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Optic nerve hypoplasia in children
- prevalence, associated disorders and genetic causes

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OPTIC NERVE HYPOPLASIA IN CHILDREN

- PREVALENCE, ASSOCIATED DISORDERS AND GENETIC CAUSES

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Karolinska Institutet

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‘There is more here than meets the eyes.’

Murasaki Shikibu

ABSTRACT

Optic nerve hypoplasia (ONH) is a congenital ocular malformation with a thin optic nerve in one or both eyes. ONH has been associated with neurodevelopmental disorders and pituitary dysfunction, but the prevalence of these comorbidities are still unknown, especially in children with less severe visual impairment and unilateral disease. The aims of this thesis were to examine the prevalence of ONH in children, to determine the prevalence of intellectual disability, autism spectrum disorder, motor impairment, and pituitary dysfunction in unilateral and bilateral disease, as well as to identify genetic variants underlying ONH.

Methods: In study I, a population-based cohort of patients with ONH was established including patients who were diagnosed with ONH, below 20 years of age, and living in Stockholm in December 2009. Ophthalmological assessments were performed, and the Five to Fifteen parent questionnaire (FTF) was used to screen for developmental problems. Subsequently, study II and study III were population-based cross-sectional cohort studies. In study II, neurodevelopmental disorders were confirmed by the FTF, neurological assessments, reviewing previous neuropsychological investigations, or conducting neuropsychological tests. In study III, motor impairments were assessed using a specific protocol and previous neuroradiology was reviewed. Blood sampling was performed to screen for pituitary dysfunction together with analysis of growth curves and medical history. Study IV included 29 patients with ONH who were analysed with array comparative genomic hybridization followed by whole genome sequencing and *in silico* filtering of single nucleotide variants in 42 candidate genes. Rare variants were verified by Sanger sequencing.

Results: The prevalence of ONH in children in Stockholm was 17.3/100 000. Unilateral ONH was almost as common as bilateral (45%). Intellectual disability was more common in bilateral ONH than in unilateral ONH (56% vs 9%, $p< 0.001$). Autism spectrum disorders were diagnosed in 17%, without correlation to laterality. Motor impairments were identified in 47% of the patients and were significantly more prevalent in bilateral ONH. In contrast, pituitary hormone deficiency (PHD) was confirmed in 29% of the cohort and 19% had multiple PHD, without correlation to laterality. Furthermore, presence of structural pituitary abnormalities had a very high sensitivity for PHD. Finally, we identified three rare variants in *COL4A1* and three rare variants in *COL4A2*, of which two were assessed as likely pathogenic. In addition, we found an interesting rare variant in *OPA1* and two other likely pathogenic variants in *SOX5* and *PAX6*.

Conclusions: ONH is a common ocular malformation in children, with a prevalence of 17.3/100 000 in Stockholm. Unilateral ONH seems to be as common as bilateral. Children with both unilateral and bilateral ONH have a high risk of neurodevelopmental disorders and PHD, but bilateral ONH increases the risk of intellectual disability, motor impairment, and brain abnormalities. These findings need to be considered when designing screening, care, and follow-up programs. Our genetic study suggests that a genetic cause of ONH is more common than previously reported and highlights *COL4A1* and *COL4A2* as candidate genes.

LIST OF SCIENTIFIC PAPERS

I. Teär Fahnehjelm K, **Dahl S**, Martin L, Ek Ulla. Optic nerve hypoplasia in children and adolescents; prevalence, ocular characteristics and behavioural problems. Acta Ophthalmol. 2014;92(6):563-570.

II. **Dahl S**, Wickström R, Ek U, Teär Fahnehjelm K. Children with optic nerve hypoplasia face a high risk of neurodevelopmental disorders. Acta Paediatr. 2018;107(3):484-489.

III. **Dahl S**, Kristoffersen Wiberg M, Teär Fahnehjelm K, Sävendahl L, Wickström R. Hormonal and neurological dysfunction in optic nerve hypoplasia: a population-based cohort study (submitted manuscript).

IV. **Dahl S**, Pettersson M, Eisfeldt J, Flögel AK, Wickström R, Teär Fahnehjelm K, Anderlid BM, Lindstrand A. Genome sequencing unveils genetic heterogeneity in optic nerve hypoplasia and highlights *COL4A1* and *COL4A2* as candidate genes (manuscript).

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADHD	Attention deficit hyperactivity disorder
ADOS	Autism Diagnostic Observation Schedule
Array-CGH	Array comparative genomic hybridization
ASD	Autism spectrum disorder
BCVA	Best-corrected visual acuity
CNV	Copy number variant
COL4A1	Collagen type IV alpha 1 chain
COL4A2	Collagen type IV alpha 2 chain
CT	Computed tomography
FSH	Follicle-stimulating hormone
FSIQ	Full scale intelligence quotient
FTF	Five to Fifteen parent questionnaire
GH	Growth hormone
GHD	Growth hormone deficiency
HESX1	HESX homeobox 1
IGF-1	Insulin-like growth factor-1
IGFBP-3	Insulin-like growth factor-binding protein 3
LH	Luteinizing hormone
MRI	Magnetic resonance imaging
OCT	Optical coherence tomography
ONH	Optic nerve hypoplasia
OTX2	Orthodenticle homeobox 2
PHD	Pituitary hormone deficiency
PVL	Periventricular leukomalacia
RGC	Retinal ganglion cell
SNV	Single nucleotide variant
SOD	Septo-optic dysplasia
SOX2	SRY-box 2

TSH	Thyroid-stimulating hormone
WGS	Whole genome sequencing
WISC-IV	Wechsler Intelligence Scale for Children, Fourth Edition

1 INTRODUCTION

Optic nerve hypoplasia (ONH) is a congenital ocular malformation with a thin optic nerve with a reduced number of retinal ganglion cell axons, resulting in a visual outcome from near normal to blindness. It is one of the most common causes of visual impairment in children in developed countries and often occurs in association with neurodevelopmental disorders and hormone deficiencies. The prevalence of these comorbidities are still unknown, especially in children with less severe visual impairment and unilateral ONH.

1.1 HISTORY

The first reported patient with ONH was a child with a small and pale optic disc, nystagmus, and blindness, described by Magnus in 1884.[1] The first illustration of ONH was by Schwarz in 1915,[2] and in 1941, Reeves published the first association of ONH and absent septum pellucidum.[3] However, this association was later incorrectly ascribed to George de Morsier, who described two patients with absent septum pellucidum, where the first patient had a unilateral vertically rotated optic tract without any history of visual impairment, and the second had a visual field defect with enlargement of the blind spot suggesting a defect optic disc. From the literature, de Morsier supplemented these two patients with eight other patients with absent septum pellucidum and other ophthalmological disorders.[4] The only patient included with ONH was the one Reeves had discovered.[5] De Morsier associated optic nerve or ocular malformations with agenesis of the septum pellucidum and postulated the term 'la dysplasie septo-optique'.[4]

Later in 1970, Ellenberger and Runyan published the first case with unilateral ONH, absent septum pellucidum, and dwarfism.[6] The same year Hoyt et al described nine additional patients with ONH and pituitary dwarfism, and made the important association between ONH and growth hormone deficiency (GHD).[7] Four of these patients also had an absent septum pellucidum and Hoyt et al described it as 'septo-optic dysplasia' (SOD), referring to de Morsier. SOD is sometimes called de Morsier syndrome although it is not known if he ever identified a case of ONH.[5]

1.2 PREVALENCE OF OPTIC NERVE HYPOPLASIA

ONH is the most common optic nerve malformation[8] and one of the most frequent causes of visual impairment and blindness in children in developed countries.[9-12] In Swedish children with visual impairment, only cerebral visual impairment and non-hereditary optic atrophy were more common, and ONH was followed by retinopathy of prematurity as a causative factor in a study from 1997.[9] The prevalence of children with ONH has been reported to be increasing, partly due to increased awareness of the condition and improved diagnostics among ophthalmologists, but it may also represent a true increase.[13] According to the Swedish Register of Visually Impaired Children, i.e. with a best-corrected visual acuity (BCVA) of < 0.3, approximately seven children per 100 000 had ONH causing visual impairment, and the prevalence had significantly increased from 1980 to 1999.[13, 14] In

Northwest England, the prevalence of ONH/SOD was reported to be 10.9/100 000 in children younger than 16 years of age in 2006.[15] However, Patel et al concluded that the prevalence was likely underestimated due to lack of patients with ONH and mild visual impairment, and they did not report the proportion of unilateral and bilateral ONH.[15] Many other studies have included only patients with bilateral ONH or had a high proportion of bilateral ONH. For instance, in a large prospective study, 82% of the children had bilateral ONH.[16] Men and females have been reported to be equally affected by ONH.[9, 17]

1.3 AETIOLOGY

1.3.1 Embryology

1.3.1.1 Development of the optic nerve

The aetiology of ONH is in the majority of cases unknown, but is considered to be multifactorial with a combination of environmental factors and genetic vulnerability. The association with pituitary dysfunction and other brain malformations makes it important to understand the underlying embryology. The optic nerves, the anterior and posterior pituitary lobes, the hypothalamus, and the forebrain develop from the anterior neural plate.[18] The cranial neuropore closes on day 24 and the forebrain develops into the telencephalon (primordia of the cerebral hemispheres) and the diencephalon. During the early fourth fetal week, the optic vesicles, which are the primordia of the retinas and optic nerves, appear on each side of the diencephalon and remain attached to the brain via the optic stalks.[19] The optic vesicle evaginates towards the surface ectoderm (the future lens placode) and thereafter invaginates to become the optic cup (Figure 1). The inner layer of the optic cup develops into the neural retina, and the outer layer give rise to the retinal pigment epithelium (Figure 2). At the posterior pole of the optic cup is the optic nerve head, which is connected to the optic stalk. The first retinal ganglion cells (RGCs) appear in the sixth fetal week in the central retina, close to the optic nerve head, and later differentiated RGCs develop in more peripheral retinal regions. Consequently, later generated RGC axons have a longer distance to travel into the optic nerve and by the eighth week a solid optic nerve (cranial nerve II) is present.[20] Remarkably, over three million RGC axons must be guided to each optic nerve, and RGC neurogenesis is limited in time.[19-21]

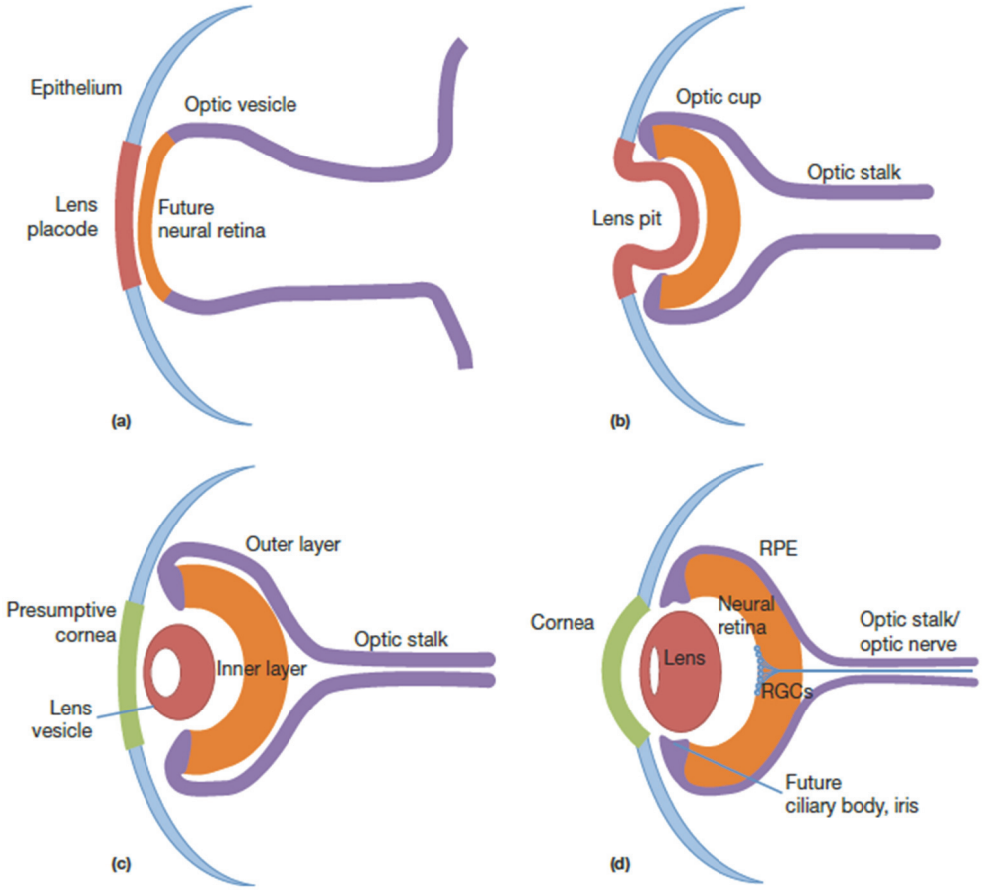

Figure 1. The development of the neural retina and the optic nerve. RPE, retinal pigment epithelium. Published with permission from the author Brown NL.[22]

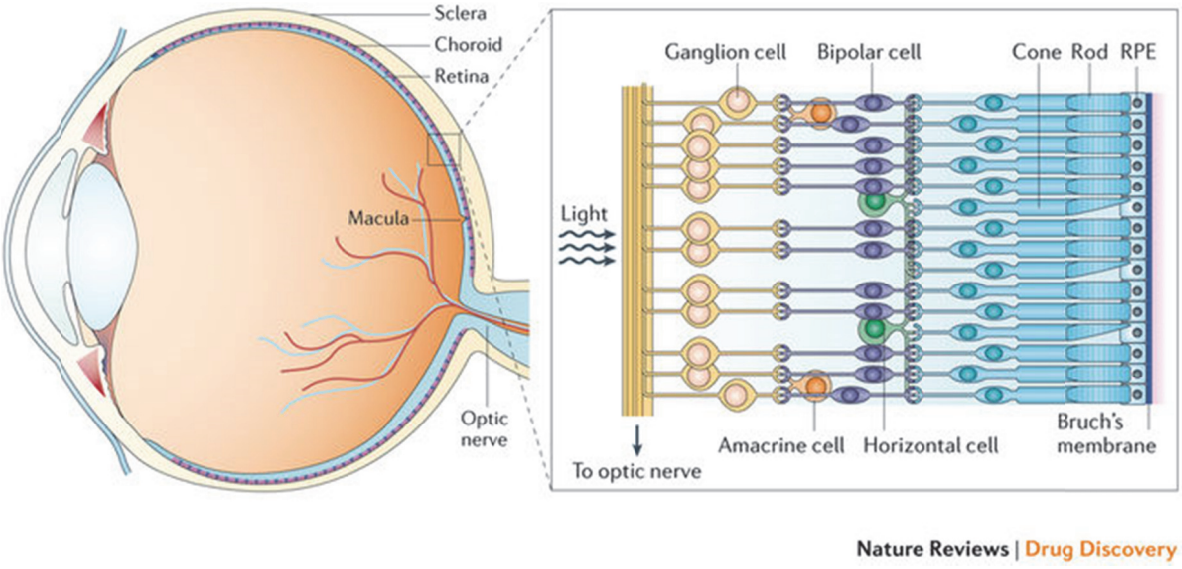

Figure 2. The cell types of the retina. RPE, retinal pigment epithelium. Reprinted by permission from Springer Nature: Nature reviews. Drug discovery. Current status of pluripotent stem cells: moving the first therapies to the clinic. Kimbrel EA, Lanza R, © 2015.

How the axon guidance is performed is not fully understood, but cadherins seem to be important in the axonogenesis of RGCs, and inhibitory chondroitin sulfate might prevent axonal growth in wrong direction. Furthermore, slit molecules are known to influence the growth cone, and within the retina they attract the RGC axons, but at the optic chiasm they work repulsive. This is explained by a mechanism where different molecules at the growth cone modulate the response. Another important strategy for the axon guidance is when later generated RGC axons find and fasciculate with earlier generated axons, which have already reached the optic nerve head.[19] Furthermore, the expression of sonic hedgehog (*Shh*) in RGCs has, in a mouse model, been shown to be important in order to extend the axons to the optic nerve.[23] The axons in the retinal nerve fiber layer are organized in the papillomacular bundle, the superior and inferior arcuate bundles, and the nasal bundle.

RGC axons in the dorsal retina are helped by the dorsal-ventral gradient of Eph and ephrin molecules. This explains why mutations in EPH receptor B2 (*Ephb2)* and *Ephb3* give rise to superior segmental ONH.[19] In this condition, there is a defect in the superior (dorsal) quadrant of the retina, resulting in an inferior visual field defect, but visual acuity is normal. In addition, there is an association between patients with superior segmental ONH and mothers with type 1 diabetes mellitus, indicating that metabolic pathways play a role in axon guidance.[24, 25]

RGC axons exit the eye through the optic disc. To pass the final exit from the retina to the optic nerve there is a netrin-dependent regulation for the RGC axons. Netrin-1 is an axon guidance molecule that binds to the DCC netrin 1 receptor (DCC), which is expressed by the RGC axons. *Netrin-1* mutant mice have ONH, which is also seen in animals without DCC function.[26] Interestingly, the absence of netrin-1 and DCC may also result in dysgenesis of corpus callosum and hypothalamus.[19]

Another gene associated with ONH, corpus callosum dysgenesis, and pituitary dysplasia is the transcription factor gene HESX homeobox 1 (*HESX1*). Many target genes for HESX1 are unknown, but probably include genes affecting axon guidance and cell migration.[19] HESX1 works like a functional antagonist to the transcription factor PROP paired-like homeobox 1 (PROP1*)*, which is also of importance in the pituitary organogenesis. *HESX1* mutations have been reported to create an inability to inhibit the activity of PROP1.[27, 28]

The growth of axons continues into the second trimester, and programmed apoptosis regulate the refinement of the connections throughout gestation. At the time of birth, approximately 1.2 million axons populate each optic nerve.[29] The optic nerves form the optic chiasm, where approximately half of the fibers cross over to the contralateral optic tract (decussation of medial fibers). The RGC axons form synapses in the suprachiasmatic nucleus of the hypothalamus, the superior colliculus and the pretectum in the midbrain, but the main relay between the retina and the visual cortex is the dorsal lateral geniculate nucleus in the thalamus (Figure 3). From there, the following optic radiation ends up in the visual cortex in the occipital lobe. Thereby, the visual pathway extends throughout the entire brain.

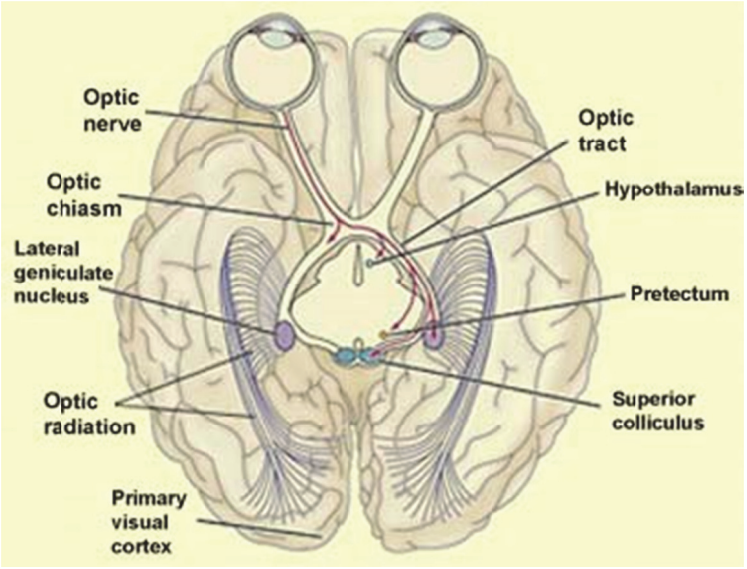

Figure 3. The visual pathway. Figure from The Brain from Top to Bottom, http://thebrain.mcgill.ca. Vision; The targets of the optic nerve. Copyleft .

In ONH, there is an underdeveloped optic nerve with less RGC axons. ONH is therefore suggested to be a result of failure of RGC neurogenesis, abnormal axonal development, dysregulation of neuronal apoptosis, or an insult leading to retrograde trans-synaptic degeneration of RGC and their axons.[19, 30, 31]

1.3.1.2 Development of the pituitary gland

At the same time as the optic nerve is developing, the formation of the pituitary gland is taking place. During the third fetal week, Rathke's pouch appears in the oral ectoderm and invaginates towards the ventral diencephalon and becomes connected to the downward extension of the hypothalamus called neurohypophysis or posterior pituitary lobe. The connection to the buccal cavity is then obliterated and Rathke's pouch becomes the adenohypophysis or the anterior pituitary lobe. Within a few days the progenitors of the five hormonal secreting cell types of the adenohypophysis start to proliferate (somatotropes, corticotropes, thyrotropes, gonadotropes, and lactotropes). The adenohypophysis and neurohypophysis form the pituitary gland in the sella turcica. This process is highly dependent on several transcription factors and signaling molecules and *HESX1* is expressed in the pituitary primordium, while Rathke's pouch is formed.[32] In addition, the signaling protein sonic hedgehog is important for the pituitary organogenesis, as well as for the development of the optic nerve.[33]

The hypothalamus orchestrates the adenohypophysis with several stimulatory releasing factors: growth hormone-releasing hormone, corticotropin-releasing hormone, thyrotropin-releasing hormone, and gonadotropin-releasing hormone. However, the hormone prolactin from the adenohypophysis is negatively stimulated by dopamine from the hypothalamus.[20] Finally, antidiuretic hormone (also called vasopressin) is released from the neurohypophysis,

which responds to signals from the neurons in the hypothalamus. These neurons connect the hypothalamus to the pituitary gland through the pituitary stalk, or infundibulum. Several transcription factors are involved in both hypothalamic and retinal development.[34]

1.3.2 Genetics

Although most cases of ONH are sporadic, there are also familial cases suggesting a genetic aetiology.[35-37] Mouse models and studies of familial cases have identified a number of genes relevant to the occurrence of ONH. Subsequently, ONH and SOD in humans have previously been associated with mutations in several developmental transcription factor genes; *HESX1*, SRY (sex determining region y)-box 2 (*SOX2*), *SOX3*, and orthodenticle homeobox 2 (*OTX2*), albeit in low frequency.[32, 36, 38, 39] Both point mutations, as well as gene dose variation have been described.[32, 39, 40]

The first homozygous mutation in *HESX1* in humans was described in two siblings with ONH, corpus callosum agenesis, absent septum pellucidum, and an ectopic neurohypophysis with panhypopituitarism.[36] Despite this interesting finding, the frequency of *HESX1* mutations in a large cohort of patients with SOD was less than 1%.[41] Until 2012, only five ONH patients with *HESX1* mutation had been described.[5] Still, this seems to be the gene which is most often analysed in children with ONH.

Furthermore, the transcription factors SOX2 and SOX3 are dose-dependent regulators of the sonic hedgehog transcription, and mouse embryos lacking the signaling protein sonic hedgehog have been reported to have severe ONH and pituitary hypoplasia.[33] In addition, mutations in the X-linked gene encoding calcium/calmodulin-dependent serine protein kinase (*CASK*) have been associated with brain malformations and ONH in humans.[42] Similarly, mutations in collagen type IV alpha 1 chain (*COL4A1*) have been associated with porencephaly, schizencephaly, cerebrovascular disease, and ophthalmological disorders including at least one patient with ONH.[43, 44] Moreover, it has been suggested that mutations in the *OPA1* gene, connected to optic atrophy 1, may underlay some cases of ONH.[45] In summary, there are an increasing number of single case-reports of mutations in different developmental genes, and besides X-linked inheritance, both autosomal dominant and autosomal recessive inheritance have been described.

ONH has also been associated with different syndromes, e.g. cardio-facio-cutaneous syndrome[46], Noonan syndrome[47], and trisomy 13.[17] An overlap in symptomatology with Kallmann syndrome (idiopathic hypogonadotropic hypogonadism and anosmia/hyposmia) has led to the detection of prokineticin receptor 2 (*PROKR2*) mutations in two patients with ONH.[48]

In conclusion, mutations in different genes have been described in only a minority of cases with ONH. However, due to the increasing rate of genetic testing of affected individuals, several developmental genes have been proposed as candidate genes for ONH.[32, 49] Nevertheless, except for the study of McNay et al,[41] there has not been any genetic cohort study of ONH. Subsequently, it is unknown to what extent genetic aetiologies contribute to

ONH and few patients with ONH are offered genetic testing. Further genetic studies would help us to better understand the development of the visual pathways, the connection to hypothalamus and pituitary abnormalities, and brain development.

1.3.3 Prenatal risk factors

Several ONH studies have reported young maternal age and primiparity as predominant maternal features.[14, 50-52] Patel et al reported a higher prevalence of ONH in urban areas with high population densities and speculated that predisposing aetiological factors such as environmental or lifestyle factors may be linked to deprivation.[15] In another cohort study, there was a high prevalence of prenatal maternal weight loss or poor weight gain, indicating an important role of nutrition.[52]

Furthermore, ONH has long been associated with alcohol and drug abuse, and in a Swedish study of 30 children with fetal alcohol syndrome (FAS), ONH was found in 48% of the eyes.[53] In addition, Ribeiro et al reported ONH in 25% of the eyes in Portuguese children with FAS.[54] The causality is supported by a recent animal study that showed that prenatal ethanol exposure cause ONH by selective inhibition of sonic hedgehog signaling in retinal progenitor cells, resulting in failure to extend axons to the optic nerve.[23] The study strongly indicates that the timing of exposure during fetal development is essential for the phenotype.[23]

Other suggested aetiological factors are prenatal exposure to prescription drugs (e.g. anticonvulsants, antidepressants), maternal smoking, viral infections (e.g. congenital cytomegalovirus infection), and maternal diabetes.[14, 31, 51] However, these prenatal exposures were not significantly increased in the large cohort study of Garcia-Filion et al.[52] These authors have suggested that future research should focus on the significance of young maternal age, primiparity, prenatal nutrition, maternal weight gain, and other factors of deprivation.[55] Recently, ONH has been described in infants with prenatal Zika virus infection and microcephaly, emphasizing the risk of ONH in certain congenital infections.[56, 57]

Another hypothesis is that ONH is a result of a vascular destructive lesion,[58, 59] and it has been speculated that early gestational bleeding as well as use of vasoconstrictors (e.g. tobacco, cocaine) may increase the risk of a vascular insult.[60] Interestingly, increased prevalence of gestational vaginal bleeding (33%) has been reported from a larger cohort study of children with ONH.[52]

1.4 OCULAR CHARACTERISTICS

1.4.1 To diagnose ONH

ONH is diagnosed when there is a small, often pale, optic disc in combination with a reduced BCVA and/or a visual field defect. The optic disc is examined by direct or indirect ophthalmoscopy and in fundus photographs. In the latter, the optic disc can be measured manually or with a digital method. The manual Zeki method involves measuring the distance between the optic disc and the macula, and then dividing it with the mean diameter of the optic disc. A ratio of three or more strongly indicates ONH.[61-64] The mean disc diameter is calculated by adding the transverse and vertical disc diameters and then dividing by two. Half of the mean diameter of the optic disc is then added to the distance between the fovea and the temporal margin of the optic disc to obtain the disc-macula distance (Figure 4).[61] Some ophthalmologists do the inverted calculation, measuring the ratio of the horizontal disc diameter to the distance between the macula and the temporal edge of the optic disc, and values below 0.35 are indicative of ONH.[65, 66] It should be noted that there are some patients with ratios of 0.30 to 0.35 who have a normal vision. Another way to estimate the optic disc is to relate the optic disc area to the size of the overlying central retinal vessels.[51]

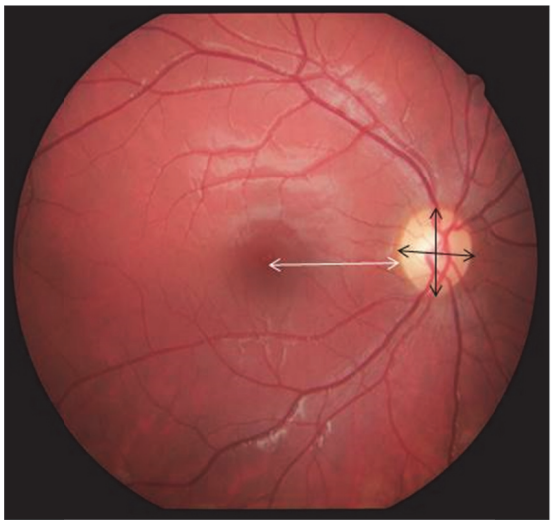

Figure 4. Demonstration of the manual Zeki method in measuring the optic disc size in a patient with a normal optic disc.[61] The mean disc diameter is calculated by adding the transverse and vertical disc diameters (black arrows) and then dividing by two. Half of the mean disc diameter is then added to the distance between the fovea and the temporal margin of the optic disc (white arrow) to obtain the disc-macula distance. The disc-macula distance divided with the mean disc diameter is less than three in a normal optic disc.

Both tortuous retinal arteries and veins, and abnormal straight vessels with less branching are well described in ONH.[31] In addition, a ring of hypo- and/or hyperpigmentation commonly surrounds the optic disc, and this is called a 'double ring sign', though not pathognomonic for ONH (Figure 5). The 'double ring sign' indicates that a thinner optic nerve, with a reduced number of axons, is in the wider scleral canal.[67] ONH may also be seen on magnetic

resonance imaging (MRI), showing thin optic nerves and/or an abnormal optic chiasm.[68, 69] The evaluation of the optic nerves on MRI is most often subjective and less reliable than ophthalmological assessments, but Lenhart et al have published an objective method to calculate the mean optic nerve diameter on high-resolution orbital MRI, which may be helpful to determine whether ONH is present.[70] In addition, spectral-domain optical coherence tomography (OCT) has lately been shown to be a useful tool in diagnosing ONH in cases of diagnostic uncertainty, demonstrating smaller optic disc diameter and thinner retinal nerve fiber layer and ganglion cell layer.[71, 72]

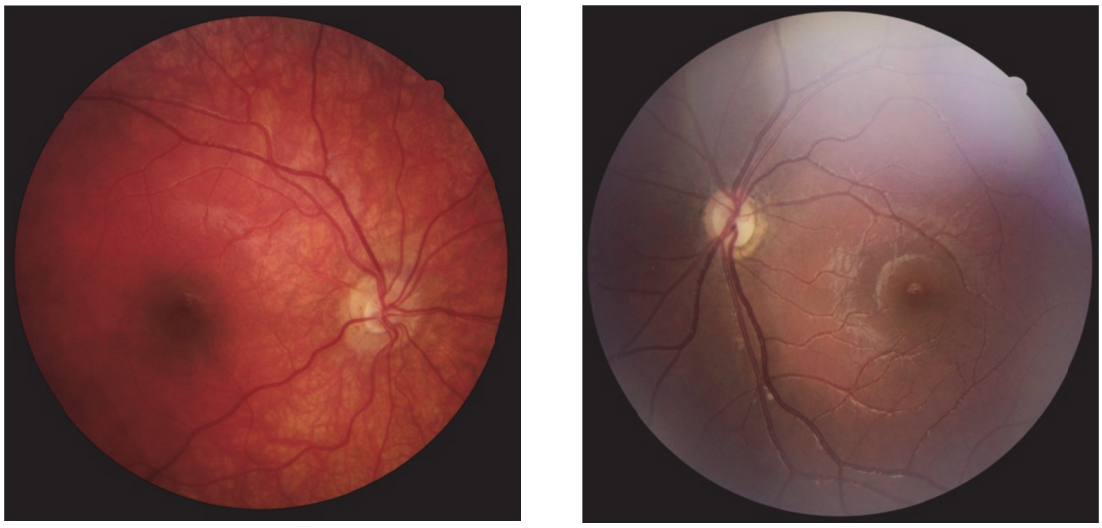

Figure 5. Two fundus photographs of ONH from two patients. The left photograph is showing a small optic disc with a surrounding scleral atrophy/'double ring sign' and the BCVA was 0.1. In the right photograph, there is a distinct 'double ring sign' and the BCVA was 0.016.

1.4.2 Special variants of ONH

The definition of ONH sometimes includes optic nerve head cupping associated with periventricular leukomalacia (PVL).[73] These patients have a normal sized optic disc with an enlarged cup, and subsequently, less axons. Another form of ONH is superior segmental ONH which results in an inferior visual field defect. These two special forms of ONH differ significantly from the more generally hypoplasia of the optic nerve regarding the risk of neurodevelopmental and endocrinological disorders.[51, 74]

1.4.3 Visual impairment

The central feature of ONH is the risk of visual impairment. In children with severe bilateral ONH, the most common presenting symptoms are lack of fixation, poor visual behaviour, roving eye movements or nystagmus, and strabismus.[31, 51] However, there are also cases with milder visual impairment presenting with strabismus or detected in visual screening at older age. The visual acuity ranges from near normal to blindness. In the study of Hellström et al,

24% of the patients were blind and 43% had severe visual impairment (visual acuity < 0.3).[31] Moreover, different patterns of visual field defects have been reported.[75]

ONH is a non-progressive disease, and in fact, the visual acuity may improve during the first four years of life, because of myelinisation and visual maturation.[76]

1.5 HYPOTHALAMIC AND PITUITARY DYSFUNCTION

1.5.1 Hypopituitarism

Since 1970, ONH has been associated with hypopituitarism.[6, 7] The main hypothesis is that a dysfunctional hypothalamus fails to orchestrate the pituitary gland,[77] but there may also be defects of the pituitary infundibulum or the pituitary gland itself.[78] Hypopituitarism or pituitary hormone deficiency (PHD) is the lack of one pituitary hormone or several hormones, i.e. multiple PHD. GHD is considered to be the most common PHD in ONH, followed by central hypothyroidism. Deficiency in adrenocorticotropic hormone (ACTH) impairs the function of the adrenal glands and leads to deficiency of corticosteroids. Cortisol deficiency can induce hypoglycemia and may be life-threatening in combination with stress. Dysfunction of gonodotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) can result in either precocious or late puberty.[77, 79] Furthermore, the water balance is disturbed when there is a deficiency of antidiuretic hormone, and may give rise to central diabetes insipidus. Hyperprolactinemia is common in ONH, and is explained by dysfunction of the dopaminergic inhibition from hypothalamus.[16, 77]

1.5.2 Prevalence of hypopituitarism

The prevalence of hypopituitarism in unilateral and bilateral ONH is still unknown despite many studies, and ranges from 28% to 72% depending on study design and population.[79-82] This is likely explained by an underrepresentation of children with unilateral ONH and milder disease in most studies. In a prospective study, including 87% bilateral ONH, endocrinopathies were described in 69% of the children with ONH.[81] GHD was reported as the most common PHD (68%), followed by central hypothyroidism (44%), ACTH deficiency (31%), and diabetes insipidus (11%).[81] In another cross-sectional study of 101 children with ONH (71% bilateral ONH), patients with bilateral ONH had a significant greater risk of PHD than unilateral ONH (35% and 10%, respectively).[80] This is in contrast to the study of Ahmad et al, who reported that hormone deficiencies were not associated with laterality of ONH.[66] To further complicate the picture, the hormonal dysfunction may be congenital or develop over time, and there is an uncertainty regarding how often late-onset hormonal dysfunction evolve, which has been shown for GHD, central hypothyroidism, ACTH deficiency, and diabetes insipidus.[83, 84] In addition, puberty disturbances are not well investigated. In a retrospective study, Oatman et al found two patients with precocious puberty (2/101, 2%) and 4/8 (50%) patients had gonadotropin deficiency.[79]

Population-based studies are needed to establish the prevalence of PHD and to conclude whether children with unilateral ONH or a structural normal pituitary have a less pronounced

risk of hypopituitarism. Unilateral ONH seems to be a milder disease, and these children may benefit from a less extensive monitoring program as well as information of a more positive prognosis.

1.5.3 Septo-optic dysplasia

Historically, the absence of septum pellucidum has been given an important clinical significance in determining the risk of PHD. However, many studies contradicts this theory,[30, 66, 77, 80, 81] and several researchers advocate abandoning the term since it has repeatedly been shown that an absent septum pellucidum does not increase the risk of hypopituitarism. Still the concept of SOD is commonly used in the clinic and in research. SOD is defined by any combination of ONH, midline neuroradiological abnormalities (including corpus callosum agenesis and absent septum pellucidum), and pituitary hypoplasia/hypopituitarism.[32] The broad inclusion criteria of SOD mean that a patient with SOD does not always have ONH. Consequently, it can be difficult to compare studies of SOD patients with ONH studies.

1.5.4 Structural pituitary abnormalities

In ONH, radiological findings of structural pituitary abnormalities have been reported to be highly predictive of PHD, but there is an ongoing debate regarding the negative predictive value of a normal pituitary gland on MRI for exclusion of PHD in children with ONH.[80, 81] This is an important clinical question, whether all children with ONH should have a thorough endocrinologically follow-up, or if those with a normal pituitary gland should be offered a less meticulous follow-up, or none at all.

Ramakrishnaiah et al have published a cross-sectional study of 101 children with ONH, showing that neurohypophyseal abnormalities (including absent pituitary infundibulum, ectopic posterior pituitary bright spot, and absent posterior pituitary bright spot) on MRI had a sensitivity of 96% for PHD and a specificity of 92% for normal pituitary function.[80] On the contrary, both Oatman et al and Garcia-Filion et al concluded from two large studies that a structurally normal pituitary is a poor predictor of normal pituitary function, and does not exclude hormonal dysfunction in children with ONH.[79, 81] This debate is ongoing.

1.5.5 Hypothalamic dysfunction

Hyperprolactinemia has been suggested to be a marker of hypothalamic dysfunction in children with ONH, due to reduced dopaminergic tone.[78] However, more recent findings challenge its role as a reliable prognosticator of pituitary dysfunction.[85] Other symptoms of hypothalamic dysfunction in ONH are hyperphagia and obesity, hypophagia, body temperature dysregulation, water-seeking behaviour, and abnormal sleep-wake cycles.[51, 86]

1.6 NEUROLOGICAL DYSFUNCTION

1.6.1 Neurodevelopmental disorders

Neurodevelopmental disorders are reported to be common in children with ONH. In 1984, Margalith et al described 51 children with ONH in a population-based study, including 92% with bilateral disease. Seventy-one percent had mental retardation, 57% cerebral palsy, 37% epilepsy, and 20% behavioural problems. The behavioural problems included attention deficit disorder, aggressive, or autistic behaviour.[50]

Another study of 93 children with ONH identified developmental delay in 46% of the group with bilateral ONH, poor vision and nystagmus, but normal development in all 11 children with unilateral ONH.[74] The authors concluded that an eye examination could predict the risk of developmental disorders.[74] Significantly more difficulties were reported from a prospective study which included 18% unilateral ONH and demonstrated developmental delay in 39% of children with unilateral ONH and 78% in bilateral disease.[16] However, being a selected cohort from a referral center in USA, there is a risk of selection bias and the prevalence may be overestimated. Still, they showed that bilateral ONH increased the risk of development delay, as did corpus callosum hypoplasia and hypothyroidism.[16]

In general, blind children have been reported to have a higher risk of autism spectrum disorders (ASD),[87] but few studies have focused on the risk of ASD in children with ONH. Two smaller Swedish studies of visually impaired children with bilateral ONH reported ASD in 29% (8/28) and 69% (9/13), respectively.[88, 89] A third study described ASD in 31% of a cohort with 83 children with bilateral ONH and profound or severe visual impairment.[90] There is a paucity of data concerning less severe and unilateral ONH.

1.6.2 Neurological impairment

The prevalence of motor impairments in children with ONH is unknown. Fahnehjelm et al identified hypotonia, dyskinesia, or motor delay in 21% (6/28) in a selected cohort with bilateral ONH and severe visual impairment,[88] and Signorini et al described a high degree of motor clumsiness in a retrospective study.[91] In addition, several studies have reported cases of cerebral palsy and epilepsy.[31, 50, 88, 91-94] For exemple, Margalith et al described epilepsy in 37% of a selective cohort with 92% bilateral ONH and 57% had cerebral palsy,[50] and Fahnehjelm et al found epilepsy in 21% of a subgroup of patients with bilateral ONH and severe visual impairment.[88] Nevertheless, population-based studies, including patients with less severe visual impairment and unilateral disease, are needed to understand the risk of motor impairment, cerebral palsy, and epilepsy in children with ONH.

1.6.3 Brain malformations

As mentioned earlier, ONH is closely associated with several brain malformations. The most commonly reported are absence of septum pellucidum, corpus callosum agenesis, and pituitary abnormalities, but also more rare conditions such as schizencephaly, porencephaly, and hydrocephalus have been reported (Figure 6).[30, 50, 74, 80, 95] Furthermore, Hellström et al

identified PVL in 10% of a large cohort with 93% bilateral ONH.[31] Depending on study design and population, previous reports have presented a prevalence of brain abnormalities of 60-74% in ONH.[81, 94] Nonetheless, it is still a clinical question whether all children with ONH should have a MRI of the brain.

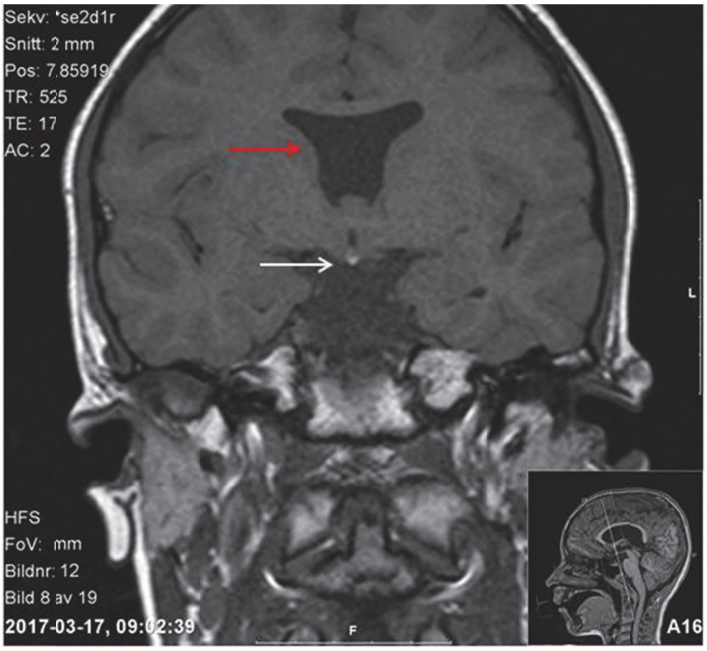

Figure 6. MRI demonstrating absent septum pellucidum in the midline (red arrow) and an ectopic posterior pituitary bright spot (white arrow). The child had bilateral ONH, multiple pituitary hormone deficiency, and a normal intelligence. The best-corrected decimal visual acuity was 0.125 right and left eye.

2 AIMS OF THE THESIS

2.1 GENERAL AIMS

The general aims of the thesis were to examine the prevalence of ONH in children and to determine the prevalence of neurodevelopmental disorders, motor impairments, and pituitary dysfunction in unilateral and bilateral disease. In addition, the aim was to identify novel genes relevant to ONH and in as many children as possible to identify a genetic cause.

2.1.1 Study I

The aims were to report the prevalence of unilateral and bilateral ONH, ocular characteristics and coexisting behavioural problems in children with ONH in Stockholm, Sweden. The hypotheses were that ONH is more prevalent and the proportion of unilateral ONH is higher than previously reported. We also hypothesized that behavioural problems are more common in bilateral disease.

2.1.2 Study II

The aim was to examine the prevalence of intellectual disability and ASD in patients with unilateral and bilateral ONH. Our hypotheses were that these problems would be common, but that unilateral disease would be associated with a less pronounced risk.

2.1.3 Study III

The aims were to describe the prevalence of neurological and hormonal dysfunction in a population-based cohort of patients with ONH, comparing unilateral and bilateral disease. Furthermore, the aims were to identify structural brain abnormalities and investigate whether these could be used prognostically. We hypothesized that patients with unilateral ONH would have a lower risk of neurological and pituitary dysfunction.

2.1.4 Study IV

The last study aimed to identify genetic variants underlying ONH in a well-characterised cohort of patients with ONH. The hypothesis was that genetic causes are more common than previously reported.

3 METHODOLOGICAL CONSIDERATIONS

In this chapter, the methods used in the four studies will be summarized, and strengths and weaknesses will be discussed.

3.1 POPULATION-BASED COHORT

The foundation of the four studies is a population-based cross-sectional cohort of children and adolescents with ONH, who were below 20 years of age and living in the county of Stockholm in December 2009. The database at the St. Erik Eye hospital was searched for diagnosed optic disc malformations (Q14.2, Q14.8) and SOD (Q0.44), according to the *International Classification of Diseases*, Tenth Revision (ICD-10). The consultants at Astrid Lindgren Children's Hospital were asked to report the same conditions. Since the St. Erik Eye hospital is the only department of paediatric ophthalmology in Stockholm along with a consultant neuropaediatric eye department at Astrid Lindgren Children's Hospital, we were able to identify all the children and adolescents with a diagnosis of ONH in our region. Altogether, 176 children and adolescents with optic nerve malformations were detected.

After excluding patients with other optic disc malformations than ONH, patients from other counties, and patients who had been incorrectly diagnosed, 79 patients with ONH were identified. Letters of invitation were sent out for enrolment together with information about the study and request for informed consent to all 79 patients. Of these, 53 families accepted clinical assessments and another 12 families gave permission to review the medical records and previous neuropsychological investigations. In addition, one family accepted review of the ophthalmological medical record. Five families declined to participate, and eight families were not reached despite a second letter and repeated phone calls. However, these 13 patients were included in the prevalence study of ONH. On the other hand, the estimate of ONH prevalence was based on children below 18 years of age, and therefore, three older patients were excluded from the prevalence study, leaving 76 patients instead of 79 (Figure 7). In study II and III, 65 patients were included, accounting for 82% of the eligible cases (Figure 7).

This is a unique population-based cohort of patients with ONH from the rather large county of Stockholm with a population of 2.1 million (Statistics Sweden 2010). Although we believe we were able to detect all children and adolescents with a diagnosis of ONH in the region, mild and asymptomatic cases may have been missed. However, a search for asymptomatic cases in the general population would require an extremely large study sample, which was not feasible.

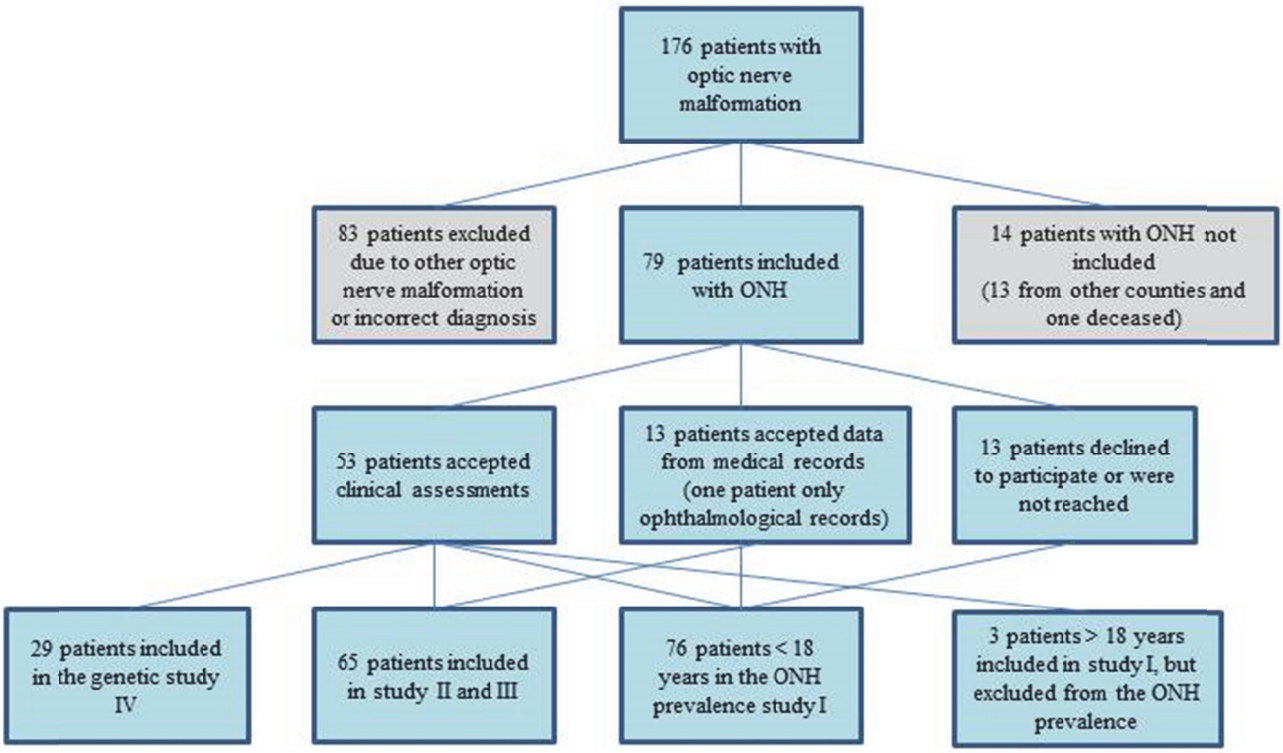

Figure 7. Flow-chart of the patients included in study I-IV.

3.2 OPHTHALMOLOGICAL ASSESSMENTS

The diagnosis of ONH was confirmed when there was a small optic disc in combination with visual deprivation and/or a visual field defect.

3.2.1 Fundus photography

Digital fundus photographs were obtained using either a Canon EOS-1 Kodak Professional DCS 520C (Rochester, NY, USA) or a Canon CRDG non-mydriatic retinal Camera (Tokyo, Japan). Two different measurement methods were used to reach high validity in the measurements of the optic disc: the manual Zeki method[61, 64] and the digital semi-automated Retinal Size Tool.[96] In the Zeki method, described in the introduction section, a ratio of ≥ 3 of the optic disc to macula distance to the mean diameter of the optic disc was considered to suggest the diagnosis of ONH (Figure 4). In the Retinal Size Tool method, the end-points of the long and short diameters of the optic disc and cup are marked by the examiner and the areas are calculated assuming elliptical shapes. The distance between the macula and the optic disc centers is used as a reference value to convert pixel number to a millimeter scale, and subsequently, compensating for the differences in magnification caused by the camera and eye optics. The disc area, cup area, and rim area were compared with reference values from a cohort of 49 healthy children with the median age of 15 years.[97] In the current study, ONH was defined as the mean disc area ≤ - 2SD, corresponding to < 1.31 mm^2 in the reference group.[97]

A study limitation is that it was not possible to obtain fundus photographs from all the patients because of poor cooperation or poor quality of the photographs (e.g. due to nystagmus). On the other hand, this study supports that the simple manual Zeki method is reliable and valuable in diagnosing ONH.

3.2.2 Best-corrected visual acuity

The patients underwent clinical assessment of BCVA using methods suitable for the age and capacity; the Teller acuity cards (Precision Vision, Woodstock, IL, USA),[98] Lea symbols (LEA-Test Ltd, Helsinki, Finland),[99] HVOT test,[100] and Konstatin Moutakis chart (Ortho KM, Lund, Sweden).[100] BCVA was classified according to the World Health Organization (WHO) as blindness (0 - < 0.05), severe visual impairment (0.05 - < 0.1), moderate visual impairment (0.1 - < 0.3), or mild or no visual impairment (≥ 0.3). In study I and II, BCVA was described and analysed as decimal acuity, but in study III, BCVA was supplemented and converted to logMAR (minimal angle of resolution) values (-0.2 to 1.5; decline with better visual acuity) in patients with BCVA ≥ 0.05 to be able to use a continuous variable in the analysis of neurological impairment. The BCVA from the totally blind patients are not represented on test charts and could not be described as either decimal BCVA or logMAR. In order to include the blind patients in the analysis of the correlation between BCVA and comorbidities, we categorized the patients in different groups according to the WHO's classification.

3.2.3 Other ophthalomological assessments including visual fields

The patients' intraocular pressures were assessed with a non-contact tonometry (Topcon CT-80 Computerized Tonometer, Japan) or a rebound tonometer, the Icare.[101] The presence of strabismus was tested and the refraction was assessed in cycloplegia. In addition, visual field examinations were performed if the patient could cooperate. Computerized Rarebit technique has been shown to be suitable for assessment of children from six years of age and was used to examine the 30 degree visual fields.[102, 103] The test consists of presentations of one or two high-contrast light spots (microdots) briefly presented against a dark background on a liquid crystal display screen. The patient responds with the corresponding single or double click on the mouse. The results are presented as the overall mean hit rate, and a hit rate ≥ 90% and < 5 depressed locations (out of 30 test areas) were used as the limit for normal visual field results.[104, 105]

A limitation is that Rarebit visual fields could be performed in only 16 patients in one or both eyes (in total, 24 eyes). Nevertheless, all these examinations, except for one, gave reliable results.

3.3 NEUROPSYCHOLOGICAL ASSESSMENTS

3.3.1 The Five to Fifteen parent questionnaire

In both study I and II, we used the Five to Fifteen parent questionnaire (FTF) to screen for developmental problems. The questionnaire was sent to the parents before the clinical

assessment and was collected during the visit. The FTF is a validated tool for assessing general development and behaviour in children five to 15 years of age.[106-108] The questionnaire consists of 181 items representing eight domains: motor skills, executive functions, perception, memory, language, learning, social skills, and emotional/behavioural problems. Each statement can be answered as either: does not apply (score of zero), applies sometimes or to some extent (score of one), or definitely applies (score of two). The mean domain scores were calculated and compared with normative data for the relevant age group.[106, 107] A score of at least the 90th percentile is indicative of definite developmental problems and a score of at least the 98th percentile indicates major developmental problems.

In study I, the FTF was used to provide an overview of the type and severity of developmental problems in children and adolescents with ONH. The eight domains are usually presented separately, but in study I, we made an attempt to combine the domains to be able to compare developmental problems between patients with unilateral and bilateral ONH. Therefore, also each domain was given a score of zero, one, or two, indicating that the domain result was below the 90th percentile, above the 90th percentile, or above the 98th percentile, respectively. Subsequently, the maximum total domain score was 8x2= 16.

This model made it possible to compare the median total domain scores (range 0-16) between unilateral and bilateral ONH, and there was a statistically significant difference, indicating that patients with bilateral ONH have more developmental problems. Nevertheless, this model has been criticized for not being validated, and in study II, we strictly followed the FTF manual.

In study II, the FTF was used to exclude intellectual disability and ASD in patients who had not had a neuropsychiatric assessment. In these patients, normal intelligence was defined as a normal neurological assessment, ordinary schooling, and a Five to Fifteen learning mean domain score below the 90th percentile (this domain is only validated for children from the age of nine). In the same way, ASD was excluded in patients with normal neurological assessment, ordinary schooling, and a Five to Fifteen social skill mean domain score below the 90th percentile. In study II, we only used the FTF for children between five to 15 years of age, and to calculate a mean domain score, more than 50% of the items within the domain needed to be answered. Borderline cases may have been missed, but the FTF has a high sensitivity for intellectual disability and ASD.[108] Nevertheless, the FTF is not validated for testing visually impaired children and was only used to exclude intellectual disability or ASD in patients with BCVA $\geq$ 0.3.

The FTF is a comprehensive questionnaire and to answer all the 181 items is time consuming. In total, forty-eight families returned the questionnaire, and after exclusion of patients younger or older than five to fifteen years of age and incomplete questionnaires, 34 questionnaires remained. The results from the learning and social skill domains were used in study II if other neuropsychological assessment was absent. A drawback from using the FTF is that some parents of children with severe developmental problems returned incomplete questionnaires. Some parents also commented that these questions were not relevant to their

child, and probably they were not motivated to fill in the lack of skills, which is understandable. This could have been improved if the parents received assistance while filling in the questionnaire. However, most families answered all the items and contributed to the data on developmental problems in patients with ONH.

3.3.2 Wechsler Intelligence Scales

In study II, we retrieved and re-evaluated previous neuropsychological investigations. Patients who had not been tested previously were tested by a psychologist with the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV),[109] or if they were older than 18 years of age, the Wechsler Adult Intelligence Scale, Fourth Edition.[110] The results were analysed with regard to the four indices: verbal comprehension, perceptual reasoning, freedom from distractibility, and processing speed, as well as for full scale intelligence quotient (FSIQ). The FSIQ is based on a mean of 100 and standard deviation of 15 and is derived from a combination of 10 core subtests. It is considered to be the most representative estimate of global intellectual functioning. The mean index scores and mean FSIQ scores, namely the mean composite scores, were compared between the patients with unilateral and bilateral ONH and correlated to BCVA. Intellectual disability was defined as an IQ of less than 70 combined with deficits in adaptive functioning.

An advantage of using the Wechsler Scales is that we could report the prevalence of intellectual disability rather than the wider concept of developmental delay reported previously.[16] Intellectual disability is by definition a dysfunctional condition that needs to be identified. The Wechsler scales are validated, have normative data, and are well-established both in the clinic and in research to evaluate global intellectual functioning. However, as with most neuropsychological methods, they are not validated for visually impaired individuals. Consequently, in children with severe visual impairment or blindness, neuropsychological tests, such as the Wechsler Scales, need modifications and various complementary tests are often used.[111] In study II, patients with severe visual impairment or blindness had previously been assessed with different neuropsychological methods in different combinations, and a skilled psychologist, experienced in testing visually impaired children, re-evaluated every neuropsychological investigation to ensure that the diagnosis of intellectual disability had been correctly made. Still, it is a study limitation that the patients were examined using different neuropsychological tests. Within the study, one patient with BCVA < 0.1 was tested with WISC-IV. Due to blindness she could not participate in the subtests for perceptual reasoning and processing speed, but performed so well in the other subtests that she was considered to have above average intelligence (but could not get a FSIQ-score).

3.3.3 Autism assessment

Patients who fulfilled several criteria for ASD (Figure 8) or attention deficit hyperactivity disorder (ADHD) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM), Fifth Edition,[112] were referred for further investigations. A diagnosis of ASD or ADHD was established in cases where thorough neuropsychiatric assessment revealed

symptoms fulfilling DSM-V[112] or the previous Fourth Edition[113] criteria for ADHD or ASD, with the latter including autistic disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified. Patients with merely autistic traits were not included as having ASD. In patients who were not neuropsychologically investigated and had BCVA ≥ 0.3, we used the FTF to exclude ASD when the social skill mean domain score was below the 90th percentile, and the neurological assessment was normal, as well as ordinary schooling.

A weakness of the study design is that no standardized autism assessment was conducted. However, there is a lack of reliable testing instruments for ASD in visually impaired children. Promising efforts have been made to modify the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview, Revised (ADI-R),[114] but these need to be validated further.

Diagnostic criteria for autism spectrum disorder (ASD)

An individual must meet criteria A, B, C, D, and E.

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history:
   1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interest, emotions, or affect; to failure to initiate or respond to social interactions.
   2. Deficits in nonverbal communicative behaviours used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understandning and use of gestures; to a total lack of facial expressions and nonverbal communication.
   3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behaviour to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

B. Restricted, repetitive patterns of behaviour, interests, or activities, as manifested by at least two of the following, currently or by history:
   1. Stereotyped or repetitive motor movements, use of objects, or speech.
   2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behaviour.
   3. Highly restricted, fixated interests that are abnormal in intensity or focus.
   4. Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment.

C. Symptoms must be present in the early developmental period (but may not become fully manifest until social demand exceed limited capacities, or may be masked by learned strategies in later life).

D. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

E. These disturbances are not better explained by intellectual disability or global developmental delay. Intellectual disability and ASD frequently co-occur; to make comorbid diagnoses of ASD and intellectual disability, social communication should be below that expected for general developmental level.

Figure 8. Diagnostic criteria for autism spectrum disorder (ASD) simplified from DSM-5.[112]

3.4 NEUROLOGICAL ASSESSMENT

A structured neurological assessment was conducted by a paediatrician, blinded to the patient's clinical characteristics. The protocol was based on motor function tests that distinguish children with deficits in motor control[115, 116] adjusted for a visually impaired population. The protocol included gross motor function tests (walking on heels, the Fog test, standing on one leg for 20 seconds, jumping on one leg 20 times, and alternating jumps) and fine motor function tests (pencil grip, drawing a person, threading beads, finger opposition test, and diadochokinesis for 10 seconds), as well as reflexes, muscle tone, head circumference, speech, and contact. To be assessed as pathological, the gross and fine motor function domains required two findings of marked motor abnormalities. Children younger than six years of age were examined according to the Swedish screening form used at the Child Health Centers including developmental assessment and neurological examination.[117] Hand preference was determined based on observation during the assessment and asked for. The parents and children/adolescents were also interviewed regarding the medical history. After the visit, data on other diagnoses (e.g. cerebral palsy and epilepsy) and results from neuroradiology were collected from medical records.

Although this is the first study which have neurologically examined and presented prevalence of motor impairment in patients with ONH, it is a study weakness that the neurological protocol we used was only partly validated. It was designed for study III, building on the work of Touwen,[118] Gillberg,[115] and Landgren et al.[116] Furthermore, the analysis of the results was in agreement with Hadders-Algra's book 'The Neurological Examination of the Child with Minor Motor Neurological Dysfunction'.[119]

We did not have a proper control group, but before the final decision if a patient had ONH or not, nine patients with other optic nerve malformations were also examined according to the neurological protocol, and could be considered a small control group regarding motor impairments. None of the nine children in the control group had impairments in gross or fine motor function. Their median age at neurological assessment was 11.5 years (range 5.6-15.8 years) and was similar to the ONH patients', but logMAR value was median 0.0 (range -0.2-0.19) and all the patients had only mild or no visual impairment, in contrast to the ONH cohort. In this small control group, three patients had optic nerve coloboma, two had morning glory syndrome, two had optic pits, one had other optic disc anomaly, and one patient was misdiagnosed and had normal optic discs. The finding that no neurological impairments were identified in the small control group indicates that the neurological protocol at least did not overestimate motor impairments. In addition, the motor impairments identified with the neurological protocol matched the result from the FTF motor skill domain in 80% of the patients with ONH. To our knowledge, there are no validated neurological protocols for visually impaired children.

3.5 NEURORADIOLOGICAL RE-EVALUATION

In study III, neuroradiological imaging was reviewed by a neuroradiologist blinded to the patient's clinical characteristics and the previous interpretation. Using a specific protocol, attention was especially directed towards the optic nerves, optic chiasm, bulbus oculi, adenohypophysis, neurohypophysis, pituitary infundibulum, hypothalamus, septum pellucidum, and corpus callosum, but other abnormalities were also sought after (e.g. schizencephaly, holoprosencephaly, hydrocephaly, cortical abnormalities including hippocampal malformations, white matter abnormalities, and PVL. The neurohypophysis was considered abnormal if the neurohypophysis bright spot was ectopic, or absent in combination with a pathological pituitary infundibulum. The neuroradiological findings were analysed in relation to laterality of ONH, neurological impairments, and PHD.

Neuroradiological imaging was available for review in 51 cases (45 MRI and 6 computed tomography (CT) scans), whereas six patients had not undergone neuroimaging. MRI is superior to CT when examining the pituitary gland, and some other studies have simply excluded CT scans when evaluating brain malformations.[81, 82] In contrast, we chose to include CT scans when they were assessable for the particular abnormality we were examining. Thereby, we could identify one patient with a large brain malformation including corpus callosum hypoplasia, schizencephaly, encephalocele, and hydrocephalus, and another patient with a hypoplastic adenohypophysis, and a third patient with corpus callosum agenesis and absent septum pellucidum. Nevertheless, the neuroimaging we reviewed was of different quality, especially with regard to the pituitary gland. The oldest CT scan was from 1994 and the oldest MRI from 1996. However, 88% of the reviewed neuroimaging was from the 2000s.

3.6 HORMONAL ASSESSMENT

In study III, the patients who had accepted clinical assessments were offered blood sampling for screening for hypopituitarism. Blood sampling was made for fasting morning glucose, cortisol, insulin-like growth factor-1 (IGF-1), insulin-like growth factor-binding protein 3 (IGFBP-3), free T4, TSH, gonadotropins (LH, FSH), testosterone, estradiol, sodium, and prolactin. Medical records were reviewed for earlier diagnoses of endocrinopathies, hormonal treatment, and pathological growth curves.

Absence of GHD was defined as normal height in relation to midparental target height (height SDS > -1.5 SD below target height), height SDS > -2.5 SD regardless of target height,[120] and IGF-1 SDS > -2 SD (https://www.endocrinesciences.com/services/tools/calculator-igf1). Patients with a height SDS ≥ 0 SD and IGF-1 SDS > - 2SD were considered to have no GHD even if target height was missing. Patients with suspected GHD underwent provocative testing or recurrent blood sampling for spontaneous growth hormone (GH) secretion profile during 12-24 hours. Cut-off for GH provocative testing was a peak GH level < 7μg/L, defining GHD. Patients treated with GH were secured to have a positive treatment response (delta-height SDS of at least +0.5 SDS over the first year) to verify the diagnoses of GHD.

ACTH deficiency was suspected in patients with low morning cortisol (< 100 nmol/L) and confirmed by a pathological stimulation test or repeated subnormal levels of cortisol. Central hypothyroidism was diagnosed if the free T4 level was below the reference range while TSH was in the lower reference range. Diabetes insipidus was suspected in cases of polydipsia, polyuria, or hypernatremia and was diagnosed with the water deprivation test or in infants, a positive response to desmopressin. Precocious puberty was defined in girls as Tanner pubertal stage B2 prior to the age of eight and in boys, a testis size of at least 4 ml prior to the age of nine. Gonadotropin deficiency was defined as no breast development by age 13 in females or testis size below 4 ml by age 14 in males.

A paediatric endocrinologist assessed the patient's pituitary function based on previous clinical evaluation, laboratory test results, and growth parameters. Subsequently, the prevalence of hormonal dysfunction was calculated for unilateral and bilateral ONH. PHDs were then analysed in relation to structural pituitary abnormalities on neuroradiological imaging, as well as to neonatal hypoglycemia (< 2.6 mmol/L) and treatment-demanding neonatal jaundice.

Hormonal assessments are often difficult to evaluate and different criteria for diagnoses of PHD is probably one reason for the shifting prevalence of PHD in previous studies. We have taken into account the growth of the child in our assessment of GHD, and hormonal replacement therapy alone was not sufficient to confirm a PHD diagnosis. As a consequence, all patients classified as having GHD had a significant GH treatment response. Current guidelines for GH provocative testing stress that the threshold that distinguish normal from partial GHD (peak GH level 5-10 μg/L) responsive to treatment is not established, and that the diagnosis of GHD should not rely only on GH provocative testing.[121] Our approach complies with these guidelines.

The population-based cross-sectional cohort is the greatest strength of this study, but also the careful re-evaluation of each patient and strict inclusion criteria for diagnoses of PHD. Study limitations are that not all the patients participated in the blood sampling and a few patients were not sufficiently investigated for PHD, so they were excluded from some analyses.

3.7 GENETIC ANALYSIS

Genomic DNA derived from whole blood from 29 patients with ONH was collected according to standard protocols for genetic analyses in study IV.

3.7.1 Array comparative genomic hybridization (array-CGH)

A high-density custom-designed array-CGH was created using Agilents online web tool for array design, eArray. The used design was Agilent 2x400K HD-CGH microarray, consisting of approximately 400 000 oligonucleotide probes. Design and laboratory protocol for the array have been published previously.[122] Detection limits for duplications were set to 0.3 and for deletions 0.6. All aberrations called by Cytosure Interpret Software were manually

inspected and classified according to the American College of Medical Genetics guidelines.[123]

This custom-designed array-CGH reveals gene dose alterations and is able to detect both large copy number variants (CNVs) and small intragenic variants in 2000 target genes.

3.7.2 Candidate gene panel

A panel of 42 candidate genes previously associated with ONH, optic nerve aplasia, optic atrophy, or foveal hypoplasia was compiled by literature review in OMIM, PubMed, and the Developmental Brain Disorders Database. The panel included transcription factor genes (e.g. *HESX1, SOX2, SOX3, SOX5, OTX2, PAX6, NR2F1, VAX1, ATOH7*), tubulin genes, genes involved in chromatin remodeling, RNA splicing, or glycosylation, and other developmental genes (e.g. *OPA1, CASK, COL4A1, COL4A2*). The detailed candidate gene list, including references, is presented in paper IV, supplementary Table S1.

The reason to include genes involved in optic nerve aplasia, optic atrophy, and foveal hypoplasia was that these disorders are closely related to ONH and may occur in the same individual. Thus, the disorders may have the same genetic aetiology. The chosen candidate genes have been associated with the above mentioned disorders in humans or in animal models. The candidate gene list has continuously been updated during the course of the study.

3.7.3 Whole genome sequencing and variant validation

Whole genome sequencing (WGS) was performed on 2.2 µg genomic DNA at National Genomics Infrastructure (NGI) Stockholm using the Illumina XTen platform. The samples were prepped using a paired end PCR-free library, resulting in an average read depth of 30X and a mean insert size of 350 base pairs. Sequencing data was processed using the NGI-piper pipeline (https://github.com/johandahlberg/piper). Structural variants were analysed using the FindSV pipeline (https://github.com/J35P312/FindSV), combining CNVnator[124] and TIDDIT.[125] Variant calling was performed using Bcftools[126] and freebayes,[127] the resulting vcf files were merged using GATK combine variants and annotated using VEP.[128] Further, single nucleotide variants (SNVs) and indels (insertions or deletions of nucleotides) in the 42 candidate genes were filtered with a maximum frequency of 0.01 in several normal variant databases including ExAC,[129] the 1000 Genomes Project (1000G),[130] and the Swedish population frequency database (SweFreq).[131]

The variants were scored using SIFT (Sorting Intolerant From Tolerant)[132] and PolyPhen,[133] the two most commonly used prediction tools for interpretation of genomic variation. Both algorithms predict whether a SNV has an effect on protein structure based on sequence conservation and the physical properties of amino acids. The PolyPhen score ranges from 0.0 (tolerated) to 1.0 (deleterious), while SIFT score ranges the opposite, from 0.0 (deleterious) to 1.0 (tolerated). The two models were interpreted together, and deleterious predictions set to moderate or high were kept. Finally, the variants were manually filtered based on their quality through inspection using IGV[134] and previous literature. The remaining candidate

variants were confirmed with Sanger sequencing and parental samples were analysed to determine the mode of inheritance.

To obtain a manageable number of variants we chose to filter the WGS data using an *in silico* panel of 42 candidate genes. Since the current knowledge of genetic causes of ONH is limited, the candidate gene list was far from complete. However, an advantage with WGS is that it enables analysis of additional candidate genes as they are discovered. For example, when we identified several candidate variants in *COL4A1* we added the gene encoding collagen type IV alpha 2 chain (COL4A2), which forms a complex with COL4A1. Subsequently, we discovered three additional candidate variants in *COL4A2*.

3.8 STATISTICAL METHODS

The statistical methods used in this thesis are described in detail in the respective paper. In all the papers, a p-value < 0.05 was considered statistically significant. Statistical analyses were performed using STATXACT 4 (CYTEL Software Corporation, Cambridge, MA, USA) and STATISTICA 10.0 (StatSoft Inc, OK, USA) in paper I and STATISTICA 13 (StatSoft Inc, OK, USA) in paper II-IV.

Independent samples *t*-test was used to compare continuous variables and when the assumptions underlying the *t*-test were not met, the Mann-Whitney U test and Kruskal-Wallis ANOVA by ranks were used instead. Analyses with chi-square test or Fisher's exact test was performed to investigate differences in proportions of categorical variables. One-way ANOVA was used to investigate group differences between the mean index scores in paper II. In multivariable analyses, logistic regression was used to investigate categorical outcome in study III. In paper I, curvilinear regression was performed to measure the association between disc size parameters from the two methods.

3.9 ETHICAL CONSIDERATIONS

Ethical approval from the regional ethics committee in Stockholm was received for study I before commencing the first study. This approval was later supplemented for study II-IV before performance of these studies. Written informed consent for participation and publication of the results was obtained from the parents and from adolescents older than 15 years of age. All studies were performed according to the Declaration of Helsinki.

4 RESULTS

4.1 PREVALENCE OF ONH

In study I, we identified 76 patients with ONH who were below 18 years of age and living in the county of Stockholm in December 2009. The corresponding population at the time was 438 897 individuals (Statistics Sweden) and the prevalence of ONH was thus determined to be 17.3/100 000. Of the 66 patients in the clinical study, 30 patients had unilateral ONH and 36 had bilateral ONH. Accordingly, 45% had unilateral ONH. The same result was demonstrated in the clinical assessment of 53 patients (24 unilateral ONH (45%) and 29 bilateral ONH (55%)). This is a high proportion of unilateral ONH, which contributed to the high prevalence of ONH and offers a good opportunity to investigate comorbidities in the unilateral group.

There were 18% (12/65) who were blind, and 28% (18/65) of the patients had a BCVA < 0.3, and all of them had bilateral ONH (Table 1). In contrast, all patients with unilateral ONH had a binocular BCVA ≥ 0.3 and thereby mild or no visual impairment.

Table 1. Best-corrected visual acuity (BCVA) in 65* patients with bilateral or unilateral optic nerve hypoplasia (ONH)

	Bilateral ONH Total number of patients = 36 BCVA binocular	**Unilateral ONH** Total number of patients = 29* BCVA in the eye with ONH
Amaurosis – < 0.05	12/36	13/29
0.05 – < 0.1	1/36	3/29
0.1 – < 0.3	5/36	8/29
≥ 0.3	18/36	5/29

*Information missing in one patient. Corrected and modified from paper I.

4.2 PREVALENCE OF NEURODEVELOPMENTAL DISORDERS

In study II, we examined the prevalence of neurodevelopmental disorders, with the focus on intellectual disability and ASD. Other conditions included in neurodevelopmental disorders are ADHD, specific learning disorders (dyslexia, dysgraphia, and dyscalculia), and communication disorders.

Analysis of 40 FTF questionnaires (22 cases of bilateral and 18 cases of unilateral ONH) revealed more developmental problems in the bilateral group (median score 3.0 vs 0.5, p< 0.05) in study I. To further investigate the prevalence of intellectual disability and ASD in patients with ONH, we collected 30 previously performed neuropsychological investigations and tested additional 15 children with the WISC-IV,[109] and one adolescent with Wechsler Adult Intelligence Scale, Fourth edition[110] in study II. Of the 16 patients tested in the study, 15 had a normal intelligence, and one patient had a mild intellectual disability. In total, composite scores from the Wechsler Scales were available for analysis in 34 patients (16

unilateral and 18 bilateral ONH), of whom 31 patients had FSIQ scores (Figure 9). The median age for testing was 10 years (range 5-19 years) and 53% were males.

Patients with bilateral ONH had lower mean FSIQ scores (mean 84.4, range 47-127) than patients with unilateral ONH (mean 99.4, range 75-133), p= 0.049 (Figure 9). However, the bilateral group displayed a wide spectrum ranging from moderate intellectual disability to above average intelligence. In contrast to FSIQ, the four Wechsler indices of verbal comprehension, perceptual reasoning, freedom from distractibility, and processing speed did not correlate to laterality.

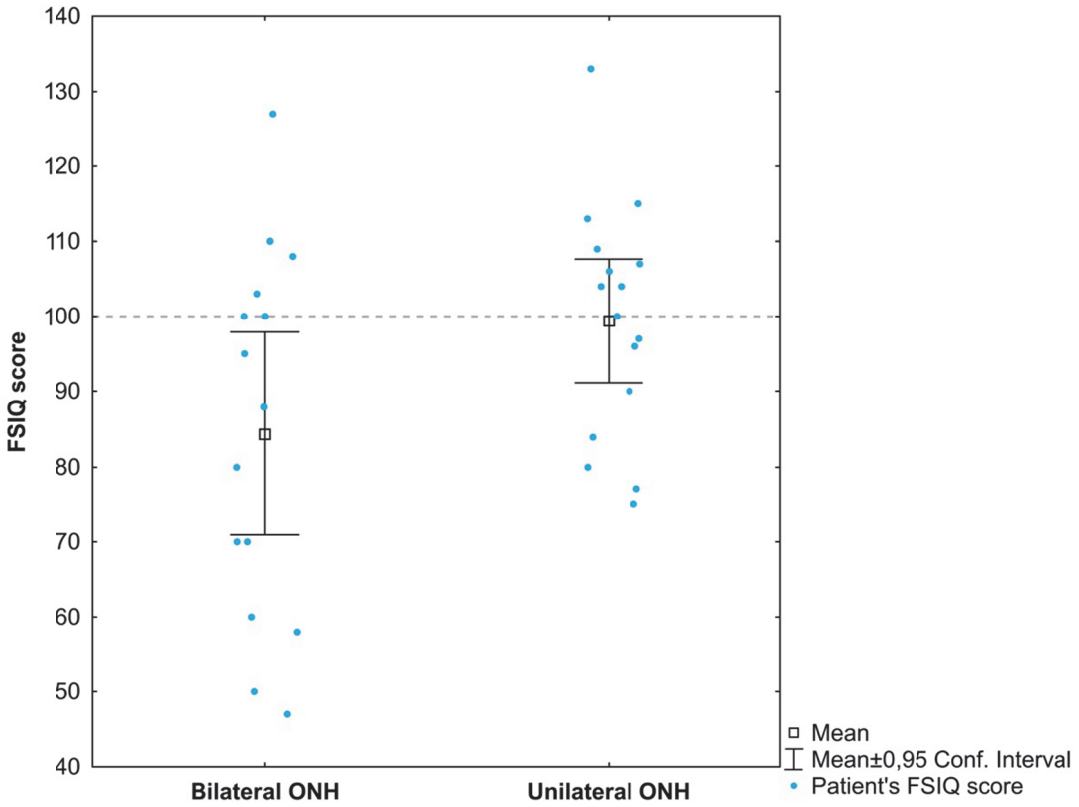

Figure 9. Results show full scale IQ (FSIQ) scores in 31 patients (15 bilateral and 16 unilateral ONH). Patients with bilateral ONH had significantly lower mean FSIQ scores compared with unilateral ONH (84.4 vs 99.4, p= 0.049).

The prevalence of intellectual disability was 36% (20/55) of the patients eligible for the analysis. The median age at diagnosis was 5.5 years (range 1.7-21.0 years) and there was an equal gender distribution. In the bilateral group, 56% (18/32) had an intellectual disability and in the unilateral group, 9% (2/23). Thus, intellectual disability was significantly more common in patients with bilateral ONH (p< 0.001) (Table 2).

ASD was diagnosed in 17% (7/42), five males and two females. The median age at diagnosis of ASD was 10.3 years (range 5.8-15.6 years). In patients with bilateral ONH, 24% (5/21) had ASD, and in unilateral ONH 10% (2/21), but there was no significant difference in prevalence (p= 0.41) (Table 2). Furthermore, there were several patients who had more than one diagnosis of neurodevelopmental disorder, and two patients had both ASD and intellectual disability. However, ASD did not correlated to lower FSIQ scores (patients with

ASD had mean FSIQ score 95.4 vs 94.0 in patients without ASD, p= 0.89). In addition, five patients (3 unilateral and two bilateral ONH) had a diagnosis of ADHD, and four of them also had an ASD.

Intellectual disability was associated with severe visual impairment (BCVA 0.05 - < 0.1) and blindness. On the other hand, all patients with a BCVA < 0.1 had bilateral ONH, and within the bilateral group, there was no significant difference between the proportion of intellectual disability in patients with BCVA < 0.1 or ≥ 0.1, respectively. This suggests that visual impairment, in itself, did not increase the risk of intellectual disability. In addition, ASD did not correlate to BCVA (BCVA < 0.1 vs ≥ 0.1, p= 1.0).

Table 2. Characteristics of the patients with ONH. Summary from paper II and III.

Variables	Total	Unilateral ONH	Bilateral ONH	P value*
Laterality	65	30/65 (46)	35/65 (54)	
Sex				0.32
Female	33/65 (51)	13/30 (43)	20/35 (57)	
Male	32/65 (49)	17/30 (57)	15/35 (43)	
Age[a], median (range), y	16.1 (8.1-27.5)	15.1 (9.9-25.5)	17.5 (8.1-27.5)	0.11
Gestational age[b], median (range), w	39 (24-43)	39 (24-43)	40 (27-43)	0.09
Intellectual disability	20/55 (36)	2/23 (9)	18/32 (56)	< 0.001*
Autism spectrum disorder	7/42 (17)	2/21 (10)	5/21 (24)	0.41
Neurological impairment	24/51 (47)	4/24 (17)	20/27 (74)	< 0.001*
Gross motor dysfunction	22/51 (43)	4/24 (17)	18/27 (67)	< 0.001*
Fine motor dysfunction[c]	15/50 (30)	1/24 (4)	14/26 (54)	< 0.001*
Cerebral palsy	6/65 (9)	1/30 (3)	5/35 (14)	0.21
Epilepsy	9/65 (14)	1/30 (3)	8/35 (23)	0.03*
Hypopituitarism				
Any PHD[d]	13/45 (29)	4/19 (21)	9/26 (35)	0.51
Growth hormone deficiency	11/52 (21)	3/22 (14)	8/30 (27)	0.32
ACTH deficiency	8/49 (16)	2/21 (10)	6/28 (21)	0.44
Central hypothyroidism	7/57 (12)	1/25 (4)	6/32 (19)	0.12
Diabetes insipidus	5/63 (8)	1/28 (4)	4/35 (11)	0.37
Precocious puberty	1/41 (2)	1/15 (7)	0/26 (0)	0.37
Gonadotropin deficiency	1/31 (3)	1/12 (8)	0/19 (0)	0.39
Multiple PHD	9/48 (19)	2/21 (10)	7/27 (26)	0.26
Hyperprolactinemia	4/32 (13)	0/13 (0)	4/19 (21)	0.13
Neuroradiological findings	27/51 (53)	7/22 (32)	20/29 (69)	0.01*
Pituitary abnormalities	12/44 (27)	4/18 (22)	8/26 (31)	0.73
Absent septum pellucidum	9/51 (18)	4/22 (18)	5/29 (17)	1.0
Corpus callosum agenesis	3/50 (6)	1/22 (5)	2/28 (7)	1.0
White matter substance loss[e]	13/46 (28)	3/18 (17)	10/28 (36)	0.20
Hippocampal malformations	10/49 (20)	1/22 (5)	9/27 (33)	0.02*

Data are n/N (%), if not otherwise specified. The total number of patients varies depending on complete assessments, available laboratory tests, and assessable neuroradiological imaging.
**P* value < 0.05 was considered statistically significant.
[a] Age at the analysis of the results in January 2018. [b] Gestational age, known in 62 patients, 29 unilateral and 33 bilateral ONH. [c] Fine motor function could not be tested in a patient with bilateral ONH and intellectual disability. [d] Any pituitary hormone deficiency (PHD) disregarding unknown pubertal disturbances. [e] White matter substance loss including periventricular leukomalacia.

4.3 PREVALENCE OF NEUROLOGICAL DYSFUNCTION

4.3.1 Motor impairments

Neurological assessment was conducted in 51 patients (27 bilateral and 24 unilateral ONH), with a median age of 10.1 years (range 2.1-19.4 years). In study III, motor impairments were identified in 47% (24/51) of the patients. In bilateral ONH, 67% (18/27) had impairments in gross motor function and 54% (14/26) in fine motor function. In contrast, among patients with unilateral ONH 17% (4/24) had impairments in gross motor function and 4% (1/24) in fine motor function. Both impairments in gross motor function and fine motor function were significantly more prevalent in bilateral ONH ($p< 0.001$) (Table 2). Furthermore, motor impairments were correlated to intellectual disability ($p< 0.001$) and visual acuity measured by logMAR or BCVA < 0.05 ($p= 0.01$ and $p= 0.001$, respectively). Consequently, patients with a more pronounced visual impairment had a higher risk of motor impairment.

In the neurological assessment, it was noted that 33% of the patients (older than four years of age) were left-handed, but left-handedness was not associated with bilateral ONH, gross or fine motor function impairment, intellectual disability, or ASD. Therefore, left-handedness does not seem to have a prognostic value.

More noteworthy, 9% (6/65) of the cohort had a diagnosis of cerebral palsy, without association with laterality of ONH, but all of them had brain abnormalities (Table 2). In addition, one patient had a hereditary spastic paraparesis and three patients with bilateral ONH had severe neurological impairments not classified as cerebral palsy, but were dependent on physical assistance and wheelchair.

4.3.2 Epilepsy

The prevalence of epilepsy was 14% (9/65) and there was a higher risk in bilateral ONH than unilateral ONH (23% vs 3%, $p= 0.03$) (Table 2). Three patients with bilateral ONH had infantile spasms.

4.4 NEURORADIOLOGICAL FINDINGS

In study III, pathological neuroradiological findings (brain malformations and white matter substance loss) were detected in 53% (27/51) (Table 2). Bilateral ONH was associated with a higher risk of brain abnormalities than unilateral ONH (69% vs 32%, $p= 0.01$). Absent septum pellucidum was found in 18%, but did not correlate to laterality, PHD, structural abnormalities, blindness, intellectual disability, ASD, or motor impairments. In contrast, absent septum pellucidum was associated with corpus callosum agenesis ($p= 0.004$). Three patients had corpus callosum agenesis and two patients had corpus callosum hypoplasia. Together these conditions constitute corpus callosum dysgenesis, which was associated with intellectual disability, motor impairment, and epilepsy ($p< 0.05$).

Structural pituitary abnormalities were identified in 27% (12/44) and did not correlate to laterality. All nine patients with PHD and assessable pituitary gland had an abnormal

pituitary, and no patient with a normal pituitary had evidence of any PHD (Table 3). Consequently, an abnormal pituitary had a very strong correlation to PHD, both single and multiple PHD ($p< 0.001$). In addition, the five patients with diabetes insipidus had an abnormal neurohypophysis (three were absent and two were ectopic).

Table 3. Hypopituitarism associated with structural pituitary abnormality, paper III

	Hypopituitarism	Normal endocrinological evaluation	Total
Pituitary abnormality	9	3	12
Normal pituitary	0	22	22
Total	9	25	34
	Sensitivity, 100%	Specificity, 88%	$p< 0.001$

Furthermore, we found hippocampal malformations in 20% (10/49) of the patients and it was more common in bilateral ONH (33% vs 5%, $p= 0.02$). Hippocampal malformations increased the risk of epilepsy ($p= 0.03$, adjusted for bilateral ONH). Finally, there was a high prevalence of white matter substance loss (28%, 13/46), including six patients with PVL. The white matter substance loss was most often located occipitally, dorso-lateral to posterior parts of the lateral ventricles.

4.5 PREVALENCE OF PITUITARY HORMONE DEFICIENCY

The prevalence of PHD in study III was 29% (13/45) and did not differ significantly between unilateral and bilateral ONH (21% vs 35%, $p= 0.51$) (Table 2). GHD was the most common PHD (21%), followed by ACTH deficiency (16%), central hypothyroidism (12%), diabetes insipidus (8%), gonadotropin deficiency (3%), and precocious puberty (2%). Multiple PHD (more than one PHD) was identified in 19% (9/48) of the patients with ONH. Of note, only 31 patients had passed the upper limit for a normal start of puberty and were clinically assessed for puberty.

The median age at diagnosis of the first PHD was 0.8 year (range 0-8.7 years), and 85% of the patients with hypopituitarism had developed their first PHD by the age of five (Figure 10). Four patients were first diagnosed with ACTH deficiency, and all eight patients with ACTH deficiency were diagnosed before five year of age (Table 4 and Figure 11).

Altogether, 33 PHD diagnoses were identified in 13 patients, and 79% of the PHDs were diagnosed before five years of age (Table 4 and Figure 11). However, the oldest patient diagnosed with a PHD was a 16-year-old boy with previous multiple PHD, who also developed central hypothyroidism. Late-onset central hypothyroidism was the most common PHD diagnosed in school age (Figure 11). Central hypothyroidism was not associated with intellectual disability ($p= 1.0$).

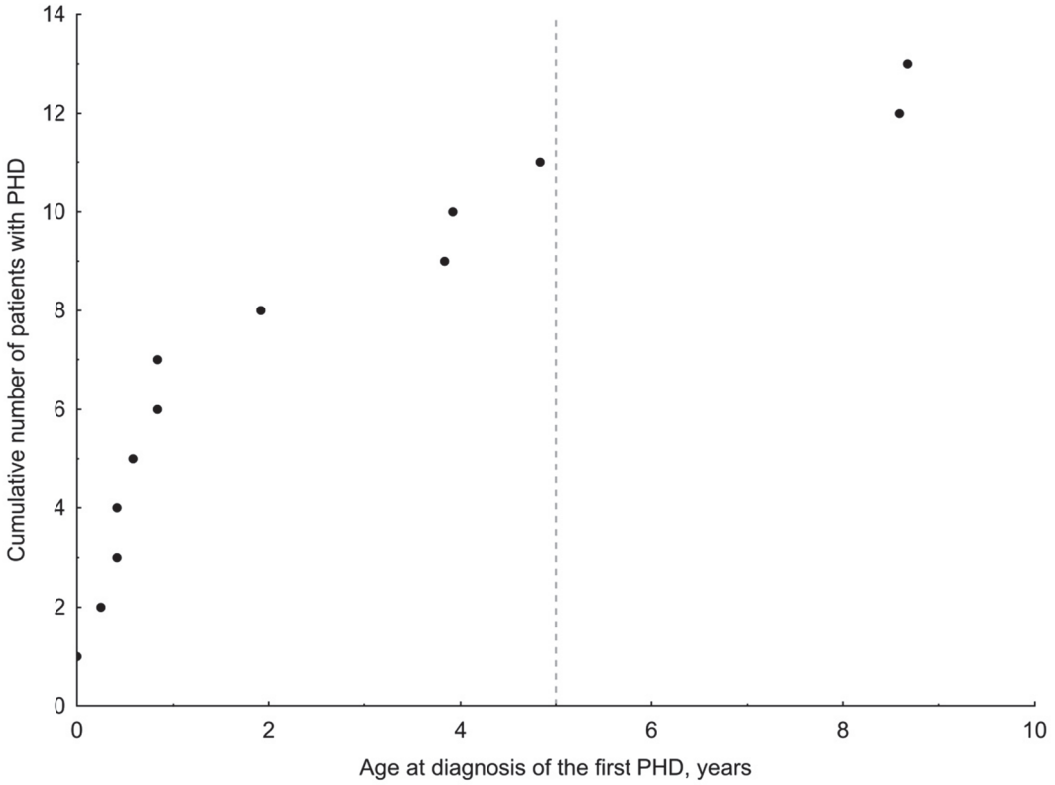

Figure 10. Age at diagnosis of the first pituitary hormone deficiency (PHD). Of 13 patients with ONH and PHD, 85% had developed their first PHD by the age of five.

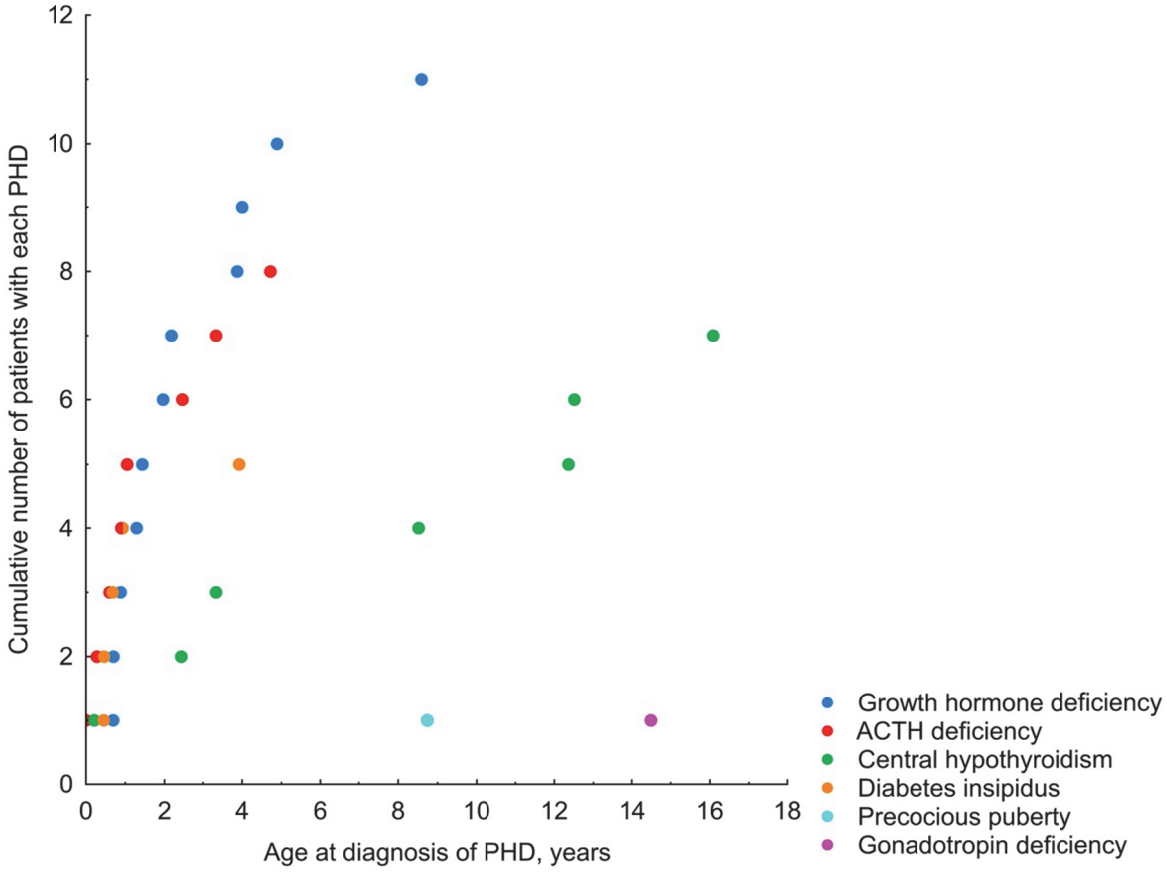

Figure 11. All 33 pituitary hormone deficiencies (PHDs) diagnosed in 13 patients, and the age at diagnosis of each PHD.

Table 4. Pattern of pituitary hormone deficiency (PHD) in 13 patients. The PHDs are numbered in order of diagnosis.

Patient	GH	ACTH	TSH	ADH	FSH/LH	Number of PHD for each patient
Patient 1	3	1	4	2		4
Patient 2	1					1
Patient 3	2	1	3			3
Patient 4	1					1
Patient 5	1		2			2
Patient 6	3	1	2			3
Patient 7	1	2	4		3	4
Patient 8	1	2	3*	4		4
Patient 9	3	1		2		3
Patient 10		2		1		2
Patient 11					1	1
Patient 12	2	3	4	1		4
Patient 13	1					1
Total number of each PHD	**11**	**8**	**7**	**5**	**2**	

*Central hypothyroidism was diagnosed at the same age as ACTH deficiency. GH, growth hormone; ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone; ADH, antidiuretic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Potential neonatal indicators of hormonal dysfunction, such as hypoglycemia and treatment-demanding jaundice were found in 16% (10/62) and 23% (14/61), respectively. Neonatal hypoglycemia was more common in the bilateral group (28% vs 3%, p= 0.01), and in patients with central hypothyroidism, ACTH deficiency, GHD, and multiple PHD (p< 0.009, adjusted for bilateral ONH). Similarly, patients who had been treated for neonatal jaundice more often had ACTH deficiency or multiple PHD (p= 0.006 and p=0.01, respectively, adjusted for gestational age), but jaundice did not correlate to laterality.

4.6 GENETIC CANDIDATE VARIANTS

In study IV, we performed a systematic screening for rare genomic variants in 29 patients with ONH and identified 13 rare SNVs in 12 patients and one rare CNV in one patient (Table 5).

Table 5. Clinical phenotypes and identified candidate genes in 13 patients with ONH. Summary from paper IV.

Case	Sex	Gene	Candidate variant	Inheritance	ONH	Neuro	PHD	Other malformations
2	M	*SPG7*	p.(Ala510Val)	Maternal	Bilat Blind	ID,CP,EP	-	Microcephaly, WMA, facial asymmetry
4	M	*COL4A2*	p.(Pro650Ser)	Paternal	Bilat	Autistic traits	-	-
5	M	*CYP26A1*	p.(Ile395Thr)	Maternal	Bilat	-	MPHD	Pituitary abnormality
9	F	*PAX6*	p.(Ter423Lys)	Ni	Unilat	-	-	-
11	M	*COL4A1*	p.(Gly948Ser)	*De novo*	Bilat Blind	ID,CP,EP	-	CD, calcification, WMA, thin CC, microcephaly
14	M	*UBE3B*	p.(Ala988Thr)	Maternal	Bilat	-	-	Unilat syndactyly toes
17	M	*COL4A2*	p.(Gly1663Ser)	Maternal, paternal	Bilat	ASD, hyposmia	MPHD	Pituitary and jaw abnormalities
18	F	*SOX5*	Heterozygous deletion exon 7-18	Not paternal	Bilat	ID, autistic traits	-	VSD
19	M	*CYP26C1*	p.(Gln284fs)	Ni	Unilat	-	-	-
20	M	*CYP26C1*	p.(Gln119Pro)	Paternal	Unilat	-	-	-
21	F	*COL4A1* *OPA1*	p.(Pro54Leu) p.(Ile382Met)	Paternal, Paternal	Unilat	-	-	-
27	F	*COL4A2*	p.(Gly729Arg)	Maternal	Unilat	ID	-	Hypertelorism
29	F	*COL4A1*	p.(Pro484Thr)	Maternal	Bilat	ID	-	Colpocephaly, CC agenesis, SP agenesis, MTS, arachnoid cyst, hypertelorism, ovarian cysts, liver and uterus abnormalities, bowel malrotation

ONH, optic nerve hypoplasia; Neuro, neurological dysfunction; PHD, pituitary hormone deficiency; Bilat, bilateral; ID, intellectual disability; CP, cerebral palsy; EP, epilepsy; WMA, white matter abnormalities; MPHD, multiple pituitary hormone deficiency; Ni, no information; Unilat, unilateral; CD, cortical destruction; CC, corpus callosum; ASD, autism spectrum disorder; VSD, ventricular septal defect; SP, septum pellucidum; MTS, molar tooth sign.

4.6.1 Single nucleotide variants (SNVs)

We identified rare heterozygous variants in *COL4A1* in three patients and variants in *COL4A2* in additional three patients. Consequently, 21% (6/29) of the patients with ONH carried a rare missense variant in either of these two genes. Three of the variants were

especially interesting. The first was the rare variant *COL4A1* p.(Gly948Ser) confirmed to be *de novo* by segregation studies. The same variant has previously been described as pathogenic in an individual with porencephaly, calcification, cerebral palsy, epilepsy, and intellectual disability.[43] Our patient had similar symptoms, which strengthens the pathogenicity of *COL4A1* p.(Gly948Ser). The second variant of interest was a homozygous mutation in *COL4A2* p.(Gly1663Ser), with each allele inherited from two unrelated parents. Interestingly, there are no homozygous individuals with this variant reported in the ExAC database.[129]

The third patient had two rare variants, a heterozygous missense variant in *COL4A1* p.(Pro54Leu) and a heterozygous missense variant in *OPA1* p.(Ile382Met). Mutations in *OPA1* are associated with optic atrophy 1, but it has previously been suggested that *OPA1* may also be involved in ONH.[45] This *OPA1* variant is enriched in individuals with autosomal dominant optic atrophy, but is also present in both the dbSNP and ExAC databases (rs143319805).[135] The rare variants in *OPA1* and *COL4A1* were inherited from the father who had strabismus as a child, suffered from a traumatic macular haemorrhage in the left eye as an adolescent and went through cataract surgery in adulthood. He was never diagnosed with ONH, but suspected to have a traumatic optic atrophy in his left eye.

Another interesting finding was a *PAX6* variant p.(Ter423Lys) which causes a lost stop codon, similar to the variant p.(Ter423Leu) that has previously been described in aniridia.[136] Although parental DNA was unavailable for analysis, this variant is a likely candidate since other variants in *PAX6* have been described in patients with ONH.[136, 137]

In summary, three of the SNVs (*COL4A1* p.(Gly948Ser), *COL4A2* p.(Gly1663Ser), and *PAX6* p.(Ter423Lys)) were assessed as likely pathogenic, and the remaining 10 were heterozygous variants of unknown clinical significance.

4.6.2 Copy number variants (CNVs)

Array-CGH revealed one rare CNV, which was a heterozygous deletion of 341 kb at 12p12.1 involving exons 7-18 of *SOX5*. *SOX5* has previously been described in Lamb-Schaffer syndrome, an autosomal dominant syndrome with varying phenotype including ONH, optic atrophy, developmental delay, behavioural problems, poor expressive speech, mild dysmorphic features, and skeletal abnormalities.[138, 139] The affected patient had bilateral ONH, intellectual disability, autistic traits, and a small ventricular septal defect. DNA was only available from the father and he did not carry the deletion. Subsequently, the *SOX5* deletion was assessed as likely pathogenic.

5 DISCUSSION

The prevalence of ONH was determined to be 17.3/100 000 in patients below 18 years of age in Stockholm. This is the highest prevalence of ONH that has been reported, and 45% of the patients had unilateral ONH. Consequently, this is a unique population-based cohort and a good foundation for further prevalence studies of associated disorders. Children with ONH had a high risk of neurodevelopmental disorders and the prevalence of intellectual disability was as high as 56% in patients with bilateral ONH and 9% in unilateral ONH. ASDs were diagnosed in 17% of the cohort and 29% had hypopituitarism.

The high prevalence of comorbidities underlines that ONH is a serious condition with a high risk of neurological and hormonal dysfunctions. Our studies clearly show that bilateral ONH is associated with a higher risk of neurodevelopmental disorders and motor impairment compared to unilateral ONH and is very likely caused by a more severe adverse impact on the developing brain. In contrast, prevalence of hormonal dysfunction and structural pituitary abnormalities did not differ between unilateral and bilateral ONH.

5.1 HIGHER PREVALENCE OF ONH

The prevalence of ONH in study I (17.3/100 000 children) was higher than the previously reported prevalence from Northwest England, which was estimated to be 10.9/100 000 in children younger than 16 years of age in 2006.[15] This may be due to a difference over time with an increasing prevalence of ONH as has previously been suggested.[13] However, the higher prevalence in our study is more likely due to the high proportion of unilateral ONH (45%), which in turn probably is a result of increased awareness of ONH and the population-based design of our study. In contrast to the study of Patel et al, the present cohort also included a high proportion of patients with bilateral ONH and mild or no visual impairment (50%).[15] On the other hand, we found that approximately 4/100 000 children had a BCVA < 0.3 (i.e. at least moderate visual impairment), which is actually lower than the previous Swedish report of 7/100 000 births.[14]

In early studies, the proportion of unilateral ONH was similar to bilateral ONH,[140, 141] but these studies were small and not population-based. Later reports have suggested that bilateral ONH is more common than unilateral ONH,[31, 74] and in a large prospective study, 82% of the patients had bilateral ONH.[16] However, depending on the study design cohorts are more or less selective, and there is a lack of population-based studies. Unfortunately, the prevalence study of Patel et al did not present the proportion of unilateral and bilateral ONH.[15] Therefore, it is very interesting that we found that unilateral ONH was almost as common as bilateral ONH. Study I supports our hypothesis that unilateral ONH as well as bilateral ONH with mild visual impairment are underrepresented in many studies. We believe that the high prevalence of ONH, the inclusion of 45% with unilateral ONH and approximately 70% with mild visual impairment are indicative of a less selected cohort.

5.2 HIGH RISK OF NEURODEVELOPMENTAL DISORDERS

We showed that children with bilateral ONH have a higher risk of intellectual disability than those with unilateral ONH (56% vs 9%). In addition, patients with bilateral disease had significantly lower mean FSIQ scores. This is consistent with the publication of Skarf et al, which described normal development in children with unilateral ONH (12% of the cohort), but in the bilateral group with poor vision, 46% had a development delay.[74] Garcia-Filion et al reported a higher prevalence of developmental delay, 78% in bilateral and 39% in unilateral ONH, using the Battelle Developmental Inventory in a prospective study from a referral center in the USA.[16] These two studies focused on the wider concept of developmental delay, while we chose to examine the prevalence of intellectual disability, as it has a larger impact on the child's everyday life. There is no other population-based study that has examined the difference in prevalence of intellectual disability in children with unilateral and bilateral ONH.

The prevalence of ASD was 24% in the bilateral group and 10% in the unilateral group. Although the difference was not statistically significant, study II was the first study to present ASD prevalence in unilateral ONH. We described a lower prevalence of ASD (17%) than previously reported, but the prevalence of ASD in the bilateral group (24%) was similar to the results of 29-33% from other studies.[88, 90, 142] We believe this discrepancy was due to the population-based design of our study.

Furthermore, it is notable that the patients in our cohort received the ASD diagnoses late, at a median age of 10.3 years (range 5.8-15.6 years). This likely reflects the lack of knowledge about the high prevalence of ASD in children with ONH, failure to recognize autistic symptoms, confusion with blindism,[143] but also a lack of validated testing instruments for visually impaired children. Our findings show that children with unilateral ONH also have a high risk of neurodevelopmental disorders compared to the general population, where the prevalence of intellectual disability is 1-2% in Sweden,[144] and the prevalence of ASD is 1% in Stockholm.[145] It therefore highlights the need of screening for neurodevelopmental disorders in all children with ONH before starting school.

Early neurodevelopmental diagnoses are important for the child's development and for the choice of an optimal school setting, and especially the dual disability of blindness and ASD is challenging.[146] In these cases, specific pedagogical support and evidence-based practices for ASD is needed and has to be adapted for the visual impairment.[146]

In general, coexistence of neurodevelopmental disorders is very common and the symptoms overlap. Therefore, the term ESSENCE (Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations) has been coined to emphasize that children with neuropsychiatric symptoms need a multidisciplinary assessment.[147] In our cohort, there were several individuals who had more than one neurodevelopmental disorder and in some patients, the diagnosis changed over time. The large amount of neuropsychiatric symptoms is

demonstrated by the fact that 46% of the patients with ONH had previously been tested neuropsychologically, once or several times.

We found that blindness and severe visual impairment was associated with an increased risk of intellectual disability, but not with ASD. However, all the patients with a BCVA < 0.1 had bilateral ONH, and within the bilateral group we found no significant difference of the proportion of intellectual disability that correlated to BCVA. This suggests that it was not the visual impairment *per se* that increased the risk of intellectual disability, but rather that it was a symptom of a more severe bilateral ONH disease. Two other reports described higher frequencies of autistic symptoms in children with ONH/SOD and profound visual impairment compared to children with ONH/SOD and better vision, but neither did they find any significantly increased risk of the diagnose ASD correlating to visual acuity.[90, 142] Our results indicate that the neurodevelopmental disorders seen in children with ONH are more likely to be a result of brain dysfunction rather than a consequence of visual impairment.

5.3 NEUROLOGICAL DYSFUNCTION

Similar to the prevalence of intellectual disability, we identified a higher risk of neurological impairments in patients with bilateral ONH in study III. In total, impairments in gross and/or fine motor function were significantly more common in bilateral ONH than unilateral ONH (74% vs 17%). There is no comparable study, but Fahnehjelm et al reported hypotonia, dyskinesia, or motor delay in 21% in a selected cohort with bilateral ONH and severe visual impairment,[88] and Signorini et al described a high degree of motor clumsiness in a retrospective study.[91] The majority of the motor impairments we detected, could be described as minor neurological dysfunction,[119] but 9% of the patients had a diagnosis of cerebral palsy and additional four patients had so severe neurological impairments that they were dependent on assistance. Several other studies have reported a similar prevalence of cerebral palsy ranging from 9-13%.[31, 92, 94] In consistence with Garcia et al, we found no statistically significant difference in the prevalence of cerebral palsy between unilateral and bilateral ONH.[94] Moreover, we found that motor impairments were associated with intellectual disability and visual impairment, which is well known and reflects that delayed motor skills are often a part of global developmental delay and severe visual impairment inhibits the child's ability to practice motor skills.

Epilepsy was diagnosed in 14% of the cohort and it was significantly more common in the bilateral group (23% vs 3%). One of the few ONH studies that has reported on this, found epilepsy in 21% of the patients with bilateral ONH and severe visual impairment, which is comparable to our bilateral group.[88] In the general population, the prevalence of epilepsy in Swedish children is much lower (0.9%).[148] Furthermore, we found that hippocampal malformations increased the risk of epilepsy.

5.4 HORMONAL DYSFUNCTION

Hypopituitarism can be life threatening, both due to ACTH deficiency and subsequent hypoglycemia and profound hypotension, as well as to diabetes insipidus causing severe

volume depletion and electrolyte abnormalities resulting in circulatory shock, seizures, and coma.[149] Garcia-Filion et al reported that 52% of the children with ONH and hypothyroidism were undiagnosed before enrollment in the study, and they reported that hypothyroidism was a risk factor for developmental delay.[16]

Despite the high risk of complications in patients with undiagnosed and untreated hormone deficiency, some ophthalmologists do not refer children with ONH to an endocrinologist, especially in unilateral disease. On the other hand, sometimes endocrinologists choose not to further investigate or follow-up children with ONH. Therefore, it is very important to determine the prevalence of PHD in ONH and to investigate if there is a difference in the frequency of PHD between unilateral and bilateral ONH.

We found a prevalence of PHD of 29%, which is in congruence with the cross-sectional study of Ramakrishnaiah et al (28%).[80] It is however, lower than in many other studies,[79, 81, 82] which is likely due to the population-based design, with 46% unilateral ONH and patients with less severe visual impairment. In addition, we have been stricter in our inclusion criteria for GHD, which is the most common PHD. In contrast to Garcia-Fillion et al,[16] we did not perform provocative testing in all the patients. We first evaluated growth curves and IGF-1/IGFBP-3 and only patients with suspected GHD was investigated further.

Several other researchers have used a cut-off level of a stimulated GH peak of 10 µg/L,[79, 81, 150] while we used the cut-off level of 7 µg/L which is more closely correlated to GHD.[121] A higher cut-off level increases the risk of capturing individuals without GHD, especially if not considering the growth curve or response to GH treatment. Furthermore, we re-evaluated all the PHD diagnoses, and hormonal replacement therapy was not considered sufficient to confirm a diagnosis. Consequently, we excluded cases of primary hypothyroidism, nocturnal enuresis, and one patient on GH treatment.

An important finding was that there was no statistically significant difference in the prevalence of PHD between unilateral and bilateral ONH. This is consistent with the studies of Ahmad and Alyahyawi and coworkers.[66, 82] Therefore, children with unilateral ONH should be followed-up endocrinologically as closely as in bilateral ONH. Our results further showed that 85% of the patients with PHD had their first PHD diagnosis before five years of age, similar to Alyahyawi et al (82%).[82] In addition, 79% of all the PHD diagnoses and 100% of the ACTH deficiencies were detected before the age of five. Ryabets-Lienhard et al recommended screening for hypopituitarism every fourth to sixth months in the first three years of life and then annually.[151] In our cohort, only 57% of the PHD diagnoses were confirmed at three years of age, and our results suggest a more frequent endocrine follow-up during the first five years of age. Due to the risk of pubertal disturbances and later evolving PHD, especially central hypothyroidism, more sparsely hormonal assessments should continue at least until puberty is completed.

Many patients in our cohort were not previously examined for PHD to a sufficient degree. In medical records, we saw examples of both good and bad endocrinological investigations,

examples of how hormonal results can be interpreted differently, and different opinions about treatment. Some parents have also chosen not to participate in screening of PHD in their children because they wanted them to stay small/short to facilitate the care of children with neurological dysfunction. In addition, we found a pattern of doctor's delay at the neurological departments, where children with severe neurological disorders too often were not endocrinologically investigated despite growth failure and where the ONH diagnosis seemed to be forgotten. It is understandable that the neurological situation, often with epilepsy, gets more attention, but this stresses the importance that these children also need to be followed-up at the endocrinological department.

5.5 BRAIN ABNORMALITIES

We detected structural pituitary abnormalities in 27% of the patients and in all of whom had PHD. Thus, the sensitivity was 100%. Ramakrishnaiah et al reported neurohypophyseal abnormalities in 33% of the cohort and described 96% sensitivity and 92% specificity for PHD,[80] while Garcia-Filion et al reported that only 9% of the children with ONH had pituitary abnormalities, with 13% sensitivity and 100% specificity for PHD.[81] In summary, there is convincing evidence that pituitary abnormalities are highly predictive of PHD, but the sensitivity for PHD is unreliable. Study III is limited by not having assessable neuroradiological imaging on all the patients, and assessment of the pituitary was missing in four patients with PHD. Still, Garcia-Filion et al have reported enough patients with hypopituitarism and a normal structural pituitary on MRI to make it impossible to rule out PHD from a patient with a normal MRI.[81] This is very important for the clinical evaluation.

Less important or not important at all, is the presence of the septum pellucidum. Still, it is evident that the concept of SOD is very much in use both clinically and in research.[152, 153] However, our results provide further evidence for the lack of clinical significance of an absent septum pellucidum as we could not find any association with laterality, PHD, structural pituitary abnormality, motor impairment, intellectual disability, or ASD. Nonetheless, absent septum pellucidum was associated with corpus callosum agenesis, which is understandable as the septum normally is attached to the corpus callosum. Corpus callosum agenesis increased the risk of intellectual disability, cerebral palsy, and epilepsy, but not the risk of PHD. In our opinion, SOD is a misleading term that increases the risk of children with ONH and a complete septum pellucidum not being properly investigated for hypopituitarism. Septum pellucidum should not be used as a risk marker for PHD.

Altogether, brain abnormalities were identified in 53% of the cohort, and bilateral ONH was associated with a higher risk than unilateral ONH. Due to our population-based design, our prevalence could be expected to be lower than previous reports of 60-74% with brain abnormalities.[81, 94] The higher frequency of brain malformations in bilateral ONH supports the theory that bilateral ONH is a result of a more severe insult to the developing brain. In addition, we detected hippocampal malformations in 20% of the patients, significantly more common in the bilateral group. This malformation has previously been noted by Riedl et al in ONH patients.[150] Finally, 28% of the patients had white matter substance loss, which could

be an indicator of a prenatal insult that may have affected the visual pathway and contributed to retrograde trans-synaptic degeneration.

5.6 RARE VARIANTS IN *COL4A1* AND *COL4A2*

Two of the rare variants in *COL4A1* and *COL4A2* were especially interesting. They were the heterozygous *de novo* variant *COL4A1* p.(Gly948Ser) previously described as pathogenic[43] and the homozygous variant *COL4A2* p.(Gly1663Ser).

COL4A1 and *COL4A2* are located on opposite strands at 13q34 and have a common promotor. *COL4A1* and *COL4A2* respectively encode the alpha-1 and alpha-2 subunits of collagen type IV. Two alpha-1 chains and one alpha-2 chain form a heterotrimer.[154] Collagen type IV is not only essential for basement membranes, but is also involved in developmental processes, such as cell adhesion, migration, proliferation, and differentiation.[154] This makes the *COL4A1* and *COL4A2* genes very interesting from a developmental perspective. *Col4a1*$^{+/\Delta ex41}$ mutant mice have been reported to have changes in the retinal inner limiting membrane besides ONH, suggesting that abnormalities of the basement membranes may cause ONH.[155]

Mutations in *COL4A1* have primarily been associated with small-vessel brain disease (e.g. porencephaly, schizencephaly, and cerebrovascular disease), but ophthalmological disorders have also been described (e.g. retinal arterial tortuosity, congenital cataract, anterior segment dysgenesis, glaucoma, microphthalmia, anophthalmia, optic atrophy, and ONH).[43, 44, 156] Similarly, mutations in *COL4A2* have been associated with intracerebral haemorrhages, porencephaly, optic atrophy, and ONH.[44, 157-160] However, only a few patients with ONH and mutations in *COL4A1* or *COL4A2* have been reported in the literature.[43, 157]

Regarding mutations in *COL4A1* and *COL4A2*, there seems to be an autosomal dominant pattern of inheritance. Therefore, our homozygous variant in *COL4A2* is of interest and further functional study is necessary to conclude whether it is pathogenic or not. Moreover, mutations in *COL4A1* and *COL4A2* show a broad phenotypic variation with reduced penetrance. For this reason, inherited rare variants could not be assessed as likely benign, and were assessed as variants of unknown clinical significance. It may be speculated if these variants could constitute a genetic vulnerability and cause ONH in combination with a second hit, genetic or environmental. Accordingly, the combination of rare variants in *COL4A1* and *OPA1* is interesting, and the affected patient will be further investigated with OCT. In general, functional studies would be helpful to determine the clinical significance of the inherited heterozygous variants.

Furthermore, the location of the variants in *COL4A1* and *COL4A2* seems to be of major importance. We found three rare variants in *COL4A1* and three rare variants in *COL4A2,* and two of the variants were located in the triple helix domain and resulted in the substitution of a glycine (Figure 12). The first was the heterozygous *de novo* variant *COL4A1* p.(Gly948Ser), and the second was a heterozygous variant *COL4A2* p.(Gly729Arg). The triple amino repeats, constituted by glycine in every third position in the triple helix domain are essential for the

proper triple helical formation. Mutations in the triple helix domain can disrupt the normal folding and protein function,[161] and especially substitutions of the glycine have been predicted to be pathogenic.[44, 154] Moreover, the homozygous variant *COL4A2* p.(Gly1663Ser) were located in the carboxy-terminal noncollagenous domain, which have an important role in regulating the stoichiometry of the heterotrimer.[154] One may hypothesize that this variant could affect the composition of the α1α1α2 heterotrimer and subsequently, the function of collagen type IV. This is another reason why a functional study could be elucidating.

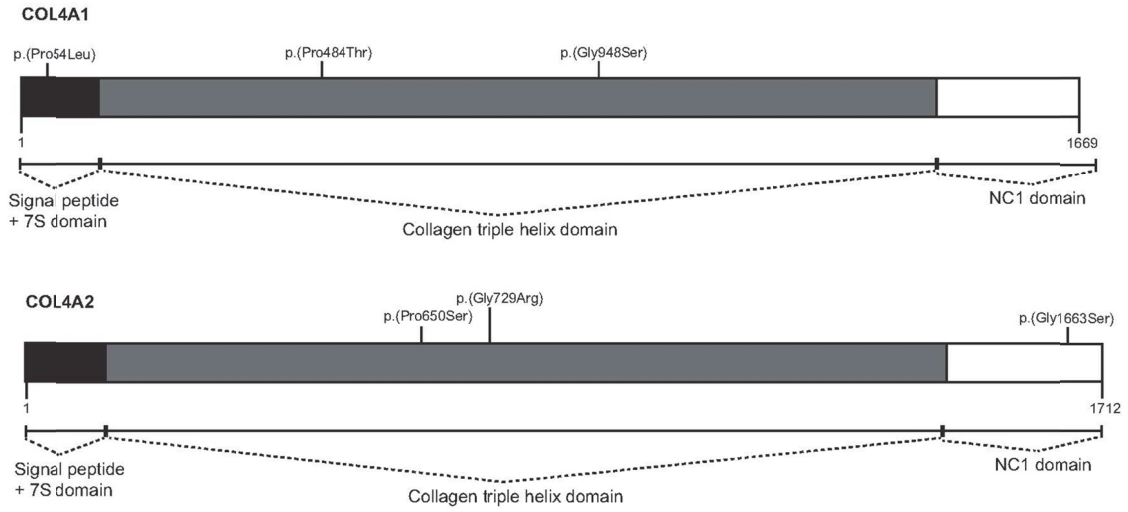

Figure 12. Six rare variants in *COL4A1* and *COL4A2* identified in the patients with ONH. Of these six variants, four affected the collagen triple helix domain of COL4A1 or COL4A2, one affected the 7S domain of COL4A1, and one affected the noncollagenous (NC1) domain of COL4A2. Figure from paper IV.

With respect to all our identified genetic variants in the patients with ONH, our results suggest that genetic causes are more common than previously described. Previous genetic research on ONH has focused on *HESX1*,[41] and array-CGH and sequencing of many candidate genes in patients with ONH have not been reported before. Interestingly, we did not identify any patients with variants in *HESX1*, *SOX2*, *SOX3*, or *OTX2*, genes earlier emphasized to be connected to ONH.[32, 36, 38, 39, 41] Instead, our research highlights *COL4A1* and *COL4A2* as candidate genes. Chen et al recently published a review of genetic causes of ONH including 16 genes.[49] To this list we suggest to add *COL4A1* and *COL4A2*.

5.7 LIMITATIONS AND STRENGTHS

Our studies' major strength is the population-based cross-sectional design. Our cohort of patients with ONH is unique by its composition of a high proportion of both unilateral and bilateral ONH and patients with mild visual impairment to blindness. The cohort is well-characterized down to the DNA level. However, there are a number of limitations. Some are already mentioned in the chapter Methodological considerations.

Although we have reported the highest known prevalence of ONH, the total number of patients was only 76 who were below 18 years of age. In the research field of ONH, this is a rather large cohort, but not in general for prevalence studies. Study I was performed in the county of Stockholm, and the cohort is likely representative for Sweden and probably at least for Scandinavia. However, a multi-center study in Sweden would have increased the power of the study, but it is uncertain if we could have had the same coverage, resulting in a high prevalence of ONH.

The prevalence of ASD and hypopituitarism did not differ significantly between unilateral and bilateral ONH, but it is possible that a larger cohort may have shown a difference in prevalence, and this should be studied further.

In study II, it is a study limitation that patients were examined using different neuropsychological tests and no standardized autism assessment was conducted. It would have been preferable to use a model of modified ADOS,[114] even though there is a lack of validated testing instruments for ASD in visually impaired children. However, it was not practically feasible at the time being, and we used the FTF to screen for ASD. Borderline cases may have been missed and might have affected the prevalence of ASD.

The neurological assessment of motor impairments would have been improved by additional assessment by a physiotherapist and an occupational therapist. But, the neurological assessment was performed at the same visit as the ophthalmological assessment and additional investigations would have required more visits for the patients, which could have affected the participation rate.

The lower prevalence of neurodevelopmental disorders, hypopituitarism and brain abnormalities in our cohort are likely a result of the population-based approach, but may also reflect that ONH is due to different aetiologies in different populations.

The endocrinological assessment was limited by not having blood sampling for evaluation of all PHDs in every patient. Thirty-one patients participated in the study blood-sampling and another 29 patients had results from clinical endocrinological blood analyses, but five patients did not have any results. A prospective design would have been preferable, and may be possible in a future follow-up program. Assessment of puberty is of particular interest since pubertal disturbances are not well studied, but clinical assessment of pubertal stages was not included in study III. Hence, for the assessment of Tanner pubertal stages and testis size, we had to rely on other clinicians' documentation in the medical records. In addition, neuroradiology was not performed in all patients, and especially assessable imaging of the pituitary was lacking in four patients with PHD.

The last limitation to be mentioned is that we performed WGS, but filtered the data for the candidate genes. By analysing the whole genome, new candidate genes probably will be found.

5.8 ONH IN A DEVELOPMENTAL PERSPECTIVE

ONH is a congenital malformation, and it is likely that monogenic, polygenic, and environmental factors, single or in combinations, can be causative. As previously described, genetic mutations and ethanol may affect the retinal progenitor cells at an early stage,[23] but other studies have indicated that ONH is more likely a result of prenatal events affecting the optic nerve, optic tract, or the optic radiation, subsequently, leading to a retrograde degeneration.[71, 162]

Furthermore, Lubinsky hypothesized that ONH/SOD were due to a vascular disruptive sequence and suggested involvement of the proximal trunk of the anterior cerebral artery.[59] Mutations in *COL4A1* and *COL4A2* may increase the risk of a vascular insult, and this could be an underlying mechanism. Both the patient with the *de novo* variant *COL4A1* p.(Gly948Ser) and the previously reported patient with the same variant had calcifications on MRI, which may indicate earlier bleeding. A vascular insult is an interesting hypothesis explaining why hypopituitarism so often occurs in patients with ONH.[59] In addition, due to the strong connection between ONH and pituitary dysfunction, there are likely undiscovered common genetic aetiologies affecting the development of the optic nerve, the pituitary gland, and hypothalamus.

Previous research suggests that the overall mutational burden matters.[163] We speculate that the broad phenotype seen in patients with ONH may be explained by the disease gene and the overall mutational burden, modified by the regulatory landscape and environmental factors. This could also be seen as an overlapping spectrum between environmental factors and genetic abnormalities. This would explain the reduced penetrance reported in many of the candidate genes and why so few family members are affected.

Neurodevelopmental disorders, motor impairments, and brain malformations were more common in bilateral ONH indicating that bilateral ONH is a more severe developmental disorder. Most likely, bilateral ONH is caused by an earlier event affecting more developmental processes. However, it should be emphasized that hypopituitarism was not associated with laterality and the results highlight the importance that all children with ONH should be followed-up endocrinologically. Isolated ONH, without any combination with neurodevelopmental disorder, other major congenital malformation, or hypopituitarism, was found in 26% (13/50) of the patients and did not differ significantly between unilateral and bilateral ONH (42% vs 16%, p= 0.054). This is because of the high prevalence of PHD in both groups.

Even though ONH may be caused by genetic mutations and environmental factors such as ethanol, many cases are probably secondary to other brain malformations, intracerebral bleeding, and white matter substance loss. The long way of connections from the retina, through the optic nerve to the visual cortex makes the optic nerve vulnerable. Despite the different aetiologies, ONH is an indicator of a deviating event during development.

5.9 CLINICAL IMPLICATIONS

The findings presented in this thesis, has several clinical implications. Firstly, it emphasizes the importance of a correct diagnosis in children with poor visual function or blindness. Not only is this important for the parents, but also since the diagnosis of ONH has a large impact on the need of further investigations. At the same time, diagnostics can be hard due to nystagmus, poor cooperation, and difficulties in getting fundus photographies to confirm the suspicion of ONH. This study suggests that the simple manual Zeki method is reliable and can be used in most patients, from a preschool age, to establish the diagnosis.

Secondly, the ophthalmologist should refer all children with ONH (i.e. both unilateral and bilateral ONH) to an endocrinologist for assessment and follow-up. In addition, a referral should be sent to a neuropaediatrician for assessment of the development and neurological function.

Thirdly, a MRI of the brain and the orbits is valuable to evaluate the optic nerves and detect other brain malformations. In particular, the assessment of the pituitary gland is important from a prognostic point of view. Therefore, it is essential to ask for pituitary abnormalities to ensure that adequate MRI protocols are used. An absent septum pellucidum, as the only finding, does not have any clinical impact. If anesthesia is necessary, ACTH deficiency must first be excluded.

When there is a structural pituitary abnormality the risk of PHD is close to 100%. Nevertheless, a normal pituitary gland does not rule out PHD and all children with ONH should have an initial and thorough endocrinological investigation, as well as a follow-up. Due to the higher risk of developing PHD during the first five years of life, the follow-up assessments during this period need to be more frequent compared to later in life. Ryabets-Lienhard et al have recommended evaluations every fourth to sixth months,[151] and this seems reasonable. In older children, it is important to keep an eye on the timing of puberty and evolving central hypothyroidism, and yearly hormonal assessments are probably sufficient. It is not known how often PHD evolve during adulthood and consequently, for how long the monitoring should continue.

From a neurological perspective, developmental problems or neurological impairments indicate whether the child needs imaging of the brain. Knowledge of major malformations and corpus callosum dysgenesis are of prognostic value. Children with motor deficits should be further assessed by a physiotherapist and/or an occupational therapist. Patients with neurodevelopmental disorders and motor dysfunction should be referred to a habilitation center. In addition, all children with ONH should be screened for neurodevelopmental disorders before starting school and screening questionnaires, such as the FTF, can be used. If developmental problems are detected, further neuropsychological testing should be performed.

Genetic testing is proposed primarily for children with ONH in combination with other malformations, neurological dysfunction (especially intellectual disability and ASD), or in

cases with related parents and affected siblings. Suggested analyses are array-CGH and sequencing of suspected candidate genes, but WGS are useful in priority cases.

6 CONCLUSIONS

The conclusions from this thesis are that the prevalence of ONH in children was 17.3/100 000 in Stockholm, and unilateral ONH was almost as common as bilateral ONH.

Children with bilateral ONH have a very high risk of neurodevelopmental disorders, especially intellectual disability (56%). Children with unilateral disease have a less pronounced risk of intellectual disability (9%), but ASDs were diagnosed in 17% of the cohort, without association with laterality of the disease. The high frequency of neurodevelopmental disorders in both unilateral and bilateral ONH warrants screening before starting school.

Motor impairments are very common in children with ONH, and more prevalent in bilateral disease. Except for neurological evaluation, children with motor deficits should be assessed by physiotherapist and occupational therapist for support to improve their motor skills.

Both children with unilateral and bilateral ONH have a significantly increased risk of hypopituitarism (29%). Structural pituitary abnormalities are highly predictive of PHD. Accordingly, MRI of the brain including the pituitary gland is of prognostic value. However, a normal structural pituitary gland does not rule out hypopituitarism.

Of the patients with hypopituitarism, 85% were diagnosed with their first PHD diagnosis before five years of age. In addition, 79% of all the PHDs were diagnosed within the age of five. Subsequently, a more frequent endocrine follow-up is warranted until five years of age and thereafter more sparsely until puberty is completed.

Our results suggest that a genetic cause of ONH is more common than previously reported and highlights *COL4A1* and *COL4A2* as candidate genes. Rare variants in *COL4A1* and *COL4A2* were identified in 6/29 of the patients, and two of the variants were assessed as likely pathogenic. We conclude that genetic testing is valuable in a substantial proportion of the children with ONH, and in the future, more of these children should be able to receive a genetic diagnosis.

7 FUTURE PERSPECTIVES

To understand a disorder, it is important to know what is causing it and how common it is. This thesis has underlined the need of population-based cohorts when studying the prevalence of ONH, but also associated comorbidities such as neurodevelopmental disorders and hypopituitarism. To confirm our results, additional population-based studies are necessary, and a future study looking at hypopituitarism should be prospectively designed and include clinical assessment of pubertal stages.

Our research group intends to design a national follow-up program for children with ONH based on the studies in this thesis, and this would be a good opportunity to organize a national prospective study.

In study II, we acknowledged the lack of validated testing instruments for ASD in visually impaired children. In general, blind children have an increased risk of ASD,[87] and in our study we could also see that children with ONH received the ASD diagnoses late. This needs to be improved to be able to give the children and families early support and a better chance for development. Clinically used testing instruments (e.g. ADOS) need to be adjusted for visually impaired children and validated. Subsequently, the prevalence of ASD in children with both unilateral and bilateral ONH should be reinvestigated.

The aetiology of ONH is still unknown in most patients. Epidemiological studies are wanted to look for risk factors, especially environmental factors that are preventable. Here, collaboration between maternity care, ophthalmologist and paediatricians is needed, and Swedish registers could be an advantage.

Genetic causes of ONH should be further studied with WGS, and we plan to analyse all our WGS data. This will very likely reveal more pathogenic variants and contribute to the understanding of ONH, but also contribute to our understanding of the developing brain. Furthermore, the candidate variants need to be confirmed with functional studies, and zebra fish could be an interesting model.

Another way to explore the impact on the brain in children with ONH is to perform diffusion tensor imaging and other functional MRI studies. Especially the high prevalence of white substance loss could be further investigated with these methods, but also different parts of the visual pathway. In fact, we already have ethical approval for this study and patients willing to participate.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Optikushypoplasi (optic nerve hypoplasia, ONH) är en medfödd synnervsmissbildning som innebär att synnerven är tunn och underutvecklad. ONH kan drabba det ena eller båda ögonen och det är en vanlig orsak till synnedsättning och blindhet hos barn i industrialiserade länder. Förekomsten av ONH är dock något oklar och tidigare studier har indikerat att förekomsten ökar. Ofta drabbas barn med ONH inte bara av synnedsättning, utan de har också en förhöjd risk för utvecklingsavvikelser och brist på hypofyshormoner. Dessa hormonbrister kan leda till tillväxthämning och kortvuxenhet, pubertetsrubbningar, vätskebalansstörningar, låga blodsocker, kramper, ytterligare hjärnskador och i värsta fall dödsfall. Om vi kan finna hormonbristen i tid så finns det dock bra behandling att erbjuda.

Det är vidare oklart hur vanligt det är med intellektuell funktionsnedsättning (tidigare benämnt utvecklingsstörning), autism och hormonbrister hos barn med ONH, och det saknas ett uppföljningsprogram. Syftet med avhandlingen var att beräkna förekomsten av ONH, liksom samsjuklighet med intellektuell funktionsnedsättning, autism, motoriska svårigheter och hormonbrister, och därtill jämföra de två grupperna med ensidig eller dubbelsidig ONH. Slutligen eftersökte vi genetiska orsaker till ONH eftersom orsaken i de flesta fall är okänd.

Studie I: I denna studie identifierade vi, via diagnosregister, alla patienter som diagnostiserats med ONH och var under 20 år samt boende i Stockholms län i december 2009. Totalt sett identifierades 79 patienter. Patienterna fick genomgå ögonundersökningar för att bekräfta diagnosen och testa synskärpan, dessutom fick föräldrarna fylla i ett ”5-15 formulär” för bedömning av utveckling och beteende. Denna populationsbaserade kohortstudie visade att förekomsten av ONH hos barn i Stockholm var 17.3/100 000. Det är den högsta förekomsten som rapporterats internationellt. Sextiosex patienter undersöktes klinisk och knappt hälften hade ensidig ONH, vilket är en ovanligt hög andel. Utvecklingsavvikelser var vanligare vid dubbelsidig ONH.

Studie II: Denna studie inkluderade 65 patienter med ONH från ovan beskrivna populationsbaserade kohort. Diagnoserna intellektuell funktionsnedsättning och autism bekräftades med neurologisk undersökning, genomgång av tidigare neuropsykiatriska utredningar och de som inte redan hade utretts erbjöds neuropsykologisk testning. Dessutom användes ”5-15 formuläret” för att utesluta intellektuell funktionsnedsättning och autism hos dem som inte genomgick någon neuropsykologisk testning men där både neurologisk undersökning och skolgång varit normal. Studie II visade att det finns en hög risk för intellektuell funktionsnedsättning vid ONH (36%) och att det är vanligare vid dubbelsidig ONH (56%) jämfört med ensidig ONH (9%). Patienterna med dubbelsidig ONH hade också lägre medelvärde på helskale-intelligenskvot (IQ) än den ensidiga gruppen (84.4 och 99.4, respektive). Förekomsten av autism var 17% och skiljde sig inte åt mellan grupperna.

Studie III: Populationsbaserad kohortstudie med 65 patienter med ONH, varav 46% hade ensidig ONH. Patienterna bedömdes med en strukturerad neurologisk undersökning med fokus på grov- och finmotorik. De fick också lämna in blodprover för screening av

hormonbrister från hypofysen. Utifrån provsvar, bedömning av tillväxtkurvor och tidigare sjukdomshistoria fastslogs om patienterna hade brist på hypofyshormon, så kallad hypopituitarism. Tidigare datortomografier (skiktröntgen) och magnetkameraundersökningar av hjärnan eftergranskades dessutom. I studie III framkom att motoriska svårigheter förekom hos 47% av patienterna med ONH och var vanligare vid dubbelsidig ONH och hos dem med sämre synskärpa. Cerebral pares (CP) återfanns hos 9% av ONH patienterna och 14% hade epilepsi. Brist på hypofyshormon förekom hos 29% av fallen och 19% hade brist på flera hypofyshormoner. Vanligast var brist på tillväxthormon (21%). Ett viktigt konstaterande var att bristen på hypofyshormon inte var relaterat till ensidig eller dubbelsidig ONH. Dessutom såg vi att 85% av dem som fick hypopituitarism hade fått sin första hormonbrist diagnostiserad före fem års ålder. Slutligen så fann vi att en strukturellt avvikande hypofys hade en stark koppling till hormonbrist.

Studie IV: Blodprover från 29 patienter med ONH från ovanstående kohort samlades in för att på DNA genomföra genetiska analyser med array-CGH, som visar gendosavvikelser i hela arvsmassan, och helgenomsekvensering med fokus på 42 kandidatgener. Dessa 42 kandidatgener har tidigare kopplats till ONH eller närliggande tillstånd. De identifierade kandidatvarianterna (mutationerna) bekräftades med Sangersekvensering och föräldraprover analyserades för att fastställa nedärvning. Vi fann sällsynta varianter i generna *COL4A1* och *COL4A2* hos sex patienter, varav två varianter bedömdes som sannolikt patogena (sjukdomsframkallande). Generna *COL4A1* och *COL4A2* kodar för två proteiner i kollagen typ IV (fiberprotein), som är nödvändigt för basalmembran och är även betydelsefullt vid tidig utveckling. Detta fynd talar för att mutationer i *COL4A1* och *COL4A2* är viktiga genetiskt bidragande faktorer vid ONH. Dessutom identifierade vi två andra sannolikt patogena varianter i generna *SOX5* och *PAX6*, samt en intressant variant i *OPA1*. Sammantaget talar detta för att genetiska orsaker till ONH är vanligare än vad som tidigare rapporterats, men dessa fynd behöver bekräftas med funktionella studier.

Slutsatser: Förekomsten av ONH hos barn i Stockholm var 17.3/100 000. Ensidig ONH var nästan lika vanlig som dubbelsidig. Både barn med ensidig och dubbelsidig ONH har en hög risk för neuropsykiatriska sjukdomar och brist på hypofyshormon, men den bilaterala gruppen har ännu högre förekomst av intellektuell funktionsnedsättning, motoriska svårigheter och hjärnmissbildningar. Våra resultat påtalar ett behov av att barn med ONH behöver screenas för neuropsykiatriska sjukdomar senast före skolstart och den hormonella uppföljningen behöver vara frekventare de fem första levnadsåren. Vidare indikerar den genetiska studien att genetiska orsaker är vanligare än vad som tidigare rapporterats och lyfter fram *COL4A1* och *COL4A2* som intressanta kandidatgener. Sannolikt är genetisk testning av värde för en betydande del av barnen med ONH, och med den snabba utvecklingen inom genetisk diagnostik så borde fler av dem kunna få en genetisk diagnos i framtiden.

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