DGAP177: 46,XX,t(1;22)(q21.3;q11.23)

DGAP177 is a X year old female with bilateral Tessier class 4 OFC and clubfoot deformity (talipes equinovarus) who carries the balanced translocation 46,XX,t(1;22)(q21;q12).Orofacial clefting is a complex disorder whose genetic resolution has been difficult. While an increasing number of genes have been linked to cleft lip and palate (CL/P), no genes have been identified for the genesis of oblique facial clefts (OFC), also known as Tessier clefts. These relatively rare uni- or bilateral facial clefts may extend into the orbit and frequently involve micro- or anopthalmia. We have used this naturally occurring human chromosomal translocation to identify a gene responsible for OFC, and have sought to establish causation through the mutational analysis of independent but phenotypically similar cases of OFC.

We delimited the 22q12 translocation breakpoint to intron 14 in the gene *SPECC1L* (also known as *Cytospin A*). Q-RT-PCR demonstrated a 40% reduction in wild type *SPECC1L* transcript levels in lymphoblastoid cells derived from the proband (Fig. DGAP177-1) suggesting haploinsufficiency as the responsible mechanism. The 1q breakpoint does not disrupt any gene. The mouse homolog, *Specc1l,* is expressed in the facial primordia, the eye and hindlimb (Fig. DGAP177-2). When overexpressed as a GFP fusion protein in cultured cells, SPECC1L co-localizes with microtubules in a nocodazole-dependent fashion (Fig. DGAP177-3). Endogenous Specc1L localizes to the mitotic spindle of anaphase nuclei and to the base of primary cilia, where its expression partly overlaps with that of gamma tubulin, a centriole marker. (Fig. DGAP177-4).

We next examined the sequence of *SPECC1L* in patients with OFC. We identified two missense sequence variants, T190M and Q415P, which respectively reside in the first and second coiled coil domains of SPECC1L. Furthermore, when transfected into cells, the Q415P mutant fails to fully assemble into a microtubular pattern, suggesting a less efficient interaction with the intracellular microtubular network (Fig. DGAP177-5). Recent Y2H experiments {Kim, 2007 #235} indicate that Specc1l interacts with the Gli family member Glis2, an effector in the Hh pathway and also an interactor with beta catenin, a key component of the canonical Wnt signaling pathway. We have confirmed the Specc1l-Glis interaction via IP-Western blot analyses. In addition, Wnt family members (Wnt4, 9) are implicated in orofacial clefting. These data thus identify a new gene responsible for OFC in humans, and suggest potential mechanisms for the pathogenesis of this disorder.

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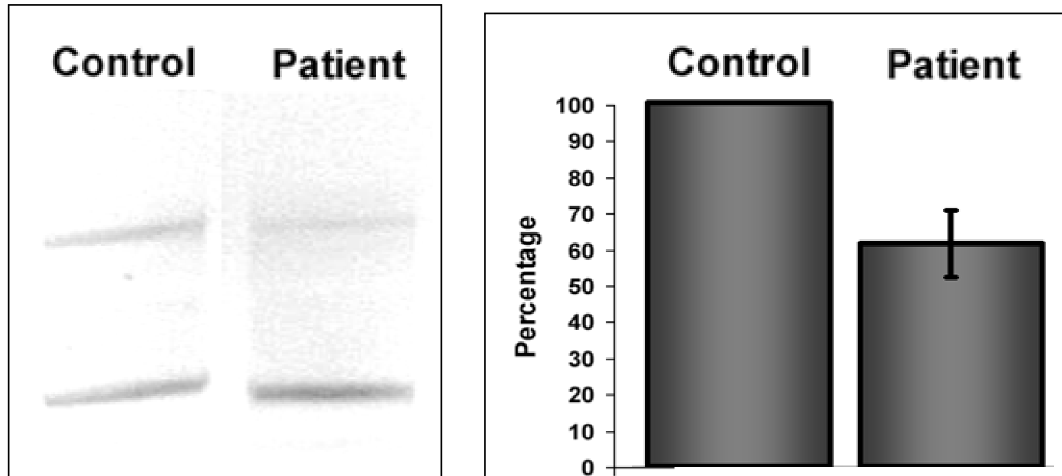
Fig. DGAP177-1. *SPECC1L* is haploinsufficient in DGAP177. *SPECC1L* was identified by FISH analysis to be disrupted by the 22q breakpoint in patient lymphoblasts. RNA from these lymphoblasts was then used to show haploinsufficiency of *SPECC1L* transcripts by standard and quantitative RT-PCR**.**



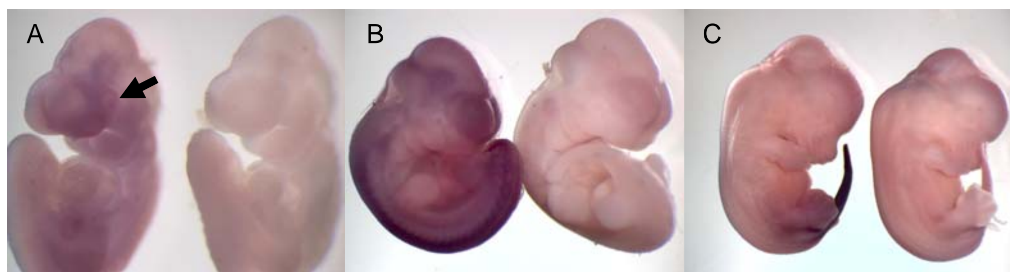


Fig. DGAP177-2. Whole Mount *in situ* Hybridization on Mouse Embryos. (A) Expression of *Cytospin A* in the eye (arrow), frontal prominence and first and second branchial arches at E9.5, and (**B)** developing vertebral column and hind limbs at E10.5. **(C)** Expression in hind limbs and caudal part of the vertebral column persists at E12.5.

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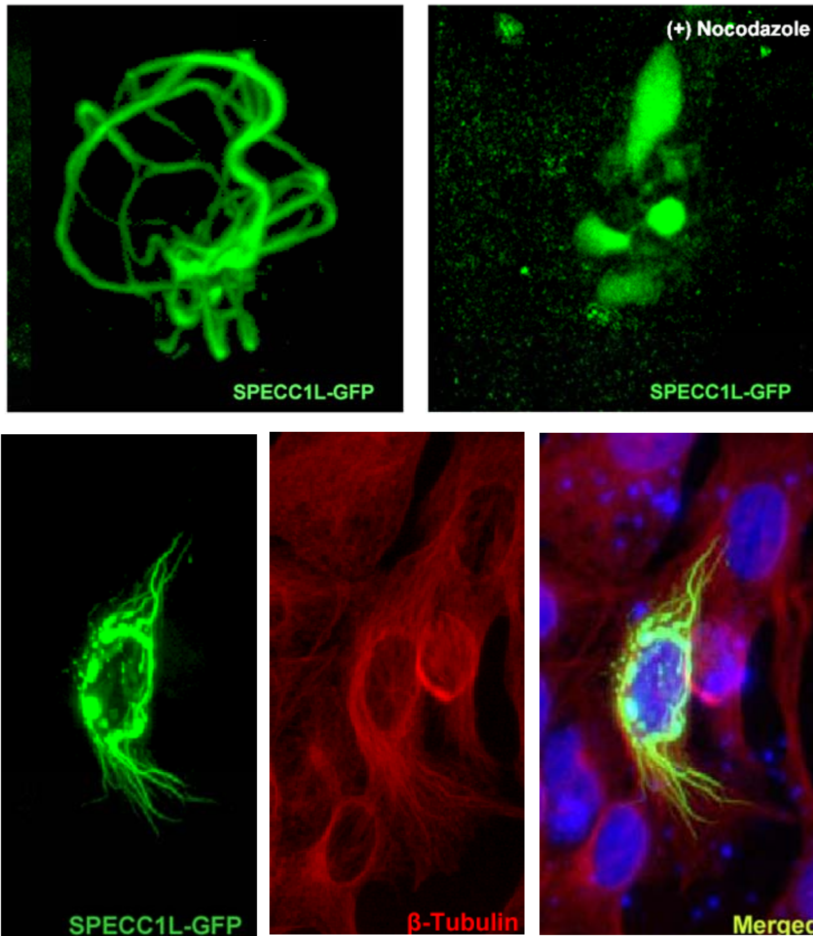

Fig. DGAP177-3. SPECC1L-GFP colocalizes with microtubules. Overexpression of SPECC1L-GFP showed a Nocodazole sensitive microtubular pattern (above). The overexpressed protein did indeed co-localize with a subset of β-tubulin fibers (below).

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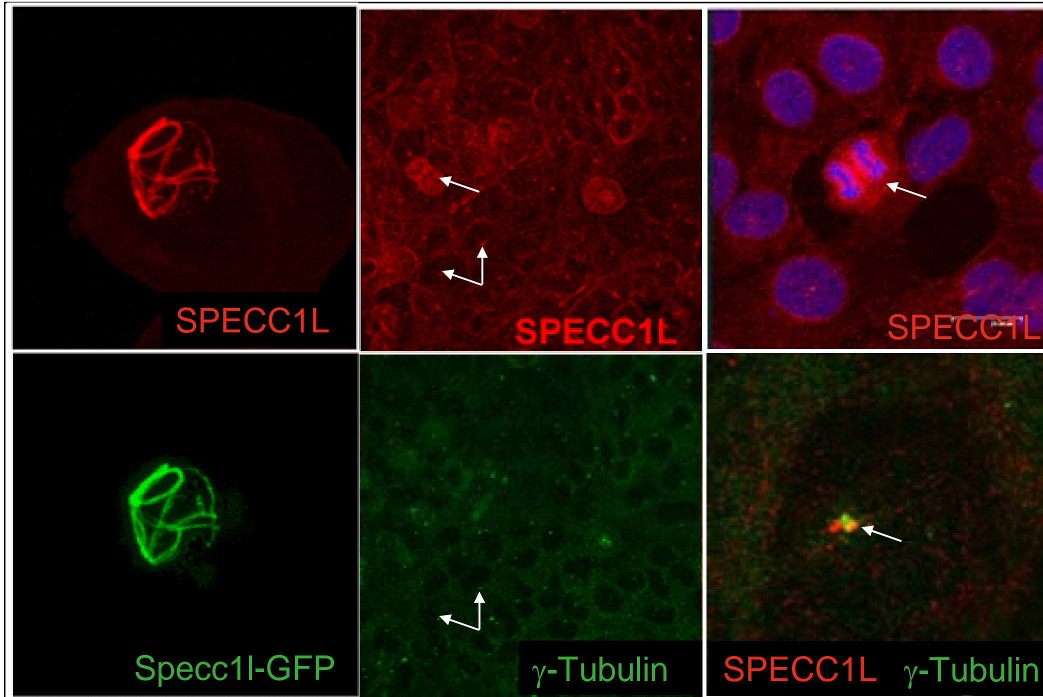

Fig. DGAP177-4. SPECC1L expression localizes to the microtubule organizing center (MTOC). We generated a SPECC1L antibody (red), which specifically recognized (left, top) the Specc1l-GFP overexpression fibers (left, below). We used this antibody to determine the endogenous expression pattern of SPECC1L (center, top), which appeared similar to the expression pattern of γ-tubulin (center, below), a centriole-specific marker in the MTOC (far right). At higher magnifications, SPECC1L expression partially overlaps with that of γ-tubulin in the MTOC (right, below) and SPECC1L shows greater accumulation during cell division (right, top).

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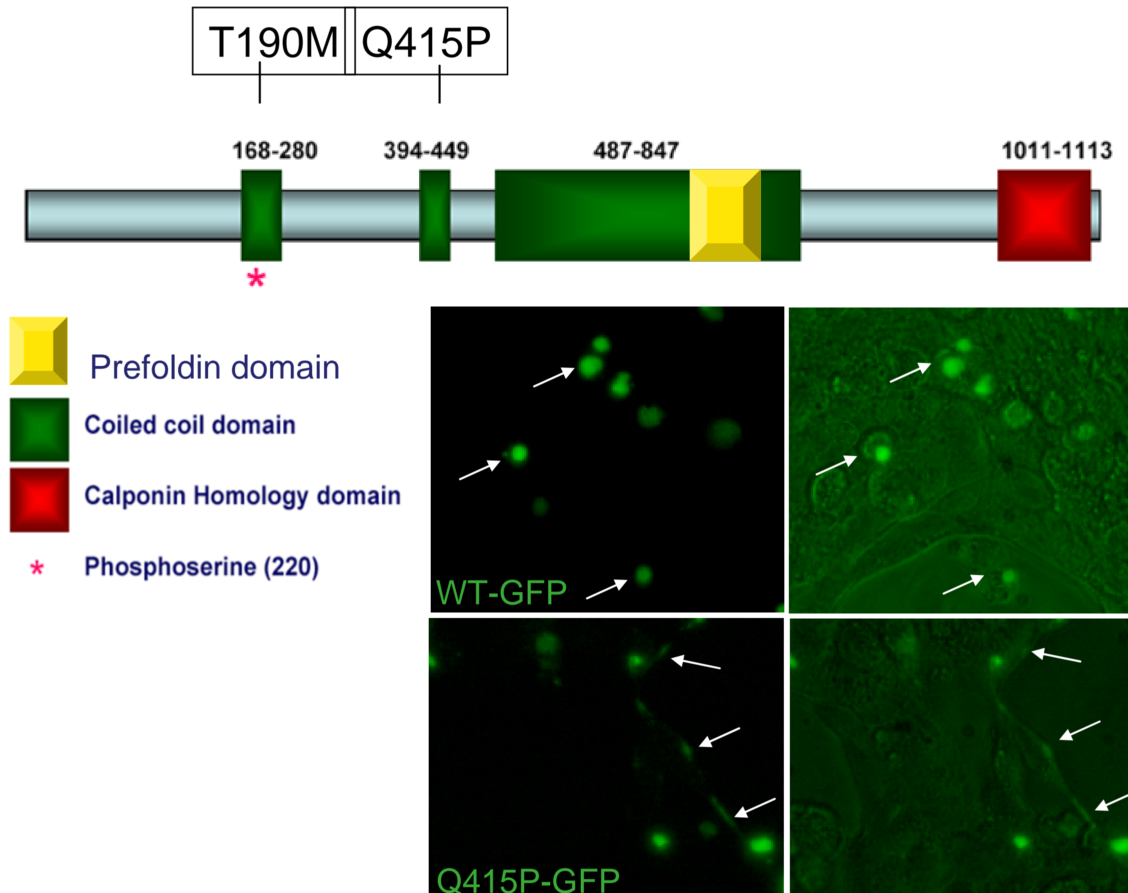
Fig. DGAP177-5. SPECC1L mutations in OFC patients. We have sequenced 22 OFC patients and have identified 3 putative mutations (shown on schematic). Wildtype Specc1l-GFP overexpression results in a rounded cell shape (middle panels). Interestingly, the Q415P-GFP mutant overexpression shows a significant number of cells that do not have an altered shape (lower panels), which suggests a reduced ability to bundle the fibers.



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