1. INTRODUCTION

Ageing leads to degenerative changes over time in an organism. Generally, it is characterized by decreasing adaptation to environmental stress, increasing homeostatic imbalance, and higher risk of diseases with death as its ultimate consequence. Ageing has been for a long time considered to be an unregulated process until recent genetic studies, particularly in *C. elegans* suggested the possibility for manipulation in order to ward off age-related diseases and thereby extend lifespan (Kenyon et al., 1993). Some organisms such as hydra are even suspected to escape ageing presumably due to some evolutionary selective pressures (Martinez, 1998). We are aware that population ageing was one of the most distinctive demographic events of the twentieth century and will remain important throughout the twenty-first century. The shift in age structure is imposing a profound impact on a broad range of economic, political, social and medical conditions.

Progeroid syndromes have been intensely studied in part under the hope that they might shed some light on the pathology and biology of ageing (Kudlow et al., 2007). The two classical diseases of premature ageing are HGPS (Hutchinson-Gilford progeria syndrome 'progeria of childhood', OMIM 176670) and Werner syndrome ('progeria of adults', OMIM 277700). HGPS symptoms usually become apparent in the first or second year of life and are characterized by sclerodermatous skin, hair loss, bone deformations as well as growth retardation (Mattout et al., 2006). Patients with Werner syndrome generally develop normally until they reach puberty. The symptoms include scleroderma-like skin, subcutaneous calcification, premature arteriosclerosis and prematurely aged facies. Both HGPS and Werner syndrome have shortened life expectancy owing to cardiovascular diseases or increased cancer incidence (Kudlow et al., 2007). The gene responsible for Werner syndrome was identified by positional cloning in 1996. It encodes a 162-kDa RecQ helicase protein (Yu et al., 1996). The genetic determinant of most cases of HGPS was found to be splicing mutations of lamin A in 2003 (Eriksson et al., 2003). Emerging functional evidence indicated that changes in DNA repair and the DNA-damage responses might be the common mechanism underlying both syndromes (Eller et al., 2006; Liu et al., 2005; Martin, 2005).

Two of the most obvious features during ageing are alterations of the skin with reduced elasticity and declining bone mass. Since skin wrinkles are mainly of cosmetic relevance, great interest has been shown in genetic conditions with lax skin. Osteoporotic fractures

frequently happen in the elderly, affecting 50% of women and 20% of men older than 50 years (Cummings and Melton, 2002). As such, monogenic human disorders characterized by osteoporosis are of particular interest. By investigating their genetic basis important insights into the molecular mechanisms that control the regulation of bone mass can often be gained, which allows potentially the identification of novel targets for the therapy of osteoporosis.

1.1 Skin and premature ageing disorders

1.1.1 Skin

The different layers of the skin are of different embryonic origin. In mammals, the neuroectodermal cells that remain at the embryo's surface become the epidermis, while mesoderm-derived cells contribute to the collagen-secreting fibroblasts of the underlying dermis (Blanpain and Fuchs, 2006).

Adult skin is made up of three distinct layers. The outer layer, called the epidermis, protects the body from invasion and infection and helps to seal in moisture. This layer is translucent and allows light to pass partially through it. Lying underneath the epidermis is the middle layer called the dermis, which together with the epidermis is termed cutis. A very thin membrane, the basement membrane, extends between these two layers. Below the dermis is the subcutis, overlying the muscles and bones, to which the whole skin structure is attached by connective tissues. The subcutis contains fat tissue important for plastering and isolation.

Whereas the epidermis does not have a direct blood supply, the dermis contains blood vessels, nerves, hair roots and sweat glands. Collagen, elastin and glycosaminoglycans are primary structural components of the dermis. Bundles of tough fibres in this layer give the skin elasticity, firmness and strength. As the most abundant protein found in humans, collagen makes up 70–80% of the dry weight of the skin and confers mechanical strength and structural support. The major collagens in skin are types I and III, while minor collagens include types V, VI, VII, XI (Baumann, 2007). Elastin is a minor component of the dermis and accounts for 2–4% of the extra-cellular matrix, but it has an important function in providing the elasticity of the skin (Uitto, 1979).

1.1.2 Skin ageing

Skin ageing, especially on the face, is associated with a progressive decline in elasticity and increase in extensibility. Upon age both collagen and elastic fibres show marked alterations in their three-dimensional arrangements and the synthesis of collagen and elastin gradually declines (Lavker et al., 1987). The ratio of collagen types in human skin also changes with age. In young skin, collagen I comprises 80% and collagen III comprises about 15% of total skin collagen; in older skin, the ratio of Type III to Type I collagen has been shown to increase (Oikarinen, 1990). The overall collagen content per unit area of skin surface declines approximately 1% every year (Shuster et al., 1975). The phenomenon of sagging skin often observed in the elderly is in large part due to the loss of elasticity. Proteoglycans in the dermal extracellular matrix also show age-related differences (Carrino et al., 2000).

1.1.3 Cutis laxa

The network of elastic fibres in the dermis is a good indicator of skin resilience, texture, and quality (Davidson and Giro, 2006). Alteration of the elastic fibres produces a dramatic ageing phenotype. Cutis laxa is a heterogeneous group of inherited and acquired connective tissue disorders characterized by loose, sagging, and redundant skin, which often leads to an appearance of premature ageing (Uitto and Pulkkinen, 2002). Histologically, elastic fibres are sparse and fragmented in the skin in this group of disorders.

1.1.3.1 Elastin

Elastin is the major component of elastic fibre that provides resilience and elasticity to many tissues such as skin and lungs, which require the ability to deform repetitively and reversibly. As the core of the elastic fibres, elastin is a highly insoluble extracellular matrix (ECM) protein. Tropoelastin, the precursor of elastin, is expressed as many isoforms. At least 11 human tropoelastin splice variants have been identified with six exons shown to be subject to alternative splicing: exons 22, 23, 24, 26A, 32 and 33 (Vrhovski and Weiss, 1998). Tropoelastin molecules are secreted from cells as a soluble form. In the extracellular space, they are joined covalently through chemical modification and cross-linking of specific lysyl residues to form mature insoluble elastin. A number of lysyl oxidases of amine oxidase family catalyze the reaction (Molnar et al., 2003). This process

is facilitated by interacting with 10–12 nm microfibrils which align cross-linking domains between individual tropoelastin molecules (Mecham and Davis, 1994).

There are numerous acquired and inherited diseases known to affect the integrity of elastic fibres (Vrhovski and Weiss, 1998). The organs most obviously affected are those rich in elastin such as skin, lungs, and aorta. Supravalvular aortic stenosis (SVAS) and Williams syndrome have been directly linked with alterations in the elastin gene. Three types of mutations have been identified in SVAS. One is a large 30-kb deletion within the gene involving exons 2-27 (Olson et al., 1995); the second involves a deletion of the 3'-end of the gene, resulting in a truncated protein missing the C-terminus from exon 28 onwards (Curran et al., 1993; Ewart et al., 1994; Morris et al., 1993). The third class of mutations comprises various point mutations, most of which result in the loss of the conserved C-terminus through introduction of premature stop or frameshift codons (Li et al., 1997; Tassabehji et al., 1997). Williams syndrome is a contiguous syndrome which results from a large (e.g. 114-kb) deletion containing elastin and adjacent genes including LIM-kinase (Ewart et al., 1993; Tassabehji et al., 1996). Frameshift mutations in ELN have been reported in individuals with autosomal dominant cutis laxa (Tassabehji et al., 1998; Zhang et al., 1999).

1.1.3.2 Fibulins

The fibulins are a family of extracellular matrix (ECM) proteins, whose members include larger and more structurally complex fibulins 1 and 2 as well as smaller and structurally simpler fibulins 3, 4, and 5. Fibulins contain sequential repeats of calcium-binding epidermal growth factor (EGF)-like (cbEGF) modules, followed by a globular C-terminal fibulin-type module. A growing number of ECM and secreted proteins have been reported to interact with fibulins. For instance, FBLN-5 interacts with tropoelastin directly in a calcium-dependent manner, with C-terminal fibulin-type module binding apolipoprotein A and lysyl oxidase-like 1 (Liu et al., 2004; Zheng et al., 2007). All these features make FBLN-5 an essential molecule for normal elastogenesis of the uterus, lung, skin and vasculature.

Several genetic disorders have been linked to members of the fibulin family including cutis laxa (Table 1). Mutations in the gene fibulin-5 can cause either autosomal recessive or autosomal dominant cutis laxa (Loeys et al., 2002; Markova et al., 2003). Investigation by

Hu et al. suggests that mutations in fibulin-5 result in misfolding and decreased secretion (Hu et al., 2006). Moreover, the mutant forms of fibulin-5 hold a reduced binding to tropoelastin and microfibrils, leading to impaired elastic fibre development (Hu et al., 2006).

One homozygous missense mutation (169G-->A; E57K) in the fibulin-4 gene was described in a child with autosomal recessive cutis laxa (Hucthagowder et al., 2006). The E57 residue is very conserved in cbEGF modules, which would be essential part for calcium binding. Importantly, there is no detectable fibulin-4 in the ECM extracts from patient fibroblasts, which indicates that mutation E57K impairs the secretion as well as matrix binding or stability of this protein (Hucthagowder et al., 2006). Highly reduced expression of fibulin-2 was also found in one patient with cutis laxa (Markova et al., 2003).

Fibulin gene	Synonyms	Alternative splice variants	Human chromosome	Heritable disease
FBLN-1	BM-90	4	22q12.31	**Synpolydactyly** **Autosomal-dominant Giant Platelet Syndrome**
FBLN-2	–	2	3p25.1	–
FBLN-3	EFEMP-1 S1-5, T16	5	2p16	**Malattia Leventinese/Doyne Honeycomb retinal dystrophy**
FBLN-4	EFEMP-2 MBP-1, H411 UPH1	2	11q13	**Cutis laxa**
FBLN-5	DANCE EVEC, UP50	None	14q32.1	**Cutis laxa** **Age-related macular degeneration**

Table 1. Fibulins and human pathologies. Modified from (Albig and Schiemann, 2005)

1.2 Bone and osteoporosis

Bone is a dynamic organ undergoing remodelling. A delicate balance between bone resorption by osteoclasts and deposition by osteoblasts is essential to maintain normal skeletal structure and function. Any disturbance of this process affects the density and architecture of bone, thereby resulting in diseases such as osteoporosis (Marks and Hermey, 1996).

1.2.1 Bone development

1.2.1.1 Skeletal patterning

Where a skeletal element forms initially depends on the patterning information, which was determined by a complex process of signals from some secreted molecules such as Wnt, Hedgehog, fibroblast growth factor (FGF) families and the TGF-β superfamily, as well as a number of transcription factors including Hox, Pax, homeodoman- containing, Forkhead and basic helix–loop–helix (bHLH) families (de Crombrugghe et al., 2001). Mutations in early patterning genes cause disorders called dysostoses; these affect only specific skeletal elements, leaving the rest of the skeleton largely unaffected (Zelzer and Olsen, 2003). For instance, Waardenburg's syndrome (MIM 148820), characterized by limb phenotype including fusion of wrist bones, syndactyly, and small or missing phalangeal bones, is caused by mutations in PAX3, a member of a family of transcription factors containing two interdependent DNA-binding domains, while in-frame expansions of a polyalanine tract in the amino-terminal region of HOXD13 are responsible for Synpolydactyly (Muragaki et al., 1996; Tassabehji et al., 1992).

1.2.1.2 Chondrocyte differentiation

Endochondral ossification occurs in most parts of the skeleton. Here, a cartilaginous template is first formed and subsequently replaced by bone. Central to this process is the formation of a growth plate, which is subdivided into three layers: reserve chondrocytes, proliferating chondrocytes, and hypertrophic chondrocytes (Kornak and Mundlos, 2003). Sox9, a member of the Sox family of transcription factors with a high-mobility-group (HMG)-box DNA binding domain, plays an essential role in chondrocyte differentiation (Akiyama et al., 2002). This was strongly supported by the skeletal anomalies in the human genetic disease campomelic dysplasia (CD) characterized by hypoplasia of practically all endochondral skeletal elements, in which heterozygous mutations in and around the SOX9 gene have been identified (Foster et al., 1994; Wagner et al., 1994). The investigation of sox9 null mouse cells indicates that Sox9 is required for an early step in the pathway of chondrocyte differentiation - mesenchymal condensation (Bi et al., 1999). Sox5 and Sox6, two other Sox family members, cooperate with Sox9 during chondrogenesis (Lefebvre et al., 1998).

1.2.1.3 Osteoblast differentiation

Osteoblasts, which originate from mesenchymal cells, are responsible for bone matrix production in both intramembraneous and endochondral bone formation. Cbfa1/Runx2, a member of a small transcription factor family that shares DNA-binding domains of homology with Drosophila Runt, is a key regulator of osteoblast differentiation and function (Ducy et al., 1997; Komori et al., 1997; Otto et al., 1997). In Cbfa1/Runx2 null mice, no endochondral or membranous bone is formed owing to an arrest during the early steps of osteoblast differentiation (Otto et al., 1997). Haploinsufficiency of Cbfa1/Runx2 causes cleidocranial dysplasia (CCD) in both mice and humans (Mundlos et al., 1997). CCD is an autosomal dominant disorder characterized by a delay in closure of cranial sutures and fontanelles, hypoplastic or aplastic clavicles, and dental anomalies (Mundlos et al., 1997).

1.2.1.4 Osteoclast formation

Osteoclasts are derived from the haematopoietic monocyte/macrophage lineage. As soon as the bone is formed, osteoclast precursors enter the ossification center through invading vessels and resorb calcified cartilage as well as mature bone to counteract the bone forming activity of osteobalsts, which is prerequisite both for bone growth and homeostasis.

Osteoclast formation and activation is regulated by preosteoblastic stromal cells through expression of receptor activator of NF-κB ligand (RANKL), a member of the tumor necrosis factor (TNF) superfamily, and macrophage colony-stimulating factor (M-CSF) (Takayanagi, 2007). Osteocytes embedded in the bone might recruit osteoclasts to sites of bone destined for removal by largely unknown mechanisms (Khosla et al., 2008).

M-CSF, a secreted protein, can bind to its receptor, c-Fms, on early osteoclast precursors and therefore provides signal for their survival and proliferation (Teitelbaum, 2000). RANKL cooperates with multiple factors to regulate osteoclasts such as NF-κB, c-Fos and NFATc1 (Boyce and Xing, 2006). NFATc1, a transcription factor, is activated by c-Fos in osteoclasts downstream from RANK, the RANKL receptor (Takayanagi, 2005). Osteoprotegerin (OPG), a truncated soluble RANK protein, expressed by more mature osteoblasts can bind to RANKL, make it thus inaccessible to the osteoclast receptor to limit osteoclast formation (see above). Antagonism between OPG and RANK was also

demonstrated by severe osteopetrosis in RANK knockouts and osteoporosis in OPG knockouts (Kong et al., 1999; Simonet et al., 1997).

1.2.1.5 Extracellular matrix

The extracellular matrix is composed of a complex mixture of proteins, proteoglycans and mineral deposits (in the case of bone). Extracellular matrix of cartilage and bone plays critical roles in skeletal development, as was demonstrated by the mutations of the matrix molecules found in numerous skeletal dysplasias (Olsen, 1995). For instance, heterozygous mutations in COL1A1 and COL1A2 are responsible for osteogenesis imperfecta (OI) (Kocher and Shapiro, 1998). Mutations in type II collagen cause a spectrum of diseases defined as type II collagenopathies (Mundlos and Olsen, 1997). Type II collagen is the major structural collagen component of cartilage. In addition to type II collagen, a number of minor collagens (types IX, X, XI, and XII) are components of cartilage. Collagen IX mutations have been identified in multiple epiphyseal dysplasia (MED), characterized by mildly short stature and early-onset osteoarthritis (Paassilta et al., 1999). Mutations in COL10A1 have been linked to the autosomal dominant disorder Schmid metaphyseal chondrodysplasia, which is characterized by bowing of the legs, growth retardation of the lower extremities, and coxa vara (Warman et al., 1993).

1.2.2 Osteoporosis

Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration, which results in higher bone fragility and thereby susceptibility to fracture (Cummings and Melton, 2002). It is correlated with increased bone turnover in which the bone-resorbing activity of osteoclasts exceeds the bone-forming activity of osteoblasts. In the Western population, about 50% of women and 20% of men older than 50 years will have a fragility fracture in their remaining lifetime (Cummings and Melton, 2002).

The human disorders with osteoporosis have been extensively studied for decades, and the identification of genes associated with these diseases has increased our understanding of molecular mechanisms that control bone development and homeostasis.

One recent example is the discovery of disease-causing mutations in the autosomal recessive osteoporosis pseudoglioma syndrome (OPPG). Children with OPPG have very low bone mass and are prone to developing fractures and deformation (Gong et al., 2001).

By genetic mapping and candidate approach, mutations were identified in the gene LRP5, encoding a Wnt co-receptor in the canonical signaling pathway that employs β-catenin as a downstream effector (Mao et al., 2001). When compared to age- and gender matched controls, all OPPG patients have an extremely low BMD. Interestingly, all heterozygous carriers of OPPG mutation also have reduced bone mass, which indicates a gene dosage effect (Gong et al., 2001).

Other well-known hereditary disorders with low bone mass include cleidocranial dysplasia (CCD; MIM 119600), hypophosphatasia (MIM 241500), aromatase deficiency (MIM 107910) and osteogenesis impefecta (OI; MIM 166200). CCD is caused by Haploinsufficiency of Runx2, the key regulator of osteoblast differentiation (see above). Deficiency of alkaline phosphatase is responsible for the low bone mineral deposition in hypophosphatasia (Mornet, 2007). While reduced production of estrogen due to aromatase deficiency leads to increased osteoclast activity (Jones et al., 2007), OI is caused by malformed collagen fibres due to mutations in type 1 collagen (Martin and Shapiro, 2007).

1.3 Geroderma osteodysplastica (GO) and its genetic basis

Monogenic disorders leading to osteoporosis are extremely rare. Geroderma osteodysplastica (GO; OMIM 231070) is an outstanding example. GO is a rare autosomal recessive disorder characterized by wrinkled skin with prematurely aged appearance as well as osteoporosis, particularly of the vertebrae, leading to frequent fractures (**Fig. 1**). Interestingly, individuals with GO have normal life expectancy without suffering complications from other organ systems, which differs from other age-associated diseases. The existence of this sort of syndrome suggests that skin wrinkling and declining bone mass during usual ageing might share a common mechanism.

1.3.1 History of GO

GO was first described by Bamatter et al. in 5 members of a Swiss family (Bamatter et al., 1950). Based on the occasional manifestation in the same family, Boreux claimed an X-linked recessive inheritance of the disorder (Boreux, 1969). But the report of 2 females among the 6 affected children in 2 Mennonite families strongly indicated that GO is an autosomal recessive disorder (Hunter et al., 1978). Subsequently, many related cases were reported all over the world (Hunter, 1988). Hunter emphasized the droopy, jowly face with a degree of malar hypoplasia and mandibular prognathism. Skin was lax but not

hyperelastic, most prominent over the extremities, and the patients were susceptible to fractures and vertebral collapse owing to osteoporosis (Hunter, 1988).

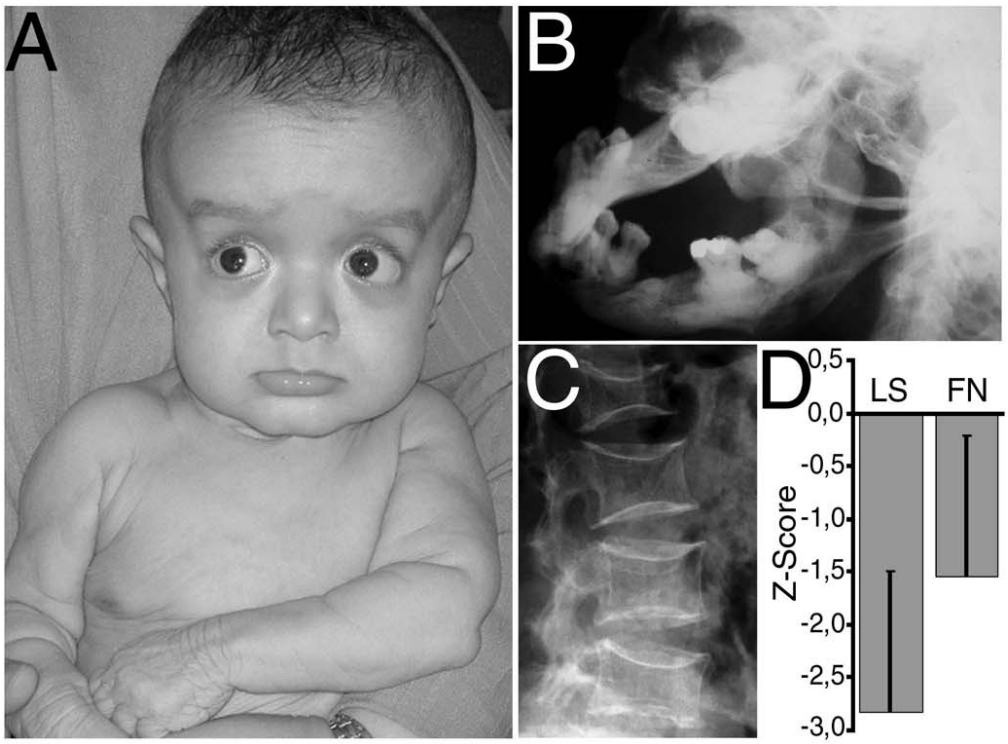

Fig. 1. Clinical features of geroderma osteodysplasticum (GO). (A) 9-month old patient with two spontaneous femoral fractures. Note the typical facial appearance with sagging cheeks and pronounced wrinkling at the chest and dorsum of the hands. (B) Skull radiograph in a 45-year old patient showing thinning of the mandibula and tooth loss. (C) X-ray of the lumbar spine from a 34-year old female patient from Oman showing low mineral density and compression fractures of two vertebrae. (D) Bone mineral density in GO shown as number of standard deviations after age-correction (Z-score). While average bone mineral density values match the criteria for osteoporosis at the lumbar spine, the femoral neck displays osteopenia.

Wrinkly skin syndrome (WSS; OMIM 278250) is a related connective tissue disorder, sharing considerable features with GO such as skin wrinkling, craniofacial dysmorphism, and skeletal anomalies. Al-Gazali suggested that both syndromes might represent the same condition with variable manifestation (Al-Gazali et al., 2001). However, we have recently demonstrated clinically and genetically that GO and WSS are separate entities (Kornak et al., 2008; Rajab et al., 2008).

1.3.2 Molecular basis of GO

By genetic mapping in several consanguineous Mennonite families from North America and Germany with multiple cases of GO, our group could identify a shared region of homozygosity on chromosome 1q24, and several different mutations in the gene Scyl1bp1 were subsequently identified by U. Kornak (Charité) and H.C. Hennies (University of Cologne).

A homozygous nonsense mutation, p.Glu123X, was present in affected individuals from all four Mennonite families (**Table 2**). Eight other homozygous or compound heterozygous mutations were found in nine further GO cases from Germany, Switzerland, Italy, Oman, Pakistan, and Libya (**Table 2**).

Scyl1bp1 encodes the soluble protein SCY1-like 1 binding protein 1 (SCYL1BP1; NTKL-binding protein 1), for which we coined the name Porostin. SCYL1BP1 is an evolutionarily conserved protein, existing in different organisms from fruit fly to human. It has been described initially as a 368-residue protein in the mouse interacting with N-terminal kinase-like protein (Scyl1) (Di et al., 2003). In human, a 394-residue protein has been predicted for Scyl1bp1 (GenBank accession no. NM_152281). SCYL1BP1 is predicted to have coiled-coil domains and multiple potential phosphorylation sites. However, little functional information was available except the two interaction partners reported recently (Di et al., 2003; Zhang et al., 2005).

To uncover the molecular mechanisms underlying GO, we generated polyclonal and monoclonal antibodies against SCYL1BP1 and started with subcelluar localization study. We also made a comparison between the fibroblasts derived from GO patients and normal controls. To get more functional information, we revealed the expression profile of this gene by RT-PCR, *in situ* hybridization, X-Gal staining and immunostaining. We also looked for its interaction partners by yeast two hybrid screening. For a systemic functional study of SCYL1BP1, we established a gene trap mouse model in which SCYL1BP1 was mutated.

Patient	Origin	Mutation	Exon	Protein	Genotype
1432	Oman	c.-1_1GA>CT	1	Met1?	homozygous
P1	Pakistan	c.-1_1GA>CT	1	Met1?	homozygous
P2	Pakistan	c.-1_1GA>CT	1	Met1?	homozygous
0754	Germany	c.136G>T	2	Glu46X	heterozygous
L1	Libya	c.190C>T	2	Gln64X	homozygous
0755	Italy	c.257delC	2	Pro86ArgfsX70	homozygous
0487	Germany	c.367G>T	2	Glu123X	homozygous
0766	Germany	c.367G>T	2	Glu123X	homozygous
0820	Canada	c.367G>T	2	Glu123X	homozygous
1209	Mexico	c.367G>T	2	Glu123X	homozygous
0978		c.662+5G>C	4	Ser175_Arg221del	heterozygous
0776	Switzerland	c.739C>T	5	Gln247X	homozygous
0978		c.784C>T	5	Arg262X	heterozygous
1124	Germany	c.784C>T	5	Arg262X	homozygous
0754	Germany	c.1050_1053delTCTT	5	Phe350LeufsX26#	heterozygous

Table 2. Mutations in Scyl1bp1 identified in 13 families with GO. The numbering of cDNA positions and deduced protein changes is based on a cDNA that starts 25 codons later than the one predicted in GenBank (accession no. NM_152281). # predicted peptide is five residues longer than SCYL1BP1.