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Is early visual intervention reasonable in patients with albinism and congenital low vision?

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To my father Eriks Brikmanis (1921-2002) in Loving Memory

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List of Abbreviations

AS	Angelman Syndrome
BBC	British Broadcasting Corporation
CD	Cysteinyl-dopaquinone
CHS	Chediak–Higashi Syndrome
DNA	Desoxyribonucleic acid
DOPA	3,4-Dihydroxyphenylalanine
DVM	Delayed visual maturation
E.C.	Enzyme Classification
EEG	Electroencephalogram
EI	Early Intervention (Frühförderung)
ERG	Electroretinogram
FF	Frühförderung
GS	Griscelli Syndrome
HPS	Hermansky-Pudlak Syndrome
Hz	Hertz
L-DOPA	Levodopa
OA	Ocular albinism
OCA	Oculocutaneous albinism
OCT	Optical coherence tomography
ONH	Optic nerve head
PPMCC	Pearson product-moment correlation coefficient
PWS	Prader-Willi Syndrome
ROS	Reactive oxygen species
UKS	Universitätsklinikum des Saarlandes
UV	Ultraviolet Radiation
UVA	Ultraviolet Radiation Range A
VA	Visual Acuity
VEP	Visually Evoked Potentials

Abstract

Albinism is an inherited congenital disorder which occurs in humans with a prevalence of around 1 in 20,000 and is linked to a disruption of melanin synthesis or transport. Associated with this disorder is a significant hypopigmentation, primarily of skin, hair and the eye, but also of parts of the brain. This hypopigmentation has various effects on the morphology and function of the eye and the brain. It affects, among others, the iris, retinal pigment epithelium and macular differentiation, Nervus Opticus, visual acuity, chiasmal crossings, binocular vision, fixation and nystagmus, refraction and cerebral maturation.

In patients affected by albinism, the treatments of eye-related disorders vary and can range from surgical intervention to milder forms of treatment. Early Intervention (EI) is a general term which subsumes various supportive, non-invasive therapies for children aiming to improve their quality of life. These strategies are known as “Fruehfoerderung” in German, and appear in the Anglo-Saxon medical literature under various names, besides ‘Early Intervention’ also as ‘visual stimulation’, ‘visual learning’ etc.

As part of the study, the specific impact of EI on visual acuity has been considered. Relevant data has been obtained from the Archive of the Department of Ophthalmology of the University Hospital of Saarland, and the visual acuity of a cohort of children aged 6 to 15 (test group, **n = 232**) has been compared to the one of a control group of patients aged over 25 (control group **n = 139**), who have not received EI.

The statistical analysis conducted as part of this thesis points towards a slight yet statistically insignificant improvement of visual acuity in girls who have received Early Intervention. This slight improvement of around 0.1 visual units is notably absent in boys. Further analysis has confirmed that this small improvement is not due to a generally better visual acuity in females, as no enhanced visual acuity could be seen in the females of the control group when compared to the corresponding males.

Nonetheless, even this small improvement of the visual acuity in the females of the test group is weak when considered on a scale from 0 to 1. Macular and optical nerve pathologies correlate significantly to visual acuity. The more severe such pathologies are, the worse the visual acuity is.

As the statistical analysis employed as part of this study only considers cohorts of patients, but cannot monitor the changes of visual acuity on an individual basis, it is possible that some patients with a particular predisposition may be more amenable to treatment by EI than others. Such individual effects ‘disappear’ in a cohort-based analysis but may be relevant. Indeed, not all patients in the two respective cohorts show similar visual acuities, and the statistical spread is considerable. Future studies should therefore investigate the impact of EI on a more personal basis, for instance by monitoring

improvements of visual acuity on an individual basis, during EI and in form of a close follow-up during the subsequent years. At the same time, the question of ‘responders’ and ’non-responders‘ is of major importance for a possible treatment and prognosis and should be addressed by accompanying biochemical and possibly even genetic analyses.

Kurzfassung

Albinismus ist eine erbliche, kongenitale Veränderung, die beim Menschen mit einer Häufigkeit von etwa 1 in 20.000 auftritt und mit einer gestörten Synthese von Melanin oder einem gestörten Melanin-Transport in Verbindung steht. Hypopigmentation geht mit dieser Störung einher und betrifft primär die Haut, Haare und das Auge, aber auch Teile des Gehirns. Hypopigmentation wirkt sich auf verschiedene Weise auf die Morphologie und Funktion des Auges und Gehirns aus. Es betrifft unter anderem die Iris, das retinale Pigment Epithelium und die makulare Differentiation, den Nervus Opticus, die Sehschärfe, chiasmale Kreuzungen, binoculare Sehweise, Fixation und Nystagmus, Refraktion und cerebrale Entwicklung.

Bei Patienten mit Albinismus variiert die Behandlung von Beeinträchtigungen des Auges und reicht dabei von einem chirurgischen Eingriff bis zu milderen Formen der Behandlung. Der Ansatz der visuellen Frühförderung (FF) subsummiert dabei verschiedene unterstützende, nicht-invasive Therapien für Kinder, die auf eine Verbesserung der Lebensqualität abzielen. Diese Strategien finden sich in der anglosächsischen Literatur unter Bezeichnungen wie „Early Intervention“ (EI), „visual stimulation“ oder „visual learning“ wieder.

Als Teil dieser Studie ist der besondere Einfluss der visuellen FF auf die Sehschärfe untersucht worden. Die entsprechenden Rohdaten sind dabei aus dem Archiv der Augenklinik des Universitätsklinikums Saarland bezogen worden. Die Sehschärfe einer Gruppe von Kindern im Alter von 6 bis 15 Jahre (Test-Gruppe, **n = 232**) ist dann mit der Sehschärfe der Kontrollgruppe von Patienten über 25 Jahren verglichen worden (**n = 139**), die keine FF erhalten hatten.

Die als Teil dieser Arbeit durchgeführte statistische Analyse weist auf eine geringe, aber statistisch nicht signifikante Verbesserung der Sehschärfe bei Mädchen mit FF hin. Diese geringe Verbesserung von etwa 0,1 Messstufen (Zeilen) fehlt interessanterweise bei den Jungen. Eine weitere Analyse der Daten hat ergeben, dass diese geringfügige Verbesserung nicht auf einer generell besseren Sehschärfe bei Frauen beruht, da bei den Frauen in der Kontrollgruppe im Vergleich zu den entsprechenden Männern keine bessere Sehschärfe ermittelt werden konnte.

Letztendlich fällt diese kleine Verbesserung der Sehschärfe bei den Patientinnen in der Test-Gruppe auf einer Skala von 0 bis 1 aber sehr gering aus. In diesem Fall dominieren letztendlich Pathologien, wie Veränderungen der Iris, der Makula und des Sehnervs, die auch signifikant mit der Sehstärke korrelieren. Je dramatischer solche Pathologien sind, desto schlechter ist die Sehschärfe.

Da die in der Arbeit verwendete statistische Analyse allerdings lediglich Gruppen von

Patienten betrachtet - und daher individuelle Veränderungen der Sehschärfe außer Acht lässt - ist es durchaus möglich, dass manche Patienten mit einer gewissen Prädisposition besser auf eine Behandlung mit FF reagieren als andere. Solch individuelle Besonderheiten „verschwinden“ bzw. gehen in einer Gruppen-basierenden Analyse quasi in der Menge unter, können jedoch relevant sein. In der Tat zeigen nicht alle Patienten in den beiden entsprechenden Gruppen jeweils ähnliche Sehschärfen und die statistische Streuung ist erheblich. Zukünftige Studien sollten daher die Auswirkung von FF eher mit Hinblick auf einzelne Patienten untersuchen, beispielsweise durch eine Beobachtung der Verbesserung der Sehschärfe auf individueller Basis, während der FF und anhand einer umfassenden Nachbetreuung in den darauffolgenden Jahren. Zugleich ist der Umstand, dass gewisse Patienten auf die Behandlung ansprechen, und andere nicht, von großer Bedeutung sowohl für die Therapie als auch für die Prognose und sollte daher von begleitenden biochemischen und womöglich sogar genetischen Studien näher beleuchtet werden.

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1. Albinism: A short introduction

Albinism is an inherited congenital disorder which occurs in humans with a prevalence of around 1 in 20,000 (Käsmann-Kellner et al. 2007). In some parts of the world, such as Africa, the prevalence is even somewhat higher (*e.g.* 1 in 10,000). Albinism is the result of disrupted or reduced melanin biosynthesis or deficient transportation (see below). Hence, persons with albinism are characterized by a distinct lack of melanin, which sometimes results in a range of obvious features, such as white skin, white hair and also absence of pigment in the eyes. Interestingly, albinism is not limited to humans but also occurs in all mammals, and "albino" rats, for instance, are well known and liked as pets (Figure 1).

From a scientific point of view, albinism is a rather complex issue which combines various aspects ranging from the genetics of inheritance and melanin biosynthesis to ophthalmological issues and, in some extreme cases, even social rejection. Before moving on to more clinical matters, it is therefore necessary for our understanding of this matter to briefly consider these genetic, biochemical and social aspects first.

Figure 1.1: Picture of two normally pigmented (left) and an albinistic guinea pig (right). The white fur and red eyes are clearly visible. Whilst some normal guinea pigs may also be almost while, pigmentation may be present in patches and the eyes are black (left). Animals and photos are kindly supplied by Elizabeth Louise Jacob.

In short, albinism is a multi-facetted inheritable condition based on specific genetic alterations with an associated, changed biochemistry, notably the one surrounding the pigment melanin. Albinism sometimes results in a specific phenotype with diverse characteristics which have an impact on the individual's visual sensory abilities and in some cases on her or his neuropsychiatric health. In addition, such changes and "unusual looks" in general terms may also raise issues of a more social nature. Here, a particular

sensitivity of skin towards sunlight and often impaired eyesight are perhaps the most significant issues affecting persons with albinism. Such sensitivities obviously demand specific precautions and types of behaviour (especially with regard to exposure to sunlight) which are not necessary for persons with normally pigmented skin or eyes. These individual layers of albinism from basic genetic changes to social behaviour will now be addressed briefly starting with the basic genetic aspects.

1.1. Genetic aspects of albinism

From a genetic point of view, albinism is not just a single disorder due to one specific mutation. It rather represents a group of slightly different recessive disorders, each with one (or several) particular mutations in the melanin biosynthesis or transport pathway. Most of these forms are related to autosomal recessive alleles which have to be passed on from both parents (see below).

As the various forms of albinism are recessive traits, the idea that albinism is an inheritable disorder is far from obvious, as it also "appears" in children of seemingly non-albinistic parents. Historically, the notion of albinism as an inheritable disorder therefore dates back to the beginning of the 20th Century, and hence to the beginnings of modern genetic research. W.C. Farabee, in 1903, was the first to suggest that albinism in humans could be considered a recessive trait, which was confirmed later on by more extensive studies conducted by W.E. Castle and colleagues (Farabee 1903, Castle 1903). As a consequence of the recessive nature of this trait, the chance of inheriting albinism is comparably low and, as already mentioned, albinism occurs despite the fact that both parents are seemingly non-albinistic.

Indeed, according to the genetics of inheritance - which dates back to Gregor Mendel (1822-1884) and the mid 19th Century - a recessive trait is defined as a particular phenotypical property **A**, which is passed on to the offspring only if *both* parents possess one allele bearing the genetic information of his trait (without, however, necessarily also showing this phenotype). In contrast to a dominant trait, recessive traits are therefore passed on to the next generation rather infrequently. Yet in contrast to dominant traits, the phenotype **A** may be passed on from non-**A** parents, *i.e.* from parents who do not show this particular phenotype. Hence albinism may occur in children of parents which both are not albinistic, and therefore for the layman also rather "surprisingly".

Whilst these considerations apply to albinism in general terms, albinism is not a simple 'one mutation' issue. At closer inspection, it becomes apparent that different forms of albinism exist which are associated with different kinds of mutations (see 1.2.). Eventually it has been shown that all forms of albinism are autosomal recessive with the exception of ocular albinism (OA), which is X-linked recessive and linked to a single gene, OA1 (Oetting *et al.* 1999). The OA1 gene is located on chromosome Xp22.32. A total of 25 missense, two nonsense, nine frameshift and five splicing mutations have been found in the OA 1 gene (Oetting 1998). As OA is passed on through X-linked inheritance, this kind of albinism is usually found in male patients. The prevalence of OA in males can be explained by considering genetics: Males possess a XY set of chromosomes (21st chromosome pair) and their Y chromosome cannot 'compensate' for an inheritable trait present on their X chromosome, whilst females have two X chromosomes and their 'unaffected' X chromosome can compensate for the mutation of the affected one (bearing in mind, of course, that the trait is recessive).

Other types of albinism, *i.e.* oculocutaneous albinism (OCA), are genetically more complex due to various distinct mutations on different genes and chromosomes. To date, 14 different genes in ten chromosomes (not including the X chromosome) have been identified which are associated with different types of OCA. These forms of OCA are briefly summarized in Table 1 (Cortes *et al.* 2005, Oetting *et al.* 2005, Rundshagen *et al.* 2002, Käsmann-Kellner *et al.* 2007, Hedge *et al.* 2002, Fitzpatrick *et al.* 1974). It should be noted that this classification is based on a rather detailed genetic analysis and as a consequence is generally open to future changes and additions.

Based on genetic considerations, oculocutaneous albinism (OCA) itself is divided into four forms (see Table 1). Whilst it is difficult to clinically distinguish these forms by phenotype alone, they differ by their distinct mutations in different proteins or enzymes (see 1.2.). Therefore genetic analysis is a highly useful tool in the precise diagnosis of the specific type of albinism (Zühlke *et al.* 2007). At closer genetic inspection, Zühlke *et al.* have found genetic variations in 70 % of a large German cohort of albinism patients. Most of these (German) patients exhibit OCA1 with mutations in the tyrosinase gene, whilst other, slightly milder forms (OCA2 - OCA4) result from mutations in genes which affect the processing of this enzyme or trafficking to the melanosomes (Costin *et al.* 2003, Toyofuku *et al.* 2010). Here, it should be noted that OCA2 - and not OCA1 - is the most frequent form of albinism worldwide as it is most prevalent in countries and continents with a high rate of consanguineous parents.

Importantly, whilst OCA1 is related to mutations in the tyrosinase gene, such mutations are not necessarily identical in all individuals with OCA1. An analysis of OCA1 patients has surfaced DNA variations in 82 unrelated individuals, and these variations include 68 single nucleotide changes, stop mutations, polymorphisms, four nucleotide insertions and six deletions (Opitz et al 2004). In any case, tyrosinase, a key enzyme in the melanin synthesis, is affected by these various mutations.

Type of Albinism	Genetic Location	Dysregulation of Melanin Synthesis	Subgroups and Syndromes of Hypopigmentation
OCA1	Mutations in tyrosinase gene 11q14-q21	Blockage of pigment formation	OCA1A tyrosinase completely blocked OCA1B tyrosinase incompletely active OCA1TS temperature-sensitive activity of tyrosinase
OCA2	Mutations in P-gene 15q11.2-q12	Formation of Melano-soms “P”: “pink eye-dilution gene” transport	OCA2 Prader-Willi-Syndrome PWS Angelman-Syndrome AS Griscelli-Syndrome GS Type1-3
OCA3	Mutations in TYRP-1-gene 9p23	Stabilization of melanosoms (“tyrosinase related protein 1”)	OCA3 so-called “Red Albinism”
OCA4	Mutations in MATP-gene 5p13.3	Transport (membrane associated transport protein)	rare in Europe, more common in Asia
Hermansky-Pudlack-Syndrome	22q11.2-q12.2,19q13,11p15-p13, 10q24.32, 10q23.1, 6p22.3,3q24	Formation of melano-soms, biogenesis of lysosoms	HPS Types 1-8 Genetically very heterogeneous
Chediak-Higashi-Syndrome	1q42.1-q42.2	Formation and size of melanosoms	very rare
OA1(OA-X)	Xp22.3	Biogenesis of melanosoms, signal transduction	Ocular Albinism Type 1

Table 1.1: Molecular genetic types of albinism, their genetic location and resulting dysregulation of intracellular processes (including melanin synthesis). Specific subgroups, their specific names and their occurrence are mentioned where applicable. (Cortes *et al.* 2005, Oetting *et al.* 2005, Rundshagen *et al.* 2002, Käsmann-Kellner *et al.* 2007, Hedge *et al.* 2002, Fitzpatrick *et al.* 1974).

1.2. Biochemistry

As the genetic considerations have indicated already, albinism is related to the synthesis and transport of the pigment melanin. Here, melanin is a general name for a group of intensively coloured, mostly reddish, brown or black pigments synthesized in the human body primarily from the amino acid tyrosine. There are various polymeric structures subsumed under the umbrella of "melanin", most of which are based on oxidized and subsequently polymerized hydroxyl-tyrosine moieties (Mason 1948, Lerner *et al.* 1950). The melanins vary as far as their chemical composition, appearance (colour) and biosynthetic pathways are concerned. Figure 2 briefly summarizes the two most important biosynthetic pathways of melanin in the human body, ultimately resulting in the "tyrosine only" polymer called eumelanin and the "tyrosine / cysteine" polymer pheomelanin (Ito and Wakamatsu 2011). Whilst both types of melanin differ considerably in their chemical structures and absorption spectra (*i.e.* colour), they share certain similarities when it comes to their biochemical pathways, which will now be considered in more detail. It should be mentioned from the outset that this overview is necessarily brief and focussed and hence cannot provide a complete overview of the rather complex (bio-) chemistry of the various forms of melanin. Recent reviews in this field may be consulted for more detail (Simon *et al.* 2009, Hearing 2011, Ito and Wakamatsu 2011).

Despite the chemical diversity of the various melanins, the initial step in the melanin biosynthesis generally involves the hydroxylation and subsequent oxidation of the amino acid L-tyrosine to L-Dopaquinone (DQ) by the enzyme tyrosinase (E.C. 1.14.18.1) in the presence of molecular oxygen. As we will see later on, this enzyme forms the key to understating the various forms of OCA. L-Dopaquinone is the last metabolite shared by the eumelanin and pheomelanin synthetic pathways. Chemically speaking, this quinone is highly reactive and can undergo two distinct electrophilic reactions, either intramolecularly or with cysteine. In the first case, the nucleophilic amino group of L-Dopaquinone reacts intramolecularly with the quinone to form a cyclic molecule called cyclodopa. The latter can oxidize, on occasion decarboxylate and eventually polymerize in the presence of dioxygen and/ or certain enzymes (such as tyrosine related protein-1 Tyrp-1) to form various forms of eumelanin.

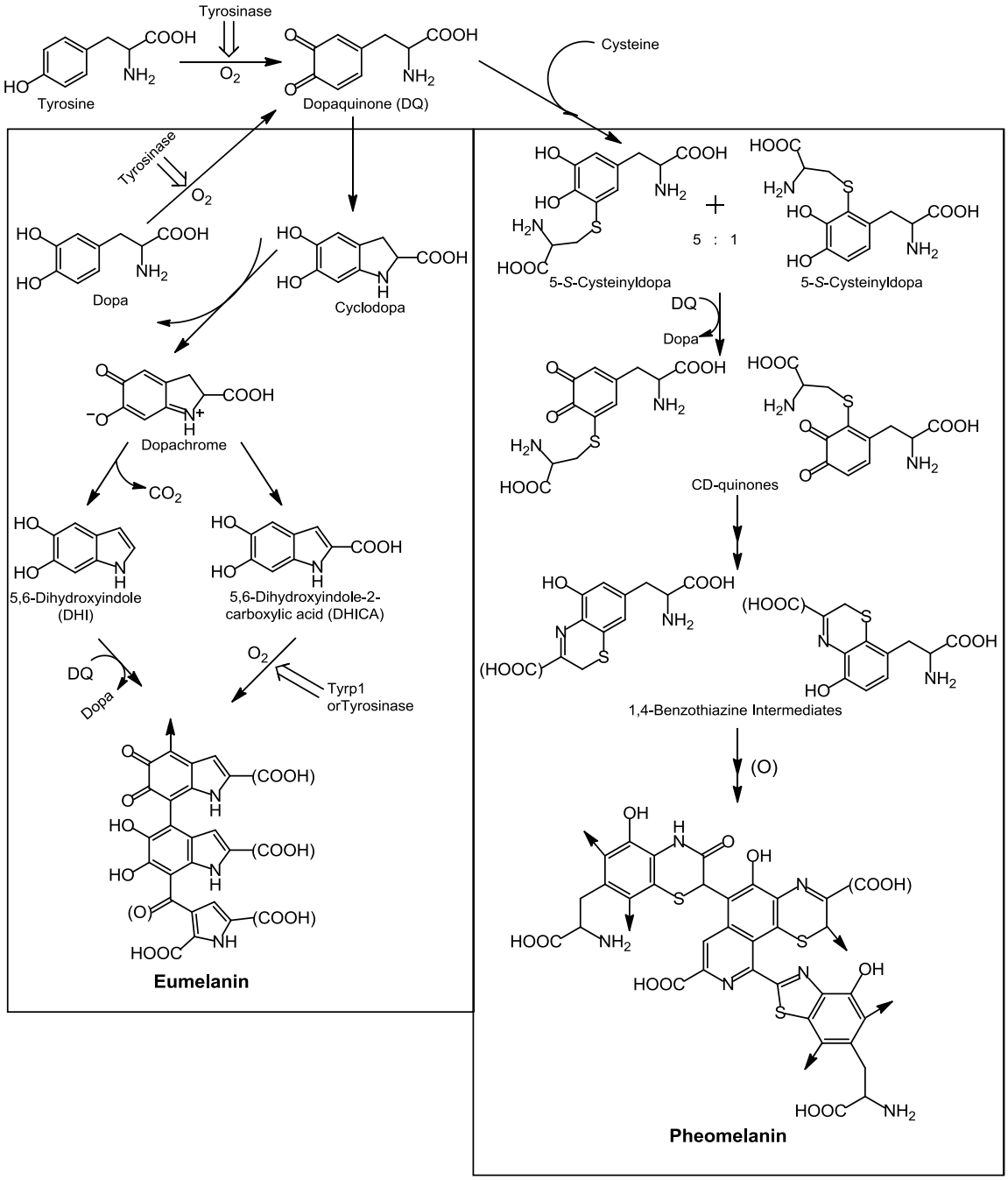

Figure 1.2: Biosynthetic pathways leading from the amino acid tyrosine to the two major forms of melanin, namely eumelanin (which is derived solely on tyrosine) and pheomelanin (which is derived from tyrosine and cysteine). CD = cysteinyl-dopaquinone, DQ = Dopaquinone.

In the second case, the nucleophilic thiol group of cysteine reacts with the quinone to form 2-*S*- or 5-*S*-cysteinyldopa in a ratio of 5:1. This adduct of two L-amino acids is subsequently oxidized to cysteinyl-dopaquinone (CD). CD is prone to a second nucleophilic attack at its quinone moiety, this time by the nucleophilic amine group of the

cysteine. This intramolecular reaction resembles the one in the eumelanin pathway (here involving the amine group of cysteine, there the amine group of tyrosine) and results in cyclic 1,4-benzothiazine intermediates which ultimately polymerize to form various types of pheomelanin.

The overarching importance of the enzyme tyrosinase in both pathways is apparent and its pivotal role in the occurrence of albinism has already been illustrated in Table 1. Interestingly, the conversion of tyrosine to L-DOPA is also a key step in the biosynthesis of the neurotransmitter dopamine, yet the biosynthetic pathways leading to the melanins on the one hand and the neurotransmitters on the other (dopamine, norepinephrine, epinephrine etc.) differ. Whilst tyrosinase is a copper-containing enzyme with the E.C. number 1.14.18.1 (see above), the tyrosine hydroxylase responsible for the hydroxylation of L-tyrosine to L-DOPA is a rather different iron-containing hydroxylase with the E.C. number 1.14.16.12, which does not subsequently oxidize the hydroquinone to the (highly reactive) quinone required for nucleophilic attacks and / or polymerization. Furthermore, tyrosinase and tyrosine hyhydroxylase are found in different types of (human) cells. Here, tyrosinase is primarily but not exclusively present in the melanosomes of pigmented cells (melanocytes). Interestingly, this enzyme has also been isolated from lower animals, plants and even fungi, including mushrooms (Bourquelot and Bertrand 1895).

Before finally turning our attention to the phenotypical and subsequently also more clinical aspects of albinism, we will briefly consider the (historical) background linking albinism to various mutations related to the tyrosinase system in general and tyrosinase in particular (in part reviewed by Hearing 2011). In 1908 A.E. Garrod was the first researcher who suggested that albinism was caused by an inborn error of metabolism (Garrod 1908). This is rather remarkable as enzymology at this time was still in its early infancy and the genetic issues surrounding albinism had also only just become apparent. A.E. Garrod mentioned tyrosinase in his publication, and hypothesized that it was the action of tyrosinase on tyrosine, possibly from protein hydrosylates, that was responsible for melanin formation. Indeed, the activity of the enzyme tyrosinase in various - lower - organisms was already known at this time (see above). At this point, A.E. Garrod did not, however, show a clear connection between lack of tyrosinase activity on the one hand and albinism on the other.

Shortly after, the DOPA reaction of B. Bloch was used and it was shown that whilst albino hair follicles in both human skin and mouse contained melanocytes, they apparently lacked tyrosinase activity (Bloch 1917). At this point, the connection between albinism and

tyrosinase became firmly established and the “hair bulb test” was introduced which measures the ability of the hairbulb to turn brown when placed into a DOPA solution (such a hairbulb obviously contains active tyrosinase) (Oetting *et al.* 2003, Witcop *et al.* 1970). According to the “hair bulb test”, different forms of albinism were therefore classified as “tyrosinase positive” or “tyrosinase negative”. “Tyrosinase negative “indicated lack of *any* tyrosinase activity (Kugelman *et al.* 1961). Unfortunately, this test was neither particularly sensitive nor specific as it could not differentiate between the genetically different forms of albinism. Furthermore, this test obviously also depended on the amount of this enzyme present in the hair follicle, which changes during adolescence. Not surprisingly, the Bloch test sometimes produced a negative result in a young child, but became positive with age. Whilst this test can be seen as an early biochemical assessment recruited for medical diagnosis, it has been superseded by modern biochemistry and especially genetics and is no longer used.

After the groundbreaking studies by enzymologists such as A.E. Garrod, the melanin biosynthesis pathway was elucidated in the 1920s by H.S. Raper and further details of this rather complex biosynthetic set of successive transformations were discovered by H.S. Mason, A.B. Lerner and T.B. Fitzpatrick in the 1940s and 1950s (Raper 1926, Mason 1948, Fitzpatrick 1958). The biosynthetic pathway leading to melanins is therefore also commonly known as the Raper-Mason pathway, which has been refined further in the 1960s and 1970s by the groups of A.B. Lerner at Yale and T.B. Fitzpatrick at Harvard (Hearing 2011). At this point, albinism was generally considered as a single gene disorder closely related to (mutations of) the enzyme tyrosinase.

Two individual families of albinists reported by P.D. Trevor-Roper and C.J. Witkop, however, apparently disagreed with this theory (Trevor-Roper 1952). In each of these families, both parents, *i.e.* mother and father, were albinistic, yet the offspring was normally pigmented. Logically, as albinism is considered a recessive trait, a *single* - *i.e.* in both parents identical - mutation in the tyrosine gene would have to be present on *both* respective chromosomes of *both* parents, and hence the offspring should also inherit the mutation on *both* of its parental chromosomes, and therefore also be albinistic. As this was clearly *not* the case, the most logical explanation was that there could be several relevant mutations leading to albinism. Hence whilst one parent exhibited one particular mutation **A** on both chromosomes leading to albinism, the other parent had a different mutation **B**, also on both of her/his chromosomes and also leading to albinism. In sharp contrast, the non-albinistic children had inherited sets of chromosomes which had mutation **A** on one chromosome and mutation **B** on another, *yet both mutations were*

counterbalanced by non-mutated versions on "healthy" chromosomes. As albinism is a recessive trait, this constellation is obviously insufficient to result in an albinistic phenotype in the children. Interestingly, the presence (and absence) of albinism in these families is a clear indication that more than one mutation exists which ultimately may result in albinism. This rather instructive finding has been confirmed subsequently by genetic analysis, as discussed already in 1.1.

Today, we know that albinism is not due to a *single* genetic / biochemical event but that different types of albinism exist which feature mutations in different genes and hence disrupt the biosynthesis of melanin at different points of the biosynthetic pathway - and with different severity. Whilst tyrosinase is clearly a key player in this pathway, and certain mutations in this enzyme may result in albinism, other mutations affecting other proteins are equally important (see Table 1.1).

1.3. Albinism as lack of pigment in skin and hair

Regardless of the precise genetic and subsequently biochemical cause or causes of albinism, the resulting phenotype is well known and also implies some specific medical "conditions". In human skin, melanin primarily serves as a natural "sunscreen" due to its ability to absorb sunlight. Without the presence of melanin, the skin is suffering from hypopigmentation, *i.e.* a pale often slightly spotted skin. The fact that the skin is not completely free of any pigmentation is due to the circumstance that melanin is not the only pigment found in skin. Therefore persons with albinism may still have some colourful (often brownish) spots in their skin due to other pigments. Furthermore, different forms of albinism differ in the severity of hypopigmentation, and some forms of albinism may not lead to a complete absence of *any* taint.

Hypopigmentation by itself is not dangerous, yet the absence of the natural sunscreen in the skin implies, of course, that energy-rich fractions of sunlight, such as ultraviolet A light (wavelength 315 - 380 nm) may enter the skin cells unfiltered and may cause severe damage to the cell, especially to proteins and DNA. Such damage is often caused by the interaction of UV radiation with chromophobic small molecules or proteins in the skin, some of which are acting as photosensitizers and trigger the generation of highly reactive oxygen species (ROS) in the cells exposed to UVA. Ultimately, exposure to UVA and associated ROS generation may result in DNA modifications and, in the worst case, in skin cancer. In fact, the risk of developing skin cancer is 30 to 40 fold higher in lighter skin

when compared to darker skin (Hearing 2011). Additional benefits of pigmentation include cosmetic appearance (see below), detoxification (of radicals) and photo-protection in humans, and even camouflage and thermal regulation in amphibians. Whilst hypopigmentation is less of a health issue in regions where the exposure to sunlight is limited (such as in Northern Europe, Siberia, Canada, Alaska), it poses a significant health risk in Africa and equatorial regions of Asia and America.

At the same time, albinism in African and Asian persons is also more obvious: despite being of a non-Caucasian origin, such albinistic persons appear as “white”. In Africa, people with albinism therefore not only possess a considerably higher risk of skin cancer but unfortunately, their appearance is also very different from the rest of the population and such persons are often socially stigmatized (see for instance BBC News, 27th of July 2008). Here, some of the most bizarre stories have recently emerged from central Africa, including the belief that persons with albinism - and their body parts - possess healing properties. Sadly, this superstition has resulted in several killings and incidences of rape and possibly even cannibalism in countries such as Tanzania, Burundi and Zimbabwe. In contrast, lack of melanin in the skin is less apparent in Caucasian people, who have a fairly white skin, anyway, and there are no major reports of social stigmatization of albinists in present-day predominantly “white” societies.

2. Albinism from the ophthalmological perspective

2.1. Albinism, syndromes and visual impairment

Besides the various medical and in some instances social issues related to “fair” skin (and hair), the most significant impact of a disturbed melanin synthesis is probably not related to the skin or hair, but to the eye, and hence falls within the remit of ophthalmology. Depending on the degree by which the eye and / or skin (hair) is affected, one may distinguish between OCA and OA as the two major forms of albinism. OA is the typical ocular albinism which primarily affects the eyes whilst OCA is a form of albinism which affects the eyes, skin and hair (see also section 1).

Notably, the effects of albinism on the eye are not just related to a “red pupil”, but also include a decreased differentiation of the central neurosensory retina and an atypical chiasmal crossing (*i.e.* the prevalence of crossed fibres). This is in part due to a hypopigmentation of the eye but also to a lack of melanin in the brain. Indeed, the occurrence of melanocytes is not limited to skin, hair and the eyes but also extends to the inner ear, the substantia nigra, the heart and even to adipose tissue (Zecca *et al.* 2001, Brito and Kos 2008, Zecca *et al.* 2008, Randhawa *et al.* 2009). Not surprisingly, there are various structural and functional pathologies associated with hypopigmentation in the eye and in the brain. Ultimately, the reduction of melanin in the eyes is characterized by several **ocular abnormalities** including foveal hypoplasia and the resulting decrease of visual acuity, misrouting of optic nerve fibres from the retina to the visual cortex, nystagmus, strabismus and translucency of the iris (Käsmann-Kellner *et al.* 2007).

Various disorders and clinical syndromes are relevant to ophthalmology and have been associated with albinism in the past (see also section 1 and Table 1 in particular). Yet most of these disorders are not limited solely to an ophthalmological issue. Such syndromes include **Hermansky-Pudlak syndrome (HPS)**, an autosomal recessive lysosomal storage disease of the reticuloendothelial system whose genetic location is on various Chromosomes (see Table 1). HPS occurs in various forms (Type 1 to 8) which are genetically very heterogeneous. In any case, HPS is characterized by OCA, platelet disorder (which leads to bleeding problems and lung fibrosis), granulomatous colitis and renal failure (Hermansky *et al.* 1959).

Chediak–Higashi Syndrome (CHS) is an autosomal recessive disorder. Its genetic

location is chromosome 1q42.1-q42.2. CHS is characterized by OCA and immunodeficiency due to an associated white blood cell disorder (the leucocytes contain gigantic peroxidase granules). Most of the patients with CHS also develop a lympho-proliferative syndrome with fever, jaundice, hepatosplenomegaly, pancytopenia and bleedings. Patients with CHS frequently require bone marrow transplantation, and without transplantation, life expectancy is only about ten years (Saez-De-Ocariz *et al.* 2008).

Prader-Willi Syndrome (PWS) can be associated with OCA2. Its genetic location is the P-gene on Chromosome 15. 75% of patients suffering from PWS have a small deletion on the proximal part of the long arm of the paternally acquired chromosome 15 (15q11-q13). Most of the remaining 25 % of PWS sufferers exhibit two apparently normal, but *maternally acquired* chromosomes 15 (uniparental disomy). The prevalence of PWS is between 1 in 25,000 and 1 in 10,000 of live births. At birth, PWS is characterized by hypotonia, failure to thrive, lethargy and feeding difficulties. Later on in life, besides OCA2 a range of additional health impediments may emerge, including hyperphagia, obesity, hypogonadism, hyperglycaemia, short stature, strabismus and intellectual delay (Cassidy 1997).

Angelman Syndrome (AS) may also be associated with OCA2. This syndrome was first described in 1965 by the British paediatrician, Dr. Harry Angelman (Angelman, 1965). AS is also a genetic disorder, with a mutation on the P-gene on Chromosome 15 (15q11-q13), similar to PWS (AS and PWS are sister syndromes). AS is characterized by intellectual and developmental delay, cognitive impairment, hyperactivity, frequent laughter and seizures (Angelman, 1965).

Griscelli Syndrome (GS) has an autosomal recessive pattern of inheritance and is associated with OCA2. As in the case of the PWS and AS, the genetic location responsible for GS is Chromosome 15. GS can be subdivided into three types (Type 1 to 3) and besides OCA2 is characterized by immunodeficiency, which often causes death in early childhood (Saez-De-Ocariz et al 2008).

Waardenburg Syndrome (WS) is a genetic condition inherited in an autosomal dominant pattern. WS appears in four types (Type 1 to 4, with Type 3 and 4 being very rare) depending on the genetic location of the mutation responsible. WS is characterized by hypopigmentation of the iris, hair and skin as well as a sensorineural hearing loss (Waardenburg 1951). The **Albinism deafness syndrome** is thought to be a form of WS Type 2. It is characterized by congenital neural deafness and a piebald-like phenotype.

Piebaldism itself is an autosomal dominant condition and, unlike albinism, does not affect the eyes (normal pigmentation). Piebaldism is rather characterized by white forelocks, normally pigmented and hypopigmented skin patches and triangular hypopigmented areas on the forehead.

While these syndromes in one aspect or another have been associated with albinism (mostly OCA2), the changes to the visual system in albinism are extensive and often also diverse. In order to understand better the impact albinism has on the eyesight of albinism patients in particular - and their quality of life in general - it is therefore necessary to literally keep a closer eye on the various changes to the visual system in albinism, which may occur besides the more obvious syndromes and OCA2.

2.2. Phenotypes of the visual system in ocular and oculocutaneous albinism

First of all, one should note that hypopigmentation has different effects on the morphology and function of the eye and the brain and, as a result, patients with albinism are not all “the same” but may exhibit a quite variable phenotype. This requires a more differentiated approach and a detailed classification of the stages and the levels of the ocular pathology. This classification assists with the individual assessment of each albinism patient and enables the ophthalmologist to assess if any (abnormal) changes are present. This classification is particularly important in the case of children, as it allows an early diagnosis and may also benefit support, such as Early Intervention which is the topic of this thesis.

In albinism, we may generally distinguish structural and functional pathologies related to:

- Iris
- Retinal pigmentepithelium and macular differentiation
- *Nervus Opticus*
- Visual acuity
- Chiasmal crossings
- Binocular vision / squint
- Angle kappa κ
- Fixation / nystagmus
- Refraction
- Cerebral maturation / myelinisation / delayed visual maturation

In the following sections, we will consider briefly the typical features observed in the eye of persons with albinism. It should be mentioned from the outset that only a selection of the most significant pathological and non-pathological hallmarks of albinism in the eye can be covered, some of which are also relevant to this work. More comprehensive discussions related to the various morphological changes in albinism can be found in the recent literature (Cortes et al. 2005, Käsmann-Kellner et al.2003, Käsmann-Kellner et al 2007).

2.2.1. Iris

In persons affected by albinism, the colour of the iris itself is not affected directly, and, similar to non-albinistic persons, albinists may have blue, blue-grey or dark brown eyes, yet with variable degrees of iris *translucency*. The translucency of the iris is due to the lack of melanin in the iris pigment epithelium. In persons with albinism, the colour of the iris therefore often appears as "lighter" when compared to the one found in their next of kin. Yet this apparent change is not related to a particular type of OCA, it must rather be seen before an ethnical background. In Caucasians affected by OCA2, for instance, the colour of the iris is blue. In the same group of African patients, the iris appears as green-brown.

Nonetheless, whilst hypopigmentation of the iris is not connected directly to OCA, it is also not just a "cosmetic" issue either, as the pigments also serve as natural filters for (energy rich, potentially damaging) light. Comparable to the situation in the skin, iris translucency results to an increased sensitivity towards light, and in the case of the eye, leads to photophobia. In the iris, there are four different degrees of hypopigmentation which are briefly summarized in Table 2.1. In this case, more severe degrees of hypopigmentation go hand in hand with a more severe sensitivity to light.

Degree of Hypopigmentation	**Hallmark**
Degree 1	Translucency in a form of dots in the periphery of the iris
Degree 2	Diffuse translucency in the periphery of the iris, pupillary margin well pigmented, not translucent
Degree 3	Confluent translucency in the periphery of the iris, the margin of the lens is well distinguished in the periphery of the iris in a circular pattern, the pupillary margin is not translucent
Degree 4	Complete translucency of the iris, the pupillary margin is translucent

Table 2.1: Classification of the four degrees of hypopigmentation of the iris (Käsmann-Kellner *et al.* 2007).

2.2.2. Retinal pigment epithelium (RPE) and differentiation of the macula

The hypopigmentation of the RPE exhibits a variable degree of hypopigmented retinal periphery and lack of foveal landmarks. The topographic differentiation of central photoreceptors depends on the synthesis of pigment. Hypopigmentation results in variable degrees of foveal dysplasia and has an influence on the visual acuity later in life. There is a correlation between the mutation in the tyrosinase gene (OCA1, see section 1) and a 30% reduction of ganglion cells. Optical coherence tomography (OCT) is able to detect a variety of foveal developmental abnormalities in albinism, from complete absence of foveal development to a central depression corresponding to a rudimentary annular reflex detected by ophthalmoscopy. Within this context, the often limited detection of foveal development (and abnormalities) in OCT as compared to other ophthalmoscopic techniques is likely due to poor fixation in patients with nystagmus (Harvey *et al.* 2006).

Accordingly, the classification of hypopigmentation of the RPE results in four stages, each associated with a more pronounced degree of macular hypoplasy (Table 2.2).

Degree of Hypopigmentation	Hallmark
Degree 1	Peripheral hypopigmentation of the RPE, foveal landmarks well structured
Degree 2	Peripheral and central hypopigmentation of the RPE, Wall-reflex positive, foveal landmarks hypoplastic
Degree 3	Significant peripheral and central hypopigmentation of the RPE, hypoplastic / dysplastic fovea and macula
Degree 4	Stage 3 plus atypical retinal vessels, failing to wreath the fovea

Table 2.2: Classification of the four degrees of hypopigmentation of the RPE and macular hypoplasy (Käsmann-Kellner et al 2007).

2.2.3. Degrees optic nerve head (ONH) pathology

In most patients with albinism, the optic nerve head is smaller than in persons not affected by albinism, and quite often also shows a hypo- or dysplasia. A dysplastic optic nerve head is more common in OCA1 patients (Käsmann-Kellner *et al.* 2007).

Such abnormalities related to the optical nerve seriously affect the eyesight of the person affected. Within this context, clinical data of over 500 patients examined in the Department of Ophthalmology of the University Hospital of Saarland (UKS) show a significant correlation between the degree of optic nerve head dysplasia on the one hand and visual acuity on the other (Käsmann-Kellner *et al.* 2007). Whilst there is also an apparent correlation between the type of albinism and the dysplasia of the optic nerve head, a correlation between the type of albinism or the stage of hypopigmentation of the iris or the retinal pigmentepithelium on the one hand, and visual acuity on the other, could not be observed in this study. These findings are rather surprising since optic nerve head dysplasia is particularly significant in the OCA1 type albinism, where it is present in 54% of OCA 1 patients, compared to a presence in only 14% of OCA2 and OA patients. Why such a strong prevalence of nerve head dysplasia in OCA1 patients does not firmly link OCA1 patients to a particularly poor visual acuity remains to be clarified. These results may require a thorough re-evaluation of the mechanisms underlying low visual acuity in albinism, that up to now were thought to be associated in one way or another with the ocular lack of pigmentation.

Nonetheless, the staging of optic nerve pathology is still a valuable tool which assists in the determination of the correct diagnosis and prognosis of albinism affecting eyesight, especially in children. The correlation between the four degrees of the optical nerve head pathology on the one side and visual acuity on the other (Table 2.3) forms part of this thesis and will be discussed in more detail in the Results and Discussion section.

Degree of ONH pathology	**Hallmark**
Degree 0	Normal ONH
Degree 1	ONH pale but of normal size
Degree 2	ONH small but vital
Degree 3	ONH pale and small
Degree 4	ONH dysplasia

Table 2.3: Classification of the four degrees of ONH pathology. The latter can be used as part of the diagnosis and prognosis of the future impact of albinism on visual acuity, especially in children (Käsmann-Kellner *et al.* 2007).

Whilst there is no direct correlation between the *type* of albinism or the stage of

hypopigmentation and visual acuity, the latter is often seriously affected by all forms of albinism. In many instances, a rather poor visual acuity across all types of albinism results in serious visual handicaps which will be reviewed in the next section.

2.3. Visual acuity and the level of visual handicap

As already mentioned, albinism in general affects the eyesight in different ways and in varying degrees of severity. This section will briefly consider the most apparent issues, problems and ultimately also pathologies associated with individual forms of albinism. Here, only a selection of the most apparent problems can be discussed, such as loss of visual acuity (which is the focus of this thesis), strabismus and nystagmus. Some of these deviations from normal eye morphology and eyesight result in distinct ophthalmic syndromes which will be mentioned and discussed briefly.

It should be noted that the data presented in this section has been obtained as part of a larger study involving over 500 patients with albinism at the Department of Ophthalmology of the University Hospital of Saarland (UKS) (Käsmann-Kellner *et al.* 2007). These values are therefore representative of persons living in (this part of) Germany, and hence are directly relevant to the research conducted as part of this thesis. One must bear in mind, however, that this data and the various correlations discussed here - do not necessarily apply in the same manner or intensity to other populations (with different habits) or to different ethnical groups or to predominantly non-Caucasian populations.

2.3.1. Visual acuity

The visual acuity in persons affected by albinism varies widely, ranging from 0.01 to 1.2 (compared to 1.0 in the case of normal visual acuity). As we will see in section 5, this wide spread of visual acuity results in considerable statistical variations and uncertainties. Hence, while the median visual acuity in patients suffering from various types of albinism is just 0.15 (Summers 1996, Käsmann-Kellner *et al.* 2007), there is a considerable spread and variability among albinists. Nonetheless, as this median value is rather low, many persons require medical assistance. The latter may include ophthalmological and refractive care and Early Intervention (see section 2.7), as well as low vision aid prescription, school, academic and professional rehabilitation services. Table 2.4 summarizes the most common types of OCA and their typical hallmarks, in the context of skin, hair, visual acuity and the incidence of nystagmus.

Type	Skin	Hair	VA	Median VA	Nystagmus (%)
OCA 1A	No tanning	Stays white	0.1 - 0.3	0.10	100
OCA 1B	Light tanning	Turns darker later on in life, blond to brown, eye lashes darker than hair	0.1 - 0.9	0.28	87
OCA 2	Light tanning	Turns darker later on in life, middle blond, eye lashes the same colour as hair	0.1 - 0.8	0.24	95
OCA 3	Pigmented	Ginger	0.1 - 0.3	0.2	90
OCA 4	Fair, no tanning	White to brown	0.1 - 0.5	0.1	100
OA	Normal or a little fair	All colours possible	0.1 - 0.8	0.12	92

Table 2.4: Summary of the various types of OCA (Types 1, 2, 3 and 4), including the dermatologic phenotypes and median visual acuity associated with these specific types of OCA. Features of persons with OA are provided for comparison. VA = visual acuity.

2.3.2. Atypical chiasmal crossings

Patients with albinism are characterized by atypical chiasmal crossings. While there are only 30 to 50% crossed chiasmal fibres normally, in patients with albinism this percentage increases to 70 to 80% (yet rarely reaches 100%). Such atypical chiasmal crossings result in a characteristic picture in the Visually Evoked Potentials (VEP) examination. The visually evoked potential is an electric signal generated by the occipital visual cortex in response to stimulation of the retina by light (Kanski, 2008). The VEP examination is used to diagnose optic nerve diseases, particularly disorders which are the result of demyelination. In the case of albinism, a special recording technique of the VEP examination indeed can be rather useful in diagnosing patients with an otherwise less typical phenotype. Here, the extent of atypical chiasmal crossings correlates rather well with the type of albinism, and such crossings are more pronounced in patients with OCA1 (Hoffmann et al.2003, Hoffmann *et al.* 2006, Käsmann-Kellner *et al.* 2007).

2.3.3. Strabismus

Strabismus is extremely common in patients with albinism. It has been found in 83.6% of the 500 patients with albinism examined as part of the above-mentioned study at the UKS (Käsmann-Kellner *et al.* 2007). The prevalence of strabismus in patients with albinism depends on the type of albinism, and strabismus is most prevalent in patients with OCA1 and OA (incidence rates in patients with OCA1: 93%, OCA2: 63%, OA: 93%, with a statistical significance of $p < 0.005$). In fact, the prevalence of strabismus is considerably higher in most patients with albinism compared to patients suffering from other congenital visual abnormalities, with the notable exception of patients with OCA2. Compared to other forms of OCA (and OA), OCA2 is associated with a markedly lower incidence of strabismus which is similar to the one found in patients with other congenital visual abnormalities, such as achromatopsia, aniridia or congenital cataract.

Interestingly, the different forms of OCA also differ with respect to the prevalence of different *sub-types of strabismus*. The incidence of *Strabismus divergens*, for instance, is significantly higher in OCA compared to other underlying conditions of squint. Furthermore, the study conducted at the UKS has observed that albinism is sometimes observed in patients with *Infantile Strabismus Syndrome*. Here a VEP examination (see above) has been particularly useful as diagnostic tool. It has been able, for instance, to identify atypical chiasmal crossings in some children with otherwise full visual acuity yet suffering from Infantile Strabismus Syndrome (Käsmann-Kellner *et al.* 2007), thus broadening the etiological aspects of infantile strabismus.

2.3.4. Positive Angle Kappa

Whilst the pupillary axis is defined as a perpendicular line through the cornea and the centre of pupil, the visual axis connects the fixation object and the fovea. The angle between these two axes is called kappa (κ). In normal patients, this angle is slightly positive as in healthy eyes the fovea is positioned slightly temporally from the pupillary axis. In contrast, a negative value for κ suggests *Strabismus convergens* and an increased, *i.e.* exceptionally large value for κ implies *Strabismus divergens*. As far as patients with albinism are concerned, an increased, highly positive κ has been found in about 50% of these patients (Brodsky *et al.* 2004, Summers *et al.* 2004, Käsmann-Kellner *et al.* 2007). As a very positive angle kappa may simulate a divergent squint, examinations related to strabismus in persons with albinism need to include an observation of the corneal reflex in monofixation of both eyes.

2.3.5. Nystagmus

Horizontal pendular and later jerk nystagmus is also linked to albinism where it generally occurs between the 8th and 12th week of life. The intensity of nystagmus (intensity = frequency x amplitude, given in Hz) changes significantly during the first four to six years of life and reaches a steady state between the sixth and eighth year. In the first years it is characterized by a change from pendular to jerk nystagmus and by a reduction of amplitude. Some patients with nystagmus adopt an abnormal head posture to take advantage of the so-called neutral zone, where nystagmus intensity is less (Käsmann 2000, Käsmann-Kellner *et al.* 2007). It should be emphasized that today it is possible to reduce nystagmus surgically in certain clinical constellations at or after five years of age.

2.3.6. Refraction

Albinism is also associated with a high prevalence of abnormalities in refraction. About two thirds of patients with albinism suffer from hyperopic astigmatism and one third from myopic astigmatism. Such anomalies with respect to refraction are already present in babies. As far as astigmatism is concerned, the grade of the optic nerve head dysplasia (see section 2.2.3.) directly correlates with a very high astigmatic refraction. In this case, the prescription of glasses is indicated as early as possible. An appropriate correction of these anomalies has a positive effect on the visual development later on in life. As albinism is also associated with a particular sensitivity to light (see section 1), two pairs of glasses are usually recommended, one with a light absorption of 20% and the other with a light absorption of 80% (Summers 1996, Käsmann-Kellner *et al.* 2007).

2.3.7. Delayed visual maturation (DVM)

A delayed visual maturation (DVM) is found in 50 to 75% of children affected by albinism. Here, a diagnosis of DVM should not lead to the premature conclusion that the child is actually blind. Indeed, the extent of DVM differs considerably between patients with albinism and its grade correlates with the abnormalities associated with the optic nerve head (Käsmann-Kellner *et al.* 2007). DVM is characterized by a delay in the onset of fixation in newborn babies. Fixation normally starts between the 4th and 8th week of life, and is occasionally delayed until the 12th week. No fixation after the 12th week is considered as delayed visual maturation and requires further evaluation. In albinism, this process can be delayed up to the 10th month of life.

The children suffering from DVM show the following signs (Duckman 2008):

- No fixation by 2 to 4 months after birth
- No eye contact
- No explorative visual behaviour
- No significant pathology in visual pathways or brain

There are four types of DVM, referred to as DVM 1 - DVM 4 (Duckman 2008). DVM 1 is characterized by:

- Neurological development and findings are normal
- No systemic abnormalities
- Electroretinogram (ERG) is often normal
- VEPs may be normal or abnormal, but present
- Electroencephalogram (EEG) is normal
- Normal ocular examination results
- Most patients show the signs by three months of age
- Resolution takes rarely longer than 6 to 7 months

DVM 1 is further divided in two subgroups, namely DVM 1A and DVM 1B. DVM 1A is a form of DVM 1 with no perinatal complications, whilst DVN 1B is a form of DVM1 with perinatal complications.

DVM 2 is characterized by DVM with systemic disease and/or neuro-developmental abnormalities.

DVM 3 is DVM associated with nystagmus and oculo-cutaneous albinism. The loss of visual function is significantly higher than would be predicted on the basis of nystagmus or albinism alone.

DVM 4 is DVM associated with congenital bilateral ocular abnormalities. The loss of visual function is significantly higher than would be predicted on the basis of the ocular abnormality alone.

After successful fixation the Sensitive Phase of visual maturation sets in, which itself consists of two phases. The Morphologically Plastic Phase usually lasts from the 8th to 10th month of age while the Functionally Plastic Phase may last up to 7 to 10 years of age. During these phases, various corrections or treatments are possible, which interfere with, guide or correct the maturation process. Here, a classical example is the treatment of amblyopia. It is therefore advisable that patients with albinism should also be involved in adequate supportive actions, such as Early Intervention, as early in life as possible (see section 2.4 and section 3).

2.4. Early Intervention ("Fruehfoerderung")

In the field of any paediatric subspecialty, Early Intervention is a general term which subsumes various supportive, non-invasive therapies for children aiming to improve the quality of life. These strategies are known as "Fruehfoerderung" in German, and appear in the Anglo-Saxon medical literature under various names, including 'visual stimulation', 'visual learning', 'early occupational therapy', 'exemplary early support', 'early education', 'Early Intervention' etc. Here, the term 'Early Intervention' (EI) will be used to represent these different strategies, as 'Early Intervention' seems to be the most adequate and also the most inclusive of these expressions.

In the German-speaking medical community, Early Intervention for visually handicapped and blind children as a comprehensive strategy can be traced back to the 1980s, for instance to the introduction of such an approach at the Hospital "Barmherzige Brueder" ("Good Samaritans") in Linz, Austria in 1986 (Priglinger *et al.* 1993). This does not, however, rule out that some related, yet probably less structured methods may have been used previously elsewhere, or that such strategies were implemented earlier on by other hospitals which failed to report them in the literature.

Today, the key elements of Early Intervention are represented by the following training schemes (Zihl *et al.* 2012):

- Visual exploration
- Visual localisation and orientation in the room
- Visual differentiation of colours and forms
- Visual differentiation of objects and faces

- Development of visual memory
- Visual differentiation of details and visual-cognitive discrimination of “Gestalt” and figure - ground - phenomenon

The impact of Early Intervention on the visual function in children has been studied soon after the introduction of this method in the 1980s. In 1993, Priglinger *et al.* have reported a comprehensive, yet from a statistical point of view rather limited study with 49 children, where the authors have evaluated visual acuity of these children in response to Early Intervention. In 34 of these patients, an improved visual function has been observed in everyday life. Yet only 15 of the 49 children showed a measurable improvement of visual acuity, which also was rather small (0.1 to 0.2). Notably, these children belonged to a group of patients suffering from an atrophic optic nerve (Priglinger *et al.* 1993).

3. Hypothesis

As mentioned in the Introduction, the genetic, biochemical and - above all - clinical evidence currently available points towards a significant correlation between albinism and a wide range of more or less severe ophthalmological disorders. In several cases, such as DVM, therapeutic interference with delayed or insufficient processes due to albinism seems to be possible, at least in theory. Not all of these interventions need to involve surgery or medication. As already indicated in the previous sections, a milder form of such an interference may involve Early Intervention (in children) in order to compensate for the delay in normal development.

The main objective of the research presented here therefore has been to explore a possible link between Early Intervention on the one side and improved visual acuity in patients having undergone this treatment as compared to patients with no Early Intervention on the other. Here, visual acuity (VA), rather than any of the other visual handicaps discussed in section 2, has been selected as an indicator of an improved eyesight, as it affects most patients with different forms of OA and OCA rather severely (the median of visual acuity in various forms of albinism is just 0.15 compared to 1.0 in normal persons (Käsmann-Kellner *et al.* 2007). VA can also be measured rather readily, mostly precisely and reliably, and allows a proper, objective statistical evaluation of the data generated. Furthermore, data on visual acuity is readily available.

The main hypothesis of this research is therefore that Early Intervention significantly improves visual acuity in children.

This general hypothesis results in a set of more specific individual research questions which can be addressed by statistical evaluation.

First and foremost, visual acuity critically depends on the morphology of the optic nerve head (Käsmann-Kellner *et al.* 2007). It is therefore of considerable interest to see if the optic nerve morphology is the same in test and control groups. If, for instance, the optic nerve morphology would be much better in the test group in comparison with the control group, it would not be possible to link any improvement of visual acuity to Early Intervention.

After clarification of this issue, the possible link between Early Intervention and visual

acuity can be examined in more general terms, *i.e.* for a cohort of children aged 6 to 15, all of whom have received Early Intervention. The visual acuity of this cohort can then be compared to a control group consisting of (older) patients who have never received any Early Intervention treatment.

Furthermore, as explained in the introduction, some of the ophthalmic disorders associated with albinism are gender specific. Hence a separate consideration of female and male patients is sensible to see if Early Intervention makes specific sense in female or male patients.

Ultimately, the following specific assumptions or questions for investigation emerge and will be addressed as part of this thesis.

- Is there is a strong correlation between optical nerve head morphology and visual acuity in patients with albinism, and if yes, is it similar to the control group?

- Is Early Intervention effective in children whose visual maturation is still ongoing, *i.e.* is there a subsequent improvement of visual acuity in children with completed Early Intervention aged 6 to 15?

- Is it possible that the benefits of Early Intervention are gender specific?

- Does any improvement achieved by Early Intervention match or even outpace the impact of various morphological pathologies (iris, macula, optic nerve) on visual acuity?

4. Methods

4.1. Samples and individual cohorts

In order to investigate the various hypotheses put forward in section 3, it is necessary to collect data from a wide range of children with albinism. Here, substantial cohorts of probands are required to achieve statistical significance, which obviously have to exceed the size of previous studies, for instance the one by Priglinger et al.1993. Therefore, the relevant data has been collected for a total of **232** patients with albinism (children, aged 6 to 15) from the Archive of the Department of Ophthalmology of the University Hospital of Saarland in Homburg/Saar. This cohort is considerably larger than the one studied in a similar context by Priglinger *et al.* in the early 1990s. In the study reported here, the test group consists of children, aged 6 to 15, who have received Early Intervention. Indeed, Early Intervention is carried out at the infantile age from age 0 to 6. In this study we are therefore focusing on the age group from 6 to 15, because these children have most recently undergone and also completed Early Intervention. The control group comprises a total of **139** of patients at the Department of Ophthalmology aged over 25 who have not received Early Intervention. The total sample size of the test and control group is therefore **371.** Table 4.1 summarizes the size of the individual cohorts.

Cohort	Female	Male	Total
Test group (aged 6-15) **With EI**	98	134	232
Control group (aged 25+) **No EI**	68	71	139
Total	166	205	371

Table 4.1: Overview of the different cohorts (and their respective sizes in number of patients) used in order to address the general hypothesis and the age- and gender-specific aspects of this study**. EI = early Intervention**.

Table 4.1 summarizes the various cohorts used for this study. These cohorts were sufficient in size in order to derive at statistically useful results. A similar separation of the total of patients with and without Early Intervention has been undertaken to investigate the correlation between visual acuity and the morphological degree of Iris, Optic Nerve and Macula pathology.

4.2. Initial assessment of the normal distribution of the data sets

The statistical evaluation of the data has been performed with the **Statistical Package for the Social Sciences (SPSS 17.0)**. In the first step the various data sets and suspected correlations have been analyzed to determine if the distributions at hand follow a *normal distribution* or not. Such an initial analysis of the data sets is required to determine which algorithm is used subsequently to calculate the correlations and statistical significances (see below). This initial assessment of the data collected has been performed in SPSS employing the *Shapiro-Wilk test*, which is based on a so-called Q-Q plot, comparing histograms and box-plots and calculating the two relevant parameters known as *skewness* and *kurtosis*.

Here, the Shapiro–Wilk test is a test of normality used in statistics. It has been developed in 1965 by Samuel Sanford Shapiro and Martin Wilk. The null-hypothesis of this test is that the population is normally distributed. If the p-value is less than 0.05 ($p_{SW} < 0.05$), the null hypothesis is rejected and the data tested are not from a normally distributed population. In other words, the data is not normal. If the p-value is greater than 0.05, then the null hypothesis is considered as “verified” and the data at hand can be analysed further using an algorithm applicable to normally distributed data sets.

Whilst the p_{SW} value provides a good indication to decide if a set of data is normally distributed or not, it is often not sufficient to arrive at a firm conclusion. Hence additional information is sought in form of a Q-Q plot, the skewness and kurtosis of the data and its distribution (Wilk et al. 1965). The *Q-Q plot* is a probability plot which allows the comparison of two probability distributions by plotting their quantiles against each other. It is a graphical method which compares the shapes of distributions in a more qualitative manner. It shows, for instance, if relevant properties of two given distributions, such as

location, scale, and skewness, are similar or different in the two distributions. A Q-Q plot is generally a better method to analyse statistical data than the common technique of comparing histograms of the two samples (Wilk et al 1968). It is often complemented by a more quantitative analysis of the “shape” of the distribution(s) at hand. Here, skewness and kurtosis are relevant parameters used in statistics to describe the shape of distributions. *Skewness* is a measure of the asymmetry of the distribution around its mean. The skewness value can be positive or negative, or undefined. *Kurtosis* is any measure of the "peakedness" of the probability distribution. It originates from the Greek word *kurtos* and means curved or arched. Together, skewness and kurtosis represent semi-quantitative descriptors of the shape of a probability distribution (Joanes et al 1998).

As part of this study, p_{SW}, skewness and kurtosis have been calculated first for each data set and correlation under investigation. Based on the outcome of this calculation, it has been decided to treat the data as either normally or non-normally distributed. Based on this decision, the correlation and its associated significance has then been calculated using either the Pearson product-moment correlation coefficient (PPMCC or PCC or Pearson's *r*) or the Spearman’s rank correlation coefficient.

4.3. Analysis using Pearson product-moment or Spearman's rank correlation coefficient

The *Pearson product-moment correlation coefficient* (PPMCC or PCC or Pearson's *r*) is used for *normally distributed data* ($p_{sw} > 0.05$, see above). It provides a measure of the linear correlation (dependence) between two variables *X* and *Y*. The value obtained resides between +1 and −1 inclusive, where 1 is totally positive correlation, 0 is no correlation, and −1 is totally negative correlation. This algorithm has been introduced by Karl Pearson in the 1880s (Fischer *et al.* 1915) and is used in Science as a measure of the degree of linear dependence between two variables.

In contrast, the *Spearman's rank correlation coefficient* or *Spearman's rho* is applicable for data which is *not normally distributed* ($p_{sw} < 0.05$). Developed by Charles Spearman at the beginning of the 20th Century, this algorithm assesses how well the relationship between two variables can be described using a monotonic function. If there are no repeated data

values, a perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other. Spearman's coefficient is calculated for continuous and discrete variables, including ordinal variables (Fieller et al. 1957).

As part of this thesis, both algorithms have been used as adequate for either normally or non-normally distributed data. It should be noted, however, that in some instances, very similar sets of data have demanded the use of either Pearson's or Spearman's analysis based on the outcome of the initial analysis described in 4.2. In such cases, a direct comparison of parameters from the Pearson analysis with the one from the Spearman analysis may have become necessary. To avoid a direct - and certainly problematic - comparison of parameters obtained by different algorithms it has been decided in these cases to perform the calculation using *both* methods for better comparison. Yet the results obtained with the "correct" algorithm are clearly marked in order to avoid any misunderstandings.

5. Results

Employing the various data sets and an appropriate statistical analysis as described in the Methods section, a number of key findings have been obtained which are presented below.

In the first instance, the "anatomical" similarity of the two cohorts, *i.e.* the test group and the control group has been confirmed to ensure that both groups do not differ *per se* regarding their morphological degree of optical nerve pathology. This investigation ensures that there is no statistically significant difference in optic nerve pathology between the test group and the control group (**Figure 5.1**). It implies that both groups are statistically comparable for the optic nerve head anatomy. Therefore it is subsequently possible to consider the effect of Early Intervention as the primary, possibly even the sole underlying cause of any changes in visual acuity which may be observed. This wouldn't be the case if optic nerve morphology were much better in - for instance - the test group.

As described in the Methods section, the individual data sets have then been analysed with the Shapiro-Wilk test to determine if the data follows a normal distribution or not. Within this context, the data sets have been tested by visual inspection of the histograms, normal Q-Q plots and box-plots and a calculation of skewness and kurtosis. As it turns out, virtually all of the data sets forming part of this study are *not normally distributed*. Therefore Spearman's rho correlation is appropriately used to evaluate any correlations and statistical significances which may exist within of these sets of data. Nonetheless, using this kind of pre-analysis "first inspection", two exceptions have been observed which ask for an analysis using the Pearson correlation and significance. One exception is found in the correlation of visual acuity and morphological degrees 1 and 2 of iris pathology in the age group 25+. The other exception is noted in the the correlation of visual acuity and morphological degrees of optic nerve pathology (Degree 0, normal optic nerve) in the same age group. These data are normally distributed.

In order to avoid any confusion, both the Pearson correlation and Spearman's rho correlation are shown in the following when discussing data sets (see section 4.3.). Based on the Shapiro-Wilk test, however, the most appropriate form of analysis (either Pearson or Spearman's rho) is shown in black whilst the less appropriate is shown in fading grey for comparison. The relevant information on the p-value of the Shapiro-Wilk analysis (p_{SW}), skewness and kurtosis is then provided in the legend of each figure for information.

5.1. Morphology of the Optic Nerve Head (ONH) in control and test groups

As already mentioned in section 2 it appears that the ONH pathology may be the most important factor in determining visual acuity. It was therefore tested whether the control and test groups are statistically comparable concerning this particular parameter. Such a similarity in ONH pathology is a prerequisite for any further analysis with regard to Early Intervention.

Correlations

		Morphological degrees of optic nerve pathology	Age
Morphological degrees of optic nerve pathology	Pearson Correlation	1	-,016
	Sig. (2-tailed)		,762
	N	371	371
Age	Pearson Correlation	-,016	1
	Sig. (2-tailed)	,762	
	N	371	371

			Morphological degrees of optic nerve pathology	Age
Spearman's rho	Morphological degrees of optic nerve pathology	Correlation Coefficient	1,000	-,003
		Sig. (2-tailed)	.	,948
		N	371	371
	Age	Correlation Coefficient	-,003	1,000
		Sig. (2-tailed)	,948	.
		N	371	371

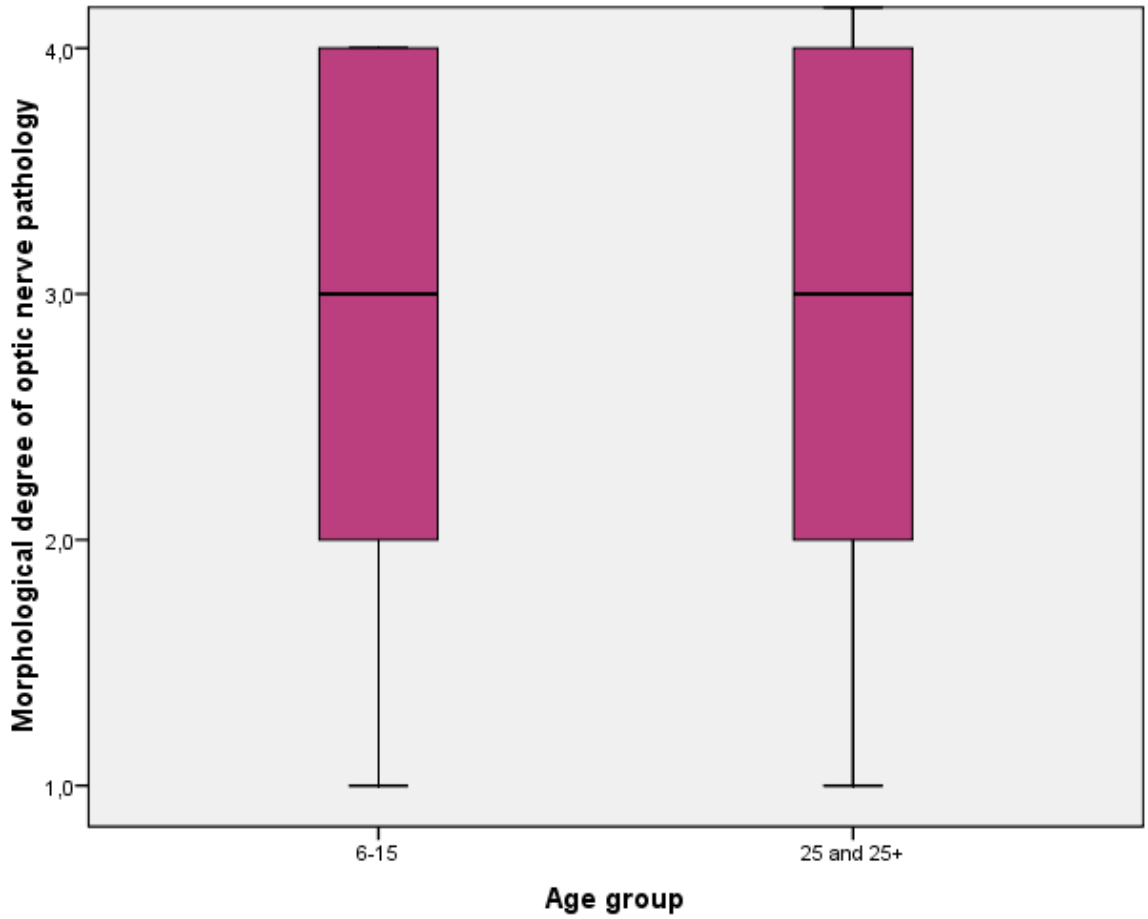

Figure 5.1: Correlation of age and morphological stages of optic nerve pathology between the two selected age groups 6 - 15 and 25+, **n = 371**. The Spearman's rho correlation coefficient is 0.003 and the (Spearman's) significance is 0.948, *i.e.* considerably higher than 0.01. The correlation and significance values indicate that the correlation is not statistically significant. Age group 6-15 and age group 25+ $p_{SW} < 0.05$. Age group 6-15 skewness -2.93, kurtosis -3.56. Age group 25+ skewness –1.158, kurtosis -3.17.

The data shown in Figure 5.1 indicates that the morphological stages of optic nerve pathology do not correlate with the age groups as the significance of 0.948 is considerably higher than 0.01. This implies that the optic nerve pathology is vastly independent of the age group, *i.e.* is "more or less the same" in both groups. Hence there are no statistically relevant differences in optic nerve pathology and any differences subsequently observed between the two cohorts must originate from another factor or factors.

5.2. Visual Acuity in control and test groups

After ensuring that the two individual groups do not differ *per se* due to differences in the morphological degree of optic nerve pathology, and hence can be compared, the relationship between visual acuity and Early Intervention has been investigated. Here, **Figure 5.2** at first "visual" inspection seems to indicate that the visual acuity (VA) in the test group (children aged 6 to 15) is slightly better in comparison with the control group (patients aged over 25). The median visual acuity in the test group is around 0.18 whereas in the control group it is slightly lower, around 0.15. At the same time, the box plot for the test group, compared to the control group, indicates that the first quartile extends to slightly higher, *i.e.* better values for visual acuity. Yet whilst the box plot graphically seems to point towards an improved visual acuity in the test group, this improvement, however, is not statistically significant. The Spearman's rho correlation coefficient is 0.003 and the significance is 0.949, *i.e.* considerably higher than 0.01, indicating that the correlation is not significant statistically.

Correlations

		Age	Visual acuity
Age	Pearson Correlation	1	,015
	Sig. (2-tailed)		,770
	N	371	371
Visual acuity	Pearson Correlation	,015	1
	Sig. (2-tailed)	,770	
	N	371	371

Correlations

			Age	Visual acuity
Spearman's rho	Age	Correlation Coefficient	1,000	,003
		Sig. (2-tailed)	.	,949
		N	371	371
	Visual acuity	Correlation Coefficient	,003	1,000
		Sig. (2-tailed)	,949	.
		N	371	371

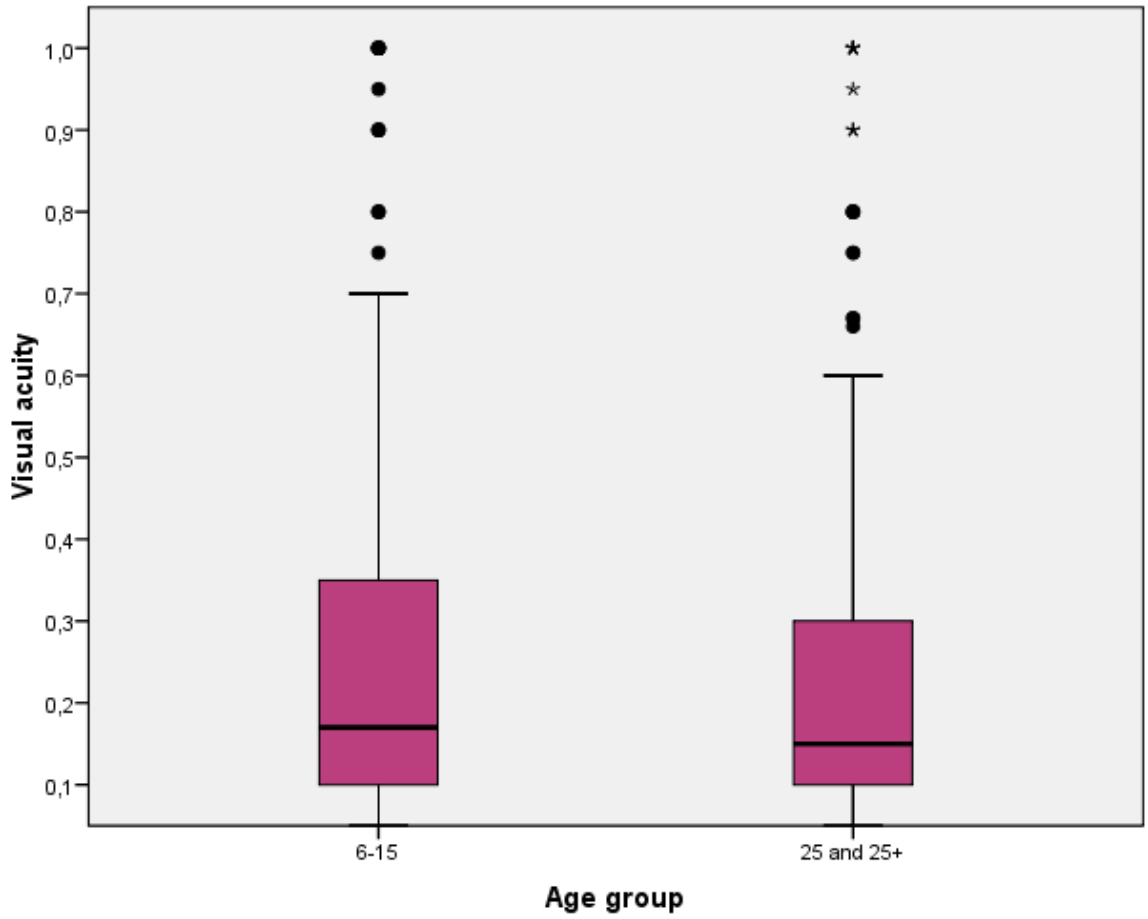

Figure 5.2: Correlation of age and visual acuity between the two selected age groups 6 - 15 and 25+, *males and females*, **n = 371**. Age group 6-15 and age group 25+ $p_{SW} < 0.05$. Age group 6-15 skewness 10.29, kurtosis 7.07. Age group 25+ skewness 7.95, kurtosis 4.29. Whilst at first glance, there seems to be a slightly improved VA in the children compared to the control group, this difference is not relevant statistically.

5.3. Gender specific improvement of visual acuity due to Early Intervention

The lack of any general and statistically significant improvement in the children compared to the control group is disappointing as it seems to refute the hypothesis that Early Intervention has a pronounced effect on (most of) the children who have previously undergone this particular treatment. Nonetheless, such a more general finding across a rather large population of 232 children in the test group and 139 older persons in the 25+ control group may conceal significant improvements of visual acuity in individual patients or sub-groups of patients. As highlighted in section 1, there are significant gender specific

differences due to the particular inheritance of albinism, and therefore a gender specific analysis of visual acuity in the control and test groups has been performed.

The results obtained for the female subgroups are shown in **Figure 5.3**. As in Figure 5.2, a generally higher visual acuity is noticed in the test group compared to the control group. At first inspection of the box plots, the median visual acuity is around 0.2 in the test group, whilst it is lower, around 0.15, in the control group. At the same time, the first quartile also extends to a higher, *i.e.* better acuity in the test group compared to the control group (0.4 and 0.3, respectively). Nonetheless, the characteristic statistical parameters still point at a rather weak, non-significant correlation. The Spearman's rho correlation coefficient is -0.085 with a significance of 0.275, indicating that indeed there is a better visual acuity in the test group compared to the control group (negative correlation values), but also that the correlation is not significant. Nonetheless, a significance of 0.275 is considerably better than in Figure 5.2 (0.949), yet still well above 0.01 and hence not significant from a statistical point of view.

Correlations

		Age	Visual acuity
Age	Pearson Correlation	1	-,092
	Sig. (2-tailed)		,238
	N	166	166
Visual acuity	Pearson Correlation	-,092	1
	Sig. (2-tailed)	,238	
	N	166	166

Correlations

			Age	Visual acuity
Spearman's rho	Age	Correlation Coefficient	1,000	-,085
		Sig. (2-tailed)	.	,275
		N	166	166
	Visual acuity	Correlation Coefficient	-,085	1,000
		Sig. (2-tailed)	,275	.
		N	166	166

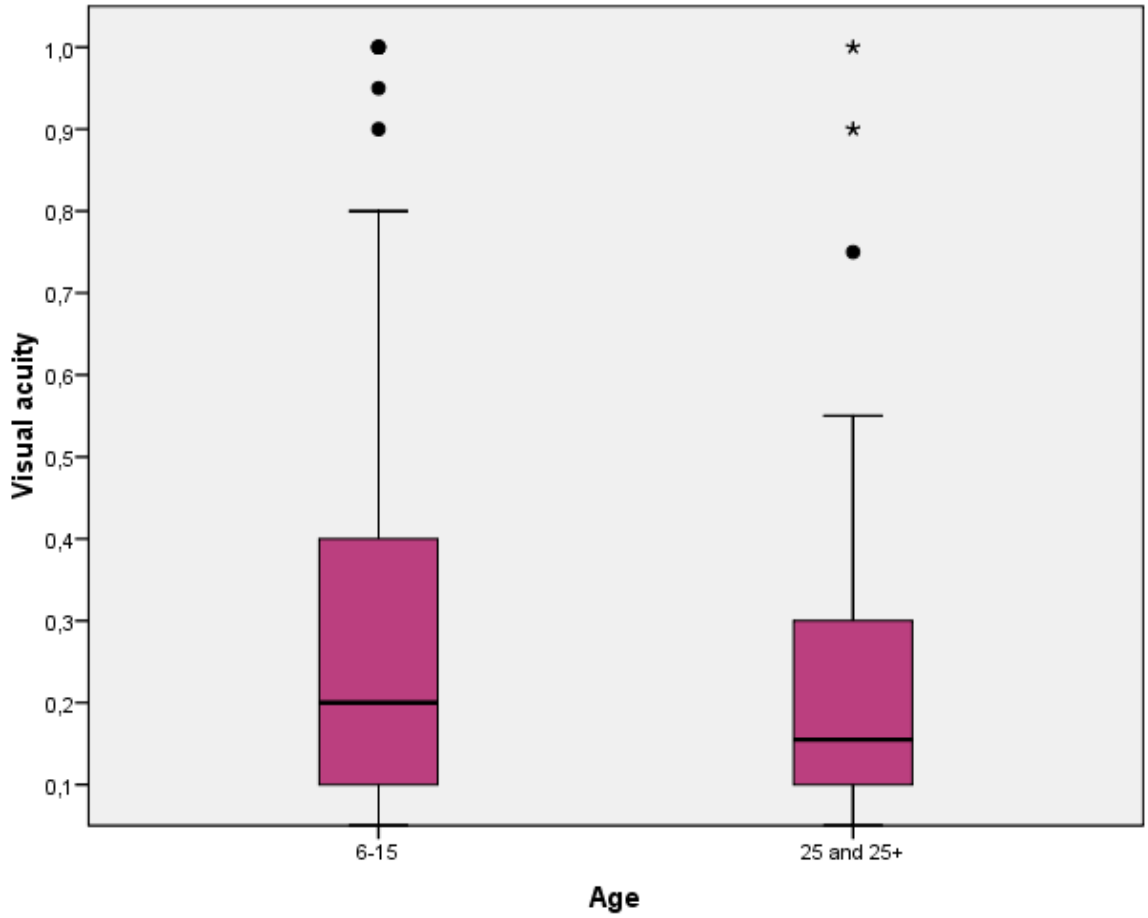

Figure 5.3: Correlation of age and visual acuity *in females* in the two selected age groups 6 - 15 and 25+, **n =166**. Age group 6-15 and age group 25+ $p_{SW} < 0.05$. Age group 6-15 skewness 5.82, kurtosis 3.18. Age group 25+ skewness 7.24, kurtosis 7.14.

Interestingly, whilst despite the lack of a *statistical* significance there seems to be a small but measurable improvement of visual acuity upon Early Intervention, this effect is notably absent in the group of males. As **Figure 5.4** indicates the median visual acuity is almost the same in both groups, *i.e.* around 0.15 in the test as well as the control group. At the same time, the height of the box plots indicative of the first quartile are almost "mirrored" compared to the female population. It therefore seems that in the case of males, the control group has the better visual acuity compared to the test group. This first impression is supported by the relevant statistical parameters. The Spearman's rho correlation coefficient is 0.067 and the significance is 0.342, pointing towards an even slightly worse (the correlation coefficient this time is positive), but again not statistically significant visual acuity in the test group. This finding will be discussed in more detail in the Discussion.

Correlations

		Age	Visual acuity
Age	Pearson Correlation	1	,102
	Sig. (2-tailed)		,146
	N	205	205
Visual acuity	Pearson Correlation	,102	1
	Sig. (2-tailed)	,146	
	N	205	205

Correlations

			Age	Visual acuity
Spearman's rho	Age	Correlation Coefficient	1,000	,067
		Sig. (2-tailed)	.	,342
		N	205	205
	Visual acuity	Correlation Coefficient	,067	1,000
		Sig. (2-tailed)	,342	.
		N	205	205

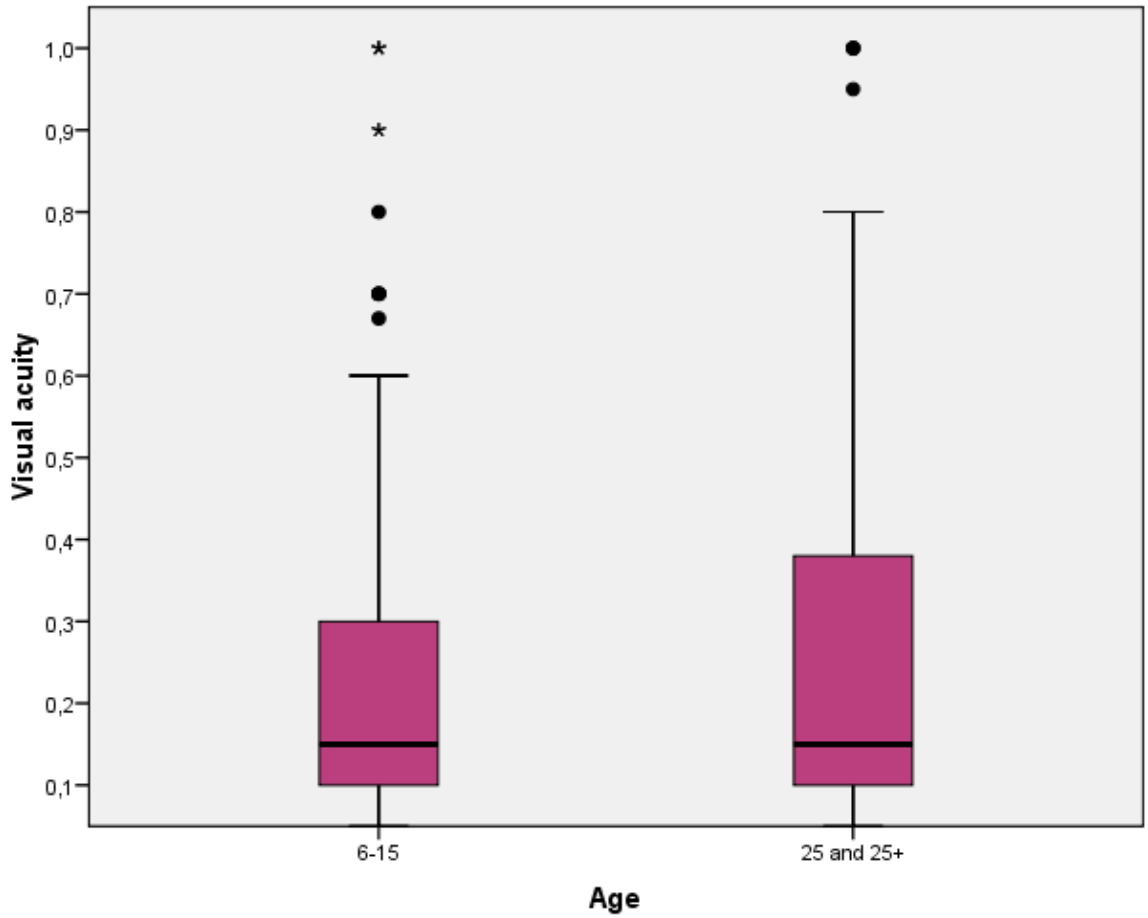

Figure 5.4: Correlation of age and visual acuity *in males* in the two selected age groups 6 - 15 and 25+, **n = 205**. Age group 6-15 and age group 25+ $p_{SW} < 0.05$. Age group 6-15 skewness 8.8, kurtosis 7.29. Age group 25+ skewness 4.59, kurtosis 1.0.

5.4. Does gender have a general influence on Visual Acuity?

Importantly, an improved visual acuity in females could also be independent of Early Intervention, *i.e.* a more "natural effect" (*e.g.* genetically linked). The visual acuity of females and males has therefore been compared in both groups in order to establish - or rule out - any possible link between gender and a generally improved visual acuity. Figure 5.5 illustrates the gender-specific distribution of visual acuity in the control group. If there would be a more general impact of gender on visual acuity, for instance because of X-chromosome linked inheritance of certain mutations, then this general influence should be apparent in the control group of persons 25+ as well.

In fact, the median visual acuity in the male population and female population of the

control group is around 0.2 and 0.15, respectively, actually pointing towards a generally better "natural" visual acuity in *males* (and not in females as may have been predicted). Furthermore, the first quartile of the box plot in the male population is somewhat higher than in female population (0.4 and 0.3, respectively). As expected from this visual inspection of the box plots, the analysis using Spearman's rho correlation is negative at -0.118, pointing towards a lower visual acuity in the female cohort, yet this is not statistically significant as the significance parameter calculated is 0.166, *i.e.* well above 0.01. In any case, whilst this decrease in visual acuity in the female cohort compared to the male cohort is not statistically significant, it clearly refutes the initial suspicion raised in Figures 5.3 and 5.4 that females may "naturally" have a better visual acuity compared to males.

Correlations

		Visual acuity	Sex
Visual acuity	Pearson Correlation	1	-,090
	Sig. (2-tailed)		,290
	N	139	139
Sex	Pearson Correlation	-,090	1
	Sig. (2-tailed)	,290	
	N	139	139

Correlations

			Visual acuity	Sex
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,118
		Sig. (2-tailed)	.	,166
		N	139	139
	Sex	Correlation Coefficient	-,118	1,000
		Sig. (2-tailed)	,166	.
		N	139	139

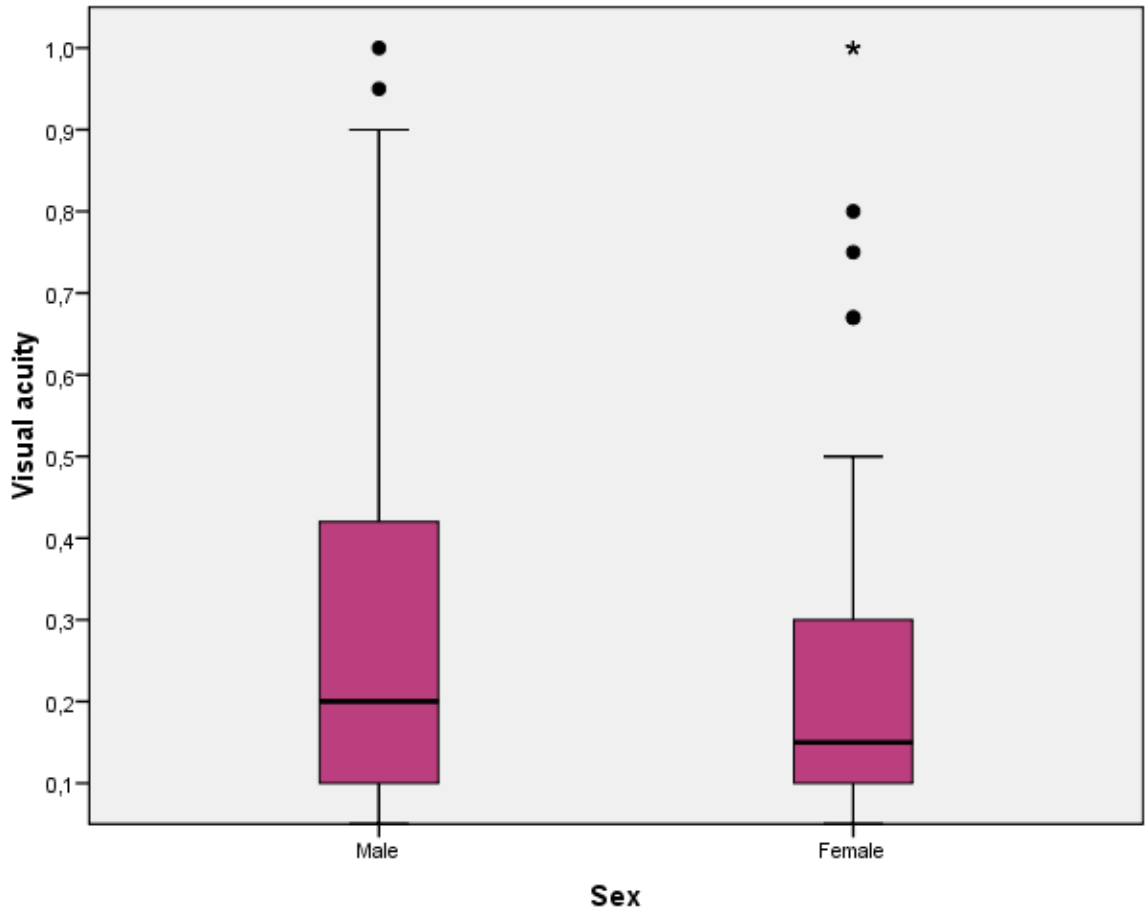

Figure 5.5: Box plot and nonparametric correlation of visual acuity and gender of the *control group*, age group 25 +, **n = 139**. Age group 25+ $p_{SW} < 0.05$. Male group skewness 4.27, kurtosis 0.69.Female group skewness 6.6, kurtosis 5.11.

Whilst the data obtained for the control group age 25+ clearly contradicts the idea that gender matters in general terms, gender indeed seems to be responsible for a considerable difference in visual acuity once the children who have undergone Early Intervention are considered. As shown in **Figure 5.6**, the median visual acuity in females of this group is considerably higher than in males, at around 0.5 compared to 0.2. In line with this visual inspection of the box plot, the Spearman's rho correlation coefficient is rather positive, at 0.058, with a significance of 0.378.

Correlations

		Visual acuity	Sex
Visual acuity	Pearson Correlation	1	,036
	Sig. (2-tailed)		,590
	N	232	232
Sex	Pearson Correlation	,036	1
	Sig. (2-tailed)	,590	
	N	232	232

Correlations

			Visual acuity	Sex
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	,058
		Sig. (2-tailed)	.	,378
		N	232	232
	Sex	Correlation Coefficient	,058	1,000
		Sig. (2-tailed)	,378	.
		N	232	232

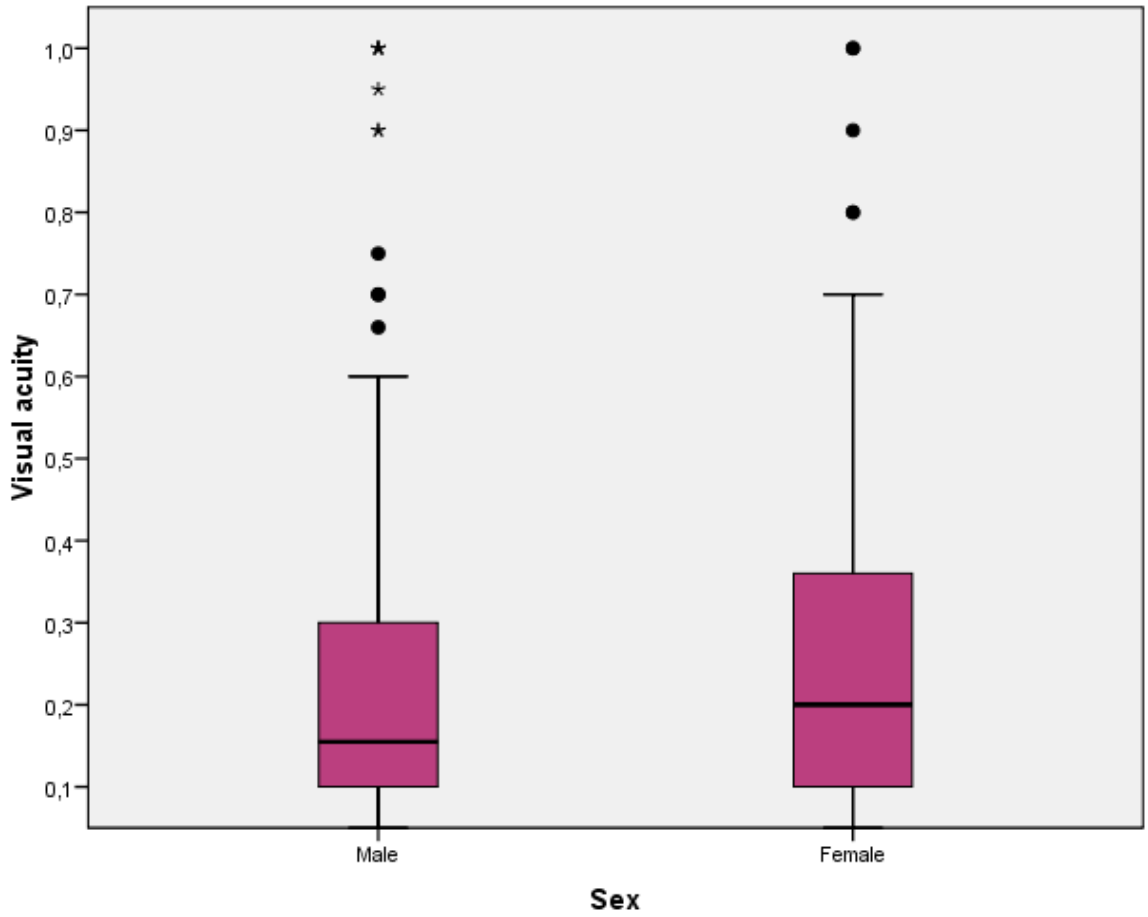

Figure 5.6: Box plot and nonparametric correlation of visual acuity and sex of the *test group*, age group 6-15, **n = 232**. Age group 6-15 $p<0.05$. Male group skewness 9.11, kurtosis 7.54.Female group skewness 5.87, kurtosis 3.76.

Whilst the significances in Figures 5.5 and 5.6 still are not sufficient to postulate a firm correlation, they nonetheless confirm that (a) the apparent improvement of visual acuity in females is not due to a generally improved visual acuity in women and that (b) there is a clear, albeit not statistically significant improved visual acuity of females with Early Intervention not only compared to the females in the control group but also compared to the males in the test group itself. These findings are of considerable interest as they point towards a better impact of Early Intervention in young girls. They will be discussed in more detail in section 6.

Despite these rather revealing findings, it seems that Early Intervention can only exert a small - and strictly speaking non-significant - effect on visual acuity. The anticipated improvements are too small to be significant, and this in turn raises the question if there are any other factors which may determine visual acuity and hence impact on the outcome of Early Intervention. Here, “anatomical” aspects of the eye first come to mind

(see also section 2). These factors include, for instance, iris morphology, macular pathomorphology, optic nerve head pathology and strabismus, which have also been analysed as part of this study.

5.5. Visual Acuity and morphological parameters in test and control group

5.5.1. Visual Acuity and Iris Pathology

Indeed, as **Figure 5.7** indicates, there is a statistically highly significant correlation between visual acuity and the morphological degrees of *iris pathology* in the test group. The Spearman's rho correlation coefficient is -0.193, pointing towards a lower visual acuity with higher degrees of iris pathology, and in this case the significance is extraordinarily high, at 0.002. When comparing these results for iris pathology with the ones for Early Intervention, it is apparent that the pathology clearly outpaces any small effects due to Early Intervention, in both respects, the extent of differences in visual acuity (0.2 units) as well as its significance.

Correlations

		Morphological stages of iris pathology	Visual acuity
Morphological degreesof iris pathology	Pearson Correlation	1	-,175**
	Sig. (1-tailed)		,004
	N	232	232
Visual acuity	Pearson Correlation	-,175**	1
	Sig. (1-tailed)	,004	
	N	232	232

**. Correlation is significant at the 0.01 level (1-tailed).

Correlations

			Morphological stages of iris pathology	Visual acuity
Spearman's rho	Morphological degrees of iris pathology	Correlation Coefficient	1,000	-,193**
		Sig. (1-tailed)	.	,002
		N	232	232
	Visual acuity	Correlation Coefficient	-,193**	1,000
		Sig. (1-tailed)	,002	.
		N	232	232

**. Correlation is significant at the 0.01 level (1-tailed).

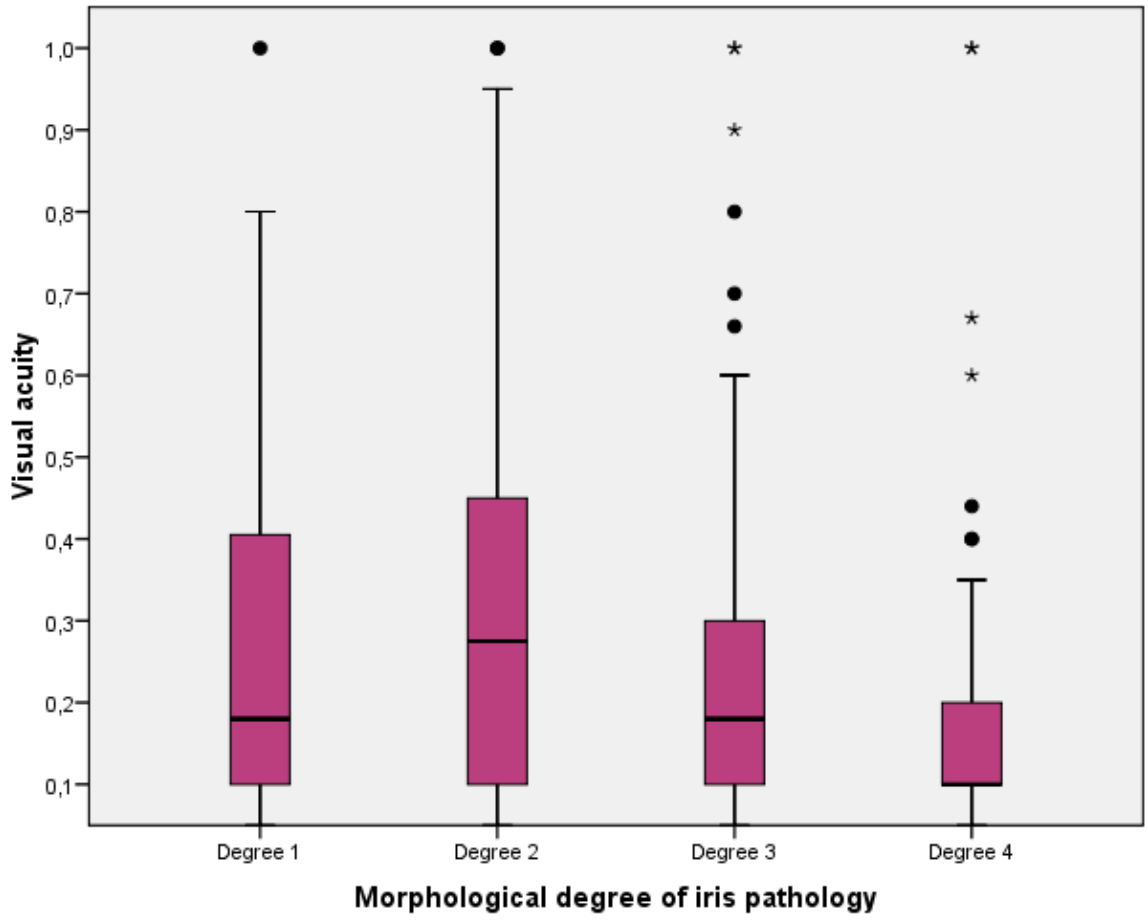

Figure 5.7: Correlation of visual acuity and morphological degrees of iris pathology age group 6-15, *test group with EI*, **n = 232**. Age group 6-15 Degree 1-4 $p_{SW} < 0.05$. Skewness Degree 1 = 3.28, Degree 2 = 3.46, Degree 3 = 7.71, Degree 4 = 9.02. Kurtosis Degree 1 = 1.65, Degree 2 = 0.96, Degree 3 = 8.4, Degree 4 = 13.77.

Indeed, the dramatic impact of iris morphology on vision is also found in the control group. As shown in **Figure 5.8**, there is a statically significant decrease in visual acuity with an increase in iris pathology. Here, the Spearman's rho correlation coefficient is -0.244 and the significance is 0.002. It must be noted, however, that the data in Figure 5.8 should, strictly speaking, be analysed using the Pearson algorithm ($p_{SW} > 0.05$). Here, a similar trend is observed. The Pearson correlation is also negative, at -0.305, with a significance of 0.000. Hence regardless of the algorithm used, the notion that the visual acuity is reduced significantly with an increasing degree of iris morphology holds true for both cohorts, the test group of children aged 6-15 and the control group aged 25+.

Correlations

		Visual acuity	Morphological degrees of iris pathology
Visual acuity	Pearson Correlation	1	-,305**
	Sig. (1-tailed)		,000
	N	139	139
Morphological degrees of iris pathology	Pearson Correlation	-,305**	1
	Sig. (1-tailed)	,000	
	N	139	139

**. Correlation is significant at the 0.01 level (1-tailed).

Correlations

			Visual acuity	Morphological degrees of iris pathology
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,244**
		Sig. (1-tailed)	.	,002
		N	139	139
	Morphological degrees of iris pathology	Correlation Coefficient	-,244**	1,000
		Sig. (1-tailed)	,002	.
		N	139	139

**. Correlation is significant at the 0.01 level (1-tailed).

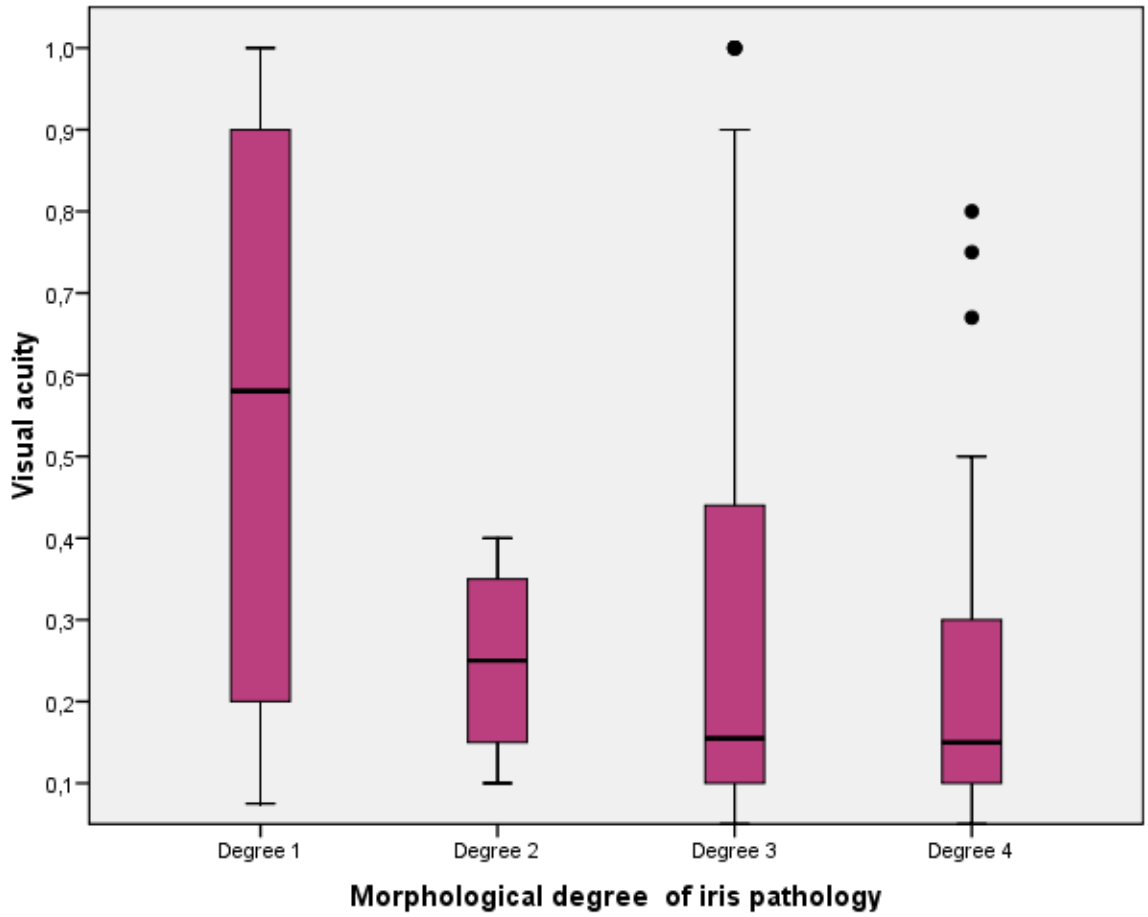

Figure 5.8: Correlation of visual acuity and morphological degrees of iris pathology Age group 25+, *control group* **, n = 139**. Age group 25+ Degree 1-2 $p_{SW} > 0.05$, Degree 3-4 $p<0.05$. Skewness Degree 1 = - 0.21, Degree 2 = 0, Degree 3 = 4.4, Degree 4 = 5.6. Kurtosis Degree 1 = -1.31, Degree 2 = - 1.11, Degree 3 = 0.87 Degree 4 = 4.94.

5.5.2. Visual Acuity and Macular Pathomorphology

The notion that morphological features ultimately determine the visual acuity of patients, and by far outpace any small effects due to Early Intervention, is supported further when considering other morphological parameters besides iris pathology, such as the morphological degrees of *macular pathology*. As **Figure 5.9** indicates, the correlation between visual acuity and the morphological degrees of macular pathology in the test group is statistically significant, whereby the Spearman's rho correlation coefficient in this case is -0.146 with a significance level of 0.013, implying that visual acuity is related inversely to an increase in the degree of macular pathology.

Correlations

		Visual acuity	Morphological degrees of macular pathology
Visual acuity	Pearson Correlation	1	-,145*
	Sig. (1-tailed)		,014
	N	232	232
Morphological degrees of of macular pathology	Pearson Correlation	-,145*	1
	Sig. (1-tailed)	,014	
	N	232	232

*. Correlation is significant at the 0.05 level (1-tailed).

Correlations

			Visual acuity	Morphological degrees of macular pathology
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,146*
		Sig. (1-tailed)	.	,013
		N	232	232
	Morphological degrees of macular pathology	Correlation Coefficient	-,146*	1,000
		Sig. (1-tailed)	,013	.
		N	232	232

*. Correlation is significant at the 0.05 level (1-tailed)

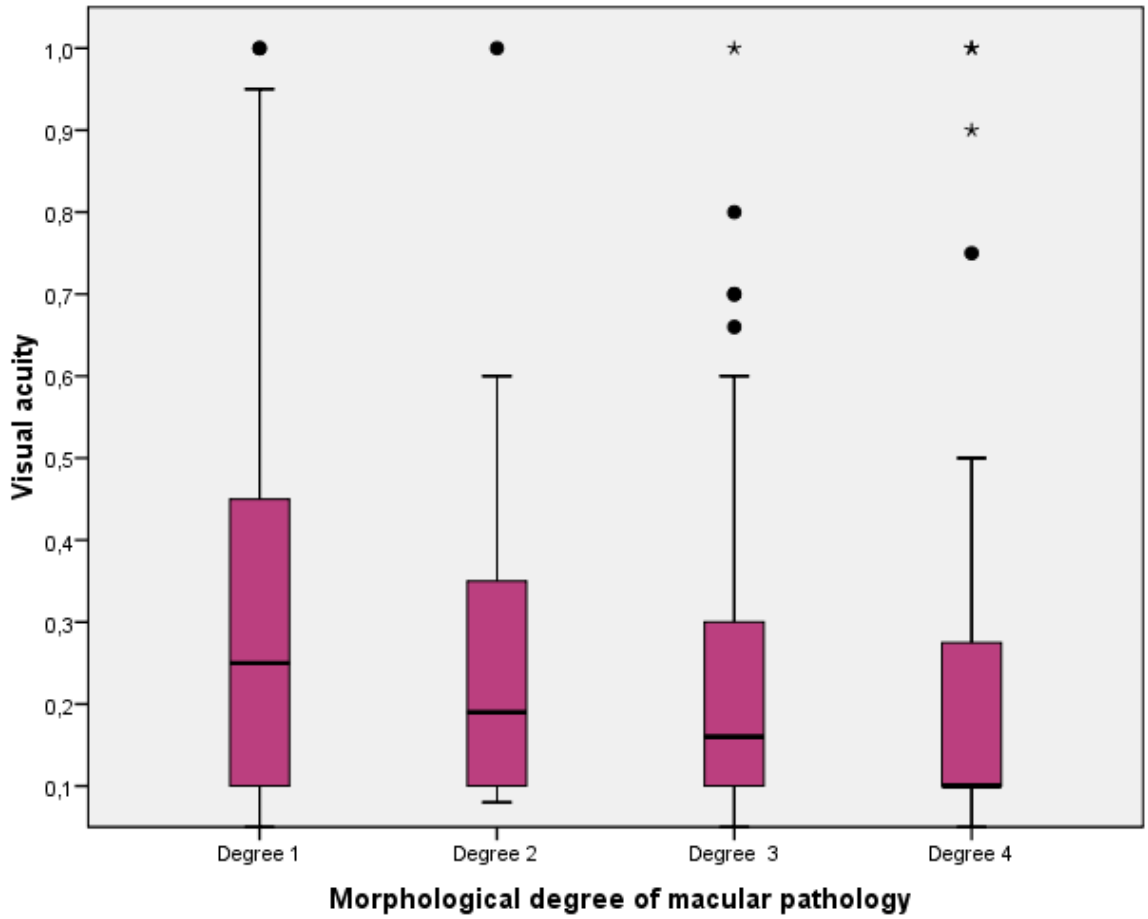

Figure 5.9: Correlation of visual acuity and morphological degrees of macular pathology Age group 6-15, *test group*, **n = 232**. Age group 6-15 Degree 1-4 $p_{SW} < 0.05$. Skewness Degree 1 = 3.07, Degree 2 = 5.42, Degree 3 = 6.77, Degree 4 = 6.39. Kurtosis Degree 1 = - 0.02, Degree 2 = 8.6 Degree 3 = 6.92 Degree 4 = 5.43

As in the case of iris pathology, the impact of macular pathology on visual acuity is independent of the cohort used, as a similar trend is also observed in the control group shown in **Figure 5.10**. Here, the correlation between a lower visual acuity and a higher degree of macular pathology is also statistically significant, with a Spearman's rho correlation coefficient of -0.216 and a significance of 0.005.

Correlations

		Visual acuity	Morphological degrees of macular pathology
Visual acuity	Pearson Correlation	1	-,142*
	Sig. (1-tailed)		,048
	N	139	139
Morphological degrees of macular pathology	Pearson Correlation	-,142*	1
	Sig. (1-tailed)	,048	
	N	139	139

*. Correlation is significant at the 0.05 level (1-tailed).

Correlations

			Visual acuity	Morphological degrees of macular pathology
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,216**
		Sig. (1-tailed)	.	,005
		N	139	139
	Morphological degrees of macular pathology	Correlation Coefficient	-,216**	1,000
		Sig. (1-tailed)	,005	.
		N	139	139

**. Correlation is significant at the 0.01 level (1-tailed).

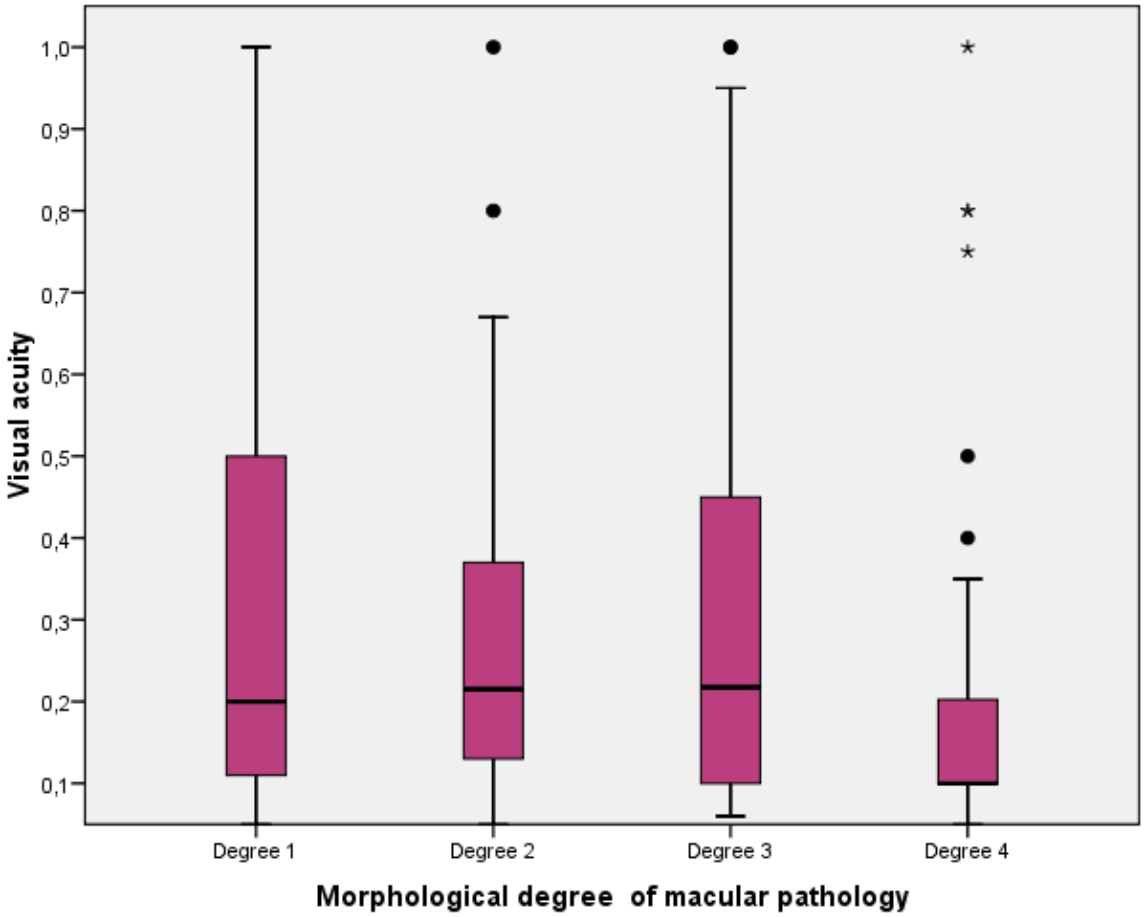

Figure 5.10: Correlation of visual acuity and morphological degrees of macular pathology Age group 25+, *control group*, **n = 139**. Age group 25+ Degree 1-4 $p_{SW} < 0.05$. Skewness Degree 1 = 2.72, Degree 2 = 2.69, Degree 3 = 3.31, Degree 4 = 7.14. Kurtosis Degree 1 = 0.97, Degree 2 = 1.35 Degree 3 = 0.3 Degree 4 = 8.21.

5.5.3. Visual Acuity and Optic Nerve Head Pathology

There are other pathologies, however, which compared to iris pathology and macular pathology, seem to be less significantly correlated with visual acuity. Here, the morphological degrees of optic nerve pathology have been considered first. **Figure 5.11** indicates that in the test group, the degree of optical nerve pathology indeed correlates with a (lower) visual acuity, yet the statistical significances are lower than in the cases described above. As expected, the Spearman's rho correlation coefficient is -0.060, yet with a significance of just 0.181. A similar correlation is also found in the control group, which – in a strict sense has to be analysed employing the Pearson algorithm (p_{SW} > 0.005). As shown in **Figure 5.12**, this correlation is statistically significant. The Pearson correlation is -0.163 with a significance of 0.028. Merely for comparison, the Spearman's rho correlation coefficient for this correlation, if applicable, would also be similarly negative, at -0.186, with a significance of 0.014.

Correlations

		Visual acuity	Morphological degrees of optic nerve pathology
Visual acuity	Pearson Correlation	1	-,117*
	Sig. (1-tailed)		,037
	N	232	232
Morphological degrees of optic nerve pathology	Pearson Correlation	-,117*	1
	Sig. (1-tailed)	,037	
	N	232	232

*. Correlation is significant at the 0.05 level (1-tailed).

Correlations

			Visual acuity	Morphological degrees of optic nerve pathology
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,060
		Sig. (1-tailed)	.	,181
		N	232	232
	Morphological degrees of optic nerve pathology	Correlation Coefficient	-,060	1,000
		Sig. (1-tailed)	,181	.
		N	232	232

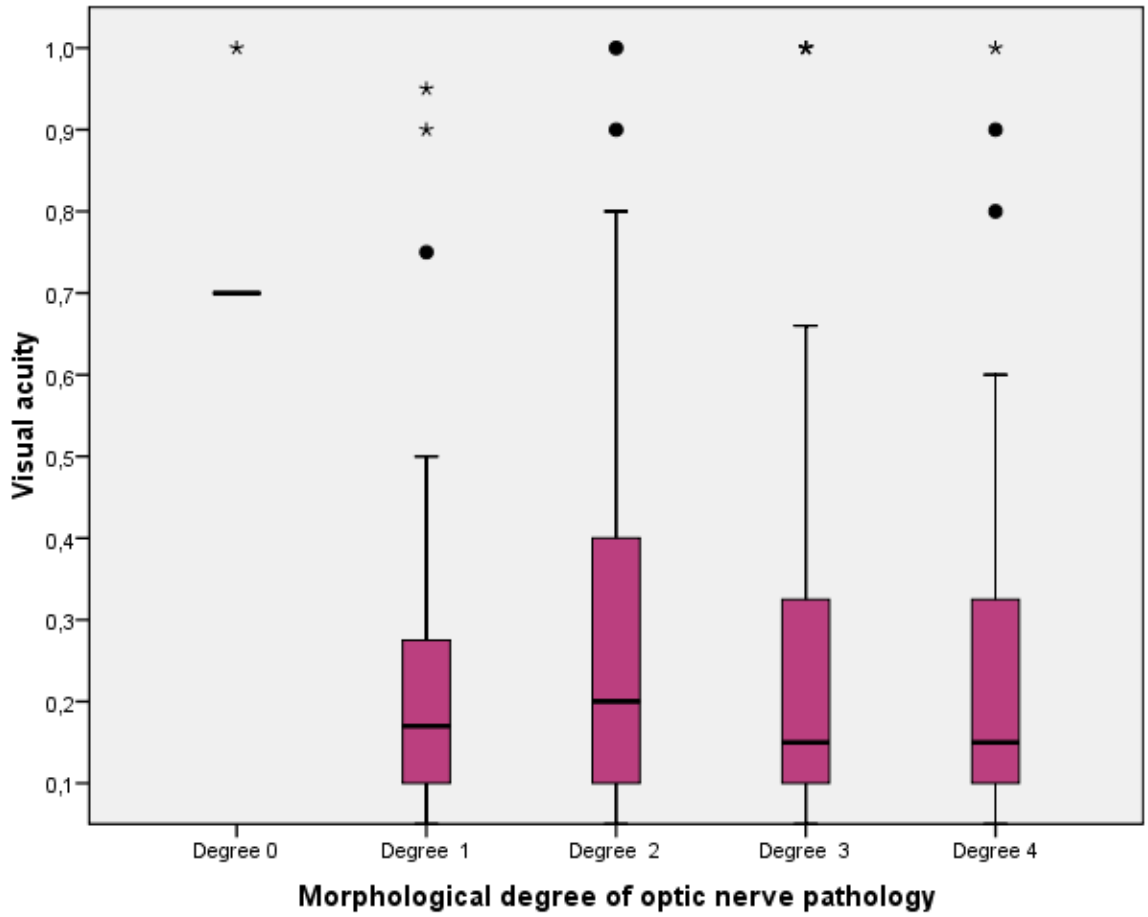

Figure 5.11: Correlation of visual acuity and morphological degrees of optic nerve pathology Age group 6-15, *test group*, **n = 232**. Age group 6-15 Degree 1-4 $p_{SW} < 0.05$. Skewness Degree 0 = 2.45 Degree 1 = 6.11, Degree 2 = 3.68, Degree 3 = 6.53, Degree 4 = 6.86. Kurtosis Degree 0 = 2.5 Degree 1 = 7.21, Degree 2 = 2.19 Degree 3 = 5.85 Degree 4 = 7.56.

Correlations

		Visual acuity	Morphological degrees of optic nerve pathology
Visual acuity	Pearson Correlation	1	-,163*
	Sig. (1-tailed)		,028
	N	139	139
Morphological degrees of optic nerve pathology	Pearson Correlation	-,163*	1
	Sig. (1-tailed)	,028	
	N	139	139

*. Correlation is significant at the 0.05 level (1-tailed).

Correlations

			Morphological degrees of optic nerve pathology	Visual acuity
Spearman's rho	Morphological degrees of optic nerve pathology	Correlation Coefficient	1,000	-,186*
		Sig. (1-tailed)	.	,014
		N	139	139
	Visual acuity	Correlation Coefficient	-,186*	1,000
		Sig. (1-tailed)	,014	.
		N	139	139

*. Correlation is significant at the 0.05 level (1-tailed).

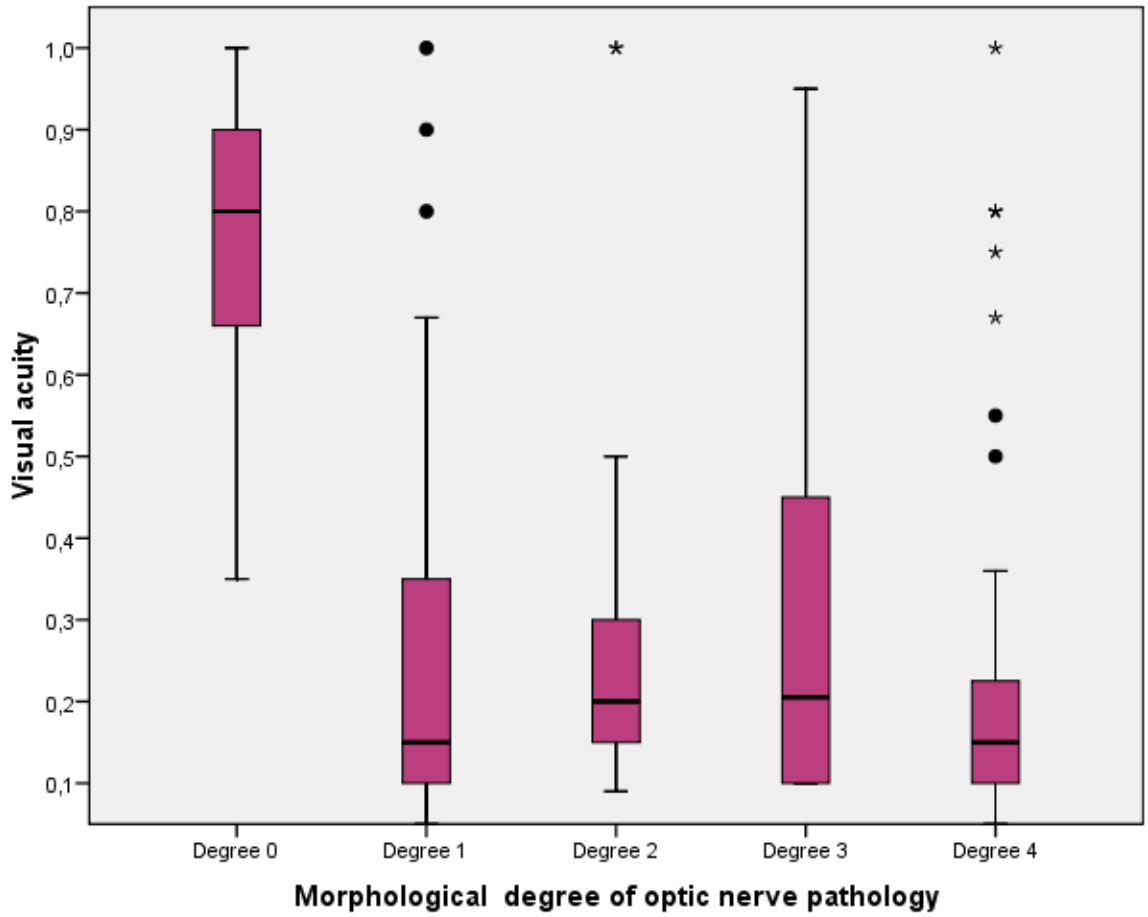

Figure 5.12: Correlation of visual acuity and morphological degrees of optic nerve pathology Age group 25+, *control group*, **n = 139**. Age group 25+ Degree 0 $p_{SW} > 0.05$, Degree 1-4 Skewness Degree 0 = -1.12 Degree 1 = 3.71, Degree 2 = 4.3, Degree 3 = 2.66, Degree 4 = 6.15. Kurtosis Degree 0 = 0.41 Degree 1 = 2.08, Degree 2 = 4.42 Degree 3 = 1.08 Degree 4 = 4.84

5.5.4. Visual Acuity and Strabismus

Finally, a possible correlation between visual acuity and squint has also been considered. Unlike the pathologies analysed before, squint is not a “pathology” in the sense of a particular degeneration (*e.g.* of iris, macular, optical nerve), but caused by an incorrect positioning of the optical axis (see section 1). Hence when it comes to persons with albinism, it is necessary to distinguish between different groups of patients. One group of patients has no squint. There are two groups with *Strabismus convergens* and *Strabismus divergens*, respectively, whilst the fourth group doesn’t have any defined data on squint in the relevant patient’s records. One may speculate that there is a correlation between

visual acuity and squint and that such a correlation may become apparent during statistical analysis.

The data for the test group is shown in **Figure 5.13**. At first inspection, there seems to be a decrease in the median visual acuity from around 0.3 in persons without squint to around 0.15 to 0.2 in persons with *Strabismus convergens* and *Strabismus divergens*. Such a correlation, however, is not statistically significant. Whilst the Spearman's rho correlation coefficient is negative with a value of -0.058, as may be expected, the significance is 0.379. A similar trend is found in the control group as illustrated in **Figure 5.14**. Here, a similar, negative correlation is found between visual acuity and different types of squint. As in the test group, the Spearman's rho correlation coefficient is negative, at -0.046, yet with a low significance of 0.590.

Correlations

		Visual acuity	Forms of squint associated with albinism
Visual acuity	Pearson Correlation	1	-,045
	Sig. (2-tailed)		,491
	N	232	232
Forms of squint associated with albinism	Pearson Correlation	-,045	1
	Sig. (2-tailed)	,491	
	N	232	232

Correlations

			Visual acuity	Forms of squint associated with albinismus
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,058
		Sig. (2-tailed)	.	,379
		N	232	232
	Forms of squint associated with albinismus	Correlation Coefficient	-,058	1,000
		Sig. (2-tailed)	,379	.
		N	232	232

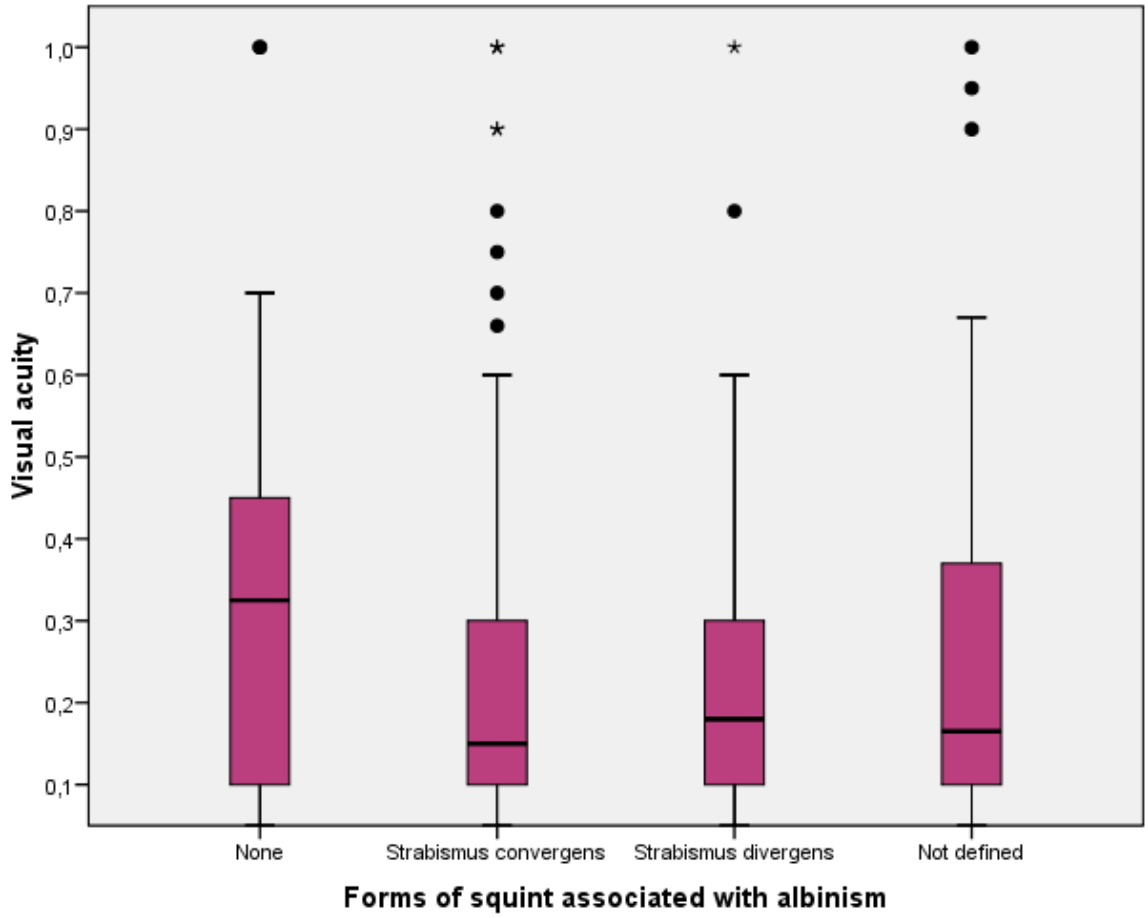

Figure 5.13: Correlation of visual acuity and forms of squint associated with albinism age group 6-15, *test group*, **n = 232**. Age group 6-15 Degree 1-4 $p_{SW} < 0.05$. Skewness No squint 2.53, Strabismus convergens 8.75, Strabismus divergens 6.17, Not defined 3.25. Kurtosis No squint 0.81, Strabismus convergens 8.12, Strabismus divergens 8.05, Not defined 1.28.

The general trend observed in the test group of children aged 6-15 is confirmed in the group of adults aged 25+. As illustrated in Figure 5.14, strabismus seems to be related to a decreased visual acuity, yet these changes are once more not statistically significant with a significance of just 0.590.

Correlations

		Visual acuity	Forms of squint associated with albinism
Visual acuity	Pearson Correlation	1	-,077
	Sig. (2-tailed)		,370
	N	139	139
Forms of squint associated with albinism	Pearson Correlation	-,077	1
	Sig. (2-tailed)	,370	
	N	139	139

			Visual acuity	Forms of squint associated with albinismus
Spearman's rho	Visual acuity	Correlation Coefficient	1,000	-,046
		Sig. (2-tailed)	.	,590
		N	139	139
	Forms of squint associated with albinismus	Correlation Coefficient	-,046	1,000
		Sig. (2-tailed)	,590	.
		N	139	139

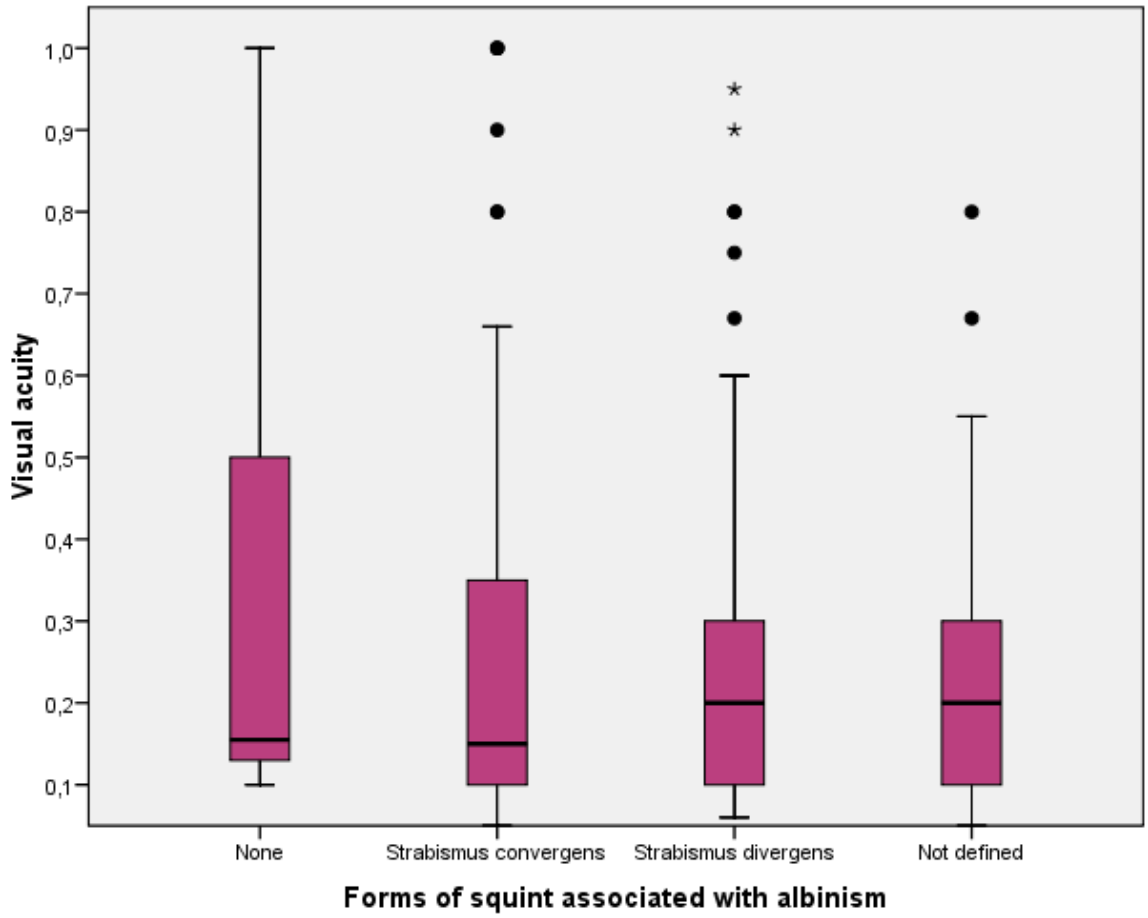

Figure 5.14: Correlation of visual acuity and forms of squint associated with albinism Age group 25+, *control group*, **n = 139**. Age group 25+ Degree 1-4 $p_{SW} < 0.05$. Skewness No squint 2.26, Strabismus convergens 4.84 Strabismus divergens 4.6, Not defined 2.53. Kurtosis No squint 0.29, Strabismus convergens 2.34, Strabismus divergens 2.21, Not defined 1.08.

6. Discussion

Overall, the results obtained as part of this study point towards a small and statistically not significant improvement of visual acuity in females who have received Early Intervention. Whilst this improvement is not due to any significant differences in the pathologies underlying the test and control groups, it is also weak and cannot overcome the various iris, macular and optical nerve pathologies which ultimately correlate with visual acuity, regardless if Early Intervention has been applied or not.

6.1. General impact of EI on visual acuity

As the primary objective of this study has been a direct comparison of patients with albinism who have - and have not - undergone EI, it has been essential to ensure upfront that both cohorts (one with, one without EI) do not differ significantly in any other relevant parameters, such as a particular morphology. As shown in **Figure 5.1**, the Optic Nerve Head is morphologically and anatomically identical in test and control groups. If this were not the case, it would be futile to statistically compare the impact of Early Intervention.

Backed with this pivotal information, it has then be possible to compare the two cohorts in question, *i.e.* the children aged 6-15 who had undergone EI with the control group of adults aged 25+ who because of their age had not been exposed to EI during their childhood. The results shown in **Figure 5.2** indicate that there may be a slight tendency to an improved visual acuity in the children with EI, but this “improvement” - if at all - is statistically not significant. Whilst this particular outcome is rather disappointing from a therapeutic point of view, the numbers shown in Figure 5.2. also provide one additional information: The visual acuity in both groups is extraordinarily low, *i.e.* around 0.2, whilst a normal visual acuity is around 1.0. This finding is relevant as it indicates that the patients in question have a very low visual acuity, which must be caused by serious morphological “defects”. In turn, such “defects”, which have been investigated and will be discussed in more detail later on, are obviously difficult to treat by non-invasive measures, such as EI. Hence it may not be that surprising that (a) the visual acuity is extremely low in both groups and (b) any milder form of intervention may not suffice to address the serious disorders at the root of this very low visual acuity. This discussion will be revisited when considering such morphological changes (see from Figures 5.7 to 5.12).

6.2. Gender-specific effects

Returning to the more general view on visual acuity and EI, it is interesting to note the difference which exists between female and male patients. As mentioned in the Results section, there is a slight improvement of visual acuity in females who have received Early Intervention (**Figure 5.3**). Whilst this improvement is statistically not significant, the trend is clearly visible, especially when compared to the male counterparts. Here, a small, but insignificant trend in the opposite direction may be perceived, whereby the control group of patients aged 25+ even appears to have a better visual acuity (**Figure 5.4**). Whilst this effect is minor and not worth discussing further, it confirms that there is certainly no trend towards an improved visual acuity in the case of males.

It seems that if Early Intervention is probably more effective in girls than boys. Whilst this improvement in visual acuity is comparably modest (around 0.1 at most) and also not seen in all participants (hence a low statistical significance), it still represents a non-invasive kind of treatment and deserves serious consideration in the future.

In order to obtain a better insight into this gender-specific effect, a few additional analyses have been carried out. As the findings shown in **Figure 5.3** consider two variables, namely EI and sex, the possibility that women generally have a better visual acuity than men has been investigated, as this may also explain the findings seen in **Figure 5.3** and **Figure 5.4**. As **Figure 5.5** illustrates nicely, this does not seem to be the case. If any trend is noticed in this figure, then it is a rather worse general visual acuity in females with albinism compared to males with albinism. Whilst a significance of 0.166 is not relevant, it is rather good and refutes the hypothesis that albinistic women in general may show a better visual acuity than albinistic men. Hence the improvement in visual acuity seen for girls with EI, but not for boys with EI, is not due to their gender but probably indeed to EI. This explanation is supported by the box plots in **Figure 5.6**. Here, the apparent improvement of visual acuity in girls (with EI) compared to boys (with EI) is visible once more. In the case of patients with EI, the dependence on gender is opposite compared to the patients without EI, hence counting against sex as the decisive factor and confirming that EI may indeed result in some improvements in visual acuity, especially - if not exclusively - in girls.

Here, the statistical methods employed as part of this study reach their own limitations, as they are used to analyse differences between cohorts, and cannot focus on changes on an individual basis. Indeed, some individual patients may benefit considerably from EI. It

seems that visual acuity of individual participants "hidden" behind the boxes of the plot is generally better in the females of the test group. Whilst the form of the box plots shows that the values of visual acuity are rather spread in females and males, with and without EI, the highest value for the spread of visual acuity in girls with EI is 0.8 whereas in the control group it is 0.5 (**Figure 5.3**). The data are almost opposite in the males in test and control groups (**Figure 5.4**). Although no firm conclusions can be based on these spreads, they may indicate that some of the girls with EI ultimately have a visual acuity which is considerably improved compared to the "baseline" of around 0.2, and in some cases even reaches values close to normal.

In the future, questions if EI is beneficial to girls and / or boys, if significant improvements of visual acuity by more than just 0.1 units can be achieved by this method, and if the key to a successful treatment with EI is a particular genetic predisposition or due to a particular morphology, need to be addressed on an individual basis (see also section 6.5.). Such studies would be rather extensive and, for instance, monitor the success of EI on the basis of individual patients and over the duration of many years. They may be rather difficult to execute, though, as appropriate "control" patients would probably be hard to find, assuming that EI is now used widely in Germany.

6.3. The overarching role of morphological changes

Whilst the previous discussion has focussed on the role of EI in - a possible improvement of - visual acuity, the trends seen in response to EI, if any, were minor and not particularly relevant from a statistical point of view. As described at length in the Introduction, patients affected by albinism exhibit a range of serious ophthalmic impediments, which in one way or another may result in a dramatically reduced visual acuity. Such impediments include various morphological changes affecting the eye and ultimately may "dominate" the patient's eyesight, rendering a milder form of intervention, such as EI, almost ineffective.

It has therefore been important to establish some of these "predominant" factors in the patients who have undergone EI and the patients of control group, to see if any of these factors, in particular, may promote or prevent the effectiveness of EI.

It is already known, for instance, that the impact of iris morphology on visual acuity in patients with albinism is less in comparison to optic nerve head and macular pathology (Käsmann-Kellner 2000). At the same time, hypopigmentation of the iris does not seem to

primarily affect visual acuity but rather causes a kind of glare.

As can be seen in **Figure 5.7** and **Figure 5.8**, the visual acuity, however, correlates highly significantly with the **morphological degree of iris pathology** in the test as well as in the control group. As expected, the more limited the changes of the iris morphology are (*i.e.* degree 1), the better is the visual acuity and *vice versa*. This correlation is immediately apparent in the age group 25+ but also holds true for the children treated with EI. It is noteworthy that the median of visual acuity in the control group with a degree 1 even exceeds 0.5, but the spread is rather large in this case. At the same time, none of the patient cohorts, regardless of the degree of iris pathology, shows a visual acuity close to normal. This underlines once more that regardless of its severity, the pathology of the iris reduces visual acuity. It is then hardly surprising that a mild form of treatment, such as EI, cannot perform “miracles” by restoring visual acuity from a value close to 0.2. or 0.3 all the way to normal.

Similar considerations apply to the correlation of the visual acuity with the **morphological degree of macular pathology**. As shown in test and control groups **Figure 5.9** and **Figure 5.10**, all patients involved, regardless of EI or not, or of the specific degree of macular pathology, exhibit a drastically reduced visual acuity of around or even below 0.2. As for the iris, the degree of pathology seems to correlate inversely with the visual acuity, which is hardly surprising as a worse pathology should also result in a worse visual acuity. Whilst this trend is interesting from a statistic point of view - these are (highly) significant correlations - it is less thrilling from an ophthalmological perspective. Even patients with a degree 1 of macular pathology show a very poor visual acuity, and therefore the correlations observed, and any small differences which might exist depending on the degree of the pathology or EI are minute when projected on the overall scale of visual acuity which reaches to 1.0 as normal.

In essence, the same applies to considerations involving the **morphological degree of optic nerve head pathology**. As is apparent from the data presented in **Figure 5.11** and **Figure 5.12**, there is a clear inverse correlation between the degree of pathology and visual acuity in both, the group of children with EI and the control group of patients aged 25+. It should be noted that in this particular case, patients with a “degree 0” have been available in both cohorts and hence a benchmark value for the visual acuity of those patients could be determined. As may be expected, a degree 0 (normal optic nerve head) is associated with a rather good visual acuity of around 0.8 in the control group and around 0.7 in the group of children with EI. In contrast, patients with a degree of 1 or

higher show a dramatically reduced visual acuity of around 0.2. Since the overall sizes of sub-groups are fairly small, these numbers for the means, and the general correlations shown in the two figures need to be taken with some care. Yet they still corroborate the general conclusion that pathologies are the overriding factors when it comes to visual acuity, and that EI can only result in small, statistically insignificant improvements - at least when entire populations rather than individuals are considered.

Overall, the various correlations seen in **Figure 5.7 to Figure 5.12** between pathologies on the one hand and visual acuity on the other seem to be fairly plausible from a medical perspective and, as mentioned already, support the notion that pathologies ultimately form the overarching determinants of visual acuity. Indeed, this notion is not new, and as mentioned above, Käsmann-Kellner *et al.* have already discussed the fact that the better the macula and Optic Nerve Head morphology are, the better their function is (Käsmann-Kellner *et al.* 2007). Whilst the results obtained in the present study in essence confirm these previous findings and trends, for instance with regard to the iris, optic nerve head and macular pathology, there are also slight differences.

6.4. Squint

The two forms of squint, *i.e. Strabismus convergens* and *Strabismus divergens* are associated with albinism (Käsmann-Kellner *et al.* 2007). Furthermore, the visual acuity could be affected by both forms of squint, as both of them may lead to amblyopia. As shown in **Figure 5.13**, the mean visual acuity in children with no squint appears to be slightly higher when compared to the children with squint (just above 0.3 compared to between 0.1 and 0.2, respectively). In contrast, no apparent impact of squint on an already poor visual acuity can be observed in the case of the control group of adults aged 25+ (**Figure 5.14**). In this group the visual acuity is again centred around a median of between 0.1 and 0.2 in patients with or without squint. Indeed, in all cases any changes that may be related to squint are not statistically significant and, from an ophthalmological perspective, certainly minor on a scale from 0 to 1.

Whilst a major impact of pathologies, such as macular and optical nerve pathologies, on visual acuity is hardly surprising, these findings also deserve some comment from a more medical perspective. Considering the data and the various correlations and significances at hand, it appears, for instance, that a hypopigmentation of iris or dysplasia of macula and optic nerve head results in an - often severely - reduced visual acuity. Moreover, iris

morphology seems to contribute the least to visual acuity when compared with the two other pathologies, in agreement with previous findings (Käsmann-Kellner 2000, see above). Yet these numbers need to be used with some caution. Whilst a statistical analysis may indeed point towards a significant correlation between two parameters, the latter may not necessarily form a causal relationship, but may both represent consequences of a third, perhaps yet unknown parameter. Whilst it is therefore safe to state that all three, *i.e.* iris pathology, macular pathology and optic nerve pathology represent parameters which allow a **prediction** of visual acuity (in these cases the more advanced the pathology, the worse the visual acuity), such low visual acuity it is not necessary ***caused*** directly by either of these pathologies. This is sensible as it is indeed unlikely that these pathologies, act on their own and that each pathology results in an isolated effect on visual acuity which can then simply be "added to the total". Albinism, loss of pigmentation and the various complex biochemical and physiological processes associated with such disturbances may manifest themselves in many different ophthalmic pathologies and problems, and not just in one pathology. There may well be additional factors further compromising visual acuity, which may be considered as independent, "additional burdens", for instance glaucoma and maybe squint. Unfortunately, as in the case of squint, the data sets available in this study are insufficient to demonstrate such a purely "additional" effect which may be causally unrelated to the other pathologies and hence could be treated separately.

Ultimately, the correlations discussed here are purely "mathematical" and do not provide any information about the (anatomical, biochemical) mechanisms which may link these pathologies to visual acuity and ultimately establish a true causal relationship. Here statistics needs to move on into the realm of (bio)medicine in order to literally fill the numbers with life.

6.5. Is Early Intervention a women's thing - and why?

These issues need to be born in mind when moving on to the question if EI itself has an impact on visual acuity. As above, any correlations seen in this case may be due to a direct causal relationship, or the consequence of a third parameter impacting at the same time on both parameters. And indeed, whilst it appears that EI overall results only in a rather limited improvement of visual acuity, the effect seems to differ once a further parameter, *i.e.* gender, is also taken into account. As shown in **Figures 5.3** and **5.4**, there is a slight, noticeable but still statistically insignificant improvement of visual acuity in

females, but not in males, who have undergone EI. This apparent improvement may have been due to a generally better visual acuity among females, yet this gender specific advantage has been ruled out by comparing females and males in the test and control groups in **Figures 5.5** and **5.6**. The improvement of visual acuity is comparably modest (around 0.1 at most) and also not seen in all participants (hence a low statistical significance). Still EI represents a non-invasive kind of treatment and deserves serious consideration in the future.

At the same time, the underlying causes of the notable differences between females and males need to be considered. As delineated above, the statistical analysis does not answer the question why such effects may occur. It may be possible, for instance, that girls of this particular age (6 to 15) are more compliant with EI than boys. It may also be possible that the causes at the root of low visual acuity are somewhat different in girls and boys (see Introduction) and hence girls do respond better to such “treatment” when compared to boys.

Moreover, it would be counter-productive to suggest that EI only “works” in females and males may not benefit from this treatment at all. Indeed, EI not only - more or less - affects visual acuity, but also assists in the development of diverse compensatory mechanisms. This in turn may or may not lead to a notably improved visual acuity in later life. Ultimately, the matter at hand is far from trivial, as visual acuity is just one of a wide range of parameters influenced by EI.

In the end, the statistical analysis performed as part of this study can only highlight some significant - and not so significant - correlations and trends, but cannot explain the underlying causes. Here, more eloquent studies will be required to determine the impact of EI on fully “characterized” individual patients, including relevant genetic information. These patients would have to be monitored throughout the time of EI and the years afterwards.

7. Conclusions and outlook

To conclude, the statistical analysis conducted as part of this thesis points towards a slight yet statistically insignificant improvement of visual acuity in females who have undergone EI. This even slight improvement of around 0.1 visual units is notably absent in males. As the cohorts used in this study are rather large, a coincidental trend is less likely. It rather appears that females seem indeed to benefit more from EI than males.

In the future, it would therefore be interesting to study in more detail *why* EI may be more effective in girls than in boys. At this point, one may speculate that either genetic causes or a better compliance could affect the ultimate outcome. It may be possible, for instance, that girls of infantile age (0 to 6) are more compliant with EI than boys. In this case, the results ultimately achieved by this approach may be better in girls than in boys.

In the future, these issues could be investigated as part of a new study which would not simply rely on “old”, “passive” data of entire patient populations, but would accompany and closely monitor the EI process on an individual basis, for instance by recording the number of sessions, the effort of the children and also other factors which may influence the outcome. As part of the follow-on process after completion of EI, visual acuity of the patients should be tested regularly and over a longer period of time. Using this rather tedious and time-consuming approach, it would then be possible to draw firm conclusions about any improvements within the same cohort of patients and possibly also to identify the factors influencing or enhancing any such improvements.

From a research point of view, possible genetic influences are also of considerable interest. It may be possible that the causes at the root of low visual acuity are somewhat different in girls and boys (see Introduction) and hence girls do respond better to such “treatment” when compared to boys. Indeed, this idea has been considered as part of the present study, but as all pathologies caused a dramatic reduction in visual acuity, a more refined analysis has not been possible. This hypothesis is not too far-fetched, as various forms of albinism are X-chromosome linked. Here, the participants of this study would have to be dissected further into sub-groups based on specific forms of albinism. Information on these underlying causes may ultimately enhance or refine treatment, and at the same time may also serve as a predictor for the outcome of such a treatment.

Ultimately, it may even be possible to perform an entirely new study using genetic

information in addition to information about specific pathologies and visual acuity. Using techniques such as the Polymerase Chain Reaction (PCR); it is nowadays possible to obtain information on genetic mutations fairly rapidly, reliably and also rather inexpensively. It would therefore be very interesting to add the various genetic mutations, *i.e.* changes to melanin synthesis and transport as additional factor(s) to the study. Such a future investigation may also be complemented by an analysis of any anatomical processes and perhaps even biochemical mechanisms which may accompany EI “on the quiet” and hence result in an improved visual acuity.

In any case, whilst this study has shown that pathologies such as the morphological pathology of the macula and the optical nerve are clearly the predominant factors influencing visual acuity in the patients, it has also lent some additional support to the idea that EI is not a miracle cure but ultimately may make at least *some* sense, especially in girls - and that this form of mild, non-invasive treatment most certainly deserves further investigation.

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