Dystroglycan in the Molecular Diagnosis of the Podocytopathies

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The dystroglycan complex, among other molecular complexes, attaches podocytes to the glomerular basement membrane (GBM). Regele *et al.* (1) previously localized α-dystroglycan and β-dystroglycan to the sole of the podocyte foot process. Furthermore, they found that in minimal-change nephropathy (MCN), protein expression was reduced by 75% for α-dystroglycan and by 50% for β-dystroglycan, whereas expression of both dystroglcyans was normal or slightly increased in FSGS. In this issue of *CJASN*, Giannico *et al.* (2) extend these findings to a somewhat different clinical setting, namely, seven patients with findings typical of MCN, including extensive foot process effacement and nephrotic proteinuria (with the exception that one patient had subnephrotic proteinuria). Because of limitations in the number of glomeruli present on these biopsies, the diagnosis of unsampled FSGS could not be safely excluded, and this group is therefore described as "undefined." Giannico *et al.* report decreased expression of α-dystroglycan by podocytes, whereas β-dystroglycan expression was numerically but not significantly decreased.

Dystroglycan assembles with other transmembrane and intercellular proteins to form the dystroglycan glycoprotein complex (DGP; Figure 1). DGPs are expressed in most, if not all, tissues (recent reviews are available [3–5]) and are expressed by kidney cells, including podocytes and tubular epithelial cells (6). The C-terminal domain of β-dystroglycan associates with dystrophin (in skeletal muscle and elsewhere) or with utrophin (in podocytes [7] and elsewhere). These molecules in turn interact with filamentous actin. Other members of the DGP include sarcoglycans, which are transmembrane proteins that interact with β-dystroglycan. Podocytes seem to express only δ-sarcoglycan (8).

Two macromolecular complexes that tether podocytes to the glomerular basement membrane (GBM) have been identified: α3β1 integrins and dystroglycans (9). Dystroglycans were named on the basis of their role in muscular dystrophy but are widely distributed outside skeletal muscle. Dystroglycan is the product of a two-exon gene, *DAG1*, located on chromosome 3p21; posttranslational processing yields α-dystroglycan and β-dystroglycan, which maintain a noncovalent association. β-Dystroglycan is an integral membrane protein. α-Dystroglycan is located in the extracellular space, extending into the lamina rara externa of the GBM. It has binding specificity for GBM components, including laminin and the agrin, the major heparan sulfate proteoglycan in GBM. Interestingly, exposure to reactive oxygen species deglycosylates α-dystroglycan *in vitro* and thereby abrogates the ability of α-dystroglycan to bind laminin and agrin, suggesting that carbohydrate residues may be required for these interactions (10). If this mechanism operates *in vivo*, then it would suggest a pathway by which reactive oxygen species might lead to podocyte loss. There remains controversy as to whether α-dystroglycan is confined to the soles of the podocyte foot processes, as Regele *et al.* (1) suggested, or α-dystroglycan expression extends to the apical domain, as Berden and colleagues (11) found; the observed differences may be due to differences in tissue preparation or antibody epitopes.

What are the functions of dystroglycan and its partners in podocyte biology? First, together with α3β1 integrin, the DGP likely anchors the podocyte to the GBM, counteracting the expansible force of hydrostatic pressure within the glomerular capillary that could detach the podocyte. Second, podocytes may deploy dystroglycan to coordinate the spatial arrangement of particular GBM proteins, as suggested by Kojima and Kerjaschki (8). Third, α-dystroglycan contains sialic acid (as does podocalyxin) and, as noted already, α-dystroglycan seems to be distributed in the apical podocyte domain. Thus, α-dystroglycan is positioned to help maintain podocyte foot process architecture and in particular maintain the patency of the filtration slit. The removal of sialic acid with neuraminidase (sialidase) (12) and the failure to synthesize sialic acid in mice with a null mutation in the gene encoding the enzyme responsible for sialic acid (13) each is associated with foot process effacement and proteinuria, suggesting that sialic residues on α-dystroglycan, podocalyxin, or other molecules, are critical to maintaining podocyte cytoarchitecture and the glomerular filtration barrier.

Is there now a role for α-dystroglycan immunostaining of kidney biopsies in clinical practice? A presentation of the semiquantitative scoring of α-dystroglycan staining carried out by Giannico *et al.* (2) is shown in Figure 2. Using a threshold score of 0.3 arbitrary units (which will of course vary from laboratory to laboratory and observer to observer), the sensitivity is 0.86, the specificity is 0.88, the positive predictive value is 0.67, and the negative predictive value is 0.95. Thus, in this series, reduced α-dystroglycan staining is modestly predictive of likely

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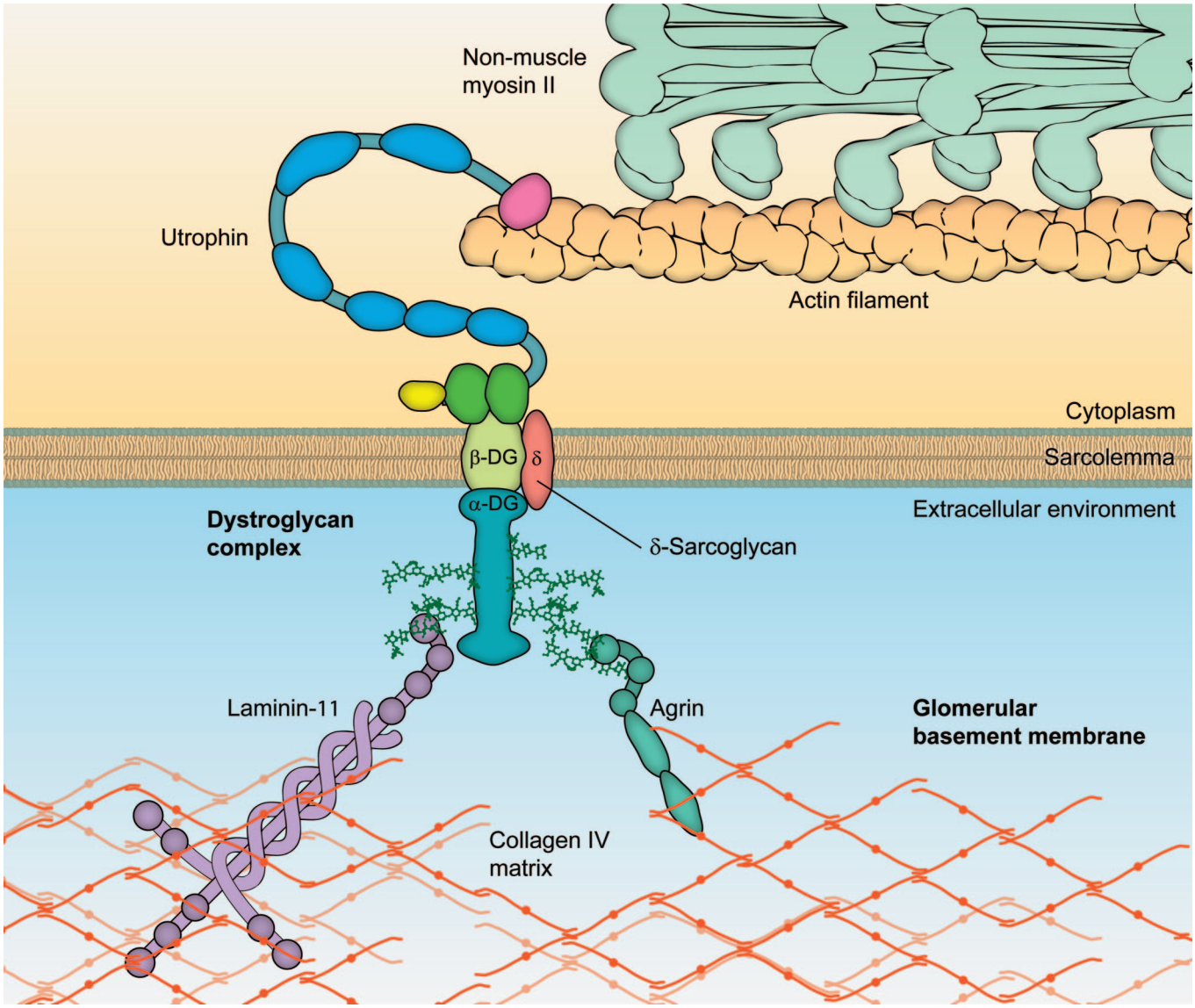

Figure 1. The dystroglycan complex of the podocyte. β-Dystroglycan is an integral plasma membrane protein; it is noncovalently associated with α-dystroglycan (shown with its glycan side chains), which extends into the lamina rara externa of the GBM. There, α-dystroglycan interacts *via* glycan chains with laminin-11 (composed of α5, β2, and γ1 chains) and the heparan sulfate proteoglycan agrin. These interactions may facilitate the role of the podocyte in maintaining the proper molecular structure of the GBM, which also contains a lattice-work of collagen IV molecules (composed in the mature kidney of α3, α4, and α5 chains). The cytoplasmic tail of β-dystroglycan binds utrophin, which in turn binds an actin filament. Utrophin structure is speculative, assembled from the determined or predicted structure of various motifs, including an actin-binding domain (red), spectrin repeats (blue), EF hands (green; proposed interaction site with α-dystroglycan), and a ZZ domain (zinc finger domain, yellow). The actinomyosin motor complex is composed of an actin filament and a multimeric myosin assembly, composed of nonmuscle myosin IIA or IIB molecules. This actinomyosin complex maintains cell tension and cytoarchitecture and facilitates contraction (perhaps in response to increased hydrostatic glomerular capillary pressure) and migration (if podocyte foot processes are indeed mobile under physiologic or pathologic conditions). The molecular images are based on solved, observed, or predicted structures, except for sarcoglycan, for which information is lacking; molecules are not drawn to scale, and the precise sites of intermolecular interactions are not well defined. Actin filaments diameters range from thin (as shown) to thick (not shown; *e.g.*, as would be present in stress fibers).

MCN (as defined by the subsequent clinical course, with complete remission induced by glucocorticoids therapy in four of six cases), whereas staining above this threshold is strongly predictive of FSGS (in cases for which the diagnosis of FSGS in this series could be made by other criteria). Of course, it is possible that those in the "undefined" group with higher α-dystroglycan scores in fact do have undiagnosed FSGS, which would account for their glucocorticoid resistance. If this were the case, then this would suggest a higher predictive power for α-dystroglycan staining to identify MCN cases.

Immunostaining is most likely to assist the pathologist in making a diagnosis when the change is dramatic, either much increased or nearly absent, because such changes are more likely to be robust when used over time and in disparate laboratories.

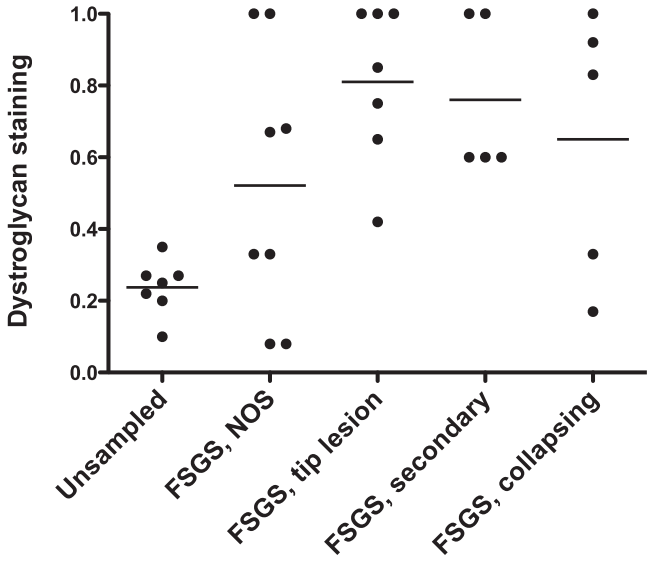

Figure 2. Immunostaining scores for α-dystroglycan are shown for 32 biopsies with diagnoses as shown, together with mean values (bar). Data are replotted from the original scoring data presented in Figure 2II (in reference 2), generously provided by Dr. Agnes Fogo.

From these data, it remains unclear whether staining for α-dystroglycan has a role in the clinical evaluation of renal biopsies in which the diagnosis of MCN *versus* FSGS remains in doubt; if used, then it may be more reliable in excluding MCN than in diagnosing MCN. It is also possible that dystroglycan gene expression will add useful information, perhaps assessed as part of a molecular profiling approach, as has been described by Kretzler and colleagues (14). A fairly common clinical dilemma, similar to that studied by Giannico *et al.* (2), is a renal biopsy obtained from a patient with nephrotic range proteinuria that manifests non-diagnostic abnormalities such as focal global glomerulosclerosis and/or focal tubular atrophy, interstitial inflammation, or interstitial fibrosis. Does this individual have MCN, or does this the patient have FSGS, unsampled because of the limitations of the biopsy sample? Further studies, suitably powered, of α-dystroglycan expression in cases such as these, as well as those studied by Giannico *et al.*, would be helpful in further defining the clinical utility of α-dystroglycan staining.

In summary, we still have much to learn about dystroglycan biology and how the vicissitudes of these and related molecules are related to podocyte injury in various clinical settings. The work by Giannico *et al.* (2) suggests that such studies may expand the molecular tool kit for the renal pathologist and allow more clinical information to be extracted from a few cubic millimeters of tissue.

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Disclosures

None.

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See related article, "Dystroglycan in the Diagnosis of FSGS," on pages 1747–1753.