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# A novel RAB33B mutation in Smith-McCort dysplasia

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**Abstract**

Smith-McCort dysplasia (SMC) is a rare autosomal recessive spondylo-epi-metaphyseal dysplasia with skeletal features identical to those of Dyggve-Melchior-Clausen syndrome (DMC) but with normal intelligence and no microcephaly. Although both syndromes were shown to result from mutations in the *DYM* gene, which encodes the Golgi protein DYMECLIN, a few SMC patients remained negative in *DYM* mutation screening. Recently, autozygosity mapping and exome sequencing in a large SMC family have allowed the identification of a missense mutation in *RAB33B*, another Golgi protein involved in retrograde transport of Golgi vesicles. Here, we report a novel *RAB33B* mutation in a second SMC case that leads to a marked reduction of the protein as shown by western blot and immunofluorescence. These data confirm the genetic heterogeneity of SMC dysplasia and highlight the role of Golgi transport in the pathogenesis of SMC and DMC syndromes.

**Key words:** Smith-McCort dysplasia, Dyggve-Melchior-Clausen syndrome, RAB33B, DYMECLIN, Golgi apparatus

Smith-McCort dysplasia (SMC; MIM# 607326) is a rare autosomal recessive skeletal dysplasia first described as an osteochondrodystrophy by R. Smith and J.J. McCort in 1958 (Smith and Mc, 1958) and later highlighted by J. Spranger who proposed the term of Smith-McCort Dwarfism (Spranger, et al., 1976). SMC is characterized by a short trunk dwarfism with a barrel-shaped chest, rhizomelic limb shortening and specific radiological features including marked platyspondyly with double-humped end-plates, kyphoscoliosis, metaphyseal irregularities, laterally displaced capital femoral epiphyses and small pelvis with a lace-like appearance of iliac crests (Nakamura, et al., 1997). These clinical and radiological features are also common to Dyggve-Melchior Clausen syndrome (DMC; MIM# 223800) and the two disorders are distinguished by the presence of mental retardation in DMC but a normal intelligence in SMC (Paupe, et al., 2004). A common gene responsible for both conditions, *DYM*, was identified in 2003 showing that SMC and DMC are allelic disorders and assigning SMC to the status of clinical variant of DMC with less severe outcome (El Ghouzzi, et al., 2003) (Cohn, et al., 2003). In the vast majority of DMC cases, identified mutations predict premature truncations of the *DYM* gene product and thus a complete loss-of-function of DYMECLIN (Paupe, et al., 2004) (Girisha, et al., 2008). By contrast, SMC was found to be associated with missense mutations thought to have a milder effect (Cohn, et al., 2003) (Santos, et al., 2009). However, *DYM* mutations have been reported in only three SMC families (Cohn, et al., 2003) (Santos, et al., 2009) among a total of twelve families reported so far (Smith and Mc, 1958) (Spranger, et al., 1976) (Koppers, 1979) (Nakamura, et al., 1997) (Bayrak, et al., 2005; Neumann, et al., 2006) (Gun, et al., 2012). In addition, the absence of mutation in *DYM* in a well-characterized SMC patient had led us to hypothesize a possible genetic heterogeneity of the disease (Neumann, et al., 2006). In support of this hypothesis, Alshammari and colleagues have recently identified a missense mutation (p.K46Q) in a new gene, *RAB33B*, using autozygosity mapping and exome sequencing in a large consanguineous Saudi family (Alshammari, et al., 2012). RAB33B (MIM# 605950) belongs to the large Rab family of small

GTP-binding proteins that play important roles at defined steps of vesicular transport in protein secretion and the endocytosis pathway (Zheng, et al., 1998) (Barr and Lambright, 2010; Stenmark and Olkkonen, 2001). Like DYMECLIN, RAB33B localizes to the Golgi apparatus and is involved in Golgi homeostasis and trafficking (Osipovich, et al., 2008) (Dimitrov, et al., 2009) (Starr, et al., 2010). The recent implication of RAB33B in SMC prompted us to sequence this gene in our previously reported *DYM*-negative SMC patient (Neumann, et al., 2006). We report here the identification of a novel homozygous missense mutation in *RAB33B* in SMC dysplasia.

The patient is a 22-year old Turkish man who was diagnosed with SMC in 2006 (Neumann, et al., 2006). He is the second child of healthy consanguineous parents and displays features typical of DMC/SMC with normal cognitive functions (Figure 1A). Height was 133cm, his head circumference was in the normal range. He had undergone genu valgum operation at the age of 12. Direct sequencing of the two exons of *RAB33B* from genomic DNA of the patient revealed a homozygous c.444T>A transversion in exon 2, predicting the substitution of the highly conserved asparagine by a lysine residue at codon 148 (p.N148K) (Figure 1B). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence for human *RAB33B* (GenBank: AF350420.1), according to journal guidelines (http://www.hgvs.org/mutnomen). The initiation codon is codon 1. This substitution was found at the heterozygous state in the genomic DNA from both parents and absent in his healthy brother (Supp. Figure S1). It was absent as well from publicly available databases of known polymorphic variants, including dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 genome (http://www.1000genomes.org/) and Exome variant server (http://evs.gs.washington.edu/EVS/). In silico prediction of pathogenicity was also very high, with a PolyPhen-2 score of 1 (predicting a mutation “probably damaging” for the protein function) and a Sorting Intolerant From Tolerant (SIFT) score of 0.05 (which predicted an intolerant substitution for the protein). Moreover, it occurred in the G-4 GTPase domain of RAB33B (GenBank:

AAL83916.1) which is responsible for the specificity of the GTPase for the binding of guanine nucleotides through its 6$^{th}$ GTP/Mg2+ binding site and which is highly conserved both in RAB33B orthologues across species and in all RAB paralogues (Figure 2A and 2D, (Sanders, 1990)). This novel c.444T>A transversion has been submitted to the Leiden Open Variation Database (www.lovd.nl/RAB33B).

In order to evaluate the consequences of this novel mutation on *RAB33B* transcript and protein stability, we extracted mRNA and proteins from the patient's fibroblasts. The c.444T>A substitution was also found at the mRNA level, indicating that the mutated mRNA was present (not shown). However, a marked reduction of the protein was observed in western blot even after loading a heavy amount of total proteins (60µg) (Figure 2B). This was also visible on fixed fibroblasts using the same anti-RAB33B antibody in immunofluorescence. The fluorescent signal was strongly decreased although not completely (Figure 2C). Moreover, co-labelling with an antibody against GIANTIN revealed a swollen and fragmented appearance of the Golgi apparatus in many cells. The loss of RAB33B fluorescence was much more pronounced in those cells, suggesting a correlation between the structure of the Golgi and the presence of RAB33B. However, this could be due also to a dilution effect in the swollen stacks. Finally, RAB33B was found present and normally localized in fibroblasts from a DMC patient bearing a well-characterized splice mutation in DYMECLIN (c.194-1G>A) that was previously shown to result in skipping of exon 4 and premature termination of the protein (Figure 2B-C, (Neumann, et al., 2006)).

This novel mutation is the second SMC mutation reported in *RAB33B*. Our findings confirm that SMC may be caused by a deficiency of either DYMECLIN or RAB33B and highlight that DMC and SMC both result from an altered Golgi trafficking. Results from this study and from Alshammari and colleagues (Alshammari, et al., 2012) show that specific missense mutations located in critical domains of RAB33B (Figure 2D) can cause a remarkable instability of the

protein. They also raise the question of whether the *RAB33B* gene would account exclusively for SMC, suggesting that mental retardation would be mainly associated with a complete loss-of-function of the *DYM* gene. However, *RAB33B* analyses in additional cases of SMC and DMC syndromes are now required to answer this question.

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**Conflict of interest statement**

The authors have declared that no conflict of interest exists

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**Legends to Figures**

**Figure 1. SMC phenotype and sequence chromatogram of the novel RAB33B mutation**

A. Photographs of the patient at the age of 22. Note the short trunk with barrel-shaped chest, pronounced curvature of the spine and normal head circumference. Written permission of the patient for reproduction of these photographs was obtained.

B. Direct sequencing of *RAB33B* revealed a homozygous T>A transversion at position 444 of exon 2, predicting the substitution of the highly conserved asparagine by a lysine residue at codon 148 (p.N148K).

**Figure 2. High conservation of the mutated asparagine residue during evolution and detection of the RAB33B protein**

A. Multiple alignment of the human G-4 GTPase domain (red frame) of human RAB33B (GenBank: AAL83916.1) with orthologues Rab33 proteins and paralogues Rab1A-3A proteins. Note the invariant conservation of the GNK motif that contains the asparagine residue (N in yellow) mutated in the SMC patient.

B. Western blot analysis of RAB33B in human fibroblasts from a control (Ctl), a DMC patient with the c.194-1G>A mutation in the *DYM* gene previously shown to result in premature termination of DYMECLIN (DMC) and the SMC patient with the novel *RAB33B* mutation reported here (SMC). Note the almost complete absence of signal detected in the fibroblasts from the SMC case. (60µg total proteins loaded in each lane, the anti-RAB33B antibody was purchased from Frontier Bioscience and used at the concentration of 1µg/ml).

C. Immunofluorescence detection of RAB33B in human fibroblasts from the control (Ctl) and same DMC and SMC patients along with DAPI and the Golgi marker GIANTIN. Note the strong

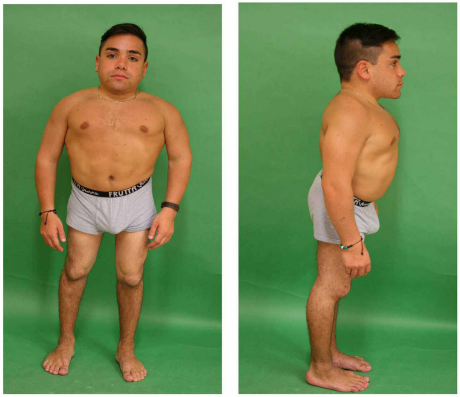
depletion of RAB33B in the fibroblasts from the SMC patient. (Anti-RAB33B and anti-GIANTIN antibodies from Abcam ab24586 were used at the concentration of 1µg/ml).

D. Schematic representations (linear, left and three-dimensional, right) of the RAB33B protein showing the localization of the 5 GTPase domains (orange boxes) along with the 8 GTP/Mg2+ binding sites (green boxes) and the position of the two known mutations causing SMC dysplasia (p.K46Q (Alshammari, et al., 2012) and p.N148K (this study)). The three-dimensional representation of the RAB33B molecule was adapted from the crystallographic structure of the Rat protein (GenBank NP_00102414.1) available in Protein Data Bank Europe (PDBe, http://www.ebi.ac.uk/pdbe-srv/view/entry/1z06/secondary.html) and modified using the PyMol Software (version 1.5). Note that the two missense mutations are spatially very close to each other.

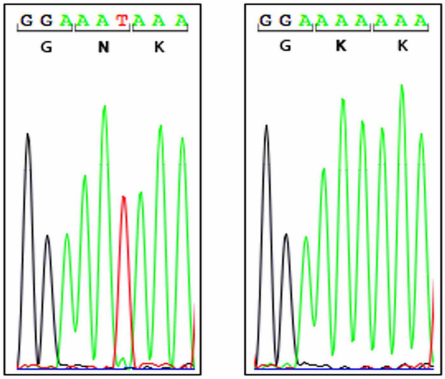
**Supplementary Figure S1. Sequence chromatogram of the healthy parents and brother**

The c.444T>A transversion in exon 2 of *RAB33B* was found at the heterozygous state in both parents but not in the healthy brother whose sequence was normal.

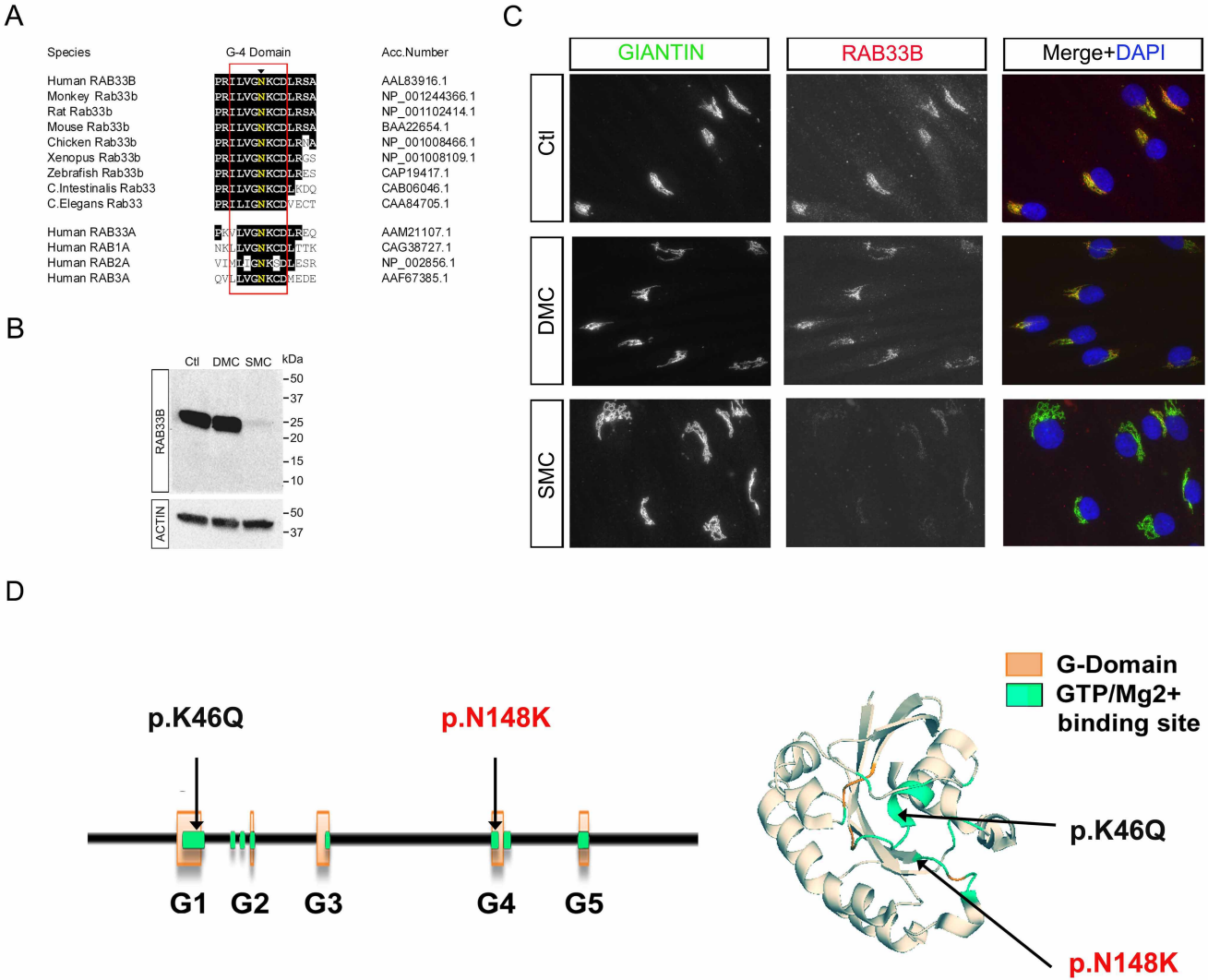
A



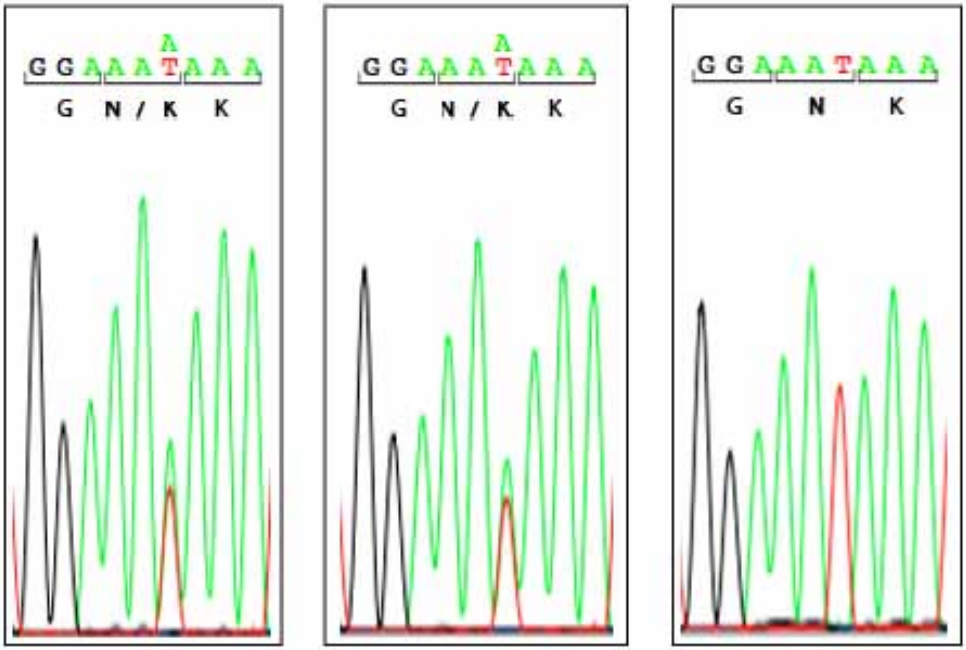
B




Dupuis et al, Figure 1




Dupuis et al, Figure 2




Dupuis et al, Supplementary Figure S1