The homeobox gene *Otx2* in development and disease

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\textbf{A B S T R A C T}

The *Otx2* gene encodes a transcription factor essential for the normal development of brain, cerebellum, pineal gland, and eye. In the retina, *Otx2* has essential functions from early embryogenesis to adulthood. As soon as the optic vesicle is formed, the gene is required for retinal pigment epithelium specification. *Otx2* is also a key regulator of photoreceptor genesis and differentiation, and is required after birth for bipolar cells terminal maturation. *Otx2* expression is maintained in the differentiated retina wherein the gene is critical for the outer retina maintenance. In the visual cortex, the gene modulates the neuronal plasticity through a paracrine mechanism. *Otx2* heterozygous mutations in humans have been linked to severe ocular malformations associated with brain abnormalities and pituitary dysfunction. Recent studies have also established the *Otx2* gene as an oncogene for medulloblastoma, a malignant brain tumour originating in the cerebellum.

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1. Structure

The *OTX2* protein (Orthodenticle homeobox 2) is a homeodomain-containing transcription factor, which plays a critical role in forebrain and eye development. The *Otx2* gene is located on human chromosome 14q22.3 and is organized into five exons of which the first two exons are noncoding. Alternative splicing results in two *OTX2* mRNA transcripts encoding two isoforms: a full-length *OTX2* protein containing 297 amino acids (NP_068374.1), and a shorter isoform, a 289 amino acid residues long protein (NP_758840.1), which appears to be the more abundantly produced. In mice, the *Otx2* gene uses at least three distinct promoters acting at different times and in different cellular types throughout development (Courtois et al., 2003). The proximal promoter dominates during early embryonic stages and becomes progressively replaced by the most distal promoter as development proceeds. The intermediate promoter is specifically activated in the developing retina where it is maintained later, accounting for more than 30% of *Otx2* transcripts (Fossat et al., 2005). The human *OTX2* protein (Uniprot Accession #P32243) is represented in Fig. 1. The homeodomain region of the protein (amino acids 39–95) is located near the N-terminal region. The protein also contains a highly conserved SIWSPA peptide sequence (150–155), and 2 tandem tail motifs (255–263, 273–281) located within the C-terminal domain. The mouse *Otx2* protein shares 100% amino-acid identity with the human *OTX2* protein. As in humans, both 289- and 297 amino-acid isoforms are produced in vivo and cannot be distinguished by their activity in vitro. The protein can bind as a monomer with high affinity to the DNA *bicoid* target sequence: 5'-TAATCC-3' through its homeodomain (Chatelain et al., 2006). It can also accommodate related motifs such as 5'-TAACC-3' or 5'-TAAGCC-3'. The *Otx* tail motifs account for most of the transactivation activity of the protein while the 35 amino-acid domain at the N-terminus is required for full activity. In the cell, the transcription factor *Otx2* adopts an intra-nuclear location which appears to be controlled by two distinct domains acting in conjunction; a nuclear localization sequence located within the homeodomain that is not affected by mutations of residues critical for DNA binding, and a nuclear retention domain located in the central part of the protein (117–146). The SIWSPA motif is involved in protein–protein interaction with repressor members of the Groucho family (Puuelles et al., 2004). The basic RKQRRER motif at the N-terminus of the homeodomain allows the protein to be captured by neuronal cells (Beurdeley et al., 2012).

2. Expression pattern and function

2.1. *Otx2* is required for forebrain induction and specification

Together with *Otx1* and the more distantly related *Crx*, *Otx2* gene is a vertebrate ortholog to the *Drosophila* orthodenticle
homeobox gene (Simeone et al., 1992). The Otx2 homeobox gene is one of the most important genes for forebrain induction and head formation in vertebrates. In mouse embryo, Otx2 is already transcribed from the embryonic morula stage, and in the murine blastula, the Otx2 expression is detected in the entire epiblast. During the gastrulation process, the expression becomes restricted to the anterior part of the mouse embryo (Ang et al., 1996; Simeone et al., 1993). At this time, Otx2 has a crucial role in specification and regionalization of the rostral central nervous system. As other genes such as Lim1 or nodal, Otx2 is early required in the anterior visceral endoderm for normal specification of the anterior neuroectoderm (Acampora et al., 1995). Later in development, the expression covers most of the developing forebrain and midbrain neuroepithelium. The gene is also expressed in a dorsal area caudal to the midbrain/hindbrain boundary, which corresponds to the prospective cerebellum. This expression is in line with another essential function of the gene, which is to delineate the anatomic border between midbrain and hindbrain territories.

At later embryonic stages, the gene is expressed in several regions of the brain (diencephalon, mesencephalon, pineal gland, plexus choroid, and cerebellum) and also in sensory organs such as the inner ear, retina, and olfactory epithelium. In the developing human brain, the expression of the gene Otx2 is in line with the expression pattern described in the mouse. From 7 to 14 weeks postconception, the expression in human fetal brain is located in the diencephalon, mesencephalon, choroid plexus, and basal telencephalon. Later, an Otx2 expression is also found in the subcommissural organ, pineal gland, and cerebellum (Larsen et al., 2010).

Complete deletion of Otx2 in mice results in embryonic lethality due to absence of the rostral neuroectoderm fated to become forebrain, midbrain and rostral hindbrain (Acampora et al., 1995). Heterozygous knockout mice are characterized by variable phenotype (accephaly, holoprosencephaly, short nose, anophthalmia/microphthalmia, agnathia/micrognathia, or normal phenotype), depending on the genetic background (Matsuo et al., 1995).

In addition to its essential function in forebrain specification, Otx2 has other crucial roles in the developing brain. The gene is required for neurogenesis of dopaminergic neurons in mesencephalon, and also for the development of the pineal gland, which is the organ involved in melanin biosynthesis and circadian entrainment. Apart from a severe impairment in retinal photoreceptors development, the Otx2-deficient mice also show a total lack of pinealocytes in the pineal gland (Nishida et al., 2003). Otx2 is also required for the normal formation of the anterior pituitary. The Otx2 protein can bind to the Hesx1 gene promoter and has been found to be critical for Hesx1 expression (Spieler et al., 2004). It has also been shown that OTX2 transactivates the promoter of the POU1F1 (POU class 1 homeobox 1, also known as PIT1) gene (Dateki et al., 2008). The Hesx1 (Hesx homeobox 1) gene and the POU1F1 gene are involved in the early and late stages of pituitary development, respectively (Dateki et al., 2008; Zhu et al., 2005). Otx2, which is also expressed in the hypothalamus, has also a transactivation function for the GnRH promoter (Kelley et al., 2000). The GnRH gene, whose proximal promoter is regulated by the Otx2 homeoprotein, is essential for the gonadotropin-releasing hormone GnRH secretion in the hypothalamus (Dateki et al., 2010).

2.2. In the eye, Otx2 is crucial for retinal pigment epithelium specification and for photoreceptors formation and maintenance

The Otx2 gene acts in the very first steps of ocular embryogenesis. It is speculated that, in mammals, Otx2 could already have a key role in eye field specification as it has been shown in teleosts and amphibians. The eye field is a presumptive eye tissue existing at the neural plate stage several hours prior to optic vesicle formation. In vertebrates, the eye field specification requires a coexpression of several transcription factors such as Six3, Pax6, Rx1, Lhx2, or Six6. Overexpression of Pax6, Six3, Rx, or Six6 can induce the
formation of an ectopic eye tissue, but such an ectopic ocular formation seems to be possible only in a competent neural region where the Otx2 gene is already expressed (Chuang and Raymond, 2002). Moreover, it has been shown that expression of the eye-field transcription factors is induced by the coordinated action of Otx2 and the neural inducer noggin (Zuber et al., 2003). Within the optic vesicle, which is a bilateral expansion of neural tissue deriving from the lateral wall of the forebrain, the Otx2 gene is strongly expressed in the retinal pigment epithelium layer and weakly in the neural retina (Fig. 2A). At the beginning of the optic vesicle formation, the RPE and neural retina are formed from common precursors. At this time, Otx2 acts in conjunction with the transcription factor Mitf for normal specification of the presumptive RPE territory. In mice deficient for either Otx2 or Mitf, the development of the RPE is abnormal and is characterized by the loss of melanogenic gene expression. Moreover, an overexpression of Otx2 in avian neural retina cells induces a pigmented phenotype and, in vitro, it has been shown that OTX2 binds specifically to a bicoid motif present in the promotor regions of three genes involved in melanogenesis and terminal differentiation of the RPE: the Tyr gene encoding the melanogenic enzyme tyrosinase, the TRP-1 gene encoding the tyrosinase-related protein-1, and the gene QNR71 encoding a melanosome glycoprotein (Martinez-Morales et al., 2003). Later in prenatal retina development, Otx2 transcripts are mainly detected in the outer part of the neuroblastic layer, which corresponds to the photoreceptor layer of the mature retina. A weak expression is also detected in the inner aspect of the neuroblastic layer. Such a strong expression in the neuroblastic layer comes along with a crucial function, which consists to control photoreceptor cell fate determination and terminal differentiation (Fig. 3). Conditional ablation of Otx2 in the developing neural retina leads to loss of photoreceptor, bipolar, and horizontal cells (Nishida et al., 2003), the differentiating photoreceptor cells being converted into amacrine-like neurons. On the contrary, introduction of the Otx2 gene into non-retinal cells such as ciliary- or iris-derived cells can induce, by itself, photoreceptor-specific phenotypes with expression of photoreceptor specific genes such as rod opsin or recoverin (Akagi et al., 2004). It has been shown that, at the time of neural retina formation, Otx2 transactivates the cone-rod homeobox gene Crx (Nishida et al., 2003), which in turn is required for terminal differentiation of photoreceptor cells and normal circadian entrainment (Furukawa et al., 1999). In the differentiating outer retina, Otx2 is expressed in progenitors of both photoreceptor and bipolar cells. Transient expression of the transcriptional repressor Blimp1 in a subset of Otx2+/− precursors orients them towards the photoreceptor cell fate and prevents them to adopt a bipolar fate (Brzezinski et al., 2010). It has also been suggested that both Otx2 and Crx play a role in the terminal differentiation of the photoreceptors during development, since downregulation of photoreceptor-specific genes is more pronounced in the compound mutant Otx2+/−; Crx−/− retinas than in Crx−/− retinas (Koike et al., 2007). After birth, a cell-specific ablation of Otx2 in postnatal retinal bipolar cells leads to an impairment of both immunohistochemical and electrophysiological maturation of these cells (Koike et al., 2007), suggesting for Otx2 a functional role in the maturation of the bipolar cells during postnatal mouse retinal development. Beyond developmental stages, Otx2 expression is abundantly maintained in the RPE, photoreceptor and bipolar cells (Fig. 2B) (Fossat et al., 2007). A study based on a conditional self-knockout strategy showed that Otx2 is also essential in the mature retina for long-term maintenance of photoreceptors. Ablation of Otx2 in the adult retina causes progressive disappearance of photoreceptor cells and rapid alterations of retinal pigment epithelium (RPE) cells consisting in reduction in melanin content, extensive vacuolization, and loss of RPE contacts with disc-containing photoreceptor outer segments (Beby et al., 2010). Interestingly, embryonic and adult Otx2 functions seem to differ greatly. For instance, while Crx expression in precursors of photoreceptor cells is controlled by Otx2, that is no longer the case in adult retina, where the gene remains expressed after Otx2 removal (Beby et al., 2010).

2.3. The homeoprotein Otx2 appears to modulate postnatal plasticity in the visual cortex

Although Otx2 is not expressed in the visual cortex, the Otx2 protein has been found in the parvalbumin cells that control plasticity onset in the visual cortex. It has been shown that in Otx2 knockout mice, the suppression of the protein in the visual cortex impairs the maturation of the parvalbin cells, and conversely, a cortical infusion of exogenous Otx2 leads to an acceleration of the parvalbin-cells development (Sugiyama et al., 2008). Considering these findings, it has been hypothesized that Otx2 can modify the postnatal visual plasticity after a cell-to-cell transfer of the protein from the retina to the visual cortex (Sugiyama et al., 2008).

![Fig. 2. Expression of Otx2 in the developing and adult mouse eye. Section of embryonic (embryonic day E10.5, A) and adult (30 days post-natal, B) mouse retina from an Otx2+/−/GFP mouse line harbouring an insertion of the GFP gene within the Otx2 coding sequence (Fossat et al., 2007). L, lens; RPE, retinal pigment epithelium; NR, neural retina; INL, inner nuclear layer; ONL, outer nuclear layer; GCL, ganglion cell layer.](image-url)
2.4. The regulation of Otx2 expression in the eye relies on complex composite elements

An elaborated control of expression underlies the numerous functions exerted by Otx2. Several enhancers driving reporter gene activity in subdomains of Otx2 expression area in the epiblast, the anterior neural plate and the early embryonic forebrain and midbrain have been localized between 90 kb 5’ upstream and 115 kb 3’ downstream the coding exons (Kurokawa et al., 2004a, 2004b). The regulation of Otx2 expression in the retina also involves a complex set of enhancers and transcription factors. Otx2 gene expression starts in retinal precursors shortly before they exit the cell cycle and is maintained throughout life (Bovolenta et al., 1997; Fossat et al., 2007). This sustained expression has been proposed to be achieved through an auto-activation loop involving the binding of Otx2 protein to a site located within a 1.8 kb fragment upstream the translation initiation codon (Martinez-Morales et al., 2003). However, unchanged expression level of the Otx2 locus in adult Otx2-ablated retinas suggests that other activating mechanisms may also be at work (Beby et al., 2010). During embryogenesis, Otx2 expression is down-regulated in the developing retina of conditional Pax6 mutants, suggesting that Pax6 could activate Otx2 gene promoter (Oron-Karni et al., 2008). Similarly, double COUP-TFII conditional ablation in the developing eye leads to down-regulation of both Pax6 and Otx2 expression, suggesting a hierarchy with COUP-TFs at the top, activating Pax6, which in turn, would positively regulate Otx2 (Tang et al., 2010). The mechanism of this regulation has not been elucidated. Two elegant studies have investigated Otx2 cis-regulatory regions. Emerson and Cepko have used in vivo DNA electroporation in neonatal retina to test the ability of evolutionary conserved regions of the Otx2 gene (ECRs) to drive expression of reporter genes (Emerson and Cepko, 2011). Two ECRs, ECR1 and ECR2, which map to previously described promoter C and B respectively (Courtois et al., 2003), exhibit retinal specific enhancer activity. Further dissection of ECR2, located 2.5 kb upstream Otx2 translation initiation codon, indicates a composite element which functions as a unit. Fate mapping of ECR2-active cells reveals that the enhancer functions mainly in photoreceptor progenitors but is no longer active as they differentiate. In keeping with its evolutionary conservation, ECR2 also drives photoreceptor-specific expression in chicken. ECR2 is not active in other Otx2-expressing cells such as the RPE or the tectum. Muranishi and co-workers have used mouse transgenesis to dissect Otx2 regulatory regions (Muranishi et al., 2011). Based on transcriptional activity and conservation across mammalian species, they identified an element called EELPOT (embryonic enhancer locus for photoreceptor Otx2 transcription), located 16 kb upstream Otx2 translation initiation codon, which recapitulates Otx2 expression in the embryonic mouse retina. Strikingly, this 500 bp composite element also functions as a unit and has its activity restricted to embryonic stages, indicating that it could control initiation but not maintenance of Otx2 expression. Functional assays show that the embryonic homeodomain transcription factor Rax is mandatory for EELPOT activity, while Notch effectors act as repressors on this enhancer. Whether ECR2 and EELPOT enhancers act in common or separate pathways is not known yet. However, both studies illustrate the intricate dynamics and complexity of the regulation of Otx2 expression.

3. Disease involvement

3.1. OTX2 mutations are associated with blinding ocular defects and brain abnormalities

The major phenotype encountered in patients with heterozygous OTX2 mutations (Table 1) consists in severe ocular defects associated with brain malformations (Ragge et al., 2005) or pituitary abnormalities (Schilter et al., 2011). The OTX2 mutations described so far (Fig. 1) seem to be sparsely distributed throughout the protein, although a majority of them involves the last exon of the gene.

For the majority of the patients, the disease can be considered as a haploinsufficiency neuro-ocular disorder, and is typically associated with OTX2 nonsense or frameshift mutations introducing a premature termination codon and resulting in a truncated protein. Functional studies have shown that the truncation of the OTX2 protein leads to a significant decrease in transactivation activity without dominant-negative effect (Table 1). More rarely, the malformative disorder has been associated with an OTX2 missense mutation affecting a conserved residue. In some cases, such a
<table>
<thead>
<tr>
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<th>Mutation description</th>
<th>Functional analysis of the mutant protein</th>
<th>Gender Patient phenotype</th>
<th>Cranio-facial malformation</th>
<th>Pituitary abnormality or dysfunction</th>
<th>Brain malformation or neurological disorder</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.81delC</td>
<td>S28PfsX23</td>
<td>Del. resulting in FS and TP Het. NS Mut.</td>
<td>Severe LF, no DNE (Tajima et al., 2009)</td>
<td>F b M, b ONH</td>
<td>–</td>
<td>–</td>
<td>nr</td>
<td>Ragge et al., 2005 (patient 3A)</td>
</tr>
<tr>
<td>c.93C &gt; G</td>
<td>Y31X</td>
<td>Het. NS Mut.</td>
<td>nr</td>
<td>F L M</td>
<td>nr</td>
<td>nr</td>
<td>No seizure disorder</td>
<td>Wyatt et al., 2008 (case 3)</td>
</tr>
<tr>
<td>c.106dupC</td>
<td>R36PfsX25</td>
<td>Het. 1 bp Dup. resulting in FS and TP</td>
<td>nr</td>
<td>M R M</td>
<td>–</td>
<td>–</td>
<td>No significant developmental delay</td>
<td>Wyatt et al., 2008 (case 4)</td>
</tr>
<tr>
<td>c.106delC</td>
<td>R36GfsX15</td>
<td>Het. Del. resulting in FS and TP</td>
<td>nr</td>
<td>F nr</td>
<td>otocephaly</td>
<td>nr</td>
<td>nr</td>
<td>Chassaing et al., 2012 (sporadic case)</td>
</tr>
<tr>
<td>c.117_118delCC</td>
<td>R40GfsX47</td>
<td>Del. resulting in FS and TP</td>
<td>Severe LF, no DNE (Chatelain et al., 2006; Dateki et al., 2008; Tajima et al., 2009)</td>
<td>M b A, b ONH</td>
<td>–</td>
<td>–</td>
<td>Anterior commissure thin, developmental delay</td>
<td>Ragge et al., 2005 (patient 5)</td>
</tr>
<tr>
<td>c.136dupA</td>
<td>T46NfsX42</td>
<td>Het. Dup. resulting in FS and TP</td>
<td>nr</td>
<td>F b M, b ONH</td>
<td>microcephaly</td>
<td>nr</td>
<td>–</td>
<td>Schilter et al., 2011 (patient 1A)</td>
</tr>
<tr>
<td>c.203G &gt; C</td>
<td>R58P</td>
<td>Het. Miss. Mut.</td>
<td>LF, reduced transactivation of the IRBP and POU1F1 promoters, weak DNE on IRBP promoter</td>
<td>M R M, R vitreous opacities, R ONH L M, L ONH</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Schilter et al., 2011 (patient 1B)</td>
</tr>
<tr>
<td>c.214_217del16GC</td>
<td>A72HfsX15</td>
<td>Het. 4-bp Del. and 2-bp Ins. resulting in FS and TP</td>
<td>Severe LF (no transactivation functions), no DNE</td>
<td>F b M</td>
<td>nr</td>
<td>Normal pituitary function</td>
<td>nr</td>
<td>Dateki et al., 2010 (case 2)</td>
</tr>
<tr>
<td>c.221_236del16K</td>
<td>K74SfsX30</td>
<td>Het. 16-bp Del. resulting in FS and TP</td>
<td>Severe LF (no transactivation functions), no DNE LF (25% of activity), no DNE (Chatelain et al., 2006)</td>
<td>M R A, L M</td>
<td>nr</td>
<td>Pituitary hypoplasia, ectopic posterior pituitary, IGHD</td>
<td>Developmental delay</td>
<td>Dateki et al., 2010 (case 1)</td>
</tr>
<tr>
<td>c.265C &gt; G</td>
<td>R89G</td>
<td>Transversion with arginine-to-glycine change in the HD</td>
<td>Het. Miss. Mut.</td>
<td>F b M, b ONA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Ragge et al., 2005 (patient 2)</td>
</tr>
<tr>
<td>c.270A &gt; T</td>
<td>R90S</td>
<td>Het. Miss. Mut.</td>
<td>LF, inhibition of both DNA binding and transactivation</td>
<td>M R A</td>
<td>–</td>
<td>Small anterior pituitary gland, invisible stalk, ectopic posterior lobe, IGHD</td>
<td>–</td>
<td>Ashkenazi-Hoffnung et al., 2010</td>
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<tr>
<td>c.289C &gt; T</td>
<td>Q97X</td>
<td>Het. NS Mut.</td>
<td>Transcription activity probably reduced by up to 80%</td>
<td>M b extreme M</td>
<td>nr</td>
<td>–</td>
<td>nr</td>
<td>Wyatt et al., 2008 (case 6)</td>
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<tr>
<td>c.292delC</td>
<td>Q98NfsX11</td>
<td>Het. Del. resulting in FS and TP NS Mut.</td>
<td>nr</td>
<td>M or F L retinal coloboma, M/A or otocephaly or M/A + otocephaly</td>
<td>–</td>
<td>–</td>
<td>Variable degree of intellectual disability</td>
<td>Wyatt et al., 2008 (case 7)</td>
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<tr>
<td>c.295C &gt; T</td>
<td>Q99X</td>
<td>Het. Del. resulting in FS and TP NS Mut.</td>
<td>Severe LF, no DNE (Tajima et al., 2009)</td>
<td>M b A, b orbital cystic remnants, b ONA</td>
<td>–</td>
<td>–</td>
<td>Seizures, hippocampal malformation, hydrocephalus Autism, mental retardation</td>
<td>Chassaing et al., 2012 (family A)</td>
</tr>
<tr>
<td>c.313C &gt; T</td>
<td>Q105X</td>
<td>Het. NS Mut.</td>
<td>nr</td>
<td>F b A, b ONA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Ragge et al., 2005 (patient 6)</td>
</tr>
</tbody>
</table>

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<tr>
<th>Nucleotide mutation(^a)</th>
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<th>Patient phenotype</th>
<th>Cranio-facial malformation</th>
<th>Pituitary abnormality or dysfunction</th>
<th>Brain malformation or neurological disorder</th>
<th>Reference</th>
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<tr>
<td>c.371_372delAG S125WfsX11</td>
<td></td>
<td>Transcription activity probably severely reduced</td>
<td>M</td>
<td>b A</td>
<td>nr</td>
<td>nr</td>
<td>Mild developmental delay</td>
<td>Wyatt et al., 2008 (case 8)</td>
<td></td>
</tr>
<tr>
<td>c.397C &gt; A</td>
<td>P133T</td>
<td>Mis. Mut.</td>
<td>Normal transactivation activity, no DNE (Chatelain et al., 2006)</td>
<td>F</td>
<td>b M, L sclerocornea, b retinal detachment</td>
<td>–</td>
<td>nr</td>
<td>–</td>
<td>Ragge et al., 2005 (patient 7)</td>
</tr>
<tr>
<td>c.400C &gt; G</td>
<td>P134A</td>
<td>Mis. Mut.</td>
<td>Normal transactivation activity, no DNE (Chatelain et al., 2006)</td>
<td>M</td>
<td>L A, cataracts, dysplastic ears, brachycephaly</td>
<td>nr</td>
<td>–</td>
<td>Attention-deficit/hyperactivity disorder</td>
<td>Ragge et al., 2005 (patient 8)</td>
</tr>
<tr>
<td>c.402_403insC S135LfsX2</td>
<td></td>
<td>Ins. resulting in FS and TP</td>
<td>Severe LF, drastically reduced transactivation functions, no DNE</td>
<td>F</td>
<td>b A, b ONH</td>
<td>Cleft palate</td>
<td>Partial GH deficiency, normal pituitary gland structure</td>
<td>Developmental retardation</td>
<td>Dateki et al., 2008</td>
</tr>
<tr>
<td>c.405_406insCT S136LfsX43</td>
<td></td>
<td>Ins. resulting in FS and TP</td>
<td>Severe LF without DNE (no activation of the promoter of the HESX1 and POU1F1 gene)</td>
<td>M</td>
<td>b A, b ONH</td>
<td>–</td>
<td>Small anterior pituitary, ectopic posterior lobe, CPHD</td>
<td>Developmental delay, Chiari malformation</td>
<td>Tajima et al., 2009</td>
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<td>c.413C &gt; G</td>
<td>S138X</td>
<td>Het. NS Mut.</td>
<td>Leber congenital amaurosis</td>
<td>M</td>
<td>Leber congenital amaurosis</td>
<td>nr</td>
<td>Pituitary insufficiency</td>
<td>–</td>
<td>Henderson et al., 2009</td>
</tr>
<tr>
<td>c.537T &gt; A</td>
<td>Y179X</td>
<td>NS Mut.</td>
<td>Severe LF, no DNE (Chatelain et al., 2006; Dateki et al., 2008)</td>
<td>F</td>
<td>b M, b iris coloboma</td>
<td>–</td>
<td>nr</td>
<td>Developmental delay, seizures</td>
<td>Ragge et al., 2005 (patient 4A)</td>
</tr>
<tr>
<td>c.556_557insTATA</td>
<td>S186IfsX2</td>
<td>Het. Ins. Mut. resulting in FS and TP</td>
<td>Small anterior pituitary and absent posterior pituitary glands</td>
<td>F</td>
<td>b M, b ONH</td>
<td>microcephaly</td>
<td>Pituitary hypoplasia, ectopic posterior pituitary, CPHD</td>
<td>Hypotonia, developmental delay</td>
<td>Schilter et al., 2011 (patient 4)</td>
</tr>
<tr>
<td>c.562G &gt; T</td>
<td>G188X</td>
<td>Het. NS Mut.</td>
<td>mild LF (50% reduction in transactivation functions), no DNE</td>
<td>M</td>
<td>b M</td>
<td>nr</td>
<td>Pituitary hypoplasia, ectopic posterior pituitary, CPHD</td>
<td>Developmental delay</td>
<td>Dateki et al., 2010 (case 3)</td>
</tr>
<tr>
<td>c.674A &gt; G</td>
<td>N225S</td>
<td>Het. Mis. Mut.</td>
<td>normal DNA binding, DNE (inhibition of HESX1 expression)</td>
<td>M</td>
<td>nr</td>
<td>–</td>
<td>Ectopic neurohypophysis, hypoplastic adenohypophysis, CPHD</td>
<td>–</td>
<td>Dateki et al., 2010 (case 4)</td>
</tr>
</tbody>
</table>

\(^a\) Nucleotide mutations are numbered according to the OTX2 cDNA sequence NM_172337.1 transcript variant 2, using +1 for the adenine of the ATG initiation codon.
missense mutation can cause impairing of the protein activity without dominant negative effect. For instance, it has been shown that the R89G point mutant protein displays only 25% of the normal protein activity (Chatelain et al., 2006). In other cases, the amino acid change leads to a mutant protein acting as a dominant negative inhibitor on gene expression. For example, the N225S mutant binds normally to target genes but acts as a dominant inhibitor of the HESX1 gene expression, which is required for normal anterior pituitary development (Diazcok et al., 2008). Whether the reported missense mutations are pathogenic is not definitively established for all the cases, taking into account that some changes (P133T, P134A, T178S, A245V) described as missense mutations could also be considered as rare polymorphisms (Dateki et al., 2008; Schilte et al., 2011; Wyatt et al., 2008). To date, no clear genotype/phenotype correlation can be established for the OTX2 mutations (Table 1).

The range of ocular disorders caused by OTX2 mutations or deletions includes anophthalmia or microphthalmia, optic nerve or optic chiasm hypoplasia, ocular coloboma, and retinal dystrophies such as Leber congenital amaurosis. Two distinct syndromic diseases are actually linked to the haploinsufficiency of the OTX2 gene: the microphthalmia syndromic type 5 (MCOPS5, OMIM 610125), and the Combined Pituitary Hormone Deficiency 6 syndrome (CPHD6, OMIM 613986). In MCOPS5, the unilateral or bilateral microphthalmia/anophthalmia (M/A) is associated with ocular and extra-ocular features such as ocular coloboma, retinal dystrophy, optic nerve hypoplasia, agenesia of the corpus callosum, and brain malformations, the later leading to seizures and developmental delay of variable degree. Considering that OTX2 heterozygous mutations are diagnosed in 2–8% of patients with M/A, the haploinsufficiency of the OTX2 gene could be considered as the second most common genetic cause of M/A after SOX2, which is mutated in 10–20% of the anophthalmic or microphthalmic patients (Schilter et al., 2011). Although most of the patients with OTX2 mutations suffer from a major malformation of the eyes, mutations in OTX2 are also a rare cause of retinal dystrophy. A recent study suggests that mutations in OTX2 are responsible for less than 1% of infantile retinal disorders such as early onset retinal dystrophy or Leber congenital amaurosis (Henderson et al., 2009). CPHD6 patients present neonatal hypoglycemia and insufficient production of growth hormone, thyroid-stimulating hormone, luteinizing hormone, follicle stimulating hormone, and adrenocorticotropic hormone. An abnormal pituitary structure or function seems to be present in 30% of patients with a mutated allele of OTX2.

3.2. OTX2 has a potential role in otocephaly

Recently, it has been hypothesized that OTX2 mutations may contribute to otocephaly-dysgnathia complex (OMIM 202650), a rare malformative syndrome characterised by mandibular hypoplasia or agenesis, ventromedial auricular malposition, microstomia, and oroglossal hypoplasia or aglossia. Indeed, Chassaing and collaborators identified a framshifting mutation in the OTX2 gene in a family in which the affected members present with variable phenotype ranging from microphthalmia/anophthalmia to otocephaly (Chassaing et al., 2012). The same investigators also found an OTX2 mutation in one of nine non-related patients with otocephaly. These data suggest a potential role of OTX2 in otocephaly, taking into account that other genetic factors are required for the manifestation of the syndrome.

3.3. Role of Otx2 in medulloblastoma

Medulloblastoma, which originates in the cerebellum, is the most common malignant brain tumour in children. To date, the 5-year survival rates reaches only 50–60%. In recent years, several studies have pointed out an oncogenic role for OTX2 in medulloblastoma. It has been shown that OTX2 is overexpressed and/or genomically amplified in medulloblastoma cell lines and primary tumours (Boon et al., 2005; de Haas et al., 2006). Moreover, knockdown of OTX2 expression by small interfering RNAs leads to an inhibition of medulloblastoma cell growth in vitro (Di et al., 2005). A recent study also demonstrates that OTX2 acts as a repressor of myogenic and neuronal differentiation in the medulloblastoma cells (Bai et al., 2012).

4. Future studies regarding Otx2 functions in the eye

To better understand the function of Otx2 during eye development and in post-natal life, it is critically needed to document the repertoire of genes controlled by this transcription factor at various stages. Several genetic mouse models have been engineered, all relying on floxed Otx2 alleles, that allow ablation of the gene in various spatial and temporal conditions. Among these, a model coined Otx2 self-knockout allows to perform full ablation of the gene in all cells where it is expressed, at any stage of development (Fossat et al., 2006). It combines a floxed allele with a driver allele where a tamoxifen-inducible Cre gene substitutes for the coding sequence. This model was successfully used to abolish Otx2 expression in adults, revealing for the first time new functions of this gene in the mature retina (Beby et al., 2010). As it elicits complete and synchronous ablation of Otx2, it provides a mean to address the molecular functions of this transcription factor by deciphering the complement of its target genes in the developing and in the mature retina. Time-series analyses of gene expression starting at the time of tamoxifen administration will allow the monitoring of genes deregulated upon Otx2 ablation without interference of compensatory mechanisms as can be observed in endpoint studies. Such data will also benefit of complementary studies of Otx2 genome occupancy using ChIP-seq analyses. The evolution of the gene repertoire controlled by this transcription factor as retinal development and maturation proceed should greatly help the understanding of mechanisms involved in human diseases caused by Otx2 mutations.

Author contributions

Francis Beby: wrote the manuscript.
Thomas Lamonerie: wrote the manuscript.

Conflict of interest

None of the authors declare any conflict of interest.

References


