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The Skeletal Manifestations of Deranged Glycosylation

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Abstract

The congenital disorders of glycosylation (CDG) are a rapidly expanding disease group with protean presentations in which the skeletal manifestations are often not appreciated. In this brief review we will discuss the skeletal manifestation of CDG patients, their potential clinical and functional impact on the CDG child and their family, and consider possible underlying mechanisms of these skeletal manifestations.

Molecular and functional insights into clinical disease phenotypes associated with a primary skeletal dysplasia phenotype eg. achondrogenesis type 1A, provides invaluable, mechanistic understanding to pathology in defects associated with primary and secondary glycan deficiencies.

Introduction

Glycosylation is a ubiquitous post translational modification (PTM) of proteins and lipids, with an estimated 1% of the human genome dedicated to the process [1]. In eukaryotes the linkage of glycans to proteins and lipids is carried out by eleven biosynthetic pathways [2]. The covalent attachment of a glycan onto a protein constitutes the glycosylation process, an important PTM involved in folding, stability and interactions of glycoproteins. In eukaryotes the linkage of glycans to proteins and lipids is carried out by eleven biosynthetic pathways [1], six of which are associated with human genetic disorders. The last decade has seen a rapid delineation of genetic glycosylation disorders which have been identified predominantly in the individual *N*-linked and *O*-linked protein glycosylation pathways (16 and 8 diseases respectively), while combined defects in both the *N*- and the *O*-glycosylation pathways, or other pathways eg *O*-Mannosylation have also been described (17 diseases). These genetic defects in PTM have multi-system clinical manifestations with the central nervous system being a consistent site for disease morbidity for the CDG child and their family.

The transfer of initial sugar(s) to glycoproteins or glycolipids occurs in the endoplasmic reticulum (ER) or on the ER membrane. The subsequent addition of the many different sugars that make up a mature glycan is accomplished in the Golgi. Golgi membranes are embedded with glycosidases, glycosyltransferases, and nucleotide sugar transporters from the *cis*-Golgi to the *trans*-Golgi network (TGN). N-glycosylation starts primarily in the ER while O-glycosylation occurs in the Golgi. Recently primary genetic defects within the Golgi network have been described in humans and animal models in whom a primary skeletal dysplasia phenotype has been the presenting feature, and even a lethal manifestation in some cases eg achondrogenesis type 1A (OMIM 200600). Hypochondroplasia (OMIM 146000) is a common skeletal dysplasia due to mutations in the Fibroblast Growth Factor Receptor 3 gene (*FGFR3)*. Novel mutations in *FGFR3* disrupt a putative N-glycosylation site and where the phenotype most probably results from altered receptor glycosylation [3]. Understanding the pathogenesis of defects in these cellular compartments is relevant to understanding the embryogeneic defects observed in children with CDG and potentially in creating targets treatment options.

While the clinical features observed in CDG are protean, those that incur the highest disease burden involve the central nervous system,

gastrointestinal and cardiac disease systems [4].A previous review reported the wide spectrum of skeletal phenotypes reported in CDG [5] which are summarised in table 1. In this review we discuss the skeletal manifestations of CDG, postulate as to their mechanism, and draw correlations from an evolving body of evidence from the primary skeletal dysplasia's.

Skeletal manifestations of the congenital disorders of glycosylation

The most common CDG is secondary to Phosphomannomutase 2 deficiency (PMM2) PMM2-CDG (CDG-Ia) (OMIM212065) [6]. The most commonly reported skeletal manifestations of CDG-Ia are those of osteopenia, thoracic cage abnormalities, short stature, kyphosis and scoliosis. Less common manifestations include a "dysostosis multiplex like phenotype", a "bone-in-bone" appearance, C1-C2 subluxation and platyspondyly, a primary skeletal dysplasia reminiscent of a type II collagenopathy [7-10]. Some of the observed defects may be multifactorial in aetiology eg. joint contractures and camptodactyly may be secondarily observed peripheral features as a consequence of central nervous system dysfunction. Table 1 summarise the more commonly reported skeletal manifestations in CDG patients, and a more comprehensive review of the delineated skeletal features are summarised in [5]

Peters Plus syndrome (OMIM 261540) is an autosomal recessive disorder with the main clinical features involving anterior eye-chamber defects, short stature, developmental delay and cleft lip/palate [11]. It is caused by mutations in beta-1,3-galactosyltransferase-like gene (*B3GALTL)* which encodes a β1,3-glucosyltransferase [11], which as

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Clinical feature
Short stature
joint contractures
rhizomelic limbs
osteopenia
kyphosis
scoliosis
C1-C2 instability
platyspondyly
Epi-metaphyseal dysplasia with cartilage herniation
dysostosis multiplex like phenotype
Brachydactyly
camptodactyly

Table 1: Commonly reported skeletal manifestations of CDG (adapted from (5)).

glucose to an O-Linked fructose and hence is a *CDG, B3GALTL-CDG*.

Multiple osteochondromas (MO) (OMIM 133700/1) is a common autosomal dominant skeletal dysplasia with an estimated incidence of 1 in 50,000. The osteochondromas are caused from an increased chondrocyte proliferation resulting in disruptive bone growth in the bony metaphyses. The ostechondromas contribute to short stature, joint deformities with functional decreases in range of movement, and carry a 1-5% life time risk of developing into a malignant chondrosacroma [12]. This clinical phenotype is created by mutations in Exostosin-1 (*EXT1*) and Exostosin-2 (*EXT2*), which are tumour suppressor genes from a multigene family that all encode glycosyltransferases [12]. This common skeletal dysplasia is a congenital disorder of glycosylation EXT1/EXT2-CDG.

Discussion

The spectrum of skeletal abnormalities observed in CDG suggests numerous potential points of bony developmental disruption including:

1. Collagen post translational modification (PTM)

2. Defects in the Golgi Apparatus and skeletal maturation

3. Notch signalling and Nuclear receptor retinoid-related orphan receptor (ROR) signalling

4. Bone Mineralisation and Remodelling – Small Integrin-Binding Ligand, N-linked Glycosylation (SIBLING) family

5. Glycosaminoglycan (GAG) metabolism

Collagen post translational modification (PTM)

The fibrillar collagens type I and II are found predominantly in the extracellular matrices of bone, tendon and skin, and epiphyseal growth plate and hyaline cartilage, respectively. Mutations in these genes underlie osteogenesis imperfecta and a varied group of conditions associated with abnormal type II collagen (*COL2A1*) (*COL1A1* (OMIM 120150) and *COL1A2* (OMIM 120160)), including achondrogenesis type II (OMIM 200610), spondyloepiphyseal dysplasia congenita (SEDC) (OMIM 183900), Kneist dysplasia (OMIM 156550) and some forms of Stickler syndrome (OMIM 108300).

Post-translational modification results in mature collagen I and II through N-glycosylation of the C-terminal procollagen and subsequent cleavage of the N- and C-terminal propeptide domains. Since molecular mutations result in skeletal dysplasia phenotypes through expression of abnormal secreted protein eg collagen I and II, it is conceivable that defective post-translational N-glycosylation of such matrix protein, or disturbed Golgi architecture or function could modify skeletal phenotypes in patients with CDG, as defects within these domains create primary human skeletal dysplasia phenotype.

Defects in the golgi apparatus and skeletal maturation

The conserved oligomericgolgi apparatus (COG) is constituted of 8 subunits (Cog1 to Cog8) and involved in retrograde vesicular Golgi trafficking. The importance of COG in Golgi glycosyltransferase localization is highlighted by the severe and multisystem clinical phenotypes associated with COG subunit efficiencies [13].

Achondrogenesis type 1A is a lethal skeletal dysplasia recently identified to be secondary to loos of function in Golgin GMAP-210 [14]. The Golgins are a family of golgi microtubule-associated proteins that sereve as tethering factors to assist in vesicle fusion and golgi architecture. Recently a member of the Golgin family of proteins, Golgin-84, has been shown to interact with COG via the COG7 subunit [15] Craniolenticular dysplasia (OMIM 607812) is an autosomal recessive disorder characterised by skeletal dysplasia due to deficiency in secretary mutant protein 23A (SEC23A), a protein involved generating vesicles to move proteins from the endoplasmic reticulum to the Golgi [16]. Mutations affecting trafficking protein particle complex 2 (TRAPPC2) is causative for X-linked spondyloepiphyseal dysplasia tarda (OMIM 313400) [17]. The gene product Sedlin, plays an important role in the transport of proteins between the endoplasmic reticulum and the Golgi apparatus [18].

The phenotypic overlap of the skeletal manifestations of CDG and conditions such as SED and type II collagenopathies, combined with common end points of glycan synthesis and processing i.e. the glogi apparatus, suggest common roles in the pathogenesis and the importance of animal models in understanding the non-skeletal pathogenesis of the CDG-COG family of diseases, especially as skeletal defects have been reported in patients with COG deficiencies [5].

Notch signalling and nuclear receptor retinoid-related orphan receptor (ROR) signalling

Normal function of the NOTCH signalling pathway is essential for correct human embryogenesis, and glycan PTM is an essential component in NOTCH regulations. Primary genetic defects in the NOTCH pathway create heterogeneous clinical phenotypes including congenital cardiac disease (tetralogy of fallot, aortic valve disease), Alagille syndrome (OMIM 118450) and Spondylocostaldystosis (SCD) (OMIM 277300) [19,20].

The SCD predominantly affect the segmental development of vertebrae and ribs. Clinical manifestations of SCD include truncal shortening, scoliosis, and restrictive lung defects. The three genes implicated in SCD are involved in NOTCH signalling, delta-like 3 gene (*DLL3*) (Type 1), mesoderm posterior 2 gene (*MESP2*) (Type 2) and lunatic fringe gene (*LNFG*) (and Type 3) [21]. The *LNFG* gene encodes an O-fucose-specific beta 1,3-N-acetylglucosaminyltransferase and is in fact classified as a CDG, SCDO3-CDG [22]. Further evidence for defective fucosylation interacting with Notch signalling is highlighted by CDG-IIc, also known as leukocyte adhesion defect II [23]. CDG-IIc is characterised by short stature, dysmorphic facial features, intellectual impairment, and a severe immune deficiency with neutropenia [24] and is due to a deficiency in the GDP-fucose transporter. GDP-fucose is essential for the fucosylation of N-linked glycans and O-fucosylation, both processes occurring in the Notch receptors [23].

Isolated brachydactyly is a common skeletal dysplasia that is genetically heterogeneous. Mutations in receptor orphan receptor tyrosine kinase 2 (*ROR2*) are associated with brachydactyly type B (OMIM 113000) [25], and ROR2 and ROR1 are N-glycosylated proteins [26]. Similar clinical phenotypes have been reported in ALG6-CDG (CDG-Ic) (OMIM 603147) [27,28]. As such it is possible that the hypoglycosylation state created by CDG may have led to decreased ROR1/2 function thus predisposing to the observed phenotype.

Bone mineralisation and remodelling –small integrin-binding ligand, N-linked glycosylation (SIBLING) family

Bone is a living dynamic entity, open to repair and remodelling by numerous mechanisms. The process of bone mineralisation is another avenue for perturbed glycosylation, both primary and secondary, to expose clinically significant skeletal phenotypes. Osteopenia is a frequently reported skeletal manifestation of PMM2-CDG, and recurrent fractures can be a clinically significant sequel [29,30]. Secondary aberrations in glycan synthesis and processing augment the clinical phenotype encountered by patients with classic Galactosaemia (OMIM 600999), in whom osteopenia is a common feature [31,32]. A potential cause for this pathology may be defective control of the bone remodelling process [32].

The extracellular matrix of dentin and bone contains numerous non-collagenous proteins, including the SIBLING family. This family includes osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE). OPN is widely expressed in many tissues and undergoes significant glycosylation [33], and may function as an inhibitor of bone mineralization [34], and as a regulator of osteoclast activity [35]. Isoform specific interactions with OPN and BSP are important isoform specific interactions partners for Mucin-type O-glycoptoteins which are abundantly expressed in the bone [36]. DMP1 is an important SIBLING protein and pathogenic mutations in this gene are causative for the autosomal recessive hyphophosphatemic rickets syndrome (OMIM 241520) [37]. We postulated that hypoglycosylation, either in a primary CDG or secondary as in Galactosaemia, may significantly interfere with the glycan PTM of the SIBLING proteins, and thus creating significant abnormalities with bone mineralization.

Glycosaminoglycan (GAG) metabolism

Glycosaminoglycans are synthesised in the Golgi and play integral roles in structural integrity of tissues, ligand binding, cell adhesion and cell singling [38]. Deficiencies in the synthesis and processing of GAG create a subgroup of lysosomal storage disease, most notably mucopolysaccharidoses (MPS).

The MPS clinical phenotype commonly involved skeletal disease in the form of dysostosis multiplex (DM). DM has been reported in PMM2-CDG, but differs from MPS, in that the acetabular roofs are not steep as in MPS-DM and in that there is no central pointing of the proximal metacarpals, with the tubular bones being undermodeled and "bullet" shaped.

Elevated lysosomal enzymes (LEL) have been observed in the serum of PM2-CDG, resembling those seen in I-Cell disease, perhaps reflecting missorting, defective uptake, or reduced stability of the enzymes as a secondary consequence of the defective glycosylation [39]. The identification of a "dysostosis multiplex like" phenotype and elevated LEL may provide mechanistic insights into the skeletal manifestations of CDG.

MO is caused by mutations in *EXT1/EXT2* which are members of the a family that encode glycosyltransferases which are involved in the adhesion and/or polymerization of heparin sulfate (HS) chains at HS proteoglycans (HSPG's) [12]. EXT1 and EXT2 form a hetero oligomeric protein complex in the endoplasmatic reticulum, after it is transferred to the Golgi apparatus it adds N-acetylglucosamine and glucuronic acid residues to the HS-chains [12]. However in the case of MO, the HSPG's accumulate in the cell cytoplasm instead of undergoing normal trafficking processes [12].

Primary abnormalities in PTM of GAGs can create clinical phenotypes that mimic a primary defect in CDG. A recent example is that of the adducted thumb-club foot syndrome (ATCFS) (OMIM 601776), an autosomal-recessive disorder characterized by dysmorphic face, progeroid appearance, congenital contractures of thumbs and feet, joint instability, facial clefting, coagulopathy, and other end organ dysfunction [40]. ATCFS is secondary to mutations in the charbohydrate sulfotransferase 14 (*CHST14*) gene which encodes N-acetylgalactosamine 4-O-sulfotransferase 1 (D4ST1) [40]. D4ST1 catalyzes 4-O sulfation of N-acetylgalactosamine, an essential step in dermatan sulfate biosynthesis [40].

Conclusion

The skeletal manifestations of CDG can be lost amongst the significant disease burden impacted by other disease systems such as the neurological manifestations. PMM2-CDG carries a 20-25% risk of lethality during infancy. Skeleton involvement in CDG has significant potential to impact negatively on the CDG child and their family, for example, reduced capacity to perform activities of daily living associated with rhizomeliic shortening of upper limbs, recurrent fractures from osteopenia, decreased mobility associated with joint contractures, and potential mortality from cervical cord compression associated with cervical spine instability.

The process of glycan PTM is also of paramount importance to proteins implicated in the development of cartilage and bone and also in skeletal patterning pathways. Aberrant glycosylation impacting on bone health and integrity, both in primary CDG conditions and secondary hypoglycosylation states provides opportunities for greater understanding of the molecular mechanisms associated with skeletal dysplasia disease, and such understanding provides possibilities for novel treatments.

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