The retinal pigmentation pathway in human albinism: Not so black and white

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1. Introduction

Albinism is a clinically and genetically heterogeneous disorder related to melanin pigment. The term ‘albinism’ is derived from the Latin word *albus*, meaning ‘white’. Ancient biblical texts mention Noah as the first person with albinism (Sorsby, 1958), and Pliny the Elder quotes from the Greek writer Isogonus of Nicaea: “a sort of people born in Albania, with eyes like owls, whereof the sight is fire-red; who from...
their childhood are grey-haired and can see better by night than by day” (Froggatt, 1960). The word “albino” itself is Portuguese and the first recorded use is by Balthazar Tellez in 1660 describing Africans with albinism that were seen along the African coast. A century later, the word “albino” came into more general use in the French, English and German languages. Nowadays, the term “people with albinism” rather than the potentially stigmatising term “albino” is commonly used. Albinism occurs throughout the animal kingdom (Bergsma and Brown, 1976).

The first scientific clinical description of albinism dates back to 1893, by G.M. Gould in the Journal of the American Medical Association (Caddy, 1894). Gould described photophobia, nystagmus, ametropia, iris transillumination, and amblyopia in human albinism. After the rediscovery of Mendel’s laws in the early 1900s, Davenport (1908) and, subsequently, Waardenburg (Van Den Bosch and Waardenburg, 1956) recognized the hereditary nature of albinism. The disease was described by Garrod as an inherited metabolic disorder and ascribed the phenotype to a biochemical defect in melanin production (Garrod, 1908). Sheridan was the first to describe paucity of uncrossed optic nerve fibres in the Norwegian albino rat (Sheridan, 1965), confirmed by Creel using electrophysiological studies (Creel, 1971; Creel et al., 1970). In 1974, Creel, Witkop and King demonstrated a significant reduction of uncrossed nerve fibres at the optic chiasm in persons with albinism, the so-called chiasmal misrouting (Creel et al., 1974). Subsequently, Tomita et al. (1989) were the first to identify a mutation in the TYR gene causing human albinism. Bassi et al. (1995) identified the gene in which mutations cause ocular albinism 1 (OA1, also known as Nettleship-Falls OA): GPR143, with albinism features limited to the eye.

While albinism has fascinated scholars for centuries, only recently major advances have been made in the understanding of its molecular and cellular pathology. In this report, to minimize complexity, we focus on the melanin pigmentation pathway in the retinal pigment epithelium (RPE) in human albinism. Nonetheless, our discussion may also have implications for other cell types and disorders where melanosomal pigment is key.

1.1. A phenotypic summary of human albinism: ocular, oculocutaneous and syndromic forms

In humans, albinism affects melanosomal pigmentation in the eye, skin and hair. In addition, patients have the following ocular abnormalities: nystagmus, iris translucency, fundus hypopigmentation, foveal hypoplasia and chiasmal misrouting. These abnormalities result in reduced visual acuity (VA) in patients. As melanin prevents excessive DNA-damage by UV radiation, a large subset of persons with albinism can be prone to skin cancer, unless adequate protective measures (sunscreen, clothing) are implemented (Halder and Bridgeman-Shah, 1995).

![Clinical presentation of two Caucasian patients with albinism](image-url)

*Fig. 1. Clinical presentation of two Caucasian patients with albinism. Top row, from left to right (A): Patient with OCA4 type albinism caused by a homozygous mutation in SLC24A5: c.264del p.(Gly89Aspfs*24); The phenotype consists of (A1): white hair and eyebrows, pale skin; visual acuity (VA) 0.8 logMAR, nystagmus, grade 4 iris translucency; (A2): grade 3 fundus hypopigmentation (OD = optic disc) (Kessel et al., 2021); A2 and A3: to ensure the foveal region was captured, we obtained 12 radial line scans (small OCT inset left from A3) (A3): grade 4 foveal hypoplasia (OD = optic disc) (Kessel et al., 2021); A3: VEP amplitudes show definite misrouting of the optic nerve fibres (Kruijt et al., 2019). Bottom row, left to right (B): patient with OCA2 type albinism with a homozygous mutation c.1037-T>A in OCA2. The phenotype consists of (B1): strawberry blond hair and eyebrows, pale skin; VA 0.3 logMAR, nystagmus, no iris translucency; (B2): no fundus hypopigmentation (grade 0) (OD = optic disc); (B2–B3): again, 12 radial line scans were made; see inset) (B3): grade 1 foveal hypoplasia (see arrowhead) (Thomas et al., 2011); (B4): definite misrouting of the optic nerve fibres (Kruijt et al., 2019). As an illustration of clinical variability, the OCA4 patient (A) has all typical features of albinism, while the OCA2 patient (B) has a less severe pigmentation phenotype, especially of the fundus (A2, B2). Foveal hypoplasia is less severe in the OCA2 patient as well, but chiasmal misrouting is present in both patients. Of note, none of the clinical features of albinism are present in all albinism patients (Kruijt et al., 2018). In general, considerable phenotypic variability is present between and within albinism subtypes, patients and families.*
The clinical diagnosis of albinism is based on examination of the eyes, skin, hair, and assessment of chiasmal misrouting. The diagnosis can be confirmed by pedigree analysis and DNA diagnostics of all known albinism disease genes. Of note, in approximately 25–30% of patients no disease-causing mutations have been identified (yet) (Kruijt et al., 2018; Lasseux et al., 2018; Montoliu et al., 2014). Ocular examination includes best corrected VA measurement, slit lamp examination to assess iris translucency, and fundoscopy to assess pigment levels. Optical coherence tomography (OCT) scans are used to determine the degree of foveal hypoplasia, measured using the Leicester grading system (Thomas et al., 2011). OCT is also used to record the asymmetry of the ganglion cell layer thickness between the nasal and temporal areas of the retina, as found in patients with albinism (Brücher et al., 2019; Woertz et al., 2020a). Analysis of visual evoked potentials (VEPs) is used to assess optic nerve misrouting (Hoffmann et al., 2005a; Kruijt et al., 2019).

In oculocutaneous albinism (OCA), the pigmented skin, eyes, and hair are all affected to a variable degree (Fig. 1). In OA1, the hypopigmentation is mostly restricted to the eyes (iris and retina) (Schiappino and Tacchetti, 2005). Nonetheless, some evidence suggests that OA1 may also be associated with mild hypopigmentation of the skin (Lewis, 1993a). In addition to OCA and OA1, Hermansky-Pudlak syndrome (HPS) and Chediak-Higashi syndrome (CHS) include albinism in their pathological spectrum (Bowman et al., 2019; Huizing et al., 2008). Albinism deafness syndrome(s), including the autosomal dominant Tietz syndrome, as well as Waardenburg syndrome type 2, caused by mutations in MITF, are clinically considered auditory-hypopigmentation syndromes (Chiang et al., 2009; Lakhdar et al., 2021; Smith et al., 2000; Vetrini et al., 2004), and are excluded from this report. MITF is only briefly mentioned in sections 8.1 and 9.2 as it is a transcription factor that affects expression of a number of melanosomal genes. Griscelli syndrome can also be considered a syndromic form of human albinism, but it has no consistent ocular phenotype (Mancini et al., 1998; Montoliu et al., 2014; Wasif et al., 2020), and is therefore excluded from this report. Finally, FHONDA (foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis) is an autosomal recessive syndrome with remarkable similarities to albinism, including nystagmus, foveal hypoplasia and misrouting of the optic nerve fibres. However, an abnormal pigmentary phenotype is absent (Poultet et al., 2013).

Taken together, the phenotypic variability in human albinism is wide, both between and within genetic albinism subtypes. As illustrated in Fig. 1B, patients with OCA2 can show a relatively mild phenotype, whereas it is often more severe in other albinism subtypes. Thus, the severity of the disease differs from patient to patient. No single pathognomonic sign defining the diagnosis or prognosis currently exists (Kruijt et al., 2018).

### 1.2. A summary of different genetic forms of human albinism

While the prevalence of human albinism in Western societies ranges from 1:14,000 to 1:17,000, in African countries it lies between 1:1500 to 1:3000.

#### Table 1

| Group                          | Disorder | Gene ID          | Gene name                                  | Mouse gene name, mouse model name | Hypopigmentation | Ocular features | Gene OMIM Number |
|-------------------------------|----------|------------------|--------------------------------------------|----------------------------------|------------------|----------------|------------------|
| Hermansky-Pudlak Syndrome     | HPS1     | HPS1/ BLOC51     | HPS1 biogenesis of lysosomal organelles complex 3 subunit 1 | Hps1, pale ear                  | Mild to severe   | Yes             | 604982           |
| HPS                           | HPS2     | AP3D1            | Adaptor related protein complex 3 subunit beta 1 | Ap3d1, pearl                     | Mild to severe   | Yes             | 603401           |
| HPS                           | HPS3     | HPS3/ BLOC51     | HPS3 biogenesis of lysosomal organelles complex 2 subunit 1 | Hps3, cocoa                      | Mild             | Severe          | 606118           |
| HPS                           | HPS4     | HPS4/ BLOC52     | HPS4 biogenesis of lysosomal organelles complex 3 subunit 2 | Hps4, light ear                  | Mild to severe   | Yes             | 606682           |
| HPS                           | HPS5     | HPS5/ BLOC52     | HPS5 biogenesis of lysosomal organelles complex 2 subunit 2 | Hps5, ruby-eye 2                | Mild             | Severe          | 607521           |
| HPS                           | HPS6     | BLOC53           | HPS6 biogenesis of lysosomal organelles complex 2 subunit 3 | Hps6, ruby-eye                   | Mild to severe   | Yes             | 607522           |
| HPS                           | HPS7     | DYN3BP1/ BLOC58  | Dystrobrevin binding protein 1             | Dnbp1, sandy                     | Mild to severe   | Yes             | 607145           |
| HPS                           | HPS8     | BLOC53           | Biogenesis of lysosomal organelles complex 1 subunit 3 | Bloc1x3, reduced pigmentation, rp | Mild to severe   | Yes             | 609762           |
| HPS                           | HPS9     | BLOC1S6          | Biogenesis of lysosomal organelles complex 1 subunit 6 | Bloc1x6, pallid                  | Mild             | Severe          | 604310           |
| HPS                           | HPS10    | AP3D1            | Adaptor related protein complex 3 subunit delta 1 | Ap3d1, mocha                     | Mild             | Yes             | 607246           |
| HPS                           | HPS11    | BLOC55           | Biogenesis of lysosomal organelles complex 3 subunit 5 | Bloc1x5, muted                   | Mild             | Yes             | 607289           |
| Chediak-Higashi Syndrome      | CHS      | LYST             | Lysosomal trafficking regulator            | Lyst, beige                      | Mild to severe   | Yes             | 606897           |
| Oculocutaneous Albinism       | OCA1     | TYR              | Tyrosinase                                 | Tyr, albino                      | Mild to severe   | Yes             | 606933           |
| OCA                           | OCA2     | OCA2             | OCA2 melanosomal transmembrane protein/P protein | Oca2, pink-eyed dilution         | Mild to severe   | Yes             | 611409           |
| OCA                           | OCA3     | TYRBP1           | Tyrosinase related protein 1               | Tyrp1, brown                     | Mild to moderate | Yes             | 115501           |
| OCA                           | OCA4     | SLC45A2          | Solute carrier family 45 member 2          | Slc45a2, underwhite              | Mild to severe   | Yes             | 606202           |
| OCA                           | OCA5     | Not defined      | Locus 4q24                                 | Moderate to severe              | Yes             |                  | 609802           |
| OCA                           | OCA6     | SLC24A5          | Solute carrier family 24 member 5          | Slc24a5                          | Moderate to severe | Yes     |                  |                  |
| OCA                           | OCA7     | LRMDA            | Leucine rich melanocyte differentiation associated | Lrmda                            | Mild             | Yes             | 614537           |
| OCA                           | OCA8     | DCT              | Dopachrome tautomerase                      | Dct, slaty                       | Mild             | Only ocular, mild | Yes             | 191275           |
| Ocular Albinism               | OCA1     | GPR143           | G protein-coupled receptor 143             | Gpr143                           | Mild             | Yes             | 300808           |
| FHONDA                        | FHONDA   | SLC38A8          | Solute carrier family 38 member 8          | Slc38a8                          | No               | Severe          | 607108 615585   |
1.3. A summary of the biochemistry of melanosomal pigmentation in the retina

Albinism is a disorder of melanin pigmentation. There are three major types of melanin pigment in the healthy human body: brown/black eumelanin, red pheomelanin, and black neuromelanin. Eumelanin and pheomelanin are present in the eye and skin; neuromelanin can only be found in the brain. In this report we mainly focus on the eye and more specifically the RPE, as it relates to retinal development in albinism. The RPE primarily contains eumelanin (Weiter et al., 1986). Interestingly, the type, amount and localisation of melanin in the choroid correlates to the colour of the iris (Sturm, 2009; Wakamatsu et al., 2008). Eumelanin absorbs free radicals and photonic energy. It dissipates vast amounts of energy as heat, and releases excess toxic radicals. In contrast, pheomelanin absorbs less light of the ultraviolet and other wavelengths, but is less cytotoxic (Ortonne, 2002). For the RPE, a high ratio of eumelanin to pheomelanin seems most important for its photoprotective properties (Vincensi et al., 1998). The basic features of the biosynthetic pathways of eumelanin, pheomelanin and neuromelanin are presented in Fig. 2, and briefly described below. In reality, pigmentation is “not so black and white”, since multiple genes, environmental factors and different (combinations of) biochemical intermediate levels or activity determine pigmentation and a myriad of associated colours in the different tissues (Ito and Wakamatsu, 2008).

In brief, eumelanin synthesis starts with the conversion of L-tyrosine into both L-DOPA and dopaquinone by the enzyme tyrosinase (TYR; OCA1) (Fig. 2; left column). Subsequently, inside the melanosome, dopaquinone spontaneously transforms into (leuco-)dopachrome. Next,
dopachrome is converted either into dihydroxyindole (DHI) followed by subsequent processing into indolequinone (IQ), again by TYR; or into dihydroxyindole carboxylic acid (DHICA) by dopachrome tautomerase (DCT; OCA8). Leucodopachrome is processed into indole-2-carboxylic acid quinone (ICAQ) by tyrosinase related protein 1 (TYRP1; OCA3). All three resulting compounds (IQ, DHICA and ICAQ) are finally polymerized into black/brown eumelanin (Hearing and Tsukamoto, 1991; Ito and Wakamatsu, 2008; Sugumaran, 2016).

Pheomelanin also relies on the TYR-dependent production of dopaquinone. Dopaquinone subsequently reacts with cysteine to produce different forms of cysteinyl-dopa. These cysteinyl-dopa variants are oxidised by TYR to produce cysteinyl-dopa quinones and, subsequently, benzothiazine intermediates. Oxidation of these intermediates results in the generation of pheomelanin polymers (Hearing and Tsukamoto, 1991). It is thought that pheomelanin can react spontaneously with sulfhydryl compounds resulting not in red, but yellowish pigment in the hair of some patients (Ray et al., 2007). In OCA2 and OCA4, the low pH in the melanosome is not optimal for TYR function: low levels of dopaquinone are produced that spontaneously result in pheomelanin production. Indeed, in OCA2 and OCA4, the reddish pheomelanin pigment is very obviously overrepresented, resulting in yellowish, sandy blond skin and hair.

The competitive metabolic activity down the eumelanin or pheomelanin pathway in melanosomes is at least determined by competition for the shared biochemical intermediate dopaquinone and the local activity of the Melanocortin 1 receptor (MC1R). The default synthesis (in the presence of MC1R antagonists) is that of pheomelanin. Independently from antagonists, this default synthesis happens also when MC1R is mutated, a common feature in red-haired people. MC1R can be activated by the α-Melanocyte-stimulating hormone that promotes the synthesis of black-brown eumelanin (Swope and Abdel-Malek, 2018). While MC1R is also expressed in primary RPE mouse cultures (Cheng et al., 2014), and may protect mouse retinal cells against oxidative stress (Maisto et al., 2017; Rossi et al., 2016), its exact role in the RPE remains to be elucidated.

The third type of melanin, neuromelanin, is also produced from L-tyrosine (Fig. 2; right column). However, tyrosine is not processed by TYR, but by tyrosine hydroxylase (TH) to DOPA. Subsequently, in neurons, L-DOPA is converted by the aromatic acid decarboxylase into the neurotransmitter dopamine, which is one of the biochemical intermediates toward the formation of neuromelanin (Rabey and Hefti, 1990). In the neural cell, a granule forms with a pheomelanin-related reactive core encapsulated by more inert eumelanin-like pigment (Ito, 2006; Zucca et al., 2014). Neuromelanin is found in the substantia nigra in the midbrain of primates (including humans) (Carballo-Carbajal et al., 2019). The substantia nigra degenerates in Parkinson’s disease. The role of neuromelanin is thought to be similar to the eumelanin and pheomelanin pigments: scavenging potentially harmful oxidative stress-generated free radicals (LaVoie et al., 2005). Neuromelanin is considered to result from a protective process against excess cytotoxic dopamine that can be cytotoxic (LaVoie et al., 2005). Interestingly, TYR (OCA1) is also expressed in the human substantia nigra (Miranda et al., 1984; Xu et al., 1997b), but its potential role in neuromelanin production is unclear. Recently, Carballo-Carbajal et al. (2019) constructed genetically modified mice in which Tyr was overexpressed in the substantia nigra, and found that neural cells started to produce neuromelanin that is normally not present in mice (Carballo-Carbajal et al., 2019). A potential relation between TYR and neuromelanin is also supported by a rare TYR mutation (p.V275F) implicated in albinism that has recently also been associated with a higher risk of developing Parkinson’s disease (Lubbe et al., 2016). Taken together, these initial data suggest that TYR may also have a role in neuromelanin production or function. While eumelanin and pheomelanin are primarily produced early in life, neuromelanin takes decades to form. As such, it is not present in most laboratory animals, for example rodents (Carballo-Carbajal et al., 2019). Whether the albinism phenotype of commonly used laboratory animals could influence neuromelanin formation is unknown. Finally, it must be noted here that, although dopaminergic neurons, such as amacrine cells, are present in the neural retina (Pourcho, 1982), there is no evidence that these cells contain neuromelanin.

1.4. A summary of cellular pathology in human albinism

Pigment cells (melanocytes and RPE) are affected in albinism. Melanocytes originate from the neural crest and are particularly abundant in the eye (choroid, iris) and skin. The RPE is a neuro-epithelial cell layer of the retina lining the back of the eye. The choroid is a highly vascular tissue underneath the RPE and Bruch’s membrane that supplies the outer retina with oxygen and nutrients, and removes metabolic waste (Brinks et al., 2021). Melanocytes are also found in other places in the body, such as the stria vascularis of the cochlea in the inner ear (Murillo-Cuesta et al., 2010) and in the heart valves (Mjaatvedt et al., 2005). In all these tissues, the melanosomal pigment is thought to reduce oxidative stress.

The intracellular site of pigmentation is the melanosome. This is an LRO in a melanocyte or RPE cell, in which melanin is produced and stored. Healthy melanosomes develop and mature through four defined subsequent stages (Schiaffino, 2010): Stage-I melanosomes are early endosomes. Stage-II melanosomes are elongated with longitudinal pre-melanosome proteins (PMEL17) fibrils but without melanin. Stage-III have clear fibrils and pigmentation (Watt et al., 2009). In the fourth and final stage, the organelles are filled with melanin and their internal structure becomes optically obscured. The primary function of melanin lies in photo-protection of (adjacent) cells and tissues against light-induced oxidative damage (Boulton and Dayhaw-Barker, 2001). The most obvious sign to the outside world is that melanin colours cells. As described in the previous section, in RPE and skin there are two types of melanin present: eumelanin (dark brown-black) and pheomelanin (yellow-red) (Boulton and Dayhaw-Barker, 2001; Strauss, 2005). Besides melanin, other pigments are also present in the retina, for example: lipofuscin in the RPE; the pigments lutein and zeaxanthin next to macular photoreceptors; and melanopsin, uniquely found in retinal ganglion cells (RGCs). This report focuses on melanin pigment because of its key role in human albinism.

2. The proposed retinal pigmentation pathway in human albinism

In this report, we propose a retinal pigmentation pathway hypothesis. As introduced above, several clinical and genetic subtypes of albinism exist, which typically have nystagmus, foveal hypoplasia and misrouting of the optic tracts in common. The first subtypes are syndromic forms of albinism that affect general LRO genesis and function (including melanosomes) in multiple cell types and tissues including the eye, skin and hair (section 3). The second subtype, OCA, affects only eye, skin and hair pigmentation, because of either specific melanosome dysfunction, defective melanin production, or pigment cell differentiation (section 4). Next, we describe OA with near normal melanin production but affected melanosome formation, with typical ocular, but not skin features of albinism (section 5). And finally, FHONDA, which resembles albinism remarkably with nystagmus, foveal hypoplasia and misrouting, but without pigimentary abnormalities (section 6).

To date, genetic mutations in at least 22 disease genes have been implicated in all forms of human albinism (Table 1). Each of these disease-causing genes has a specific function, with the pathology ranging from general to specific: from general syndromic pathology, to more specific oculocutaneous and ocular albinism, to remarkably restricted in FHONDA. Guided by our own data, combined with those of the literature (Bassiri et al., 1995; Fernández et al., 2021; Figueroa and McKay, 2019; McKay, 2019), we propose a new relationship between disease genes and function across various types of albinism,
Two syndromic forms of human OCA exist: HPS and CHS. Typically, patients with these syndromes all have key, although variable, characteristics of OCA plus a number of additional clinical features. HPS and CHS patients typically present with bleeding problems, with the latter accompanied by a serious predisposition to infections and neurodegeneration. Both syndromes are characterised by a general cellular defect in the genesis or function of LROs (Olkkonen and Ikonen, 2006; Shiflett et al., 2002; Shiotelersuk and Gahl, 1998). LROs are derived from the endosomal system and form specialised organelles in specific cell types, including melanosomes in pigment cells (Luzio et al., 2014; Marks et al., 2013). Consequently, in HPS and CHS, cells other than pigment cells are also affected. HPS and CHS and their wide range of underlying molecular and cellular mechanisms have recently been excellently reviewed (Bowman et al., 2019; Fernández et al., 2021). The relevant information in the context of albinism is summarised and updated below. For each of the syndromes, the specific phenotype, pathology and underlying molecular and cellular mechanisms in (retinal) pigment cells are consecutively discussed.

Fig. 3. Proposed retinal pigmentation pathway in human albinism connecting genotype (top row), cellular defects (middle row) and phenotypes (bottom row) into a single framework. Disease genes of different types of albinism affect increasingly restricted cellular and molecular mechanisms and result correspondingly in increasingly specific phenotypes. Left column: the syndromic forms of albinism (HPS1-11, CHS) involve a generalised lysosome-related organelle (LRO) defect in different cell types, which also affects melanosomes in the RPE and melanocytes. Second column (OCA1-8 downward): the different types of OCA (OCA1-8) cause specific defects of the melanosome. Some of the implicated genes encode enzymes involved in melanogenesis or pigment cell differentiation, others appear to be involved in trafficking of melanosomal proteins or regulate melanosome physiology essential for melanin formation. According to the proposed retinal pigmentation pathway, HPS1-11, CHS and OCA1-8 defects all result in lower L-DOPA production and subsequent reduced activation of its receptor GPR143 (OA1). Third column (OA1 downward): OA1, with a phenotype restricted to the eye, and a feedback loop that results in macro-melanosomes formation, and melanin pigment abnormalities in the RPE. If GPR143 is dysfunctional, no downstream paracrine signalling to the neural retina occurs through this pathway, affecting retinal development, as illustrated by the OA1 pathology of the neural retina and optic nerve. Fourth column (SLC38A8 downward): mutations in SLC38A8, downstream in the proposed pathway have been implicated in the FHONDA phenotype, which has no pigmentation related defects at all and has a consistent albinism-like severe pathology of the neural retina (foveal hypoplasia) and optic nerve (misrouting).

3. Genetic defects affecting LROs, including the melanosome, lead to syndromic forms of albinism

3.1. Syndromic forms of human albinism: common features of the phenotype and pathology

Two syndromic forms of human OCA exist: HPS and CHS. Typically, patients with these syndromes all have key, although variable, characteristics of OCA plus a number of additional clinical features. HPS and CHS patients typically present with bleeding problems, with the latter accompanied by a serious predisposition to infections and neurodegeneration. Both syndromes are characterised by a general cellular defect in the genesis or function of LROs (Olkkonen and Ikonen, 2006; Shiflett et al., 2002; Shiotelersuk and Gahl, 1998). LROs are derived from the endosomal system and form specialised organelles in specific cell types, including melanosomes in pigment cells (Luzio et al., 2014; Marks et al., 2013). Consequently, in HPS and CHS, cells other than pigment cells are also affected. HPS and CHS and their wide range of underlying molecular and cellular mechanisms have recently been excellently reviewed (Bowman et al., 2019; Fernández et al., 2021). The relevant information in the context of albinism is summarised and updated below. For each of the syndromes, the specific phenotype, pathology and underlying molecular and cellular mechanisms in (retinal) pigment cells are consecutively discussed.

3.2. Syndromic forms of human albinism: disease genes, proteins and molecular mechanisms

3.2.1. Hermansky-Pudlak syndrome (HPS)

HPS is a heterogeneous autosomal recessive syndrome (Oh et al., 1998; Seward and Gahl, 2013). In the general population, the prevalence of HPS is approximately 1:1,000,000. However, in some more genetically isolated populations the prevalence is much higher; for example: up to 1:1800 in Puerto Rico (Seward and Gahl, 2013), likely due to founder effects. HPS consists of at least 11 genetic subtypes (HPS1-11; Table 1). Next, we describe the features that all HPS subtypes have in common, as well as the characteristics that are unique for one or more subtypes.

HPS patients have characteristic but variable features of OCA, such as mild to severe hypopigmentation of skin, hair and eye, and including a reduced VA. In addition, patients experience excessive bleeding and bruising due to the absence of platelet-dense granules (Seward and Gahl, 2013). Some subtypes also include immune deficiencies, lung defects and granulomatous colitis (Bowman et al., 2019). In HPS, the production of a number of organelles including melanosomes is affected in cells of different organs, such as the eye (RPE and choroid), and skin and hair melanocytes. The HPS proteins can be divided in four functional complexes, called the Biogenesis of LROs Complex (BLOC) –1, –2 and –3, as well as the Adaptor Protein (AP) –3 complex (Bowman et al., 2019). An overview of these complexes and their function is presented in Fig. 4. The role of (specific subunits of) BLOC and AP complexes may be cell-type specific, which could partially explain the variability of the HPS albinism syndrome features. Interestingly though, clinical features are remarkably similar for genetic subtypes associated with the same complex. Therefore, the BLOC and AP related pathology can be best understood by discussing the four different complexes (BLOC-1 and AP-3) in-depth, below.

The BLOC-1 protein complex consists of 8 subunits: BLOC1S1-8 (Table 1). Mutations in the BLOC-1 genes encoding DTNBPI (BLOC1S8; HPS7), BLOC1S3 (HP58), BLOC1S6 (HPS9), BLOC1S5 (HPS11) have been implicated in human HPS (Gwynn et al., 2004;
Patients with HPS3, 5 and 6 present with a relatively mild pigmentation intracellular destination. Recently, BLOC-2 was implicated in the for BLOC-2, endosomal tubules make less stable connections with cellular and melanosome clumping in choroidal melanocytes (Dennis et al., 2015). Relatively little is yet known about BLOC-2 function. Without BLOC-2, cargo can arrive at the wrong destination, such as the Golgi or plasma membrane instead of the melanosome. The BLOC-3 complex controls antero- and retrograde trafficking between the melanosome and other cellular compartments. Finally, the AP-3 complex is involved in the budding of the endosomal vesicles used to transport cargo to other LROs.

Huang et al., 1999; Li et al., 2003; Pennamen et al., 2020). Patients with BLOC-1 mutations have a relatively mild pathology without severe immune deficiency or lung defects (Cullinane et al., 2012; Li et al., 2003). BLOC-1 mediates budding and extension of membrane tubules from the endosome to LROs including the melanosome, enabling transport of essential cofactors and enzymes (Delevoye et al., 2016; Dennis et al., 2016). These include cargos that have been implicated in albinism, such as TYR (OCA1), OCA2 and TYRP1 (OCA3) (Delevoye et al., 2009; Setty et al., 2007; Sitaram et al., 2012).

The BLOC-2 protein complex consists of three subunits: BLOC2S1-3. Mutations in these genes have been implicated in, respectively, HPS3, 5 and 6 (Anikster et al., 2001; Di Pietro et al., 2004; Gautam et al., 2004). Patients with HPS3, 5 and 6 present with a relatively mild pigmentary and bleeding phenotype (Huizing et al., 2001, 2009; Michaud et al., 2017). Neither granulomatous colitis nor lung disease are present. Mice with mutations in BLOC-2 subunits exhibit hypopigmentation in the RPE and melanosome clumping in choroidal melanocytes (Dennis et al., 2015). Relatively little is yet known about BLOC-2 function. Without BLOC-2, endosomal tubules make less stable connections with cellular vesicles, including the melanosome, and cargo can end up in the wrong intracellular destination. Recently, BLOC-2 was implicated in the formation of so-called Weibel-Palade bodies (Karampini and Voorberg, 2020; Sharda et al., 2020), an essential compartment for haemostatic and inflammatory deposits in platelets and endothelial cells, possibly explaining the bleeding endo-phenotype. BLOC-1 and BLOC-2 can physically interact and possibly share some functionalities (Di Pietro et al., 2006). Indeed, in cultured BLOC-1 and BLOC-2 defective mouse or human melanocytes, trafficking of the same set of proteins (TYRP1, OCA2, TYR) is affected (Di Pietro et al., 2006) resulting in similar disease pathology.

The BLOC-3 protein complex has two subunits: BLOC3S1 and BLOC3S2 implicated in specific forms of HPS (respectively: HPS1 and HPS4) (Martina et al., 2003). HPS1 and 4 patients have nystagmus and hypopigmentation of the RPE and skin. In the choroid and hair follicles, enlarged melanosomes have been observed (Di Pietro and Dell’Angelica, 2005; Erickson, 1997). Whether the RPE has enlarged melanosomes, or not, is not clear. In addition to albinism features, HPS1 and HPS4 patients eventually develop lung fibrosis that is ultimately fatal without a lung transplant (Young et al., 2012). Around 20 to 30 percent of HPS1 and HPS4 patients additionally suffer from granulomatous colitis, also known as Crohn’s disease (Seward and Gahl, 2013). The BLOC-3 complex is involved in intracellular cargo travelling to LROs, including the melanosome (Gerondopoulos et al., 2012; Wasmier et al., 2006). In BLOC3S1 (Hps1) and BLOC3S2 (Hps4) deficient mutant mice, the RPE and skin melanocytes contain smaller and less pigmented melanosomes (Gardner et al., 1997; Nguyen and Wei, 2007; Suzuki et al., 2002). In contrast, the choroid and hair bulb melanocytes contain enlarged melanosomes with normal to high levels of pigment (Nguyen et al., 2002). Apparently different cell types are affected in different ways. In BLOC-3 deficient cultured mouse melanocytes, there is a decreased presence of tubules extending from the melanosome and reduced cargo trafficking (Dennis et al., 2016). A lack of BLOC-3 activity may lead to reduced anterograde trafficking and recycling of specific components, resulting in small hypopigmented melanosomes.

Aside from the three BLOC protein complexes, a fourth HPS protein complex, called AP-3 exists. AP-3 consists of four subunits, two of which have been implicated in human HPS: AP-3 Subunit Beta 1 (AP3B1; HPS2) and AP-3 Subunit Delta 1 (AP3D1; HPS10) (Dell’Angelica, 2009). Apart from a “typical” OCA and HPS phenotype, HPS2 and HPS10 patients have an impaired immune system and lung defects (Ammann et al., 2016; Enders et al., 2006; Fontana et al., 2006). The AP-3 complex regulates the budding of endosomal vesicles to traffic specific cargos to multiple LROs, including the melanosomes (Drake et al., 2000; Peden et al., 2004; Theos et al., 2005). Specificity of AP-3 trafficking depends on AP-3 binding to dileucine motif sorting sequences of melanosomal proteins such as TYR and OCA2 and results in accumulation of these proteins in transport vesicles (Honing et al., 1998; Janvier and Bonifácino, 2005; Kelly et al., 2008). Remarkably, AP-3 does sort TYR, but not TYRP1, although these related proteins share a similar dileucine targetting motif (Huizing et al., 2001; Vijayasairadi et al., 1995). Finally, the AP-3 complex also interacts with BLOC-1, suggesting a shared functionality in both trafficking pathways (Di Pietro et al., 2006; Salazar et al., 2006; Sitaram et al., 2012).

3.2.2. Chediak-Higashi syndrome (CHS)

CHS is extremely rare, with fewer than 500 cases reported in the literature. CHS is an autosomal recessive syndrome, which involves OCA features: patients have hypopigmentation of skin, hair and eyes (Sharma et al., 2020) as well as limited VA (Kritzler et al., 1964). Affected individuals have typical grey/silver hair, different compared to other types of albinism. Giant melanosomes are found in patient melanocytes and RPE (Boissy and Nordlund, 1997; Valenzuela and Morningstar, 1981). Remarkably, the CHS RPE is (almost) devoid of pigment, while the choroid seems relatively unaffected (Ben-Ezra et al., 1980). Apart from OCA features, CHS patients suffer from bleeding and bruising problems and are prone to infections (Introne et al., 2017). Approximately 90% of patients with CHS suffer from severe immune system dysfunction, namely hemophagocytic lymphohistiocytosis (Toro et al., 1993), an overproduction of immune cells. CHS patients eventually develop lung fibrosis that is ultimately fatal without a bone marrow transplantation at an early age to overcome immune deficiencies. However, this treatment does not prevent CHS-associated neurodevelopmental or neurodegenerative problems (Tardieu et al., 2005).

CHS is caused by mutations in the lysosomal trafficking regulator (LYST) gene. LYST encodes a large protein of around 4000 amino acids of largely unknown function (Nagle et al., 1996; Ward et al., 2003). It
4. Genetic defects specifically affecting melanosome structure or function lead to OCA

4.1. Genetic melanosome defects: the phenotype and pathology

While syndromic forms of albinism affect the generation and function of LROs, including the melanosome in a wide range of cells, OCA involves more specific abnormalities affecting pigment cells only in the eye, skin, and hair. In OCA1, OCA3, and OCA8, synthesis of melanin is directly reduced as enzymes in the melanin biosynthetic pathway are mutated (Fig. 2). In OCA2 and OCA4, trafficking of specific melanosomal proteins is disrupted which leads to altered melanosome formation (Bin et al., 2015; Puri et al., 2000). This is accompanied (possibly also in mutated (Fig. 2). In OCA2 and OCA4, trafficking of specific melanosomal proteins is disrupted which leads to altered melanosome formation (Bin et al., 2015; Puri et al., 2000). This is accompanied (possibly also in mutated (Fig. 2). In OCA2 and OCA4, trafficking of specific melanosomal proteins is disrupted which leads to altered melanosome formation.

4.2. Genetic melanosome defects: disease genes, proteins and genetic variation

4.2.1. OCA1: TYR, tyrosinase

OCA1 is an autosomal recessive disorder with a prevalence of around 1:36,000 in Western countries (Kruijt et al., 2018). The specific associated phenotype, which is highly variable, ranges from complete absence of pigmentation to (almost) normal pigmentation in hair, skin, and eyes. Occular features include nystagmus, reduced VA, iris transillumination, foveal hypoplasia, and abnormal axonal crossing at the optic chiasma (King et al., 2003). TYR, located on human chromosome 11q14-q21 (Sartori et al., 1989), contains five exons, resulting in a 2062 bp transcript (NM_000372.5). The TYR promoter region contains many binding sites for regulatory proteins, such as the melanocyte inducing transcription factor (MTF) that influences the expression of the gene and, partially, the activity of the encoded protein (Ray et al., 2007). Most of what we know about the key evolutionarily conserved regulatory regions in the human TYR gene originates from the numerous studies carried out with the Týr/-/ mouse (Serugia et al., 2021).

4.2.1.1. Protein structure and function. Human TYR (OCA1) encodes a protein of 529 amino acids, resulting in a ~80 kDa glycoprotein. It is a membrane-bound mono-phenol mono-oxidase. The 2D structure of the human protein is presented in Fig. 5. The N-terminus of the protein consists of an 18 amino acid signalling domain targeting the protein to the endoplasmic reticulum (ER). Next, to it, the protein carries two highly conserved cysteine clusters, the second of which is an epidermal growth factor (EGF)-like region, most likely involved in protein-protein interactions (Davis, 1990; García-Borron and Solano, 2002). TYR has seven N-glycosylation sites and two central Cu²⁺-binding sites (CuA and CuB) with a conserved cysteine cluster in between (García-Borron and Solano, 2002). Towards the C-terminus, the CuB binding site is followed by a transmembrane domain and a dileucine and tyrosine-based motif essential for melanosomal targeting (Calvo et al., 1999; García-Borron and Solano, 2002; Höning et al., 1996; Sandoval et al., 1994).

TYR expression is primarily seen in melanoctyes and RPE cells. A complex process is needed to produce and deliver fully functional TYR to the melanosomes. First, release from the ribosome into the ER lumen is accompanied by cleavage of an N-terminal signal sequence (Bouchard et al., 1989; Ewton et al., 1987; Yamamoto et al., 1989). Next, TYR moves from ER to the Golgi, where it reaches its active configuration by folding and glycosylation (Wang and Hebert, 2006). Mediated by ATP7A (MNK), mutations in TYR are the most frequently found cause of human albinism. Missense mutations in TYR are clustered to six domains of the encoded protein: A cluster affecting conserved cysteine residues at the N-terminal side, one around the EGF-like domain, two spanning, and one in between the two Cu²⁺-binding domains, and one located between the second Cu²⁺-binding and the transmembrane domain (Fig. 5). These clusters seem to be essential for protein function and likely affect proper folding of the protein, with mutations leading to retention and degradation in the ER (Dolinski et al., 2017; Halaban and Moellmann, 1990; Toyofuku et al., 2002). One of the most common TYR mutations found in humans (p.T373K) alters a TYR glycosylation site, resulting in ER degradation (Dolinski and Sergeev, 2017; Lasseaux et al., 2018), which is also found in the extreme dilution mottled mouse model (Lavado et al., 2005). Yet other (missense) mutations found in humans (p.T373K) alters a TYR glycosylation site, resulting in ER degradation (Dolinski and Sergeev, 2017; Lasseaux et al., 2018), which is also found in the extreme dilution mottled mouse model (Lavado et al., 2005). Yet other (missense) mutations found in humans (p.T373K) alters a TYR glycosylation site, resulting in ER degradation (Dolinski and Sergeev, 2017; Lasseaux et al., 2018), which is also found in the extreme dilution mottled mouse model (Lavado et al., 2005). Yet other (missense) mutations found in humans (p.T373K) alters a TYR glycosylation site, resulting in ER degradation (Dolinski and Sergeev, 2017; Lasseaux et al., 2018), which is also found in the extreme dilution mottled mouse model (Lavado et al., 2005). Yet other (missense) mutations found in humans (p.T373K) alters a TYR glycosylation site, resulting in ER degradation.
4.2.2. OCA2: OCA2 (formerly: the P protein)

The prevalence of autosomal recessive OCA2 ranges from approximately 1:40,000 in Western countries (Kruijt et al., 2018) to 1:1450 in Africa (Rooryck et al., 2014). The OCA2 gene, cause Angelman syndrome or Prader-Willi syndrome (Sakazume et al., 2012; Spritz et al., 1997). A 2.7 kb deletion spanning exon 7 (Stevens et al., 1997). Interestingly, genetic defects, such as uniparental disomic inheritance, imprinting and de novo large deletions of the chromosome 15q11.2-q13 region, encompassing the OCA2 gene, cause Angelman syndrome or Prader-Willi syndrome (Sakazume et al., 2012; Spritz et al., 1997). A proportion of patients with the aforementioned syndromes have an albinism-like phenotype. Further sources of genetic variation influencing the OCA2 phenotype are copy number variations (Akahoshi et al., 2001) and variations outside the coding region (Donnelly et al., 2012; Han et al., 2008). A remarkable example is the single nucleotide polymorphism (SNP) (rs12913832) located 21.5 kb upstream of OCA2 in an intron of HERC2 (Eiberg et al., 2008). This SNP contributes to blue eye colour in the European population. However, HERC2 itself has no known role in pigmentation and the region around the SNP is hypothesised to be a distant regulatory region containing MITF motifs, which affects OCA2 expression (Eiberg et al., 2008; Levy et al., 2006; Sturm, 2009; Sturm et al., 2008; Visser et al., 2014).

4.2.2.1. Protein structure and function. OCA2 encodes a melanosome membrane protein of 838 amino acids with a mass of 110 kDa (Rosenblat et al., 1994). The OCA2 protein has 12 transmembrane domains (Fig. 5) (Rinchik et al., 1993) and has two dileucine motifs in its cytoplasmic tail. These dileucine motifs are essential for melanosomal localisation of the protein. Interestingly, the motifs interact with ubiquitous cytoplasmic adaptors AP-1 and AP-3 implicated in HPS (section 3.2.1) (Sitaram et al., 2009). Electrophysiological patch clamping of skin cells suggested that the OCA2 protein is a Cl− channel (Bellono et al., 2014). The channel is involved in regulation of the pH (Puri et al., 2000), which is essential for proper TYR function and melanin formation (Bin et al., 2015; Brilliant, 2001).

4.2.2.2. Genetic variation and mutations. Missense mutations in OCA2 are somewhat clustered in the central region of the second and fifth transmembrane domains, but are also present in the central region elsewhere (Fig. 5) (Oetting and King, 1999). There are also a few loss of function nonsense mutations in different locations of the coding region (Oetting and King, 1999). An estimated 77% of OCA2 patients in sub-Saharan Africa carry a 2.7 kb deletion spanning exon 7 (Stevens et al., 1997). Interestingly, genetic defects, such as uniparental disomic inheritance, imprinting and (de novo) large deletions of the chromosome 15q11.2-q13 region, encompassing the OCA2 gene, cause Angelman syndrome or Prader-Willi syndrome (Sakazume et al., 2012; Spritz et al., 1997). A proportion of patients with the aforementioned syndromes have an albinism-like phenotype. Further sources of genetic variation influencing the OCA2 phenotype are copy number variations (Akahoshi et al., 2001) and variations outside the coding region (Donnelly et al., 2012; Han et al., 2008). A remarkable example is the single nucleotide polymorphism (SNP) (rs12913832) located 21.5 kb upstream of OCA2 in an intron of HERC2 (Eiberg et al., 2008). This SNP contributes to blue eye colour in the European population. However, HERC2 itself has no known role in pigmentation and the region around the SNP is hypothesised to be a distant regulatory region containing MITF motifs, which affects OCA2 expression (Eiberg et al., 2008; Levy et al., 2006; Sturm, 2009; Sturm et al., 2008; Visser et al., 2014).

4.2.3. OCA3: TYRP1, tyrosinase related protein 1

OCA3 has a prevalence of approximately 1:8500 individuals in Southern Africa, but in Western countries it is virtually absent (Rooyrick et al., 2008). OCA3 was originally called ‘rufous’ or ‘red’ ocular albinism, since its phenotype is characterised by a copper-red skin and increased pheomelanin production (Boissy et al., 1996). Ocular defects are, in general, not as severe as in OCA1 or OCA2 (Kromberg et al., 1999). The OCA3 gene, TYRP1, located on the human chromosome 9p23 (Chintamaneni et al., 1991; Manga et al., 1997), consists of 8 exons, encoding 2896 bp of RNA (NM_000550.3). TYRP1 is the human homolog of the mouse Tyrp1 in the brown locus (Jackson, 1988).
4.2.3.1. **Protein structure and function.** **TYRP1** encodes a membrane-bound protein of 537 amino acids. The TYRP1 protein structure is presented in Fig. 5. The N-terminal end of TYRP1 consists of a highly conserved signalling domain. TYRP1, like TYR, contains two clusters of five cysteine residues, one of which, close to the C-terminus, is an EGF-like domain. TYRP1 also has a C-terminal end transmembrane domain and a tyrosine based dileucine motif for melanosomal targeting (García-Borrón and Solano, 2002; Vijayasankari et al., 1995). In contrast to the TYR Cu\(^{2+}\) binding sites, TYRP1 has Zn\(^{2+}\) binding sites that are presumed catalytically inactive in humans (Decker and Tuzcek, 2017; Lai et al., 2017). TYRP1 residues are frequently glycosylated, but potential functional consequences are yet understudied (Xu et al., 1997a).

TYRP1 and TYR are evolutionarily conserved homologues which interact and stabilise each other at the cell membrane of the melanosome (Kobayashi and Hearing, 2007; Kobayashi et al., 1998; Orlov et al., 1994). In murine melanocytes, Typr1 functions as a DHICA oxidase during the biosynthetic conversion of tyrosine to eumelanin (Boissy et al., 1998; Sarangarajan and Boissy, 2001). Apparently, it has a different, currently unknown function in human melanocytes (Sarangarajan and Boissy, 2001). In addition, in the mouse, Typr1 modulates Tyr activity (Sarangarajan and Boissy, 2001).

4.2.3.2. **Genetic variation and mutations.** There are only a few known missense mutations in **TYRP1**, which are present in the last two thirds of the coding region (Fig. 5). Most importantly, three missense mutations were identified in the first and second metal binding domains. These mutations possibly influence the ability of the protein to be properly loaded with zinc ions, rendering it inactive. Nonsense mutations in **TYRP1** leading to premature chain termination and loss of protein are relatively common. There are three hotspots of these in **TYRP1**. The first is around the first cysteine cluster, thought to be involved in protein-protein interaction. The second one is just after the first metal binding domain, and the third hotspot is located the second metal binding domain. These hotspots possibly locate to regions of the gene that are sensitive to replication errors.

4.2.4. **OCA4: SLC45A2, solute carrier family 45 member 2**

OCA4 has a prevalence of around 1:100,000 in Caucasian populations. However, OCA4 is very common in East Asia and Japan, where it accounts for up to 24% of all albinism cases (Inagaki et al., 2004; Suzuki and Tomita, 2008). Patients with OCA4 can be as severely hypopigmented as OCA1 patients, but in some patients pigmentation levels increase in the first decade (Inagaki et al., 2004; Rundshagen et al., 2004). Most patients present with foveal hypoplasia and chiasmal misrouting as well as nystagmus and poor VA, but some have only mild ocular features (Krujit et al., 2021). OCA4 is caused by mutations in the human **solute carrier family 45 member 2** (SLC45A2) gene (Newton et al., 2001). SLC45A2 is located at Giemsa band 5p13.3. The gene has seven exons, which produces three alternatively spliced transcripts: a long transcript and two shorter ones (NM_001012509.4), and two shorter ones (NM_016180.5, 1728 bp transcript; NM_001297417.4, 1122 bp transcript). SLC45A2 encodes an integral melanosomal membrane protein (Bin et al., 2015). The human gene is the orthologue of the mouse **underwhite (uw)** gene (Newton et al., 2001).

Indeed, melanocytes of the mutant mouse orthologue for **SLC45A2, uw**, show defective TYR trafficking and a lower melanosomal pH (Bin et al., 2015; Le et al., 2020; Puri et al., 2000). A low melanosomal pH is detrimental for TYR activity.

4.2.4.2. **Genetic variation and mutations.** Missense and nonsense mutations in **SLC45A2** have been identified but do not appear to be clustered to particular domains of the protein (Fig. 5). Mutations affect protein stability and transport activity that result in complete loss of melanin production in vitro (Cook et al., 2009). Cultured human melanocytes with common African or Caucasian SLC45A2 SNPs express different levels of SLC45A2 transcript and show differential TYR activity (Cook et al., 2009). A number of OCA4 mutations were reproduced in the evolutionarily conserved homologous regions of a rice sucrose transporter, and confirmed a corresponding severe decrease in transport activity (Reinders and Ward, 2015). Finally, the SLC45A2 gene and its promoter region have also been implicated in natural skin and eye pigmentation variation (Graf et al., 2007; Mengel-From et al., 2010; Sturm and Larsson, 2009).

4.2.5. **OCA5: chromosomal locus 4q24**

The OCA5 locus (4q24) was found in a family presenting white skin, golden yellow hair, low VA, nystagmus and foveal hypoplasia (Kausar et al., 2013). So far, the causative gene has not been identified. The relevant genomic bin includes at least 14 candidate OCA5 genes. However, none of these genes has, apparently, a direct functional relationship with the melanogenesis pathway. One candidate gene, MANBA, has been implicated in lysosomal function, while other genes, SLC9B1, SLC9B2, and SLC9A8 are of the solute carrier family. Screening of candidate genes (SLC9B1 and SLC9B2) did not yield any pathogenic variants (Kausar et al., 2013), so further research is warranted to identify the OCA5 disease gene.

4.2.6. **OCA6: SLC24A5, NCCX5, Na\(^{+}/\)Ca\(^{2+}\)-K\(^{+}\) exchanger 5**

OCA6 patients have light hair that darkens over time. They also present with foveal hypoplasia, nystagmus and limited VA (Morice-Picard et al., 2014; Wei et al., 2013). The OCA6 phenotype is caused by mutations in **solute carrier family 24 member 5** (SLC24A5), located on human chromosome 15q21.1. The gene contains nine exons encoding a 1879 bp transcript (NM_205850.3).

4.2.6.1. **Protein structure and function.** **SLC24A5** encodes a 500 amino acid solute carrier protein that functions as a K\(^{+}\) dependent ion exchange transporter for Na\(^{+}/\)Ca\(^{2+}\) (NCKX5). SLC24A5 contains two clusters of five transmembrane domains and a large cytosolic loop (Fig. 5). Its proper localisation is dependent on the cytosolic loop (Schnetkamp, 2004, 2013). The SLC24A5 protein is located in the trans-Golgi network in cultured melanocytes, HEK293 cells, and the melanoma cell line MNT1, but its location in the RPE has not been studied (Ginger et al., 2008; Lamason et al., 2005; Rogasevskaja et al., 2019).

While there is a pigmentation phenotype in OCA6 patients, it is remarkable that there is no clear evidence for presence of SLC24A5 in wild-type melanosomes. Possibly it has an early vesicle function in the development of melanosomes. Alternatively, SLC24A5 may regulate transcription of MC1R or affect cholesterol homeostasis, both affecting melanogenesis with an apparently severe effect on melanin (Wilson et al., 2013). Epidermal melanocytes from OCA6 patients have a relatively high number of immature melanosomes (Wei et al., 2013), suggesting melanosomal maturation is disturbed. Of note, zebrafish OCA6 models with mutations in the slc24a5 orthologue are called the golden mutants, exhibiting fewer and smaller melanosomes in the eye and skin (Lamason et al., 2005). A similar phenotype was observed in Slc24a5\(^{-/-}\) mice (Vogel et al., 2008). Thus, SLC24A5 may have an opposite regulatory role compared to GPR143, which is involved in macro-melanosome formation.
4.2.6.2. Genetic variation and mutations. Only three SLC24A5 missense mutations were studied in the closely related protein family member NCKX4, using a Ca\textsuperscript{2+} transport assay (Jalloul et al., 2016). While protein expression levels were not affected, the p.R174K mutation resulted in a more than 70% decrease in Ca\textsuperscript{2+} transport, while the other two variants showed no exchanger activity at all. Defective SLC24A5 leads to reduced pigment biosynthesis (Lamason et al., 2005). Ginger et al. (2008) overexpressed SLC24A5 carrying a p. A111T missense variant in “High Five” insect cells, resulting in a reduced Ca\textsuperscript{2+} exchange over the plasma membrane. SLC24A5 maintains the proper melanosomal ion concentrations which possibly, like OCA2 and SLC45A2, contributes to melanosomal pH. Chain-terminating nonsense mutations, resulting in loss of protein function, have been identified across the entire SLC24A5 coding region. Like other albinism genes, genetic variation in SLC24A5 contributes also to variation in natural human pigmentation. For example, the SLC24A5 missense variant, p.A111T in Caucasians has been implicated in light skin (Ginger et al., 2008; Stokowski et al., 2007).

4.2.7. OCA7: LRMDA, leucine-rich melanocyte differentiation related

Mutations in the OCA7 gene (LRMDA) were found in a Faroese albinoism family and confirmed in a Lithuanian patient (Grønskov et al., 2013). OCA7 patients are not completely devoid of pigmentation but usually have lighter skin than healthy relatives. In addition, typical ocular albinoism features have been reported, including nystagmus, reduced VA, and misrouting of the optic nerves (Grønskov et al., 2013; Kessel et al., 2021; Krujit et al., 2018). OCA7 is associated with mutations in the leucine-rich melanocyte differentiation related (LRMDA); also known as C10orf11 gene located to human chromosomal region 10q22.2-q22.3. Q. The gene has seven exons coding for two transcripts, one of 3662 bp (NM_001305581.2) and one of 3692 bp (NM_0032045.5).

4.2.7.1. Protein structure and function. The LRMDA gene encodes a protein of 198 amino acids. It is a member of the leucine-rich repeat (LRR)-containing protein family (CT-LRR (Fig. 5) (Bella et al., 2008; Grønskov et al., 2013). LRMDA localises to melanoblasts and melanocytes of foetal skin epidermis (Grønskov et al., 2013). CT-LRR proteins have multiple functions, including RNA processing, extracellular matrix assembly, cell adhesion or signalling and neuronal development (Bella et al., 2008). Knockdown of the LRMDA zebrafish homolog c10orf11 (lrmda) resulted in reduced pigmentation and fewer pigmented melanocytes (Grønskov et al., 2013). The phenotype in the fish was rescued by wild-type, but not mutant c10orf11 mRNA. Intriguingly, LRMDA expression was neither found in embryonic RPE of seven weeks, nor in our own retinal organoid dataset (section 7.2; Supplementary Fig. 2) (Wagstaff et al., 2021b). This is remarkable because a neural retinal albinism phenotype is present in these patients, and warrants further investigation.

4.2.7.2. Genetic variation and mutations. Only a few mutations in LRMDA causing albinoism have been found. A study in 23 unrelated Iranian albinism patients reported a p.N89K missense mutation (Fig. 5) (Khordadpoor-Deilamani et al., 2016). The functional consequence of these mutations remains to be elucidated.

Next to the aforementioned missense mutation, both LRMDA stop and frameshift mutations have also been implicated in OCA7. The stop mutation is located very close to the C-terminus at residue 194 of 198 (p. R194*) (Grønskov et al., 2013). The frameshift mutation is located at residue 23 of the protein (p.A23Rfs*39) likely resulting in loss of functional protein (Grønskov et al., 2013).

4.2.8. OCA8: DCT, dopachrome tautomerase

Recently, Pennamen et al. (2021) identified a new OCA disease gene on human chromosome 13q32.1, DCT (OCA8) in previously genetically unresolved patients (Pennamen et al., 2021). OCA8 patients show typical ocular features of albinism and relatively mild skin and hair pigmentation abnormalities. DCT contains 13 exons encoding seven transcripts (NM_001129889.3, 5173 bp; NM_001322182.2, 4289 bp; NM_001322183.2, 4343 bp; NM_001322184.2, 4643 bp; NM_001322185.2, 4589 bp; NM_001322186.2, 4858 bp; NM_001922.5, 5074 bp) through alternative splicing.

4.2.8.1. Protein structure and function. DCT (also known as tyrosinase related protein 2, TYRP2) encodes four protein isoforms of 552, 519, 456 and 208 amino acids. In Fig. 5, the DCT structure is given for isoform 1 (519 aa). DCT is highly similar to TYR and TYRPI. The three proteins contain an evolutionarily conserved EGF-like domain (Fig. 5). DCT, like TYRPI, also has two Zn\textsuperscript{2+} -binding sites essential for catalytic activity (Olivares and Solano, 2009). A transmembrane domain is located at the C-terminal end of the protein followed by two tyrosine-based motifs for melanosomal targeting (Garcia-Borron and Solano, 2002). Finally, there are several sites for possible glycosylation. Knockout of Dct in mice results in altered pigmentation, which is called the slaty phenotype (Budd and Jackson, 1995; Jackson et al., 1992). Just as in OCA8 patients, pigmentation of the RPE is decreased in Dct\textsuperscript{-/-} mice (Fig. 2). DCT functions as a biochemical intermediate in the eumelanin biosynthetic pathway (Tsukamoto et al., 1992) (Fig. 2; section 1.3).

4.2.8.2. Genetic variation and mutations. So far, five mutations in DCT, associated with albinism, have been published. Pennamen et al. (2021) described two missense mutations affecting conserved cysteine residues (p.C40S and p.C61W) in the first EGF-like domain. One additional missense mutation was recently identified: p.G59V, that most likely affects TYRP2 glycosylation and results in ER retention of the protein. So far, two DCT nonsense mutations have been found: a 14 bp frameshift deletion in exon 9, p.F469* (Pennamen et al., 2021), and the nonsense p.Y292* mutation (Volk et al., 2021).

5. Genetic defects in GPR143 lead to ocular albinism (OA1)

5.1. OA1: the specific phenotype

OA1 is the only proven form of albinism inherited in an X-linked recessive fashion. This disease subtype has a prevalence of at least 1:60,000 (Rosenberg and Schwartz, 1998). The phenotype of OA1 is mainly restricted to the eye, while pigmentation of skin and hair are normal. Nonetheless, anecdotal evidence suggests that male OA1 patients might have somewhat pale skin as well (Lewis, 1993a). Typically, ocular pathology in affected males is on average more severe than in OCA1 or OCA2, and characterised by iridal translucency, foveal hypoplasia and chiasmal misrouting (Schiavino, 2010). On a cellular level, fewer and abnormally enlarged melanosomes are present in the RPE as well as in skin, iris and choroidal melanocytes (Garner and Jay, 1980; O’Donnell et al., 1976; Wong et al., 1983). Intriguingly, while pathology is limited to the eye, macro-melanosomes are present in both eye and skin (Schiavino, 2016; Schiavino and Tacchetti, 2005). In female carriers, punctate iris translucency and a typical pattern of peripheral mottled retinal pigment changes probably due to random X chromosome inactivation can be seen in the fundus (Lang et al., 1990; Oetting, 2002).

In Gpr143\textsuperscript{-/-} mice, the hair colour pigmentation is indistinguishable from that of wild-type pigmented mice. However, in mice, coat colour is governed by hair pigmentation, unlike humans, where the skin pigmentation is primarily determined by melanocyte-keratinocyte interactions outside the hair bulb. This suggests that also hair bulb melanosomes are not affected in OA1 subjects (Incerti et al., 2000). Below we discuss the OA1 gene, its protein function, and the relevant effects of
mutations in this gene.

5.2. OA1: GPR143, G protein-coupled receptor 143

The OA1 gene (GPR143) in band Xp22.3 was identified more than two decades ago (Bassi et al., 1995). GPR143 contains nine exons resulting in a 1645 bp RNA product (NM_000273.3). There are no known splice variants.

5.2.1. Protein structure and function

GPR143 consists of 404 amino acids and is a G protein-coupled receptor (GPCR) (Innamorati et al., 2006) with seven membrane-spanning domains (Sone and Orlow, 2007). Similar to other GPCR family members, GPR143 has two extracellular loops, two evolutionarily conserved cysteine residues (Schiapfino et al., 1999), and a DRY-like motif in both the third cytosolic loop and sixth luminal/extracellular loop (Sone and Orlow, 2007), and a tryptophan-glutamic acid doublet in the C-terminal tail (Piccirillo et al., 2006). Relevant for downstream GPCR signalling, both the second and third cytosolic loops as well as the C-terminal tail of the G protein-coupling domains (Innamorati et al., 2006; Schiaffino et al., 1999).

Localisation of GPR143 was studied in both pigmented and non-pigmented cells. In general, GPCRs are localised to the plasma membrane (classical signalling), or the endosomal compartment (endosomal signalling) (Liccardo et al., 2022; Pavlos and Friedman, 2017). The localisation(s) of GPR143 in the human RPE cell in vivo remains to be elucidated. In non-pigmented cultured cells (COS-7; HeLa; COS-1; CHO) transfection of recombinant GPR143 showed various possible subcellular localisations of the gene product, probably depending on culture conditions and difference in study design. GPR143 was not only localised to intracellular vesicles such as late endosomes/lysosomes (Burgoine et al., 2013; Piccirillo et al., 2006; Schiaffino et al., 1999; Sone and Orlow, 2007), but also to the plasma membrane (De Filippo et al., 2017a, 2017b; Innamorati et al., 2006; Khristov et al., 2018; Lopez et al., 2008). Supporting these data, recombinant GPR143 localisation in yeast Saccharomyces cerevisiae was found in both late endosomes and the plasma membrane (Staleva and Orlow, 2006). Others however showed only intracellular localisation of GPR143, regardless of expression level and culturing in the absence or presence of tyrosine (De Filippo et al., 2017a).

Multiple GPR143 localisation studies were also performed in cultured pigmented cells, including human pigmented melanoma cells, healthy human and murine melanocytes. In these cells, GPR143 showed a granular cytoplasmatic appearance, more concentrated in the perinuclear area and spread toward the periphery (Palmasano et al., 2008; Piccirillo et al., 2006; Samaraweera et al., 2001; Schiaffino et al., 1996, 1999; Shen et al., 2001). It localises to the membrane and the interior of melanosomes, organelles which originate from endosomal precursors (Schiaffino et al., 1996). Histological and proteomics studies suggested that GPR143 localises mainly to late endosomes and/or melanosomes (Basarut et al., 2003; Piccirillo et al., 2006; Samaraweera et al., 2001). Cell sorting studies suggested plasma membrane localisation in murine melanocytes (De Filippo et al., 2017b).

In human RPE, in situ labelling of GPR143 showed positive signals both intracellularly and on the plasma membrane, a typical result for GPCR localisation studies to date (Lopez et al., 2008; McKay, 2019; Pavlos and Friedman, 2017). In human donor RPE, 3.5 ± 0.7% of the total GPR143 was present on the apical plasma membrane surface of RPE (Lopez et al., 2008). In cell cultures, intracellular localisation apparently depends on the level of tyrosine in the medium (Lopez et al., 2008; Schiaffino et al., 1996). GPR143 localisation studies in GPR143 KO mice were not conclusive (Bassi et al., 1996; Cortese et al., 2005; Incerti et al., 2000).

Recently, L-DOPA was identified as one of the ligands and dopamine as a competitive inhibitor of GPR143 (Goshima et al., 2014; Lopez et al., 2008; McKay, 2019). The N-terminal ligand binding part of GPR143 extends into the extracellular space or melanosomal lumen, while its C-terminal is in the cytoplasm. (Extracellular) activation of the GPR143 RPE receptor results in downstream intracellular GPR143 signalling. The central role of L-DOPA and GPR143 signalling in albinism pathology and regulation of the retinal pigmentation pathway is further explored in sections 5.3 and 8-11 below.

Possibly, the G protein subunit Goi3 (GNAI3) may be essential for GPR143 downstream signalling through a second messenger cascade. Indeed, in mice, Goi3 interacts with GPR143, and the independent knockout of Goi3 and GPR143 results in a similar phenotype (Young et al., 2008, 2011). Constitutively active Goi3 can rescue the GPR143 knockout phenotype. While mouse Gpr143 interacts with Goi3 (Young et al., 2008, 2011, 2013), the G protein with which human GPR143 interacts remains unclear (Innamorati et al., 2006; Schiaffino et al., 1999). A potential direct physical interaction between GPR143 and TYR has also been proposed, since these proteins co-immuno-precipitate in a biochemical assay (Cortese et al., 2005). Whether this is the case in physiological conditions remains to be elucidated. In any case, GPR143 probably does not regulate TYR activity directly as in Gpr143 mutant mice Tyr activity is not impaired (Cortese et al., 2005).

5.2.2. Mutations

A variety of mutation types has been identified along the whole sequence of GPR143. GPR143 missense mutations can functionally be divided in two types: the first alters cellular distribution, the second type affects receptor signalling. Examples of the first type are: p.Q124R, p.A138V, p.S152N, p.G229V, p.T232K, p.E235K, p.E271G (Fig. 5) (d’Addio et al., 2000). The second type of mutations (p.R5C, p.G35D, p.D78N, p.G84D, p.C116S, p.G118E, p.W133R, p.A173D, p.I261N, p.W292G and p.T290del) are mainly found in the second and third cytosolic loop (Fig. 5) (d’Addio et al., 2000; Innamorati et al., 2006; Schiaffino et al., 1999). Functional in vitro studies using COS-7 cells showed that GPR143 ligand subunits Gp and Goi did not bind at all (p.C116S), or hardly (p.D78N) to mutant proteins (Schiaffino et al., 1999). 23 nonsense and frameshift mutations are archived in the Leiden Open Variome Database (LOVD). They are found across the gene and most likely result in premature chain termination and loss of protein function.

5.3. GPR143: the molecular mechanism of ocular albinism

As described above, the OA1 phenotype, caused by mutations in GPR143, is limited to the eye. Both OA1 RPE and skin have relatively few enlarged macro-melanosomes (Lewis, 1993a; Schiaffino and Tacchetti, 2005), but only the RPE has decreased melanin content (Cortese et al., 2005; Schiaffino, 2010). The (defective) GPR143 receptor plays a key role in ocular albinism (McKay, 2019). In this section, we first discuss the ligands activating or inhibiting this receptor and next potential downstream events, such as secretion of growth factors and exosomes as well as the formation of macro-melanosomes.

One important ligand of GPR143 is L-DOPA (Lopez et al., 2008; McKay, 2019) (section 1.4, Fig. 2). We are aware of the multiple potential mechanistic actions of L-DOPA, which are reviewed elsewhere (De Deurwaerdere et al., 2017). However, in the context of this report, we only consider the role of L-DOPA in the proposed retinal pigmentation pathway. L-DOPA is produced when tyrosine is converted to melanin in the RPE melanosome (Hearing and Tsukamoto, 1991). L-DOPA interacts with GPR143, most likely at the apical surface of the RPE or at the luminal side of the melanosomal membrane (Basrur et al., 2003; Lopez et al., 2008; Piccirillo et al., 2006). The fundamental role of L-DOPA in albinism had been anticipated by several studies from Glen Jeffery’s laboratory since 1998, stating its deficit in the eyes of persons with albinism and its role as a cell-cycle regulator (Jeffery, 1998). Thereafter, using rats as experimental model, they proposed that
L-DOPA might regulate the time at which retinal cells exit mitosis (Ilia and Jeffery, 1999), rod photoreceptor production (Ilia and Jeffery, 2000), and cell cleavage orientation in the developing retina (Tibber et al., 2006). They also confirmed similar properties associated with dopamine (Kralj-Hans et al., 2006). Experiments by Lavado et al. (2006) elegantly illustrated the role of L-DOPA in albinism associated retinal pathology. They constructed transgenic mice overexpressing TH under control of the Ty promoter, in a spontaneously mutated Tyr defective strain (NMRI) (Lavado et al., 2006). These mice are not able to convert tyrosine into melanin but are able to produce L-DOPA in the RPE (Fig. 2). Indeed, introduction of TH in the aforementioned mice rescued the neural retinal albinism pathology (Lavado et al., 2006). Thus, the lack of L-DOPA production in the RPE appears to be crucial in the pathology. They constructed transgenic mice overexpressing dopamine (Kralj-Hans et al., 2006). Experiments by Lavado et al. (2006) elegantly illustrated the role of L-DOPA in albinism associated retinal pathology. They constructed transgenic mice overexpressing TH under control of the Ty promoter, in a spontaneously mutated Tyr defective strain (NMRI) (Lavado et al., 2006). These mice are not able to convert tyrosine into melanin but are able to produce L-DOPA in the RPE (Fig. 2). Indeed, introduction of TH in the aforementioned mice rescued the neural retinal albinism pathology (Lavado et al., 2006). Thus, the lack of L-DOPA production in the RPE appears to be crucial in the albinism retinal phenotype, while the lack of melanin is not. This hypothesis was corroborated by Marullo-Cuesta et al. (2010), who found, in the same albinism mouse strain that L-DOPA, produced through TH in the cochlea, rescued age-related and noise-induced hearing loss associated with albinism (not systematically described in albinism patients).

L-DOPA is a ligand of GPR143 (Lopez et al., 2008). Genetic defects in GPR143 cause OA1, while retinal melanin is present (Cortese et al., 2005; O’Donnell et al., 1976; Wong et al., 1983). Given the aforementioned phenomena, we hypothesise that defects in GPR143 or its activation by L-DOPA is central in the retinal pathology of albinism.

The regulation of GPR143 activity is complex: L-DOPA (from RPE and neural retina) and dopamine (from neural retina) can modulate GPR143 activity in an excitatory or inhibitory way, respectively (Lopez et al., 2008). L-DOPA is present in embryonic stages of healthy retinal development in WT mice but not in albino animals (Roffler-Tarlov et al., 2013). Dopamine production starts postnatally in the neural retina (Roffler-Tarlov et al., 2013), when, at the same time, retinal development and maturation still continues (Booth et al., 1985; Hendrickson et al., 2012; Tian, 2004). In addition, L-DOPA and dopamine production in the retina appears to be under circadian control (Baba et al., 2017). These regulatory aspects of the retinal pigmentation pathway are discussed in detail in section 9. We reviewed a number of clinical trials testing L-DOPA treatments in section 12.

6. The FHONDA phenotype explained: “albinism” without pigmentation abnormalities

6.1. Phenotypic characteristics of FHONDA

The FHONDA albinism-related ocular phenotype is generally more severe and more consistent between patients than any of the albinism subtypes. VA is worse than in OCA: median VA is 0.7 logMAR (IQR 0.6–0.8) for FHONDA while median VA for OCA1 is 0.5 logMAR (IQR 0.3–0.7) and for OCA2 0.5 logMAR (IQR 0.2–0.7). While foveal hypoplasia grades in OCA range from 0 to 4, foveal hypoplasia in FHONDA is limited to grade 3 and 4 (Kruit et al., 2022; Thomas et al., 2011). Nystagmus and chiasmal misrouting have been uniformly present in reported FHONDA cases, compared to 93% and 84% detected in albinism respectively (Forsius et al., 1964). Anterior segment dysgenesis is only present in 19% of FHONDA patients, while general prevalence in ophthalmology clinic patients is between 7 and 32% (Forsius et al., 1964; Ozeki et al., 1997; Rennie et al., 2005). Thus, the association of this sub-phenotype is most likely due to chance. No pigment abnormalities are present in FHONDA patients. Our hypothesis is that FHONDA is at the end the proposed retinal pigmentation pathway (Fig. 3), suggesting that retinal development may be more severe and consistently affected, lacking potential upstream compensatory mechanisms present in (other types of) albinism. This is also in line with the clinical observations in FHONDA. Below we discuss what is known about the gene implicated in FHONDA, the protein and known pathogenic variants.

6.2. FHONDA: SLC38A8, solute carrier family 38 member 8

6.2.1. Protein structure and function

Autosomal recessive FHONDA is caused by mutations in SLC38A8 (Poulter et al., 2013). The gene has nine exons resulting in a 1672 bp transcript (NM_001080442.3). It encodes a 435 aa protein with 11 transmembrane domains (Fig. 5). SLC38A8 belongs to the SLC38 family of sodium-coupled neutral amino acid transporters (Mackenzie and Erickson, 2004). Indeed, SLC38A8 has a preference for sodium dependent transport of glutamine, alanine, arginine and histidine in the central nervous system (CNS) (Hågglund et al., 2015). In mice, RNA expression of SLC38A8 was found in the eye but not in the skin (in contrast to many albinism disease gene products) (Perez et al., 2014). In human donor eyes, immunohistochemical staining showed protein expression of SLC38A8 in the neural retina and possibly the RPE. In addition, protein expression was found in human foetal and adult brains, in neuronal cell bodies and axons as well as in the spinal cord (Poulter et al., 2013). Expression in the adult retina, and other parts of the CNS, suggests that SLC38A8 function is not limited to neural development alone. In genetically modified mice, Slc38a8 mutations recapitulate the phenotype observed in humans, with distorted optical tract but without pigmentary alterations (Lluis Montoliu, unpublished data).

6.2.2. Genetic variation, mutations and mechanism

So far, 37 mutations in SLC38A8 have been implicated in FHONDA (Fig. 5). 21 of them are missense mutations. 16 out of 21 missense mutations possibly affect transport function, as they are found in or close to transmembrane domains. Three missense mutations, p.T87I, p.E233K and p.D283A, affect hydrophobicity near the channel pore, potentially disrupting sodium dependent transport. Yet another mutation, p.E233K, affects hydrogen bonding capabilities and possibly protein folding (Poulter et al., 2013; Toral et al., 2017). Two mutations, p.D283A and p.A282del, are located in the fourth extracellular loop and may affect subcellular localisation of the protein (Poulter et al., 2013; Toral et al., 2017). In total, 14 loss of function mutations have been identified, leading to premature chain termination (Kruit et al., 2022).

7. Expression of genes implicated in albinism in human RPE, neural retina and retinal organoids

In the previous sections we discussed all currently known disease genes implicated in (ocular) albinism and FHONDA, and their respective phenotypes. In order to have an impression when function or molecular pathology could set in, we summarised and compared five RNA expression datasets of (disease) genes involved in the different types of albinism. Three out of five sets contain data from all major human retinal cell types together, including RPE, during development (Fig. 6 and Fig. 7). Two out of these three are derived from wild-type human retinal organoid in vitro models (GSE119274) (Kim et al., 2019; Wagstaff et al., 2021b), and one out of three is a healthy human foetal retina developmental control data set (GSE104827) (Hoshino et al., 2017). Note that in these samples the relative size of the whole retina increases over time compared to the RPE and thus in these datasets the relative expression of RPE genes will also generally decrease. It is difficult to directly compare the expression data sets, since the two human retinal organoids sets were generated with different protocols and the human foetal expression data suffers from limitations intrinsic to using human donor material. Also, in all three datasets, not all relevant genes were present, expression was calculated in different ways, and time points studied varied. Next to the aforementioned organoid and foetal datasets, we also include embryonic stem cell (ESC) derived RPE-only expression data sets to which the same study limitations apply (Supplementary Fig. 1 and 2) (Bennis et al., 2017; Smith et al., 2019). Obviously, the expression data presented are important for comparison of human organoid models with the physiological situation, and are relevant for the construction of new models. Adding more -omics datasets like this in
the future will give a more complete picture of the aetiology of human albinism and related disorders.

7.1. RNA expression of genes implicated in syndromic albinism

As proposed in section 3, syndromic forms of albinism involve a disruption of general cellular processes related to LROs generation or function. LROs are present in many different cell types and tissues resulting in a wide range of symptoms affecting patients. From the earliest stages of (retinal) development onward, all disease genes implicated in syndromic forms of albinism seem to be consistently expressed, albeit at different levels, in all datasets (Fig. 6; Supplementary Fig. 1). Mostly, this expression does not change dramatically over time. In the full retina entities, relatively high expression is found for AP3D1 and increasingly for LYST, and perhaps BLOC1S6. Tentative lower expression is found for HPS1, HPS6, DTNBPI and BLOC1S3 (Fig. 6). Data from the RPE-only expression datasets suggest that AP3D1 and BLOC1S6 are relatively high expressed in RPE (Supplementary Fig. 1). These data confirm the very early onset of expression of disease genes involved in syndromic albinism in human (retinal) development. Indeed, as these genes have a role in multiple LROs in multiple cell types, it is expected that they are ubiquitously present.

7.2. RNA expression of genes implicated in oculocutaneous albinism

The genes implicated in OCA, described in section 4, are most likely involved in the function and pathology of pigment cells. Using the same aforementioned gene expression datasets, we analysed the timing and level of OCA disease gene expression in the developing retina (Fig. 7) and RPE (Supplementary Fig. 2). In contrast with the disease genes involved in syndromic forms of albinism, expression of OCA genes appears to be relatively low and remains so during early stages of retinal
development (Fig. 7). For example, the expression of TYR, SLC45A2 and SLC24A5 in all datasets is somewhat variable but generally low during all developmental stages studied. This might confirm that, in OCA, the pathology of pigment cells uniquely is implicated, which develop at later stages. Apparent exceptions are: DCT, which is relatively highly expressed at all time points in all data sets; and OCA2, which is relatively highly expressed in two out of three datasets. The high expression of DCT in comparison to, for example, TYR that functions in the same melanin production pathway, might reflect the hypothesis that DCT is involved in additional functionalities. These possibly include the clearing of toxic quinone metabolites in the retina (Michard et al., 2008), or neural progenitor proliferation (Jiao et al., 2006). Interestingly, although OCA2 levels are different in the models, the OCA2 expression pattern seems to rise, peak, fall, and then perhaps stabilise during development. It is tempting to speculate that this is correlated with the transient role OCA2 plays in melanosome development (Bellono et al., 2014). While most OCA genes are expressed in the RPE-only data sets (Supplementary Fig. 2) and, most recently, in preliminary expression data provided by George et al. (2022) and Bakker et al. (data not shown) in iPSC derived RPE cultures, no further firm conclusions or hypotheses can be drawn on the basis of currently available RNA data.

7.3. RNA expression of GPR143

The GPR143 protein is localised to the RPE apical membrane or to the RPE melanosomal compartment, or both (for a full description see section 5.2). GPR143 controls melanosome formation in pigment cells and is implicated in the OA1 phenotype that is largely restricted to the eye. As we discussed elsewhere in this report (sections 2, 5.3, 8-10) the protein plays a central role in the retinal pigmentation pathway.

We observed a temporal decrease of GPR143 gene expression in all aforementioned datasets during full retinal development (Fig. 8). However, in the RPE-only datasets, GPR143 expression seems to be stable during retinal development. Possibly these expression patterns...
reflect the expanding cellular contribution of the neural retina (where GPR143 is not present) compared to the RPE monolayer (and thus GPR143 gene expression) to the developing retina. Obviously, further studies are needed to shed light on this issue.

7.4. RNA expression of SLC38A8

SLC38A8 localisation and expression has been found in the neural retina (Poulter et al., 2013). When mutated in FHONDA patients, it causes a severe albinism-like phenotype restricted to the neural retina. Pigmentation is not affected in FHONDA. Therefore, we hypothesised it...
to function downstream from GPR143 in the retinal pigmentation pathway (section 2). Again, we reviewed and compared the temporal retinal RNA-seq expression data of SLC38A8 during development in two organoid and one foetal datasets (Fig. 8) (Hoshino et al., 2017; Kim et al., 2019; Wagstaff et al., 2021b). Remarkably, no SLC38A8 expression was detected in our own retinal organoid differentiation data (Wagstaff et al., 2021b) while it is temporally variable in the second organoid expression data set (Fig. 8B1) (GSE119274) (Kim et al., 2019), as well as in foetal retina (Fig. 8B2) (GSE104827) (Hoshino et al., 2017). Given the potential role of SLC38A8 cone specification in patients (Ruht et al., 2020), it is of interest to speculate that the expression of SLC38A8 is a bit elevated in the cone-rich organoid data (Kim et al., 2019), compared to the Wagstaff retinal organoid data, where it is virtually absent (not shown); an issue that needs further investigation. Perhaps even more surprisingly, in the RPE-only dataset of Smith et al. (2019) RNA expression of SLC38A8 was detected. This raises the intriguing possibility that SLC38A8 may be transcribed in the RPE, and that transcripts or protein is transferred to the neural retina. Alternatively, SLC38A8 may be transcribed in more than one retinal cell type. Future detailed studies need to be performed to shed light on this issue. Of note, SLC38A8 expression is below detection level at some time points in foetal retina, so it may be that expression in the other datasets is temporarily present between the actual time-points measured.

8. GPR143 controls RPE signalling in albinism

8.1. GPR143-activated paracrine RPE growth factor signalling

In the retina, expression of GPR143 is limited to the RPE (Bharti et al., 2006; Surace et al., 2000). However, foveal hypoplasia and chiasmal misrouting are deficiencies of the neural retina. In this section, we describe the potential (indirect) regulatory role of GPR143 in paracrine signalling from the RPE to the neural retina. Paracrine signalling in the proposed retinal pigmentation pathway may consist of PEDF, VEGF secretion and exosome release from the RPE, which are discussed one by one below.

In the healthy RPE, both GPR143 and PEDF RNA expression are present during retinal development in retinal organoids (Wagstaff and Bergen, unpublished). In the healthy (foetal) situation, PEDF is secreted mainly from the apical RPE toward the neural retina (Fig. 9) (Fields et al., 2017; Maminishkis et al., 2006). We hypothesise that this is (in part) stimulated by GPR143 activation (Falk et al., 2012; Lopez et al., 2008). In syndromic or oclocutaneous albinism, the conversion of tyrosine to L-DOPA (Fig. 2) is decreased and local L-DOPA levels are depleted. We hypothesise that this leads to reduced activation of the GPR143 receptor and less apical PEDF secretion from the RPE. Indeed, in human primary RPE cultures, reduction in PEDF secretion caused by pharmacological blocking of TYR can be rescued by L-DOPA supplementation (Falk et al., 2012; Lopez et al., 2008).

PEDF is a neurotrophic and antiangiogenic factor primarily secreted apically by foetal RPE (Fields et al., 2017; Maminishkis et al., 2006). Its antiangiogenic role may be important for the development of the foveal avascular zone and the subsequent formation of the fovea (Provis et al., 2013). Consequently, the potential lack of PEDF secretion in albinism may contribute to an underdeveloped macular area and foveal hypoplasia. Furthermore, PEDF has also been implicated in long range axon formation (Barnstable and Tombran-Tink, 2004). In addition, PEDF promotes cell survival in immature cerebellar granule cell cultures from chick spinal cord but not in mature granule cells (Araki et al., 1996). This suggests a potential neurotrophic role for PEDF in neuronal and possibly retinal development.

In contrast to increased apical PEDF secretion, activation of GPR143 may lead to decreased VEGF secretion from human primary RPE cultures (Blauwgeers et al., 1999; Falk et al., 2012). VEGF is secreted mainly from the basolateral side of the foetal RPE (Fields et al., 2017; Maminishkis et al., 2006). In the healthy developmental situation, VEGF secretion has been implicated in the development of the choroid, and, in low levels, in development and maintenance of the foveal avascular zone (Provis, 2001). In the adult situation, basolaterally secreted VEGF acts on the choroid and maintains retinal differentiation. In the elderly, basolateral RPE secreted VEGF plays a key role in chorioidal neo-vascularization (in age-related macular degeneration; AMD) (Adams et al., 1993; Blauwgeers et al., 1999; Witmer et al., 2003). Whether VEGF is also secreted in significant levels from the apical RPE, and what the implications would be for neural retinal development and disease, is not clear. In albinism, we hypothesise that low L-DOPA levels could lead to less activation of the GPR143 receptor, and therefore locally increased VEGF secretion. In albinism, excess VEGF could possibly promote vascularisation, impede formation of the avascular zone, and contribute to hypoplasia of the fovea. Of note, GPR143 signalling also regulates MITF expression in the RPE. MITF is a transcription factor not only controlling melanogenesis but also PEDF expression (Zhu et al., 2011). Thus, the potentially increased release of PEDF seen after GPR143 activation is possibly also modulated by MITF.

8.2. Apical RPE exosome release and GPM6A

Following the retinal pigment pathway hypothesis, GPR143 activation possibly leads to a decrease of secreted exosomes from primary RPE cultures (Locke et al., 2014; Lopez et al., 2008). Exosomes develop through fusion of intracellular multivesicular endosomes with the plasma membrane that result in release of vesicles from the cell (Lo Cicero et al., 2015; Raposo and Stoorvogel, 2013). PEDF exosomes carry a diverse range of biomolecules (lipids, proteins, RNAs) that are key to
intercellular signalling and processing of cellular waste (Colombo et al., 2014; van der Pol et al., 2012). In the healthy condition, the diameter of exosomes ranges between 30 and 100 nm (Lo Cicero et al., 2015).

RPE exosomes are released both apically, toward the neural retina, and basolaterally toward the Bruch’s membrane and choroid (Klingeborn et al., 2018). The release of exosomes from (adult primary porcine) RPE is polarised as many proteins found in apical exosomes are different from basolateral ones (Klingeborn et al., 2017). Whether RPE exosomes are released at similar rates and with similar contents during development remains to be elucidated. For our present analyses, we used the best data sets available on RPE exosome contents. Possibly, apical RPE exosome release and signalling are involved in proper retinal development and function and they are, at least in part, likely to be relevant to the ocular albinism phenotype. Given the abnormal development of the neural retina in albinism, we focus here on the apical RPE exosome release towards the neural retina.

Interestingly, RPE cells, like other cell types, increase release of exosomes upon stress (Atezcan-Aroca et al., 2016; King et al., 2012). The RPE in albinism may be in a continuously stressed state due to its inability to form pigment that otherwise photo-protects the tissue. In donor eyes with vitreous and neural retina removed, exosome release is halted by supplementation of excess L-DOPA possibly through GPR143 activation (Locke et al., 2014). Note that this study is performed in adult donor eyes and the developmental situation may differ. Conversely, GPR143 may be inactivated by genetic modification or dopamine supplementation, which may ultimately lead to increased exosome release. What role these exosomes may play in healthy retinal development and in albinism depends on their content. Below we use a bioinformatics approach to identify RPE exosome constituents potentially relevant for the ocular pathology of albinism.

Limited by the data yet available, we aim here to identify potential relevant exosome bio-molecules in the context of albinism. Klingeborn et al. (2017) previously identified 55 unique proteins in the apical RPE exosomes of first passage primary porcine RPE cultures (obtained from adult eyes) (Supplementary Table 1). In order to identify albinism relevant entries, we compared this apical exosome protein set (Supplementary Table 1) with a list of gene ontology entries related to both neural development and potentially involved in albinism pathology (Supplementary Tables 2A and 2B). The overlap between these data sets (Supplementary Tables 1 and 2B) yielded a single entry: Glycoprotein M6A (GPM6A) (Fig. 10). Interestingly, substantial in vitro and in vivo experimental evidence support the notion that GPM6A may be highly relevant for retinal and CNS development and maturation. Additionally, taken the bioinformatic and experimental data together, GPM6A may also potentially be relevant for neural albinism pathology. This evidence consists of two relevant groups: (1) studies directly involving the developing (neural) retina or optic nerve and (2) neuronal studies involving that other part of the CNS: the brain.

Overexpression of Gpm6a in mouse retinal progenitor cells resulted in enhanced neurite outgrowth (Zhao et al., 2008). Indeed, in mice, mutations in Gpm6b resulted in hypoplasia of axon tracts in the brain (Mita et al., 2015). In the neonatal mouse, Gpm6a protein is present in RGC axons (Zhao et al., 2008). In adult mouse retina, Gpm6a has been localised to the inner retinal layers (Zhao et al., 2008). In a study involving of the Drosophila Gpm6a orthologue M6, mutants showed a defective visual system development: reduced M6 levels triggered mild eye defects, including a disorganised ommatidium array, defects in ommatidium shape and/or ommatidium fusion, defective or missing bristles and a reduced phototactic response to light (Zappia et al., 2012).

In culture, GPM6A has been implicated in filopodia formation in neuronal cells (Honda et al., 2017). Filopodia are cellular projections that have been linked to neural differentiation and projection as well as dendrite and synapse formation (Brocco et al., 2010). Genetically modified cultured mouse stem cells showed that Gpm6a is involved in the differentiation of neurons (Michibata et al., 2008, 2009). Also, Gpm6a dynamically supports dendritic spine and synapse formation in mouse primary hippocampal neuronal cultures (Brocco et al., 2010; Formoso et al., 2016).

Taken together, experimental in vitro and in vivo as well as in silico data support our hypothesis that GPM6A is involved in retinal or optic tract (projection) development and can thus be relevant for the development of the neural pathology in albinism, downstream in the proposed retinal pigmentation pathway. Obviously, this hypothesis needs further substantiation in future studies.

8.3. GPR143-activated autocrine RPE growth factor signalling

According to our proposed retinal pigmentation pathway, downstream GPR143 signalling may result in PEDF and VEGF secretion from the RPE. This has not only an effect on the adjacent tissues, but also an autocrine effect on the RPE itself. These effects have been both studied in in vitro and in vivo models. Obviously, in vitro data should be considered with caution, and results obtained depend on research questions posed, study design and biomarkers used. For example, cultured ARPE-19 cells may not respond to a VEGF stimulus in the same manner as primary foetal human RPE cultures do. Also, ARPE-19 cells possibly behave more like aged RPE, while foetal (human) RPE appears to be more representative for healthy young human RPE (Ablonczy et al., 2011). Nonetheless, both are relevant to study. The autocrine effects of PEDF and VEGF on the RPE are summarised below (Supplementary Fig. 3).

Autocrine effects of RPE PEDF secretion may be beneficial in development and homeostasis of the retina. We will discuss first the role of PEDF in development and next its role in homeostasis. Beneficial developmental effects of PEDF supplementation to primary rat RPE cultures include larger RPE cell bodies, enhanced development of tight junctions to neighbouring cells, increased number of phagocytic vesicles and more melanosomes with increased maturity, and PEDF expression was correlated with human embryonic stem cell-derived RPE maturity (Al-Ani et al., 2020; Malchiodi-Albedi et al., 1998). Experimental evidence also suggests that PEDF positively affects mature RPE homeostasis: first, PEDF protects cells including rescued barrier function and mitochondrial function from sodium-iodate or H2O2-induced oxidative stress (He et al., 2014; Ho et al., 2006; Nadal-Nicolas and Becerra, 2018; Subramanian et al., 2016; Wang et al., 2019). Second, PEDF allows the RPE to form a stable monolayer by inhibiting proliferation and migration in vitro (Farnoodian et al., 2015; Ma et al., 2012b). Finally, in PEDF receptor (PNPLA2) knockout models, including mice and choroidal or RPE explants, photoreceptor outer segment phagocytosis was low
compared to controls (Bullock et al., 2021). Taken together, the autocrine effect of PEDF signalling on the RPE itself appears essential for proper function and homeostasis of the RPE.

Not only PEDF, but also mechanistically coupled VEGF secretion from the RPE plays an important role in both the healthy and diseased RPE state. Indeed, some VEGF autocrine stimulation of RPE seems essential and has a (positive) effect on RPE homeostasis, as illustrated by the following examples: First, VEGF plays a critical role in survival and maintenance of RPE integrity shown in murine retina eye developmental studies (Ford et al., 2011). Anti-VEGF treatment of ARPE-19 and long-term anti-VEGF treatment of primary porcine RPE in vitro cultures cause a decrease in cell viability (but also reduces RPE65 expression) (Brinkmann et al., 2022; Tsujinaka et al., 2015). Last, VEGF may enhance the intrinsic protective capability of the RPE to deal with oxidative stress. On one hand exposure to cigarette smoke, advanced glycation end products and lipid peroxidation products induced oxidative stress and also induced expression of VEGF (Bergmann et al., 2011; Bertram et al., 2009; McFarlane et al., 2005; Tsujinaka et al., 2015). Additionally, the oxidative stress stimulated VEGF induced its own expression in an autocrine manner (Rossino et al., 2020). On the other hand, in oxidatively stressed ARPE-19 cell cultures (by cigarette smoke), VEGF treatment increased phagocytosis and rescued cell death (Chu et al., 2013). Indeed autocrine VEGF signalling enhanced ARPE-19 cell survival under oxidative stress (by H$_2$O$_2$) (Byeon et al., 2010).

In contrast to the aforementioned positive effects, increased VEGF expression may also have detrimental effects on barrier function and maintenance of cellular RPE identity: Indeed, VEGF reduces RPE barrier function via VEGF receptor 2 (VEGFR2) activation in primary porcine RPE and ARPE-19 cells, as measured using trans-epithelial resistance (Ablonczy and Crosson, 2007). There is a molecular cross-talk in the RPE between PEDF and VEGF inside the RPE, with PEDF inhibiting VEGFR2 signalling, securing its barrier function (Ablonczy et al., 2009, 2011) and also VEGF induces PEDF expression via VEGFR1 (Ohno-Matsui et al., 2001) suggesting a regulatory interaction between the two systems. This counterbalance was shown in RPE from smoker patients, where VEGF-to-PEDF ratio was increased in human RPE from smoker patients with AMD (Pons and Marin-Castaño, 2011). Further, dysregulation of RPE barrier function by VEGF can result in retinal oedema in rabbits (Dahrouj et al., 2011). Finally, in proliferative vitreoretinopathy, the RPE contracts, proliferates and migrates. In adult human primary RPE cultures, VEGF induces contraction, proliferation and migration of RPE cells and the RPE acquires a more mesenchymal phenotype (Hoffmann et al., 2005b; Kehler et al., 2012; Ma et al., 2012a).

9. Regulatory aspects of the retinal pigmentation pathway

Above, we proposed a new functional genetic classification of albinism, and formulated a retinal pigmentation pathway (section 2, Fig. 3). We also described details of this pathway one by one. It is now of interest to elaborate further on the regulatory aspects that, in part, determine the activity of the proposed pathway (Fig. 11) and may contribute to the variability seen in the albinism phenotype. The activity of regulatory hubs in the pathway may be driven by variation in (a combination of) genetic factors, as well as biochemical, metabolic and environmental cues. Main regulatory entities include competitive activation and inhibition of GPR143 by L-DOPA/dopamine, as well as the regulation of (macro)melanosomes formation and oxidative stress. Below, we will discuss the former two entities, while oxidative stress is discussed in the context of AMD and retinopathy of prematurity (ROP) in section 12.4.

9.1. Competitive inhibition and activation of the GPR143 receptor

As described briefly above (section 5.3), the activation of the GPR143 receptor by its ligand L-DOPA, and competitive inhibition by dopamine could play an important role in the proposed retinal pigmentation pathway (Fig. 11) (Falk et al., 2012; Locke et al., 2014). Please note that dopamine is not present during embryonic stages of retinal development (Roffler-Tarlov et al., 2013). In addition, we assume most activity of GPR143 takes place at the plasma membrane as that is typical for GPCRs (Pavlos and Friedman, 2017). However, if the main site of activation of GPR143 in vivo is in the melanosome competitive inhibition by dopamine may be less or not present. Previous studies in

![Fig. 11. Four (potential) regulatory aspects of the retinal pigmentation pathway (itself depicted in Fig. 2): (half shaded boxes) circadian control of the activity of the pathway; (1) variation in L-DOPA and dopamine release, and their effect on GPR143 signalling; (2) regulation of melanosome formation through GPR143 signalling and circadian rhythm; (3) reduced capacity of the RPE to handle and process oxidative stress (over time). Obviously, the regulation of the activity of the proposed pathway involves many other factors, including intrinsic (age-related factors, pleiotropic effects and additional feedback loops), genetic (MITF activity, relevant genetic variants in disease genes or regulatory sequences), and environmental or metabolic factors (diet-driven), which are areas of future research. Here, we focus on the aforementioned aspects. Half shaded boxes: L-DOPA and dopamine are, respectively, receptor activating and inhibiting ligands of GPR143. L-DOPA is produced by the RPE melanosome as tyrosine is converted into melanin. L-DOPA and/or dopamine is also secreted from the (adult) neural retina to the RPE during the day. The aforementioned processes are under circadian control (Hardman et al., 2015) (shaded boxes) and therefore may regulate the activity of the retinal pigmentation pathway. (ad 1) L-DOPA and dopamine competitively modulates GPR143 signalling. (ad 2) GPR143 intra-cellular feedback signalling in RPE cells result in the production of macro-melanosomes and reduced melanin production, which also affects the local L-DOPA production. Thus, the effectiveness of this feedback loop (different in retina and skin) may constitute a regulatory aspect of the activity of the pathway. (ad 3) Finally, oxidative stress, partly due to reduced pigmentation, affects general health of the RPE by oxidising a large range of local biomolecules (discussed in section 12.6). However, oxidative stress may also harm the specific structure or function of the melanosome, and thus the specific (protein) activity of the proposed retinal pigmentation pathway.
foetal retinal tissue and animal models showed that L-DOPA has an essential regulatory role regarding cell fate and differentiation in retinal development (Kralj-Hans et al., 2006; Reis et al., 2007). Excess L-DOPA produced in the initial step of melanogenesis is released from the RPE to the subretinal space. To complicate matters, in the adult situation, L-DOPA and dopamine can also be released from the neural retina to the subretinal space (Reis et al., 2007). In the neural retina, L-DOPA is a precursor for the neurotransmitter dopamine. Indeed, in contrast to the neuro-epithelial RPE, dopaminergic neurons in the retina express the enzyme TH. This enzyme is essential for the direct conversion of L-DOPA into dopamine (Relaï et al., 2007). If dopamine is released from the neural retina and reaches the RPE (Rajan et al., 2000), it could compete with and block binding of L-DOPA to GPR143 and inhibit downstream signalling (Lopez et al., 2008). At the same time, L-DOPA could be released from the neural retina and stimulate GPR143. Unlike L-DOPA, dopamine does not activate a downstream G protein-coupled receptor response along with characteristic Ca2+ influx into the cell (Lopez et al., 2008). Taken together, we hypothesise that L-DOPA is released into the subretinal space by both the RPE and neural retina, activating GPR143, while dopamine is released from the neural retina, competitively inhibiting GPR143. L-DOPA and dopamine, exerting opposite effects, are therefore locally competing for the GPR143 binding site. Uptake of dopamine by the RPE would remove it from the subretinal space where the ligand-binding domain of GPR143 is located and decrease its inhibitory effect. A shift in L-DOPA to dopamine ratio in the subretinal space could possibly result in a shift in activation/inhibition of GPR143.

Dopamine release from retinal neurons follows a circadian rhythm (Jackson et al., 2011; Jin et al., 2015). During the day, active neurons secrete dopamine that builds up in the retina and is (partially) released toward the RPE (Rajan et al., 2000). On the surface of the RPE, dopamine can inhibit GPR143 activity. During the night, neurons are less active and produce less dopamine. Possibly, this mechanism controls the activity of the pigmentation pathway to uniquely accommodate for light induced stresses of the retina during the day. After all, during the night the retina does not have to process light-induced stresses. In addition, both L-DOPA and melanin production by the RPE follow a circadian rhythm. Expression of melanogenic enzymes (TYR, TYRP1, DCT) and production of melanin in hair follicle melanocytes are under circadian control, regulated by the light dependent expression of the clock genes BMAL1 and PER1 (Hardman et al., 2015). These circadian clock genes are active during the day, and melanogenesis is suppressed during this period. The circadian clock appears to be active in the RPE as well and could therefore possibly regulate L-DOPA and melanin production (Bagchi et al., 2020; Milicevic et al., 2021). Whether the aforementioned clock regulated processes act in sync, is currently not clear.

Nonetheless, this circadian control should be taken into account when designing experiments studying the regulation and activity of the proposed retinal pigmentation pathway or experimental therapies. Of note: since albinism pathology starts early in retinal development in the womb, and relevant foveal maturation occurs after birth, the circadian regulation of the retinal pathway may be different in these two phases of life. Clearly, this issue also warrants further investigations.

9.2. GPR143 and its role in formation of macro-melanosomes

GPR143 activation and downstream signalling most likely controls intracellular formation of vesicles, as highlighted by at least two pathological OA1 features. These are the formation of fewer and abnormally enlarged melanosomes (Cortese et al., 2005; Schiaffino and Tacchetti, 2005) and, possibly, the affected RPE exo- and endocytosis (recently reviewed by Locke et al., 2014). The macro-melanosome pathology appears to be more severe in RPE and choroid than in hair or skin (O’Donnell et al., 1976). In Gpr143−/− mutant mice, fewer early melanosomes are produced which subsequently show overgrowth as they mature (Cortese et al., 2005). As previously published, GPR143 may control early melanosome formation in two ways: first, in early stages of healthy melanosome development, GPR143 signalling prevents lysosomal fusion of late endosomes or melanosome precursors (Burgoyne et al., 2013), so that multiple distinct melanosomes can form. Consequently, GPR143 inactivation (in OA1) would lead to fewer enlarged melanosomes. Second, GPR143 signalling may also regulate the expression of MITF, a master regulator of the RPE and melanocyte development (Levy et al., 2006). MITF (co-)controls the expression of genes encoding melanosomal proteins, such as TYR and the structural fibril protein PMEL. In theory, lower levels of GPR143 signalling and a subsequent decrease of MITF activation can lead to lower amounts of PMEL, which allows fewer melanosomes to be formed (Burgoyne et al., 2015; Levy et al., 2006; Ma et al., 2019). Falletta et al. (2014) proposed that fewer early-stage melanosomes, as controlled by GPR143 signalling, lead to an increased ratio of melanosomal proteins to melanosome number. This would lead to proteins accumulating in fewer melanosomes with more pigment production and increased growth compared to normal sized melanosomes (Cortese et al., 2005; Falletta et al., 2014).

10. Do mechanisms underlying foveal hypoplasia and optic tract misrouting overlap?

Obviously, foveal hypoplasia and misrouting are two distinct entities with many differences. These include clinical, anatomical, cellular, molecular and functional phenomena. Molecular differences relate to molecular and developmental cues affecting the development of the retina and optic tracts (Fig. 12). Functional differences can be defined as developmental timing and projection of the optic tracts. The subject of foveal formation (Provis et al., 2013) and optic nerve (mis-)routing (Hoffmann et al., 2005a) has been reviewed elsewhere in depth.

Considering the clinical and pathological overlap between the various forms of albinism in the context of our single proposed pathway, the question arises whether foveal hypoplasia and optic nerve misrouting share one or more common molecular or cellular mechanisms. Indeed, these two entities are consistent defects observed among all genetic albinism subtypes, suggesting that these are intrinsically linked in albinism disease pathology and part of a final common pathway. FHONDA, with its most restricted but severe albinism-like phenotype and with its corresponding SLC38A8 disease gene expression in the neural retina and not in the RPE (Poulter et al., 2013), suggests a (partly) shared mechanism for foveal hypoplasia and chiasmal misrouting originating in the neural retina.

A vast amount of data on this subject comes from previous animal studies. It should be noted here that in a number of animal models of albinism investigated, the ocular phenotype is slightly different from the one observed in humans. For example, in rodents, the fovea does not exist. However, there is an increase of photoreceptors in the central retina, which can be quantified and used to compare differences between albino and wild-type murine retinas (Jeffery et al., 1997). In rabbits, the fovea is represented by a visual streak, whose presence is significantly reduced in albino animals (Jeffery et al., 1997). Various degrees of optic tract misrouting exist in different animal models which are usually investigated by anterograde labelling methods (Jeffery et al., 1994) or optokinetic tests (Lavado et al., 2006; Lavado and Montoliu, 2006). In the context of this article, we only summarise the most relevant aspects of the development of the optic nerve (section 10.1), misrouting of the optic tracts (section 10.2), development of the fovea (section 10.3), and its hypoplasia in albinism (section 10.4) to address the main specific question here: In the context of the proposed retinal pathway, do a number of mechanisms underlying foveal hypoplasia and optic tract misrouting overlap?

10.1. A summary of molecular/cellular mechanisms underlying development of the optic nerve

The retina transmits visual information to the brain by the RGCs.
RGCs are situated in the innermost layer of the retina, and receive their input from the photoreceptors via the bipolar and amacrine cells. They possess long axons encapsulated by glia to form the optic nerve. The optic nerves extend to the optic chiasm where they partially decussate. Most of the axons of the optic nerve terminate in the lateral geniculate nucleus. There, information is relayed to the visual cortex, while other axons terminate in the pretectal area. In humans, RGCs are one of the earliest-born cell types of the future neural retina, developing between foetal week five and foetal week 18 (Pacak and Bremner, 2014). RGC genesis follows a wave of Sonic hedgehog (Shh) signalling (Wagstaff et al., 2021a) developing first in the central retina before spreading to the periphery (Erskine and Herrera, 2014; McCabe et al., 1999). Once born, RGC axons need to be guided out of the retina into the optic nerve. This is achieved using a spectrum of intra- and extracellular signalling molecules alongside physical cues that attract, repel or guide RGC axons (Deiner et al., 1997; Erskine and Herrera, 2014). Examples of these signalling molecules are the aforementioned Shh; the family of Slit proteins, Netrin-1, Sema5A (Fig. 12) (Deiner et al., 1997; Erskine and Herrera, 2014) and possibly, as described in section 8.2 of this report: GPM6A.

Fig. 12. Overview of axon guidance throughout the retina and optic tract. As ganglion cells are born, guidance cues are present already in the retina to direct outgrowing axons through the proper path. First, the Slit2 protein is expressed just below the ganglion cell layer, which restricts axons from growing down into the other layers of the retina. More repellent cues are expressed toward the retinal periphery, while attractant cues are expressed towards the optic disc. At the optic disk, Netrin-1 is highly expressed, channelling the axons out through the back of the retina and down the optic tract. Slit2 is also found on either side of the optic chiasm, possibly ensuring that no crossing occurs other than at the chiasmal midline. Once axons reach the optic chiasm, they are divided into the contralateral and ipsilateral by further guidance cues. Contralateral ganglion cells (shown in red) respond to attractants such as VEGF, Sema6D, Plexin-A1 and NrCAM. Ipsilateral ganglion cells (shown in blue) are repelled at the midline due to the interaction of Ephrin-B2 and its ligand, found only in ipsilateral ganglion cells. The ipsilateral axons instead travel straight down towards the lateral geniculate nucleus.
Once RGCs arrive at the optic chiasm, axons usually cross at the midline and grow towards the contralateral side of the brain towards their target. However, in species with binocular vision, including humans, a subset of axons do not cross the chiasmal midline and instead grow towards the ipsilateral side of the brain. The nasal side of the retina gives rise to contralateral RGCs, while the temporal side gives rise to ipsilateral RGCs. Although not unquestioned (Larsson, 2011), the amount of crossing appears to reflect the importance of binocular vision: in most fish and birds, where binocular vision is often not present, all of the RGC projections cross at the optic chiasm and the excellent binocular vision of birds of prey is achieved by converging retinal information in the brain by way of the thalamofugal pathway (Güntürkün and Hahmann, 1999). However, recent data does suggest that in some species of fish, ipsilateral projections do exist that do not correlate with the traditional positioning of the eyes in binocular vision (Vigouroux et al., 2021).

In mice, only around 3–5% of RGC axons do not cross the midline, whereas in humans it rises to roughly 50% (Erskine et al., 2011; Kuwajima et al., 2012). VEGF acts through the receptor neuropilin1 (Nrp1) (Erskine et al., 2017). This receptor is expressed by contralateral RGCs only, and is crucial for midpoint crossing: Nrp1−/− mice present with smaller proportion of contralateral RGCs (Erskine et al., 2011, 2017). Sema6D specifically attracts contralateral axons, via interaction with Plexin-A1 and NrCAM (Kuwajima et al., 2012). Indeed, a loss of Sema6D results in a decreased number of contraterally projecting RGC axons (Kuwajima et al., 2012).

Ipsilateral RGCs respond to repellent cues, such as resulting from the interaction between tyrosine kinase receptor EPHB1 and its ligand, Ephrin-B2. EPHB1 is uniquely expressed in ipsilateral RGC axons, and once bound to its ligand, creates a repellent response that results in ipsilateral axons not crossing and growing away from the chiasmal midline (Petros et al., 2010). The expression of EPHB1 is controlled by ZIC2, a known marker and transcription factor in ipsilateral RGCs (García-Frigola et al., 2008). Taken together, RGC axons appear to cross the chiasm depending on their retinal area of origin, which receptors they express and the resulting attractant or repellent processes at different stages. Once the RGCs exit the optic chiasm, they continue down the optic tract towards their specific targets in the brain. Although guidance molecules have been implicated in this process, brain targets of the optic tracts fall outside the scope of this review.

10.2. A summary of misrouting in albinism

Numerous albinism studies have been carried out in mice, for obvious reasons, but it is also clear that chiasm formation is fundamentally different in mice and men (Neveu and Jeffery, 2007). Consequently, translation of animal model findings to the human situation remains a challenge.

In albinism, the crossing of RGC axons at the optic chiasm is severely altered (Ather et al., 2019; Puzniak et al., 2019). The optic chiasm in albino animal models usually exhibits an increased number of contralateral RGCs crossing the chiasmal midline at the expense of ipsilaterally projecting RGCs. Previous studies demonstrated that the albinism-related defects in axon guidance are primarily due to the location and/or properties of retinal cells, not to midline cues: retinal explants from pigmented healthy mice grew normally in the presence of either pigmented or albino midline cells. In contrast, retinal explants from albino mice grew differently compared to pigmented controls (Marcus et al., 1996). This was corroborated by the observation that albino mouse models have a decreased number of RGCs expressing EPHB1, an ipsilateral-specific receptor responsible for repelling axons in the chiasm (Rebsam et al., 2012). However, the effects observed in albino models are not identical to EPHB1 null models (Rebsam et al., 2009). Thus, this observation suggests that a lack of EPHB1 expression is not the sole reason for a reduction in ipsilateral projecting RGCs. Interestingly, Liwai-Takekoshi et al. (2018) found that activation of Wnt signalling reduces ipsilateral RGCs in pigmented wild-type retina. Thus, evidence exists that the origin or location of retinal cells, rather than the guidance cues, determines the (non) crossing fate of RGC axons at the optic chiasm. During normal retinal development, ipsilateral RGCs develop earlier and during a shorter time interval compared to contralateral ganglion cells (Drager, 1985).

In albino mice retinas, there is a temporal shift in neurogenesis resulting in uneven development of retinal cell types (ganglion cells and cone photoreceptors) compared to wild-type (Drager, 1985). Indeed, this initially altered molecular specification might correlate with a decrease of ipsilateral axon specific transcription factor ZIC2 positive RGCs in albinism (Herrera et al., 2003). In addition, the aforementioned temporal shift in retinal development could also explain a decrease in photoreceptors in the albino retina, as the timing determines ultimate retinal cell fate. A delayed peak in albino outer nuclear layer cell proliferation compared to pigmented retinal cells may account for altered cone numbers (Ilia and Jeffery, 2000). This animal model based delay-in-neurogenesis-hypothesis is further supported by the fact that the first RGCs that reach the optic chiasm early during development are more likely to stay ipsilateral (Baker and Reese, 1993). Consequently, an albinism-related temporal shift in retinal development would result in RGCs arriving later at the optic chiasm, meaning fewer would stay ipsilateral. Whether this is due to a different fate determined at birth of the RGC, processes at the chiasm, or both, is presently unclear.

10.3. A summary of molecular/cellular mechanisms underlying development of the fovea

The fovea is the cone-rich area of the retina, located in the centre of the macula, developing from foetal week 12 in humans. The highest densities of cone photoreceptors and RGCs are found in the fovea. Every foveal cone connects to roughly 1–3 ganglion cells, whereas in the peripheral retina the ratio increases to 4–6 cones per ganglion cell (Ahmad et al., 2003; Sjöstrand et al., 1999). Together, this enables highly detailed central vision (Curcio and Allen, 1990; Wässle et al., 1989). The fovea can be separated into three different regions. The fovea centralis, also known as the foveal pit, is the centre of the fovea where the density of cone photoreceptors is highest. The fovea centralis is also the location of the rod-free zone and the foveal avascular zone, an area including and surrounding the foveal pit that is devoid of blood vessels and is critical for the formation of the foveal pit. The foveal avascular zone is defined before the foveal depression is formed, and indeed, previous research suggested that a fovea develops only in combination with an avascular zone (Provis et al., 2013). Surrounding the foveal pit is the parafovea, which extends roughly 1–3 mm from the central fovea and is home to the foveal walls. The walls are the thickest part of the retina, as it includes all of the cells that have been displaced from the foveal pit (Bringmann et al., 2018). The fovea is surrounded by the perifovea, located roughly 3–5 mm from the centre (Turan-Vural et al., 2014).

The fovea develops initially in utero by displacing inner retinal layers to form the foveal pit around foetal week 25 (Hendrickson et al., 2012). Foveal maturation extends long after birth and continues until 13 years of age. At that time, cone numbers have increased in the area and become tightly packed in a hexagonal configuration (Thomas et al., 2020). Indeed, foveal cone density of young adult retinas can be 20–30 times higher compared to foetal cone density (Yuodelis and Hendrickson, 1986). The complete development and maturation of the fovea is poorly understood, but it is thought that the formation of the foveal pit...
and the central packing of cone cells are independent of each other.

10.4. A summary of foveal hypoplasia in albinism

In albinism, developmental retinal abnormalities occur, including foveal hypoplasia (lack of foveal pit). This can be clearly seen using OCT (section 1.1). Possible explanations for hypoplasia as a result of the dysfunctional activity of the central pigmentation pathway, in particular the activity of L-DOPA/GPR143 and possible subsequent PEDF, VEGF and exosome signalling, are discussed next.

In albinism, the fovea is characterised by the absence of a foveal avascular zone. Interestingly, PEDF (part of the retinal pigmentation (section 1.1). Possible explanations for hypoplasia as a result of the pathway) is known to be a potent inhibitor of angiogenesis (Kozulin et al., 2009b; Xi, 2020). In the developing retina, PEDF is not only intrinsically expressed by RGCs located at the emerging fovea when compared to other portions of the retina (Kozulin et al., 2010), illustrating the importance of PEDF signalling in the macular area. Therefore, we hypothesise that, if PEDF expression is decreased in albinus retina compared to healthy controls, this lack of PEDF could contribute to underdevelopment of the foveal avascular zone, and, ultimately, foveal hypoplasia in these patients (Krujit et al., 2022).

In contrast with PEDF, VEGF promotes angiogenesis, and a balance of these growth factors is essential for maintaining the avascular areas of the retina (Ohno-Matsui et al., 2001). Indeed, similarly to PEDF, VEGF is also expressed by the RGCs in the central retina before the formation of the fovea (Sandercoe et al., 2003). This suggests an early expression of these two factors in the immediate area of the yet-to-develop fovea plays a crucial role in the development of the important foveal avascular zone. Currently, there is no indication for a potential role for exosomes or GPM6A in foveal development. Finally, it should be noted that, in conditions other than albinism, such as aniridia caused by mutations in the PAX6 gene, foveal hypoplasia can occur in the absence of optic nerve misrouting (Neveu et al., 2005).

10.5. Potential common mechanisms

Persons with albinism have a decreased foveal cone density (Wilson et al., 1988) and a lack of a rod-free zone (Fulton et al., 1978; Woertz et al., 2020b). Albino animals show lower rod numbers in general (Donatien and Jeffery, 2002; Grant et al., 2001) as well as a decreased number of ipsilateral projecting RGCs. This can (at least in part) be attributed to delayed neurogenesis in the neural retina (Baker and Reese, 1993; Dräger, 1985; Ilia and Jeffery, 2000). This could take place during the first wave of neural retina cell development since that includes, in the healthy retina, simultaneous genesis of RGCs and photoreceptors. This delay could be caused by (a genetically determined) lack of L-DOPA, since that molecule not only activates GPR143, but is also involved in the cell-cycle exit (Ilia and Jeffery, 2000; Lavado et al., 2006; Tibber et al., 2006). Thus, theoretically, a lack of L-DOPA signalling could result in a delayed genesis or differentiation of specific neural retinal cells affected in albinism. However, in the case of FHONDA, pigmentation is normal and we hypothesise that L-DOPA and GPR143 signalling are unaffected. Although this hypothesis has not been studied specifically, FHONDA patients do present with foveal hypoplasia and optic nerve misrouting. In addition, SLC38A8 appears not to be present in the RPE, but in the neural retina (Poulter et al., 2013). Thus, the most likely explanation is that a common pathological mechanism for foveal hypoplasia and misrouting in albinism exists, downstream of RPE/GPR143 mediated signalling and FHONDA action in the neural retina, and which probably originates in the developing early neural retina. At the same time, it is not clear whether candidate signalling cues identified, such as L-DOPA, EphA6, PEDF/VEGF balance, and GPM6A (also) affect the expression or function of SLC38A8 (FHONDA) or even more downstream mechanisms and restricted phenotypes directly.

The potential action of L-DOPA/GPR143, GPM6A is discussed above (section 8.2). Three (additional) possible molecular mechanisms governing both foveal development and chiasmal misrouting are discussed next. Two proposed mechanisms originate in the neural retina probably downstream of SLC38A8, one of them is RPE based.

First, Poulter et al. postulated a general hypothesis how SLC38A8 mutations may lead to the FHONDA phenotype (Poulter et al., 2013): in summary, glutamate transporters, such as SLC38A8, play a role in the recycling of (glutamate and GABA) neurotransmitter metabolites in the CNS, including possibly also in the retina. Indeed, synaptic activity does play a role in the developing retina and refinement of retinal projections including those of RGCs (Koch et al., 2011). Additionally, neurotransmitters may be involved in retinal progenitor proliferation regulation before synapses are even present (Martins and Pearson, 2008). Taken together, these phenomena may affect the temporal timeline of retinal cell generation or differentiation, which is thought to be crucial to the proper proportion of crossing axons at the optic chiasm (Martins and Pearson, 2008) and thus may contribute to the albinism-related FHONDA phenotype.

The second hypothesis regarding the SLC38A8-related mechanism causing both foveal hypoplasia and chiasmal misrouting was recently postulated by Krujit and co-workers (Krujit et al., 2022): SLC38A8 may be involved in PEDF and EphA6 signalling in the developing retina. Interestingly, PEDF and EphA6 are involved in formation of the foveal avascular zone (Kozulin et al., 2009a), which is essential for formation of the foveal pit (Provis et al., 2013). High levels of EphA6 are present in the macula during development and foveal cone specialisation (Kozulin et al., 2009a, 2009b, 2010). In addition, the temporal retina, where ipsilateral retinal projections originate, exhibits high expression of EphA6 (Lambot et al., 2005; Provis et al., 2013). Aberrant regulation of EphA6, possibly in the case of albinism and FHONDA, could therefore potentially explain the combination of foveal hypoplasia and chiasmal misrouting.

Finally, altered PEDF/VEGF balance in the developing (albino) retina could also be involved in both foveal development and regulation of RGC projections. Altered PEDF and VEGF signalling can account for the absence of a foveal avascular zone (Ohno-Matsui et al., 2001; Provis et al., 2013). The foveal avascular zone is hypothesised to be essential for the formation of the foveal pit (Provis et al., 2013). At the same time, PEDF is involved in RGC neurite development and the neuroprotection of RGCs after damage to the optic nerve (Vigneswaran et al., 2013). In addition, VEGF acts as an attractant for contralateral RGCs in the optic chiasm through the Nrp1 receptor (Erskine et al., 2011, 2017). Possibly, VEGF secreted by the RPE can have a similar effect in the neural retina. However, this needs to be confirmed. Obviously, the precise role of each of the (combination of) many factors controlling these processes needs to be further elucidated.

11. The pigmentation pathway in the skin: why is the OA1 phenotype restricted to the retina?

As described above (section 5), the OA1 albinism phenotype is caused by mutations in GPR143, and its pathiology is largely restricted to the visual system (Lewis, 1993a). The GPR143 receptor is not only implicated in OA1, but also one of the key features of the proposed retinal pigmentation pathway potentially resulting in both abnormal growth factor secretion as well as vesicle formation and release (Fig. 3) (Falk et al., 2012; Locke et al., 2014; Lopez et al., 2008; McKay, 2019). OA1 patient RPE is hypopigmented because (macro-)melanosomes numbers are low and not evenly dispersed compared to healthy RPE (Cortese et al., 2005; Garner and Jay, 1980; Wong et al., 1983). Intriguingly, however, GPR143 is also expressed in skin melanocytes in mice (Bassi et al., 1996). In OA1 patients, skin macro-melanosomes are also present, but the colour of the skin is (nearly) normal (Garner and Jay, 1980).

These observations raise the question: what are the pigmentation
signalling differences between retina and skin, and what role does GPR143 play in skin development, pigmentation or homeostasis? Two clear differences between retina and skin are that mature skin melanosomes, as opposed to those in the RPE, traffic out of the melanocytes into skin keratinocytes, upon which local intracellular melanosome formation largely ends. Skin melanocytes are also constantly producing melanosomes, whereas RPE melanocytes only produce these during development (Boulton, 2014; Obbayashi and Fukuda, 2020). Again, we discuss three potentially relevant features of the proposed pigmentation pathway (Fig. 3): melanosome formation, PEDF/VEGF secretion and exosome release.

Macro-melanosome formation in the RPE occurs through the GPR143 signalling feedback loop (section 9.2) (Cortese et al., 2005; Schiaffino and Tacchetti, 2005). Possibly, GPR143 cannot (fully) exert its action in skin, most likely due to subtle molecular differences between RPE and skin melanocytes: Further investigation is warranted whether macro-melanosomes can only form when multiple melanosomes are co-maturing as is the case in RPE, but not in skin as the melanosomes are trafficked out of the cell.

Paracrine signalling (PEDF, VEGF and exosome release) may also be different between (OA1) RPE and skin. The role of PEDF, VEGF and exosome release in the retina is discussed above (section 8.1-3). In the skin, it is not clear whether GPR143 signalling modulates skin physiology and disease through control of PEDF and VEGF secretion at different stages in life. PEDF is produced by skin melanocytes and contributes to skin homeostasis (Zhang et al., 2009). This growth factor does have a role in physiology of hair follicles where it affects cell motility and possibly inhibits angiogenesis (Li et al., 2013). PEDF has also been implicated in melanoma tumour growth (Chi et al., 2006). Next to PEDF, VEGF also plays a role in skin homeostasis. In this context, VEGF has been implicated in angiogenesis, melanoma formation and wound healing (DiPietro, 2016). Interestingly, VEGF receptor expression increases after UVB exposure and promotes melanogenesis and melanocyte proliferation (Zhu et al., 2020). Like PEDF, VEGF has a role in melanoma development: higher VEGF expression in melanocytes is indicative of neoplastic changes and the development of melanoma (Einspahr et al., 2007; Gajarin et al., 2011; Stefanou et al., 2004), which may underlie the aforementioned link between albinism and skin cancer. Unfortunately, VEGF release from healthy skin melanocytes has been understudied.

Finally, potential exosome release from the RPE (in albinism) via GPR143 signalling is discussed extensively above (section 8.3). In the skin, it is presently not clear whether GPR143 signalling in melanocytes regulates exosome release. Healthy skin melanocytes do release exosomes which play a role in skin physiology: Upon UVB stimulation, exosome secretion from skin melanocytes increases (Shen et al., 2020). Exosomes released from melanocytes probably do have an influence on skin homeostasis. Finally, a potential role for GPM6A in the development or homeostasis of the skin is presently unclear.

Taken all available data together, we hypothesise that a molecular pigmentation pathway in the skin is present, that resembles the retinal pigmentation pathway postulated above. There are a number of molecular and cellular differences between the two aforementioned pathways that warrant further in-depth investigation. One of these differences seems to be that in skin, melanosomes are trafficked out of the cell, whereas in the RPE they remain inside the cell. Another difference seems to be that, in skin, GPR143 feedback signalling to form macro-melanosomes may not be fully active, while GPR143 downstream signalling, potentially related to PEDF, VEGF secretion and exosome secretion is. In-depth further research on both albinism retina and skin (models) regarding these issues will yield further detailed knowledge about the aetiology of albinism and the development of potential future therapies.

12. Implications for clinical classification, research and therapeutics of albinism

12.1. A summary of concepts and findings

In this report, we proposed one central pathway for retinal pigmentation implicated in the pathogenesis of human albinism. In the context of this pathway, we connected disease gene, gene function, and cellular pathology to phenotypes. This suggests a correlation between increasingly specific cellular and molecular pathology and increasingly restricted phenotypes (Fig. 3): We formulated that defects affecting the genesis or function of multiple cellular organelles, including melanosomes, cause syndromic forms of albinism (HPS, CHS). Specific defects in melanosome function and pigment cell differentiation cause specific forms of OCA (OCA1-8). Further, we incorporated that GPR143 mutations uniquely causes OA1, resulting in macro-melanosome formation and a restricted ocular pigmentation phenotype. Defective GPR143 signalling may affect paracrine signalling in the RPE resulting in an abnormal (developmental) neural retinal phenotype. Finally, we highlighted the involvement of SLC38A8, in mutated form leading to a very restricted neural retinal “albinism” phenotype (FHONDA), without pigment abnormalities. The retinal pigmentation pathway, based on albinism gene involvement, beginning upstream and continuing downstream, can be defined as: HPS1-9, CHS; OCA1-8; GPR143/OA1; SLC38A8/FHONDA (Fig. 3).

In terms of biochemical action and physiological signalling, the proposed retinal pigmentation pathway can be described as follows: The amino acid L-tyrosine is converted to melanin in RPE melanosomes under release of L-DOPA. Subsequently, L-DOPA from the RPE and L-DOPA/dopamine from the neural retina activates or inhibits the subsequent signalling from the apical GPR143 RPE receptor. Next, GPR143 signalling may have multiple effects: via a regulatory feedback loop it has an effect on maturation of the (macro-)melanosome, and downstream it possibly modulates PEDF, VEGF and exosome secretion from the RPE. Less local tyrosine-melanin conversion leads to less local L-DOPA production and subsequently, less GPR143 activation. A lack of functional GPR143, for example, due to mutational events, may have a similar effect in the proposed retinal pigmentation pathway; and both can potentially result in altered paracrine signalling to the neural retina. The resulting alterations in neurogenesis in the neural retina may contribute to the ocular pathology: foveal hypoplasia and chiasmal misrouting. We have described the FHONDA syndrome and how the corresponding protein may act downstream of GPR143 signalling in the albinism pathway to produce such a remarkably albinism-like phenotype.

Next, we suggested three features of this pathway that go beyond direct albinism disease gene involvement: first, we noted that the regulation of the activity of the (retinal) pathway proposed is under circadian control. Second, we bioinformatically compared (GPR143-mediated) exosome content release from the apical RPE and proteins known to be involved in neural development. That analysis yielded one particular new signalling protein of interest: GPM6A. We suggest that this protein may contribute, at least in part, to neural retinal development and, perhaps, albinism pathology. We also explore how the retinal pigmentation pathway could partly explain foveal hypoplasia and chiasmal misrouting. RGC genesis and the timing of RGC axon development may be key to proper crossing of the RGC axons at the optic disc and the formation of the foveal avascular zone for foveal development. Both could be modulated via the retinal pigmentation pathway. Finally, we compared retinal pigmentation in skin and eye, as far as data is available.

12.2. Future perspectives: research

In this section, we will discuss possible directions of new research into the pathophysiological mechanisms of albinism. The
conceptualization of one retinal pigmentation pathway together with new technological possibilities, opens up many new avenues for systematic future research. These topics include at least: further clinical (sub-)classification in relation to genotype, the role of specific (candidate) regulatory or signalling molecules (L-DOPA, PEDF, VEGF, GMP6A) along the proposed pathway and exosome signalling in the developing (albinism) retina, the similarities and differences between molecular and cellular mechanisms underlying foveal hypoplasia and chiasmal misrouting and, even, the molecular mechanisms of nystagmus in albinism. New technological possibilities include advanced in vitro modeling of albinism using stem cell-derived (knock-out) RPE cultures and retinal organoids, and the development and characterization of new animal models. These issues are described in summary below.

There is a clear need in albinism and pigmentation research for further detailed clinical and molecular genetic investigations. It is not only essential to determine further phenotype-genotype relationships and to develop early DNA diagnostic tests, for example in new-born screenings. Most importantly, emerging new therapies can only enter clinical trials when the natural history of disease subtypes is established together with timely treatment opportunities.

As described in sections 3-6, there are, so far, 22 disease genes implicated in (the retinal pathology) of albinism (including FHONA/SLC38A8). Between the different genetic causes there is considerable variability in the phenotypic severity, except for very limited variability in the more consistently severe FHONA phenotype. Further characterization of albinism (sub-)phenotypes and their correlation to genotypes enhances understanding of patient-specific pathologies and treatments. In addition, it is important to mention that additional genetic causes of albinism still have to be identified, with approximately 25% of patients remaining genetically unresolved (Kruit et al., 2018). Some candidate genes have been proposed in the literature. A downstream effector of GPR143, GNAI2 (discussed briefly in section 5.2), has been raised as a potential additional candidate gene for OA (Young et al., 2016). Interestingly, animal models with mutations in BLOC-1 subunits (BLOC1S4, -5 and SNAPIN) that have not been implicated in HPS in humans, as well as a protein that interacts with BLOC-3 (RAB38), show HPS-like phenotypes (Bowman et al., 2019; Lootus et al., 2002; Montoliu and Marks, 2017; Oiso et al., 2004; Zhang et al., 2014). Finally, there are three more genes (VPS33A, RabGGTA, and SLC7A11) involved in LRO trafficking of associated with pigmentation phenotypes in mice (Chintala et al., 2005; Detter et al., 2006; Suzuki et al., 2003). Consequently, the aforementioned genes are excellent candidates to elucidate the yet unresolved molecular pathology in albinism patients.

The currently available models of human retinal development and albinism have a number of limitations. As discussed in section 10 above, these include the absence of a true fovea in most animal models used, and significant differences in contralateral and ipsilateral (non-)crossing RGC axons at the optic chiasm. With the development of patient-derived induced pluripotent stem cells and methods to derive RPE cells and 3D retinal organoids, possibilities have opened up to study cellular and molecular processes in human-representative albinism. As discussed in section 10 above, the aforementioned genes are excellent candidates to elucidate the yet unresolved molecular pathology in albinism patients.

Fig. 13. Rows: WT hESC (A1-2) and OCA1 iPSC-derived RPE (B1-2) cultures. Column, left (A1-B1) corresponds with culture time point day 25, which coincides with the start of pigmentation in the WT cultures. Column, right (A2-B2), day 60: full pigmentation can be seen in the WT cells (A2), but not in the OCA1 line (B2). Both lines exhibit the typical hexagonal RPE morphology (Bakker et al., unpublished data). Data presented here corroborate those previously described (George et al., 2022; Schaub et al., 2020).

The conceptualization of one canonical retinal pigmentation pathway provides the opportunity for further systematic investigation. Subject for further study is the subcellular location(s) of the GPR143 receptor in the in vivo human RPE. Further, the regulation and signalling of the proposed retinal pigmentation pathway is, most likely, complex, and partly needs to be elucidated. More specifically, further research in this area includes the exact role of circadian rhythms, effects of L-DOPA signalling, growth factor action, SLC38A8 function and exosome release, and the potential role(s) of Epha6 and GPM6A. Further downstream, the intrinsic neural properties and neural guidance cues (specified in section 10) that determine proper retinal and optic nerve development are also an exciting area of ongoing research. A subject of future research may be the release of exosomes from the RPE, not only in human albinism, but also in other retinal disease entities. Indeed, the role of exosomes in various physiological contexts recently gained attention: exosomes are not just a way of cellular waste secretion but also an important signalling vehicle between cells (Colombo et al., 2014; van der Pol et al., 2012). Interestingly, L-DOPA treatment of cultured primary porcine RPE cells (likely, but not per se exclusively, via GPR143 activation) halt their apical RPE exosome release (Locke et al., 2014). If we translate this finding to current concepts in human albinism, where L-DOPA is not sufficiently produced or GPR143 itself is not functional, we hypothesise that subsequent exosome release may increase. Another specific question to be answered is: what do these albinism driven exosomes carry compared to healthy controls and does the (abnormal) biomolecular content play a role in (defective) development of the neural retina? Our analysis of the available proteome datasets of apically released exosomes from RPE cultures and genes related to neural retina development yielded one interesting protein: GPM6A, that is an excellent candidate to modify neural retina phenotype of albinism (as described in section 8.2). Furthermore, in addition to proteins, exosomes also carry signalling lipids, RNAs and small molecules (Colombo et al., 2014; van...
der Pol et al., 2012). All these molecules can have their own effects in health and disease. Taken together, functional analysis of exosome release is a promising new frontier in albinism research.

12.3. Future perspectives II: therapies

12.3.1. Previous and ongoing clinical trials

Before discussing potential new experimental therapies, it is of interest to make a short inventory of recent and ongoing clinical trials in the context of the retinal pigmentation pathway: https://www.clinicaltrials.gov/ct2/results?cond=Albinism. In summary, the resulting entries are oral L-DOPA treatment, dietary supplementation of zeaxanthin and lutein, and, finally, nitisinone treatment. These are mostly based on repurposing drugs; i.e. existing drug FDA/EMA approved for another indication, and subsequently approved in clinical trials for albinism. These trials are summarised below.

A phase 2 clinical trial, administering L-DOPA orally to albinism patients has been performed (ClinicalTrials.gov Identifier: NCT01176435) (Summers et al., 2014). L-DOPA supplementation is a potentially promising potential therapy since, in most forms of albinism, with ocular albinism and FHONDA as the exceptions, local L-DOPA production is probably limited, resulting in pathological effects in the neural retina via the proposed retinal pigmentation pathway. In this clinical trial, the age of the participants ranged from 3.5 to 57 years old (mean age: 14.5). Participants included OCA1, OCA2 and HPS1 patients. The result of the trial showed that there was no significant effect on VA after 20 weeks of oral L-DOPA treatment (Summers et al., 2014). One possible explanation for the lack of significant results was the recruitment of albinism patients with different gene mutations, whose response to L-DOPA are not necessarily comparable. Another explanation could be that people with more mature visual systems were included, and once the development of RGCs and the fovea is complete, it is unlikely that a prominent effect would be seen following L-DOPA treatment. A stratification of future clinical trials according to albinism genotypes could provide more precise results.

Given the proposed retinal pigmentation pathway (Fig. 3), the results of this L-DOPA treatment trial may, in retrospect, be explained as follows. As albinism mechanistically seems to be a very early onset disorder, treatment at later ages may have no effect. In this trial, L-DOPA supplementation, which in principle can activate the GPR143 receptor to rescue the albinism phenotype, was given orally to albinism patients over the age of 3.5. At that age the initial formation of the foveal pit and retinal projections to the chiasm are already largely completed. The foveal pit is forming already at 25 weeks of gestation, and foveal cone specialisation and packing starts from birth and continues at least until four years of age. The fovea is still developing up to 13 years of age (Hendrickson et al., 2012). A potential large treatment effect in individuals with a considerably matured macula is unlikely. Nonetheless, because cone packing and specialisation correlates with VA, aiding this process with L-DOPA supplementation could still be beneficial (Provis et al., 2013). Finally, it should be noted that L-DOPA release from the neural retina, and possibly the RPE, is, at least postnatally, under circadian control (section 9.1) (Bagchi et al., 2020). Thus, a potential L-DOPA supplementation treatment should not only start early in life, but also take circadian timing into account. Prenatal or perinatal treatment could perhaps still be an option when clinical genetic diagnosis is already clear in the family, taking ethical and safety considerations into account.

Another relevant (pre-)clinical albinism study using oral L-DOPA supplementation in the form of Levodopa is currently carried out by Helena Lee, Andrew Lotery and co-workers in Southampton, UK (http://gtr.ukri.org/projects?ref=MR%2FR007640%2F1; the OLIVIA study). Levodopa is a drug that is currently successfully used to treat (three-month-old) infants with infantile dystonia, which is now being repurposed for human albinism. Indeed, in contrast with similar previous studies and trials, the OLIVIA study includes infants from three months onward. The purpose of the OLIVIA study is threefold: (1) prove that Levodopa supplementation at early age (up to postnatal day 15) can rescue retinal development, morphology and visual function in a mouse model of human albinism. This was reported to be successful (Lee et al., 2019); (2) Analyse the response of the developing retina after use of different doses of L-DOPA at multiple stages of development; (3) Test the feasibility of Levodopa intervention and determine inclusion criteria for a future randomised controlled clinical trial in children with albinism. As the clinical phase of the study is planned to end in 2024, no additional public data is yet available.

A completely different approach was to test oral nitisinone supplementation in OCA1 patients with residual pigmentation, in a combined phase 1/2 clinical trial (ClinicalTrials.gov Identifier: NCT01838655) (Adams et al., 2019). Nitisinone (an FDA-approved drug for hereditary tyrosinemia type I) raises plasma levels of tyrosine through inhibition of tyrosine catabolizing enzymes. This approach may be particularly effective in forms of albinism where a residual function of TYR remains. Obviously, some residual TYR function is essential for L-DOPA production in the RPE (Fig. 2). This hypothesis was tested by preclinical supplementation of nitisinone in an OCA1 mouse model (with residual TYR activity) which increased pigmentation in coat, iris and RPE (Onojafe et al., 2011). Similar experimental treatment of an OCA3 mouse model with nitisinone yielded a significant positive pigmentation effect, but in the iris only (Onojafe et al., 2018). Exposing other genetically modified OCA1 mouse models to nitisinone (Onojafe et al., 2011, 2018) also did not result in significant re-pigmentation (Lluis Montoliu, unpublished results). It is not clear whether treatment of the aforementioned mice had an effect on RPE L-DOPA production and subsequent neural retina development or optic nerve decussation.

The participants in the human clinical nitisinone supplementation trial indeed had some residual TYR activity, and the increase in the substrate tyrosine could thus possibly raise L-DOPA and melanin production closer to normal levels. No adverse safety issues of the trial were reported. At the same time, no statistically significant difference in skin and eye pigmentation or VA was found. In some subjects there appeared to be a slight increase of skin pigmentation. Consequently, these results were in line with the aforementioned pre-clinical studies. In view of the retinal pigmentation pathway proposed, in some severe cases of albinism nitisinone treatment would be most effective if supplemented at a relatively young age when the affected developmental processes are still happening in the retina. A challenge in this context is that the VA would have to be assessed very early, possibly through grading of foveal hypoplasia, as this correlates with VA. In this stage, the fovea is still in development and higher tyrosine levels inducing higher L-DOPA production could possibly aid in foveal maturation and improve VA. Again, while this treatment could in principle have some effect in a subset of patients, treatment at early age raises ethical and safety questions.

Yet another albinism trial has been listed by investigators of the Johns Hopkins University (ClinicalTrials.gov Identifier: NCT02200263). They performed a dietary supplementation trial with the macular carotenoids pigments zeaxanthin and lutein in a group of genetically undefined albinism patients aged 12 and older. This can be considered a low-risk trial, given that these carotenoids generally are taken up from a general dietary source of eggs, fruits and vegetables. The function of these non-melanosomal macular pigments is to absorb excess light and to increase the protection of photoreceptors from oxidative damage. The overall reason for this clinical trial was to investigate a potentially (proved) correlation between pigmentation of the fundus and visual function. The potential effect was expected to be optical, reducing light scatter and chromatic aberration. The goal was not to rescue the physical abnormalities of the retina, such as foveal hypoplasia or chiasmal misrouting. So far, to the best of our knowledge, no results of this trial have been published in any form.

To summarise all published therapeutic trials, the L-DOPA and nitisinone studies both attempt to restore the L-DOPA levels in the retina, albeit in different ways. Most studies included adult subjects, i.e. with
little ongoing foveal maturation. A significant effect of the treatment in humans was not observed. No major safety issues of the treatments were identified in the published trials. A next step could be perinatal administration, which, obviously, raises considerable ethical and safety issues that need to be addressed first. Zeaxanthin and lutein administration to albino patients has not been reported to elicit an effect.

### 12.3.2. Development of potential new therapies

On the basis of the proposed retinal pigmentation pathway, there may be a few new therapeutic options to consider. Treatment of (albinism) eye defects has many advantages over many other organ systems. These include potential local application and non-invasive monitoring of the treatment, the fact that the eye is a closed system, and that one eye can sometimes be used as an untreated control. Obviously, early treatment in albinism is essential but experimentation and invasive treatments on young children are subject to ethical and safety concerns. If possible, local treatment is preferred over systemic treatment to limit potentially harmful side effects. Therapeutic options for albinism may potentially also be applicable to other diseases involving melanosome pigment defects or early onset retinal disease.

New potential albinism treatment options include gene therapy, and small molecule or growth factor administration (Ruan et al., 2017). Gene therapy application in the eye is promising. Adeno-associated virus (AAV) gene therapy treatment for patients with RPE65-related retinal dystrophies was recently approved by FDA and EMA (Russell et al., 2017). Many new preclinical ocular studies and trials, including modern CRISPR/Cas9 approaches, are well under way. As genetic defects in patients can now be analysed better, faster and cheaper than in the past, gene therapy replacing the defective disease gene could potentially treat albinism (Dunbar et al., 2018). As with previous clinical trials for albinism (section 12.3.1), a challenge would be to administer the therapy in an early stage of pathology since intact retinal cells are needed to accommodate gene therapy. So far, gene therapy is not considered suitable for infants and young children, and therefore may also be too late for foveal development to be rescued AAV gene replacement therapy.

Small molecule intervention affecting the regulation and activity of the retinal pigmentation pathway could also be considered. As described above, L-DOPA and nitisnone treatment are examples of small molecules that can potentially have an effect on (the development of) albinism pathology. Several types of albinism appear to be associated with impaired proteins regulating the internal pH of melanosomes affecting TYR activity. Therefore, one could also imagine the discovery and application of small molecules that could also counteract the pH distortions and, thus, restore pigmentation levels. Further (pre-) clinical research into other small molecules regulating the activity of (parts of the) retinal pigmentation pathway should be considered as an experimental therapeutic option.

### 12.3.3. A potential role for the retinal pigmentation pathway in other retinal diseases, including AMD

In addition to albinism, L-DOPA/GPR143 signalling (Figueroa and McKay, 2019) and the proposed retinal pigmentation pathway (section 2) could play a role in other retinal pathologies. Alterations in melanin pigmentation and L-DOPA could have an effect on downstream RPE signalling, contributing to pathological outcomes. The activity of the proposed retinal pigmentation pathway may be not exclusively genetically driven, but the mechanism itself can also be affected by environmental factors, genetic background, diet or drugs, metabolic differences, disease and ageing, resulting in melanosomal pigmentation differences. The retinal pigmentation pathway could also (partly) play a role in non-albinism pathologies in various stages of life. It is outside of the scope of this report to address all potential retinal pathologies in which melanosomal pigment changes play a role, but to show the reasoning, we briefly describe the examples of AMD and ROP below.

AMD is a disease affecting 4% of the population over 60 and almost a third of individuals over the age of 75 (Klein et al., 2011; Wong et al., 2014). AMD is characterised by central vision loss initially characterised by macular pigment alterations and the formation of subretinal deposits, called drusen. AMD consists of a common dry form (retinal atrophy) and a more rare wet form (neovascularization). AMD patients experience a severe loss of quality of life and lose their independent lifestyle (Klein et al., 2011). There is no (effective) cure for at least 90% of patients (Wong et al., 2014). The disease is caused by (combinations of) environmental factors, such as smoking; and genetic risk factors, most importantly genetic variation in genes that determine the activity of the complement system (de Jong et al., 2021). Mechanistically, abnormalities of the complement system, the lipid metabolism, and extracellular matrix have been implicated in the disease (Fritsche et al., 2016). Oxidative stress is probably a major driver of the AMD (Abokyi et al., 2020), affects RPE health, and basolaterally secreted oxidatively modified biomolecules may invoke a complement response, leading to further local pathology.

Much attention has been given to the photoprotective role of specific macular pigments in AMD (Chew et al., 2013, 2014). Also, a decline in the optical density of non-melanosomal macular pigment is part of normal ageing, and significantly less macular pigments are observed in AMD compared to healthy individuals (Beatty et al., 2001). However, the potential role of melanosomal pigments in the RPE (and choroid) in AMD is relatively understudied. Here, we focus on melanosomal pigment abnormalities accompanying AMD and how the proposed retinal pigmentation pathway may be implicated in part of the disease: Does (reduced) melanosomal pigmentation have a direct (declining) protective antioxidant effect or is there an indirect effect due to altered signalling through the proposed retinal pigmentation pathway, or both? Below, we will first discuss the antioxidant activity (defect) of melanin in the RPE in AMD, and subsequently the potential indirect effects via the retinal pigmentation pathway.

The ageing RPE (and choroid) is severely burdened by oxidative stress and resulting free radicals (Mettu et al., 2012; Miceli et al., 1994). Apart from other (macular) pigments, melanin pigmentation in the RPE has also been directly implicated in protecting the AMD macula from oxidative damage (Bergen et al., 2019; de Jong et al., 2021). Furthermore, epidemiological evidence suggests that less pigmented individuals may have increased risk for oxidative stress-related diseases. Indeed, in a prospective cohort study with subjects of many different ethnicities within the United States, Caucasian participants were 5-fold more likely to suffer from AMD than participants of African descent (Klein et al., 2011, 2013). Participants of Hispanic and Asian descent showed an intermediate risk. Controlling for other known risk factors like body mass index, hypertension, diabetes, smoking and alcohol consumption did not explain the differences (Klein et al., 2011, 2013). Albinism as a risk factor for developing AMD would support this hypothesis, but whether albinism patients have an (slightly) increased risk for developing AMD is unknown. The possible late-onset clinical diagnosis of AMD in albinism patients may go unnoticed since these patients are rare and have a low VA already early in life.

We next consider the potential role of the retinal pigmentation pathway in AMD pathology. Even in the presence of protective pigments, oxidative stress harms many molecular compounds and microstructures of the RPE cell, including the melanosomes. Thus, we can hypothesise that the RPE and individual features of the retinal pigmentation pathway may be directly affected by oxidative stress. Alternatively, this stress may primarily affect TYR activity and melanin production, and, through L-DOPA, indirectly, downstream signalling and functionalities, such as GPR143 signalling and potentially PEDF and VEGF secretion and exosome release (Falk et al., 2012; Locke et al., 2014). Indeed, it may affect the resilience of the ageing retina and development of AMD (McKay, 2019).

To the best of our knowledge, a direct effect of subretinal L-DOPA/dopamine levels on AMD pathology has not been investigated. Nonetheless, if GPR143 signalling protects from AMD damage during ageing,
L-DOPA supplementation treatment may exert protective effects. Interestingly, oral L-DOPA treatment for Parkinson patients reduces, in parallel, risk for AMD or delays onset of the disease (Brilliant et al., 2016). Further, in a pilot study, patients with wet AMD (who received no anti-VEGF treatments) were treated with L-DOPA, which increased VA and delayed the necessity of anti-VEGF treatment (Figueroa et al., 2021). The possible role of these growth factors (PEDF and VEGF) as well as exosomes (including GPM6A) will be discussed next.

According to the proposed pigmentation pathway, decreased pigmentation (in areas of the RPE) may lead to less L-DOPA, less GPR143 activity and increased VEGF secretion (Falk et al., 2012; Holekamp et al., 2002). Increased local VEGF secretion enhances angiogenesis as seen in neovascular AMD. Similarly, decreased pigmentation, L-DOPA and GPR143 activity may lead to less PEDF secretion, which also has been implicated in AMD (Barnstable and Tombran-Tink, 2004; Farnoodian et al., 2017; Holekamp et al., 2002). GPR143 activation/signalling may possibly increase the amount of exosome secretion (Locke et al., 2014). Interestingly, there are a number of independent relevant leads in the literature implicating basolateral RPE exosomes in AMD: First, exosomes from stressed ARPE-19 cells may enhance the sensitivity of the receiving cells for VEGF signalling, possibly facilitating pathological events (Atienzar-Aroca et al., 2016; Fukushima et al., 2020). Second, RPE exosomes, released upon oxidative stress, contain a collection of phosphoproteins that can activate inflammation, autophagy, apoptosis, and degeneration pathways in receiving RPE (and other cells) (Biasutto et al., 2013; Gao et al., 2015; Ke et al., 2020; Shah et al., 2018; Wang et al., 2009b; Yang et al., 2020). This may facilitate spread of the AMD pathology. Third, a role of RPE exosomes in AMD may come from trafficking of (complement) proteins, or lipids that can protect both RPE and photoreceptor cells from oxidative stress and prevent apoptosis (Anderson et al., 2010; Boon et al., 2008; Klingborn et al., 2017; Sreekumar et al., 2010; Wang et al., 2009a, b). Fourth, exosomes could contribute to the formation of drusen, a hallmark of the AMD pathology (Bergen et al., 2019; Boon et al., 2013; Flores-Bellver et al., 2021).

Finally, the potential future treatments discussed in section 12.3.2, based on manipulation of L-DOPA or other compounds part of the retinal pigmentation pathway could possibly also be of interest for treatment of other disorders, such as AMD. Indeed, L-DOPA administration for AMD patients could be beneficial: a retrospective study of patients that took L-DOPA (for other indications than AMD) showed that treated individuals have a smaller risk for developing AMD or develop symptoms later (Figueroa et al., 2021). A potential role of the exosome protein GPM6A has not been studied in AMD.

To illustrate further that the proposed retinal pigmentation pathway does not have to be driven by genetic factors (alone), we describe the example of ROP next. ROP is an important cause of infant blindness. Risk factors to develop ROP are low birth weight and/or premature birth. In ROP, the blood vessel system in the retina is not fully developed yet, frequently resulting in fragile and leaky blood vessels, retinal scarring and detachment. When children are born prematurely, ROP can occur in two major phases. In the first phase, hyperoxia causes VEGF levels to decrease, which leads to a halt in the development of the retinal vasculature (Cavallaro et al., 2014; Hellström et al., 2013). In the second phase, the rising metabolism of the developing retina and resulting hyperoxia lead to a rise in VEGF levels and abnormal production of blood vessels in the vitreous (Cavallaro et al., 2014). Both phases may lead to changes with ROP, presenting subtle changes to their foveae (Krumova et al., 2019; Martínez-Córdoba et al., 2021). How does the proposed retinal pigmentation pathway potentially play a role in this? Does the level of activity of the retinal pigmentation pathway, which possibly modulates local VEGF, PEDF and exosome secretion, affect ROP development? Indeed, evidence from the literature suggests so: first, children born to mothers with dark pigmented skin are less likely to develop ROP (Husain et al., 2013). Next, it is clear that VEGF secretion plays a central role in ROP pathology (Cavallaro et al., 2014; Zhu et al., 2015). Third, PEDF is considered an inhibitor of angiogenesis and could protect against abnormal vessel growth (Xi, 2020). Indeed, PEDF administered to the eyes of an ROP mouse model reduced neovascularization (Zhang et al., 2016). Finally, while research concerning the role of exosomes in ROP is lacking, they possibly play a mechanistic role in ROP: previous research, albeit in AMD context, showed that VEGFR and VEGF mRNA containing exosomes could exacerbate avascular vasculature formation (Atienzar-Aroca et al., 2016).

13. Conclusion

We propose here a retinal pigmentation pathway linking all (known) albinism causing genes, their function and their (ocular) phenotype together into one cohesive framework. We have explored the possible processes up and downstream that could influence neural retinal development and the albinism phenotype. Upstream, the role for TYR, L-DOPA and GPR143 in the pathology has been reasonably documented. Downstream of GPR143, SL3C8A8 (PHONDA), together with a range of (candidate) signalling molecules that play an essential role in retinal development, optic nerve guidance and albinism. Taken together, this report opens new and systematic avenues for research into albinism and signalling from RPE to neural retina during retinal development. We suggest that the proposed retinal pigmentation pathway opens new avenues for development of new therapeutics in human albinism, but may also have an impact on other retinal pathologies involving melanosomal pigment abnormalities.

Author statement

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Appendix A. Supplementary data

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References

Ablonczy, Z., Crosson, C.E., 2007. VEGF modulation of retinal pigment epithelium resistance. Exp. Eye Res. 85, 762–771.
Ablonczy, Z., Prakash, A., Fant, J., Faqu, A., Crosson, C., Sambamurti, K., 2009. Pigment epithelium-derived factor maintains retinal pigment epithelium function by inhibiting vascular endothelial growth factor-R2 signaling through gamma-secretase. J. Biol. Chem. 284, 30177–30186.
Ablonczy, Z., Dahrouj, M., Tang, P.H., Liu, Y., Sambamurti, K., Marmorstein, A.D., Crosson, C.E., 2011. Human retinal pigment epithelium cells as functional models for the RPE in vivo. Invest. Ophthalmo. Vis. Sci. 52, 8614–8620.
Abohyi, S., To, C.H., Lam, T.T., Tse, D.Y., 2020. Central role of oxidative stress in age-related macular degeneration: evidence from a review of the molecular mechanisms and animal models. Cell. Mol. Life Sci. 77, 2590–2570.

Adams, A.P., Shima, D.T., Yeo, K.T., Yeo, T.K., Brown, L.F., Beren, B., D’Amore, P.A., Folkman, J., 1993. Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. Biochem. Biophys. Res. Commun. 196, 631–638.

Adams, D.R., Menezes, S., Jauregui, R., Valivullah, Z.M., Power, B., Abraham, M., Adams, A.P., Solc, V., 2000. Lysosomal stress and lipid peroxidation in the retina of 5-fluorouracil treated mice. Invest. Ophthalmol. Vis. Sci. 41, 499–506.

Bergsma, D.R., Brown, K.S., 1976. Animal models of albinism. Birth Defects Orig. Artic. Ser. 12, 409–413.

Bergstrom, L., Lindberg, M., Leidinger, B., 2016. Identification of a retinal pigment epithelium (RPE) exosome containing signaling phosphoproteins affected by oxidative stress. Exp. Eye Res. 147, 57–62.

Bonilha, L., Shigemasa, D., Lopes, J.B., Vaz, M.J.J., Lopes, F.L., Donoso, M.J., 2015. Oxidative stress and retinal pigment epithelium cell aging: a review. Front. Aging Neurosci. 7, 45.

Bonne, S., Jeges, D., Auerbach, E., 2006. Ocular melanin pigment and risk for age-related macular degeneration in subjects from a Northern European population. Invest. Ophthalmol. Vis. Sci. 47, 439–446.

Boothe, R.G., Dobson, V., Teller, D.Y., 1985. Postnatal development of vision in human term neonates. J. Neurosci. Res. 13, 735–742.

Bouw, J., Dijk, J., Kijlstra, A., van Hinsbergh, V.W., Schlingemann, R.O., 1999. Membrane-associated transporter protein (MATP) regulates melanosomal pH and influences tyrosinase activity. PLoS One 10, e0129273.

Bouwmeester, T., Roider, J., Klettner, A., 2022. Effect of long-term VEGF treatment on viability and function of RPE cells. Curr. Eye Res. 47, 127–134.

Briand, A., Winkelmann, M., Toepfer, J., Roeder, K., Klettner, A., 2002. Effect of long-term VEGF treatment on viability and function of RPE cells. Curr. Eye Res. 47, 127–134.

Briand, A., Winkelmann, M., Toepfer, J., Roeder, K., Klettner, A., 2002. Effect of long-term VEGF treatment on viability and function of RPE cells. Curr. Eye Res. 47, 127–134.

Briand, A., Winkelmann, M., Toepfer, J., Roeder, K., Klettner, A., 2002. Effect of long-term VEGF treatment on viability and function of RPE cells. Curr. Eye Res. 47, 127–134.
Budd, P.S., Jackson, I.J., 1995. Structure of the mouse tyrosinase-related protein-2/dopachrome tautomerase (Tyrp2/Dct) gene and sequence of two novel slaty alleles. Pigment Cell Res. 8, 29–43.

Bullock, J., Polato, F., Abu-Asab, M., Bernardon-Col, A., Aflaki, E., Agbaga, M.P., Becerra, S.P., 2021. Degradation of photoreceptor outer segments by the retinal pigment epithelium requires pigment epithelium-derived factor receptor (PEDF-R). Invest. Ophthalmol. Vis. Sci. 62, 30.

Burgess, A., Mormon, J.P., de Saint-Basile, G., Callebaut, I., 2009. A concanavalin A lectin domain in the CHS/LYST protein, shared by members of the BEACH family. Biochim. Biophys. Acta 1795, 122–132.

Burgoyne, T., Jolly, R., Martin-Martin, B., Seabra, M.C., Piccirillo, R., Schiaffino, M.V., Davis, C.G., 1990. The many faces of epidermal growth factor repeats. N. Engl. J. Med. 323, 1278–1284.

Bennett, D.C., Park, Y.M., Gahl, W.A., Huizing, M., Spritz, R.A., Ben, S., Novak, E.K., 1991. Mapping the human CAS2 gene, the homologue of the mouse brown (b) locus, to human chromosome 1. Nature 349, 75–78.

DiPietro, S.M., Dell’Angelica, E.C., 2004. Characterization of BLOC-1 in patient-derived organoids at single-cell resolution. Cell 182, 1623–1644 e1634.

De Pietro, S.M., Falccioni-Perez, J.M., Perez, J.D., Dettie, G., Schulze, A., Huhová, B., Pínska, J., Štoll, B., Cerný, I., Sirot, I., Hudák, J., Štoll, A., Cerný, M., 2016. BLOC-1 interacts with BLOC-2 and the AP-3 complex to facilitate protein trafficking on endosomes. Mol. Biol. Cell 27, 4533–4544.

De Pierto, A., 2016. Angiogenesis and wound repair: when enough is enough. J. Leukoc. Biol. 99, 264–308.

De Pierto, A., Falccioni-Perez, J.M., Perez, J.D., Dettie, G., Schulze, A., Huhová, B., Pínska, J., Štoll, B., Cerný, I., Sirot, I., Hudák, J., Štoll, A., Cerný, M., 2016. BLOC-1 interacts with BLOC-2 and the AP-3 complex to facilitate protein trafficking on endosomes. Mol. Biol. Cell 27, 4533–4544.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.

Dettie, G., Zang, K., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurry, E.B., Tchernova, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T., Kingsmore, S.F., 2000. Rab geranylgeranyl transferase alpha mutation in the gunmetal mouse reduces Rab GTPase function and results in increased Rab localization to endosomes and from endosomes via distinct tubular transport carriers. J. Cell Biol. 149, 293–308.
Kuht, H.J., Han, J., Maconachie, G.D.E., Park, S.E., Lee, S.T., McLean, R., Sheth, V., Kritzler, R.A., Terner, J.Y., Lindenbaum, J., Magidson, J., Williams, R., Presig, R., Kralj-Hans, I., Tibber, M., Jeffery, G., Mobbs, P., 2006. Differential effect of dopamine on heterogeneous cohort. Invest. Ophthalmol. Vis. Sci. 60, 3963–4009.

Klingeborn, M., Dismuke, W.M., Skiba, N.P., Kelly, U., Stamer, W.D., Bowes Rickman, C., Klein, R., Li, X., Kuo, J.Z., Klein, B.E., Cotch, M.F., Wong, T.Y., Taylor, K.D., Rotter, J.I., King, R.A., Pietsch, J., Fryer, J.P., Savage, S., Brott, M.J., Russell-Eggitt, I., Summers, C. R. Bakker et al. 2013. Associations of candidate genes to age-related macular degeneration among human retinal organoids. Proc. Natl. Acad. Sci. U. S. A. 116, 10824–10833.

Lim, H.T., Thomas, M.G., 2020. SLC38A8 mutations result in arrested retinal development with loss of cone photoreceptor specialization. Hum. Mol. Genet. 29, 2989–3002.

Kupfer, C., Hambly, L., Downer, J.C., 1967. Quantitative histiometry of optic nerve, optic and lateral geniculate nucleus of man. J. Anat. 101, 393–401.
Luzio, J.P., Hackmann, Y., Dieckmann, N.M., Griffiths, G.M., 2014. The biogenesis of lysosomes and lysosome-related organelles. Cold Spring Harb Perspect Biol. 6, a016846.

Lyons, M.F., King, T.R., Gondo, Y., Gardner, J.M., Nakatsu, Y., Eicher, E.M., Brilliant, M.H., 1992. Genetic and molecular analysis of recessive alleles at the pink-eyed dilution (p) locus of the mouse. Proc. Natl. Acad. Sci. U. S. A. 89, 6698-6792.

Ma, J., Zhong, Q., Moe, M.C., Zhu, L., 2012a. Reduced collagen cell contraction in human retinal pigment epithelial cells by vascular endothelial growth factor compared with transforming growth factor-β. Clin. Exp. Ophthalmol. 40, 195-205.

Ma, X., Pan, L., Jin, X., Dai, X., Li, H., Wen, B., Chen, Y., Ma, A., Qu, J., Hou, L., 2012b. Microphthalmia-associated transcription factor activity is regulated by PEGF to regulate RPE cell migration. Exp. Cell Res. 318, 251–261.

Ma, H., Li, H., Chen, Y., Yang, J., Chen, H., Arveller, H., Hou, L., 2019. The transcription factor MITF in RPE function and dysfunction. Prog. Retin. Eye Res. 73, 100766.

Mackenzie, B., Erickson, J.D., 2004. Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. Pfluegers Archiv 447, 784-795.

Maguire, J.A., Lu, L., Mills, J.A., Sullivan, L.M., Gadue, P., French, D.L., 2016b. Generation of Hermansky Pudlak syndrome type 2 (HPS2) induced pluripotent stem cells (iPSCs). Stem Cell Res. 16, 287-289.

Maguire, J.A., Lu, L., Mills, J.A., Sullivan, L.M., Gadue, P., French, D.L., 2016b. Generation of Hermansky Pudlak Syndrome type 1 (HPS1) induced pluripotent stem cells (iPSCs). Stem Cell Res. 16, 233–235.

Maisto, R., Gesualdo, C., Trotta, M.C., Grieco, P., Testa, F., Simonelli, F., Barcia, J.M., Arveiler, B., 2014. SLC24A5 mutations are associated with non-syndromic ocular albinism in the mouse. PloS One 9, e95111.

Martinínez-C, B., Sierra-Bernal, R., 2014. Tyrosinase-like activity of melanosome proteins in melanocytes from mice with oculocutaneous albinism type 2. Exp. Eye Res. 2, 695-710.

Marcus, R.C., Wang, L.C., Manon, C.A., 1996. Retinal axon divergence in the optic chiasm: midline cells are unaffected by the albinism mutation. Development 122, 859-866.

Marks, M.S., Heijnen, H.F., Raposo, G., 2013. Lysosome-related organelles: unusual lysosomes and lysosome-related organelles: unusual compartments become mainstream. Curr. Opin. Cell Biol. 25, 495-505.

Martina, J.A., Moriyama, K., Bonifacio, J.S., 2003. BLOC-3, a protein complex containing the Hermansky-Pudlak syndrome gene products HPS1 and HPS4. J. Biol. Chem. 278, 29076-29082.

Martínez-Córdoba, C.J., Quijano-Nieto, B.A., Echeverría-González, C.L., Sierra-Bernal, R. M., 2021. A comparison of posterior segment optical coherence tomography findings that in the mouse. Invest. Ophthalmol. Vis. Sci. 62, 2362-2367.

Mancini, A.J., Chan, L.S., Paller, A.S., 1998. Partial albinism with immunodeficiency: griezel syndrome: report of a case and review of the literature. J. Am. Acad. Dermatol. 38, 295–300.

Mango, P., Kromberg, J.G., Box, N.F., Sturm, R.A., Jenkins, T., Ramsay, M., 1997. Rufous ocularocutaneous albinism in southern African Blacks is caused by mutations in the TYRP1 gene. Hum. Mol. Genet. 6, 1095–1100.

Mango, P., Boisvy, R.E., Pikó-Hirt, S., Zhou, B.K., Orlov, S.J., 2001. Mislocalization of melanosomal proteins in mice from with oculocutaneous albinism type 2. Exp. Eye Res. 72, 695-671.

Marcus, R.C., Wang, L.C., Manon, C.A., 1996. Retinal axon divergence in the optic chiasm: midline cells are unaffected by the albinism mutation. Development 122, 859-866.

Ma, X., Hejnowicz, P., Raposo, G., 2013. Lysosome-related organelles: unusual lysosomes and lysosome-related organelles: unusual compartments become mainstream. Curr. Opin. Cell Biol. 25, 495-505.

Martina, J.A., Moriyama, K., Bonifacio, J.S., 2003. BLOC-3, a protein complex containing the Hermansky-Pudlak syndrome gene products HPS1 and HPS4. J. Biol. Chem. 278, 29076-29082.

Martínez-Córdoba, C.J., Quijano-Nieto, B.A., Echeverría-González, C.L., Sierra-Bernal, R. M., 2021. A comparison of posterior segment optical coherence tomography findings that in the mouse. Invest. Ophthalmol. Vis. Sci. 62, 2362-2367.

McCabe, K.L., Gunter, E.C., Rea, T.A., 1999. The development of the pattern of retinal ganglion cells in the chick retina: mechanisms that control differentiation. Development 126, 5713-5724.

McFarlane, S., Glenn, J.V., Lichanska, A.M., Simpson, D.A., Stitt, A.W., 2005. Melanosome morphologies in murine models of hermansky-pudlak syndrome reflect blocks in organelle development. J. Invest. Dermatol. 119, 1156-1164.

O'Donnell Jr., F.E., Hamburg, J.F., Green, W.R., Hill, W.J., Stone, D.L., 1976. X-linked ocular albinism. An ocularocutaneousmelanosomal disorder. Arch. Ophthalmol. 94, 833–838.

Oetting, W.S., 2002. New insights into ocular albinism type 1 (OAI): mutations and polymorphisms of the OAI gene. Hum. Mutat. 19, 85-92.

Oetting, W.S., King, R.A., 1999. Molecular basis of albinism: mutations and polymorphisms of pigmentation genes associated with albinism. Hum. Mutat. 13, 99-115.

Ohno-Matsui, K., Fujiki, H., Morita, I., Tombran-Tink, J., Mrazek, D., Onodera, M., Uetama, T., Shinoda, M., Takahashi, Y., Nakamura, K., et al., 1999. Molecular basis of albinism: mutations and polymorphisms of the OA1 gene. Hum. Mutat. 19, 85-92.

Ohno-Matsui, K., Morita, I., Tombran-Tink, J., Mrazek, D., Onodera, M., Uetama, T., Shinoda, M., Takahashi, Y., Nakamura, K., et al., 1999. Molecular basis of albinism: mutations and polymorphisms of the OA1 gene. Hum. Mutat. 19, 85-92.
Oiso, N., Riddle, S.R., Kuramoto, T., Spritz, R.A., 2004. The rat Ruby (R) locus is Rhb38: identical mutations in Fawn-hooded and Tester-Montgomery rats result from a single mutation in Evans rats. Genetics 155, 307–314. Olives, C., Solano, F., 2009. New insights into the active site structure and catalytic mechanism of tyrosinase and its related proteins. Pigment Cell Melanoma Res. 22, 760–762. Oikonomou, V.M., Ikonen, E., 2006. When intracellular logistics fails–genetic defects in membrane trafficking. J. Cell Sci. 119, 5031–5045. Onojafe, I.F., Megan, L.H., Melch, M.G., Aderemi, J.O., Alur, R.P., Abu-Asab, M.S., Onojafe, I.F., Adams, D.R., Simeonov, D.R., Zhang, J., Chan, C.C., Bernardini, I.M., 2006. An unconventional dileucine-based motif and a novel cytosolic motif are required for the lysosomal and melanosomal targeting of OA1. J. Cell Sci. 119, 5031–5041. Ossandón-Kellner, B., 2004. An unconventional dileucine-based motif and a novel cytosolic motif are required for the lysosomal and melanosomal targeting of OA1. J. Cell Sci. 119, 5031–5041. Overy, C.J., Murphy, J., 2003. Induction of the ganglion cell differentiation program in fetal mouse retina. Dev. Neurobiol. 70, 781–794. Oveisi, A., Malinovsky, R., 2004. The prevalence and associated features of posterior embryotoxon in the general ophthalmic clinic. Eye 19, 396–399. Pajusi, A., Maroller, J., Sohn, E.H., Mahajan, V.B., Pfeifer, W., Weckmann, M., Johnson, C., Pappas, J., Elci, O., McCague, S., Cross, D., Marshall, K.A., Walshine, J., Kehoe, T.J., Reichert, H., Davis, M., Raffini, L., George, L.A., Hudson, F.P., Dingfeld, L., Zhu, X., Haller, J.A., Surace, F., Testa, F., Simonelli, F., Greco, P., Merlino, F., Perretti, M., D’Amico, M., Di Filippo, C., 2016. Activation of melanocortin receptors MC1 and MC5 attenuates retinal damage in experimental diabetic retinopathy. Mediat. Inflamm. 2016, 2016. Provis, J.M., Dubis, A.M., Maddess, T., Carroll, J., 2013. Adaptation of the central retina for high acuity vision: cones, the fovea and the avascular zone. Prog. Retin. Eye Res. 39, 1–81. Pryor, P.R., Murdock, B.M., Bright, N.A., Lindsay, M.R., Gray, S.R., Richardson, S.C., Stewart, A., James, I.E., Piper, R.C., Luzio, J.P., 2004. Combinatorial SNARE complex formation with VAMP8 defines late endocytic fusion events. EMBO Rep. 5, 590–595. Puri, N., Gardner, J.M., Brilliant, M.H., 1992. Ophthalmic, M., Zühlke, C., Opitz, S., Schwinger, E., Kiasmann-Kellner, B., 2004. Mutations in the MATP gene in five German patients affected by oculocutaneous albinism type 4. Hum. Mutat. 37, 226–233. Poves, M.S., van Meel, E., Oorschot, V., ten Brink, C., Fukuda, M., Swetha, M.G., Mayor, S., Meuleman, J., Stuver, M., van der Kooi, A.W., Stroobants, S., 2015. Investigating polymorphisms in membrane-associated transporter protein SLC45A2, using sucrose transporters as a model. Mol. Membr. Biol. 32, 20. Petric, M.J., Struan, D., Mercer, J.F., 2000. The Menkes copper transporter is required for the activation of tyrosinase. Hum. Mol. Genet. 9, 2845–2851. Petros, T.J., Blyson, J.B., Mason, C., 2010. Ephrin-B2 elicits differential growth cone collapse and axon retraction in retinal ganglion cells from distinct retinal regions. Dev. Neurobiol. 70, 768–780. Pickuth, J., Böck, J., 2003. Tyrosinase activity is regulated by the autocrine loop of VEGF in the developing retina. J. Invest. Dermatol. 121, 1992–1998. Rizkalla, S.W., de la Sizeranne, N., Madea, B., Durand, C., Rosenbloom, K., 2006. Oxidative stress induces a VEGF autocrine loop in the retina: relevance for diabetic retinopathy. Cells 9, 26–33. Ross, S.C., Bright, N.A., Pappas, J., Elci, O., McCague, S., Cross, D., Marshall, K.A., Walshine, J., Kehoe, T.J., Reichert, H., Davis, M., Raffini, L., George, L.A., Hudson, F.P., Dingfeld, L., Zhu, X., Haller, J.A., Surace, F., Testa, F., Simonelli, F., Greco, P., Merlino, F., Perretti, M., D’Amico, M., Di Filippo, C., 2016. Activation of melanocortin receptors MC1 and MC5 attenuates retinal damage in experimental diabetic retinopathy. Mediat. Inflamm. 2016, 2016. Ruins, E.A., Arkadiusz, B., Angelica, E.C., Peden, A.A., Werner, E., Faundez, V., 2006. CRISPR/Cas9-mediated genome editing as an alternative to homologous recombination for in vivo gene therapy. Pigment Cell Melanoma Res. 21, 583–587. Rossini, S., Masino, R., Genualdo, C., Trota, M.C., Ferracanno, F., Keneva, M.K., Getting, S., Scott, K., Rundshagen, U., Zühlke, C., Opitz, S., Schwinger, E., Kiasmann-Kellner, B., 2004. BLOC0357 variant in patients with oculocutaneous albinism. Genet. Med. 23, 479–487. Rossini, S., Masino, R., Genualdo, C., Trota, M.C., Ferracanno, F., Keneva, M.K., Getting, S., Scott, K., Rundshagen, U., Zühlke, C., Opitz, S., Schwinger, E., Kiasmann-Kellner, B., 2004. BLOC0357 variant in patients with oculocutaneous albinism. Genet. Med. 23, 479–487. Rossini, S., Masino, R., Genualdo, C., Trota, M.C., Ferracanno, F., Keneva, M.K., Getting, S., Scott, K., Rundshagen, U., Zühlke, C., Opitz, S., Schwinger, E., Kiasmann-Kellner, B., 2004. BLOC0357 variant in patients with oculocutaneous albinism. Genet. Med. 23, 479–487. Rossini, S., Masino, R., Genualdo, C., Trota, M.C., Ferracanno, F., Keneva, M.K., Getting, S., Scott, K., Rundshagen, U., Zühlke, C., Opitz, S., Schwinger, E., Kiasmann-Kellner, B., 2004. BLOC0357 variant in patients with oculocutaneous albinism. Genet. Med. 23, 479–487. Rossini, S., Masino, R., Genualdo, C., Trota, M.C., Ferracanno, F., Keneva, M.K., Getting, S., Scott, K., Rundshagen, U., Zühlke, C., Opitz, S., Schwinger, E., Kiasmann-Kellner, B., 2004. BLOC0357 variant in patients with oculocutaneous albinism. Genet. Med. 23, 479–487.
Wassle, H., Grünert, U., Rohrenbeck, J., Boycott, B.B., 1989. Cortical magnification factor and the ganglion cell density of the primate retina. Nature 341, 643-646.

Welti, R., Niel, G., Fowler, D.M., Burhan, I., Luk, K.C., Stayrok, S.E., Lemmon, M.A., Raposo, G., Shorter, J., Kelly, J.W., Marks, M.S., 2009. N-terminal domains elicit formation of functional Pmel17 amyloid fibrils. J. Biol. Chem. 284, 5543-5555.

Wei, A.H., Zhang, D.J., Zhang, Z., Liu, X.Z., He, X., Yang, L., Wang, Y., Zhou, Z.Y., Zhang, M.R., Dai, L., Li, S., 2013. Exome sequencing identifies SLC24A5 as a candidate gene for nonsyndromic oculocutaneous albinism. J. Invest. Dermatol. 133, 1843-1840.

Weiter, J.J., Delori, F.C., Wing, G.L., Fitch, K.A., 1986. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. Invest. Ophthalmol. Vis. Sci. 27, 145-152.

Wilson, H.R., Mets, M.B., Nagy, S.E., Kressel, A.B., 1988. Albino spatial vision as an indicator of the presence of arrested viral infection. J. Clin. Pathol. 37, 591-596.

Wilson, S., Ginger, R.S., Dadd, T., Gunn, D., Lim, F.L., Sawicka, M., Sandel, M., Schnetkamp, P.P., Green, M.R., 2013. NCKX5, a natural regulator of human skin colour variation, regulates the expression of key pigment genes MC1R and alpha-MSH and alters cholesterol homeostasis in normal human melanocytes. Adv. Exp. Med. Biol. 961, 95-107.

Witmer, A.N., Vrensen, G.F., Van Noorden, C.J., Schlingemann, R.O., 2003. Vascular endothelial growth factors and angiogenesis in eye disease. Prog. Retin. Eye Res. 22, 1-29.

Woertz, E.N., Obmsa, B.S., Dunn, T.M., Chiu, S.J., Farsiu, S., Strul, S., Summers, C.G., Drack, A.V., Carroll, J., 2020a. Assessing ganglion cell layer topography in human albinism using optical coherence tomography. Invest. Ophthalmol. Vis. Sci. 61, 36. 2020b. Exome sequencing identifies GNAI3: another candidate gene to screen in persons with ocular albinism. PLoS One 5, e94157.

Yang, C., Shani, S., Tahiti, H., Ortiz, C., Gu, M., Laveije, J.C., Croteau, S., Hardy, P., 2020. A role for the membrane protein M6 in the Drosophila visual system. BMC Neurosci. 21, 291-298.

Yamamoto, H., Takeuchi, S., Takeda, M., Fujimura, T., Takasawa, S., Ogata, N., 2015. Human retinal pigment epithelium-derived factor inhibits oxygen-induced retinal neovascularization in mice. Mol. Med. Rep. 19, 563-569.

Yamamoto, R., Takeda, M., Fujimura, T., Takasawa, S., Ogata, N., 2015. Human retinal pigment epithelium (RPE)-derived factor inhibits oxygen-induced retinal neovascularization in a murine model. Pediatri. 35, 173-185.

Yamamoto, R., Takeda, M., Fujimura, T., Takasawa, S., Ogata, N., 2015. Human retinal pigment epithelium (RPE)-derived factor inhibits oxygen-induced retinal neovascularization in a murine model. Pediatri. 35, 173-185.

Yamamoto, R., Takeda, M., Fujimura, T., Takasawa, S., Ogata, N., 2015. Human retinal pigment epithelium (RPE)-derived factor inhibits oxygen-induced retinal neovascularization in a murine model. Pediatri. 35, 173-185.

Yamamoto, R., Takeda, M., Fujimura, T., Takasawa, S., Ogata, N., 2015. Human retinal pigment epithelium (RPE)-derived factor inhibits oxygen-induced retinal neovascularization in a murine model. Pediatri. 35, 173-185.
Zhao, H., Boissy, Y.L., Abdel-Malek, Z., King, R.A., Nordlund, J.J., Boissy, R.E., 1994. On the analysis of the pathophysiology of Chediak-Higashi syndrome. Defects expressed by cultured melanocytes. Lab. Invest. 71, 25–34.
Zhao, J., Iida, A., Ouchi, Y., Satoh, S., Watanabe, S., 2008. M6a is expressed in the murine neural retina and regulates neurite extension. Mol. Vis. 14, 1623–1630.
Zhao, J., Deng, X., Spec, C., Sonoda, S., Hsieh, C.L., Barron, E., Pera, M., Hinton, D.R., 2011. Polarized secretion of PEDF from human embryonic stem cell-derived RPE promotes retinal progenitor cell survival. Invest. Ophthalmol. Vis. Sci. 52, 1573–1585.
Zhao, J., Chen, C., Shi, W., 2015. Variations of vascular endothelial growth factor and pigment epithelial-derived factor Are related to retinopathy of prematurity in human babies. W. Indian Med. J. 65, 251–255.
Zhu, J.W., Ni, Y.J., Tong, X.Y., Guo, X., Wu, X.P., 2020. Activation of VEGF receptors in response to UVB promotes cell proliferation and melanogenesis of normal human melanocytes. Exp. Cell Res. 387, 111796.
Zucca, F.A., Basso, E., Cupakoli, P.A., Ferrari, E., Sulzer, D., Casella, L., Zecca, L., 2014. Neuromelanin of the human substantia nigra: an update. Neurotox. Res. 25, 13–23.