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Review

Missense mutations resulting in type 1 lissencephaly

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Abstract. Proper human brain formation is dependent upon the integrated activity of multiple genes. Malfunctioning of key proteins results in brain developmental abnormalities. Mutation(s) in the *LIS1* gene or the X-linked gene *doublecortin* (DCX) results in a spectrum of disorders including lissencephaly, or 'smooth brain', and subcortical band heterotopia, or 'doublecortex'. Here, we will focus on a particular subset of missense mutations in these two genes and their effect on protein structure and function.

Key words. Brain development; lissencephaly; LIS1; doublecortin; neuronal migration; microtubules.

Introduction

The fact that a single amino acid change may result in a genetic disease was first documented in 1949 when Pauling and colleagues isolated hemoglobin from a sickle-cell patient [1]. The abnormality was due to a valine-to-glutamic acid substitution described by Ingram only in 1956 [2]. The amino acid change was due to a missense mutation, with one DNA base change in the coding region, resulting in a changed codon for amino acid. Although more than 50 years of research have passed since this initial discovery, our ability to predict the outcomes of specific missense mutations has not increased in a significant way. In this review, we focus on missense mutations, which dramatically affect neuronal migration during brain development, and result in lissencephaly ('smooth brain') or subcortical band heterotopia ('double cortex').

The functional complexity of the vertebrate cerebral cortex is facilitated by an intricate structural organization. Brain morphogenesis involves multiple steps, including cell proliferation, cell fate identity, cell migration and cell differentiation implicating growth of axons and dendrites. The migratory pathway of individual neurons can be extremely long, especially in primates (reviewed by [3]). Neurons in the neocortex are organized into six layers that share characteristic dendritic morphologies, axonal connections and physiological properties (reviewed in [4, 5]). The neocortex is also regionalized into areas characterized by distinctive cytoarchitectonic features [6] that house specific cognitive, sensory or motor functions [7]. The axonal connections of neurons in different areas reflect their functional specificity. The six layers of the neocortex are formed during development mainly by inward-to-outward migration of neurons [8] born in the ventricular zone to the cortical plate [9, 10]. Our knowledge regarding key molecules participating in brain lamination has increased significantly during the last decade, with the discovery of disease genes associated with abnormal cortical layering (reviewed in [11]). The disorders of neuroblast migration are customarily divided according to the anatomical sites of arrested migration: (i) in the periventricular region (embryonic subventricular zone) from where they never initiate their migration; (ii) in the subcortical white matter where their journey is arrested before reaching the cortical plate; and (III) disturbance of organization of the cortical plate in terms of lamination and orientation of neurons, which

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also affects their synaptic circuitry. In many cases the phenotype does not correspond only to one category, but is rather a mixture (as described below). Abnormalities in the migration of neuroblasts into the embryonic cortex lead in extreme cases to loss of normal convolutions of the cortex in humans, known as lissencephaly (smooth brain). There are two types of lissencephaly: type I or 'classical lissencephaly', where four layers of abnormally positioned neurons are observed in the neocortex, and type II or 'cobblestone' lissencephaly, where the cortex is unlayered [12]. Mutations in two different genes may result in type I lissencephaly: *LIS1*, an autosomal gene located on chromosome 17p13.3 [13], and *doublecortin*, an X-linked gene [14, 15].

Lissencephaly is characterized by absent (agyria) or decreased (pachygyria) convolutions, producing a smooth cerebral surface with thickened cortex [16]. Subcortical band heterotopia (SBH) is a related disorder in which there are bilateral bands of gray matter interposed in the white matter between the cortex and the lateral ventricles [17]. SBH is very common among females with mutations in DCX [14, 15]. Lissencephaly and SBH have been observed in different regions of the same brain, defining an 'agyria–pachygyria-band' spectrum. Thus, it is possible to observe SBH, pachygyria/lissencephaly and poor cortical architecture in the same brain. So far, to the best of our knowledge, a clear genetic-neuropathological correlation in this regard has not been determined. A grading system characterizes the malformations from grade 1 (most severe) to grade 6 (least severe) [18]. Grades 1–4 are all lissencephalies of decreasing severity. Grade 5 is mixed pachygyria and SBH, whereas grade 6 is SBH alone.

LIS1

Deletions involving *LIS1* are far more common than other mutations [19, 20], 88% of the mutations result in a truncated or internally deleted protein. Until now, only six missense mutations were found in patients [20–22]. Nevertheless, truncated and internally deleted proteins are not likely to reveal much information regarding protein function since these proteins are unlikely to fold correctly [23, 24]. With the goal of learning more about *LIS1* protein function, a group of five missense mutations described in patients leading to variable phenotypes were analyzed [25]. The fact that single amino acid substitutions can result in different phenotypes may help us to address the complicated task of elucidating the mechanisms behind brain development. Prior to discussing mutation analysis, we shall outline basic information regarding the *LIS1* protein structure and function.

The deduced amino acid sequence places *LIS1* as a member of the WD (tryptophan-aspartic acid) repeat family of proteins [13]. A hallmark of this family is their involvement in multiple protein-protein interactions [26], and *LIS1* is not an exception (reviewed by [27]). *LIS1* contains seven WD repeats in its C-terminal region and a distinct N-terminal region of 95 amino acids. Previously, this region was found to include a LisH (*LIS1* homology domain amino acids 8–37) [28] and a coiled-coil domain (amino acids 51–79) important for dimerization [29]. Recently, the structure of the N-terminal region was solved [30]. The structure revealed that the LisH motif is a novel, thermodynamically very stable dimerization domain. Thus, the N-terminal region contains two domains, which both contribute to *LIS1* dimerization.

LIS1-protein interactions may be grouped conceptually into two classes: evolutionarily conserved and relatively new interactions. Much has been learnt from the interactions that have been conserved throughout evolution [31]. NudF, a LIS1 homolog in *Aspergillus nidulans*, was identified in a screen for mutants defective in nuclear migration [32]. All the mammalian homologs of genes involved in this pathway have been shown to interact with *LIS1*. Such is the case for the mammalian homolog of NudC, a protein that controls the levels of NudF in *Aspergillus nidulans* [32], and was shown to interact with LIS1 [33]. This is consistent with a similarity of mechanism between nuclear movement in the fungus and neuronal migration in the brain, since nuclear or somal translocation is one of the three main types of radial migration during the neocorticogenesis (reviewed by [34]). A tight relationship between LIS1 and microtubule (MT) regulation and MT-based motor proteins has been suggested for many organisms. In *Aspergillus nidulans* a mutation in the alpha tubulin gene suppresses mutations in *nudA*, *nudC*, *nudG* and *nudF* [35]. We have shown that LIS1 interacts with tubulin and can modulate MT dynamics in vitro [36], suggesting an evolutionarily conserved LIS1 function. This interaction and probably others are regulated by phosphorylation [37]. Several of the nud genes (*nudA*, *nudI* and *nudG*) encode subunits of cytoplasmic dynein (hereafter called dynein), a MT-based motor protein (reviewed by [38]), while NudK and NudM are part of the dynein regulatory complex dynactin [39–42]. A genetic interaction of LIS1 with the dynein/dynactin/MT-mediated pathway has also been suggested from work on early development in *Drosophila* [43–45], demonstrating that *LIS1*, similar to dynein heavy chain, is essential for germ-line division, nuclear positioning and oocyte differentiation. Moreover, also in *Saccharomyces cerevisiae*, a LIS1 homolog is involved in nuclear migration [46]. LIS1 was found to interact directly with three distinct subunits of the dynein and dynactin complexes, representing both motor and cargo binding regions [47, 48]. Indeed, it has been shown to directly interact with the dynein heavy chain (in the P1 loop region, and another region involved in the interaction with light intermediate chains, LICs)

[47, 48], the dynein intermediate chain [47, 48] and the dynamitin (p50) [47, 48]. Furthermore, dynein, dynactin and LIS1 coimmunoprecipitated and colocalized both in tissue culture and in the brain [49, 50]. In addition, overexpression [49, 50] as well as reduced expression [50, 51] of LIS1 interfered with dynein function. An additional member of this pathway is *nudE*, a multicopy suppressor of a mutation in the *nudF* gene [52]. The interaction is evolutionary conserved: LIS1 interacts with two mammalian homologs of NUDE (NUDEL and mNudE or rNudE, 85% similarity in the coiled-coil domain) [47, 52–54].

An additional LIS1 interacting protein that is part of dynein/dynactin complex is cytoplasmic linker protein (CLIP-170) [55, 56]. CLIP-170 is a MT-plus-end tracking protein [57], which may act as a rescue factor [58]. It has been proposed to mediate the association of dynein/dynactin to MT plus ends [59–62], and it also binds to kinetochores in a dynein/dynactin-dependent fashion [63], both via its C-terminal domain. We recently demonstrated a direct interaction between these two proteins and suggested that LIS1 is a regulated adapter between CLIP-170 and the dynein/dynactin complex at sites involved in cargo-MT loading and/or in the control of MT dynamics [60].

Furthermore, LIS1 was found to be a regulatory subunit of platelet-activating factor acetylhydrolase (PAF-AH) Ib [64, 65], and this interaction is a relatively new event in evolution based on the homolog isolated from *Drosophila*. This protein lacked both catalytic activity and the ability to interact with LIS1 [66]. In spite of that, the *Drosophila* LIS1 protein does interact with mammalian PAF-AH subunits [66].

In addition, we detected an interaction between LIS1 and doublecortin (DCX) [67]. Our results suggest that LIS1 and DCX are coexpressed, interact and can function in the same protein complex in the developing brain [67]. Addition of both proteins promotes MT polymerization in an additive manner in vitro [67]. Interestingly, overexpression of DCX rescued the migration defect observed in *LIS1*$^{-/+}$ neurons [68]. The same study indicated an association of DCX with the dynein complex [68].

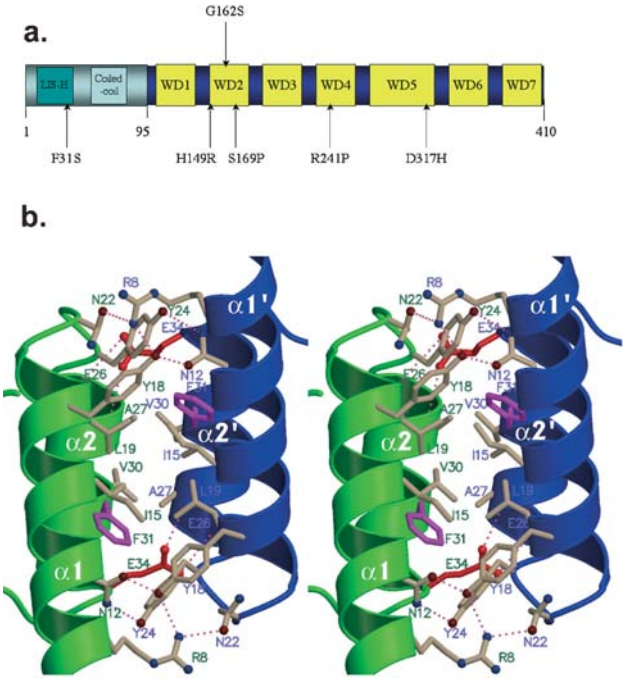

Figure 1 (*a*) Schematic presentation of LIS1. The known protein motifs are indicated (from the N-terminus to the C-terminus) LisH (green), coiled-coil (light blue), both located in the N-terminal region (grey), and seven WD repeats (yellow). The known missense mutations are indicated. (*b*) Intermolecular interface of the LisH dimer. A stereo side view between two LisH motifs showing one dimer in green and the other in blue. The most critical side chain contacts involved in the dimer interface are shown. F31 is highlighted in purple, The hydrophobic bonding network at each end of the interface is shown, as is the helix capping hydrogen bonds of E34 in red. Reprinted from Kim M. Y, Cooper D. R., Olekey A., Devedjiev Y., Derewenda U., Reiner O.et al., The structure of the N-terminal domain of the product of the lissencephaly gene *LIS1* and it functional implications, Structure 12: 987–998 Copyright (2004), with permission from Elsevier.

LIS1 missense mutations

We tested five different missense mutations for their gross biochemical properties, their subcellular localization, as well as their ability to retain interactions with known protein partners [25]. A schematic presentation of the LIS1 protein and the position of the known missense mutations are shown in figure 1. The summary of our results indicates that no single structural, biochemical, or subcellular localization assay can point out to the predicted lissencephaly severity. The biochemical profile included a protein-folding assay using resistance to trypsin cleavage, measuring of protein-protein interactions using recombinant proteins, yeast two-hybrid assays and co-immunoprecipitations. Subcellular localizations were assessed by immunostaining of mitotic and interphase cells. Therefore, it is likely that the basic biochemical and cellular pathways affected by each mutation are different.

Mutation H149R causes the most severe phenotype among the lissencephaly patients analyzed in this study. The biochemical profile of the mutated protein, including misfolding (as showed by complete degradation by trypsin) and short half-life, can provide the explanation. In addition, this mutation was examined previously, demonstrating unstability, misfolding [23] and abolishment of protein interactions [69–71]. Hence, the substitution of residue 149 from histidine to arginine resulted in a devastating malformation seen in the patient. The biochemical profile of mutation S169P is even worse than the one seen in mutation H149R. Mutation S169P is

the only substitution that is in clear contrast to the observed phenotype. The mutated protein is folded incorrectly, and its stability is extremely reduced. Its interaction with other proteins is the same as mutation D317H, which results in a much more severe phenotype. This mutation has also been investigated in relation to its interaction with PAF-AH subunits [69], NudEL [70] and NudE [71], and no interactions were observed [70]. These results can only be settled if we accept the biochemical predictions and the authors' [72] notion that the patient is a mosaic. In such a case, the severely defected protein (substitution of residue 169 from serine to proline) is expressed in only some of the cell population of the brain. In other cells the expression of wild-type LIS1 protein can diminish the resulted malformation. There are several documented cases of LIS1 mosiacism, for example patients with a missense mutation R241P, or a truncation mutation R8X, both exhibiting a mild phenotype of SBH [73]. Two of the mutations – F31S and D317H – cause the same lissencephalic grade (4a1); indeed, the total biochemical grade is also similar (18). However, looking closely at the experimental data reveals different biochemical properties, suggesting that diverse mechanisms are responsible for the observed phenotypes. Mutation F31S resides in the N-terminal region of LIS1 protein within the LisH domain [28] in the intermolecular interface of the structure (fig. 1b). This amino acid residue is highly conserved and has been proposed to play an integral role in the formation of the hydrophobic core and the dimerization interface. It is expected that removal of the phenyl ring of F31 would create two hydrophobic cavities in the heart of this dimer, destabilizing the complex [30]. Since the N-terminal region is predicted to remain external to the β-propeller structure characteristic of WD proteins Q it is not surprising that the mutated protein, was partially protected from trypsin cleavage, as the globular WD domain is most likely still intact. However, the mutation severely affects protein stability, and interaction abilities are reduced. The aggregates that are formed in cells may suggest nonfunctional mutated protein. We reported such protein aggregations of LIS1 mutated in F31S. The failure of polypeptides to adopt their proper structure is a major threat to cell function and viability, and may result in a toxic gain of function or be associated with a loss of function. In the case of the F31S mutation, we propose a loss of function mechanism, since the aggregated mutated protein does not interact with several cellular proteins. It is well known that molecular chaperones promote correct folding and prevent harmful interactions. Indeed, the PAF-AH catalytic subunit was identified as a potential LIS1 chaperone. Even so, once the aggregates are solubilized, the mutated LIS1 still does not properly localize and does not interact with other normal interacting proteins. The unfolded proteins are degraded primarily by the ubiquitin-proteasome system, and failure to detect and eliminate misfolded proteins may contribute to the pathogenesis of neurodegenerative disease or in this case to lissencephaly. Partial phenotypic rescue was observed with overexpression of Nudel or NudE, but not with overexpression of other LIS1 binding proteins such as CLIP-170 and dynein.

Although exhibiting the same lissencephaly phenotype, the obstructed pathway with the D317H mutation is different. Mutation D317H is located in WD5, which is unique among the WD repeats of LIS1 in that it contains a hinge region – rich in serine residues. The mutated protein is most likely missfolded, though the half-life assay may imply that some chaperone aid helps a portion of the protein to avoid degradation in the cell. Evidently, the two mutated proteins (F31S and D317H) behave in a different biochemical and cellular context. However, each of the two separate pathways eventually leads to the same lissencephaly phenotype. Mutation G162S located in the WD2 repeat of LIS1 manifests a rather mild phenotype exhibited by the patient. This outcome is supported by the biochemical data. Even though the protein is probably missfolded in vitro, it clearly maintains its stability. Furthermore, interactions with other proteins were only slightly affected, giving the highest interaction score. Overall, the results presented here are consistent with the weak manifestation of lissencephalic syndrome.

Among the five point mutations analyzed, three (H149, S169, D317) are conserved in all species that contain a LIS1, homolog, including *Drosophila, Caenorhabditis elegans, Dictyostelium discoideum, A. nidulans* and *S. cerevisiae*. The N-terminal region (where mutation F31S resides) is somewhat less conserved, and in *A. nidulans* and *S. cerevisiae,* the phenylalanine residue is changed to leucine. Glycine in position 162 in the patient is asparagine in *A. nidulans* but is a serine residue in *S. cerevisiae* thus identical to the mutation found in the patient with the least severe phenotype. Therefore, it could be expected that the LIS1 protein G162S should be capable of supporting the functions of LIS1 that are conserved in yeast.

DCX

As mentioned, a spectrum of neuronal migration defects including double cortex and lissencephaly result from mutations in LIS1 [13], or X-linked DCX [14, 15]. In the mouse, DCX mutants exhibit a lamination defect only in the hippocampus [74] identical to that described in *LIS1*$^{-/+}$ mice [75], suggesting that the two gene products participate in the same pathway. Interestingly, RNA interference (RNAi) of DCX has been shown to cause migrating neurons to arrest in migration, thus mimicking the double-cortex syndrome [76]. DCX [77–80] and its related gene product DCLK [81, 82] exhibit the proper-

ties of classical MT-associated proteins (MAP), and contain two tubulin binding repeat sequences which are evolutionarily conserved. Interestingly, most missense mutations (but not nonsense mutations) that are found in lissencephaly patients cluster in this defined DCX (double-cortin) domain [83, 84]. The repeated protein motifs are 11 kDa and located in the N-terminal region of DCX, which is 30 kDa. The C-terminal region is 10 kDa and rich in serine and threonine residues. The structure of the DCX domain is quite different from that of classical MAPs, and they adopt globular structures with a ubiquitin-like fold [85]. The nature of DCXs binding to MTs was studied using electron microscopy [86]. DCX binds selectively 13 protofilaments of MTs and also promotes assembly of MTs composed 13 protofilaments. DCX binds to MTs in a novel site, in between the protofilaments. This binding site also explains the stabilization of MTs by DCX.

DCX-interacting proteins

Although DCX is a classical MAP, it interacts with multiple additional proteins. Its interaction with clathrin adaptor proteins, the μ subunits of AP-1/-2, suggests a potential involvement of DCX in protein sorting or vesicular trafficking [87]. The region of interaction involves the C-terminal domain of DCX, where a tyrosine-based sorting signal (YLPL) was detected. A specific missense mutation (so far not detected in patients) in this sequence abolished this interaction. Tyrosine-based sorting signals, recognized by μ subunits, have previously been reported to be important for the recruitment of integral membrane proteins into clathrin-coated vesicles [88].

A significant portion of DCX is membrane bound, and it interacts in a phosphospecific manner with neurofascin [89]. Neurofascin is a transmembrane protein of the immunoglobulin (Ig) superfamily that engage in protein interactions as well as signaling pathways [90, 91]. The interaction with phospho-neurofascin requires the full-length DCX.

DCX is connected not only to the MT cytoskeleton, but may also link to the actin filament through its interaction with neurabin II, an actin binding protein [92]. The interaction site of DCX with neurabin II was mapped to a region extending between the C-terminal portion of the second part of the DCX motif and the N-terminal part of its Ser/Pro-rich region.

We detected an interaction between DCX and JNK interacting proteins, JIP1 or JIP2 [93]. These proteins serve as scaffold JNK proteins, and bind both activating and inhibitory components of this signaling pathway. JIP1/2 bind among their multiple interacting proteins JNK, MKK7 and members of the mixed-lineage kinase (MLK) group of MAP3K [94, 95]. Furthermore, both can bind the MAPK phosphatase MKP7 [96]. The C-terminal region of JIP1/2 provides a link with a molecular motor, as its last 11 amino acids are essential for interaction with kinesin light chain [97]. Indeed, we demonstrated that the distribution of DCX to the proximal parts of neurites is dependent upon its interaction with JIP1/2 and of the latter with kinesin [93]. JIP1/2 are structurally similar and contain a SH3 domain and a PID (protein interacting domain) domain in the C-terminal region [94, 95, 98–100]. The PID domain interacts with p190 RhoGEF [101], with amyloid precursor protein [102–104] and with members of the low-density lipoprotein receptor family, including ApoER2 and megalin [105, 106]. ApoER2 as one of the receptors for reelin is of particular interest, since it provides a link between DCX and the JNK pathway with the reelin pathway. The same cortical layering phenotype is observed in cases of mutations in the extracellular matrix protein reelin [107–109], or in two of its multiple receptors (ApoER2 and VLDL) [110, 111] or in the intracellular adaptor protein mDab1 [112]. JIP1/2 interacts with DCX in the PID, and the same point mutation in the PID, previously reported to affect the interaction of JIP-1 with RhoGEF [101], affected the interaction with DCX [93]. The JIP proteins also bind the Rac exchange factors Tiam1 and Ras-GRF1, although the interaction with JIP2 is significantly stronger than the interaction with JIP1 [113]. In addition, members of the fibroblast growth factor homologous protein family (FHF) have been reported to selectively interact with JIP2 [114].

Protein phosphorylation is a way to control transient protein interactions, which are typically used in signal transduction pathways. Recently, phosphorylation of DCX by at least three different kinases was demonstrated; JNK [93], Cdk5 [115], protein kinase A (PKA) and/or the MARK/PAR-1 family of protein kinases [116] (reviewed by [34, 117]). JNK phosphorylates DCX on at least three different sites, T321, T331 and S334 [93]. Cdk5 phosphorylates DCX on S297 [115] or, as other results indicate, S28, and S339 as the main phosphorylated sites [118], and PKA and/or the MARK kinase phosphorylates DCX on several sites, with the most significant one being S47 [116]. In vitro analysis indicated that DCXs phosphorylation by Cdk5, PKA and MARK reduced the affinity of DCX to MTs [115, 116]. We postulated that phosphorylation of DCX by different kinases may facilitate changes between different modes of neuronal migration occurring in the developing brain.

DCX missense mutations

To date, more than 25 missense mutations in DCX were detected in patients [14, 15, 72, 119–130], interestingly, several of these sites were independently mutated several times. Our discussion will focus on those mutations

which have been analyzed by functional studies. A schematic presentation of DCX and the position of the missense mutations to be discussed are shown in figure 2. A point mutation in serine 47 to arginine was detected in a lissencephaly patient and his mother [15]. Overexpression of the mutated protein in COS-7 cells resulted in apparent MT bundles, suggesting increased stability [83]. Overexpression of S47R in PC12 cells completely blocked neurite outgrowth [131]. The same serine residue was identified as required for the regulation of DCX MT binding by MARK and PKA in vitro [116]. Arginine in position 59 was mutated in a mother and her daughters with double-cortex syndrome to leucine [15], and in a sporadic case to histidine [121]. Overexpression of DCX R59L resulted in MT bundles [83]. The protein mutated in the same position to histidine demonstrated reduced in vitro and in vivo MT polymerization [84]. In addition, DCX R59H did not bind to phospho-FIGQY neurofascin [89]. A mutation in amino acid 62 changing aspartic acid to asparagine was observed in a mother with double cortex and her lissencephalic son [14]. Overexpression of D62N did not localize well to MT, and at high levels of overexpression changed cell morphology [83]. Another study indicated that the general structure of MTs did not change [80]. Similar to the previous mutation, D62N did not bind to phospho-FIGQY neurofascin [89]. An extended family carrying an alanine-to-serine (A71S) mutation was reported [129]. The mutated protein was studied by overexpression in COS-7 cells [125]. In that study, the authors noted that overexpression of wild-type DCX interfered with normal mitosis. Overexpression of the A71S mutated protein did not interfere with normal mitosis and the MT bundles were less obvious, thus suggesting that the mutated protein lacked part of the normal DCX activities. A mutation in arginine (R89G) was reported in a familial case of SBH, and was detected both in the mother and son [124]. The mutated protein demonstrated reduced in vitro and in vivo MT polymerization [84]. The tyrosine in position 125 was mutated independently to histidine (familial case) [14] or asparagine (sporadic case) [119]. Mutation of this site had a dramatic effect on MTs. When Y125H was overexpressed in COS-7 cells, MTs were disrupted [80, 83]. The same cellular phenotype was observed with the Y125D mutation, and with two artificial mutations, Y125S or Y125T. Nevertheless, Y125F resulted in normal MT appearance, suggesting that the local conformation, but not the phosphorylation of this tyrosine is important for functional binding to MTs [80]. When the recombinant Y125H protein was incubated with MTs, it caused severing of MTs at high molar concentrations [83].

A familial mutation found in several different families was detected at amino acid 192 (R192W) [14, 15, 125]. Overexpression of the mutated protein in COS cells resulted in quite normal cell morphology (in comparison to overexpression of the wild-type DCX) [80, 83, 84, 125]. Expression of the mutated protein demonstrated reduced in vitro and in vivo MT polymerization in comparison to wild-type [84], and also its effect upon mitotic impairment was reduced in comparison to wild-type DCX [125].

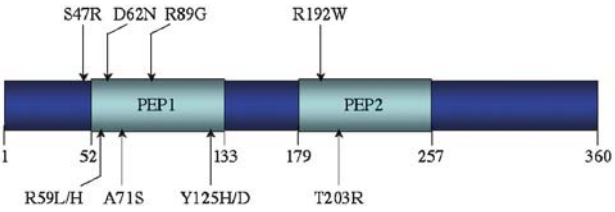

Figure 2 Schematic presentation of DCX. The two tandem DCX motifs (grey) are indicated. The position of missense mulations discussed in this manuscript are indicated.

The final missense mutation to be discussed is a change from threonine to arginine at position 203, found in a family with lissencephaly and double cortex [15]. In one study overexpression of the mutated protein resulted in MT bundles [83], while in the other, it did not localize with MTs in vivo, nor promoted MT assembly in vitro [84]. The results of the latter study suggested that this mutation is a complete null allele.

Discussion

The study of missense mutations identified in patients suffering from lissencephaly or SBH is of a great interest since it provides us with the opportunity for better understanding of molecular and cellular pathways in which LIS1 and DCX are involved. These pathways are likely to be essential for brain development and in particular are involved in migration of post-mitotic neurons. Both of these proteins are multifunctional, and as such the understanding of abnormal processes is a daunting task. Nevertheless, the mutational profile and the possible functional output of the studied mutated proteins differ considerably.

DCX missense mutations are rather frequent and have the tendency to cluster in the identified DCX motif. This protein motif is best known for its MT-related functions and thus links lissencephaly and SBH to probable MT assembly and dynamics defects. Since MTs are one of the major cytoskeletal components, it is expected that associated functions such as intracellular trafficking, mitosis and cell motility will be affected as well.

On the other hand, *LIS1* missense mutations are sparse and not very common. Our work led us to define a spectrum of biochemical and cellular defects for each mutation, suggesting that numerous LIS1 functions are disrupted, but possibly at different levels, leading to various lissencephaly/SBH severities.

At present, we are probably lacking the most relevant information regarding the function of the mutated proteins, since all of the studies we have discussed were conducted in vitro, in ectopic cells systems and by overexpression. This is probably less of concern in the case of LIS1, which is a ubiquitous protein, with a mutation involving only one allele, and these lissencephaly patients may have some subtle cell biology abnormalities in cells other than neurons. However, DCX is a neuronal specific gene, and analysis of its functions will probably be most relevant in neurons. Current technological developments allowing for gene inactivation and overexpression in utero will facilitate studies of mutant proteins in the developing brain.

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1 Pauling L., Itano H., Singer S. and Wells I. (1949) Sickle cell anemia: a molecular disease. Science **110:** 543
2 Ingram V. M. (1956) A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. Nature **178:** 792–794
3 Rakic P. (1997) Intra- and extracellular control of neuronal migration: relevance to cortical malformations. In: Galabura A. M. and Christen Y. (eds), Normal and Abnormal Development of Cortex, pp. 81–98, Springer, Berlin
4 McConnell S. K. (1988) Development and decision-making in the mammalian cerebral cortex. Brain Res. Rev. **13:** 1–23
5 McConnell S. K. (1991) The generation of neuronal diversity in the central nervous system. Annu. Rev. Neurosci. **14:** 269–300
6 Brodmann K. (1909) Lokalisationslehre der Grosshirnrinde in ihren Principen dargestellt aus Grund des Zellen baue. Leipzig, Barth
7 Payne B. R., Lomber S. G., Villa A. E. and Bullier J. (1996) Reversible deactivation of cerebral network components. Trends Neurosci. **19:** 535–542
8 Takahashi T., Goto T., Miyama S., Nowakowski R. S. and Caviness V. S. Jr (1999) Sequence of neuron origin and neocortical laminar fate: relation to cell cycle of origin in the developing murine cerebral wall [In Process Citation]. J. Neurosci. **19:** 10357–10371
9 Angevine J. B. and Sidman R. L. (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. Nature **192:** 766–768
10 Rakic P. (1974) Neurons in Rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. Science **183:** 425–427
11 Gupta A., Tsai L. H. and Wynshaw-Boris A. (2002) Life is a journey: a genetic look at neocortical development. Nat. Rev. Genet. **3:** 342–355
12 Barkovich A. J., Kuzniecky R. I., Dobyns W. B., JacksonG. D., Becker L. E. and Evrard P. (1996) A classification scheme for malformations of cortical development. Neuropediatrics **27:** 59–63
13 Reiner O., Carrozzo R., Shen Y., Whenert M., Faustinella F., Dobyns W. B. et al. (1993) Isolation of a Miller-Dieker lissencephaly gene containing G protein β-subunit-like repeats. Nature **364:** 717–721
14 des Portes V., Pinard J. M., Billuart P., Vinet M. C., Koulakoff A., Carrie A. et al. (1998) A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar hetrotropia and lissencephaly syndrome. Cell **92:** 51–61
15 Gleeson J. G., Allen K. M., Fox J. W., Lamperti E. D., Berkovic S., Scheffer I. et al. (1998) *doublecortin*, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell **92:** 63–72
16 Dobyns W. B., Reiner O., Carrozzo R. and Ledbetter D. H. (1993) Lissencephaly: a human brain malformation associated with deletion of the LIS1 gene located at chromosome 17p13. J. Am. Med. Assoc. **270:** 2838–2842
17 Barkovich A. J., Guerrini R., Battaglia G., Kalifa G., N'Guyen T., Parmeggiani A. et al. (1994) Band heterotopia: correlation of outcome with magnetic resonance imaging parameters. Ann. Neurol. **36:** 609–617
18 Dobyns W. B., Truwit C. L., Ross M. E., Matsumoto N., Pilz D. T., Ledbetter D. H. et al. (1999) Differences in the gyral pattern distinguish chromosome 17-linked and X-linked lissencephaly. Neurology **53:** 270–277
19 Pilz D. T., Macha M. E., Precht K. S., Smith A. C., Dobyns W. B. and Ledbetter D. H. (1998) Fluorescence in situ hybridization analysis with LIS1 specific probes reveals a high deletion mutation rate in isolated lissencephaly sequence. Genet. Med. **1:** 29–33
20 Cardoso C., Leventer R. J., Dowling J. J., Ward H. L., Chung J., Petras K. S. et al. (2002) Clinical and molecular basis of classical lissencephaly: mutations in the LIS1 gene (PAFAH1B1). Hum. Mutat. **19:** 4–15
21 Cardoso C., Leventer R. J., Matsumoto N., Kuc J. A., Ramocki M. B., Mewborn S. K. et al. (2000) The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the LIS1 gene. Hum. Mol. Genet. **9:** 3019–3028
22 Torres F. R., Montenegro M. A., Marques-De-Faria A. P., Guerreiro M. M., Cendes F. and Lopes-Cendes I. (2004) Mutation screening in a cohort of patients with lissencephaly and subcortical band heterotopia. Neurology **62:** 799–802
23 Sapir T., Eisenstein M., Burgess H. A., Horesh D., Cahana A., Aoki J. et al. (1999) Analysis of lissencephaly-causing LIS1 mutations. Eur. J. Biochem. **266:** 1011–1020
24 Fogli A., Guerrini R., Moro F., Fernandez-Alvarez E., Livet M. O., Renieri A. et al. (1999) Intracellular levels of the LIS1 protein correlate with clinical and neuroradiological findings in patients with classical lissencephaly. Ann. Neurol. **45:** 154–161
25 Caspi M., Coquelle F. M., Koifman C., Lev, T., Arai H., Aoki J. et al. (2003) LIS1 missense mutations: variable phenotypes result from unpredictable alterations in biochemical and cellular properties. J. Biol. Chem. **278:** 38740–38748
26 Neer E. J., Schmidt C. J., Nambudripad R. and Smith T. F. (1994) The ancient regulatory-protein family of WD-repeat proteins. Nature **371:** 297–300
27 Reiner O. (2000) LIS1: Let's interact sometimes…(part 1). Neuron **28:** 633–636
28 Emes R. D. and Ponting C. P. (2001) A new sequence motif linking lissencephaly, Treacher Collins and oral-facial-digital type 1 syndromes, microtubule dynamics and cell migration. Hum. Mol. Genet. **10:** 2813–2820

29 Cahana A., Escamez T., Nowakowski R. S., Hayes N. L., Giacobini M., von Holst A. et al. (2001) Targeted mutagenesis of LIS1 disrupts cortical development and LIS1 homodimerization. Proc. Natl. Acad. Sci. USA **98:** 6429–6434

30 Kim M. H., Cooper D. R., Oleksky A., Devedjiev Y., Derewenda U., Reiner O. et al. (2004) The structure of the N-terminal domain of the product of the lissencephaly gene LIS1 and its functional implications. Structure **12:** 987–998

31 Morris N. R., Efimov V. P. and Xiang X. (1998) Nuclear migration, nucleokinesis and lissencephaly. Trends Cell Biol. **8:** 467–470

32 Xiang X., Osmani A. H., Osmani S. A., Xin M. and Morris N. R. (1995) NudF, a nuclear migration gene in *Aspergillus nidulans*, is similar to the human LIS-1 gene required for neuronal migration. Mol. Biol. Cell. **6:** 297–310

33 Morris S. M., Albrecht U., Reiner O., Eichele G. and Yu-Lee L.-y. (1998) The lissencephaly gene product LIS1, a protein involved in neuronal migration, interacts with a nuclear movement protein, NudC. Curr. Biol. **8:** 603–606

34 Reiner O., Gdalyahu A., Ghosh I., Levy T., Sapoznik S., Nir R. et al. (2004) DCX's phosphorylation by not Just aNother Kinase (JNK). Cell Cycle **3:** 747–751

35 Willins D. A., Xiang X. and Morris N. R. (1995) An alpha tubulin mutation suppresses nuclear migration mutations in *Aspergillus nidulans*. Genetics **141:** 1287–1298

36 Sapir T., Elbaum M. and Reiner O. (1997) Reduction of microtubule catastrophe events by LIS1, platelet-activating factor acetylhydrolase subunit. EMBO J. **16:** 6977–6984

37 Sapir T., Cahana A., Seger R., Nekhai S. and Reiner O. (1999) LIS1 is a microtubule-associated phosphoprotein. Eur. J. Biochem. **265:** 181–188

38 Vallee R. B., Williams J. C., Varma D. and Barnhart L. E. (2004) Dynein: an ancient motor protein involved in multiple modes of transport. J. Neurobiol. **58:** 189–200

39 Beckwith S. M., Roghi C. H., Liu B. and Ronald Morris N. (1998) The '8-kD' cytoplasmic dynein light chain is required for nuclear migration and for dynein heavy chain localization in *Aspergillus nidulans.* J. Cell Biol. **143:** 1239–1247

40 Xiang X., Beckwith S. M. and Morris N. R. (1994) Cytoplasmic dynein is involved in nuclear migration in *Aspergillus nidulans.* Proc Natl Acad Sci USA **91:** 2100–2214

41 Xiang X., Han G., Winkelmann D. A., Zuo W. and Morris N. R. (2000) Dynamics of cytoplasmic dynein in living cells and the effect of a mutation in the dynactin complex actin-related protein Arp1. Curr. Biol. **10:** 603–606

42 Zhang J., Li S., Fischer R. and Xiang X. (2003) Accumulation of cytoplasmic dynein and dynactin at microtubule plus ends in *Aspergillus nidulans* is kinesin dependent. Mol. Biol. Cell **14:** 1479–1488

43 Liu Z., Xie T. and Steward R. (1999) LIS1, the *Drosophila* homolog of a human lissencephaly disease gene, is required for germline cell division and oocyte differentiation. Development **126:** 4477–4488

44 Swan A., Nguyen T. and Suter B. (1999) *Drosophila* Lissencephaly-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. Nat. Cell Biol. 1**:** 444–449

45 Lei Y. and Warrior R. (2000) The drosophila lissencephaly1 (DLIS1) gene is required for nuclear migration. Dev. Biol. **226:** 57–72

46 Fujiwara T., Tanaka K., Inoue E., Kikyo M. and Takai Y. (1999) Bni1p regulates microtubule-dependent nuclear migration through the actin cytoskeleton in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **19:** 8016–8027

47 Sasaki S., Shionoya A., Ishida, M., Gambello M., Yingling J., Wynshaw-Boris A. et al. (2000) A LIS1/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. Neuron **28:** 681–696

48 Tai C. Y., Dujardin D. L., Faulkner N. E. and Vallee R. B. (2002) Role of dynein, dynactin, and CLIP-170 interactions in LIS1 kinetochore function. J. Cell Biol. **11:** 11

49 Faulkner N. E., Dujardin D. L., Tai C. Y., Vaughan K. T., O'Connell C. B., Wang Y. et al. (2000) A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. Nat. Cell Biol. **2:** 784–791

50 Smith D. S., Niethammer M., Ayala R., Zhou Y., Gambello M. J., Wynshaw-Boris A. et al. (2000) Regulation of cytoplasmic dynein behaviour and microtubule organization by mammalian LIS1. Nat. Cell Biol. **2:** 767–775

51 Liu Z., Steward R. and Luo L. (2000) *Drosophila* LIS1 is required for neuroblast proliferation, dendritic elaboration and axonal transport. Nat. Cell Biol. **2:** 776–783

52 Efimov V. P. and Morris N. R. (2000) The LIS1-related NUDF protein of Aspergillus nidulans interacts with the coiled-coil domain of the NUDE/RO11 protein. J. Cell Biol. **150:** 681–688

53 Niethammer M., Smith D. S., Ayala, R., Peng, J., Ko J., Lee M.-S. et al. (2000) Nudel is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. Neuron **28:** 697–711

54 Kitagawa M., Umezu M., Aoki J., Koizumi H., Arai H. and Inoue K. (2000) Direct association of LIS1, the lissencephaly gene product, with a mammalian homologue of a fungal nuclear distribution protein, rNUDE. FEBS Lett. **479:** 57–62.

55 Rickard J. E. and Kreis T. E. (1996) CLIPs for organelle-microtubule interactions. TIC **6:** 178–183

56 Galjart N. and Perez F. (2003) A plus-end raft to control microtubule dynamics and function. Curr. Opin. Cell Biol. **15:** 48–53

57 Schuyler S. C. and Pellman D. (2001) Microtubule 'plus-end-tracking proteins': the end is just the beginning. Cell **105:** 421–424

58 Komarova Y. A., Akhmanova A. S., Kojima S., Galjart N. and Borisy G. G. (2002) Cytoplasmic linker proteins promote microtubule rescue in vivo. J. Cell Biol. **159:** 589–599

59 Valetti C., Wetzel D. M., Schrader M., Hasbani M. J., Gill S. R., Kreis T. E. et al. (1999) Role of dynactin in endocytic traffic: effects of dynamitin overexpression and colocalization with CLIP-170. Mol. Biol. Cell **10:** 4107–4120

60 Coquelle F. M., Caspi M., Cordelieres F. P., Dompierre J. P., Dujardin D. L., Koifman C. et al. (2002) LIS1, CLIP–170's key to the dynein/dynactin pathway. Mol. Cell. Biol. **22:** 3089–3102

61 Hoogenraad C. C., Koekkoek B., Akhmanova A., Krugers H., Dortland B., Miedema M. et al. (2002) Targeted mutation of Cyln2 in the Williams syndrome critical region links CLIP-115 haploinsufficiency to neurodevelopmental abnormalities in mice. Nat. Genet. **32:** 116–127

62 Sheeman B., Carvalho P., Sagot I., Geiser J., Kho D., Hoyt M. A. et al. (2003) Determinants of *S. cerevisiae* dynein localization and activation. implications for the mechanism of spindle positioning. Curr. Biol. **13:** 364–372

63 Dujardin D., Wacker U. I., Moreau A., Schroer T. A., Rickard J. E. and De Mey J. R. (1998) Evidence for a role of CLIP-170 in the establishment of metaphase chromosome alignment. J. Cell Biol. **141:** 849–62

64 Hattori M., Adachi H., Tsujimoto M., Arai N. and Inoue K. (1994) Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor. Nature **370:** 216–218

65 Manya H., Aoki J., Watanabe M., Adachi T., Asou H., Inoue Y. et al. (1998) Switching of platelet-activating factor acetylhydrolase catalytic subunits in developing rat brain. J. Biol. Chem. **273:** 18567–18572

66 Sheffield P., Garrard S., Caspi M., Aoki J., Inoue K., Derewenda U. et al. (2000) Homologues of the α- and β-subunits of mammalian brain platelet-activating factor acetyl-

hydrolase Ib in the *Drosophila melanogaster* genome. Proteins **39:** 1–8
67 Caspi M., Atlas R., Kantor A., Sapir T. and Reiner O. (2000) Interaction between LIS1 and doublecortin, two lissencephaly gene products. Hum. Mol. Genet. **9:** 2205–2213
68 Tanaka T., Serneo F. F., Higgins C., Gambello M. J., Wynshaw-Boris A. and Gleeson J. G. (2004) LIS1 and doublecortin function with dynein to mediate coupling of the nucleus to the centrosome in neuronal migration. J. Cell Biol. **165:** 709–21
69 Sweeney K. J., Clark G. D., Prokscha A., Dobyns W. B. and Eichele G. (2000) Lissencephaly associated mutations suggest a requirement for the PAFAH1B heterotrimeric complex in brain development. Mech. Dev. **92:** 263–271
70 Sweeney K. J., Prokscha A. and Eichele G. (2001) NudE-L, a novel LIS1-interacting protein, belongs to a family of vertebrate coiled-coil proteins. Mech. Dev. **101:** 21–33
71 Feng Y., Olson E. C., Stukenberg P. T., Flanagan L. A., Kirschner M. W. and Walsh C. A. (2000) LIS1 regulates CNS lamination by interacting with mNudE, a central component of the centrosome. Neuron **28:** 665–679
72 Pilz D. T., Kuc J., Matsumoto N., Bodurtha J., Bernadi B., Tassinari C. A. et al. (1999) Subcortical band heterotopia in rare affected males can be caused by missense mutations in DCX (XLIS) or LIS1. Hum. Mol. Genet. **8:** 1757–1760
73 Sicca F., Kelemen A., Genton P., Das S., Mei D., Moro F. et al. (2003) Mosaic mutations of the LIS1 gene cause subcortical band heterotopia. Neurology **61:** 1042–106
74 Corbo J. C., Deuel T. A., Long J. M., LaPorte P., Tsai E., Wynshaw-Boris A. et al. (2002) Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. J. Neurosci. **22:** 7548–7557
75 Hirotsune S., Fleck M. W., Gambello M. J., Bix G. J., Chen A., Clark G. D. et al. (1998) Graded reduction of Pafah1b1 (LIS1) activity results in neuronal migration defects and early embryonic lethality. Nat. Genet. **19:** 333–339
76 Bai J., Ramos R. L., Ackman J. B., Thomas A. M., Lee R. V. and LoTurco J. J. (2003) RNAi reveals doublecortin is required for radial migration in rat neocortex. Nat. Neurosci. **6:**1277–1283
77 Horesh D., Sapir T., Francis F., Caspi M., Grayer Wolf S., Elbaum M. et al. (1999) Doublecortin, a stabilizer of microtubules. Hum. Mol. Genet. **8:** 1599–1610
78 Gleeson J. G., Lin P. T., Flanagan L. A. and Walsh C. A. (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuron **23:** 257–271
79 Francis F., Koulakoff A., Boucher D., Chafey P., Schaar B., Vinet M. C. et al. (1999) Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. Neuron **23:** 247–256
80 Yoshiura K., Noda Y., Kinoshita A. and Niikawa N. (2000) Colocalization of doublecortin with the microtubules: An ex vivo colocalization study of mutant doublecortin. J. Neurobiol. **43:** 132–139
81. Burgess H. A. and Reiner O. (2000) Doublecortin-like kinase, is a microtubule-associated protein kinase expressed in growth cones. MCN **16:** 529–544
82 Lin P. T., Gleeson J. G., Corbo J. C., Flanagan L. and Walsh C. A. (2000) DCAMKL1 encodes a protein kinase with homology to doublecortin that regulates microtubule polymerization. J. Neurosci. **20:** 9152–9161
83 Sapir T., Horesh D., Caspi M., Atlas R., Burgess H. A., Grayer Wolf S. et al. (2000) Doublecortin mutations cluster in evolutionary conserved functional domains. Hum. Mol. Genet. **5:** 703–712
84 Taylor K. R., Holzer A. K., Bazan J. F., Walsh CA. and Gleeson J. G. (2000) Patient mutations in doublecortin define a repeated tubulin-binding domain. J. Biol. Chem. **275:** 34442–34450
85 Kim M. H., Cierpicki T., Derewenda U., Krowarsch D., Feng Y., Devedjiev Y. et al. (2003) The DCX-domain tandems of doublecortin and doublecortin-like kinase. Nat. Struct. Biol. **10:** 324–33
86 Moores C. A., Perderiset M., Francis F., Chelly J., Houdusse A. and Milligan R. A. (2004) Mechanism of microtubule stabilization by doublecortin. Mol. Cell. **14:** 833–839
87 Friocourt G., Chafey P., Billuart P., Koulakoff A., Vinet M. C., Schaar B. T. et al. (2001) Doublecortin interacts with mu subunits of clathrin adaptor complexes in the developing nervous system. Mol. Cell. Neurosci. **18:** 307–319
88 Ohno H., Stewart J., Fournier M. C., Bosshart H., Rhee I., Miyatake S. et al. (1995) Interaction of tyrosine-based sorting signals with clathrin-associated proteins. Science **269:** 1872–1875
89 Kizhatil K., Wu Y. X., Sen A. and Bennett V. (2002) A new activity of doublecortin in recognition of the phospho-FIGQY tyrosine in the cytoplasmic domain of neurofascin. J. Neurosci. **22:** 7948–7958
90 Brummendorf T., Kenwrick S. and Rathjen F. G. (1998) Neural cell recognition molecule L1: from cell biology to human hereditary brain malformations. Curr. Opin. Neurobiol. **8:** 87–97
91 Hortsch M. (2000) Structural and functional evolution of the L1 family: are four adhesion molecules better than one? Mol. Cell. Neurosci. **15:** 1–10
92 Tsukada M., Prokscha A., Oldekamp J. and Eichele G. (2003) Identification of neurabin II as a novel doublecortin interacting protein. Mech. Dev. **120:** 1033–1043
93 Gdalyahu A., Ghosh I., Levy T., Sapir T., Sapoznik S., Fishler Y. et al. (2004) DCX, a new mediator of the JNK pathway. EMBO J. **23:** 823–832
94 Whitmarsh A. J., Cavanagh J., Tournier C., Yasuda J. and Davis R. J. (1998) A mammalian scaffold complex that selectively mediates MAP kinase activation. Science **281:** 1671–1674.
95 Yasuda J., Whitmarsh A. J., Cavanagh J., Sharma M. and Davis R. J. (1999) The JIP group of mitogen-activated protein kinase scaffold proteins. Mol. Cell. Biol. **19:** 7245–7254
96 Willoughby E. A., Perkins G. R., Collins M. K. and Whitmarsh A. J. (2003) The JIP-1 scaffold protein targets MKP-7 to dephosphorylate JNK. J. Biol. Chem. **10:** 10
97 Verhey K. J., Meyer D., Deehan R., Blenis J., Schnapp B. J., Rapoport T. A. et al. (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. J. Cell Biol. **152:** 959–970
98 Bonny C., Nicod P. and Waeber G. (1998) IB1, a JIP-1-related nuclear protein present in insulin-secreting cells. J. Biol. Chem. **273:** 1843–1846
99 Dickens M., Rogers J. S., Cavanagh J., Raitano A., Xia Z., Halpern J. R. et al. (1997) A cytoplasmic inhibitor of the JNK signal transduction pathway. Science **277:** 693–696
100 Negri S., Oberson A., Steinmann M., Sauser C., Nicod P., Waeber G. et al. (2000) cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein. Genomics **64:** 324–330
101 Meyer D., Liu A. and Margolis B. (1999) Interaction of c-Jun amino-terminal kinase interacting protein-1 with p190 rhoGEF and its localization in differentiated neurons. J. Biol. Chem. **274:** 35113–35118
102 Matsuda S., Yasukawa T., Homma Y., Ito Y., Niikura T., Hiraki T. et al. (2001) c-Jun N-terminal kinase (JNK)-interacting protein-1b/islet-brain-1 scaffolds Alzheimer's amyloid precursor protein with JNK. J. Neurosci. **21:** 6597–6607
103 Scheinfeld M. H., Roncarati R., Vito P., Lopez P. A., Abdallah M. and D'Adamio L. (2002) Jun NH2-terminal kinase (JNK) interacting protein 1 (JIP1) binds the cytoplasmic domain of the Alzheimer's beta-amyloid precursor protein (APP). J. Biol. Chem. **277:** 3767–3775

104 Taru H., Kirino Y. and Suzuki T. (2002) Differential roles of JIP scaffold proteins in the modulation of amyloid precursor protein metabolism. J. Biol. Chem. **277:** 27567–27574
105 Gotthardt M., Trommsdorff M., Nevitt M. F., Shelton J., Richardson J. A., Stockinger W. et al. (2000) Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. J. Biol. Chem. **275:** 25616–25624
106 Stockinger W., Brandes C., Fasching D., Hermann M., Gotthardt M., Herz J. et al. (2000) The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. J. Biol. Chem. **275:** 25625–25632
107 D'Arcangelo G., Miao G. G., Chen S. C., Soares H. D., Morgan J. I. and Curran T. (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature **374:** 719–723.
108 Ogawa M., Miyata T., Nakajiman K., Yagyu K., Seike M., Ikenaka K. et al. (1995) The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron **14:** 899–912
109 Hirotsune S., Takahara T., Sasaki N., Hirose K., Yoshiki A., Ohashi T. et al. (1995) The reeler gene encodes a protein with an EGF-like motif expressed by pioneer neurons. Nat. Genet. **10:** 77–84
110 D'Arcangelo G., Homayouni R., Keshvara L., Rice D. S., Sheldon M. and Curran T. (1999) Reelin is a ligand for lipoprotein receptors. Neuron **24:** 471–479
111 Hiesberger T., Trommsdorff M., Howell B. W., Goffinet A., Mumby M. C., Cooper J. A. et al. (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. Neuron **24:** 481–489
112 Hong S. E., Shugart Y. Y., Huang D. T., Shahwan S. A., Grant P. E., Hourihane J. O. et al. (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat. Genet. **26:** 93–96
113 Buchsbaum R. J., Connolly B. A. and Feig L. A. (2002) Interaction of Rac exchange factors Tiam1 and Ras-GRF1 with a scaffold for the p38 mitogen-activated protein kinase cascade. Mol. Cell. Biol. **22:** 4073–4085
114 Schoorlemmer J. and Goldfarb M. (2001) Fibroblast growth factor homologous factors are intracellular signaling proteins. Curr. Biol. **11:** 793–797
115 Tanaka T., Serneo F. F., Tseng H. C., Kulkarni A. B., Tsai L. H. and Gleeson J. G. (2004) Cdk5 phosphorylation of doublecortin ser297 regulates its effect on neuronal migration. Neuron **41:** 215–227
116 Schaar B. T., Kinoshita K. and McConnell S. K. (2004) Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. Neuron **41:** 203–213
117 LoTurco J. (2004) Doublecortin and a tale of two serines. Neuron **41:** 175–177
118 Graham M. E., Ruma-Haynes P., Capes-Davis A. G., Dunn J. M., Tan T. C., Valova V. A. et al. (2004) Multi-site phosphorylation of doublecortin by cyclin-dependent kinase 5. Biochem. J. **381**(Pt 2)**:** 471–481
119 des Portes V., Francis F., Pinard J. M., Desguerre I., Moutard M. L., Snoeck I. et al. (1998) doublecortin is the major gene causing X-linked subcortical laminar heterotopia. Hum. Mol. Genet. **7:** 1063–1070
120 Pilz D. T., Matsumoto N., Minnerath S., Mills P., Gleeson J. G., Allen K. M. et al. (1998) LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. Hum. Mol. Genet. **7:** 2029–2037
121 Gleeson J. G., Minnerath S. R., Fox J. W., Allen K. M., Luo R. F., Hong S. E. et al. (1999) Characterization of mutations in the gene doublecortin in patients with double cortex syndrome. Ann. Neurol. **45:** 146–153
122 Kato M., Kimura T., Lin C., Ito A., Kodama S., Morikawa T. et al. (1999) A novel mutation of the doublecortin gene in Japanese patients with X-linked lissencephaly and subcortical band heterotopia. Hum. Genet. **104:** 341–344
123 Gleeson J. G., Minnerath S., Kuzniecky R. I., Dobyns W. B., Young I. D., Ross M. E. et al. (2000) Somatic and germline mosaic mutations in the doublecortin gene are associated with variable phenotypes. Am. J. Hum. Genet. **67:** 574–581
124 Matsumoto N., Leventer R. J., Kuc J. A., Mewborn S. K., Dudlicek L. L., Ramocki M. B. et al. (2001) Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia. Eur. J. Hum. Genet. **9:** 5–12
125 Couillard-Despres S., Uyanik G., Ploetz S., Karl C., Koch H., Winkler J. et al. (2004) Mitotic impairment by doublecortin is diminished by doublecortin mutations found in patients. Neurogenetics **5:** 83–93
126 Guerrini R., Moro F., Andermann E., Hughes E., D'Agostino D., Carrozzo R. et al. (2003) Nonsyndromic mental retardation and cryptogenic epilepsy in women with doublecortin gene mutations. Ann. Neurol. **54:** 30–37
127 des Portes V., Abaoub L., Joannard A., Souville I., Francis F., Pinard J. M. et al. (2002) So-called 'cryptogenic' partial seizures resulting from a subtle cortical dysgenesis due to a doublecortin gene mutation. Seizure **11:** 273–277
128 Kato M., Kanai M., Soma O., Takusa Y., Kimura T., Numakura C. et al. (2001) Mutation of the doublecortin gene in male patients with double cortex syndrome: somatic mosaicism detected by hair root analysis. Ann. Neurol. **50:** 547–551
129 Aigner L., Fluegel D., Dietrich J., Ploetz S. and Winkler J. (2000) Isolated lissencephaly sequence and double-cortex syndrome in a German family with a novel doublecortin mutation. Neuropediatrics **31:** 195–198
130 Gleeson, J. G. (2000) Classical lissencephaly and double cortex (subcortical band heterotopia): LIS1 and doublecortin. Curr. Opin. Neurol. **13:** 121–125
131 Shmueli O., Gdalyahu A., Sorokina K., Nevo E., Avivi A. and Reiner O. (2001) DCX in PC12 cells: CREB-mediated transcription and neurite outgrowth. Hum. Mol. Genet. **10:** 1061–1070