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Podocyte proteins in Galloway-Mowat syndrome

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Abstract Galloway-Mowat syndrome is an autosomal recessive disorder characterized by early onset nephrotic syndrome and central nervous system anomalies. Mutations in podocyte proteins, such as nephrin, α-actinin 4, and podocin, are associated with proteinuria and nephrotic syndrome. The genetic defect in Galloway-Mowat syndrome is as yet unknown. We postulated that in Galloway-Mowat syndrome the mutation would be in a protein that is expressed both in podocytes and neurons, such as synaptopodin, GLEPP1, or nephrin. We therefore analyzed kidney tissue from normal children (*n*=3), children with congenital nephrotic syndrome of the Finnish type (CNF, *n*=3), minimal change disease (MCD, *n*=3), focal segmental glomerulosclerosis (FSGS, *n*=3), and Galloway-Mowat syndrome (*n*=4) by immunohistochemistry for expression of synaptopodin, GLEPP1, intracellular domain of nephrin (nephrin-I), and extracellular domain of nephrin (nephrin-E). Synaptopodin, GLEPP1, and nephrin were strongly expressed in normal kidney tissue. Nephrin was absent, and synaptopodin and GLEPP1 expression were decreased in CNF. The expression of all three proteins was reduced in MCD and FSGS; the decrease in expression being more marked in FSGS. Synaptopodin, GLEPP1, and nephrin expression was present, although reduced in Galloway-Mowat syndrome. We conclude that the reduced expression of synaptopodin, GLEPP1, and nephrin in Galloway-Mowat syndrome is a secondary phenomenon related to the proteinuria, and hence synaptopodin, GLEPP1, and nephrin are probably not the proteins mutated in Galloway-Mowat syndrome.

Keywords Galloway-Mowat syndrome · Synaptopodin · GLEPP1 · Nephrin · Immunohistochemistry

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Introduction

Galloway-Mowat syndrome (GMS) was first described by Galloway and Mowat in 1968 in two siblings who presented with early onset nephrotic syndrome, microcephaly, and hiatus hernia [1]. The syndrome is characterized by nephrotic syndrome associated with central nervous system anomalies. In GMS the onset of nephrotic syndrome occurs early in life (0–34 months, median 3 months), with no response to treatment and progressive deterioration of renal function [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The kidney biopsy findings described in GMS range from minimal change disease (MCD), mesangioproliferative glomerulonephritis, focal segmental glomerulosclerosis (FSGS) to diffuse mesangial sclerosis (DMS) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The neurological manifestations include microcephaly, psychomotor retardation, and structural central nervous system anomalies that on gross anatomy include abnormal sulci and gyri, pachygyria, cortical atrophy, and cerebellar dysgenesis/hypoplasia, and on histopathology migrational anomalies of the neurons (neuronal heterotopias, abnormal or failed lamination of cortex) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The syndrome is an autosomal recessive disorder, in which the genetic defect is not known.

In search of the pathophysiology of the syndrome, Cohen and Turner [11] using antibodies to glomerular basement membrane, tubular basement membrane, type IV collagen, and laminin, and staining kidney tissue with Congo red, thioflavin T, and phosphotungstic acid-hematoxylin could not find any abnormality in the glomerular basement membrane. Podocyte cells are an integral part of the filtration mechanism of the glomerulus, resulting in formation of urine and retention of proteins. In GMS, effacement of foot processes of the podocytes is seen on electron microscopy. Mutations in podocyte proteins are responsible for several entities of nephrotic syndrome, such as nephrin in congenital nephrotic syndrome of the Finnish type (CNF) [17], podocin in autosomal recessive steroid-resistant nephrotic syndrome [18], and α-actinin 4 in familial FSGS [19]. As GMS is characterized by early onset nephrotic syndrome and brain anomalies, we postulated that the mutation in this syndrome could involve a protein that is expressed both in the podocyte cell of the kidney and in the neuronal cell of the brain. Synaptopodin, glomerular epithelial protein 1 (GLEPP1), and nephrin are three such proteins that are expressed both in the kidney and brain [20, 21, 22, 23, 24]. We studied the expression of these three proteins by immunohistochemistry in kidney tissue of four children with GMS.

Materials and methods

Case reports

Case 1

Patient 1 was a Caucasian girl, born to non-consanguineous parents, who presented at 1 month of age with nephrotic syndrome. The kidney wedge biopsy showed more than 50 glomeruli. The glomeruli were small and immature in appearance with no evidence of sclerosis or mesangial proliferation. The glomeruli on light microscopy showed minimal changes. There was microcystic dilatation of the tubules with mild patchy interstitial fibrosis. Immunofluorescence showed deposits of IgM. Electron microscopy showed extensive effacement of foot processes and a normal glomerular basement membrane. Histologically it was difficult to differentiate the findings from CNF and the diagnosis was initially reported as CNF by the pathologist. At presentation, she was microcephalic and hypotonic. Computed tomography (CT) of the brain showed cerebellar hypoplasia/dysgenesis. She was managed supportively for her nephrotic syndrome with correction of hypoalbuminemia, and thyroid, vitamin, and mineral supplementation. On follow-up she showed marked psychomotor retardation and seizures. She died at the age of 10 months. The family refused autopsy.

Case 2

Patient 2 was a Palestinian Arab boy, born to consanguineous parents, who presented at 8 months of age with nephrotic syndrome. The kidney biopsy showed 14 glomeruli. The glomeruli were immature in appearance with no evidence of inflammation, hypercellularity, or sclerosis. On light microscopy they showed minimal changes. There was microcystic dilatation of several tubules with mild mononuclear cell infiltrate in the interstitium. Immunofluorescence showed deposits of IgM and C3. Electron microscopy was not performed. Histologically again it was difficult to differentiate the findings from CNF and it was initially reported as such by the pathologist. At presentation, he had microcephaly, spastic quadriparesis, severe psychomotor retardation, and seizures. CT of the brain showed cortical atrophy. He was treated with steroids with no response and died at the age of 41 months. The family refused autopsy. He had a brother who died at 18 months of age with steroid-resistant nephrotic syndrome. The neurological details of the sibling were not available.

Case 3

Patient 3 was a Palestinian Arab girl, born to consanguineous parents, who presented at 18 month of age with nephrotic syndrome. The kidney biopsy showed FSGS involving approximately 30% of the glomeruli. The tubulo-interstitial compartment showed moderate fibrosis. Immunofluorescence did not reveal a glomerulus. Electron microscopy was not performed. At presentation, she was microcephalic, spastic with psychomotor retardation, and suffered from seizures. Magnetic resonance imaging of the brain showed diffuse cortical atrophy and cerebellar hypoplasia. She failed to respond to treatment with steroids and died at the age of 48 months. The family refused autopsy. She had two other siblings with microcephaly, severe psychomotor retardation, abnormal neuroimaging studies, and early onset nephrotic syndrome who died at 30 and 32 months of age.

Case 4

Patient 4 is an African-American girl, born to a non-consanguineous marriage, who presented at 3 months of age with nephrotic syndrome. The kidney biopsy showed 31 glomeruli with DMS. The glomeruli showed significant chronic glomerular alterations with variable degrees of mesangial expansion and obliteration of the peripheral capillary loop lumen. The podocyte cells were hypertrophic and prominent. There was marked interstitial fibrosis and irregular dilatation of the tubules. Immunofluorescence showed deposits of IgM and C3. Electron microscopy showed extensive effacement of foot processes. She rapidly progressed to end-stage renal failure and was placed on chronic peritoneal dialysis at 5 months of age. At presentation, she had microcephaly, psychomotor retardation, and cortical blindness. CT of the brain showed diffuse cortical atrophy. She is currently 22 months old and on chronic peritoneal dialysis.

Immunohistochemistry

Control tissue (normal) obtained from nephrectomy specimens of children with Wilms tumor (n=3), kidney tissue from children with MCD (n=3), FSGS (n=3), CNF (n=3), and GMS (n=4) were studied by immunohistochemistry. Serial 3-μm sections were obtained from the patients listed above, air dried, and heat fixed on slides. The sections were deparaffinized with xylene and iodine, and rehydrated in a graded series of alcohol. The sections were treated with Target Retrieval Solution (Dako no. S1700, Dako, Carpinteria, Calif., USA) in a steamer at 90–95°C for 20 min and then cooled for 15 min. The sections were stained on the automated Dako Autostainer 3400 using Dako's LSAB+ immunoperoxidase kit with streptavidin conjugated to horseradish peroxidase. Antibody to synaptopodin (ARP, Mass., USA) is a mouse monoclonal antibody reactive with human synaptopodin. Antibody to GLEPP1 (gift from R.C. Wiggins) is a mouse monoclonal antibody raised against human extracellular domain of GLEPP1 [25]. Nephrin-I is a polyclonal rabbit antibody against the intracellular domain (amino acids 1,054–1,241) of nephrin and nephrin-E is a mouse monoclonal antibody against the extracellular domain of nephrin [26]. Primary reactions for immunohistochemistry with synaptopodin and GLEPP1 antibodies were performed at 37°C for 30 min, while staining for the intracellular domain of nephrin

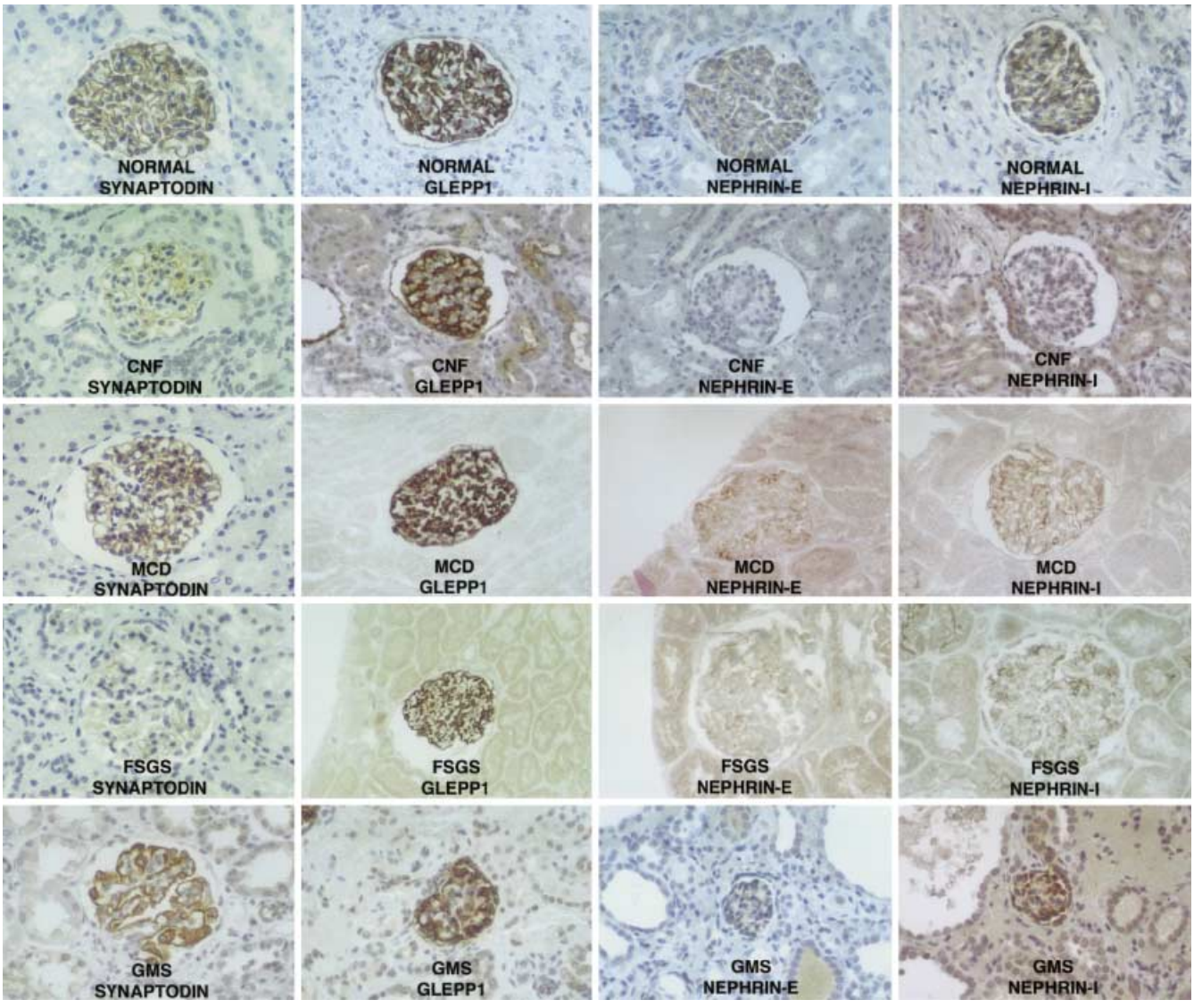

Fig. 1 Expression of synaptopodin, GLEPP1, and nephrin in kidneys from children with Wilms tumor (normal), congenital nephrotic syndrome of the Finnish type (CNF), minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and Galloway-Mowat syndrome (GMS). Diaminobenzidine was used as the chromogen for the immunohistochemical reaction, which gives a brown color to the podocyte protein under study. The *top row* shows full expression of synaptopodin, GLEPP1, and nephrin in normal kidney tissue. The nephrin expression is absent in kidney tissue from CNF (*row 2*), while that of synaptopodin and GLEPP1 is decreased. The expression of all three podocyte proteins is reduced in MCD (*row 3*) and more profoundly reduced in FSGS (*row 4*). Synaptopodin, GLEPP1, and nephrin are all present in kidney tissue from GMS (*bottom row*), albeit decreased in expression

(nephrin-I) and the extracellular domain of nephrin (nephrin-E) was performed overnight at 4°C. Diaminobenzidine was used as the chromogen. The staining on immunohistochemistry was graded from 0 to 3+: 0 being absent, ± trace, + and 2+ being decreased, and 3+ normal. The grading was performed by the pathologist (R.E.G.) who was not blinded to the diagnoses due to the nature of light microscopy findings. The grading was then re-evaluated and confirmed by two other participants (T.S. and U.S.A.).

Results

Synaptopodin, GEPP1, and nephrin were fully expressed in normal kidney tissue (Table 1, Fig. 1). In contrast, in all 3 CNF cases, nephrin expression was completely absent, while the expression of synaptopodin and GLEPP1 was reduced in the kidney tissue. In tissue specimens from children with MCD and FSGS, the expression was reduced for all of the podocyte proteins; the decrease being more marked in FSGS. In all 4 tissue samples from children with GMS, the expression of synaptopodin, GLEPP1, and nephrin, although decreased, was still present. The decrease in protein expression was more profound in tissues from the 2 GMS patients with FSGS and DMS compared with the other 2 with minimal changes on light microscopy.

Table 1 Expression of synaptopodin, GLEPP1, and nephrin in kidneys from children with Wilms tumor (normal), congenital nephrotic syndrome of the Finnish type (CNF), minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and Galloway-Mowat syndrome (GMS) on immunohistochemistry (*DMS* diffuse mesangial sclerosis)

	Histology	Synaptopodin	GLEPP1	Nephrin-E	Nephrin-I
Normal (*n*=3)	Normal	3+	3+	3+	3+
CNF (*n*=3)	CNF	±	+	0	0
MCD (*n*=3)	MCD	2+	2+	+	+
FSGS (*n*=3)	FSGS	±	+	±	±
GMS (patient 1)	Minimal changes	2+	2+	+	+
GMS (patient 2)	Minimal changes	2+	2+	+	+
GMS (patient 3)	FSGS	±	2+	±	±
GMS (patient 4)	DMS	±	2+	+	±

Discussion

GMS is a lethal autosomal recessive disorder that has been reported in different racial groups from across the world [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12 , 13, 14, 15, 16]. Using criteria suggested by Meyers et al. [27] we identified 27 children with GMS in the English medical literature (Table 2). The onset of nephrotic syndrome in GMS occurs early in life, it is unresponsive to treatment, and shows progressive deterioration of renal function. The kidney biopsy findings can be those of MCD, mesangioproliferative glomerulonephritis, FSGS, or DMS (Table 2). The histological findings can change from MCD or mesangioproliferative glomerulonephritis to DMS on subsequent biopsy [11, 16]. At times, the biopsy can show microcystic tubular dilatations that can make the differentiation from CNF difficult. Clinically CNF lacks the severe neurological manifestations in GMS (Table 2). There have been additional findings described in GMS, such as hiatus hernia, disorders in the anterior cleavage of the eye, visual and hearing impairment, thyroid dysplasia, adrenal hypoplasia, ovarian agenesis, and several dysmorphic features (Table 2).

Synaptopodin is a proline-rich actin-associated protein that is expressed exclusively in podocyte cells and dendritic spines of telencephalic synapses, and plays a role in modulating actin-based shape and motility of podocyte foot processes and dendritic spines [20]. GLEPP1 is a receptor-like membrane protein tyrosine phosphatase, which is expressed in podocyte cells and postmitotic maturing neurons of the olfactory bulb, developing neocortex, hippocampus, and thalamus [21, 22]. The receptor-like structure of GLEPP1 suggests that it functions by signaling either from outside into the cell or from inside out of the cell, or both [25]. In a gene knock-out mouse model for GLEPP1, the gross and light microscopy of the kidney and glomerular structure was normal, but scanning and transmission electron microscopy showed podocytes to be amoeboid in shape with blunted and widened foot processes rather than the octopoid structure seen in the wild-type mouse [28]. Nephrin is a component of the glomerular podocyte slit diaphragm and is essential for the normal renal filtration process [29, 30]. Mutations in the nephrin gene (*NPHS1*) have been reported in children with CNF [17, 31]. Until recently nephrin was believed to be expressed exclusively in the podocyte cell, but recent reports describe expression of nephrin, in an intense and highly restricted pattern, in cells of the hindbrain and spinal cord [24], and in discrete areas of the medulla in transgenic mice [23].

The early onset of nephrotic syndrome in GMS, with lack of histopathological evidence of an immune-mediated etiology, implies an abnormality in the podocyte cell or the glomerular basement membrane. However, Cohen and Turner [11] failed to find any abnormality in the glomerular basement membrane of patients with GMS. The expression of synaptopodin, GLEPP1, and nephrin in both the podocyte cells and neurons stimulated us to look for expression of these proteins in kidney tissue of children with GMS to see whether a defect in one or more of these proteins could be the etiology of the syndrome.

On immunohistochemistry, staining for synaptopodin, GLEPP1, and nephrin was intense and linear along the glomerular basement membrane in normal kidney tissue (Table 1, Fig. 1). In CNF, the expression of nephrin was totally absent, while that for synaptopodin and GLEPP1 was reduced. Patrakka et al. [32] reported complete absence of nephrin in CNF. Srivastava et al. [33] showed synaptopodin to be fully expressed in normal children while reduced in children with CNF, and Sharif et al. [34] demonstrated the presence of GLEPP1 in CNF. Thus our findings in normal children and in those with CNF were consistent with previous reports. In children with MCD and FSGS we found synaptopodin, GLEPP1, and nephrin expression to be reduced. Consistent with our previous report, the decrease in expression was more marked in FSGS than MCD, reflecting the magnitude of damage suffered by the podocyte cell [33]. GLEPP1 is reported to be reduced in FSGS, redistributed in the podocyte cell away from the glomerular basement membrane in MCD, and decreased in both human and animal models of crescentic glomerulonephritis, suggesting GLEPP1 to be a sensitive marker of podocyte injury [34, 35]. Nephrin is decreased but not totally absent in proteinuric states in both human [36] and animal models of proteinuria [37, 38, 39]. Patrakka et al [40] did not find major alteration in nephrin expression in children with MCD and FSGS; however they reported some attenuation which was not

Table 2 Clinical profile and biopsy findings in children with GMS

Reference no.		Year	Sex	Race	Onset of nephrotic syndrome and kidney biopsy finding	Age at death	Central nervous system	Other findings
1	1	1968	M	Caucasian – UK	7 days – MCD	9 months	Microcephaly, psychomotor retardation, hypotonia, gyral anomalies	Hiatus hernia, receding forehead, flat occiput, platybasia
	2	1968	F	Caucasian – UK	24 months – MCD with tubular dilatation	30 months	Microcephaly, psychomotor retardation	Hiatus hernia, receding forehead, flat occiput, calcification of intervertebral disc
2	3	1976	F	Caucasian – USA	1 day – FSGS with microcystic dysplasia	14 days	Microcephaly	Hiatus hernia, large and floppy ears, corneal opacity, hypoplastic iris
	4	1976	F	Caucasian – USA	5 days – Microcystic dysplasia	36 months	Microcephaly, psychomotor retardation, seizure disorder	Hiatus hernia, large and floppy ears, micrognathia
3	5	1983	F	France	1 day – DMS	3 months	Microcephaly, psychomotor retardation, seizure disorder, gyral anomalies	Neuronal heterotopias, disordered migration of neurons
4	6	1986	M	Sweden	5 months – Mesangial proliferation	34 months	Psychomotor retardation, spastic tetraplegia, gyral anomalies	Aqueductal stenosis with hydrocephalus, neuronal heterotopias
	7	1986	M	Sweden	Abortus	Abortus	Gyral anomalies	Central canal malformation, neuronal heterotopias
5	8	1987	M	Netherlands	12 months – FSGS	36 months	Microcephaly, psychomotor retardation, hypotonia, seizure disorder, gyral anomalies, cortical atrophy	Club feet
	9	1987	M	Netherlands	12 months – FSGS	18 months (alive)	Microcephaly, psychomotor retardation, seizure disorder, gyral anomalies	
6	10	1989	F	USA	9 months – FSGS	12 months	Microcephaly, psychomotor retardation, hypotonia, seizure disorder, lissencephaly, cerebellar hypoplasia	Atrophic thymus, neuronal heterotopias, fusion of leptomeninges
7	11	1991	M	Finland	6 days – DMS	2 months	Microcephaly, hypotonia, hypoplasia of cerebellum	Eventration of diaphragm, wide-set eyes, small nose, visual and hearing impairment
8	12	1993	M	Caucasian – USA	14 months – Mesangial proliferation	18 months	Microcephaly, psychomotor retardation, hypertonia, seizure disorder, gyral anomalies	Micrognathia, hypoteleroism, large ears, optic atrophy
	13	1993	F	Caucasian – USA	9 months – Not done	36 months	Microcephaly, psychomotor retardation, hypertonia, seizure disorder, gyral anomalies	Small midface, large ears, optic atrophy, visual and hearing impairment
	14	1993	F	Caucasian – USA	3 months – Mesangial proliferation	8 months	Microcephaly, psychomotor retardation, hypotonia, gyral anomalies	Sloping forehead, notched ear, anteposed anus
9	15	1994	F	Turkey	20 months – FSGS	22 months	Microcephaly, psychomotor retardation, gyral anomalies	Frontal bossing, large and low-set ears, flat occiput
10	16	1994	M	North Africa	1 month – DMS	24 months	Microcephaly, psychomotor retardation, hypertonia, cortical atrophy, cerebellar hypoplasia	Large and low-set ears, Small nose, high-arched palate, micrognathia
	17	1994	F	North Africa	12 months – DMS	15 months	Microcephaly, psychomotor retardation, hypertonia	Large and low-set ears
	18	1994	M	North Africa	Not known	36 months	Microcephaly, psychomotor retardation, hypertonia	–

Table 2 (continued)

Reference no.		Year	Sex	Race	Onset of nephrotic syndrome and kidney biopsy finding	Age at death	Central nervous system	Other findings
11	19	1994	M	Hispanic – USA	1 day – Mesangial proliferation (21 days and autopsy)	3 months	Microcephaly, hypotonia, seizure disorder, sclerosis of white matter	Anterior cleavage syndrome of eye, miosis
	20	1994	F	Hispanic – USA	14 days – Mesangial proliferation (66 days) DMS (autopsy)	24 months (dialysis-4 months)	Microcephaly, psychomotor retardation, hypotonia, cerebral atrophy	Anterior cleavage syndrome of eye, miosis, saddle nose, anteverted nose, high-arched palate, hypertelorism
	21	1994	F	Hispanic – USA	14 days – No biopsy	3 months	Microcephaly, hypotonia	Saddle nose, high-arched palate, hypertelorism
12	22	1995	F	Japan	34 months – FSGS	Alive 35 months	Microcephaly, psychomotor retardation, hypotonia, seizure disorder, cerebral atrophy	Micrognathia, almond-shaped eyes, large ears, low nasal bridge, pinched nose, thin lips, abnormal skull, coarse hair, narrow forehead
13	23	1995	F	Chinese	3 months – FSGS	4 months	Microcephaly, Dandy-Walker anomaly, abnormal EEG, brain and spinal cord atrophy	Micrognathia, epicanthal fold, contracture of digits, large ears, broad nasal bridge, sloping forehead
	24	1995	F	Chinese	6 months – Not done	Alive	Microcephaly, brain atrophy	Micrognathia, microophthalmos, contracture of joints, large ears, sloping forehead, hypertelorism
14	25	1997	M	Caucasian – Canada	14 days – DMS	1 month	Microcephaly, hypotonia, abnormal EEG, gyral anomalies	Hiatus hernia, micrognathia, low-set ears, high-arched palate, fusion of leptomeninges, heterotopic neurons, abnormal/failed lamination of cortex, frontal pachygyria, thyroid dysplasia, adrenal hypoplasia
15	26	1998	F	Lebanese	1 day – DMS	15 months (dialysis)	Microcephaly, psychomotor retardation, hypotonia, Dandy-Walker anomaly, gyral anomalies	Broad philtrum, retrognathia, preauricular fistula, miosis, corneal clouding, post-axial hexadactly, nail hypoplasia, rocker bottom feet, atrial septal defect
16	27	2000	F	Unknown – USA	9 months – MCD Autopsy – DMS	42 months	Microcephaly, psychomotor retardation, hypertonia, abnormal EEG, cerebellar atrophy	Micrognathia, large ears, cerebral dysplasia, deep-set eyes, broad nasal bridge, hypoplasia nails, optic nerve hypoplasia

quantitated. Thus podocyte injury in idiopathic nephrotic syndrome is associated with a decrease in synaptopodin, GLEPP1, and nephrin expression. In an event of podocyte dysregulation from either a genetic mutation or an immunological injury, podocyte dysfunction is clinically manifested as proteinuria and at the molecular level proteins such as synaptopodin, GLEPP1, and nephrin are disorganized in the podocyte cell.

In our patients with GMS, synaptopodin, GLEPP1, and nephrin were reduced in expression but none was completely absent (Table 1, Fig. 1). This decreased expression of synaptopodin, GLEPP1, and nephrin in GMS is most likely related to the proteinuria associated with this syndrome, as the findings were similar to those seen in children with MCD and FSGS (Table 1). It is unlikely that the decrease in the expression of the three proteins resulted from a mutation in the genes of synaptopodin, GLEPP1, or nephrin. In severe autosomal recessive disorders, truncating mutations generally lead to complete absence of the protein, as shown in the case of nephrin in CNF. However, it is conceivable that some milder mutations may lead to a low level of protein expression; such genetic heterogeneity has been documented in the literature [41]. In all our patients with GMS, all three podocyte proteins studied were expressed. The probability of a mutation in these three genes occurring in four different families from different racial backgrounds, in which protein expression in the kidney is present but decreased, is extremely low. Rather, the reduced expression of the three proteins seems to be a secondary phenomenon, as observed in children with MCD and FSGS. Indeed there was a good correlation between the severity of the glomerular histology and expression of the three proteins in both children with idiopathic nephrotic syndrome and those with GMS. Positional cloning by linkage analysis would be an ideal approach to find the genetic defect in this autosomal recessive disorder, but the lack of large families appropriate for such studies has limited such an approach. We had to approach the problem with study of suspect candidate genes. The expression of synaptopodin, GLEPP1, and nephrin in the kidney tissue by immunohistochemstry in GMS makes a mutation in the genes encoding these proteins unlikely. Although our study did not indicate an absent protein, its findings probably exclude synaptopodin, GLEPP1, and nephrin as candidate genes for this syndrome.

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