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Geroderma osteodysplastica is caused by mutations in SCYL1BP1, a Rab-6 interacting golgin

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Abstract

Geroderma osteodysplastica (GO) is an autosomal recessive disorder characterized by wrinkly skin and osteoporosis. Here we demonstrate that GO is caused by loss-of-function mutations in *SCYL1BP1*, which is expressed at high levels in skin and osteoblasts. The protein localizes to the Golgi apparatus and interacts with Rab6, identifying *SCYL1BP1* as a novel golgin. These results associate abnormalities of the secretory pathway with age-related changes in connective tissues.

Characteristic changes associated with human aging are a lax and wrinkly skin as well as a reduction of bone mass. Although the heritability of peak bone mass has been estimated to

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Statement

The study has been approved by the local institutional review board and followed the Declaration of Helsinki protocols. Samples were taken after obtaining written informed consent. The permission to publish photos was given.

range between 60 and 70%, only a few genetic determinants have been identified so far. Skin wrinkling is a feature of several uncommon monogenic disorders so far mainly assigned to mutations in extracellular matrix components (Supplementary References). In this study we investigated the molecular cause of geroderma osteodysplastica (also: geroderma osteodysplasticum, GO; OMIM 231070), a rare autosomal recessive disorder characterized by lax, wrinkled skin, joint laxity, and a typical face with a prematurely aged appearance. Skeletal signs include severe osteoporosis leading to frequent fractures, malar and mandibular hypoplasia, and a variable degree of growth retardation^{1,2}. According to the current definition, GO can be regarded as a segmental progeroid disorder involving bone and skin³.

Our study involved patients from thirteen families all showing the typical GO phenotype (Fig. 1a, Supplementary Methods, and Supplementary Fig. 1). Bone mineral density was significantly reduced in all patients, frequently resulting in fractures of long bones or vertebrae (Supplementary Fig. 1). We performed linkage analysis initially in four Mennonite pedigrees with a total of twelve affected individuals from Germany, Mexico, and Canada without known relationship to each other (Supplementary Methods). The anabaptist community of the Mennonites was founded in the 16th century in Friesland in the north of the Netherlands, and after periods of prosecution, a large part of the community migrated to Northern and Middle America. Many of them kept a common, specific language, which is an ancient German dialect also known as Mennonite Low German.

A genome-wide scan identified a homozygous region on chromosome 1q24 with a combined multipoint lod score of 12.0 (Supplementary Fig. 2). The shared homozygous haplotype in all affected persons from the Mennonite pedigrees was consistent with a founder effect and defined a candidate region between markers D1S1569 and D1S218 (Fig. 1b), which is 5.1 cM in length corresponding to 5.7 Mb containing 102 genes. In a systematic sequencing approach we detected a homozygous nonsense mutation, p.E123X, in the gene *SCYL1BP1* (*FLJ11752*, *NTKLBP1*) in all affected individuals from all four Mennonite families (Table 1, Supplementary Methods, and Supplementary Table 1). We were not able to trace back the Canadian and Mexican patients to their European ancestors. Our results also demonstrate the importance of religious beliefs and a common, specific language for the preservation of an isolated population over several generations and even large distances, and the power of homozygosity mapping in such populations.

In nine additional GO patients from Germany, Italy, Oman, Pakistan, Libya, Mexico, and the U.S. we found eight other mutations, including five nonsense mutations, two frameshift mutations, one splice site mutation, and one mutation of the deduced methionine start codon (Fig. 1c and Table 1). Obligatory carriers were all heterozygous for the respective mutation, and the mutations were not found on 100 chromosomes from ethnically matched control persons. A reading frame of 394 codons has been predicted for *SCYL1BP1* (GenBank accession no. NM_152281). However, the sequence of *SCYL1BP1* is highly conserved among species, and a comparison with orthologous sequences makes a later translation start much more likely resulting in a 369-codon reading frame. This translational start is further corroborated by the prediction of translation using the algorithm NetStart⁴.

SCYL1BP1 encodes the soluble protein SCY1-like 1 binding protein 1 (*SCYL1BP1*; *NTKL-binding protein 1*). The most notable features of its structure are a large number of charged amino acids, many predicted phosphorylation sites, and two or three predicted coiled-coil domains (Fig. 1c). Using antibodies against *SCYL1BP1* we identified a band of 47–50 kDa in Western blot analysis (Supplementary Methods). In all available patient fibroblasts, we were not able to detect significant changes in *SCYL1BP1* mRNA quantities by qRT-PCR, however, *SCYL1BP1* protein was completely absent in these cases, including the case OA

with the mutation p.M1? (Fig. 1d), pointing to loss-of-function mutations through instability of the protein.

Staining with markers for cellular compartments revealed a clear localization of SCYL1BP1 to the Golgi apparatus (Fig. 1e and Supplementary Methods). No significant alterations in Golgi morphology or in the distribution of different Golgi marker proteins were detected in patient fibroblasts (Supplementary Fig. 3). *Scyl1bp1* showed high expression in osteoblasts and in the skin (Supplementary Fig. 4), which correlates with the main sites of disease manifestation. Protein expression was shown to increase during osteoblast differentiation. Expression was also present in osteoclasts albeit at lower levels.

Since SCYL1BP1 is a soluble protein with coiled-coil domains known to mediate protein-protein interaction, we sought for binding partners in a library of mammalian small G-proteins of the Rab, ARF, and Arl families that are known as important determinants of intracellular membrane traffic⁵ using a yeast two-hybrid interaction screen. A specific interaction of SCYL1BP1 with Rab6 was identified (Fig. 1f). Pull-down experiments confirmed this interaction. The interaction was additionally shown to be dependent on the activation state of Rab6 thus further corroborating its specificity (Fig. 1g). In contrast, an interaction with Rab1 was not detected, confirming the specificity of the yeast two-hybrid screening (Supplementary Fig. 5). Golgi localization, the presence of coiled-coil domains, and the interaction with a Rab protein are the hallmarks of the golgin protein family⁶. Therefore, SCYL1BP1 can be classified as a golgin. To our knowledge, this is the first example of a golgin causing a hereditary human disorder.

Mouse *Scyl1bp1* was identified initially as a binding partner of the N-terminal kinase-like protein (*Scyl1*) in a yeast two-hybrid analysis⁷. *Scyl1* was shown to be mutated in the *mdf* mouse mutant that shows spinocerebellar neurodegeneration⁸. Human SCYL1 was found to bind to adaptor proteins of clathrin-coated vesicles⁹, and a recent search for binding partners of *Scyl1* by mass spectrometry identified an interaction with components of coatamer complex I (COPI) coats¹⁰. This observation fits very well with our finding that SCYL1BP1 is associated with the Golgi apparatus and involved in the secretory pathway.

The three Rab6 isoforms are central components of cellular membrane trafficking mainly residing in the trans-Golgi network⁵. One important function of Rab6 is the recruitment of motor proteins that promote vesicle movement. Rab6 is known to be involved in the retrograde transport from endosomes to the Golgi apparatus and plays a crucial role in the secretory pathway from the Golgi to the plasma membrane^{11,12}. Interestingly, a functional interaction of Rab6 with the conserved oligomeric Golgi complex (COG) has been demonstrated recently¹³. This is intriguing since patients with mutations in COG7, a subunit of this complex, show glycosylation defects and wrinkled skin¹⁴. Impaired glycosylation, wrinkly skin, and bone defects are also features observed in autosomal recessive cutis laxa type 2 (ARCL2; OMIM 219200). ARCL2 was recently shown to be due to mutations in the vacuolar-type H⁺-ATPase subunit gene *ATP6V0A2*¹⁵. Given the phenotypic similarities between GO and ARCL2, we analyzed the glycosylation pattern of serum transferrin from GO patients but did not detect any glycosylation abnormalities².

Our results show that two common aging phenomena, skin wrinkling and osteoporosis, can be associated with mutations in a novel golgin, SCYL1BP1. Future studies are needed to investigate the pathophysiological similarities between GO and overlapping disorders to identify a putative common mechanism regulating connective tissue homeostasis during aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Clinical features of geroderma osteodysplastica (GO), identification of mutations in *SCYL1BP1*, and characterization of *SCYL1BP1* as a golgin

(a) 9-month old patient. Note the typical facial appearance with sagging cheeks and pronounced wrinkling at the chest and dorsum of the hands giving him a prematurely aged appearance. (b) Haplotype analysis in four Mennonite pedigrees in the candidate region on chromosome 1q24. The shared haplotype defined a 5.1-cM candidate interval and indicated homozygosity by descent and a common founder in the patients from Germany, Canada, and Mexico. The boxes mark the respective homozygous intervals. (c) Schematic representation of the *SCYL1BP1* structure showing coiled-coil domains (CC), the uncharacterized domain DUF622, and the nine different mutations found in GO families. (d) GO mutations lead to a complete loss of the protein as shown in a Western blot analysis of patient fibroblast lysates. C1-C3, control fibroblasts. (e) Co-staining of *SCYL1BP1* with various Golgi marker proteins in control cells. Signals of γ -adaptin were in the same compartment but did not overlap completely. Co-localization with Rab6 pointed to the localization in the trans-Golgi network. Co-staining with Golgi matrix protein GM130 excluded a localization in the cis-Golgi compartment. Scale bar 10 μ m. (f) Yeast two-hybrid screening against a library of ARF, Arl, and Rab small GTPases. Growth on selective medium indicated interaction with Rab6a and Rab6b. (g) Confirmation of the Rab6 interaction by pull-down experiments. A GST-Rab6 fusion protein was used to pull down *SCYL1BP1* from HeLa lysates without or in the presence of GTP. Active GTP-bound Rab6 pulled down *SCYL1BP1* efficiently while the GDP-locked T27N mutant was unable to interact.

The numbering of cDNA positions and deduced protein changes is based on a transcript that starts 25 triplets later than predicted in GenBank (accession no. NM_152281).

Table 1
Mutations in SCYL1BP1 identified in 13 families with GO

Patient	Origin	Mutation	Exon	Protein	Genotype
OA	Oman	c.-1_1GA>CT	1	p.Met1?	homozygous
P1	Pakistan	c.-1_1GA>CT	1	p.Met1?	homozygous
P2	Pakistan	c.-1_1GA>CT	1	p.Met1?	homozygous
GT1	Germany	c.136G>T	2	p.Glu46X	heterozygous
LS	Libya	c.190C>T	2	p.Gln64X	homozygous
IG	Italy	c.257delC	2	p.Pro86ArgfsX70	homozygous
GT2 ^b	Germany	c.367G>T	2	p.Glu123X	homozygous
GD1 ^b	Germany	c.367G>T	2	p.Glu123X	homozygous
CD1 ^b	Canada	c.367G>T	2	p.Glu123X	homozygous
Mp ^b	Mexico	c.367G>T	2	p.Glu123X	homozygous
UR42	USA	c.662+5G>C	4	p.Ser175_Arg221del	heterozygous
IR	Italy	c.739C>T	5	p.Gln247X	homozygous
UR42	USA	c.784C>T	5	p.Arg262X	heterozygous
UT	USA	c.784C>T	5	p.Arg262X	homozygous
GT1	Germany	c.1050_1053delTTCTT	5	p.Phe350LeufsX26 ^a	heterozygous

^a predicted peptide is five residues longer than SCYL1BP1

^b Memmonite pedigrees