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***THE PLEIOTROPIC TRANSCRIPTIONAL REGULATOR COUP-TFI PLAYS MULTIPLE ROLES  
IN NEURAL DEVELOPMENT AND DISEASE***

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**ABSTRACT**

Transcription factors are expressed in a dynamic fashion both in time and space during brain development, and exert their roles by activating a cascade of multiple target genes. This implies that understanding the precise function of a transcription factor becomes a challenging task. In this review, we will focus on COUP-TFI (or NR2F1), a nuclear receptor belonging to the superfamily of the steroid/thyroid hormone receptors, and considered to be one of the major transcriptional regulators orchestrating cortical arealization, cell-type specification and maturation. Recent data have unraveled the multi-faceted functions of COUP-TFI in mouse brain development, including the neocortex, hippocampus and ganglionic eminences. However, despite NR2F1 mutations and deletions in humans have been linked to a complex neurodevelopmental disease mainly associated to optic atrophy and intellectual disability, its role during the formation of the retina and optic nerve remains unclear. In light of its major influence in cortical development, we predict that its haploinsufficiency might be the cause of other cognitive diseases, not identified so far. Mouse models offer a unique opportunity of dissecting COUP-TFI function in different regions during brain assembly; hence, the importance of comparing and discussing common points linking mouse models to human patients' symptoms.

**RUNNING TITLE**

COUP-TFI in development and disease

**KEYWORDS**

COUP-TFI, NR2F1, cortical area mapping, neurogenesis, cell migration, temporal competence, topography, chromatin remodeling, eye development, Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS)

## INTRODUCTION

In mammals, the rostral-most region of the developing neural tube, named the prosencephalon, is composed of the telencephalon (subdivided into dorsal and ventral) and diencephalon (forming the epithalamus, thalamus and hypothalamus). The dorsal telencephalon or pallium contains four main regions and each one will give rise to distinct cortical structures: the dorsal pallium develops into the neocortex, the medial pallium into the hippocampus, the lateral and ventral pallium into the olfactory cortex and amygdala. The ventral telencephalon or subpallium is subdivided into three highly proliferative regions, the lateral, medial and caudal ganglionic eminences (LGE, MGE and CGE, respectively), the preoptic area and the subpallial septum. The neocortex represents the structure that underwent the most dramatic expansion and complexity during evolution (Lui et al., 2011; Super and Uylings, 2001). While its role is principally based on the elaboration of sensory and motor inputs in lower vertebrates, the computational power of this structure has dramatically improved in mammals becoming thus the most important center for memory and language and, in humans, abstract thoughts and consciousness (Fernandez et al., 2016). Distinct populations of neural progenitors, located in the ventricular and sub-ventricular zones (VZ and SVZ, respectively) of the dorsal telencephalon, are responsible for the production of cortical neurons *via* direct or indirect neurogenesis (Florio and Huttner, 2014; Noctor et al., 2004; Ostrem et al., 2017). Shortly after birth, neocortical neurons migrate radially to the cortical plate and create a stereotyped radial structure consisting of six layers (de Juan Romero and Borrell, 2017; Greig et al., 2013; Kwan et al., 2012). The neocortical surface is also tangentially subdivided into different functional areas, each delegated to the analysis of different stimuli, and in which the six-layer structure and the circuitries are perfectly adapted to the specific tasks deputed to each functional area (Alfano and Studer, 2012; Grove and Fukuchi-Shimogori, 2003; O'Leary et al., 2007; Rakic, 1988). The "arealization" of the cortex implies thus the process leading to the functional partition of distinct neocortical tangential regions, each different in terms of cytoarchitecture and connectivity (Alfano and Studer, 2012; O'Leary and Sahara, 2008; Rash and Grove, 2006). Finally, even if placed more in "periphery", the neural retina and the optic nerve belong to the forebrain, as they are originating by a process of ventral evagination of the early prosencephalic wall. The evaginating area, called optic vesicle, undergoes an invagination event, which results in the formation of a lens vesicle surrounded by a dual-layered optic cup (Chow and Lang, 2001). As in the neocortex, neural progenitors differentiate into post-mitotic retinal cell types in an ordered fashion, and create six neuronal cell types, organized into three layers. Retinal ganglion cells are the only long-range projecting neurons of the retina, and send their axons towards the brains constituting the optic nerve (Heavner and Pevny, 2012).

In this review, we will focus on COUP-TF transcriptional regulators, which are orphan nuclear receptors belonging to the superfamily of the steroid/thyroid hormone receptors (Qiu et al., 1995; Ritchie et al., 1990; Wang et al., 1989), also called NR2F for Nuclear Receptor Family 2 (Nuclear Receptors Nomenclature,

1999). Two major homologs of COUP-TFs have been identified in vertebrates: COUP-TFI (also known as EAR-3 or NR2F1) and COUP-TFII (also named ARP-1 or NR2F2) (Alfano et al., 2013; Pereira et al., 2000; Wang et al., 1991). We will briefly introduce the molecular function of COUP-TFI as a transcriptional regulator able to both activate and repress target gene expression, and describe its expression pattern in the mouse developing brain. We will then explore COUP-TFI specific functions in different contexts: acquisition of areal identity, neurogenesis governing layer formation in the neocortex, long-range migration and fate acquisition of interneuron subtypes, balancing proliferation and migration in the telencephalon and hippocampus, and finally specification of different domains in the developing eye.

Recent reports show that COUP-TFI/NR2F1 follows a similar expression profile in the mouse and human brain (Alzu'bi et al., 2017a; Alzu'bi et al., 2017b; Yang et al., 2017), and is highly conserved in evolution with high protein homology between mice and humans (Alfano et al., 2013). Deletion and point mutations in the NR2F1 locus have been recently identified in patients with optic nerve atrophy associated with global developmental delay and intellectual disability (Al-Kateb et al., 2013; Bosch et al., 2014; Brown et al., 2009; Cardoso et al., 2009; Chen et al., 2016; Chen et al., 2017; Kaiwar et al., 2017; Martin-Hernandez et al., 2018), but also in children with infantile spasms and epileptic encephalopathies (Dimassi et al., 2016; Hino-Fukuyo et al., 2015). At present, COUP-TFI haploinsufficiency in humans represents a rare disease, recently named as Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS), and leading to a broad range of clinical phenotypes associated to syndromic and non-syndromic visual and cognitive deficits (Yang et al., 2017). When possible, we will compare data obtained by mouse studies with the clinical features described in human patients to highlight possible genotype-phenotype correlations, and discuss future approaches necessary to unravel the neuropathogenic mechanisms for refining the diagnosis of COUP-TFI haploinsufficient patients and proposing possible therapeutic treatments.

### **COUP-TF structure, molecular action and chromatin remodeling function**

The acronym COUP-TFs stands for “Chicken Ovalbumin Upstream Promoter Transcriptional Factors”, since these transcriptional regulators were originally found to regulate the expression of the chicken ovalbumin gene by specifically binding to its promoter region (Pastorcic et al., 1986; Sagami et al., 1986). A unified nomenclature system has renamed them as “NR2F” for subfamily 2 group F members of the steroid thyroid hormone superfamily of nuclear receptors (Nuclear Receptors Nomenclature, 1999). COUP-TFs are also defined “orphan” receptors, since the existence of their physiological ligands is still elusive (Pereira et al., 2000; Qiu et al., 1995; Wang et al., 1989; Wang et al., 1991). Two major homologs of COUP-TFs have been identified in vertebrates: COUP-TFI/NR2F1 and COUP-TFII/NR2F2 (Qiu et al., 1995; Wang et al., 1989). Their molecular structure is similar to that of other nuclear receptors of the same family, encompassing two highly conserved domains (**Figure 1A**): the DNA binding domain (DBD; consisting of two conserved Zinc-finger motifs) and the ligand-binding domain (LBD). The homology between COUP-TFI and COUP-TFII in humans is very high in both the DBD and LBD (see **Figure 1A**: 97% and 99% homology, respectively (Alfano et al., 2013; Qiu et al., 1995), suggesting that their function and targets might be very similar. Based on their highly conserved properties (Pastorcic et al., 1986), orthologs in mice, rats, *Xenopus*, chicken, hamster, *Drosophila*, zebrafish and sea urchin have also been cloned and their function characterized (reviewed in (Alfano et al., 2013). However, the homology of the N-terminus activation domain (AF-1), which is necessary for co-factor recruitment, is lower between COUP-TF members and between members of different species, suggesting that COUP-TF factors can bind to similar cis-responding elements on the DNA, but then greatly differ in their molecular interactions with co-factors, an important aspect for obtaining cellular specificity.

As transcriptional regulators, COUP-TFs can both function as activators or repressors to respectively promote or inhibit gene expression. This double action on their downstream target genes is obtained through different molecular and cellular mechanisms (Alfano et al., 2013; Cooney et al., 1992; Leng et al., 1996; Park et al., 2003). To act as repressors (**Figure 1B**), COUP-TFs can either homodimerize or heterodimerize with other members of the nuclear receptor superfamily, such as retinoic acid receptors (RAR), thyroid hormone receptors (TR), and vitamin D receptors (VDR), and compete for occupancy of their binding sites. COUP-TFs can bind to and sequester retinoid X receptors (RXR), universal heterodimeric partners for several other nuclear receptors (Evans and Mangelsdorf, 2014), reducing their availability and, consequently, the activation of their target genes. Alternatively, COUP-TFs can repress transcriptional activity by binding to the LBD of other nuclear hormone receptors, a process termed trans-repression (Leng et al., 1996). Finally, COUP-TFs can regulate gene expression in a more direct way by binding to two GGTC motifs, named directed repeats (DR), separated by a variable number of nucleotides (1-5) (Cooney et al., 1992; Hwung et al., 1988; Montemayor et al., 2010). Although COUP-TF members show the highest binding

affinity for the imperfect direct repeat AGGTCA separated by one nucleotide (DR1), a large-scale genome-wide study has challenged several of the published putative COUP-TFI binding sites *via* a bioinformatics approach unraveling a much higher diversity of binding sites in the non-coding genomic regions than previously described (Montemayor et al., 2010). Other studies have showed that COUP-TFs tend to recruit *in loco* other co-repressors, such as N-Cor, SMRT and histone deacetylases, *via* interaction with their C-terminal repression domain (Shibata et al., 1997; Smirnov et al., 2000). However, COUP-TFs can also positively regulate gene expression (**Figure 1C**) by directly binding to DR elements and/or indirectly influence the expression of a gene in the context of several other transcription factors (TFs), acting thus as an accessory co-factor in a transcriptional complex (Hall et al., 1995). Finally, COUP-TFs can activate transcription by protein-protein interaction, for example *via* Sp1, as is the case for OTX2 in the eye (Tang et al., 2010) or for NGFIA/EGR1/ZIF268 in cell lines (Pipaon et al., 1999). In summary, COUP-TFI binds to the DNA as a dimer, interacts with the basal transcriptional machinery either directly or indirectly *via* co-factors, and acts differently on transcriptional activity depending on two major factors: the cell context and the presence of distinct co-activators or co-repressors.

Interestingly, the majority of COUP-TF target sites are associated with increased H3K9 acetylation and enrichment for co-activators, such as CBP and SRC1, creating in this way an open chromatin environment (Montemayor et al., 2010). The interaction of COUP-TFI with chromatin remodelers adds a further step of complexity to its cellular function, as chromatin remodeling increases the effects on gene expression, influences a broader plethora of targets and fine-tunes expression levels. As an example, genomic regions co-bound by COUP-TFI and the master neural crest regulator TFAP2A are characterized by elevated percentage of active enhancer histone marks (Rada-Iglesias et al., 2012), concurring to the maintenance of a permissive chromatin state and providing docking sites for the co-activator p300 (Rada-Iglesias, 2013). Furthermore, the DNA methyltransferases DNMT3a/b cooperate with COUP-TFI to enhance gene expression by chromatin active demethylation (Gallais et al., 2007). However, COUP-TFI action can also change in different systems, such as dormant cancerous cells (Sosa et al., 2014) or virally Ad12-infected human cells (Smirnov et al., 2000), where it acts mainly as global chromatin repressor. A ligand-activated conformational change, triggering the displacement of co-repressors (HDAC complex) and the recruitment of co-activators, demethylases and histone acetyltransferases (HAT complex) could explain this change of function, since it is a common mechanism for nuclear receptors (Cooney et al., 2001). . Thus, on one side, binding sites occupied by COUP-TFs are subordinated to chromatin modifications and may be functional only at a certain stage of development, aging or disease state; on the other side, COUP-TFI itself acts as a chromatin remodeler, contributing *via* the interactions with co-factors to the chromatin state, and acting as an activator or a repressor in a tissue- or developmental time- specific manner.





### **Gradients in the brain: COUP-TFI is expressed at different levels in regionalized prosencephalic regions**

COUP-TFI and its homolog COUP-TFII are expressed in distinct, but also overlapping domains in the developing mouse brain, and their patterns are highly dynamic during development (Armentano et al., 2006; Flore et al., 2017; Qiu et al., 1994; Tripodi et al., 2004). In the mouse prosencephalon, COUP-TFI is expressed in the neuroectoderm as early as the anterior neural plate closes (Armentano et al., 2006; Qiu et al., 1994). At later stages of development, COUP-TFI expression expands in several telencephalic regions: the cerebral cortex (archicortex, neocortex, and paleocortex primordia), the preoptic area, the thalamus and the lateral, medial and caudal ganglionic eminences (LGE, MGE and CGE, respectively) (Armentano et al., 2006). As a general trend, COUP-TFI expression peaks at mid-gestation, declines before birth, but is still maintained in the postnatal and adult brain in restricted regions (Alfano et al., 2013; Flore et al., 2017; Liu et al., 2000; Zhou et al., 1999).

One of the most characteristic features of COUP-TFI is its expression in gradients in the majority of structures in which it is present. In the dorsal telencephalon, the future cerebral cortex, COUP-TFI expression is detected in a high caudal to low rostral gradient (**Figure 2A**) (Armentano et al., 2006; Liu et al., 2000; Tomassy et al., 2010; Zhou et al., 2001), whereas its homolog COUP-TFII remains restricted to the caudal-most telencephalic regions (Armentano et al., 2006; Qiu et al., 1994). A second COUP-TFI gradient follows the latero-medial neurogenic gradient with a high lateral to low medial expression profile (**Figure 2A,B**). The neocortical COUP-TFI expression gradient, initially established in progenitor cells, is maintained in post-mitotic young neurons that differentiate and migrate to the cortical plate (Armentano et al., 2007; Liu et al., 2000; Zhou et al., 2001). As described below, the progenitor COUP-TFI gradient plays a key role in the specification of areal identities of the neocortical map. However, it is only a draft for early protomap organization, which is mainly controlled by COUP-TFI post-mitotic expression and function (Alfano et al., 2014).

A characteristic COUP-TFI expression gradient is also observed in other brain structures, such as the archicortex during all phases of hippocampal and dentate gyrus development (Flore et al., 2017; Parisot et al., 2017), the ventral telencephalon in which COUP-TFI is highest in the caudal-most ganglionic eminence, called CGE (Flames et al., 2007; Touzot et al., 2016), the thalamus with high expression in the sensory thalamic nuclei (Armentano et al., 2006; Chou et al., 2013), and the retina with a clear ventro-dorsal gradient maintained during eye development (Tang et al., 2010; Tang et al., 2015). We will go in more details in all these structures in the sections below. Furthermore, COUP-TF genes are expressed in other regions of the central and peripheral nervous system (such as the cerebellum, the spinal cord and the peripheral ganglia; (Qiu et al., 1994; Yang et al., 2017), as well as during organogenesis of non-neural structures, including testes, ovary, prostate, skin, kidney, lung, stomach, pancreas and salivary gland

(Pereira et al., 1995). Little is still known on the function of COUP-TFI in all these structures, but they might influence the phenotype observed in null mutant mice and in human patients, since high human NR2F1 expression levels have been confirmed by transcriptomic analysis not only in the brain, but also in adrenal gland, bladder, kidney, lung, ovary, placenta and prostate, as reported on NCBI (<https://www.ncbi.nlm.nih.gov/gene/7025>).

### **Cortical arealization: when the neocortex specializes into functional primary areas**

The mammalian neocortex is the seat of the most complex cerebral functions, such as abstract thinking, speech and consciousness. The “arealization” or area parcellation of the neocortex is the biological process leading to the tangential partitioning and specialization of different cytoarchitectures along the neocortical surface aimed at adapting the basic six-layered cortical structure to the functionality of individual areas (Alfano and Studer, 2012; O'Leary and Nakagawa, 2002; O'Leary and Sahara, 2008). Four primary areas constitute the basic plan of the mammalian neocortex: motor (M1), somatosensory (S1), auditory (A1), and visual (V1). Two major steps, responsible for shaping the position and size of neocortical areas, have been proposed to date: an early protomap of gradient patterning genes and an activity-dependent refinement of cortical area functionality (**Figure 2C**).

Several morphogens such as Fibroblast Growth Factors (FGFs), Sonic Hedgehog (SHH), Retinoic acid (RA), Bone Morphogenetic Proteins (BMPs) and WNTs orchestrate the patterning of the mammalian forebrain; they are produced by signaling centers and can diffuse in several directions (O'Leary and Nakagawa, 2002; Sansom et al., 2005; Shimogori et al., 2004; Shimogori and Grove, 2005). By antagonizing each other and by functioning in a dose-, context- and time-dependent manner, these signaling pathways establish proper coordinates along the anterior-posterior (A-P) and dorso-ventral (D-V) axes (Fukuchi-Shimogori and Grove, 2001; Greig et al., 2013; Grove and Fukuchi-Shimogori, 2003; Sansom and Livesey, 2009; Sur and Rubenstein, 2005), instructing cells to “know” where they are, how much they need to proliferate and which identity to adopt. To this purpose, signaling morphogens either repress or promote area patterning genes, such as Pax6, Sp8, Emx2 and COUP-TFI, among others (**Figure 2C**), which can thus be considered effector genes of this process (Alfano and Studer, 2012; Grove and Fukuchi-Shimogori, 2003; O'Leary and Sahara, 2008). Neocortical progenitors express different levels of a distinct combination of area patterning genes able to provide precise spatial coordinates that cells can translate into a positional code. Thanks to this “cooperative concentration model”, a primordial rough areal map of cortical coordinates, termed “protomap” can be established (O'Leary and Sahara, 2008; Sansom et al., 2005). Gradient expression of area patterning genes in progenitors will however give a pre-identity to different areas, with still no clear-cut boundaries and functions (Greig et al., 2013).

Among FGF members, FGF8 plays a major role during the establishment of the neocortical area map, in part, by inducing rostral and repressing caudal genes. COUP-TFI gradient expression is modulated by FGF8 along the rostro-caudal telencephalic axis *in vivo* (Garel et al., 2003; Storm et al., 2006; Toyoda et al., 2010) and in organotypic explants (Sansom et al., 2005). On the other side, COUP-TFI inhibits FGF signaling (but not FGF8 expression) and antagonizes the expression of the major FGF8-activated gene, Sp8 (Borello et al., 2013; Faedo et al., 2008; Sahara et al., 2007), building thus a reciprocal antagonistic modulation. The molecular network downstream of COUP-TFI-mediated regulation of FGF8 signaling has started to emerge: overexpressing high COUP-TFI levels will reduce phosphorylation of ERK (a player of the MAPK cascade, activated by FGFs) and expression of Ets genes (downstream targets of MAPK signaling), whereas two inhibitors of the MAPK signaling, Sprouty 1 and 2 are activated (Faedo et al., 2008; Faedo et al., 2010). However, little is known on how FGF8 inhibits COUP-TFI. We recently found that FGF8 controls COUP-TFI gradient protein levels *via* the upregulation of *miRNAs* targeting its 3'UTR (MB, MS, unpublished). This is reminiscent of the modulation of PAX6 protein gradient in neural stem cells of the lateral ventricles by means of *mir7a* action (de Chevigny et al., 2013). Thus, besides morphogens, *miRNA*-mediated fine-tuning of protein expression can be considered a common mechanism for modulating spatial and regionalized information of key patterning genes.

The second step of the arealization process is controlled postnatally, when boundaries of neocortical areas become more defined (**Figure 2C**) thanks to an activity-mediated refinement following the arrival of thalamocortical afferences (TCAs) relaying sensory inputs to their respective cortical targets (Alfano and Studer, 2012; Rakic et al., 2009; Rubenstein et al., 1999; Sur and Rubenstein, 2005). COUP-TFI is expressed in thalamic progenitors and in differentiating thalamic neurons from mid-gestation, and maintained at high levels in the lateral geniculate and ventro-posterior nuclei of the dorsal thalamus at perinatal stages (Armentano et al., 2006). While constitutive and cortex-specific COUP-TFI deletion leads to abnormal patterning of primary areas and thalamocortical mis-wiring (Armentano et al., 2007; Zhou et al., 2001), selective deletion of COUP-TFI in sensory thalamic nuclei affects the organization of higher order areas (Chou et al., 2013). Therefore, COUP-TFI gradient expression seems to act on both mechanisms: (i) to specify a proto-sensory neocortical map in response to FGF8, and (ii) to define primary and secondary cortical maps by guiding TCAs to their topographic cortical targets. These two mechanisms are fundamental in determining area-specific functional properties (Alfano and Studer, 2012; Chou et al., 2013; O'Leary and Sahara, 2008; Sur and Rubenstein, 2005).

### **COUP-TFI, a key transcriptional regulator imparting cortical sensory identity**

The direct role of COUP-TFI in cortical area specification was initially unclear, as this factor is expressed in the cortex and thalamus and involved in a wide range of neural developmental processes possibly influencing neocortical patterning (reviewed in (Alfano et al., 2013)). In the constitutive *COUP-TFI null (KO)* mouse, TCAs failed to reach the cortical plate leading to the gradual death of layer IV neurons (Zhou et al., 1999). Thus, the strong caudal shift of sensory areas initially described in these mutants (Zhou et al., 2001) could have been influenced by diminished thalamocortical inputs. Furthermore, due to feeding problems, the majority of COUP-TFI mutants die perinatally (Qiu et al., 1997), which makes it difficult to study cortical arealization at postnatal stages. COUP-TFI selective ablation in the cortex (*COUP-TFI CKO*) has overcome both the animal survival problem and a potential influence from impaired thalamo-cortical innervation demonstrating an intrinsic role for COUP-TFI in patterning the developing neocortex in proto-areas, independently from thalamic inputs (Armentano et al., 2007). Moreover, while the neocortex of *COUP-TFI KO* mutants was described as a uniform “area-less” cortex (O'Leary and Sahara, 2008; Zhou et al., 2001), careful analysis on *CKO* mutants revealed that all sensory areas were present, even if substantially altered in size and position (Armentano et al., 2007). Indeed, while frontal motor areas expanded to occupy most of the cortical surface, primary sensory areas were severely reduced, and aberrantly compressed caudally (**Figure 2D**). Nonetheless, the miniaturized sensory cortex retained its normal genetic identity, was properly connected to the thalamus by area-specific thalamic inputs (Armentano et al., 2007) and maintained in adult cortices (MS, unpublished). This demonstrates that area mapping is an intrinsic event independent of thalamic inputs and that COUP-TFI plays a key role in establishing the border between presumptive motor and sensory cortex. Furthermore, in *COUP-TFI CKO* mutants the presumptive somatosensory region changed its laminar organization and topographic thalamic connectivity to mimic fronto-motor area structures (Tomassy et al., 2010). In physiological conditions, neurons of S1 correctly differentiate thanks to both the COUP-TFI-mediated inhibition of an intrinsic “motorizing” genetic program, and the direct induction of a sensory one (Alfano et al., 2014). This implies that radial (laminar) specification of neocortical layers is tightly linked to tangential (areal) specification, and that COUP-TFI, amongst other factors, couples these two events to allow proper functioning of primary sensory areas (Alfano et al., 2014; Armentano et al., 2007; Greig et al., 2013; Tomassy et al., 2010).

### **FGF8/COUP-TFI antagonism shapes the protomap *via* multiple effectors**

Area patterning genes are expressed along the ventricular proliferative region, each one with its specific gradient. While Sp8 and Pax6 display a higher expression in the frontal cortex and promote the growth and specification of rostral areas, Emx2, strongly expressed in caudal regions, helps the formation of caudal sensory areas (reviewed in (Sansom and Livesey, 2009)). Notably, the defects in cortical arealization observed in COUP-TFI mutant brains (increase of fronto-motor features and shrinkage of caudal areas;

(Armentano et al., 2007) are massive compared to less severe area shifts resulting from the absence of other area patterning genes: constitutive Pax6 loss-of-function results in a small reduction of frontal areas (Bishop et al., 2000; Bishop et al., 2002), and Pax6 cortical inactivation or gain-of-function do not seem to change area positioning or thalamo-cortical mis-wiring (Manuel et al., 2007; Tuoc et al., 2009), even if the overall S1 area is reduced and the thalamus re-patterned upon Pax6 cortical inactivation (Zembrzycki et al., 2013). On the contrary, Emx2 loss- and gain-of-function affect caudal area size, even if moderately (Bishop et al., 2000; Hamasaki et al., 2004; Mallamaci et al., 2000). The role of Sp8 in areal mapping is still controversial, as it promotes frontal identity (Zembrzycki et al., 2007) or inhibits frontal growth (Sahara et al., 2007), depending on the experimental procedure used to test its function. Sp8, which responds to FGF8, seems to act mainly on the cell growth of the rostro-medial and ventral telencephalon, resulting in severe gene expression deregulation, most probably due to secondary size defects (Zembrzycki et al., 2007).

*Why does COUP-TFI have such a strong effect, compared to other area patterning genes?* A possible explanation is that COUP-TFI might be at the top of the hierarchy governing early area patterning (**Figure 3**). In this sense, Pax6 and Emx2 will act as two effectors, which mutually regulate each other, among the several genes possibly regulated by COUP-TFI to impart cortical identity (Greig et al., 2013; O'Leary and Sahara, 2008). Even if Pax6 expression is slightly increased and Emx2 decreased upon COUP-TFI loss, these changes are too small to account for the huge area identity shift observed in mutants (Alfano et al., 2014; Armentano et al., 2007; Faedo et al., 2008). Other genes belonging to the FGF pathway act in strong connection with COUP-TFI and could have a stronger influence on area patterning. FGF8 and its downstream target Sp8 promote rostral identity and perform this function also by inhibiting COUP-TFI, which in turns antagonistically represses FGF signaling (Borello et al., 2013; Faedo et al., 2008; Faedo et al., 2010; O'Leary et al., 2007). While the exact molecular cascade activated by COUP-TFI to impart sensory identity remains still unclear, it is reasonable to hypothesize that FGF signaling in general, rather than single specific patterning genes, plays a pivotal role by influencing a broader group of target genes, cooperatively with COUP-TFI (**Figure 3**).

### **Post-mitotic control of area identity**

Another possible explanation about the strong role of COUP-TFI during area mapping is its maintained gradient expression in post-mitotic cells. Indeed, COUP-TFI differs from other area patterning genes expressed in progenitors (Pax6, Emx2), in intermediate progenitors (Tbr2, Ap2y) or solely in post-mitotic cells (Tbr1, Lhx2, Pbx1), since its expression gradient is established in progenitors and maintained in the cortical plate in post-mitotic neurons (Alfano et al., 2014). Differentiation of neuronal subtypes is in large part regulated in early differentiating neurons by a series of transcriptional regulators, possibly also involved in area specification (Fishell and Hanashima, 2008; Greig et al., 2013). For example, the genetic

inactivation of *Bhlhb5* and *Tbr1* slightly impairs neocortical arealization beside cell type specification, in line with the concept that laminar and areal identity are tightly linked during corticogenesis (Bedogni et al., 2010; Joshi et al., 2008). This implies that positional regionalized identity initially specified in mitotic progenitors (the “protomap”) is only a first raw map waiting for further cues acting in neuronal progenies. Even more interestingly, as soon as newborn neurons leave the cell cycle, they are still plastic enough to change their identity independently of the protomap gradient established in progenitors (Alfano et al., 2014). Indeed, COUP-TFI inactivation solely in cortical post-mitotic cells reproduces the severe areal defect obtained upon loss of COUP-TFI in progenitors, and reciprocally overexpression of post-mitotic COUP-TFI in a constitutive KO model, completely rescues sensory identity, laminar specification and topographic thalamocortical connectivity in S1 (Alfano et al., 2014) (**Figure 2D**). Remarkably, ectopic COUP-TFI expression in rostral cortex will impart sensory identity in motor areas, resulting in an abnormally expanded sensory neocortex with proper topographic connectivity. This implies that COUP-TFI post-mitotic expression is necessary and sufficient to specify sensory (caudal) areal identity in the developing neocortex. Similar mechanisms for the consolidation of neocortical arealization have started to emerge for other post-mitotically-expressed genes, such as *Lhx2* (Zembrzycki et al., 2015), *Pbx1* (Golonzhka et al., 2015) and *Ctip1* (Greig et al., 2016), indicating that genes expressed in young post-mitotic neurons play a fundamental role in neuronal and area specification, independently of progenitor identity.

### **Reprogramming cell identity by manipulating COUP-TFI levels**

Once considered irreversible processes, cell differentiation and lineage commitment have now been proved to be “re-programmable” by forced expression of TFs (Masserdotti et al., 2016). Cell identity can be reconverted in a “direct” way, without passing through an undifferentiated state, by over-expressing the right combination of factors: the gene *Ascl1* is potent enough to directly convert fibroblasts into functional neurons (Vierbuchen et al., 2010), but not into distinct subclasses of neurons, which probably need more specialized factors expressed in early post-mitotic neurons. Indeed, over-expression of *Fezf2* re-program young layer II-IV neurons into layer V identity (De la Rossa et al., 2013; Rouaux and Arlotta, 2013; Vierbuchen et al., 2010). As previously mentioned, high ectopic expression of COUP-TFI in the rostral mouse cortex, in a region that normally shows low levels of COUP-TFI, is sufficient to reprogram motor/rostral to sensory/caudal identity, to induce expression of sensory markers and importantly to re-wire topographic thalamo-cortical connectivity (Alfano et al., 2014). This suggests that COUP-TFI could be potentially used as a reprogramming TF, whose forced expression confers specific neuronal and regionalized identities. Interestingly, this was tested in embryonic stem cells, where COUP-TFI over-expression promoted the direct acquisition of neuronal identity in a very efficient way, comparable to the results obtained with *Ascl1*-mediated reprogramming (Yamamizu et al., 2013). In these first assays, COUP-TFI-induced neural progenitors adopted a GABAergic-like identity (Teratani-Ota et al., 2016; Yamamizu et

al., 2013), but one can speculate that the combinatorial expression with other TFs would direct the acquisition to specific neuronal subtype identities, as it happens for COUP-TFI-expressing neural progenitors *in vivo* (Alfano et al., 2014). Since COUP-TFI has been proposed to induce a permissive chromatin environment (Montemayor et al., 2010), it becomes an interesting candidate for cell reprogramming allowing proper re-expression of cell-type specific neuronal determinant genes (Smith et al., 2016).

### **Regulation of temporal competence in neural progenitors**

Besides controlling neuronal identity in post-mitotic cells, the next question is whether COUP-TFI expressed in mitotic progenitors would be involved in regulating the number and/or identity of neocortical neurons produced at specific times during neurogenesis. Master genes required in forebrain patterning often operate a multi-step control of several processes at the same time, for example by linking neurogenesis and proliferation to identity specification. Accordingly, COUP-TFI can control neurogenesis (Faedo et al., 2008) and link this process to cell identity acquisition during the transition between neurogenesis and gliogenesis (Naka et al., 2008) and to the sequential generation of distinct neuronal subtypes (Tomassy et al., 2010). This link between neurogenesis and identity specification has been conserved during evolution and has been described for COUP-TFI orthologs in *Drosophila* and *C. elegans*.

During corticogenesis, high COUP-TFI levels promote cell cycle exit: early-born neurons are produced in high numbers at the expense of the progenitor pool, which gets progressively depleted (Faedo et al., 2008). This suggests that COUP-TFI might regulate neural progenitor competence at different developmental time to generate distinct neuronal subtypes. The first data arguing for COUP-TFs as a temporal controller during CNS development came from studies in the fly (reviewed in (Pearson and Doe, 2004)). In *Drosophila*, the COUP-TF homolog SVP is well known to regulate cell fate acquisition in differentiating neuroblasts, acting thus as a “switching factor” controlling the temporal sequence by which neuronal subtypes are generated (**Figure 4A**). The temporal specification of *Drosophila* neuroblasts is controlled by the sequential expression of TFs, where single factors are sufficient to trigger the generation of a distinct neuronal subtype at a given-time point (Benito-Sipos et al., 2011; Kanai et al., 2005; Li et al., 2013). In rodents, knocking-down COUP-TFI and COUP-TFII induces the generation of early- at the expense of late-born neurons, indicating that COUP-TFs might promote acquisition of upper layer neuronal identity (**Figure 4B**)(Naka et al., 2008). However, genetic inactivation of mouse COUP-TFI in cortical progenitors leads to an overproduction of layer V neurons at the expense of layer VI, which are normally born one day before (Tomassy et al., 2010). This implies that COUP-TFI is also a fine temporal regulator of early-born neuronal subpopulations by delaying the acquisition of a subcerebral fate identity (layer V) and promoting a corticothalamic layer VI cell identity. This is reminiscent of the *C.elegans* Unc55 ortholog, which discerns two classes of motor neurons (Zhou

and Walthall, 1998), and of *Drosophila* SVP, which controls photoreceptor cell identity (Mlodzik et al., 1990) or Ap neuron sub-type specification (Benito-Sipos et al., 2011). Thus, these nuclear receptors can regulate the temporal identity of neuronal precursors by allowing them to become specified into distinct neuronal subtypes in a very narrow time window, a role that has been evolutionarily conserved from invertebrates to vertebrates (Alfano et al., 2013; Jacob et al., 2008).

As part of their role in regulating cell identity in a spatially-controlled manner, COUP-TFs also control the gradual acquisition of gliogenic competency in progenitor cells, allowing them to become either neurons or glia during development (**Figure 4B**) (Okano and Temple, 2009). When both COUP-TF genes are knocked down in neurospheres, neurogenesis is sustained in time and the onset of gliogenesis is delayed (Naka et al., 2008), suggesting a synergistic effect of COUP-TFI and COUP-TFII in gliogenic competence. Interestingly, this effect seems to be controlled by epigenetic mechanisms: COUP-TF genes can release epigenetic silencing of the GFAP promoter, a glia master gene, allowing it to respond to astrocyte-inducing environmental signals such as gliogenic cytokines (LIF and BMP2), indicating that COUP-TFs are required to confer responsiveness to gliogenic inducing signals (Naka et al., 2008; Okano and Temple, 2009). In summary, the COUP-TFI-mediated temporal specification function in neural progenitors controls key biological transitions, such as neurogenesis versus gliogenesis, generation of early- versus late-born neurons, but also more subtle events, such as the identity acquisition of distinct neuronal subtypes generated within few hours.

#### **Can regionalized progenitor competence affect the size and shaping of neocortical proto-areas?**

Proper area- and laminar-specification implies a spatiotemporal control on the lateral expansion of individual cortical areas coupled to radial growth, which will determine cortical thickness (Pierani and Wassef, 2009). COUP-TFI is expressed in a high ventral to low dorsal gradient in cortical progenitors (Armentano et al., 2006), which perfectly correlates with the neurogenetic gradient along this axis. Loss- and gain-of-functions of COUP-TFI influence the balance between apical and basal progenitors and thus the progression into neurogenesis (Faedo et al., 2008). By fine-tuning this balance in a regionalized-specific manner, COUP-TFI could modulate neuron production and ultimately affect cortical area size. We can hypothesize two different scenarios (schematized in **Figure 4C**). First, a region with a higher rate of asymmetric divisions undergoing neurogenesis would increase neuronal production locally, and thus a larger number of neurons would reach the cortical plate, increasing the thickness of a given area (**Figure 4C**, upper scheme). As an alternative, a cortical region with increased symmetric proliferative divisions would amplify the progenitor pool, leading to a lateral (or “tangential”) expansion of the ventricular surface (**Figure 4C**, lower scheme). This would translate into an increased number of neuronal columns ultimately leading to an enlarged area surface.



Regionalized altered growth has been already described in mutants in which FGF signaling is perturbed. Targeted deletion of FGF10 extends the period of symmetric progenitor divisions by delaying neurogenesis, and ultimately leads to an overproduction of neurons and tangential expansion of the frontal/motor cortex (Sahara and O'Leary, 2009). On the contrary, constitutive activation of the FGF receptor 3 (FGFR3) results in expansion of the surface area and cortical thickness exclusively in the caudo-lateral cortex (Thomson et al., 2009). This suggests that development of anterior and posterior areas is regulated by different FGF signaling during area mapping. COUP-TFI expression is repressed by FGF8 and regulates FGF signaling, and might probably control the size of the sensory cortex, besides imparting a sensory identity. The other area patterning genes, such as Emx2 (Heins et al., 2001) or Pax6 (Asami et al., 2011; Georgala et al., 2011; Sansom et al., 2009), are involved in balancing symmetric (non-neurogenic) versus asymmetric (neurogenic) divisions of the apical progenitor pool. These genes together with COUP-TFI probably work in a dose-dependent manner, allowing cortical progenitors to acquire different neurogenic potentials depending on their coordinates along the A-P and D-V axes. A regional control of cortical progenitor proliferation has been demonstrated for Pax6 (Mi et al., 2013), but whether COUP-TFI function in progenitors also controls neocortical area size, is still not known.

#### **COUP-TFI is required in regionalized radial and tangential neuron migration**

In the neocortex, radial migration allows newly produced neurons to settle beyond their predecessors following an inside-outside order ultimately leading to the six-layered structure. The rate of radial migration follows a rostral-low to caudal-high gradient, in which late-born neurons migrate at a higher rate in the caudal cortex, possibly reflecting a difference in neurogenesis and axogenesis between anterior and posterior regions (Alfano et al., 2013). As COUP-TFI transcriptionally regulates the expression level of the Rho GTPase Rnd2, which is required in cell migration and actin cytoskeletal reorganization (Azzarelli et al., 2015), and is normally expressed in an opposite gradient to COUP-TFI, i.e. high rostral and low caudal, loss of COUP-TFI affects the Rnd2 expression gradient and ultimately impairs the neuronal migration rate along the A-P cortical axis (**Figure 5**) (Alfano et al., 2011; Alfano et al., 2014). Abnormally high Rnd2 protein levels together with altered distribution of cytoskeletal proteins were also observed in primary hippocampal neurons and in the embryonic cortex lacking COUP-TFI (Armentano et al., 2006). Since migrating neurons have a characteristic leading process that extends over relatively long distances away from the soma with which they detect guidance cues (Marin, 2013), it is not surprising that axon growth and cell migration share similar mechanisms and are controlled by related molecular pathways. A role for COUP-TFI in cell migration and axonogenesis through an integrin-dependent mechanism was already suggested from *in vitro* studies (Adam et al., 2000).

Besides being involved in cortical radial migration, COUP-TFI, together with COUP-TFII, also control tangential interneuron migration. Overexpression of COUP-TFI in telencephalic explants promotes basal telencephalic cells to migrate dorsally and ventrally, whereas COUP-TFII guides cells to take principally a dorsal direction (**Figure 6A**) (Tripodi et al., 2004), suggesting a different intrinsic role of COUP-TFs in driving migrating neurons towards their target regions. This was also confirmed *in vivo* thanks to the *5HT3aR-GFP* mouse transgenic line (Lee et al., 2010; Vucurovic et al., 2010), in which two novel streams originating from the CGE were identified and shown to be dependent on COUP-TFs, Sp8 and Prox1 (**Figure 6B**) (Touzot et al., 2016). Inactivation of COUP-TFI affects the rate of Sp8+ migrating cells in the lateral migratory stream (LMS) and COUP-TFII+ cells in the medial migratory stream (MMS) (Touzot et al., 2016), whereas COUP-TFII seems to be mainly involved in driving CGE cells to caudal targets (Kanatani et al., 2008). Thus, immature interneurons will disperse tangentially along different caudal and rostral directions before reaching the cortex by dorsal and radial migration and settle into appropriate cortical and sub-cortical structures (Ruiz-Reig and Studer, 2017). The choice of the path seems to be under the control of a defined combination of intrinsic factors, which most probably determine also identity and final cortical location of differentiating interneurons. Overall, COUP-TFI might regulate similar target genes, which act during tangential and radial cell migration by contributing to the proper distribution of late-born glutamatergic cortical neurons and GABAergic interneurons in the developing cortex.

### **Specification of mature interneuron molecular identity and implications for epilepsy**

Similarly to the dorsal telencephalon, COUP-TFI delineates different sub-domains of the ventral telencephalon (Flames et al., 2007), from which GABAergic interneurons are born, specified and then migrate toward the cortex before being integrate into the brain circuitry. While COUP-TFI extends to all ganglionic eminences at early phases, its expression becomes gradually restricted to the dorsal-most region of the MGE and maintained at high levels in the ventral CGE, depicting again a high caudal to low rostral expression profile (Lodato et al., 2011; Touzot et al., 2016).

GABAergic progenitors and migrating interneurons express COUP-TFI and also COUP-TFII not only in rodents but also in human embryos (Alzu'bi et al., 2017a; Reinchisi et al., 2012; Varga et al., 2015), and expression is maintained in mature cortical interneuron subtypes (Lodato et al., 2011), with a preference to CGE-derived interneurons. Loss of COUP-TFI in interneuron precursors alters the delicate balance between MGE- and CGE-derived populations, without affecting the overall interneuron number (**Figure 6C**). Late-born, CGE-derived VIP+ and CR+ interneurons, are decreased in the mutant, compensated by the concurrent increase of early-born, MGE-derived PV-expressing interneurons, possibly due to increased proliferation in the MGE (Lodato et al., 2011; Touzot et al., 2016). Surprisingly, while alteration in the balance of interneuron subtypes is often correlated with epileptic phenotypes (Powell et al., 2003), this is

not reproduced in COUP-TFI interneuron-specific *CKOs*, which on the contrary result more resistant to pharmacologically induced seizures, possibly due to the increase of PV+ inhibitory interneurons (**Figure 6C**) (Lodato et al., 2011). The epileptic-resistant phenotype of mouse conditional mutant is strikingly different to what reported for haploinsufficient COUP-TFI patients, which often show epileptic spasms (Chen et al., 2016). At a first glance, this difference might be confusing. However, in mouse studies, COUP-TFI expression was inactivated only in interneuron precursors by leaving its expression in glutamatergic cortical neurons unaltered (Lodato et al., 2011). This is strikingly different from what happens in human patients, where one allele is mutated/deleted in all cells. Since COUP-TFI is also required in cortical glutamatergic neuron development, the epileptic features described in some human patients might be due to alterations in the balance between excitation and inhibition of developing circuits. Studies on membrane excitability and cortical activity in a mouse model reproducing the human phenotype might help in understanding the causes of seizures observed in some young patients (Dimassi et al., 2016; Hino-Fukuyo et al., 2015).

#### **COUP-TFI in adult neurogenesis is regulated by activity-dependent mechanisms**

Expression of COUP-TFI is maintained in the adult brain, suggesting a role in later events of brain maturation, such as assembly and/or maintenance of mature neuronal circuits. Together with COUP-TFII, it is highly expressed in both neurogenic niches of the adult rodent brain: the subventricular zone (SVZ) of lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Bovetti et al., 2013; Zhou et al., 2015). Moreover, COUP-TFI function seems to depend on neural activity, since its expression in the visual cortex is downregulated in enucleated mice (Abbott et al., 2015), and dependent on external odor stimuli in the mouse olfactory bulb (OB) (Bovetti et al., 2013).

The OB is a highly plastic telencephalic region where newly SVZ-generated inhibitory neurons integrate into local circuitry throughout life (Lledo et al., 2008). COUP-TFI is expressed at relatively low levels in the adult neurogenesis niche of the SVZ, with a low-rostral to high-caudal expression gradient, and in the rostral migratory stream (Zhou et al., 2015); expression however increases in the OB, and particularly in the tyrosine hydroxylase (TH)-positive dopaminergic interneuron population of the adult OB glomerular layer (**Figure 7**) (Bovetti et al., 2013; Zhou et al., 2015). To maintain TH expression, adult dopaminergic cells need to be stimulated by sensory inputs; odor deprivation causes TH and COUP-TFI downregulation (Bovetti et al., 2013; Cave and Baker, 2009). However, this regulation turns out to be indirect *via* the modulation of ZIF268/EGR1 expression, an immediate early gene responding to activity, and a possible target for COUP-TFI *via* Sp1 (Pipaon et al., 1999). This suggests that COUP-TFI cell-intrinsically controls interneuron responsiveness both in basal conditions and following acute stimulation (**Figure 7**), and plays a key role in the maintenance, rather than establishment, of the dopaminergic phenotype by modulating TH expression in an activity-dependent manner.

Besides being involved in OB dopaminergic interneuron maturation (Bovetti et al., 2013), COUP-TFI is also required in SVZ cell proliferation, migration and survival, as a way to provide new cells into the adult OB (Zhou et al., 2015). However, this latter study failed to specifically challenge COUP-TFI in adult neurogenesis, since early loss of COUP-TFI *via* the use of the *hGFAP Cre-recombinase* mouse line will influence the behavior of the adult population in an indirect way (Zhou et al., 2015). The same applies for a recent paper showing a drastic decrease of proliferating cells in the adult DG upon COUP-TFI inactivation in the hippocampal primordium (Parisot et al., 2017). To ultimately prove that COUP-TFI acts on the maintenance pool of adult stem cells, inducible *Cre-recombinases* and/or viral-mediated approaches acting exclusively at adult stages must be used to definitely discern its role from any secondary effect resulting from impaired embryonic development.

### **Graded COUP-TFI expression in distinct structures of the developing visual system**

Similarly to other regions of the mouse brain, COUP-TFI is expressed in a dorsal-low to ventral-high gradient in the developing retina, whereas COUP-TFII displays an opposite gradient (Tang et al., 2015) (**Figure 8A**). The dynamic expression profile described for both mouse COUP-TF homologs in the early optic system is tightly correlated with eye and optic nerve morphogenesis (Tang et al., 2010; Tang et al., 2015). While neither COUP-TFI nor COUP-TFII single *KO* mice seemed to develop major eye abnormalities, double *KO* mice showed severe coloboma and a microphthalmic eye (Tang et al., 2010), suggesting a compensatory effect of both homologs during eye development. This is quite surprising since COUP-TFI haploinsufficient patients show ocular impairments with a high penetrance (see more below). Mouse studies showed that both COUP-TF genes act on the early domain of retinal/optic nerve identities and orchestrate eye development (**Figure 8B**). Furthermore, COUP-TFs link the D-V retinal identity (imparted *via* modulation of *Vax* genes) to the regional expression of different subtypes of photoreceptors, as revealed by Opsin expression (Sato et al., 2009). In particular, COUP-TFI suppresses S-opsin expression in the dorsal retina and M-opsin expression in the ventral retina, providing a molecular link between BMP-mediated D-V patterning and consequent Opsin expression (**Figure 8C**).

After birth, COUP-TFI continue to be expressed in a gradient in the adult mouse retina throughout the ganglion cell layer and the inner nuclear layer (Inoue et al., 2010); however, little is known about its function on the maintenance and functionality of mature retinal subpopulations. The presence of a distinct D-V gradient and the ability to be modulated by activity would suggest a contribution for COUP-TFI in the formation of retino-topics visual maps by guiding retinal ganglion cells, the only long-range projecting neuron of the retina, to their proper targets (Assali et al., 2014). However, this has not been investigated so far.

### **COUP-TFI influences complex animal behaviors by controlling the development of hippocampal and neocortical circuitries**

As mentioned above, COUP-TFI acts as a key gene in several neuronal populations and at different developmental times, and its full *KO* leads to death at birth. However, the use of cell-specific *CKOs* has allowed deciphering its role in distinct neuronal populations and studying the consequences of its absence in adult behavior. We previously reported that the parietal cortex changes its laminar and connectivity organization and resembles more as a frontal-motor than a somatosensory S1 cortex, when COUP-TFI is inactivated in glutamatergic projection neurons (Alfano et al., 2014; Armentano et al., 2007). As a consequence, the “motorized” S1 cortex shows a mis-specification of two classes of corticofugal neurons, the *Tbr1*+ corticothalamic (layer VI) and the *Fezf2*+/*Ctip2*+ subcerebral projection neurons (layer V), as well as improper projection of layer V neurons to subcerebral targets, such as the spinal cord and pons (**Figure 9**) (Tomassy et al., 2010). Defects in the development of the sensorimotor cortex results in impaired voluntary and coordinated movements (Gharbawie et al., 2007). While simple motor tasks were perfectly executed, fine-motor skilled performances showed a remarkable impairment in adult *COUP-TFI CKO* mutant mice (Tomassy et al., 2010). Other not yet reported behavioral abnormalities, such as hyperactivity and repetitive movements, can also be observed in mutant mice (MS, unpublished). Thus, COUP-TFI not only regulates area identity and layer specification: it allows the development of complex cortical circuitries, which are necessary for distinct sensorimotor behaviors in adult animals. The nature of these circuits still needs to be uncovered.

Besides cortical defects, COUP-TFI conditional mice show other cognitive impairments, such as learning and memory deficits, mainly controlled by the hippocampus (Flore et al., 2017). COUP-TFI is expressed from the earliest stages of hippocampal development to adulthood, from cycling progenitors to mature neurons (Flore et al., 2017; Parisot et al., 2017), thus representing one of the few TFs expressed throughout all stages of hippocampal morphogenesis. Along the hippocampal longitudinal axis, COUP-TFI is expressed in a low dorsal to high ventral gradient during postnatal development (Flore et al., 2017; Parisot et al., 2017). The structure, connectivity and functions of the hippocampus are not homogeneous along its longitudinal axis, referred to as septo(dorso)-temporal(ventral) axis in rodents and A-P axis in primates (Strange et al., 2014). While the septal part is more involved in spatial learning and memory (Moser et al., 1993; Moser et al., 1995), the temporal region is required for non-spatial aspects of learning and emotional behaviors (Kheirbek et al., 2013; Wang et al., 2013). Loss of COUP-TFI in the hippocampal primordium affects its overall growth, shape and structure: COUP-TFI adult mutant mice have a severe dysmorphic hippocampus, which is strongly affected in its dorsal pole (**Figure 10A**) (Flore et al., 2017). Connectivity between the dorsal hippocampus and entorhinal cortex was also severely impaired and associated with selective spatial

learning and memory impairments, whereas emotional memory, mainly controlled by the ventral pole, was not affected (Flore et al., 2017). Overall, COUP-TFI plays an important role in hippocampal morphogenesis by controlling cell proliferation and migration (**Figure 10B, C**), and its graded expression is involved in setting up the anatomical and functional architecture along the longitudinal axis, ultimately leading to the assembly of brain circuitries which are fundamental for complex animal behaviors. Since COUP-TFI controls spatial learning and memory *via* regulation of hippocampal development, it is tempting to speculate that hippocampal defects could also explain some intellectual deficits described in COUP-TFI haploinsufficient human patients (Chen et al., 2016).

### **COUP-TFI/NR2F1 haploinsufficiency hides behind the unknown etiology of rare human syndromes**

Gradient COUP-TFI expression in human fetal brain highly resembles its murine counterpart (Alzu'bi et al., 2017b), and both mouse and human COUP-TFI proteins share a highly conserved molecular structure (**Figure 1A**). This suggests that COUP-TFI, better known as NR2F1 in humans, might be also involved in human brain development and, consequently, might lead to pathological conditions when deleted or mutated.

The first hint of NR2F1 as a potential cognitive disease gene came from three independent studies of patients carrying a genomic deletion in the 5q14-q15 regions encompassing NR2F1 among other genes (Al-Kateb et al., 2013; Brown et al., 2009; Cardoso et al., 2009). All patients showed a global developmental delay and intellectual disability (from mild to severe), some had nodules of ectopic neurons along the ventricles (called periventricular heterotopia, PH; (Cardoso et al., 2009) and epileptic seizures as infants. Most cases had difficulties in feeding and speaking, and one patient had also hearing problems (Brown et al., 2009). The severe syndromic features were associated with NR2F1 haploinsufficiency, as the other allele was not affected. Despite other genes being deleted in the same region, NR2F1 was the most likely responsible one. This was subsequently supported by comparing overlapping deleted regions with new patients (Al-Kateb et al., 2013), and by the identification of small INDELS and missense mutations in the DNA (DBD)- or Ligand-Binding Domains (LBD) of the NR2F1 gene in later characterized patients (Chen et al., 2016) (see Table 1). The causative link between the patient clinical features and NR2F1 dysfunction is nowadays very strong, thanks to the identification of more than 30 heterozygous pathogenic missense variants mainly located in the DBD, even if some mutations are found in the start codon and LBD. Interestingly, LBD variants seem to lead to a milder phenotype than DBD ones, suggesting that the DBD represents a mutational hotspot for this gene (**Figure 11**). The translational initiation mutations have shown to decrease NR2F1 protein and *mRNA* (Chen et al., 2016), while the DBD and some LBD mutations impair the capacity to transactivate a reporter gene, implying that the transcriptional activity of NR2F1 variants is strongly compromised (Bosch et al., 2014). Furthermore, given that NR2F1 binds to DNA in the form of

dimers, or heterodimers with other nuclear receptors (**Figure 1B,C**), the presence of one mutated protein within this complex could have a dominant negative effect on the final activity of multiple nuclear receptors besides NR2F1, further amplifying the severity of the transcriptional effects (Chen et al., 2016). This is also supported by the fact that patients with whole-gene deletion of one allele seem to have milder phenotypes compared to those carrying missense variants (Chen et al., 2016, Table1).

Patients with mutations/deletions in this gene are at present diagnosed for the *Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS)*, a recently described autosomal-dominant disorder (OMIM: 615722), characterized by optic atrophy associated to intellectual disability, classified as a rare disease (ORPHA: 401777) and named according to the authors of the paper reporting for the first time point mutations in the *NR2F1* gene (Bosch et al., 2014; Chen et al., 2016). However, the 38 patients described so far exhibit a wide array of clinical features, in which global developmental delay (DD, 89%) and intellectual disability (ID, 84%) seem to prevail on the presence of an optic atrophy (OA, 68%) associated in some cases with cerebral visual impairments (CVI, 18%), abnormal optic disk, reduced optic chiasma and ocular defects (Table 1). Other common clinical features describing these patients are hypotonia (66%), oromotor dysfunctions (sucking, chewing, swallowing and feeding) (OD, 45%), hearing defects (HD, 16%), speech difficulties (delayed speech development, DSD; 29%) and motor apraxia/delayed motor development. Even if the majority of patients do not show any obvious morphological brain defects by MRI (apart from the nodular heterotopias and polymicrogyria described in the patients carrying big deletions (Cardoso et al., 2009), 9 patients have a thinning of the corpus callosum (CC) (Table 1), a clinical feature that could contribute to the ID, visual problems, speech delay and seizures described in NR2F1 haploinsufficient patients (Paul et al., 2007; Schell-Apacik et al., 2008). Of note, 14 patients exhibited significant repetitive behaviors, such as rocking, head-banging or hand-flapping, and were diagnosed as being autistic, in line with a large study of *de novo* single nucleotide variants associated with autism (ASD), in which mutations in the *NR2F1* gene were classified as “damaging” and possibly ASD-correlated (Cotney et al., 2015; Sanders et al., 2012). Other patients with aberrant behaviors revealed different degree of hyperactivity and were classified as children with an attention deficit hyperactivity disorder (ADHD, 16%) (Chen et al., 2016). NR2F1 has also been independently identified as a disease gene in studies of Infantile Spasm (IS) syndrome (Dimassi et al., 2016; Hino-Fukuyo et al., 2015; Michaud et al., 2014), while searching for rare inherited or *de novo* mutations. Infantile spasms (IS) is an early-onset epileptic encephalopathy, which frequently leads to long-term neurological impairments, including DD, ID, ASD and chronic refractory epilepsy, suggesting that some of the cognitive features described in NR2F1 patients might derive from brain damages due to infantile spasms. Consistently, early onset epilepsy and cases of IS are reported in 40% of all patients identified so far (Table 1). As the reported cases increase, new syndromic symptoms, such as low levels of serotonin and dopamine metabolites, delayed bone age, stroke-like episodes and deficiency of the mitochondrial

respiratory chain, have been proposed to represent a phenotypic expansion of the BSSOAS syndrome (Kaiwar et al., 2017; Martin-Hernandez et al., 2018). This implies that NR2F1 haploinsufficiency leads not only to optic neuropathy, as originally proposed, but also to a much broader spectrum of phenotypic features more related to broad brain abnormalities than to specific visual deficits. Since optic atrophy seems to be a less common feature of the BSSOAS syndrome compared to ID and DD, it would be preferable to rename this syndrome as BBSS or other. In conclusion, the disruption of fundamental neurodevelopmental processes controlled by NR2F1 converges to epilepsy, cognitive impairments and visual deficits in the context of different syndromes, such as IS syndrome, ASD or the newly defined BBS optic atrophy syndrome.

### **Modeling COUP-TFI-related diseases: can mouse models reproduce human syndrome features?**

The use of animal models lacking NR2F1/COUP-TFI could greatly help to further characterize patients' phenotypical features. The clinical approach, based on the identification of a patient cohort characterized by distinct symptoms and thus associated to a specific disease, has created heterogeneous groups of individuals whose symptoms derive from the mutations of different (sometimes unrelated) genes. The use of animal models in which NR2F1 mutations can be inserted into the genome would help in characterizing the pathological features and biochemical alterations directly linked to the pathogenic mutations and strengthen the genotype-phenotype correlation. For example, introducing the most disrupting DNA binding domain mutations in the mouse COUP-TFI locus would reproduce the patient phenotype and ultimately allow to evaluate the impact of such mutations on molecular mechanisms, and more generally on neurodevelopmental and metabolic processes.

To date, there are several mouse mutants that have shown to reproduce, at least in part, some of the clinical features described in patients, and that could be successfully used as a paradigm to study NR2F1-related syndromes. Since the majority of missense mutations seem to affect NR2F1 levels and transcriptional activity (Chen et al., 2016), it is reasonable to hypothesize that heterozygous COUP-TFI constitutive mice (Armentano et al., 2006; Qiu et al., 1997) represent the most adequate mouse model so far, to reproduce clinical features associated with NR2F1 haploinsufficiency. However, even if all studies dealing with COUP-TFI mouse mutants have not taken into account heterozygous animals, the phenotypic abnormalities described in either constitutive *null* or *conditional (CKO)* mouse mutants have in most cases been consistent with the symptoms described in patients (see Table 2). For example, *null* mutants have multiple defects in cranial nerve formation and projections, particularly in the glossopharyngeal IX nerve deputed to innervate the parotid gland and thus to induce salivary secretion essential for swallowing (Qiu et al., 1997). Problems in feeding have been described in several patients (Table1), particularly in infants,



and might be related to morphological defects of the IX cranial nerve. Furthermore, a reduced corpus callosum (described in 24% of patients) has been found in the majority of both COUP-TFI *null* mutants and cortical *CKOs*, and results from abnormal migration of late-born callosal projection neurons (Alfano et al., 2011; Armentano et al., 2006). Moreover, skilled motor development or more generally motor apraxia diagnosed in some NR2F1 patients has been tested *via* the single-pellet reaching task in mice and found impaired in *COUP-TFI CKO* mutants (Tomassy et al., 2010). However, no obvious epileptic events have been described so far in COUP-TFI mutant mice, and on the contrary resistance to epileptic seizures has been described in *CKO* mice in which COUP-TFI is inactivated in interneurons (Lodato et al., 2011). But, as mentioned above, this mouse does not reproduce the human syndrome since, differently from the human condition, COUP-TFI is still highly expressed in glutamatergic cortical neurons in these mutants.

The big exception, however, lies in the optic phenotype. While a high number of haploinsufficient patients show several defects in the visual system, comprising optic atrophy, pale optic disk with severe excavations and CVI (Chen et al., 2016), in the mouse, major optical defects have been found only in double COUP-TFI/COUP-TFII mutant mice (Tang et al., 2010; Tang et al., 2015). One reason could be that development of the visual system in humans is more sensitive to COUP-TF dosage and their target genes than in the mouse, or alternatively, that the *CKO* mouse model used by Tang and colleagues (a *Rax-CRE* conditional KO) (Tang et al., 2010) does not reproduce the human genotype in which the gene is affected from the earliest stages of eye development and in all cells where COUP-TFI is expressed. A detailed analysis of optic development in a mouse model more closely recapitulating the human condition (such as heterozygotes, or mice encompassing point-mutations in the DBD of the COUP-TFI gene) will be necessary to address this point. Finally, although some of the human phenotypic abnormalities have been reproduced in mice, there are still some discrepancies between the deficiencies described in the mouse and in human patients lacking COUP-TFI/NR2F1 function that need to be further elucidated (see Table 2).

## **DISCUSSION AND OPEN QUESTIONS**

### **Different roles in different brain regions and cellular contexts**

The dissection of the cellular role of transcription factors is a difficult task, as they (i) are expressed in a dynamic fashion both in time and space, (ii) control hundreds or thousands of different target genes, and (iii) can act differently depending on the cellular context. The pleiotropic regulator COUP-TFI/NR2F1 makes no exception in this sense, as it plays multi-faceted roles during the developmental processes assembling different brain structures, such as the neocortex, the hippocampus and the retina. Despite this, several studies are beginning to unravel its complex function, especially in the context of neural progenitors and their progeny. At a cell-intrinsic level, COUP-TFI has been demonstrated to be involved in (i) regulating

migration (Adam et al., 2000; Alfano et al., 2011; Parisot et al., 2017; Touzot et al., 2016), (ii) directing axonal elongation and arborization (Adam et al., 2000; Armentano et al., 2006; Qiu et al., 1997), (iii) controlling identity and temporal competency of neuronal progenitor cells (Faedo et al., 2008; Naka et al., 2008; Okano and Temple, 2009) and (iv) establishing area-specific identity in progenitors and neurons (Alfano et al., 2014; Armentano et al., 2007; Harb et al., 2016; Tomassy et al., 2010).

Available data are sometimes apparently contradictory. For example, COUP-TFI promotes cell cycle exit in the neocortex (Faedo et al., 2008), and in the MGE (Lodato et al., 2011) and CGE (Touzot et al., 2016). Post-mitotically, COUP-TFI controls radial migration of late-born cortical neurons (Alfano et al., 2011) and dictates areal identity in young neurons (Alfano et al., 2014), independently of its expression in progenitors. However, the situation is completely reversed in the hippocampal dentate gyrus, an archicortical structure with different neurogenic dynamics, where COUP-TFI acts primarily in mitotic cells by increasing the proliferative capacity of the progenitor pool with no obvious roles in post-mitotic cells (Parisot et al., 2017). Even if COUP-TFI apparently seems to exert opposite roles in the cortex and archicortex, the two structures differ in the way neurogenesis occur: in the neocortex, neurogenesis and cell migration are two distinct processes occurring at different times and in distinct radial compartments, whereas dentate gyrus progenitors proliferate and produce new neurons as they migrate to their target region, where they continue to differentiate (Urban and Guillemot, 2014). Thus, COUP-TFI exerts different roles depending on the context in which it acts: a purely pro-mitotic factor in the dentate gyrus, where cell proliferation and migration are two highly linked processes, or a pro-differentiate and post-mitotic acting factor in the neocortex, where cell proliferation and migration are independent.

Furthermore, COUP-TFI shows different, sometimes controversial functions also in cancerogenesis, where the cell cycle is abnormally re-activated and/or accelerated. COUP-TF genes have been implicated in cancer progression (Boudot et al., 2011), and orphan receptors in general have been recognized as potential drug targets for tumor treatment (Dhiman et al., 2018; Safe et al., 2014). In some tumors, COUP-TFI is re-expressed in the process of de-differentiation associated with the tumoral phenotype (Kieback et al., 1993; Le Dily et al., 2008), and strongly upregulated compared to normal tissue samples (Boudot et al., 2011; Boudot et al., 2014; Le Dily et al., 2008), whereas in other cases COUP-TFI expression is lower in the tumoral tissue compared to the normal one (De Sousa Damiao et al., 2007; Ham et al., 2008). In breast cancer, high COUP-TFI expression is associated with increased cell proliferation and migration rate (Le Dily et al., 2008). On the contrary, high COUP-TFI acts as a cell cycle break in other tumor types, such as head and neck squamous cell carcinoma, or prostate cancer, inducing long-term quiescence in dormant cancerous cells (Sosa et al., 2015). Thus, even for tumor tissues, COUP-TFI has multi-faceted functions: its expression levels and molecular roles in tumor progression vary depending on the context and the tissue

type, and whether it controls cell proliferation or migration. A better comprehension of COUP-TFI molecular effects in tumorigenesis could contribute in providing novel therapeutic targets for cancer treatment.

Focusing on the function of COUP-TFI at a molecular level (**Figure 1**), this transcription factor interacts with different co-factors and epigenetic modifiers, and plays multiple roles, as both an activator and a repressor of target gene transcription (Alfano et al., 2013; Cooney et al., 1993; Park et al., 2003). Thus, COUP-TFI could play different roles in a tissue-specific way, depending on the presence or absence of key co-factors. In this way, the expression of interactors at different levels could create a combinatorial and dose-specific code of different factors together with COUP-TFI, and thus driving the regional- and cell-specific action of COUP-TFI. In this sense, the “contradiction” of discrepant roles played by COUP-TFI would only be apparent, depending instead from a still incomplete comprehension of complicated multifactorial interactions directing its activity in different brain regions. This implies that the investigation of the molecular pathway downstream of COUP-TFI, which is still elusive to date, should be performed only after a careful selection of specific cell populations at distinct ages and in different tissue types, since the downstream network regulated by COUP-TFI could greatly vary depending on the cellular type and context. Performing deep DNA sequencing methods, such as ChIP- and *RNA-Seq*, of specific cell populations isolated thanks to the presence of a reporter protein could be a winning approach. Since chromatin interactions play a critical role in transcriptional regulation and thus serve in modulating gene expression, it would be also useful to identify COUP-TFI target genes on distal regions *via* a *ChIA-PET* approach, in which chromatin interaction is analyzed by paired-end tag sequencing, a robust method for capturing genome-wide chromatin interactions (Zhang et al., 2012). This has been successfully applied to the oestrogen receptor  $\alpha$  in the human genome (Fullwood et al., 2009).

A careful analysis of the cellular and molecular context in which COUP-TFI is acting would increase our understanding of its pleiotropic action, and help to model COUP-TFI/NR2F1-dependent human diseases in a predictable and reliable way. Since a human model of BBS optic atrophy syndrome is still missing, this could be replaced by patient-derived induced pluripotent stem (iPS) cells and generation of brain organoids, which would allow deciphering several neuronal aspects controlled by NR2F1 during human brain development, and explore *in vitro* cellular and molecular mechanisms at the basis of patients’ symptoms (Quadrato and Arlotta, 2017). Alternatively, mouse models integrating the most disruptive (DBD-located) missense NR2F1 mutations could recapitulate the BBS syndrome and allow a better exploration of clinical features in an *in vivo* context. A comparison between COUP-TFI-deficient mice (such as heterozygous and/or *null* mutants, having less or no protein, respectively) with COUP-TFI-mutated mice (having an

abnormal amount of aberrant protein) could help in unraveling possible toxic gain-of-function effects of COUP-TFI mutated protein, which to date is still not known.

#### FIGURE LEGENDS

**Figure 1. COUP-TF gene structure and molecular function. (A)** *Schematic representation of the homologies between COUP-TFI and COUP-TFII genes in human (h) and mouse (m).* The DNA-binding domain (DBD) and the Ligand-binding domain (LBD) are shown in red and blue, respectively. Numbers in the protein domains indicate the percentage of homology with respect to the corresponding human COUP-TFI protein sequence. **(B)** *COUP-TF-mediated repression of gene expression.* By forming complexes with SMRT and N-CoR co-factors, COUP-TFs can silence gene expression in a direct way, binding to two GGTC A motifs, named direct repeats (DR), separated by a spacer consisting of 1 to 5 nucleotides. The highest binding affinity for COUP-TFI is, in most cases, DR1 (direct repeats separated by 1 nucleotide). Below is shown the general consensus binding site sequence for COUP-TFI (adapted from Montemayor et al., 2010). COUP-TFs can also act as indirect repressors by competing for the binding of other nuclear receptors (RXRs, etc) and for the occupancy of target DNA binding sites such as the HRE (Hormone Response Element) sequence. Schema adapted from Tang et al., 2015. **(C)** *COUP-TF-mediated activation of gene expression.* Direct and indirect mechanisms of gene activation triggered by COUP-TF genes. COUP-TFs directly act as activators by binding to direct repeat (DR) sites. Alternatively, COUP-TFs, indirectly activate transcription acting as accessory factors together with HNF4, or relying on DNA-bound tethering cofactors such as Sp1 to access and co-activate target sequences. DR: direct repeats site; RE: response element. Schema adapted from Park et al., 2003.

**Figure 2. COUP-TFI gradient expression is a key factor for neocortical arealization. (A)** Graded red color reproduces the rostral-low to caudal-high COUP-TFI gradient in the E12.5 mouse brain. Below, three schematic sections at anterior (A), medial and posterior (P) levels highlight the high lateral to low medial cortical gradient and the low rostral to high caudal gradient in both cortex and ventral telencephalon. Ctx: cortex; LGE: lateral ganglionic eminence; MGE: medial ganglionic eminence; CGE: caudal ganglionic eminence; Tha: thalamus. **(B)** Immunofluorescence of COUP-TFI showing the protein gradient (in red) at three A-P levels on E12.5 mouse brain sections. **(C)** Two major steps are responsible for cortical arealization: an intrinsic protomap, i.e. an early coordinate map created by TF gradient expression (Pax6, Emx2, Sp8 and COUP-TFI are shown), and an extrinsic activity-dependent refinement controlled by thalamo-cortical afferents. Note that COUP-TFI is expressed not only in progenitors, but also in postmitotic cells. **(D)** *COUP-TFI controls the position and size of neocortical areas.* Neocortical primary areas are schematized as they develop in different mouse transgenic models. The rostral motor area is greatly expanded in COUP-TFI null and cortex-specific conditional mutants (*COUP-TFI KO* and *COUP-TFI fl/fl; Emx1-*

*Cre*, respectively) with a slight shift already visible in heterozygotes (*COUP-TFI fl/+; Emx1-Cre*). A similar phenotype is reproduced in mice lacking COUP-TFI only in post-mitotic cells (*COUP-TFI fl/fl; Nex-Cre*). Vice versa, COUP-TFI over-expression in the whole cortical field leads to the expansion of caudal sensory areas (*KO;iz/hCOUP-TFI*). COUP-TFI is thus necessary and sufficient to impart sensory identity in early postmitotic cortical neurons.

**Figure 3. Molecular network controlling neocortical area mapping via COUP-TFI/FGF8 antagonism.** A COUP-TFI/FGF signaling antagonism triggers the acquisition of distinct neocortical identities by regulating multiple downstream effectors: *Emx2* promotes the acquisition of caudal identity, while *Sp8* and *Pax6* promote rostral properties. The dominant action of COUP-TFI is obtained by regulating multiple, yet-unknown effectors, and further strengthened by post-mitotic gradient expression. F/M: fronto/motor cortex; S1: primary somatosensory area; V1: primary visual area.

**Figure 4. Temporal specification of neural progenitors regulated by COUP-TFI and SVP.** (A) The temporal specification of *Drosophila* neuroblasts is controlled by the sequential expression of several TFs, including *Hunchback (Hb)*, *Krüppel (Kr)*, *Pdm1/Pdm2*, *Castor (Cas)*, and *Grainyhead (Grh)*. The *Drosophila* COUP-TFI homologue, *Seven-up (Svp)*, acts as a regulator of neuroblast identity transition, inhibiting *Hb* and allowing the expression of *Kr*. When *Svp* function is lost (*Svp -/-*), *Hb* expression is prolonged and the normal birth-order program is altered, resulting in increased early-born neurons. Nb: neuroblast; GMC: ganglion mother cell. (B) Wild-type neural progenitors (upper line) produce early neurons (green), followed by late neurons (blue) and glia (yellow). Loss of COUP-TFI in progenitors (lower line) extends early-neuron generation and delays gliogenesis. (C) *Schematic prediction of morphological consequences of COUP-TFI-controlled neurogenesis.* COUP-TFI normally triggers neurogenesis and production of *Tbr2+* intermediate progenitors (IPs; yellow cells) and neurons (blue cells), thus regulating neocortical thickness (radial expansion). On the contrary, COUP-TFI removal promotes *Pax6+* neural progenitor self-renewal (green radial cells), which could lead to cortical surface increase (lateral expansion). COUP-TFI could thus affect cortical area size by regionally controlling progenitor dynamics. CP: cortical plate; IZ: intermediate zone; SVZ: sub-ventricular zone; VZ: ventricular zone.

**Figure 5. COUP-TFI controls radial migration of late-born cortical neurons by modulating Rnd2 expression levels.** The Rho-GTPase *Rnd2* expression is represented in red at different rostro-caudal levels and in the *COUP-TFI KO* mutant. COUP-TFI normally represses *Rnd2* along the A-P axis and allows proper rate of cell migration. In the absence of COUP-TFI, *Rnd2* expression abnormally increases, resulting in impaired cell morphology conversion and delayed migration of late-born neurons. MSC: multipolar shaped cells; BSC: bipolar shaped cells.

**Figure 6. Migrating routes and molecular identity of interneurons in the ventral telencephalon. (A)** *Interneuron migratory streams along the dorso-ventral axis of the mouse telencephalon.* COUP-TFI-positive interneuron precursors travel from the medio-lateral ganglionic eminence (MLGE) and caudal ganglionic eminence (CGE) dorsally to the cortex (ctx), laterally to the piriform cortex (pcx) and ventrally to the preoptic area (POa) and hypothalamus (hypo). Ctx: cortex; Ms: medial stream; LS: lateral stream; OB: olfactory bulb; ant hypo: anterior hypothalamus. **(B)** *Novel antero-posterior migratory streams distributing interneurons in the developing mouse telencephalon.* COUP-TFI and COUP-TFII are expressed in different percentages in the interneuron populations migrating from the CGE to their final targets along the caudal (CMS), lateral (LMS) and medial (MMS) migratory stream, as visualized in a schematic horizontal brain section, adapted from Touzot et al., 2016. **(C)** *Proportion and molecular identity of different interneuron subpopulations in the adult cortex.* MGE- or CGE-derived interneurons are identified by the expression of specific proteins (left scheme). In absence of COUP-TFI, the balance between different classes of interneurons is altered (right scheme); PV-expressing interneurons are increased at the expense of CR- and VIP-positive ones. Scheme adapted from Lodato et al., 2011. SST: Somatostatin; PV: Parvalbumin; CR: Calretinin; NPY: Neuropeptide Y; VIP: Vasoactive Intestinal Polypeptide.

**Figure 7. Activity-dependent control of dopaminergic interneuron function in the adult olfactory bulb.** A schematic representation of the mouse olfactory bulb (OB) shows how new dopaminergic interneurons are produced and integrated during the whole lifespan of the animal. COUP-TFI is first expressed at low levels in the lateral ventricle (LV) neural stem cells and rostral migratory stream (RMS), but then strongly upregulated in the majority of tyrosine hydroxylase (TH)-expressing interneurons. By responding to sensory inputs and activating the Zif268 expression, COUP-TFI mediates an activity-dependent control of TH expression in adult dopaminergic interneurons, as schematized in the different situations depicted on the right: presence of sensory input, absence of sensory input and without COUP-TFI function.

**Figure 8. COUP-TF genes in retinal development. (A)** COUP-TFI graded expression (red color) is shown in coronal sections of mouse neural retina and optic stalk (future optic nerve) at different embryonic ages (from E10.5 to E12.5, as indicated). Four distinct domains can be identified along the dorso-distal and the ventro-proximal axes of the early optic vesicle: the presumptive dorsal optic stalk (pdOS), the presumptive retinal pigmented epithelium (pRPE), the presumptive neural retina (pNR) and the presumptive ventral optic stalk (pvOS). Of note, COUP-TFI strong expression (in red) in the pOS (both dorsal and ventral) and in the ventral NR progenitors. Nuclei counterstaining was obtained with DAPI. Cr: crystalline lens; NR: neural retina; Pr: proximal; Di: distal; D: dorsal; V: ventral. **(B)** By interacting with key genes of retinal development (scheme on the left), COUP-TF genes play multiple roles: the specification of the presumptive dorsal optic

stalk (pdOS) identity, which is consequently lost in double mutants (1); the specification of the presumptive RPE domain, which is strongly reduced in mutants (2); and the settling of a proper boundary between the pNR and the pvOS, which results shifted proximally in the double mutant (3). Upon loss of COUP-TFI and COUP-TFII, the Pax6-positive neural retinal domain is expanded at the expense of the other domains (scheme on the right). COUP-TFs confer D-V identity to the mouse retina (4), by modulating expression of the D-V determinant genes Pax6 and Vax1/2. Green and red arrows indicate the up- and down-regulation of D-V retinal, RPE and optic stalk genes. **(C)** Fine-tuned by BMP and possibly other morphogens (RA and Shh), COUP-TFI and COUP-TFII graded retinal expressions (in ventral and dorsal retina, respectively) control the regional abundance of M-Opsin cones (red) and S-Opsin cones (blue). COUP-TFI suppresses S-opsin expression in the dorsal retina and M-opsin expression in the ventral retina.

**Figure 9. The specification of deep layer neurons is under the control of COUP-TFI.** Schematics showing a comparison between the laminar structure of a wild-type S1 cortex (left) and the equivalent rostro-caudal level of a COUP-TFI mutant cortex (right). The presumptive S1 cortex of the conditional mutant is “motorized” (“mS1”), since it acquires several molecular and anatomical characteristics of a motor (M1) cortex. This area shift (upper schemes) is reflected at the laminar and connectivity level. Layer VI cortico-thalamic neurons (CTh; violet cells, left scheme), normally projecting to the thalamus, are partially re-specified to layer V pyramidal neurons (cortico-spinal motor neurons, CSMN; violet pyramidal-shaped cells, right scheme) projecting towards the pons and the spinal cord. However, the molecular identity of genuine layer V pyramidal neurons (red pyramidal cells) is also affected in the mutant (pink cells); the majority of mis-specified CSMN axons fail to reach the spinal cord, and accumulate in the pons region.

**Figure 10. COUP-TFI acts on the morphology of the hippocampus and differentiation of dentate gyrus (DG) granule cells.** **(A)** At post-natal day 8 (P8), COUP-TFI deficient hippocampus displays poor development of the dorsal/septal portion (indicated by red arrow), which is involved in spatial learning and memory. Hp: hippocampus; DG: dentate gyrus. **(B)** COUP-TFI acts as a pro-mitotic factor in DG progenitors during development by promoting the mitotic potential of both Pax6+ and Tbr2+ DG progenitors. IPC: intermediate progenitor cell. **(C)** Schematic representation of the main developmental abnormalities found in the developing *COUP-TFI* *CKO* DG at post-natal ages. Mitotic DG cells (in red) normally proliferate both in 1ry matrix and during their migration in 2ry and 3ry matrices; some 3ry matrix cells differentiate to populate the hilus (green cells). DG cell proliferation is compromised in *COUP-TFI* *CKO* in which the progenitor cell pool is precociously exhausted in all three matrices. Additionally, migration pathways are aberrant, possibly due to altered guiding glia scaffold (represented by green lines, while black arrows highlight migration routes). Finally, the output cell number is further decreased by apoptotic events (black cells). As a result, the DG of post-natal animals (P7-P14) is reduced in size and altered in morphology. The

progenitor pool residing in the granule cell layer (gcl) is severely reduced, while misrouted progenitors differentiate along their migration, originating ectopic neuronal clusters. Increased expression of CXCR4 (shown in grey) might explain some of the defects. Scheme adapted from Parisot et al., 2017. CA1-3: cornus ammonis 1 and 3; DMS: *dentate migratory stream*, DG: *dentate gyrus*, gcl: *granule cell layer*, h: *hem*, hi: *hilus*, Hp: *hippocampus proper*, IPC: *intermediate progenitor cells*, ml: *molecular layer*; 1ry: primary matrix; 2ry: secondary matrix; 3ry: tertiary matrix.

**Figure 11. Mutations and variants compromising COUP-TFI function in humans.** Schematic representation of the human COUP-TFI protein sequence, where point mutations and short deletion/insertion (indel) identified in human syndromic patients are highlighted. Note that the mutation hotspot region is the DNA binding domain (DBD) as missense mutations in this area compromise the stability of the zinc finger domain and consequently binding to appropriate targets. Missense mutations in the ligand-binding domain (LBD) can instead disrupt the dimerization interface and/or impede the interaction with the protein physiological ligand(s), even if patients with variants in the LBD have milder phenotypes. Some patients carry mutations in the initiation codon, compromising protein production; the third codon also contains an ATG, thus alternative translation is possible. The scheme was adapted from Chen et al., 2015, and completed according to Yang et al., 2017 and Kaiwar et al., 2017. Aminoacid numbers indicating the position of DBD and LBD were updated based on the current NR2F1 human protein annotation ([https://www.ncbi.nlm.nih.gov/protein/NP\\_005645.1](https://www.ncbi.nlm.nih.gov/protein/NP_005645.1)).

**Table 1. Updated list of published NR2F1 variants and clinical description of patients.**

**Table 2. Major similarities between human patients' symptoms and deficits in mouse mutants.** The table compares symptoms found in COUP-TFI-mutated human patients (left column), with data coming from mouse models in which COUP-TFI is genetically ablated (right column). Despite the clear similarities between human patients and mouse models, many points still need to be further investigated for a better understanding of COUP-TFI-related human syndromes (see "missing points" in red).

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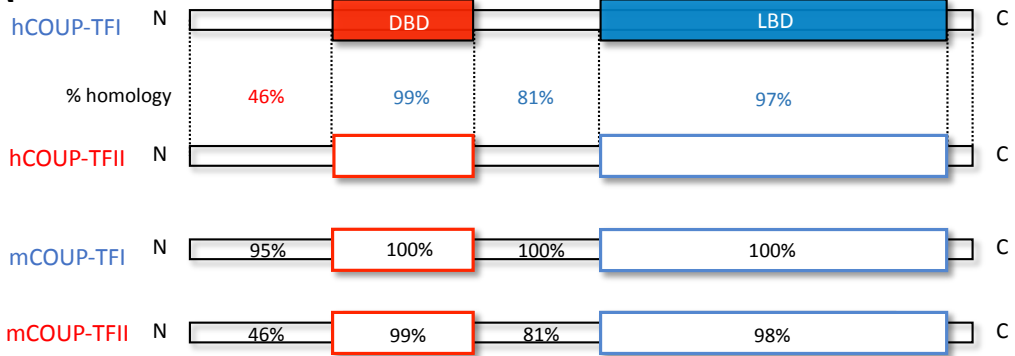
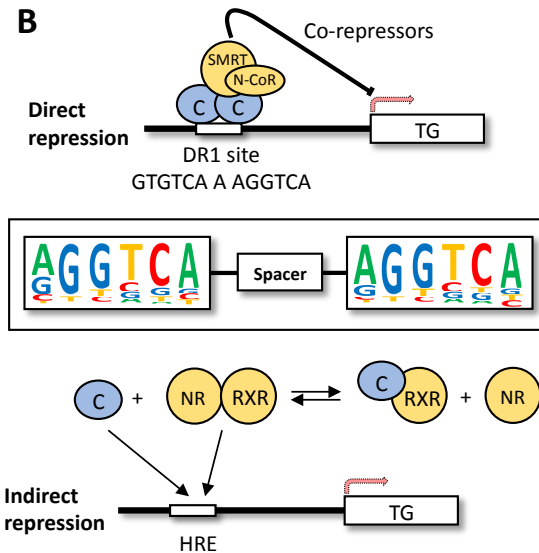
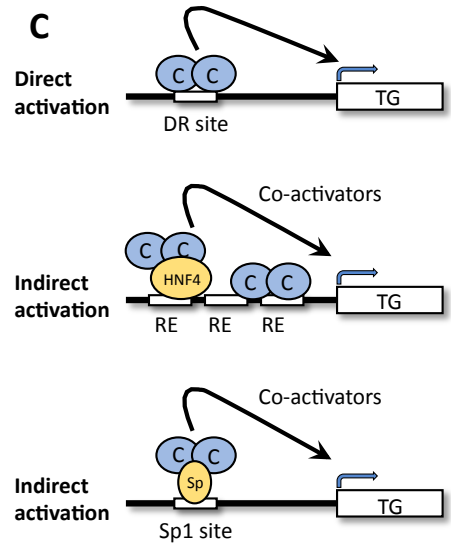
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COUP-TF



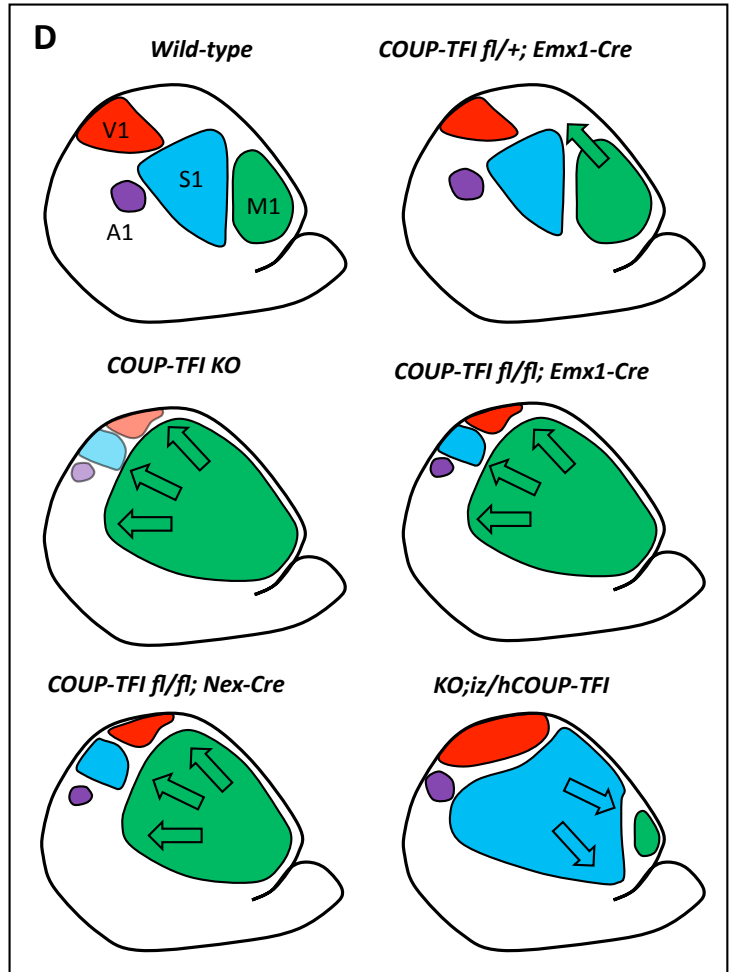
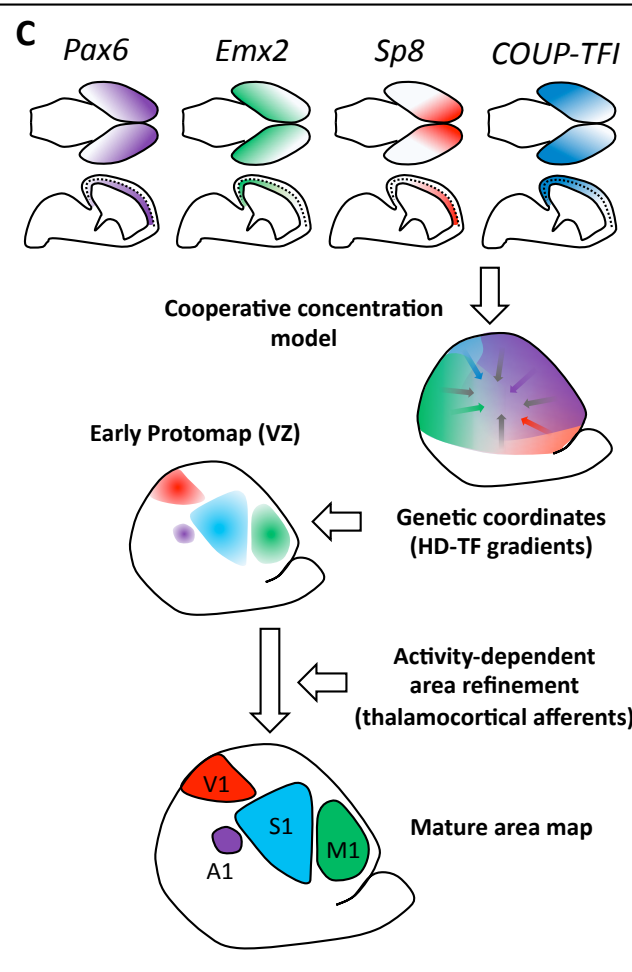
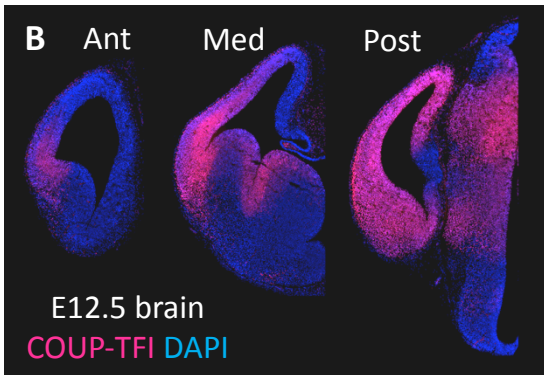
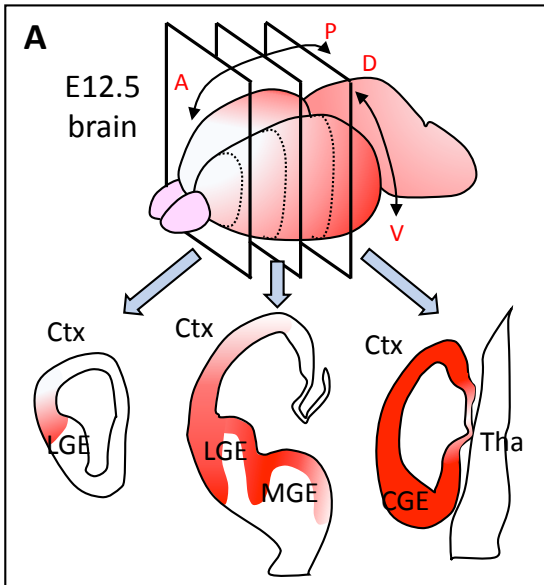
Target gene

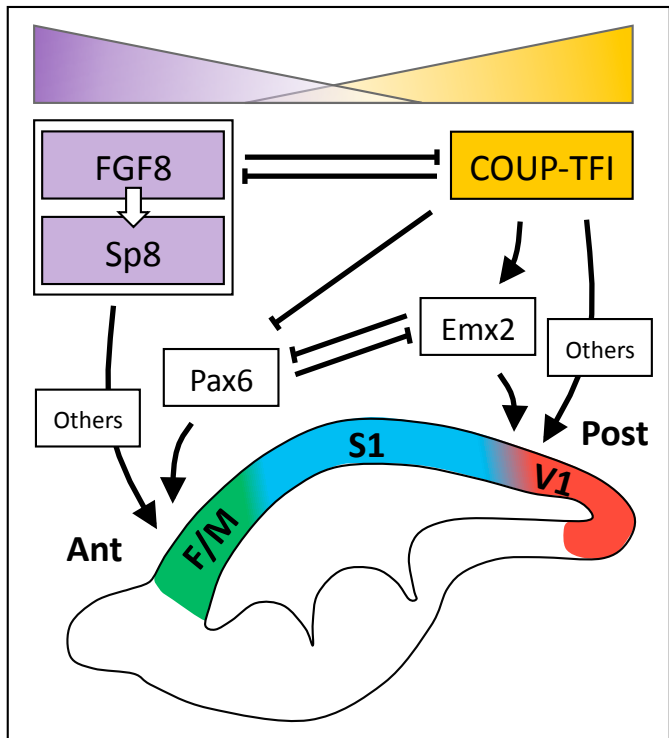


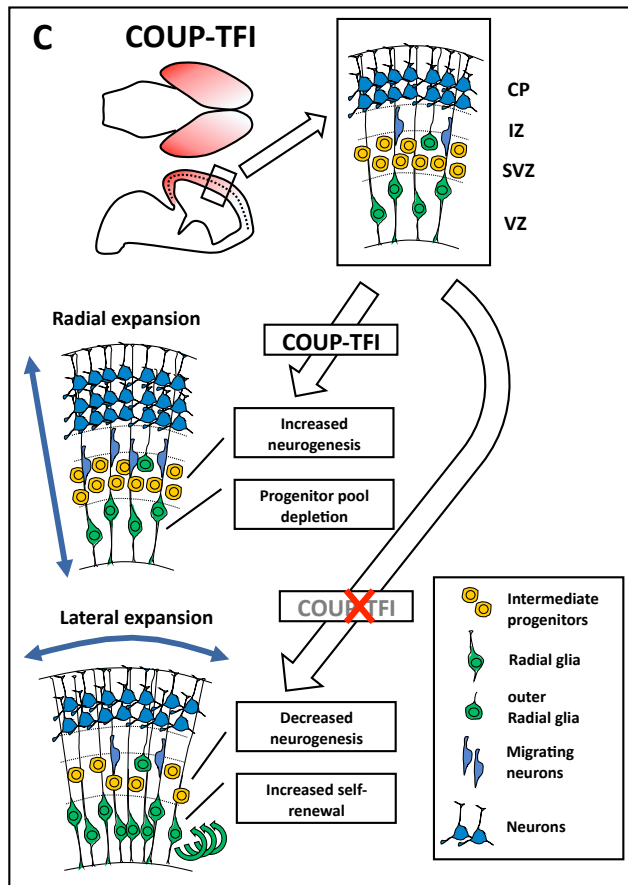
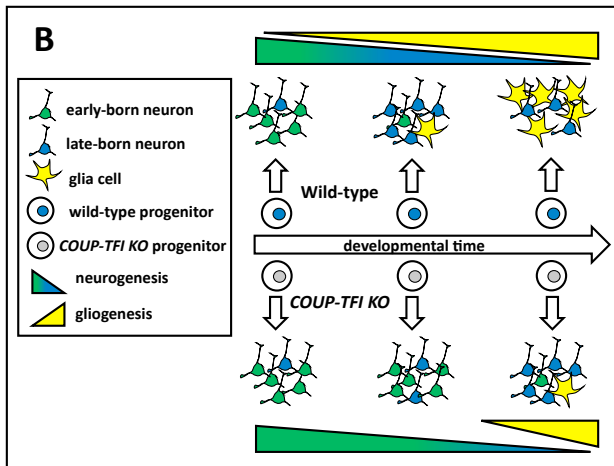
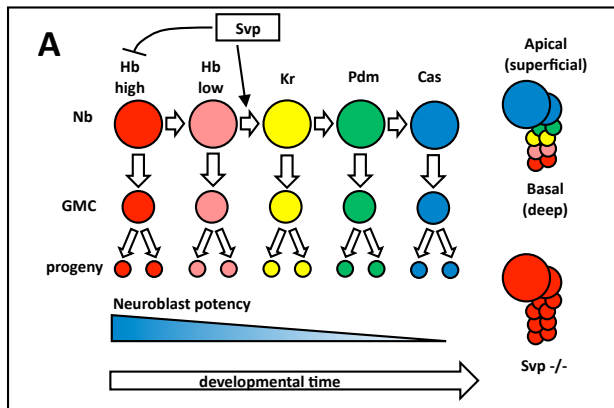
Nuclear receptor



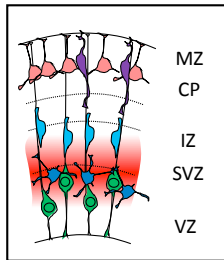
Co-factors





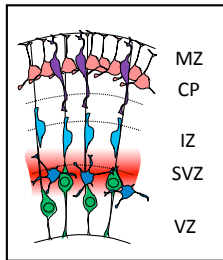


### *Wild-type rostral*



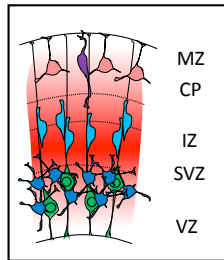
- High Rnd2
- Low migration rate

### *Wild-type caudo-medial*

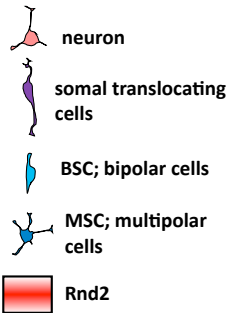


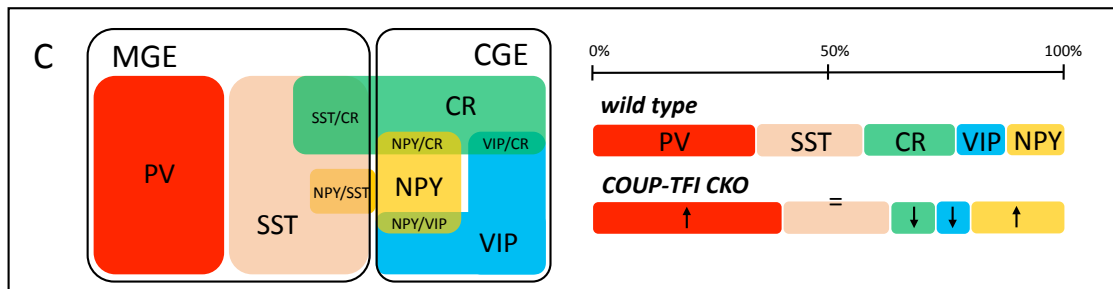
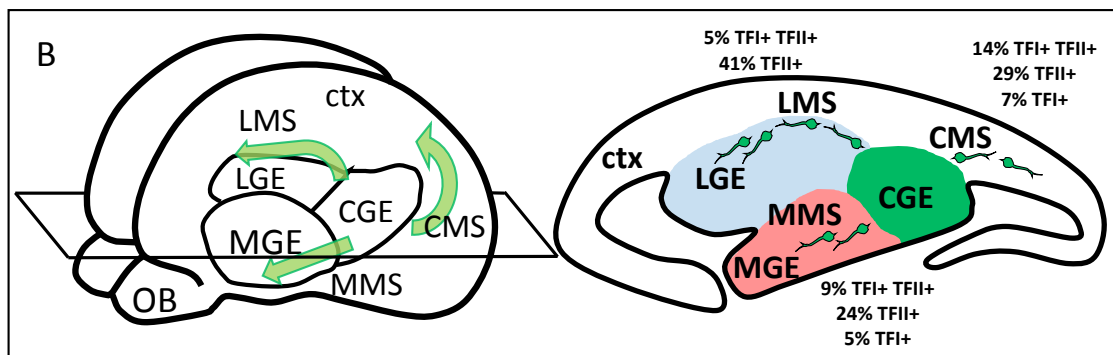
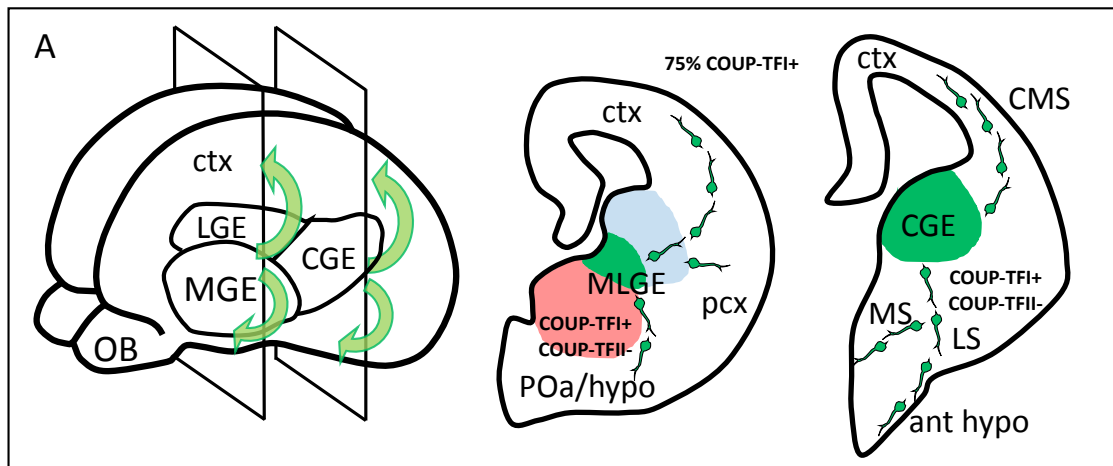
- Low Rnd2
- High migration rate

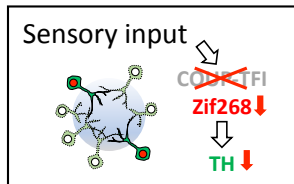
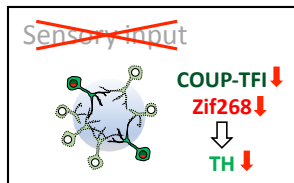
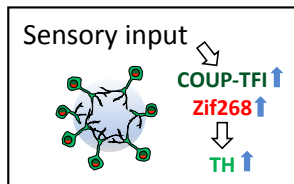
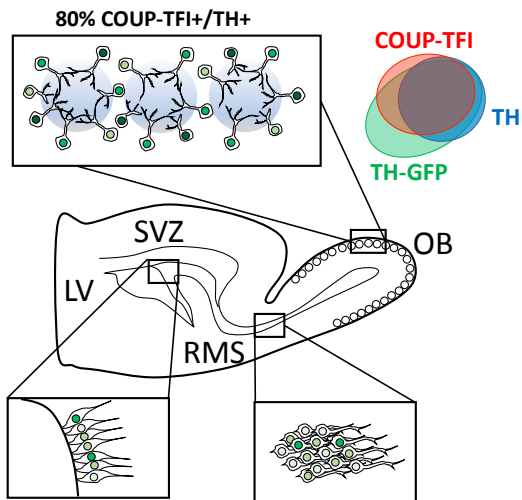
### *COUP-TFI KO*

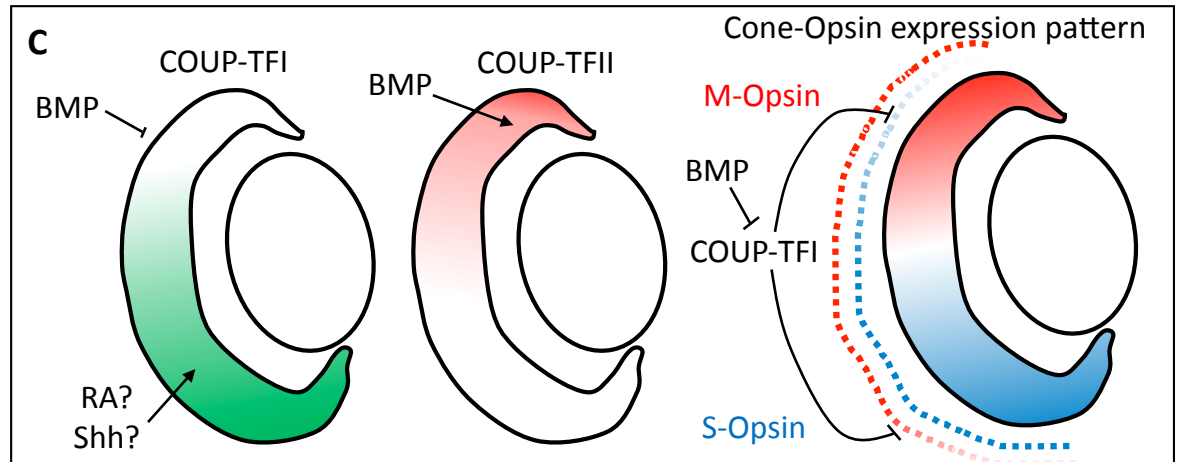
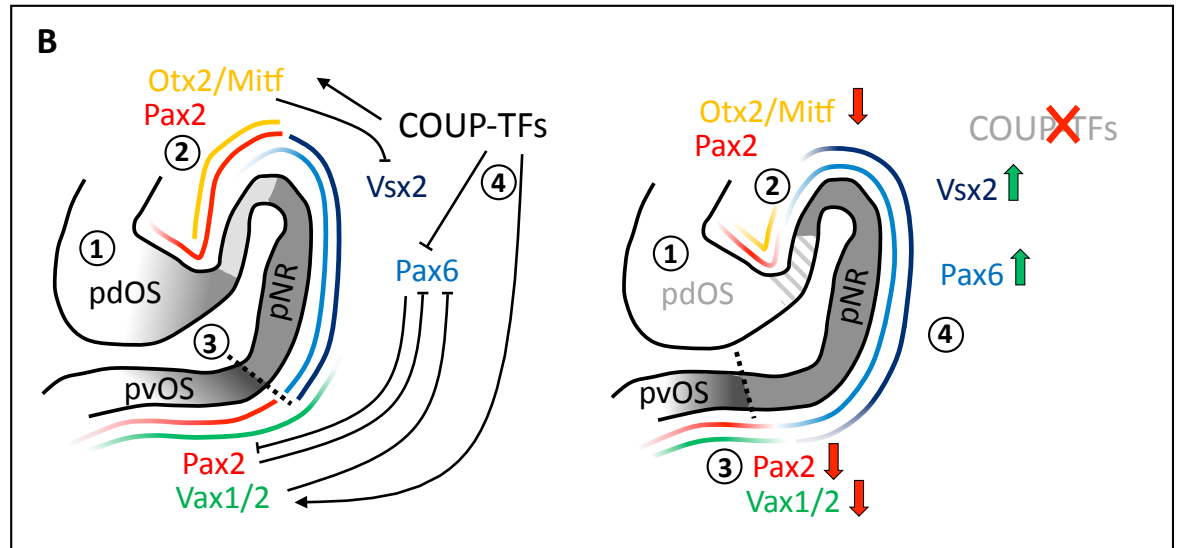
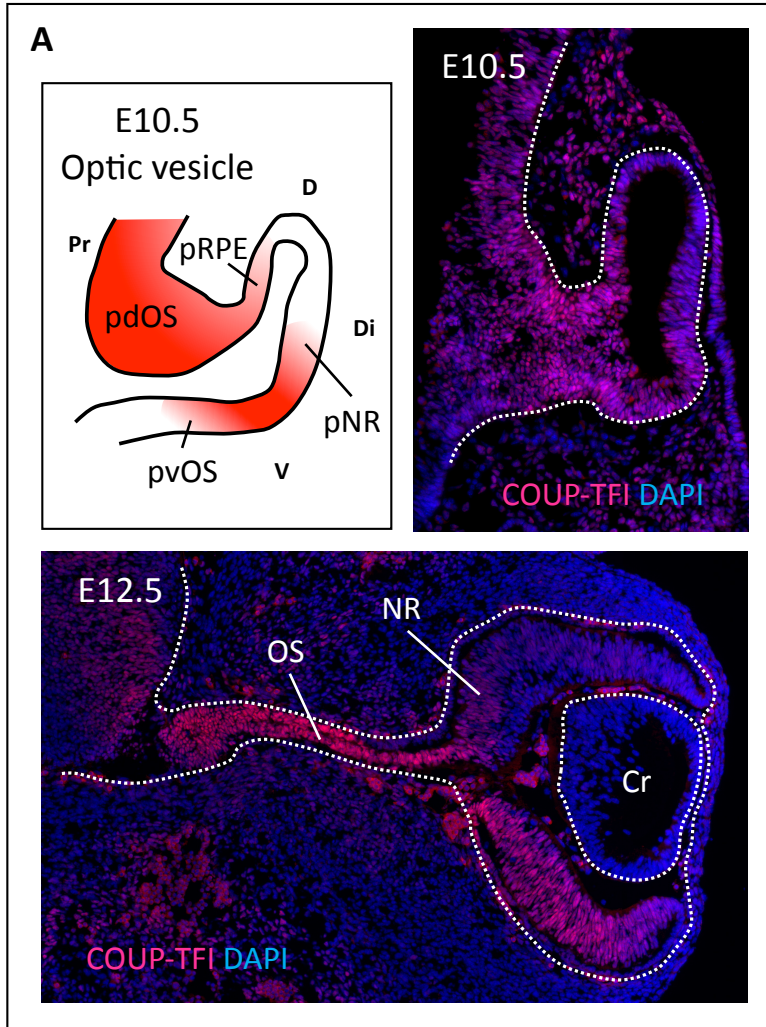


- Very high Rnd2 (even caudal)
- Impaired migration
- MSC increase
- Stalled MSC and BSC in SVZ/IZ



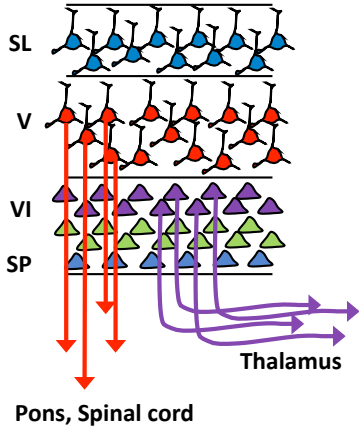
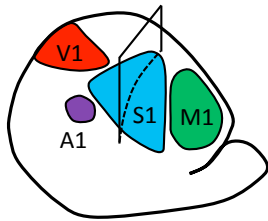




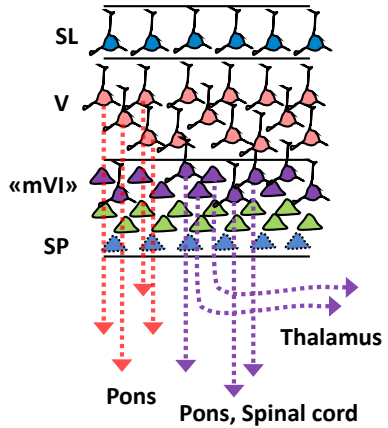
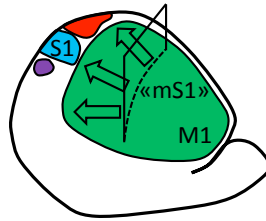








**Wild-type S1 cortex**



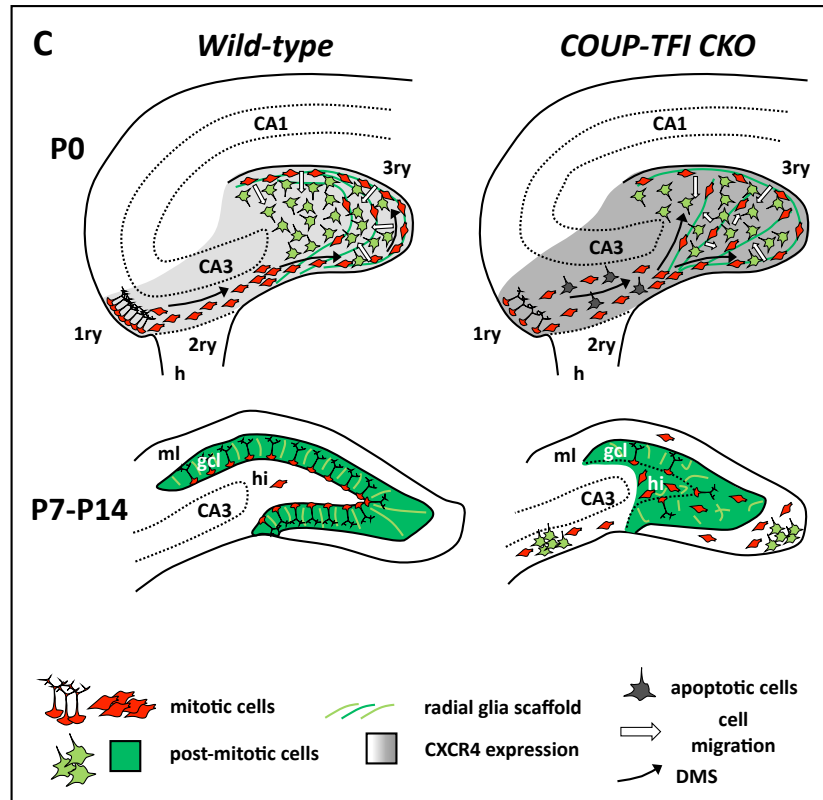
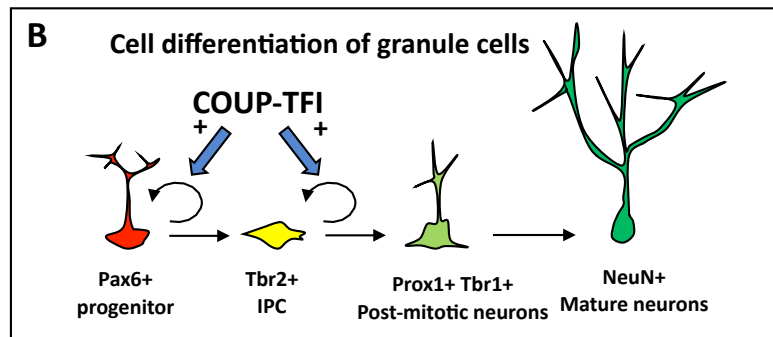
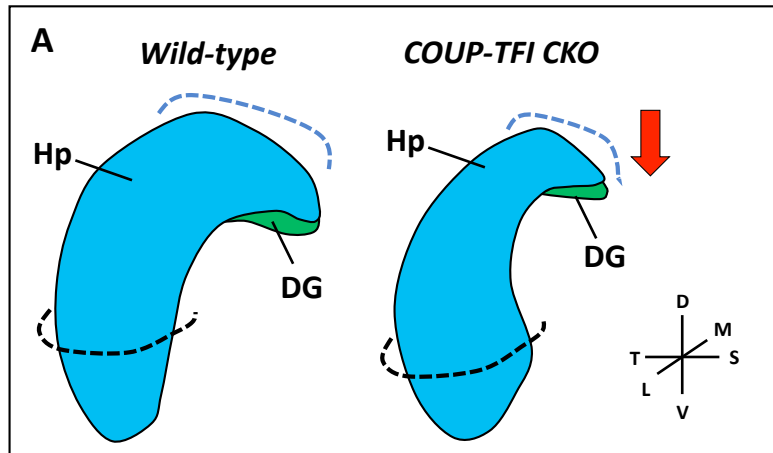
**COUP-TFI CKO «motorized» S1 cortex**



 CPN (layers II and III)  
 Pyramidal CSMN

 CThPN  
 SPN

 Layer VIb neurons



Reference	Patients	Age	Sex	Variant	Type	MRI (excluded OA)	DD	ID	EOE/S	Hypotonia	OA	CVI	FD	OD	ASD	ADHD	Other clinical features
<b>Brown et al. 2009</b>	Patient 1	4 yrs	F	5q15 MD	de novo	Cranial nerve abnormalities	yes	ND	ND	yes	ND	ND	yes	yes	ND	ND	HD, strabismus, malformed ears
<b>Cardoso et al. 2009</b>	Patient 2	7 yrs	M	5q14.3-q15 D	de novo	PH	yes	yes	FS	yes	coloboma	ND	yes	ND	ND	ND	DSD, exotropia
	Patient 3	5 yrs	F	5q14.3-q15 D	de novo	PH	yes	yes	IS	ND	ND	ND	minor	ND	ND	ND	DSD, bilateral pes talus
	Patient 4	5 yrs	M	5q14.3-q15 D	de novo	PH, macrocephaly, polymicrogyria, HPM	yes	yes	yes	yes	ND	ND	yes	ND	ND	ND	DSD
<b>Al-Kateb et al. 2013</b>	Patient 5	8 yrs	M	5q15 MD	de novo	Small optic chiasm	yes	no	ND	yes	yes	ND	yes	yes	ND	yes	DSD, strabismus, HD, DMD, uteropelvic obstruction
<b>Sanders et al. 2013</b>	Patient 6	child	ND	MM	de novo	ND	ND	ND	ND	ND	ND	ND	ND	ND	yes	ND	ND
<b>Bosch et al. 2014</b>	Patient 7	12 yrs	M	MM in DBD	de novo	normal	no	yes	ND	ND	yes (+P/SOD)	yes	yes	ND	ND	ND	ND
	Patient 8	2 yrs	F	MM in DBD	de novo	Small optic chiasm	yes	no	ND	yes	yes (+P/SOD)	yes	yes	ND	ND	ND	ND
	Patient 9	18 yrs	F	MM in LBD	de novo	ND	yes	yes	ND	yes	(P/SOD)	yes	yes	ND	ND	ND	ND
	Patient 10	24 yrs	F	5q15 MD	unknown	ND	no	mild	ND	ND	(P/SOD)	yes	yes	ND	ND	ND	ND
	Patient 11	4 yrs	F	5q15 MD	de novo	normal	yes	no	ND	ND	(P/SOD)	yes	yes	ND	ND	ND	ND
<b>Michaud et al. 2014</b>	Patient 12	35 yrs	F	MM in DBD	de novo	normal	yes	yes	IS	ND	yes	no	yes	ND	OCB, ASD	ND	ND
<b>Hino-Fukuyo et al. 2015</b>	Patient 13	ND	ND	MM	de novo	ND	yes	yes	IS	ND	ND	ND	ND	ND	ND	ND	ND
<b>Dimassi et al. 2015</b>	Patient 14	adult	F	MM	de novo	normal	yes	yes	ND	ND	yes	ND	ND	ND	ND	ND	ND
<b>Chen et al. 2016</b>	Patient 15	ND	ND	FI	de novo	mild LV asymmetry	yes	yes	IS	yes	ND	ND	ND	ND	ND	ND	ND
	Patient 16	6 yrs	M	MM in DBD	de novo	thin CC, white matter reduction	yes	yes	yes	yes	yes	no	yes	yes	RB, ASD	ND	HD
	Patient 17	4 yrs	F	MM in DBD	de novo	thin CC, DM	yes	yes	IS	yes	mild	thin CC	yes	yes	no	ND	esotropia and amblyopia
	Patient 18	15 yrs	M	MM in DBD	de novo	normal	yes	yes	FS	no	yes	no	yes	no	RB, ASD	ND	ND
	Patient 19	4 yrs	F	MM in DBD	de novo	thin CC	yes	yes	yes	yes	yes	no	yes	yes	no	ND	Nystagmus
	Patient 20	11 yrs	M	MM in DBD	de novo	thin CC	yes	yes	no	yes	yes	no	yes	yes	RB	ND	DSD
	Patient 21	10 yrs	M	MM in DBD	de novo	normal	no	no	no	yes	no	no	yes	no	no	ND	DSD
	Patient 22	21 yrs	M	MM in LBD	de novo	normal	yes	yes	yes	no	no	no	yes	no	RB, ASD	ND	ND
	Patient 23	6 yrs	F	TIV	de novo	thin CC	yes	yes	IS	yes	yes	no	yes	no	no	ND	ND
	Patient 24	30 yrs	M	TIV	de novo	normal	yes	yes	yes	yes	yes (+P/SOD)	no	mild	yes	RB, ASD	yes	HD, oculomotor disturbances
	Patient 25	21 yrs	M	TIV	de novo	ND	yes	yes	no	yes	yes	no	yes	yes	OCB/RB, ASD	yes	DSD, strabismus
	Patient 26	7 yrs	M	TIV	de novo	normal	yes	yes	no	yes	yes	no	yes	yes	RB, ASD	ND	HD, spasticity
	Patient 27	4 yrs	F	TIV	de novo	thin CC	yes	yes	no	no	yes (+P/SOD)	no	yes	yes	no	ND	ND
	Patient 28	3 yrs	F	FI	de novo	ND	yes	yes	no	yes	no	yes	yes	no	no	ND	esotropia and hyperopia
	Patient 29	12 yrs	F	FI	de novo	thin CC and CS tract	yes	yes	yes	yes	yes	no	yes	yes	OCB	yes	HD, DSD
	Patient 30	43 yrs	M	NFI	de novo	stenogryria, HPM	yes	yes	yes	yes	yes	no	yes	yes	no	ND	DMD, DSD
	Patient 31	8 yrs	F	WGD	de novo	ND	yes	yes	no	yes	yes (+P/SOD)	no	yes	no	no	yes	DSD, strabismus, nystagmus, hyperopia, chiasmal misrouting
Patient 32	35 yrs	M	WGD	ND	ND	yes	yes	no	no	yes (+P/SOD)	no	yes	no	no	yes	strabismus, red-green color blindness	
Patient 33	2 yrs	M	WGD	paternal	CC agenesis, DM	yes	yes	no	yes	yes (+P/SOD)	no	yes	yes	no	ND	DMD, nystagmus	
Patient 34	37 yrs	M	WGD	ND	ND	yes	yes	no	no	yes	no	yes	no	ASD	ND	ND	
Patient 35	6 yrs	F	WGD	ND	normal	yes	yes	no	yes	no	no	yes	yes	no	ND	macrocephaly	
<b>Kaiwar et al. 2017</b>	Patient 36	14 yrs	M	MM in DBD	de novo	macrocephaly	yes	yes	IS	yes	yes	ND	yes	yes	RB	ND	DMD, DSD, short stature, low levels of serotonin + dopamine metabolites in CSF
	Patient 37	5 yrs	F	MM in LBD	de novo	ND	yes	yes	ND	yes	no	ND	mild	yes	RB	ND	heart murmur, amblyopia
<b>Martin-Hernandez et al. 2018</b>	Patient 38	17 yrs	F	MM in DBD	de novo	thin CC	yes	yes	no	yes	mild	ND	no	yes	ND	ND	stroke-like event + mitochondrial complex IV deficit

PREVALENCE	34/38	32/38	15/38	25/38	26/38	7 out of 38	33/38	17/38	13/38	6 out of 38	HD: 6 out of 38	DSD: 11 out of 38
	89%	84%	40%	66%	68%	18%	87%	45%	34%	16%	16%	29%

<b>CC</b>	Corpus Callosum	<b>LBD</b>	Ligand binding domain
<b>CS</b>	Corticospinal tract	<b>LV</b>	Lateral ventricle
<b>D/MD</b>	Deletion / Microdeletion	<b>MM</b>	Missense mutation
<b>DBD</b>	DNA binding domain	<b>OA</b>	Optic atrophy
<b>DD</b>	Developmental delay	<b>OCB/RB</b>	Obsessive-compulsive/Repetitive behaviors
<b>DM</b>	Delayed myelination	<b>OD</b>	Oromotor dysfunctions
<b>DMD</b>	Delayed motor development/Poor coordination	<b>ON</b>	Optic nerve
<b>DSD</b>	Delayed speech development	<b>P/SOD</b>	Pale/small optic disc
<b>EOE/S</b>	Early onset epilepsy/Seizures	<b>PH</b>	Bilateral Periventricular Heterotopia
<b>FD</b>	Facial dysmorphism	<b>TIV</b>	Translation initiation variants
<b>FI/NFI</b>	Frameshifting indel/Nonframeshifting indel	<b>WGD</b>	Whole gene deletion
<b>FS</b>	Febrile seizures		
<b>HD</b>	Hearing defects		
<b>HP</b>	Hippocampus		
<b>HPM</b>	Hippocampal malrotation		
<b>ID</b>	Intellectual disability		
<b>IS</b>	Infantile spasms		

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<b>Cardoso et al. 2009</b>	Patient 2	7 yrs	M	5q14.3-q15 D	de novo	PH	yes	yes	FS	yes	coloboma	ND	yes	ND	ND	ND	DSD, exotropia	
	Patient 3	5 yrs	F	5q14.3-q15 D	de novo	PH	yes	yes	IS	ND	ND	ND	minor	ND	ND	ND	DSD, bilateral pes talus	
	Patient 4	5 yrs	M	5q14.3-q15 D	de novo	PH, MA, polymicrogyria, HPM	yes	yes	yes	yes	ND	ND	yes	ND	ND	ND	DSD	
<b>Al-Kateb et al. 2013</b>	Patient 5	8 yrs	M	5q15 MD	de novo	Small optic chiasm	yes	no	ND	yes	yes	ND	yes	yes	ND	yes	DSD, STR, HD, DMD, uteropelvic obstruction	
<b>Sanders et al., 2013</b>	Patient 6	child	ND	MM	de novo	ND	ND	ND	ND	ND	ND	ND	ND	ND	yes	ND	ND	
<b>Bosch et al. 2014</b>	Patient 7	12 yrs	M	MM in DBD	de novo	normal	no	yes	ND	ND	yes (+P/SOD)	yes	yes	ND	ND	ND	ND	
	Patient 8	2 yrs	F	MM in DBD	de novo	Small optic chiasm	yes	no	ND	yes	yes (+P/SOD)	yes	yes	ND	ND	ND	ND	
	Patient 9	18 yrs	F	MM in LBD	de novo	ND	yes	yes	ND	yes	(P/SOD)	yes	yes	ND	ND	ND	ND	
	Patient 10	24 yrs	F	5q15 MD	unknown	ND	no	mild	ND	ND	(P/SOD)	yes	yes	ND	ND	ND	ND	
	Patient 11	4 yrs	F	5q15 MD	de novo	normal	yes	no	ND	ND	(P/SOD)	yes	yes	ND	ND	ND	ND	
	Patient 12	35 yrs	F	MM in DBD	de novo	normal	yes	yes	ND	ND	yes	no	yes	ND	OCB, ASD	ND	ND	
<b>Michaud et al. 2014</b>	Patient 13	ND	ND	MM	de novo	ND	yes	yes	IS	ND	ND	ND	ND	ND	ND	ND	ND	
<b>Hino-Fukuyo et al. 2015</b>	Patient 14	adult	F	MM	de novo	normal	yes	yes	ND	ND	yes	ND	ND	ND	ND	ND	ND	
<b>Dimassi et al. 2015</b>	Patient 15	ND	ND	FI	de novo	mild LV asymmetry	yes	yes	IS	yes	ND	ND	ND	ND	ND	ND	ND	
<b>Chen et al. 2016</b>	Patient 16	6 yrs	M	MM in DBD	de novo	thin CC, white matter reduction	yes	yes	yes	yes	yes	no	yes	yes	RB, ASD	ND	HD	
	Patient 17	4 yrs	F	MM in DBD	de novo	thin CC, DM	yes	yes	IS	yes	mild	yes	yes	yes	no	ND	esotropia and amblyopia	
	Patient 18	15 yrs	M	MM in DBD	de novo	normal	yes	yes	FS	no	yes	no	yes	no	RB, ASD	ND	ND	
	Patient 19	4 yrs	F	MM in DBD	de novo	thin CC	yes	yes	yes	yes	yes	no	yes	yes	no	ND	Nystagmus	
	Patient 20	11 yrs	M	MM in DBD	de novo	thin CC	yes	yes	no	yes	yes	no	yes	yes	RB	ND	DSD	
	Patient 21	10 yrs	M	MM in DBD	de novo	normal	no	no	no	yes	no	no	yes	no	no	ND	DSD	
	Patient 22	21 yrs	M	MM in LBD	de novo	normal	yes	yes	yes	no	no	no	yes	no	RB, ASD	ND	ND	
	Patient 23	6 yrs	F	TIV	de novo	thin CC	yes	yes	IS	yes	yes	no	yes	no	no	ND	ND	
	Patient 24	30 yrs	M	TIV	de novo	normal	yes	yes	yes	yes	yes (+P/SOD)	no	mild	yes	RB, ASD	yes	HD, oculomotor disturbances	
	Patient 25	21 yrs	M	TIV	de novo	ND	yes	yes	no	yes	yes	no	yes	yes	OCB/RB, ASD	yes	DSD, STR	
	Patient 26	7 yrs	M	TIV	de novo	normal	yes	yes	no	yes	yes	no	yes	yes	RB, ASD	ND	HD, spasticity	
	Patient 27	4 yrs	F	TIV	de novo	thin CC	yes	yes	no	no	yes (+P/SOD)	no	yes	yes	no	ND	ND	
	Patient 28	3 yrs	F	FI	de novo	ND	yes	yes	no	yes	yes	yes	yes	no	no	ND	esotropia and hyperopia	
	Patient 29	12 yrs	F	FI	de novo	thin CC and CS tract	yes	yes	yes	yes	yes	no	yes	yes	OCB	yes	HD, DSD	
	Patient 30	43 yrs	M	NFI	de novo	stenogyria, HPM	yes	yes	yes	yes	yes	no	yes	yes	no	ND	DMD, DSD	
	Patient 31	8 yrs	F	WGD	de novo	ND	yes	yes	no	yes	yes (+P/SOD)	no	yes	no	no	yes	DSD, STR, nystagmus, hyperopia, chiasmal misrouting	
	Patient 32	35 yrs	M	WGD	ND	ND	yes	yes	no	no	yes (+P/SOD)	no	yes	no	no	yes	STR, red-green color blindness	
	Patient 33	2 yrs	M	WGD	paternal	CC agenesis, DM	yes	yes	no	yes	yes (+P/SOD)	no	yes	yes	no	ND	DMD, nystagmus	
	Patient 34	37 yrs	M	WGD	ND	ND	yes	yes	no	no	yes	no	yes	no	ASD	ND	ND	
	Patient 35	6 yrs	F	WGD	ND	MA	yes	yes	no	yes	no	no	yes	yes	no	ND	ND	
	<b>Kaiwar et al. 2017</b>	Patient 36	14 yrs	M	MM in DBD	de novo	MA	yes	yes	IS	yes	yes	ND	yes	yes	RB	ND	DMD, DSD, short stature, low serotonin + dopamine in C
		Patient 37	5 yrs	F	MM in LBD	de novo	ND	yes	yes	ND	yes	no	ND	mild	yes	RB	ND	heart murmur, amblyopia
	<b>Martin-Hernandez et al. 2018</b>	Patient 38	17 yrs	F	MM in DBD	de novo	thin CC	yes	yes	no	yes	mild	ND	no	yes	ND	ND	stroke-like event + mitochondrial complex IV deficit
<b>PREVALENCE</b>							<b>34/38</b> <b>89%</b>	<b>32/38</b> <b>84%</b>	<b>15/38</b> <b>40%</b>	<b>25/38</b> <b>66%</b>	<b>26/38</b> <b>68%</b>	<b>7/38</b> <b>18%</b>	<b>33/38</b> <b>87%</b>	<b>17/38</b> <b>45%</b>	<b>13/38</b> <b>34%</b>	<b>6/38</b> <b>16%</b>	<b>HD: 6/38</b> <b>16%</b>	<b>DSD: 11/38</b> <b>29%</b>

<b>CC</b>	Corpus Callosum	<b>FI/NFI</b>	Frameshifting indel/Nonframeshifting indel	<b>MM</b>	Missense mutation	
<b>CS</b>	Corticospinal tract	<b>FS</b>	Febrile seizures	<b>OA</b>	Optic atrophy	<b>ND=Non Detected</b>
<b>D/MD</b>	Deletion / Microdeletion	<b>HD</b>	Hearing defects	<b>OCB/</b>	Obsessive-compulsive/Repetitive behaviors	
<b>DBD</b>	DNA binding domain	<b>HP</b>	Hippocampus	<b>OD</b>	Oromotor dysfunctions	
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<b>DM</b>	Delayed myelination	<b>ID</b>	Intellectual disability	<b>P/SO</b>	Pale/small optic disc	
<b>DMD</b>	Delayed motor development/Poor coordination	<b>IS</b>	Infantile spasms	<b>PH</b>	Bilateral Periventricular Heterotopia	
<b>DSD</b>	Delayed speech development	<b>LBD</b>	Ligand binding domain	<b>STR</b>	Strabismus	
<b>EOE/S</b>	Early onset epilepsy/Seizures	<b>LV</b>	Lateral ventricle	<b>TIV</b>	Translation initiation variants	
<b>FD</b>	Facial dysmorphism	<b>MA</b>	Macrocephaly	<b>WGD</b>	Whole gene deletion	

CSF