36 years and range 3–88 years). Of these 90 subjects in the *FRMD7* group, 88 had familial nystagmus whilst two were sporadic. The phenotypes of these subjects were compared to 48 subjects with IIN not caused by mutations of this gene (mean age 29 years and range 4–79 years). Of the 48 subjects in the non-*FRMD7* group, 33 were sporadic, whilst the remaining 15 had at least two affected members in the family. Affected males and females were compared to see if there were any differences in clinical features and eye movements. Eye movement recordings were obtained in 52 affected subjects in the *FRMD7* group and 29 affected subjects in the non-*FRMD7* group.

We also evaluated 27 obligate female carriers of *FRMD7* mutations who were clinically unaffected comparing them to age matched healthy controls. In addition to the clinical data, eye movement recording were analysed in a subgroup of these unaffected carriers (n = 14), in particular to look for differences in smooth pursuit eye movements and optokinetic nystagmus (OKN).

3.3.1.1 Visual Acuity and Colour Vision

In the *FRMD7* group, the visual acuity was tested in 83 of the 90 subjects. (We could not obtain accurate visual acuity measurements in seven children). Most of the subjects with mutations in *FRMD7* had visual acuities better than logMAR 0.301 (Snellen equivalent 6/12) (Figure 3P.1, top panel). The median visual acuity of this group was logMAR 0.176 (6/9) with upper and lower quartiles of logMAR 0.301 (6/12) and logMAR 0.097 (6/7.5), respectively.

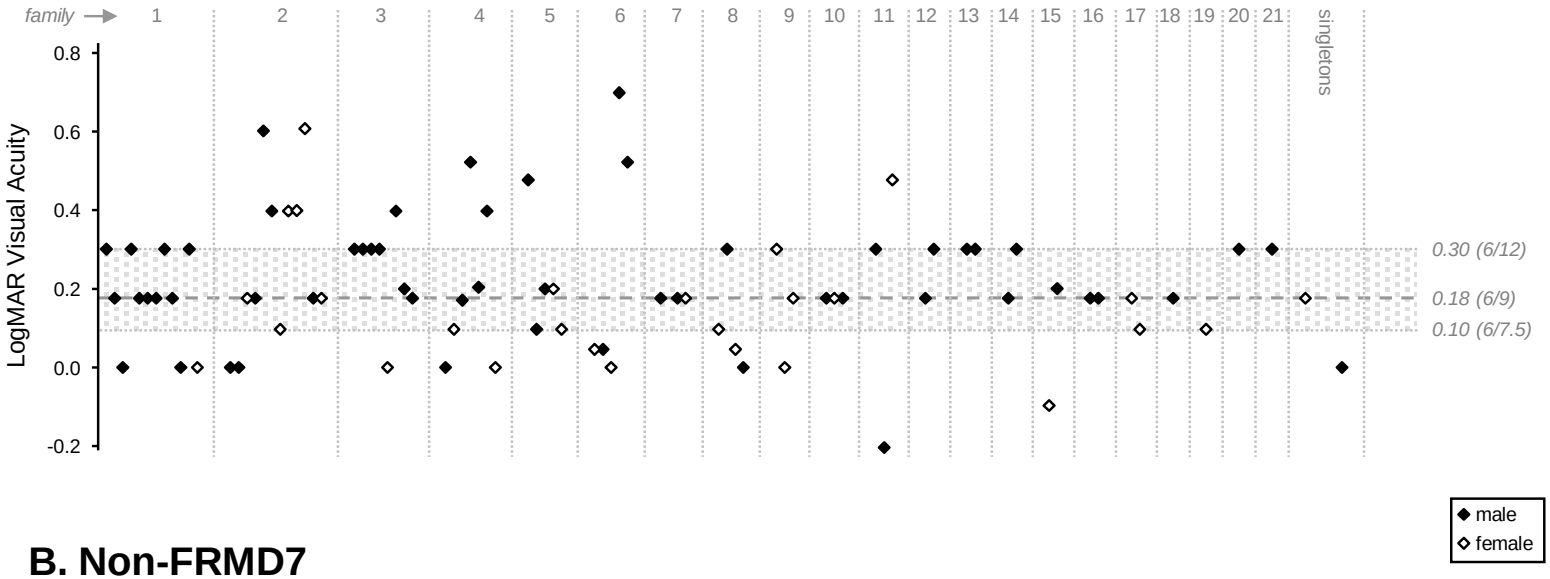
In the non-*FRMD7* group, visual acuity was obtained in 45 of the 48 subjects, the data not being available in three children. (Figure 3P.1, bottom panel). The visual acuity distribution was similar to the *FRMD7* group, the median being logMAR 0.176 (6/9) with upper and lower quartiles of logMAR 0.301 (6/12) and 0.0 (6/6) respectively. Mutations in the *FRMD7* gene did not have any significant effect on visual acuity of patients with IIN (Mann-Whitney U test p= 0.143).

In the *FRMD7* group, there was a mild but significant difference in visual acuity between affected males and females, the visual acuity being better in females (Mann-Whitney U test, p=0.014: median = 0.098 in females and 0.188 in males). In contrast, there were no significant differences between males and females in the non-*FRMD7* group (Mann-Whitney U test, p=0.36: median = 0.176 in males and females).

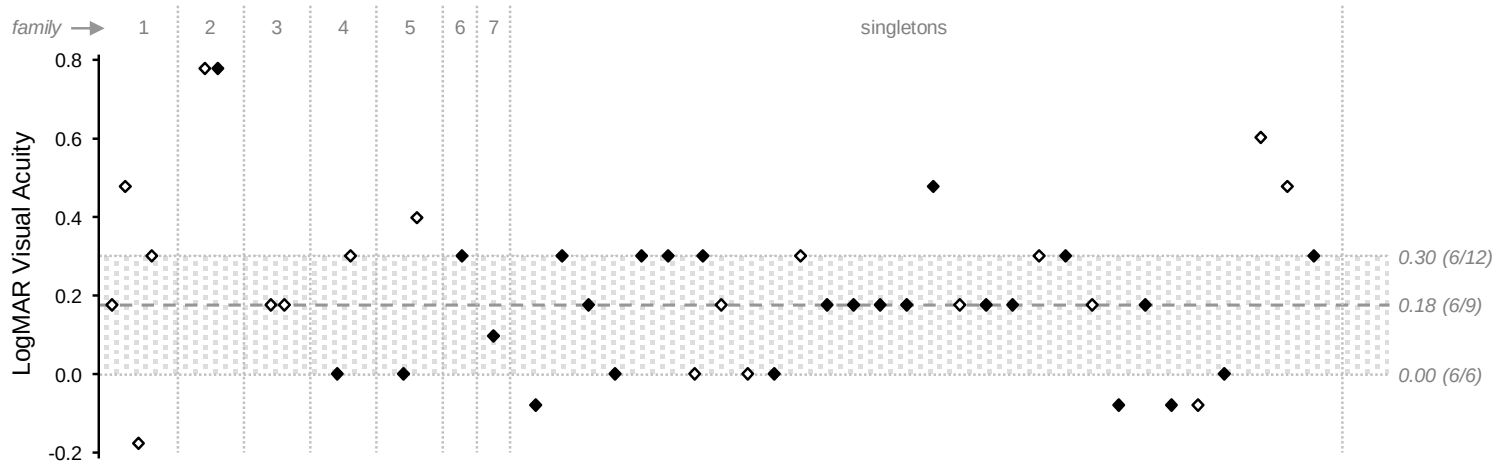
Colour vision was normal in all the subjects from both groups.

Figure 3P.1: Visual acuity (Log MAR on the left and the Snellen equivalent on the right) plotted on the Y-axis against the individual subjects on the x-axis. *The top panel shows the subjects with mutation in FRMD7 grouped into 21 different families (left side of panel) and 2 singletons (right side of panel. As shown, the median log MAR visual acuity is 0.176. The bottom panel shows the subjects with no mutations in the above gene (grouped in 7 families on the left side and 31 singletons on the right side). The median visual acuity is 0.176. The males are represented with filled diamonds and females with open diamonds. The shaded area represents the data between the upper and lower quartiles.*

A. FRMD7



B. Non-FRMD7



3.3.1.2 Stereopsis

Of the 90 subjects in the *FRMD7* group, 76 were tested for stereo-visual acuity. Most of the subjects (93.4%) had good binocular vision demonstrable on Lang test. 71 subjects who were Lang positive were examined using the Frisby test and were found to have a median stereopsis of 150". Of the 5 subjects who were Lang negative, 4 were Bagolini positive. The Bagolini negative subject had alternating esotropia. Six subjects with manifest strabismus detected on cover test were found to have binocular vision on Bagolini test.

In the non-*FRMD7* group, binocular vision was evaluated in 37 of the 48 subjects. 29 subjects (78.4%) were Lang positive and their median stereopsis on Frisby test was also 150". Eight subjects were Lang negative, of which two were Bagolini positive. Pearson Chi-square test was used to compare the relative proportions of subjects who were Lang positive in the *FRMD7* and non-*FRMD7* groups and it showed a significantly higher proportion in the *FRMD7* group (p=0.019).

3.3.1.3 Strabismus

Strabismus was detected in 7 of the 90 subjects (7.8%) in the *FRMD7* group. Three subjects had esotropia and three exotropia; one subject had left hypertropia. Six of these seven subjects with manifest strabismus had at least gross binocular vision (Bagolini positive). In the non-*FRMD7* group, 5 of 48 (10.4%) had manifest strabismus, all of them having esotropia. (Pearson Chi-square test between the two groups, p=0.61).

3.3.1.4 Latent Nystagmus

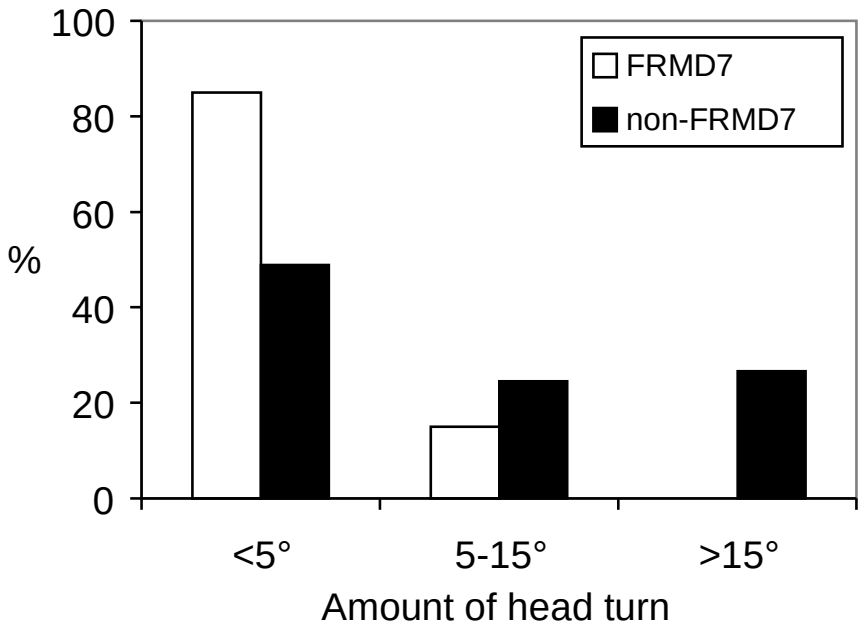
None of the subjects from the *FRMD7* group had latent nystagmus. However, one subject with strabismus in the non-*FRMD7* group was found to have a latent component to the nystagmus.

3.3.1.5 Anomalous Head Posture (AHP)

Head posture was recorded in 80 subjects in the *FRMD7* group (Figure 3P.2). 68 of the 80 subjects (85%) did not have significant anomalous head posture, i.e., AHP less than 5°. Twelve subjects (15%) had AHP of 5-15°. None of the subjects in this group had vertical head posture and the horizontal AHP in this group never exceeded 15°. None of these subjects had had surgery for abnormal head posture.

Head posture was recorded in 45 of the 48 subjects in the non-*FRMD7* group. 22 of 45 (49%) did not have significant AHP, i.e.; AHP less than <5° whereas 11 subjects (24 %) had moderate (5-15°) AHP. Twelve of the 45 subjects (27%) had AHP of more than 15° of which 8 subjects had undergone Kestenbaum procedure for AHP in the past. Of these 12 subjects with AHP more than 15°, 4 had vertical head postures (2 chin down and 2 chin up). The comparison of the AHP in the two groups is shown in figure 3. Gamma statistic was used to compare the relative proportions between the groups and showed a highly significant difference between *FRMD7* and non-*FRMD7* groups (p=5.7x10^{-6}).

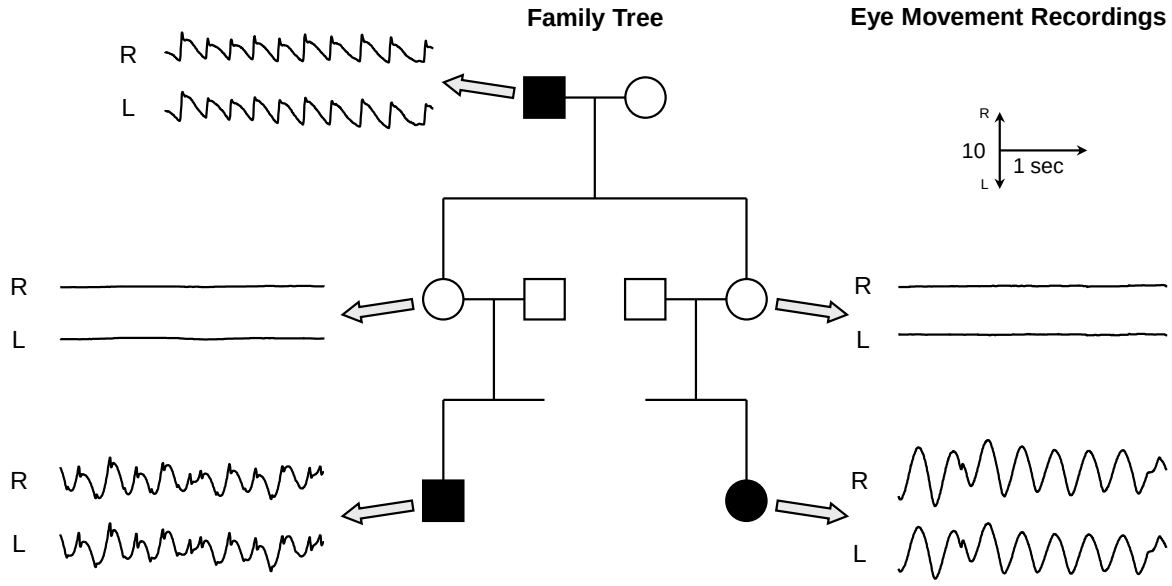
Figure 3P.2: Degree of head turn in subjects in the FRMD7 and non-FRMD7 groups. *X-axis shows the three subgroups with different degrees of abnormal head position (AHP); the Y-axis the percentage of subjects. Open bars represent FRMD7 and the filled bars denote the non- FRMD7 group.*



3.3.1.6 Nystagmus Form and Eye Movement Recording

In the group with mutations in *FRMD7*, all subjects had conjugate horizontal nystagmus as well as most of the subjects in the non-*FRMD7* group with the exception of three subjects who had conjugate vertical nystagmus. The amplitude, frequency and waveform of nystagmus varied considerably within families in both groups. Original eye movement recordings of subjects from a family which is representative of the *FRMD7* group is shown in Figure 3P.3.

Figure 3P.3: Representative original eye movement recordings of 5 subjects from a family with FRMD7 mutation, plotted with the family tree. *Three affected subjects have different waveforms whilst the carriers are unaffected.*



The author evaluated the amplitude and frequency of nystagmus in three positions of gaze and noted the waveform of nystagmus in these positions.

3.3.1.7 Amplitude of nystagmus

There was considerable intra-family and inter-family variability in the amplitude of nystagmus. The amplitude of nystagmus in 15° left gaze, primary position and 15° right gaze of the subjects are shown in Figure 3P.4A. The mean amplitude (±SD) of nystagmus in primary position for males in the *FRMD7* group was 3.86° (±3.52°), while the mean amplitude in the females was 3.27° (±2.44°). In the *FRMD7* group there was a reduction of amplitude in primary position of gaze with increase of amplitude in 15°gaze to the right

and left while no significant differences in amplitudes between primary position of gaze and eccentric position of gaze was found for the non-*FRMD7* group (Figure 3P.4 C). In the non-*FRMD7* group the mean amplitude of nystagmus in primary position was 5.76° (±3.89°).

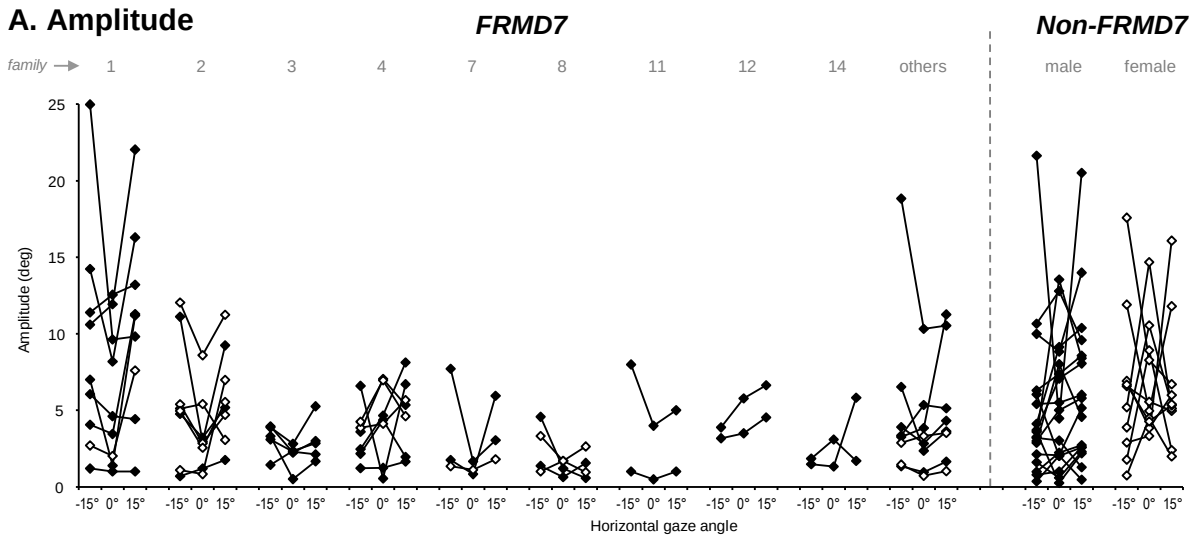
In the *FRMD7* group, gender (F=0.03, p=0.87) and family (F=1.72, p=0.09) were not significant predictors of log nystagmus amplitude whereas eccentricity (F=11.6, p=2.9x10^{-5}) was highly significant. In contrast, eccentricity (F=0.18, p=0.83) was not a significant predictor of log amplitude in the non-*FRMD7* group. Gender (F=3.35 , p=0.07) was also not a significant predictor of log amplitude in the non-*FRMD7* group. The effect of eccentricity on amplitude can be clearly seen in Figure 3P.4C where a marked reduction in the mean log amplitude is evident at primary position (0°) in the *FRMD7* group but not in the non-*FRMD7* group. Consequently, there was a significant difference in log amplitude between *FRMD7* and non-*FRMD7* groups at primary position (F=7.29, p=0.009) but not at –15° (F=0.14, p=0.70) or 15° (F=0.55, p=0.45) eccentricity.

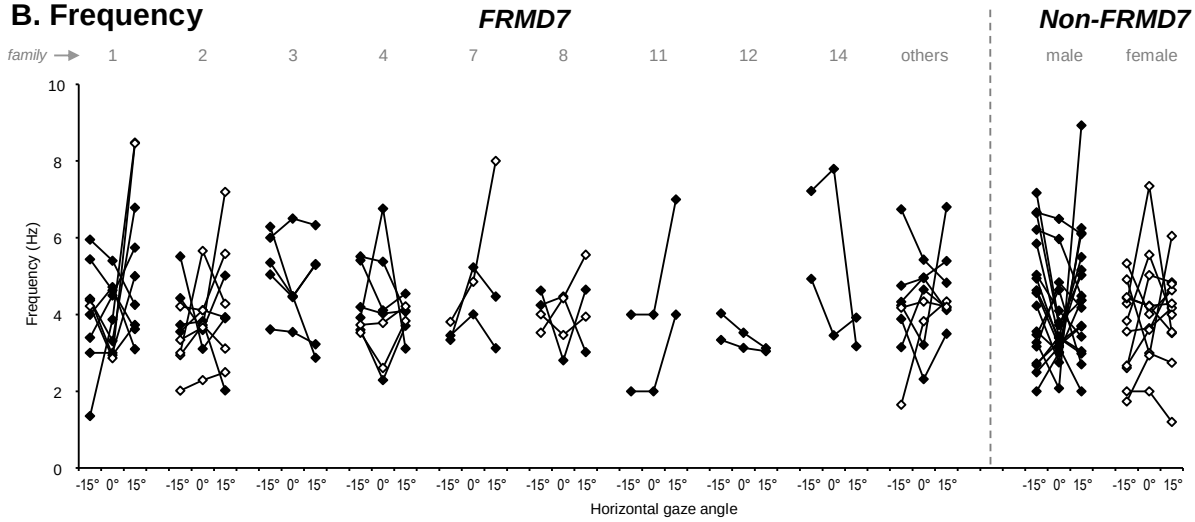
3.3.1.8 Frequency of nystagmus

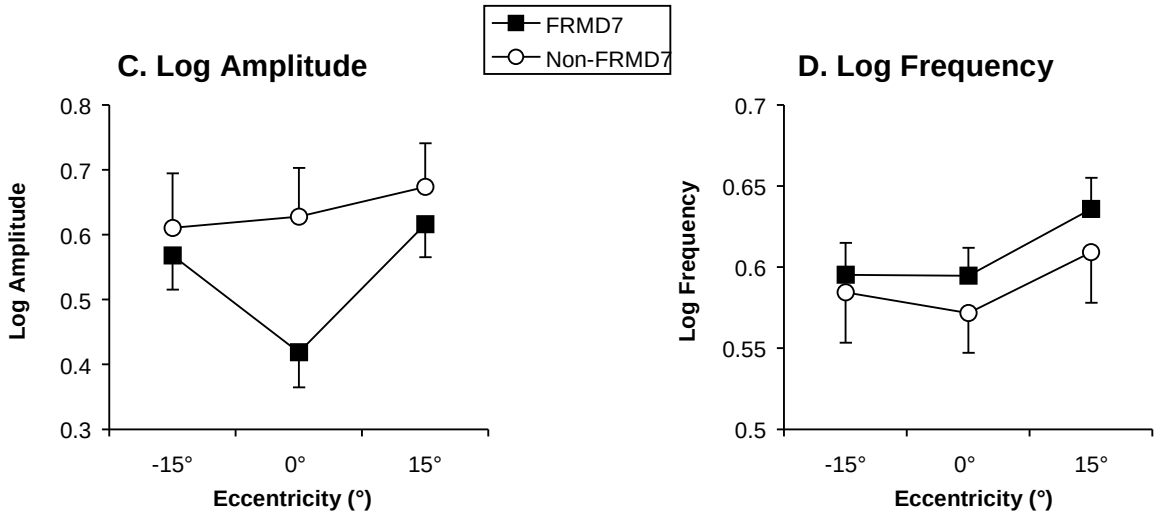
The mean frequency of nystagmus in primary position was 4.08Hz (±1.16) for the *FRMD7* group and 3.89Hz (±1.24) for non-*FRMD7* group. Mean log of the frequency showed similar patterns in both groups (Figure 3P.4D). Eccentricity was not a significant predictor for log nystagmus frequency in either group (F=1.86, p=0.16 for *FRMD7* and F=0.44, p=0.65 for non-*FRMD7*) (Fig 4B). Log frequency of nystagmus was not dependant on

gender (F=0.007, p=0.93 for *FRMD7* and F=1.11, p=0.30 for non-*FRMD7*). There was no significant difference between the log frequencies of the *FRMD7* group and non-*FRMD7* group overall (*F*=0.73, *p*=0.39).

Figure 3P.4: (A) Amplitude and (B) Frequency of nystagmus in degrees (Y-axis) plotted against the horizontal gaze angle on the X-axis. (–15°=15° left gaze; 0= primary position and +15°= 15° right gaze). *Fourteen larger families in the FRMD7 group are represented separately, while the smaller families are grouped together as 'others'. The non-FRMD7 group is shown on the right hand side of the figure. The subjects are sub-grouped into males and females. The males are represented with dark diamonds whilst the females are represented with open white diamonds.* ***(C)*** *Logarithm of the mean amplitude (±SEM) and* ***(D)*** *mean frequency (±SEM) of nystagmus in the FRMD7 group and the non-FRMD7 group in the three positions of gaze (-15°, 0 and +15°). The FRMD7 group is represented with black squares and the non-FRMD7 group is shown in white circles. The mean amplitude is significantly lower in primary position in the FRMD7 group.*


A. Amplitude
FRMD7
Non-FRMD7
family
1
2
3
4
7
8
11
12
14
others
male
female
Amplitude (deg)
25
20
15
10
5
0
-15° 0° 15°
Horizontal gaze angle

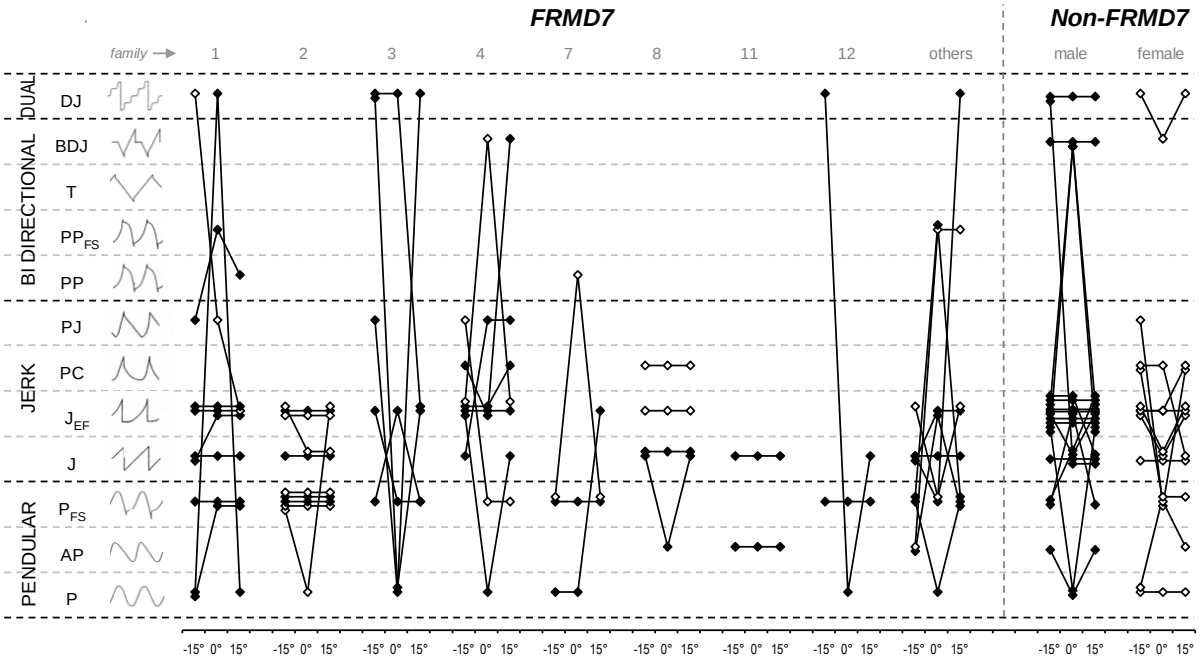

B. Frequency
FRMD7
Non-FRMD7
family
1
2
3
4
7
8
11
12
14
others
male
female
Frequency (Hz)
10
8
6
4
2
0
-15° 0° 15°
Horizontal gaze angle


FRMD7
Non-FRMD7
C. Log Amplitude
Log Amplitude
0.8
0.7
0.6
0.5
0.4
0.3
-15°
0°
15°
Eccentricity (°)
D. Log Frequency
Log Frequency
0.7
0.65
0.6
0.55
0.5
-15°
0°
15°
Eccentricity (°)

3.3.1.9 Waveform characteristics

The waveforms of nystagmus were compared with the 12 forms described by Dell'Osso et al.[208] (Figure 3P.5). The waveforms varied with direction of gaze with some degree of intra- and interfamily difference. The frequency of pendular and jerk nystagmus was compared between the groups using the Pearson Chi-square test. Pendular related waveforms were more commonly associated with the *FRMD7* group (p=0.0025; pendular waveforms account for 45.3% in *FRMD7* group and 28.3% in the non-*FRMD7* group).

Figure 3P.5: The waveforms of nystagmus are shown according to the 12 waveforms described by Dell'Osso et al. 1975. *The three positions of gaze (–15°=15° left gaze; 0= primary position and +15°= 15° right gaze) are shown on the X-axis. Y-axis shows the waveform of nystagmus. P=pendular, AP=asymmetric pendular, P_{FS}=Pendular with foveating saccades, J=Jerk, J_{EF}=Jerk with extended foveation, PC=Pseudo cycloid, PJ=Pseudo jerk, PP= Pseudo pendular, PP_{FS}= Pseudo pendular with foveating saccades, T=Triangular, BDJ= Bi-directional Jerk, DJ=Dual Jerk). A diagrammatic representation of each waveform is shown at the left of the figure. FRMD7 is sub-grouped into 12 families with the males shown in dark diamonds and females in white open diamonds. The non-FRMD7 group is sub-classified into males and females as most of them were non-familial.*



3.3.2 Comparison of phenotype between Missense and Truncating mutations

We compared the phenotypical features of the affected subjects based on the type of mutation. Fifty-six subjects with nystagmus had a mutation predicted to result in a truncated protein whereas fourty-six patients had a missense mutation. However we do not have data on all clinical features in every subject. The number of subjects in each family and the type of mutation are shown in Table 3P.01. The details of each mutation is given in Table 3L.01

Table 3P.01: *The number of affected subjects (n) in each family and the type of mutation is given in this table. Details of each mutation is given in table 3L.01 (in section 3.2.5)*

Family	Mutation type	n
N1	Truncating	14
N2	Truncating	7
N3	Missense	9
N4	Truncating	5
N5	Missense	2
N6	Truncating	4
N7	Truncating	3
N9	Truncating	3
N10	Truncating	3
N11	Missense	12
N12	Missense	5
N13	Truncating	6
N14	Truncating	4
N15	Missense	5
N16	Missense	4
F1	Missense	2
F2	Missense	3
F4	Missense	2
F5	Missense	2
F6	Truncating	2
F7	Truncating	2
F8	Truncating	3

3.3.2.1 Clinical Features

Visual acuity

There was no significant difference in the visual acuity between the two groups. The median logMAR visual acuity in both groups were 0.18 with upper and lower quartiles of 0.09 and 0.30. (Mann-Whittney U test- p=0.464).

Stereopsis

Two of 36 subjects with a truncating mutation and three of 31 subjects with a missence mutation were Lang negative. Pearson Chi square test was used to compare the relative proportion of subjects who were Lang positive in each group. There was no significant difference in the degree of binocular vision between the two groups. (p= 0.522)

Strabismus

Four of 42 subjects with a truncating mutation had manifest strabismus (two with exotropia and two with esotropia). Three of 34 subjects in the group with missense mutation had manifest strabismus (one with esotropia, one exotropia and one hypertropia). There was no significant difference between the groups. (Pearson Chi Square test- p= 0.916).

Anomalous Head Posture (AHP)

Thirty-two subjects with a truncating mutation had no significant AHP while ten had moderate AHP. In the group with missense mutation, twenty-nine subjects had no

significant AHP and two subjects had moderate AHP. These difference between the two groups were statistically significant. (Pearson Chi Square test- p= 0.048). However if we exclude family N13, which was phenotyped by a clinical collaborator, the difference between the two groups become less significant (Pearson Chi Square test- p= 0.505). Even though same methods were used to assess the degree of head posture, observer bias could not be ruled out in assessing the degree of AHP.

Amplitude and Frequency of Nystagmus

The mean log amplitude of nystagmus in primary position was 0.45° for the group with truncating mutation and 0.38° for the group with missense mutation. (p= 0.669). In both groups eccentricity was significantly related to the amplitude of nystagmus, amplitude of nystagmus being minimal at primary position of gaze. (p= 8.64 x 10^{-5}).

The log frequency of nystagmus was similar in both the groups in all positions of gaze. Eccentricity was not a significant predictor for the frequency of nystagmus, the mean log frequency being 0.61 Hz in the group with truncating mutations and 0.60 Hz in the group with missense mutations. (p= 0.313).

3.3.3 Obligate Female Carriers of a *FRMD7* Mutation

The criteria used to define an unaffected obligate carrier were

1. Unaffected daughter of an affected male subject
2. Unaffected mother of an affected child if there is family history of IIN in the previous generation
3. Unaffected mother of an affected child who has a sister with affected children (e.g. lab number 388 and 387 in family N12)
4. Unaffected mother or grand mom of an affected person in whom sequencing data is present (lab number 7671 and 476 in family F9)

As mentioned in section 3.3, the only significant difference between the affected females and males was the slightly better visual acuity in the females. The nystagmus characteristics (amplitude, frequency and the waveform) were similar in both the groups (affected females and affected males).

3.3.3.1 Penetrance of the disease in Obligate Female Carriers

Sixty nine obligate female carriers were identified in the study, of which 36 (52.17%) were affected clinically.

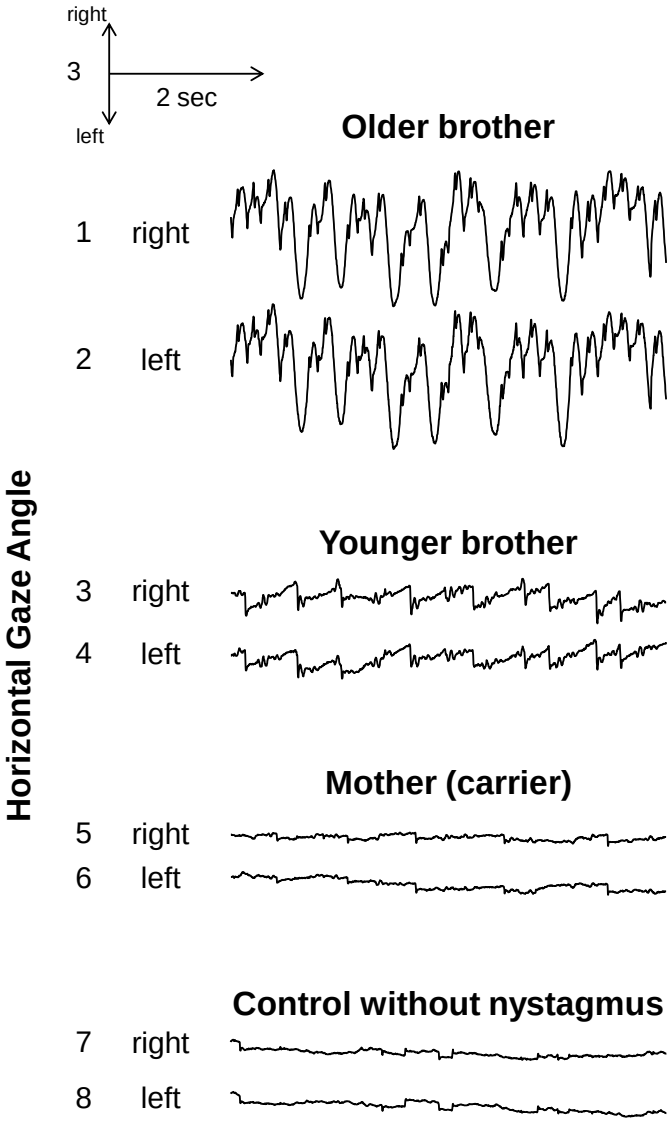
There were thirty nine obligate female carriers with a truncating mutation of which 19 were affected clinically. There were 30 subjects in the group with a missense mutation, of which 17 were affected clinically. The penetrance of the disease in obligate female carriers with a truncating mutation was 48.72% compared to 56.67% in the group with a missense

mutation. This difference was not statistically significant. (Pearson Chi Square test- *p*= 0.474).

3.3.3.2 Comparison between Unaffected Carriers and Normal Controls

Eye movement recordings were obtained from 14 clinically unaffected female obligate carriers of a *FRMD7* mutation. Their mean age was 53.2 (range= 29-81). They were compared to 20 age-matched female control subjects who did not have any ophthalmological disease and were not related to the subjects with nystagmus. They had normal Snellen visual acuity (mean = 6/5). None of the carriers or controls had any strabismus or abnormal head posture. All of the carriers had normal stereo-acuity (Lang test). Eye movement recording of the clinically unaffected carriers revealed that one of them had sub-clinical nystagmus (amplitude 0.43° and frequency 2.86 Hz) as shown in Figure 3P.6

Figure 3P.6: Infra red eye movement recordings form three subjects from a family (mother and two children) showing the variability of nystagmus. *The older brother has horizontal nystagmus of ~5 degrees (traces 1 and 2), the younger brother has small amplitude nystagmus (~ 1.5 degrees, traces 3 and 4). Fine subclinical nystagmus with slow rightward drifts and fast beats to the left of the mother who is a carrier (~0.5 degrees nystagmus) is shown in traces 5 and 6). Traces 7 and 8 depict eye movement recordings from a control subject without nystagmus. Small oscillations can be seen; these are square wave-jerks and are normal.*



Analysis of optokinetic nystagmus in the unaffected obligate carriers showed a bimodal distribution, i.e.; some of the carriers had good OKN gain whilst the others had poor responses to optokinetic stimulus in most directions. An example of original eye movement recordings of OKN of a carrier and a control subject is shown in Figure 3P.7. This difference was significant for rightward (Mann Whitney U, *p=0.03*) and downward (*p= 0.002*) movement of the stimulus as shown in Figure 3P.8A. The author also analysed the horizontal and vertical smooth pursuit eye movements in clinically unaffected carriers and

controls. There was no significant difference between the groups (p>0.05) in the smooth pursuit gain and the number of catch-up saccades/second as shown in Figure 3P.8B.

Figure 3P.7: *Original eye movement recording of a 33 year old obligate carrier (upper panel) and that of a 32 year old control (lower panel) which shows the optokinetic nystagmus (OKN) response to a target moving at 20°/second in four directions. The OKN gain is poor in the carrier compared to that in the control.*

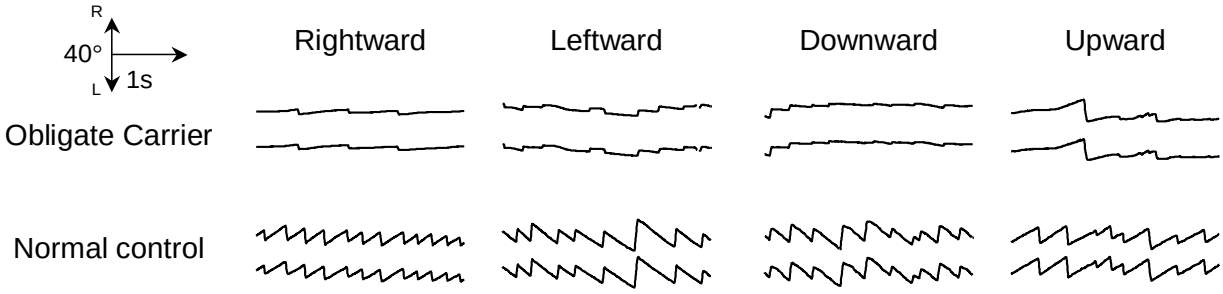
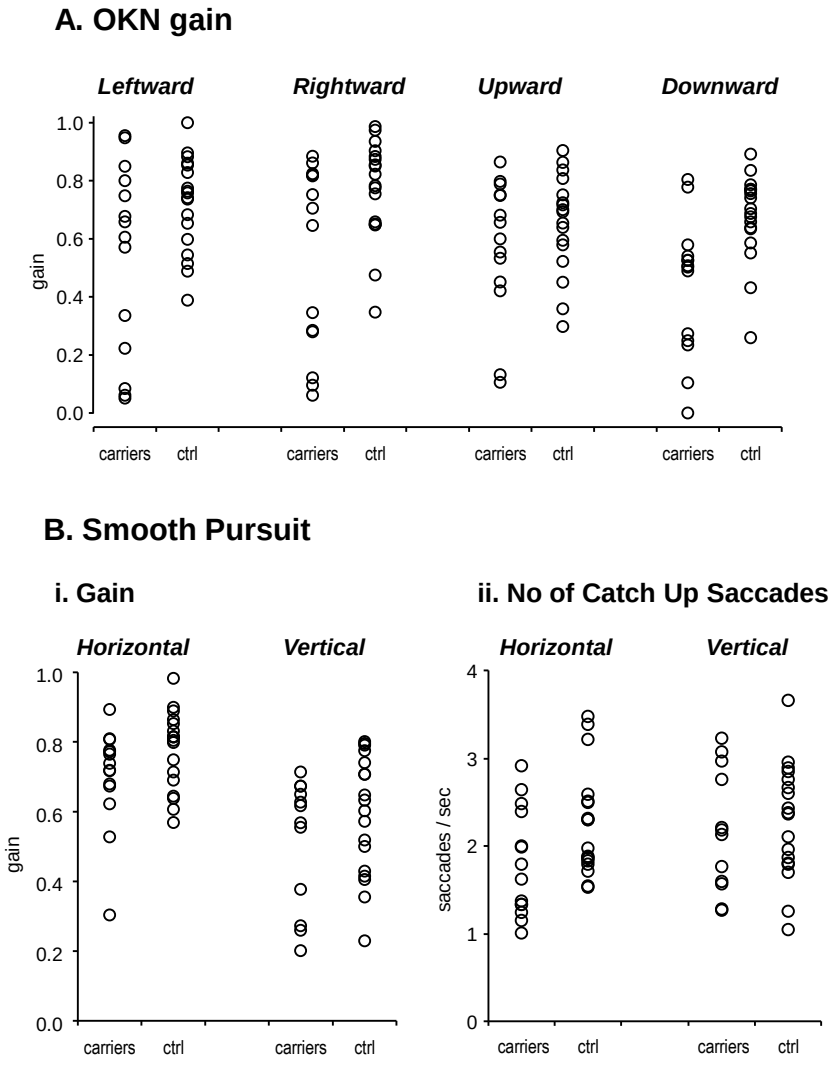


Figure 3P.8: (A) Optokinetic nystagmus gain of the obligate carriers and controls in the four directions of movement of the stimulus (leftward, rightward, upward and downward) moving at 20°/s. *X-axis shows the two groups (carriers and controls) whist the Y-axis depicts the OKN gain. OKN gain shows a bimodal distribution in the carriers especially in the leftward, rightward and downward gazes.* ***(B)(i) Horizontal and vertical smooth pursuit gain in the obligate carriers and controls to stimuli moving at a linear velocity of 20°/s.*** *X-axis shows the two groups (carriers and controls) and the Y-axis shows the smooth pursuit gain. In* ***(ii)*** *the number of catch up saccades per second is shown on the Y-axis.*



4 Discussion

4.1 Mutations in *FRMD7* – Major cause of X-linked IIN

Fifteen of the sixteen linked families and eight of the ten smaller families in whom haplotype analysis had been performed were found to have mutations in *FRMD7*. This would suggest that mutations in *FRMD7* is the major cause of familial X-linked IIN. This was confirmed by other recent studies.

Schorderet et.al found mutations in *FRMD7* in five of six families with X-linked IIN which confirmed the role of this gene in the pathogenesis of X-linked IIN. One of these families was from Switzerland (family A), two were of German origin (families B and C) and two were of Caucasian origin living in USA (families D and E). The clinical features of subjects were similar to those described in this thesis. The Swiss family (family A) harboured a missense mutation (c.673T>G) in exon 8 resulting in the hemizygous replacement of tryptophan at position 225 by glycine (p.W225G). In family B, a nonsense mutation in exon2 resulted in a stop codon at positon 20. (C58T, Q20X). All the screenced members of Family C had splice site mutation at the 5' region of intron 1 (c.57+5G>A). A missence mutation was observed in subjects from family D (A824C, H275P). In the last family (family E) described in this paper, the splice junction at the 3' region of intron 6 was abolished by mutation at position -2 (c.676-2A>G). They also screened fourteen subjects with sporadic IIN, however none of them were found to have mutations in the gene.[209]

In another study, four different mutations in *FRMD7* were detected in four of fourteen Chinese families with X-linked IIN.[210] Two of these mutations were known and two were novel (C436T, R146W) in exon 6, and (C685T, R229C) in exon 8.

Self et.al identified mutations in *FRMD7* in two of ten families (20%) with apparent X-linked IIN and one of twenty-eight (3.6%) sporadic IIN.[211] This included one novel mutation involving a base insertion ('A') after base 880 designated 880insA, 293fs. This frame shift mutation is predicted to cause a stop codon at amino acid position 301. The other mutation (IVS4 + 1G→A) detected by Self et.al (in an English family and a singleton) is the same as of family N1 in this study.

In another recent study by Zhang B et.al, three mutations were detected in three of three Chinese families with X-linked IIN.[212] Two of these missence mutations were novel (C781G, R261G and G886C, G296R). The third mutation detected in a Chinese family (C1003T, R335X) is reported in this thesis and was found in an English family (N10), a family of Indian origin (F6) and a sporadic subject with IIN.

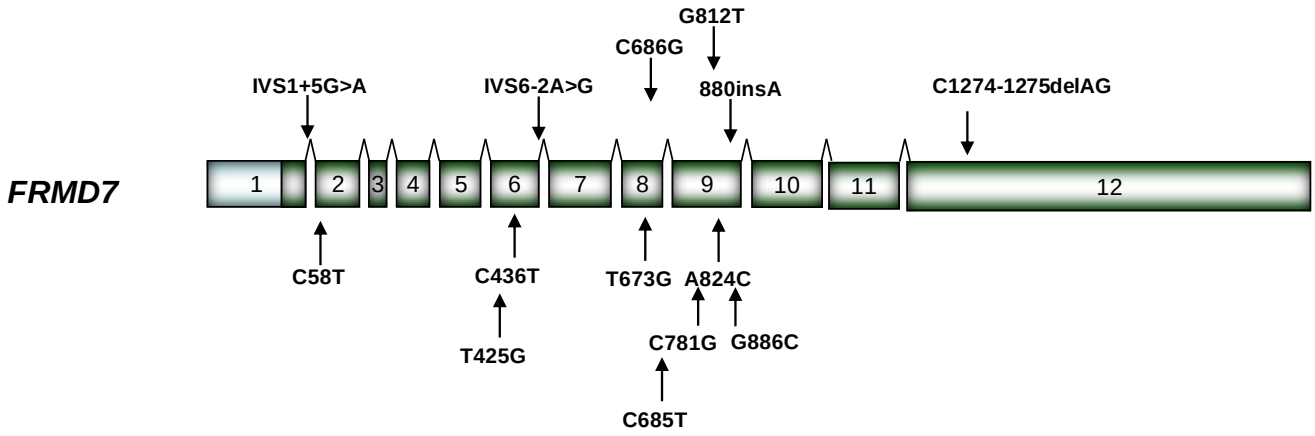
There are a few more recent publications about mutations in *FRMD7* causing nystagmus in families of various ethnic origins. He et.al found a novel mutation in the gene (G812T, C271F) in a large Chinese family with IIN. In addition, a frameshift mutation (1274-1275 delTG, 426fs) was detected in six Chinese pedigrees with X linked IIN by the same author.[213, 214] Kaplan et.al detected a missense mutation (C686G, R229G) in the gene in a large Turkish family.[215] Shiels et.al found another mutation (T425G, L142R) in two unrelated Caucasian American families with X linked IIN.[216] This mutation was present in one of the families of Irish origin (N16) described in this thesis.

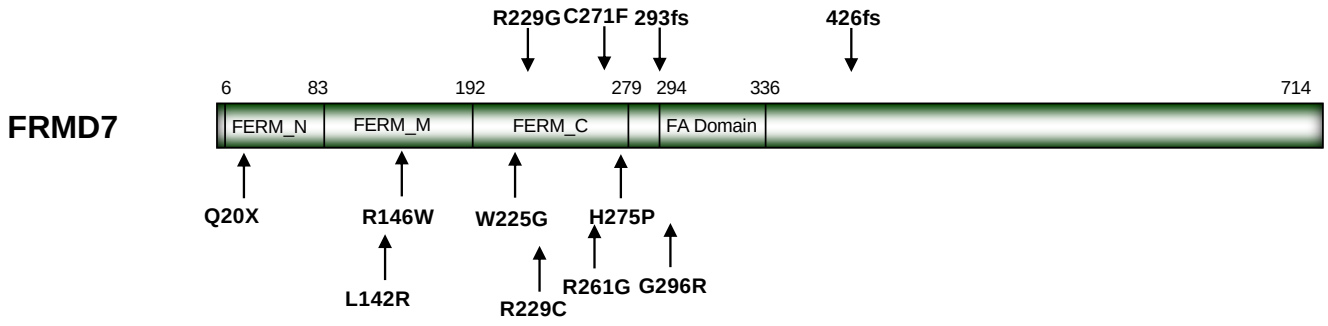
A list of novel mutations detected by other groups are shown in Table 4.1 and Figure 4.1.

Table 4.1: Shows the new mutations detected by other groups after the identification of FRMD7*. The sequence change, amino acid change, class of mutation and the list of authors are shown.*

Number	Identified by	Sequence change	Amino acid change	Class
1	Schorderet et.al	T673G	W225G	Missense
2	Schorderet et.al	C58T	Q20X	Nonsense
3	Schorderet et.al	A824C	H275P	Missense
4	Schorderet et.al	IVS1+5G>A		Truncating
5	Schorderet et.al	IVS6-2A>G		Truncating
6	Zhang B et.al	C781G	R261G	Missense
7	Zhang B et.al	G886C	G296R	Missense
8	Zhang Q et.al	C436T	R146W	Missense
9	Zhang Q et.al	C685T	R229C	Missense
10	Self et.al	880insA	293fs	Truncating
11	He et. al	G812T	C271F	Missense
12	He et. al	1274-1275 delTG	426fs	Truncating
13	Kaplan et.al	C686G	R229G	Missense
14	Shiels et.al	T425G	L142R	Missense

***Figure 4.1**: **A schematic representation of novel mutations in FRMD7 detected in other studies**. **Upper panel:** The 12 exons and the introns are shown. Non-coding region of exon1 is lightly shaded. **Lower panel:** Diagram of the protein* FRMD7*, with its functional domains. The mutations are concentrated around the FERM domains at the N-terminal end.*





4.2 Clinical features of *FRMD7* related IIN

Most of the clinical and eye movement characteristics were similar in the subjects with IIN irrespective of the presence or absence of mutations in *FRMD7*. Vision, stereopsis and ocular alignment were very good with a median visual acuity of logMAR 0.176 (6/9) in both the groups. However, the *FRMD7* group had a significantly lower number of subjects with pronounced head turn and, correspondingly, had relatively smaller nystagmus amplitudes in primary position of gaze. Nystagmus waveforms showed large inter- and intra familiar variation in both the groups. However, pendular waveforms were significantly more common in the *FRMD7* group. Subtle abnormalities in optokinetic nystagmus were found in unaffected carriers.

The visual acuity in both the groups were similar. The median Snellen visual acuity in subjects with IIN was found to be 6/9 and possibly this is because of the careful elimination of subjects with other nystagmus forms such as retinal diseases or albinism which are associated with poorer visual acuity. This study suggests that visual acuity of less than 6/18 (see Figure 3P.1) should raise the suspicion of other underlying disease.

It is interesting to note that patients with the mutations in this gene have good visual acuity, suggesting that the retinal function is not severely affected. However, retinal involvement causative to nystagmus cannot be ruled out as it was evident from *in-situ* hybridization studies that *FRMD7* is expressed in the retina as well as other parts of the central nervous system. The cerebellum has been associated with the neural integrator[217] and a number of acquired nystagmus forms[218] and therefore it is plausible that cerebellar dysfunction related to the *FRMD7* gene may be the pathology underlying IIN.

The prevalence of strabismus in general population has been reported as between 3% and 6%. Graham[219] reported that the prevalence of strabismus is 5.66% based on a study done on 4784 children.

4.2% of 1187 children were found to have strabismus in a study done by Chew et al.[220] In a study done in Denmark, the prevalence of strabismus was found to be 3.2 %.[221]

Compared to the prevalence of strabismus in general population, its prevalence in IIN has been reported higher in the previous literature. In a study by Forssman[222] the prevalence of strabismus in IIN is reported to be 16%. He reported that more than one-third of the subjects with IIN had normal or near normal visual acuity. In another study[223] the prevalence of strabismus in IIN was reported to be 17%. Self et al [211] reported that the prevalence of strabismus is 44% in IIN due to mutations in *FRMD7* based on a study which involved 9 affected subjects. In this study, compared to previous studies the prevalence of strabismus was found to be lower in the *FRMD7* group (7.8%) and in the non- *FRMD7* group (10%). These differences could be explained by the fact that patients with *FRMD7* mutations form a homogeneous group. In addition, the lower prevalence of strabismus in our study may be partly attributable to our extensive examination of family members allowing better exclusion of patients with albinism and other eye diseases. Previous studies[223, 224] included mainly infants and children in whom it may have been more difficult to diagnose strabismus in the presence of nystagmus. Hertle et.al[224] in their study, included patients with albinism where strabismus is more common than in IIN. The low incidence of strabismus in this study indicates that despite constant eye oscillations, subjects with IIN have good ability to fuse and so strabismus is only slightly more frequent

compared to the general population.[219, 220] Subjects without associated strabismus had good stereo-acuity in both groups. Only a small insignificant increase in the prevalence of squint was found in the non-*FRMD7* group when compared to the *FRMD7* group. However the *FRMD7* group had good stereovision more frequently (on the Lang test) than the non-*FRMD7* group. This discrepancy could be explained by the observation that small squints are more difficult to measure on cover test and that stereovision might be a more sensitive measure for eye alignment in nystagmus.

None of the subjects in the *FRMD7* group had latent nystagmus. However, one subject with strabismus in the non-*FRMD7* group had a latent component to the nystagmus.

In their study on the clinical features of infantile nystagmus in the first six months of life, Hertle et al.[224] have reported that 19% (5 of 27 subjects) had an anomalous head posture. In a series by Abadi et al. 9 of 16 (at least 53%) subjects (15 patients with IIN and one with albinism) were found to have AHP.[225] However in this study, the prevalence of significant AHP was found to be much less in the *FRMD7* group than the non-*FRMD7* group. This suggests that the null region of the nystagmus is central in subjects with *FRMD7* mutations. However the underlying anatomical basis of the null region characteristic of IIN is poorly understood.

There was significant intra-family and inter-family variability in the amplitude and frequency of nystagmus in both the *FRMD7* and non-*FRMD7* group. Most of the waveforms described by Dell'Osso et al. were found in both groups. This is in accordance with the published literature. Kerrison et al. found that the waveform of nystagmus can differ in members of the same pedigree.[195] Abadi et al. has reported dissimilar waveforms

in monozygotic twins with nystagmus.[226] Hertle et al.[227] published the clinical features of a family with periodic alternating nystagmus. He reported the presence of subclinical nystagmus in one member of the family in addition to the intra-familial variability of nystagmus.

The variable phenotype in subjects with *FRMD7* mutations was not affected by the type of mutation. In this study, there are families with different types of mutations i.e., truncating and missence mutations; however the type of mutation did not predict the severity of clinical presentation. As in many other Mendelian disorders there is a high level of intrafamilial variability of phenotype in our patients. This could probably be explained by the effect of other unlinked modifier genes and/or the effect of environmental factors.

The presence of many nonsense and truncating mutations in the affected families suggests that nystagmus is caused by loss of function of FRMD7; however the mechanism in which the missense mutations affect the function of the protein is not known.

The penetrance of the gene was 53% among the carriers. It was interesting to note that one of the obligate carriers had sub-clinical nystagmus on eye movement recording. X-chromosome inactivation (XCI) could be a reason behind incomplete penetrance of this disease in females. However a study by Self et.al[211] did not find any definitive pattern of X inactivation in unaffected carriers or manifesting females. In another study by Kaplan et.al, skewed XCI was evident in four affected females with FRMD7 related IIN; however, in two affected females the inactivation pattern was random.

The other possible explanation for incomplete penetrance is that it could be a dominant disease with reduced female penetrance due to genetic or environmental factors.

The optokinetic responses were subnormal in a subgroup of obligate carriers and this finding was not age related. However, smooth pursuit gain in obligate carriers and normal controls was similar. Smooth pursuit stimulus causing reversal of slow phase of nystagmus[228] and inversion of optokinetic responses[229] has been reported in patients with congenital nystagmus. In this context, absence of OKN response in these obligate carriers could be considered as a subtle subclinical manifestation of nystagmus. Eye movement recordings in obligate female carriers show a continuum between subjects having large amplitude nystagmus, small amplitude nystagmus, subclinical nystagmus, OKN abnormalities and normal eye movements.

In summary, the clinical and eye movement data of a large homogeneous group of patients with nystagmus due to mutations in *FRMD7* were analysed, comparing them to a group of patients with IIN not due to mutations in this gene. The median visual acuity was 6/9 in both groups and in patients with a visual acuity less than 6/18, suspicion of the nystagmus not being idiopathic should be raised. Good ocular alignment and binocular vision were present in patients with IIN. Interestingly more patients in the non-*FRMD7* group had anomalous head posture. There was wide variation of nystagmus waveforms even within families. Approximately half of the female carriers were clinically affected and some of the unaffected female carriers had subnormal OKN mainly in the horizontal direction. This most likely represents a subclinical affection of the eye movement system. These clinical characteristics can be used to distinguish IIN from other forms of nystagmus, provide guidance as to what further investigations may be helpful, and assist in genetic counselling.

4.3 Families with IIN not associated with mutations in *FRMD7*

As it was discussed elsewhere in this thesis, twenty-three of the thirty families with possible X linked with IIN were found to have mutations in the *FRMD7* gene. Nine families with IIN (prefixed with 'E') were excluded from the study. Families with IIN not associated with mutations in *FRMD7* are discussed below.

4.3.1 Smaller Families without mutations in *FRMD7*

In the sixteen families were linkage analysis was done one family (N8) did not have mutations in the gene. This could be because of mutations in the promoter/regulatory regions of the gene which could have been missed. Other possible explanations include copy number variation in *FRMD7* or the presence of another gene causing the disease. Eight of the 10 families recruited in the second phase in whom haplotype analysis was done had mutations in the gene. Family F3 and F10 did not have mutations in the gene. Family F3 have two affected members and a third subject with end point nystagmus who share the haplotype.

It is interesting to note that in family F10 all the four affected subjects are females. Moreover one of the subjects (8072) was diagnosed with choroidal coloboma. The unaffected male subject (8073) seems to share the haplotype with the affected female (subject 7311) suggesting that the genetic locus lies elsewhere.

DNA samples from four smaller families who were recruited into the study were sent for sequencing of the FRMD7 gene. However none of the subjects from these four families

(Family G1, G2, G3, and G4) were found to have mutations in the gene. Family G1 has only two affected members. Family G2 has also two affected members and has history of consanguinity in the family.

Family G3 has only two affected siblings. Members of family G4 have vertical nystagmus unlike most of the subjects from other families.

4.3.2 Families with associated iris anomalies

At least one subject of the three families in the study was noted to have iris abnormality. These include family F10 and E2. Subject 8366 of family E2 was diagnosed with IIN. However his mother (lab number 304) was noted to have complete aniridia. Iris anomalies in these two families make them phenotypically different from IIN due to mutations in *FRMD7*.

4.3.3 Family with IIN and cataract

Subjects in family E7 had a different phenotype compared to the rest. The phenotype appears to be inherited as an autosomal dominant trait. Subjects have infantile nystagmus, the waveform characteristic of IIN; however, three subjects of this family had vertical nystagmus.

Of the 45 subjects examined, nine had manifest strabismus. Two subjects had bilateral blepharoptosis. Eight subjects had either cataract or were pseudophakic. Typically cortical lens opacities begin to appear during the 2nd decade of life and these subjects go on to

have cataract surgery in the 3rd or 4th decade of life. Optical coherence tomography was done in a few affected subjects which showed foveal hypoplasia.

Syndrome of infantile nystagmus and foveal hypoplasia have been reported earlier in literature (MIM136520). Missense mutations in PAX6 is known to cause autosomal dominant foveal hypoplasia.[230] Mutations in PAX6 associated with autosomal dominant infantile nystagmus, congenital cataract, foveal hypoplasia, peripheral corneal vascularisation and corneal epithelial changes has been reported in literature.[34]

Even though phenotypically dissimilar, many of the clinical features in this family are similar to the syndrome of autosomal dominant foveal hypoplasia (MIM136520) and this might warrant screening for missence mutations in PAX6.

A mother and daughter from family E8 were diagnosed with IIN and macular coloboma.

Only two subjects from family E9 were examined by the author. Subject 810 was found to have bilateral cortical cataracts.

4.4 Importance of this work

This work identified the first gene implicated in the pathogenesis of IIN. We were able to describe the phenotypical characteristics of a homogeneous group of subjects with IIN which would help in the clinical diagnosis of *FRMD7* related IIN.

In this study we found that many of the subjects with an *FRMD7* mutation had good visual acuity (within the driving standards in the UK). In addition we had subjects from all walks of life. These findings will help a clinician to reassure a family with a subject with *FRMD7* related IIN.

Identification of *FRMD7* has raised a new platform for research into the mechanisms behind control of ocular movements. It was found that the expression of FRMD7 in developing human embryo (at 37 days post ovulation) was limited to the areas involved in the ocular motor control and the developing neuro-retina.

In a recently published study which investigated the role of *FRMD7* in neural development using *in situ hybridisation* and immunohistochemistry, it was found that expression of *FRMD7* is spatially and temporally regulated in both the human and mouse brain during embryonic development. The subcellular localisation of FRMD7 in NEURO2A cells was found to be restricted to the cell body of the neuronal cell, the area of the primary dendrite extensions and the distal tip of the growth cone in dendrites. In addition the down-regulation of FRMD7 disturbs neurite outgrowth during retinoic acid-induced differentiation of NEURO2A cells.[231] Further studies into the spatial and temporal expression of FRMD7 and studies in animal models will help to further delineate the function of the gene and understand the mechanisms behind ocular motor control.

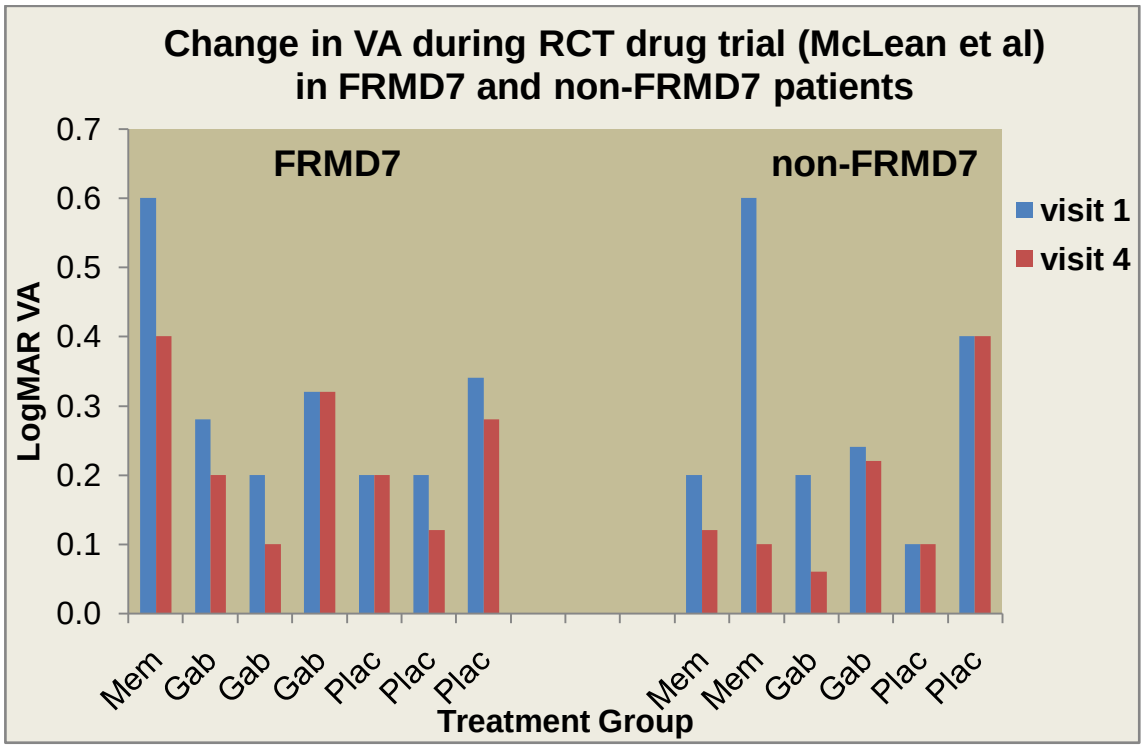
Another important aspect is identification of other genes involved in the control eye movements. Identification of *FRMD7* will help in screening for other genes based on their homology to *FRMD7*.

Several drugs are being used on an empirical basis to treat nystagmus, which forms a heterogeneous group of disorders. The discovery of *FRMD7* has identified a new homogeneous cohort of subjects with IIN which might be amenable to pharmacological treatment.

The author went through the data of a previous randomized double blind drug trial on the efficacy and safety of gabapentin and memantine carried out at the University of Leicester.[158] Forty-eight subjects were enrolled into that study, of which one subject in the placebo group dropped out. Subjects were randomized into either a memantine group (n=16), or a gabapentin group (n=16) or a placebo group (n=15). The study concluded that pharmacological agents such as memantine and gabapentin can improve visual acuity, reduce nystagmus intensity, and improve foveation in congenital nystagmus.

Subjects with mutations in *FRMD7* who participated in the above trail were identified. The only subject in the memantine group, two of three subjects in the gabapentin group and two of the three subjects in the placebo group showed improvement in vision as shown in the chart below (Figure 4.2). However the sample size was too small to make any comments on the efficacy of these drugs.

Figure 4.2: Pharmacological treatment of FRMD7-related IIN*. Visual acuities of subjects with IIN on visit1 (before commencing on drug) and visit4 (whilst on drug treatment) in the FRMD7 group and non-FRMD7 group. The number of subjects in each group is small. (Data collected retrospectively from McLean et al, 2007).*



4.5 Future work

With the discovery of *FRMD7*, many new avenues of clinical, molecular and genetic study have opened up in recent years.

As discussed in the previous chapter, it was found that *FRMD7* is expressed in the developing neuro-retina. The 1st, 2nd and 3rd order neurons of the afferent visual pathway reside at least partly in the retina and it gives us the unique opportunity to visualise and study it in detail. Recent advances in retinal imaging has enabled us to study the retinal layers in detail and imaging techniques such as high resolution Optical Coherence Tomography (OCT) and Heidelberg Retinal Tomography (HRT) could be used to study subtle pathologies in the retina.

In this study, we were not able to detect a mutation in *FRMD7* in a few linked families many other smaller families and a large cohort of sporadic cases.

Genetic abnormalities such as deletions, copy number variations and any pathogenic changes in the promoter region of the gene, if present in a family, would have been missed in this study. Further testing using array-CGH (array-comparative genomic hybridisation) and MLPA (multiplex ligation-dependant probe amplification) would be useful to detect such abnormalities. Identification and characterisation of the regulatory regions of *FRMD7* might provide candidates for mutation screening, thus increasing the yield of mutations in the gene.

Subjects from the autosomal dominant family (Family E4) could be checked for linkage to the known autosomal loci (NYS2 at 6p12, NYS3 at 7p11.2 and NYS4 at 13q31-q33) for IIN.

As detailed in chapter 3, two other members of the protein 4.1 superfamily show significant homology, particularly to the FERM domains of the *FRMD7* gene. These are *FARP1* (FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)) and *FARP2* (FERM, RhoGEF and pleckstrin domain protein 2). The cohort of sporadic cases could be used to screen for mutations in these genes which has close homology to *FRMD7.*

Other proteins with significant homology to FRMD7 include ezrin, radixin, moesin, and merlin.

Recent studies using *in-situ* hybridisation and immunohistochemistry have shed light on the sub cellular expression of FRMD7 and the effect of its down-regulation in NEURO2A cells.[231] Another valuable resource for further study will be animal knock-outs of the gene. Generation of knockout mouse is in the pipeline at the NIH backed knockout mouse project (KOMP) (http://www.komp.org). Anatomical and physiological studies on the neural and retinal tissues of *FRMD7* knockout mouse will identify any differences between the *FRMD7* mutants and their non-mutant siblings.

Many drugs have been found to be useful in the management of IIN. One drug of particular interest in memantine, an uncompetitive NMDA receptor antagonist. Used in the treatment of dementia, this drug was found to be useful in the management of IIN (including those associated with *FRMD7* mutations).[158] A randomised double blind drug trial on the efficacy of this drug in the management of *FRMD7* related IIN would prove useful. Baclofen (GABA agonist) and gabapentin (a GABA analogue with varied

mechanisms of action in the nervous system) which have been found useful in controlling nystagmus[158, 232] are also good candidates to consider for treatment trials.

5 Conclusions

This study evaluated the clinical features of subjects with familial and sporadic IIN. Familial IIN with compatible with X-linked inheritance was selected. Genotyping with microsatellite makers was carried out which reduced the potential genetic interval of 'NYS1 locus' at Xq26-q27. A new gene called *FRMD7* (Xq26.2) was identified within the critical region of NYS1.

Mutations in *FRMD7* is the major cause of X-linked IIN. It may be possible to distinguish a subject with IIN due to mutations in *FRMD7* from history and clinical examination, however for accurate diagnosis, genetic testing is required.

Subjects with *FRMD7* related IIN have good visual acuity and in many of them the visual acuity is within the driving standards in UK.

There are number of pharmacological agents used to manage acquired and congenital nystagmus with limited benefit. It is not known if any of these drugs have any particular benefit in the management of IIN due to mutations in *FRMD7.*

Further studies on the expression and function of *FRMD7* and discovering other genes involved in the pathogenesis of IIN should help to understand the pathogenesis nystagmus and to develop better treatments in the future.

Appendix 1

Publications

i) Tarpey P#, **Thomas S**#, Sarvananthan N, Mallya U, Lisgo S, Talbot CJ, Roberts EO, Awan M, Surendran M, McLean RJ, Reinecke RD, Langmann A, Lindner S, Koch M, Jain S, Woodruff G, Gale RP, Degg C, Droutsas K, Asproudis I, Zubcov AA, Pieh C, Veal CD, Machado RD, Backhouse OC, Baumber L, Constantinescu CS, Brodsky MC, Hunter DG, Hertle RW, Read RJ, Edkins S, O'Meara S, Parker A, Stevens C, Teague J, Wooster R, Futreal PA, Trembath RC, Stratton MR, Raymond FL, Gottlob I.# These authors contributed equally to this work Mutations in FRMD7, a newly identified member of the FERM family, cause X-linked idiopathic congenital nystagmus.
Nature Genetics. 2006 Nov; 38(11):1242-4.

ii) **Thomas S**, Proudlock FA, Sarvananthan N, Roberts EO, Awan M, McLean R, Surendran M, Kumar ASA, Farooq SJ, Degg C, Gale RP, Reinecke RD, Woodruff G, Langmann A, Lindner S, Jain S, Tarpey P, Raymond FL, Gottlob I. Phenotypical characteristics of Idiopathic Infantile Nystagmus with and without mutations in FRMD7.
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Mutations in *FRMD7*, a newly identified member of the FERM family, cause X-linked idiopathic congenital nystagmus

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Idiopathic congenital nystagmus is characterized by involuntary, periodic, predominantly horizontal oscillations of both eyes. We identified 22 mutations in *FRMD7* in 26 families with X-linked idiopathic congenital nystagmus. Screening of 42 singleton cases of idiopathic congenital nystagmus (28 male, 14 females) yielded three mutations (7%). We found restricted expression of *FRMD7* in human embryonic brain and developing neural retina, suggesting a specific role in the control of eye movement and gaze stability.

The prevalence of idiopathic congenital nystagmus (ICN) is estimated to be 1 in 1,000. In ICN, visual function can be significantly reduced owing to constant eye movement, but the degree of visual impairment varies[1,2]. The disease is likely to be due to abnormal development of areas in the brain controlling eye movements and gaze stability[3]. ICN is distinct from other hereditary causes of nystagmus and ocular pathology, including ocular albinism, congenital stationary night blindness, achromatopsia, blue cone monochromatism and sensory visual defects of early childhood such as congenital cataract, retinitis pigmentosa, cone-rod dystrophy and optic nerve hypoplasia[4].

ICN is usually inherited as an X-linked trait with incomplete penetrance in females. Most families map to Xq26–q27 and the locus (known as *NYS1*) has previously been mapped to a ~12-Mb interval between markers DXS9909 and DXS1211 (refs. 5,6). Others have proposed a further reduction of the candidate region to an interval between DXS8033 and DXS8043 based on a recombination event in a clinically unaffected female[7]. X-linked genetic heterogeneity has been suggested on the basis of a single ICN family that is reported to map to Xp11.4– Xp11.3 (ref. 8).

We screened 16 families with X-linked ICN using 17 markers extending from Xq26–Xq27 (**Supplementary Fig. 1** online)[6]. In these families, the disease was fully penetrant in males and ~50% penetrant in females. The phenotype was variable even within families (**Supplementary Fig. 2** and **Supplementary Videos 1**, **2** and **3** online). In all 16 families, marker haplotypes were compatible with linkage to Xq26–q27 (**Supplementary Methods** online). Recombinant events in affected males in family N1 refined the location of *NYS1* to a ~7.5-Mb interval between markers DXS1047 and DXS1041 (**Fig. 1a**,**b**).

The candidate interval contained >80 genes. We performed high-throughput DNA sequence analysis of all coding exons of all genes within this interval[9]. DNA from one affected male individual from each of the 16 linked families was screened for mutations.

We detected mutations in *FRMD7* (FERM domain–containing 7, previously known as *LOC90167*) at Xq26.2 in 15/16 of the linked families after screening >40 genes by sequence analysis (**Fig. 1c**). *FRMD7* has 12 exons and encodes a previously unidentified member of the protein 4.1 superfamily (RefSeq accession number NM_194277). All mutations identified in *FRMD7* cosegregated with disease in the linked families and were absent from 300 male control chromosomes (**Table 1**). The nonsense mutations leading to Q201X and R335X predict truncated proteins containing 28% and 47% of the protein, respectively. Four of the five splice site mutations were at conserved splice donor residues (position +1 and +2) and are thus predicted to be pathological by classical exon skipping and

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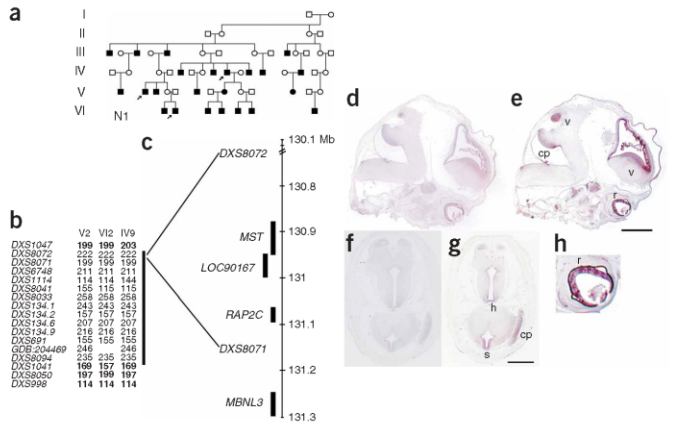

Figure 1 Refinement of the linkage interval to Xq26 and the pattern of expression of *FRMD7* in brain of human embryos ~56 d.p.o. by *in situ* hybridization. (**a**) Pedigree of family N1. Arrow marks critical individuals whose haplotypes define the minimum critical linkage interval. (**b**) Allele size for marker *DXS1047* in individual IV9 is discordant compared with the other affected males, V2 and VI2, and the allele size at markers *DXS1041* and *DXS8050* in individual VI2 is discordant compared with individuals V2 and IV9. (**c**) Location of *LOC90167* (*FRMD7*) relative to the linkage markers *DXS8072* and *DXS8071*. (**d**–**h**) A 599-bp and a 681-bp probe from unique sequence in the 3′ UTR of *FRMD7* were used as probes for *in situ* hybridization. **d** and **f** show *in situ* hybridization results using sense probes, and **e** and **g** use antisense probes in similar anatomical locations. **d** and **e** are sagittal sections showing the lateral ventricle (v), cerebellar peduncle (cp) and developing neural retina (r). **h** is an enlargement of **e**, illustrating the retina. **f** and **g** are transverse sections showing the spinal cord (s), hypothalamus (h) and cerebellar peduncle (cp). Scale bars: **e**, 2,000 μm; **g**, 1,800 μm. The study was approved by the University of Leicester Ethics Committee. Written informed consent was obtained from all study participants.

nonsense-mediated decay. In family N7, whose members had the mutation IVS2 +5G→A, we detected negligible amounts of transcript in the proband by amplification of exons 1–5 in lymphocytes compared with quantities in controls, suggesting that this is also disease associated (**Supplementary Fig. 3**, **Supplementary Methods** and **Supplementary Table 1** online). The silent variant, G252A (V84V in family N5), created a new splice acceptor site within exon 4 that resulted in the loss of transcript containing the sequence of exons 1–5 and the rare presence of a transcript with exon 4 skipped in lymphocytes (**Supplementary Fig. 3**).

The six missense mutations resulting in amino acid substitutions at positions 24, 142, 231, 266, 271 and 301 in the linked families not only involve highly conserved residues that are invariant in *Rattus norvegicus*, *Mus musculus*, *Gallus gallus* and *Xenopus tropicalis* but are also located within invariant blocks of highly conserved residues, suggesting that mutations at these locations are critical to the normal function of the protein. Residues at position 142, 231, 271 and 301 are further conserved in *Tetraodon nigroviridis*. Furthermore, with the exception of L231V, the mutations lead to changes in amino acids that are largely nonconservative in function (G24R, L142R, A266P, C271Y and Y301C). We modeled the effects of these mutations on the three-dimensional structure of the protein by mapping them onto the closest ortholog of known structure: the core domain of the cytoskeletal protein 4.1R (1GG3 http://www.pdb.org/pdb/navbarsearch.do?=All&inputQuickSearch=1gg3 in the Protein Data Bank)[10,11]. The crystal structure extends from residues 1–279, and therefore we were able to map all the changes in amino acids resulting from missense mutations in *FRMD7* except the one leading to Y301C in the linked families. We inspected the structural environment of the disease-associated missense mutations using the program Coot, which allows the identity and conformation of residues to be manipulated easily[12]. Although the effect on the structure of L231V is not clearly apparent, mutations leading to G24R, L142R and C271 are likely to destabilize the protein by the introduction of larger amino acids within restricted areas of the protein, and the introduction of a proline residue at position 266 (A266P) will disrupt a helical domain in the wild-type structure.

From the results above, we concluded that mutations in *FRMD7* are a major cause of familial X-linked congenital motor nystagmus. We then assessed the prevalence of mutations in *FRMD7* in smaller families in which linkage data was not available and in a cohort of males and females with ICN but without a family history of the condition. We screened 14 families with two or more affected individuals of either sex and found mutations in 8/14 (57%). We also identified mutations in 3/42 (7%) individuals without a family history who had undergone careful clinical and electrophysiological investigation to exclude other causes of inherited congenital nystagmus (**Table 1**). We identified mutations in 1/14 female singletons and 2/28 male singletons, and none of the new mutations was found in samples from 300 male control individuals. The results suggest that mutation analysis of *FRMD7* may be considered of diagnostic value even in isolated cases of either sex.

Expression analysis of *FRMD7* shows that the mRNA is present in most tissues at low levels (http://symatlas.gnf.org/SymAtlas/). We confirmed this by RT-PCR, detecting expression in human adult kidney, liver, pancreas and, at low levels, heart and brain (data not shown). Using this method in human fetal tissue, we detected the transcript only in kidney.

We then performed *in situ* hybridization experiments in human embryonic brain to investigate whether expression of *FRMD7* was localized or restricted. In embryos ~56 d post-ovulation (d.p.o.), there is expression in the ventricular layer of the forebrain, midbrain, cerebellar primordium, spinal cord and the developing neural retina. In earlier embryos (~37 d.p.o.) the expression is restricted to the mid- and hindbrain, regions known to be involved in motor control of eye movement (**Fig. 1d–h**).

The functional role of FRMD7 protein is unknown, but we detected close amino acid sequence homology to FARP1 (FERM, RhoGEF and pleckstrin domain protein 1; chondrocyte-derived ezrin-like protein; NM_005766) and FARP2 (NM_014808) by BLAST search analysis (http://www.ncbi.nlm.nih.gov/). The homology is concentrated at the N terminus of the protein, where B41 and FERM-C domains are present. The B41 domain is located at residues 1–192, and the FERM-C domain is located at residues 186–279 in FRMD7. The location of mutations relative to these domains is shown in **Supplementary Figure 3**. The homologous protein FARP2 modulates the length and the degree of branching of neurites in rat embryonic cortical neurons

Table 1 Mutations in *FRMD7* and variations in FRMD7

Sample	Class	Mutation/variation	Origin
N15	M	G70A, G24R	Ireland
N7	T	IVS2+5G→A	England
N4	T	IVS3+2T→G	England
N5	S	G252A, V84V	England
N1, F26	T	IVS4+1G→A	England, England
N16	M	T425G, L142R	Ireland
N13	T	IVS7+1G→C	Madagascar
N2	T	C601T, Q201X	Italy-Germany
N3	M	T691G, L231V	Ireland-Germany
N6, SF21	M	G796C, A266P	England, England
N11	M	G812A, C271Y	Scotland
N14	T	887delG, G296fs	Austria
N12	M	A902G, Y301C	England
N10, F24, SM08	T	C1003T, R335X	England, India, England
N9	T	IVS11+1G→C	Germany
F31	del	41_43delAGA, 14delI	England
F21	M	G71A, G24E	Austria
F28	T	479insT, 160fs	England
F15	M	A661G, N221D	England
F16	M	G676A, A226T	England
F20	M	C1019T, S340L	Romania
SM10	T	1262delC, 421fs	England

Families where linkage was performed are prefixed by N; those that are familial but for which no linkage data were available are prefixed F. Singleton males are prefixed by SM and singleton females SF. The class of mutation is categorized as T (truncating), M (missense), S (silent) and del (deletion). The country of origin is denoted. All mutations identified were not found in 300 control male chromosomes. The reference cDNA sequence NM_194277 is used as a basis for numbering the nucleotide of the mutation. All mutations are located relative to the A of the first coding ATG at position 179. The reference protein sequence NP_919253 is used as the basis for numbering the amino acid variation starting from the first methionine at position 1. The reference sequence for the genomic sequence is AL49792.

and reorganizes the cytoskeleton. Overexpression of FARP2 results in increased numbers of lateral growth cones extending from neurites and associated decrease in total length of the neurites per neuron[13,14]. Whether the function of FRMD7 is similar to FARP2 in specialized neuronal pathways governing integration and coordination of eye movements remains to be proved. The hypothesis that null mutations in *FRMD7*, as found in families with X-linked congenital motor nystagmus, alter the neurite length and degree of branching of neurons as they develop in the midbrain, cerebellum and retina is a plausible explanation of how defects in the protein coded for by *FRMD7* causes disease.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

This study was designed by I.G., R.C.T., E.L.R., M.R.S., S.T. and N.S.; phenotype assessment was performed by I.G., S.T., N.S., E.O.R., M.A., M.S., R.J.M., R.D.R., A.L., S.L., M.K., G.W., R.P.G., C.D., K.D., I.A., A.A.Z., C.P., O.C.B., S.J., M.C.B., D.G.H. and R.W.H.; DNA extraction, linkage analysis, sequencing, *in situ* hybridization and molecular modeling were performed by E.L.R., P.T., S.T., R.C.T., U.M., S.L., C.J.T., E.O.R., M.S., C.D.V., R.D.M., L.B., C.S.C., R.J.R., S.E., S.O., A.P., C.S., J.T., R.W. and P.A.P. and the paper was written by E.L.R., I.G., P.T., M.R.S. and S.T.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Phenotypical characteristics of idiopathic infantile nystagmus with and without mutations in *FRMD7*

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Idiopathic infantile nystagmus (IIN) consists of involuntary oscillations of the eyes. The familial form is most commonly X-linked. We recently found mutations in a novel gene *FRMD7* (Xq26.2), which provided an opportunity to investigate a genetically defined and homogeneous group of patients with nystagmus. We compared clinical features and eye movement recordings of 90 subjects with mutation in the gene (*FRMD7* group) to 48 subjects without mutations but with clinical IIN (non-*FRMD7* group). Fifty-eight female obligate carriers of the mutation were also investigated. The median visual acuity (VA) was 0.2 logMAR (Snellen equivalent 6/9) in both groups and most patients had good stereopsis. The prevalence of strabismus was also similar (*FRMD7*: 7.8%, non-*FRMD7*: 10%). The presence of anomalous head posture (AHP) was significantly higher in the non-*FRMD7* group ($P < 0.0001$). The amplitude of nystagmus was more strongly dependant on the direction of gaze in the *FRMD7* group being lower at primary position ($P < 0.0001$), compared to non-*FRMD7* group ($P = 0.83$). Pendular nystagmus waveforms were also more frequent in the *FRMD7* group ($P = 0.003$). Fifty-three percent of the obligate female carriers of an *FRMD7* mutation were clinically affected. The VA's in affected females were slightly better compared to affected males ($P = 0.014$). Subnormal optokinetic responses were found in a subgroup of obligate unaffected carriers, which may be interpreted as a sub-clinical manifestation. *FRMD7* is a major cause of X-linked IIN. Most clinical and eye movement characteristics were similar in the *FRMD7* group and non-*FRMD7* group with most patients having good VA and stereopsis and low incidence of strabismus. Fewer patients in the FRMD7 group had AHPs, their amplitude of nystagmus being lower in primary position. Our findings are helpful in the clinical identification of IIN and genetic counselling of nystagmus patients.

Keywords: X-linked idiopathic infantile nystagmus; *FRMD7*; obligate carrier; clinical characteristics; eye movements

Abbreviations: AHP = anomalous head posture; IIN = idiopathic infantile nystagmus; OKN = optokinetic nystagmus; VA = visual acuity

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Introduction

Nystagmus consists of involuntary to and fro eye movements and it can have a severe effect on vision and social function (Pilling *et al.*, 2005). The onset of idiopathic infantile nystagmus (IIN) is usually in the first months of life. IIN is characterized by an absence of other ocular

pathology such as albinism, congenital stationary night blindness or achromatopsia. Pharmacological and surgical treatments of IIN are just emerging (Hertle *et al.*, 2004; McLean *et al.*, 2007). In a recent survey of nystagmus in Leicestershire, UK, the prevalence of nystagmus has been estimated to be 2.4 in 1000 (Sarvananthan *et al.*, 2006). IIN can be sporadic or hereditary. The most common mode of inheritance is X-linked and the gene has been localised on the long arm of chromosome X (NYS1) (Kerrison *et al.*, 1998, 1999). We have recently found multiple mutations in a novel gene called *FRMD7* (Xq26.2) (NYS1), which is a major cause of X-linked IIN (Tarpey *et al.*, 2006) and has also been confirmed by others (Schorderet *et al.*, 2007; Self *et al.*, 2007; Zhang *et al.*, 2007*a*, *b*).

Several families with nystagmus of autosomal dominant inheritance have been described in the literature (Allen, 1942; Dichgans and Kornhuber, 1964). Kerrison *et al.* (1998) localized a gene for autosomal dominant nystagmus (NYS2) to chromosome 6p12. Other loci for nystagmus with autosomal inheritance include NYS3 (MIM 608345) (Patton *et al.*, 1993) and NYS4 (MIM 193003) (Ragge *et al.*, 2003). Nystagmus of autosomal recessive inheritance (MIM 257400) has been described by Waardenburg in 1961.

There have been attempts in the literature to cluster different types of congenital nystagmus together, based on the waveforms obtained through eye movement recordings. Twelve characteristic waveforms have been described in congenital nystagmus (Dell'Osso and Daroff, 1975). Hertle and Dell'Osso (1999) proposed that infantile nystagmus is a single clinical entity regardless of associated sensory abnormalities.

There are only few published data on the clinical characteristics of IIN. In most published reports, all forms of infantile nystagmus have been pooled together as a single entity irrespective of the aetiology (von Noorden and La Roche, 1983; Hertle and Dell'Osso, 1999). Abadi and Bjerre in 2002 described the motor and sensory characteristics of 224 subjects with infantile nystagmus of various aetiologies. He classified infantile nystagmus into three categories, i.e., idiopathic, associated with albinism and due to other causes and did not find differences in waveforms.

The detection of *FRMD7* allows, for the first time, the analysis of a large group of IIN patients, where the cause of disease is homogeneous. In this study we describe the clinical and eye movement characteristics of 90 subjects with IIN due to mutations in *FRMD7* and 48 subjects with IIN not caused by mutations in *FRMD7*. In addition, eye movement recordings of unaffected obligate female carriers of mutations in the *FRMD7* gene are analysed.

Patients and Methods

This work is based on a previous study in which we found mutations in the *FRMD7* gene in 23 families with X-linked IIN (Tarpey *et al.*, 2006). We identified 90 subjects with IIN due to mutations in *FRMD7* gene (mean age 36 years and range 3–88 years). Of these 90 subjects in the *FRMD7* group, 88 had familial nystagmus whilst two were sporadic. The phenotypes of these subjects were compared to 48 subjects with IIN not caused by mutations of this gene (mean age 29 years and range 4–79 years). Of the 48 subjects in the non-*FRMD7* group, 33 were sporadic, whilst the remaining 15 had at least two affected members in the family. Affected males and females were compared to see if there were any differences in clinical features and eye movements. Eye movement recordings were obtained in 52 affected subjects in the *FRMD7* group and 29 affected subjects in the non-*FRMD7* group.

We also identified and evaluated 27 obligate female carriers of *FRMD7* mutations who were clinically unaffected comparing them to age matched healthy controls. In addition to the clinical data, eye movement recording were analysed in a subgroup of these unaffected carriers ($n = 14$), in particular to look for differences in smooth pursuit eye movements and optokinetic nystagmus (OKN).

Detailed ophthalmological examination was carried out on each subject. Slit lamp bio-microscopy was performed in the dark to rule out iris transillumination. The visual acuity (VA) was recorded using Snellen visual acuity charts. Binocular vision was examined using the Lang test. If the Lang test was positive, the Frisby test was used to investigate the level of stereopsis. Bagolini striate glasses were used when the Lang test was negative. Panel desaturated D15 and Ishihara's chart were used to detect/rule out colour vision abnormalities. The presence or absence of anomalous head posture (AHP) was analysed while patients were reading a distance visual acuity chart and was classified into three groups (No AHP: i.e. $<5^\circ$ of head turn, moderate: i.e. AHP of 5–15°, and large AHP: $>15^\circ$).

Electro-diagnostic tests (electroretinogram and visual evoked potential to detect/rule out retinal disease and albinism) were done on all sporadic subjects and at least one member of each family from the familial subjects according to ISCEV standards (Marmor *et al.*, 2004; Odom *et al.*, 2004). All subjects and families with abnormal electrophysiology were excluded from the study.

Eye movements were recorded (250 Hz; EyeLink pupil tracker, SMI GmbH, Berlin, Germany) while viewing stimuli projected on a rear projection screen (1.8×1.2m) using a video projector (Hitachi CP-X958). A 17" LCD monitor (Samsung SyncMaster 710 V) placed at a viewing distance of 40 cm was used during field trips. Each eye was calibrated separately offline by selecting foveations when fixating horizontal and vertical points at $\pm 15^\circ$ eccentricity and 0°. Visual tests were performed in horizontal and vertical directions including saccades (following targets from -20° to 20° in 10° steps moving every 1.5 s), smooth pursuit (20°/s velocity, $\pm 20^\circ$ amplitude: 10° and 40°/s velocities also performed if time permitted), OKN (20°/s velocity, square wave contrast gratings of 2.2° cycle size, Michelson contrast 0.88 cd/m^2: 10° and 40°/s velocities also performed if time permitted) and steady fixation (up to 1 min recording at -15°, 0° and 15°).

Linear mixed models were used to statistically compare the effects of *FRMD7* and non-*FRMD7* inheritance including family, gender and eccentricity (for eye movements) as fixed factors excluding any non-significant interactions from the final models. Non-parametric data were either log transformed (i.e. amplitudes and frequencies) or analysed using Mann–Whitney U-tests (i.e. comparing VAs between *FRMD7* and non-*FRMD7* groups and also OKN and smooth pursuit eye movements of *FRMD7* carriers to age-matched controls). The Pearson chi-squared test and the γ-statistic were used to compare relative proportions between groups.

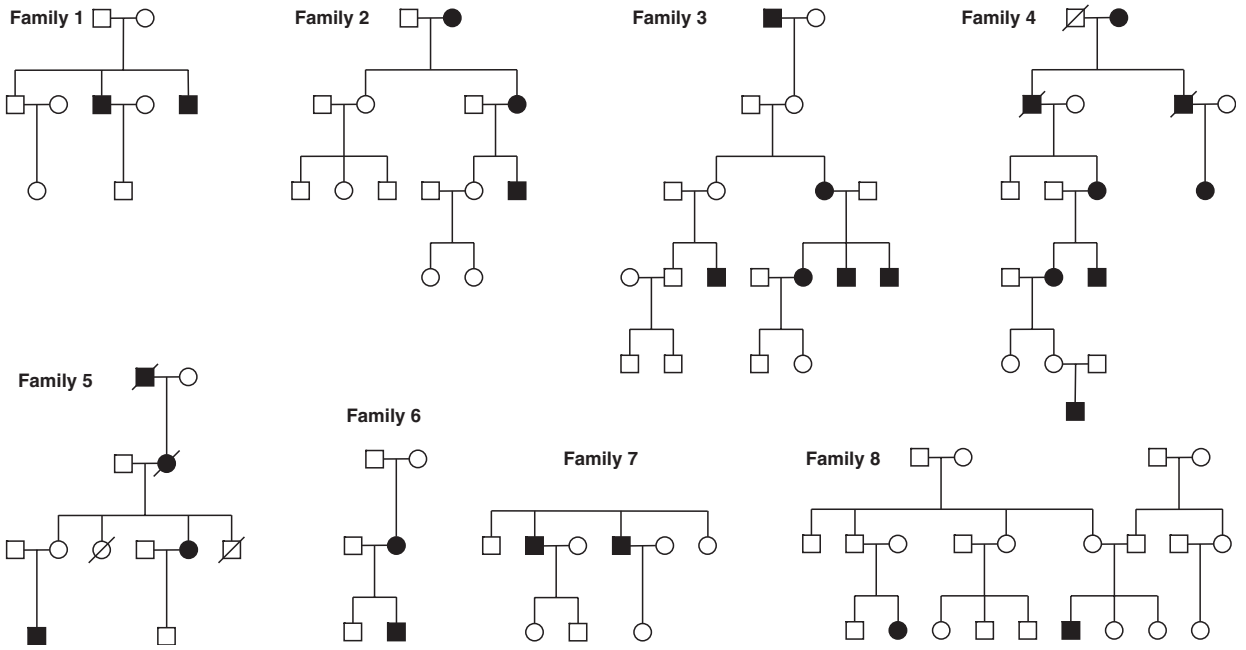

Fig. 1 Eight families with mutations in FRMD7 gene in whom the linkage analysis was not performed in our previous study (Tarpey *et al*., 2006).

Results

Frequency of FRMD7 mutations

Mutations were detected in 15 of 16 (94%) families, which were linked to the NYS1 (Xq26-27) region and another 8 of 14 (57%) families in whom linkage data were not available (Tarpey *et al*., 2006). The eight family trees which were not shown in our previous publication are shown in Fig. 1.

VA and colour vision

In the *FRMD7* group, the VA was tested in 83 of the 90 subjects. (We could not obtain accurate VA measurements in seven children.) Most of the subjects with mutations in *FRMD7* had VAs better than logMAR 0.301 (Snellen equivalent 6/12) (Fig. 2, top panel). The median VA of this group was logMAR 0.176 (6/9) with upper and lower quartiles of logMAR 0.301 (6/12) and logMAR 0.097 (6/7.5), respectively.

In the non-*FRMD7* group, VA was obtained in 45 of the 48 subjects, the data not being available in three children (Fig. 2, bottom panel). The VA distribution was similar to the *FRMD7* group, the median being logMAR 0.176 (6/9) with upper and lower quartiles of logMAR 0.301 (6/12) and 0.0 (6/6), respectively. Mutations in the *FRMD7* gene did not have any significant effect on VA of patients with IIN (Mann–Whitney U-test, $P = 0.143$). Colour vision was normal in all the subjects from both groups.

In the *FRMD7* group, there was a mild but significant difference in VA between affected males and females, the visual acuity being better in females (Mann–Whitney U-test, $P = 0.014$: median = 0.098 in females and 0.188 in males). In contrast, there were no significant differences between males and females in the non-*FRMD7* group (Mann–Whitney U-test, $P = 0.36$: median = 0.176 in males and females).

Stereopsis

Of the 90 subjects in the *FRMD7* group, 76 were tested for stereo-VA. Most of the subjects (93.4%) had good binocular vision demonstrable on Lang test. Seventy-one subjects who were Lang positive were examined using the Frisby test and were found to have a median stereopsis of 150''. Of the five subjects who were Lang negative, four were Bagolini positive. The Bagolini negative subject had alternating esotropia. Six subjects with manifest strabismus detected on cover test were found to have binocular vision on Bagolini test.

In the non-*FRMD7* group, 37 of the 48 subjects were checked for binocular vision. Twenty-nine subjects (78.4%) were Lang positive and their median stereopsis on Frisby test was also 150''. Eight subjects were Lang negative, of which two were Bagolini positive. Pearson chi-squared test was used to compare the relative proportions of subjects who were Lang positive and showed a significantly higher proportion in the *FRMD7* group ($P = 0.019$).

Strabismus

Strabismus was detected in seven of the 90 subjects (7.8%) in the *FRMD7* group. Three subjects had esotropia and three exotropia; one subject had left hypertropia. All the subjects (except one) with manifest strabismus had at least gross binocular vision (Bagolini positive). In the non-*FRMD7* group, five of 48 (10.4%) had manifest strabismus,

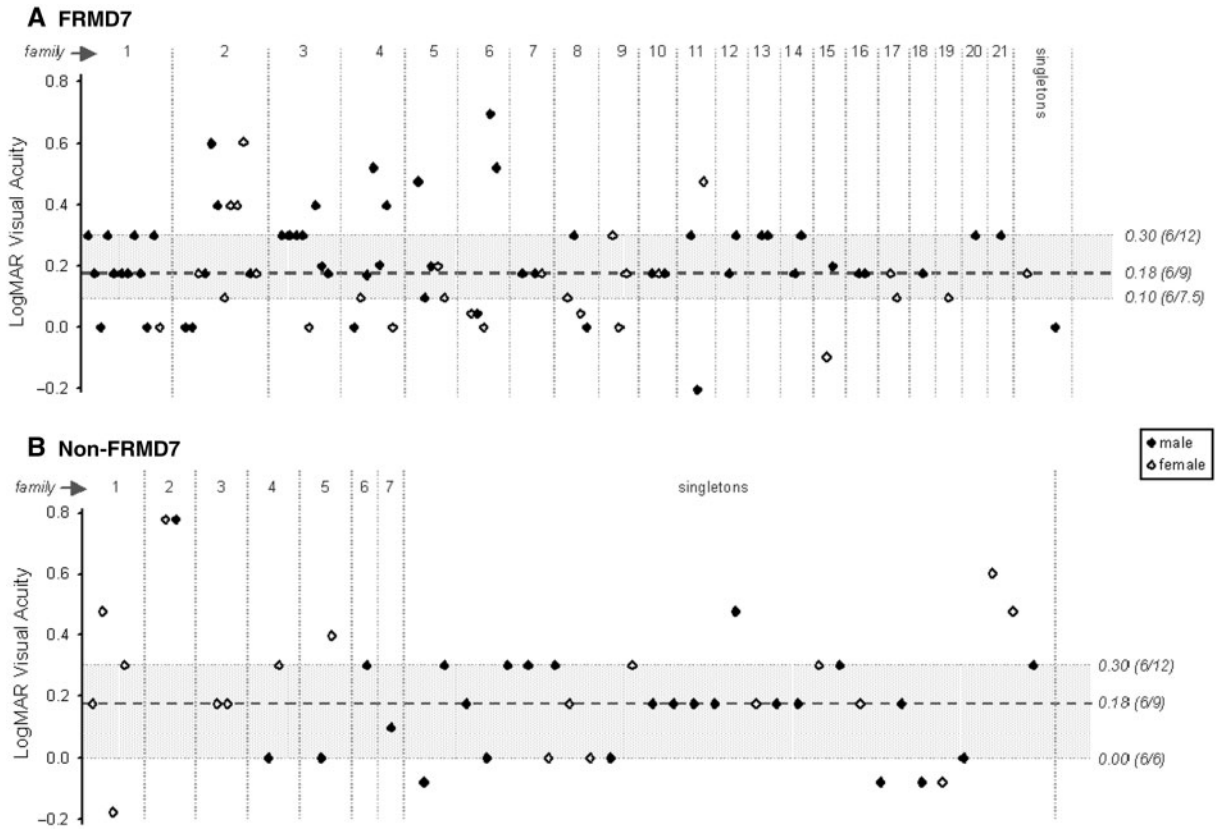

Fig. 2 VA (LogMAR on the left and the Snellen equivalent on the right) plotted on the *y*-axis against the individual subjects on the *x*-axis. The top panel shows the subjects with mutation in FRMD7 grouped into 21 different families (left side of panel) and two singletons (right side of panel). As shown, the median log MAR VA is 0.176. The bottom panel shows the subjects with no mutations in the above gene (grouped in seven families on the left side and 31 singletons on the right side). The median VA is 0.176. The males are represented with filled diamonds and females with open diamonds. The shaded area represents the data between the upper and lower quartiles.

all of them having esotropia (Pearson chi-squared test between the two groups, $P = 0.61$).

Latent nystagmus

None of the subjects from the *FRMD7* group had latent nystagmus. However, one subject with strabismus in the non-*FRMD7* group was found to have a latent component to the nystagmus.

AHP

We recorded head posture in 80 subjects in the *FRMD7* group (Fig. 3). Sixty-eight of the 80 subjects (85%) did not have significant AHP, i.e. AHP <5°. Twelve subjects (15%) had AHP of 5–15°. None of the subjects in this group had vertical head posture and the horizontal AHP in this group never exceeded 15°. None of these subjects had had surgery for abnormal head posture.

Head posture was recorded in 45 of the 48 subjects in the non-*FRMD7* group. Twenty-two of 45 (49%) did not have significant AHP, i.e. AHP <5°, whereas 11 subjects (24%) had moderate (5–15°) AHP. Twelve of the 45 subjects (27%) had AHP of >15° of which eight subjects had undergone Kestenbaum procedure for AHP in the past. Of these 12 subjects with AHP >15°, four had vertical head postures (two chin down and two chin up). The comparison of the AHP in the two groups is shown in Fig. 3. Gamma statistic was used to compare the relative proportions between the groups and showed a highly significant difference between *FRMD7* and non-*FRMD7* groups ($P = 5.7 \times 10^{-6}$).

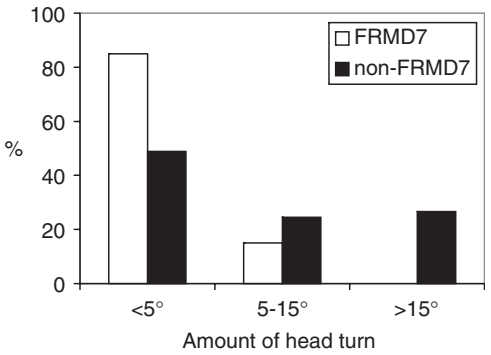

Fig. 3 Degree of head turn in subjects in the *FRMD7* and non-*FRMD7* groups. *X*-axis shows the three subgroups with different degrees of abnormal head position; the *Y*-axis the percentage of subjects. Open bars represent *FRMD7* and the filled bars denote the non-*FRMD7* group.

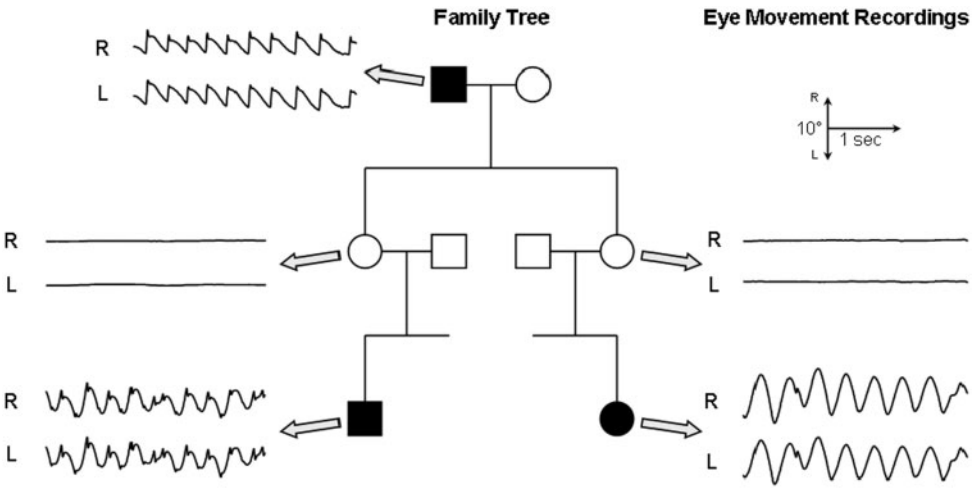

Fig. 4 Representative original eye movement recordings of five subjects from a family with FRMD7 mutation, plotted with the family tree. Three affected subjects have different waveforms whilst the carriers are unaffected.

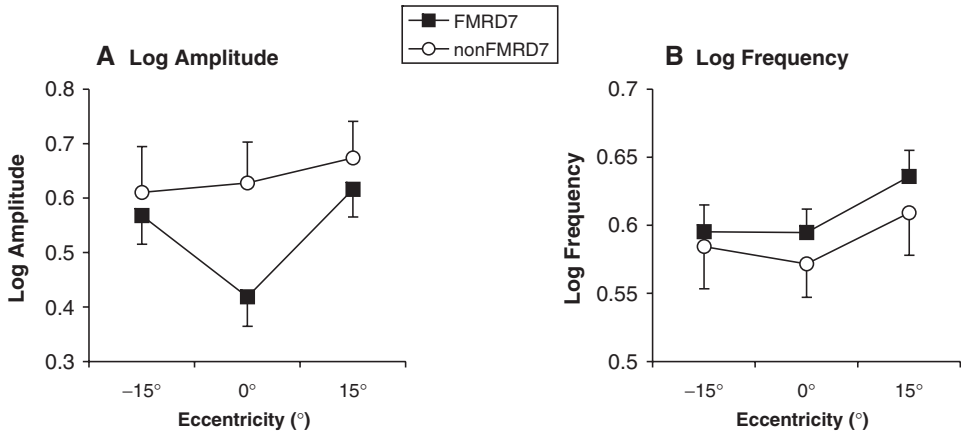

Fig. 5 (**A**) Logarithm of the mean amplitude (± SEM) and (**B**) Mean frequency (± SEM) of nystagmus in the *FRMD7* group and the non-*FRMD7* group in the three positions of gaze (−15° = 15° left gaze; 0 = primary position and +15° = 15° right gaze). The *FRMD7* group is represented with black squares and the non-*FRMD7* group is shown in white circles. The mean amplitude is significantly lower in primary position in the *FRMD7* group.

Nystagmus form and eye movement recording

In the group with mutations in *FRMD7*, all subjects had conjugate horizontal nystagmus as well as most of the subjects in the non-*FRMD7* group with the exception of two subjects who had conjugate vertical nystagmus. The amplitude, frequency and waveform of nystagmus varied considerably within families in both groups. Original eye movement recordings of subjects from a family which is representative of the *FRMD7* group is shown in Fig. 4.

We evaluated the amplitude and frequency of nystagmus in three positions of gaze and the waveform of nystagmus in these positions were noted.

Amplitude of nystagmus

There was considerable intra- and inter-family variability in the amplitude of nystagmus. The amplitude of nystagmus in 15° left gaze, primary position and 15° right gaze of the subjects are shown in Fig. 5A. The mean amplitude (±SD) of nystagmus in primary position for males in the *FRMD7* group was 3.86° (±3.52°), while the mean amplitude in the females was 3.27° (±2.44°). In the *FRMD7* group, there was a reduction of amplitude in primary position of gaze with increase of amplitude in 15°gaze to the right and left, while no significant differences in amplitudes between primary position of gaze and eccentric position of gaze was found for the non-*FRMD7* group (Fig. 5A, supplementary material: Fig. 1A). In the non-*FRMD7* group, the mean amplitude of nystagmus in primary position was 5.76° (±3.89°).

In the *FRMD7* group, gender ($F = 0.03$, $P = 0.87$) and family ($F = 1.72$, $P = 0.09$) were not significant predictors of log nystagmus amplitude, whereas eccentricity ($F = 11.6$, $P = 2.9 \times 10^{-5}$) was highly significant. In contrast, eccentricity ($F = 0.18$, $P = 0.83$) was not a significant predictor of

log amplitude in the non-*FRMD7* group. Gender ($F = 3.35$, $P = 0.07$) was also not a significant predictor of log amplitude in the non-*FRMD7* group. The effect of eccentricity on amplitude can be clearly seen in Fig. 5A where a marked reduction in the mean log amplitude is evident at primary position (0°) in the *FRMD7* group but not in the non-*FRMD7* group. Consequently, there was a significant difference in log amplitude between *FRMD7* and non-*FRMD7* groups at primary position ($F = 7.29$, $P = 0.009$) but not at −15° ($F = 0.14$, $P = 0.70$) or 15° ($F = 0.55$, $P = 0.45$) eccentricity.

Frequency of nystagmus

The mean frequency of nystagmus in primary position was 4.08 Hz (±1.16) for the *FRMD7* group and 3.89 Hz (±1.24) for non-*FRMD7* group (Fig. 5B, supplementary material: Fig. 1B). Mean log of the frequency showed similar patterns in both groups (Fig. 5B). Eccentricity was not a significant predictor for log nystagmus frequency in either group ($F = 1.86$, $P = 0.16$ for *FRMD7* and $F = 0.44$, $P = 0.65$ for non-*FRMD7*) (Fig. 5B). Log frequency of nystagmus was not dependent on gender ($F = 0.007$, $P = 0.93$ for *FRMD7* and $F = 1.11$, $P = 0.30$ for non-*FRMD7*). There was no significant difference between the log frequencies of the *FRMD7* group and non-*FRMD7* group overall ($F = 0.73$, $P = 0.39$).

Waveform characteristics

The waveforms of nystagmus were compared with the 12 forms described by Dell'Osso and Daroff (1975) (Supplementary material: Fig. 2). The waveform varied with direction of gaze with some degree of intra- and inter-family difference. The frequency of pendular and jerk nystagmus was compared between groups using the Pearson chi-squared test. Pendular-related waveforms were more commonly associated with the *FRMD7* group ($P = 0.0025$; pendular waveforms account for 45.3% in *FRMD7* group and 28.3% in the non-*FRMD7* group).

Obligate female carriers of a FRMD7 mutation

Fifty-eight obligate female carriers were identified clinically. Female carriers were identified as unaffected when they were mothers or daughters of affected males who did not have nystagmus on clinical examination (slit lamp and fundus examination). Thirty-one (53.4%) of the 58 carriers, were affected clinically. As mentioned earlier, the only significant difference between the affected females and males was the slightly better VA in the females. The nystagmus characteristics (amplitude, frequency and the waveform) were similar in both the groups (affected females and males). We were able to obtain eye movement recordings of 14 clinically unaffected female obligate carriers of a *FRMD7* mutation. Their mean age was 53.2 (range = 29–81). They were compared to 20 age-matched female control subjects who did not have any ophthalmological disease and were not related to subjects with nystagmus. They had normal Snellen VA (mean = 6/5). None of the carriers or controls had any strabismus or abnormal head posture. All of the carriers had normal stereo-acuity (Lang test). Eye movement recording of the clinically unaffected carriers revealed that one of them had sub-clinical nystagmus (amplitude 0.43° and frequency 2.86 Hz) (Tarpey *et al.*, 2006).

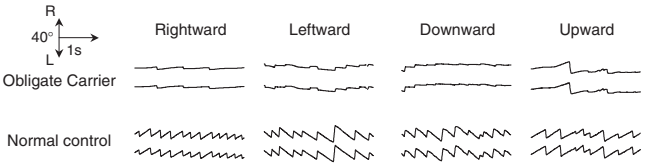

Fig. 6 Original eye movement recording of a 33-year-old obligate carrier (upper panel) and an 32-year-old control (lower panel), which shows the OKN response to a target moving at 20°/s in four directions. The OKN gain is poor in the carrier compared to the control.

Analysis of OKN in the unaffected obligate carriers showed a bimodal distribution, i.e. some of the carriers had good OKN gain, whilst the others had poor responses to optokinetic stimulus in most directions. An example of original eye movement recordings of OKN of a carrier and a control subject is shown in Fig. 6. This difference was significant for rightward (Mann–Whitney U-test, $P = 0.03$) and downward ($P = 0.002$) movement of the stimulus as shown in Fig. 7A. We also analysed horizontal and vertical smooth pursuit eye movements in clinically unaffected carriers and controls. There was no significant difference between the groups in the smooth pursuit gain and the number of catch-up saccades/second as shown in Fig. 7B ($P > 0.05$).

Discussion

We detected mutations in 15 of 16 (94%) families with IIN, which were linked to the NYS1 (Xq26-27) region and in another 8 of 14 (57%) families in whom linkage data were not available (Tarpey *et al.*, 2006). Mutations in *FRMD7* are likely to be the major cause of inherited IIN and this finding has been shared by a number of recent publications. Schorderet *et al.* (2007) detected mutations in five of six families with X-linked IIN. Three of three Chinese families with congenital motor nystagmus were found to have mutations in *FRMD7* (Zhang *et al.*, 2007*a*) A large Turkish family was also reported to have mutations of the gene (Kaplan *et al.*, 2007).

There are a few published studies in which the prevalence of these mutations was found to be lower. Zhang *et al.* (2007*b*) detected mutations in *FRMD7* in 4 of 14 (28.5%) Chinese families with X-linked nystagmus. In another study from the UK, only 20% of the families with IIN were found

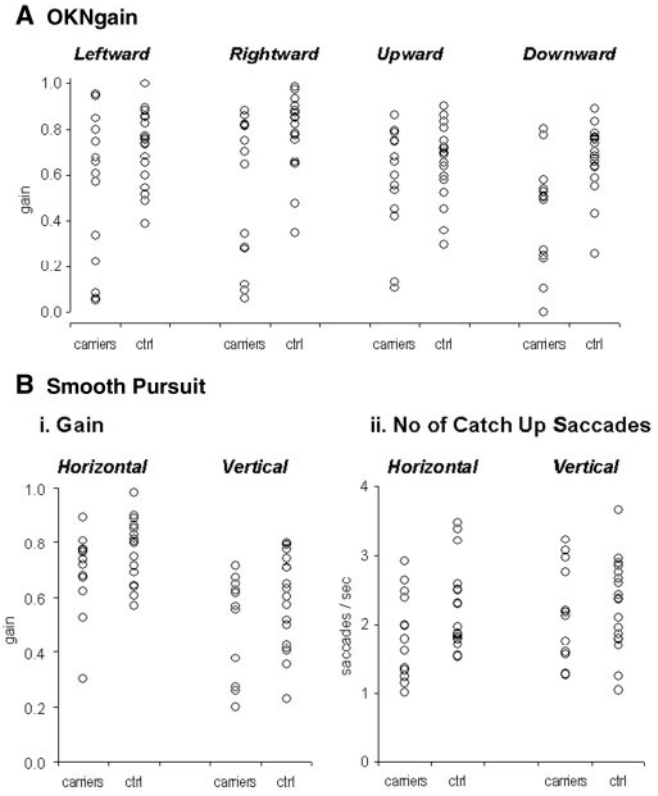

Fig. 7 (**A**) OKN gain of the obligate carriers and controls in the four directions of movement of the stimulus (leftward, rightward, upward and downward) moving at 20°/s. *X*-axis shows the two groups (carriers and controls), whilst the *Y*-axis depicts the OKN gain. OKN gain shows a bimodal distribution in the carriers especially in the leftward, rightward and downward gazes. (**B**) (i) Horizontal and vertical smooth pursuit gain in the obligate carriers and controls to stimuli moving at a linear velocity of 20°/s. *X*-axis shows the two groups (carriers and controls) and the *Y*-axis shows the smooth pursuit gain. In (ii) the number of catch up saccades per second is shown on the *Y*-axis.

to have mutations in the gene (Self *et al*., 2007). In our study, we examined several people from each family and if iris transillumination was detected in at least one subject, that family was excluded from the study to minimize the risk of including subjects with albinism, iris transillumination being one of the signs associated with ocular albinism. We believe that the utmost care taken in phenotyping has resulted in a higher prevalence of *FRMD7* mutations in our study. In the singletons with IIN, we cannot be certain that we might have overlooked the possible subtle iris transillumination in some subjects, especially in children where the examination is difficult, possibly resulting in an apparent lower frequency of mutations (7%) of this gene.

We did not find a difference in most clinical and eye movement characteristics in patients with and without a mutation in *FRMD7*. Vision, stereopsis and ocular alignment was very good with a median VA of logMAR 0.176 (6/9) in both groups. However, the *FRMD7* group had a significantly lower number of subjects with pronounced head turn and, correspondingly, had relatively smaller nystagmus amplitudes in primary position of gaze. Nystagmus waveforms showed large inter- and intra-familiar variation in both groups. However, pendular waveforms were significantly more common in the *FRMD7* group. Affected females had similar clinical and eye movement characteristics as affected males except for VA, which was slightly better in females. In unaffected carriers we found subtle abnormalities in OKN.

The prevalence of strabismus in general population has been reported to be between 3% and 6%. Graham (1974) reported that the prevalence of strabismus is 5.66% based on a study done on 4784 children. 4.2% of 1187 children were found to have strabismus in a study done by Chew *et al*. (1994). In a study done in Denmark, the prevalence of strabismus was found to be 3.2% (Kvarnstrom *et al*., 2001).

Compared to the general population, the prevalence of strabismus in IIN has been reported higher in the previous literature. In a study by Forssman (1971), the prevalence of strabismus in IIN is reported to be 16%. He reported that more than one-third of the subjects with IIN had normal or near normal visual acuity. In another study (Brodsky and Fray, 1997), the prevalence of strabismus in IIN was reported to be 17%. Recently, Self *et al*. (2007) reported that the prevalence of strabismus is 44% in IIN due to mutations in *FRMD7*. However, this study was only based on nine affected subjects. Compared to these previous studies the prevalence of strabismus in our study was lower in the *FRMD7* group (7.8%) and in the non-*FRMD7* group (10%). These differences could be explained by the fact that patients with *FRMD7* mutations are a homogeneous group. In addition, the lower prevalence of strabismus in our study may be partly attributable to our extensive examination of family members allowing better exclusion of patients with albinism and other eye diseases. The low incidence of strabismus in our study indicates that despite constant eye oscillations, subjects with IIN have good ability to fuse and so strabismus is only slightly more frequent compared to the general population (Chew *et al*., 1994; Graham, 1974). Subjects without associated strabismus had good stereo-acuity in both groups. The *FRMD7* group had good stereovision more frequently (on the Lang test) than the non-*FRMD7* group. This discrepancy could be explained by the observation that small squints are more difficult to measure on cover test and that stereovision might be a more sensitive measure for eye alignment in nystagmus.

It was interesting to note that none of the subjects in the *FRMD7* group had latent nystagmus. However, one subject with strabismus in the non-*FRMD7* group had a latent component to the nystagmus.

We found a median Snellen VA of 6/9 in subjects with IIN. Interestingly the VA in the two groups was very similar. We believe that we found a high level of VA because we carefully excluded other nystagmus forms such

as retinal diseases or albinism. Our study suggests that acuity below 6/18 (Fig. 2) should raise the suspicion of other underlying disease.

In their study on the clinical features of infantile nystagmus in the first 6 months of life, Hertle *et al.* (2002) have reported that 19% (five of 27 subjects) had an AHP. In a series by Abadi *et al.*, nine of 16 (at least 53%) subjects (15 patients with IIN and one with albinism) were found to have AHP (Abadi and Whittle, 1991). We found that the prevalence of significant AHP was much less in the *FRMD7* group than the non-*FRMD7* group. This suggests that the null region of the nystagmus is central in subjects with *FRMD7* mutations. However, the underlying anatomical basis of the null region characteristic of IIN is poorly understood. With *in situ* hybridization experiments we found that *FRMD7* is expressed in the human cerebellum, the developing neural retina and the lateral ventricles (Tarpey *et al.*, 2006). Possibly, these structures are involved in the formation of the null point position.

We found that there is intra- and inter-family variability in the amplitude and frequency of nystagmus in both the *FRMD7* and non-*FRMD7* group. Most of the waveforms described by Dell'Osso *et al.* were found in both groups. This is in accordance with the published literature. Kerrison *et al.* (1998) found that the waveform of nystagmus can differ in members of the same pedigree. Abadi *et al.* (1983) has reported dissimilar waveforms in monozygotic twins with nystagmus. Hertle *et al.* (2005) published the clinical features of a family with periodic alternating nystagmus. He reported the presence of subclinical nystagmus in one member of the family in addition to the intra-familial variability of nystagmus.

The variable phenotype in subjects with *FRMD7* mutations was not affected by the type of mutation. In this study, we have families with different types of mutations, i.e. truncating and missence mutations; however, the type of mutation did not predict the severity of clinical presentation. As in many other Mendelian disorders there is a high level of intra-familial variability of phenotype in our patients. This could probably be explained by the effect of other unlinked modifier genes and/or the effect of environmental factors. At present we are not sure if nystagmus is caused by a loss or gain of function of *FRMD7*.

We have shown that *FRMD7* is expressed in the retina and cerebellum (Tarpey *et al.*, 2006). It is interesting to note that patients with the mutations in this gene have good VA, suggesting that the retinal function is not severely affected. However, retinal involvement causative to nystagmus cannot be ruled out. The cerebellum has been associated with the neural integrator (Glasauer, 2003) and a number of acquired nystagmus forms (Leigh *et al.*, 2002) and therefore it is plausible that cerebellar dysfunction related to the *FRMD7* gene may be the pathology underlying IIN.

The penetrance of the gene was 53% among the carriers. It was interesting to note that one of the obligate carriers had sub-clinical nystagmus on eye movement recording. The basis of incomplete penetrance of this disease in females is currently not explained.

The optokinetic responses were subnormal in a subgroup of obligate carriers and this finding was not age related. However, smooth pursuit gain in obligate carriers and normal controls was similar. Smooth pursuit stimulus causing reversal of slow phase of nystagmus (Kelly *et al.*, 1989) and inversion of optokinetic responses (Halmagyi *et al.*, 1980) has been reported in patients with congenital nystagmus. In this context, absence of OKN response in these obligate carriers could be considered as a subtle sub-clinical manifestation of nystagmus. Eye movement recordings in obligate female carriers show a continuum between subjects having large amplitude nystagmus, small amplitude nystagmus, sub-clinical nystagmus, OKN abnormalities and normal eye movements.

In this article, we describe for the first time a clinical and eye movement analysis of a large homogeneous group of patients with nystagmus due to mutations in the *FRMD7* gene, comparing them to a group of patients with IIN not due to mutations in this gene. The median VA was 6/9 in both groups and in patients with a visual acuity less than 6/18, suspicion of the nystagmus not being idiopathic should be raised. We also found good ocular alignment and binocular vision in patients with IIN. Interestingly, more patients in the non-*FRMD7* group had AHPs and no decrease of nystagmus amplitude in primary gaze. We found that there is a wide variation of nystagmus waveforms even within families. Approximately half of the female carriers were clinically affected. However, some of the unaffected female carriers had subnormal OKN mainly in the horizontal direction. This most likely represents a sub-clinical affection of the eye movement system.

The clinical characteristics we have identified can be used to distinguish IIN from other forms of nystagmus, provide guidance as to what further investigations may be helpful, and assist in genetic counselling.

Supplementary material

Supplementary material is available at *Brain* online.

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