Biallelic loss-of-function variations in SMO, encoding the key transducer of the Sonic Hedgehog pathway, cause a broad phenotypic spectrum of hedgehogopathies

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subjected to whole genome sequencing (Ilumina, 30x coverage, paired-end, 150bp). Data were analyzed for both coding and non-coding single nucleotide variants and structural variants using the SODAR and VarFish pipelines.

Results: Overall we identified coding pathogenic single nucleotide variants in 9 cases (14%). We found mutations in 7 known disease genes including one repeat expansion in HOXD13. In two unrelated cases of ectrodactyly we identified likely pathogenic variants in UBA2 establishing it as a novel disease gene. In addition we found two pathogenic structural variants (3%) : one inversion segregating with the phenotype and one de novo deletion. Furthermore, we identified variants in 3 novel high confidence candidate genes. We also describe and perform a framework to identify potential non-coding pathogenic variants.

Conclusions: Whole genome sequencing is a powerful strategy to identify all types of genomic variants associated with congenital limb malformation. It also detects repeat expansions and copy number neutral inversions that are missed by most other approaches.

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C19.2

Biallelic mutations in *TOGARAM1* cause a novel primary ciliopathy

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Background: Dysfunction in non-motile cilia is associated with a broad spectrum of developmental disorders characterized by clinical heterogeneity. Despite over one hundred genes have been associated with primary ciliopathies, with wide phenotypic overlap, some patients still lack a molecular diagnosis. The aim of this work was to investigate and functionally characterize the molecular cause underlying a Meckel-Gruber-like phenotype in two sibling foetuses.

Methods: we used a trio-based whole exome sequencing (WES) strategy to identify candidate variants in *TOGARAM1*. *In silico*, in vitro and in vivo (*C. elegans*) studies were carried out to explore the impact of mutations on protein structure and function, and relevant biological processes.

Results: *TOGARAM1* encodes a member of the Crescerin1 family of proteins regulating microtubule dynamics. Its orthologue in *C. elegans*, *che-12*, is expressed in the cilium of a subset of sensory neurons, where it is required for chemosensation. Nematode lines harbouring the missense variant in *TOGARAM1* were generated by CRISPR/Cas9 technology. Although chemotaxis ability on a NaCl gradient and lipophilic dye-uptake were not impaired, *che-12* knock-in mutants displayed a shorter cilium in sensory neurons, and even shorter cilia in hemizygous worms, which recapitulate the genotype observed in the affected foetuses. Finally, in vitro analysis of microtubule polymerization in the presence of the wild-type or mutant TOG2 domain revealed a faster polymerization associated with the mutant protein, indicating aberrant tubulin binding.

Conclusions: Our data suggest a causative role of *TOGARAM1* variants in the pathogenesis of this novel disorder, connecting this gene with primary ciliopathy.

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C19.3

Biallelic loss-of-function variations in *SMO*, encoding the key transducer of the Sonic Hedgehog pathway, cause a broad phenotypic spectrum of hedgehogopathies

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The evolutionarily conserved Hedgehog (Hh) pathway is essential for organogenesis and plays critical roles in postnatal tissue maintenance and renewal. A unique feature of vertebrate Hh pathway is that signal transduction requires the primary cilium (PC) where major pathway components are dynamically enriched. These factors include Smoothened (SMO) and Patched, that constitute the core reception system for Sonic Hedgehog (SHH) as well as GLI transcription factors, the key mediators of the pathway. Here, we report biallelic loss-of-function variations in *SMO* in seven cases from five independent families causing a wide phenotypic spectrum of hedgehogopathies affecting development of the brain (hypothalamic hamartoma, microcephaly), heart (atrioventricular septal defect), skeleton (postaxial polydactyly, narrow chest, shortening of long bones) and the enteric nervous system (aganglionosis). Patient-derived cells showed normal ciliogenesis but severely altered Hh signal transduction due to either altered PC trafficking or abnormal activation of the pathway downstream of SMO. In addition, Hh-independent GLI2 accumulation at the PC tip in patient cells suggest a potential novel function of SMO in regulating basal ciliary trafficking of GLI2 when the pathway is off. Thus, loss of SMO function in humans results in abnormal PC dynamics of key components of the Hh signaling pathway and leads to a large continuum of malformations in humans.

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C19.4

Bi-allelic Variants in *RALGAPA1* Cause Profound Neurodevelopmental Disability, Muscular Hypotonia, Infantile Spasms, and Feeding Abnormalities

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Introduction: Ral (Ras-like) GTPases play an important role in the control of cell migration and have been implicated in Ras-mediated tumorigenicity. Recently, variants in *RALA* were described as a cause of intellectual disability and developmental delay, indicating the relevance of this pathway to neuropediatric diseases.

Materials and Methods: Exome sequencing was performed in four affected individuals. Immunoblotting, qRT-PCR, immunofluorescence, flow cytometry and a drug screen pipeline using cell viability were used to functionally characterize patient derived fibroblasts.

Results: Biallelic variants in *RALGAPA1* were identified in four unrelated individuals with profound neurodevelopmental disability, muscular hypotonia, feeding abnormalities, recurrent fever episodes, and infantile spasms. RalGAPA1 was absent in the fibroblasts derived from two affected individuals and levels of RalGAPB, a scaffolding subunit of the RalGAP complex, were reduced. RalA activity was increased in these cell lines, implying that RalGAPA1 deficiency causes a dysfunctional RalGAP complex and a constitutive activation of RalA. Additionally, RalGAPA1 deficiency increased cell-surface levels of lipid raft components in detached fibroblasts, indicating that anchorage-dependence of cell growth signaling is disturbed. The drug screen assay showed altered survival rates of cells upon treatment with pathway specific compounds.

Conclusions: Our findings indicate that the dysregulation of the RalA pathway has an important impact on neuronal function and brain development. In light of the partially overlapping phenotype between *RALA* and *RALGAPA1*-associated diseases, it appears likely that dysregulation of the RalA signaling pathway leads to a distinct group of