Retinal On-Pathway Deficit in Congenital Disorder of Glycosylation Due to Phosphomannomutase Deficiency

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Objective: To describe novel electroretinographic (ERG) findings associated with congenital disorder of glycosylation due to phosphomannomutase deficiency (PMM2-CDG) (previously known as congenital disorder of glycosylation type 1a).

Methods: Two male siblings with genetically confirmed PMM2-CDG underwent full-field ERG to a range of scotopic and photopic flash luminances that extended the International Society for Clinical Electrophysiology of Vision standard protocol and included scotopic 15-Hz flicker and photopic prolonged on-off stimulation.

Results: Photopic prolonged ERGs were profoundly electronegative with absent b-waves but preserved oscillatory potentials. Prolonged off-responses and off-oscillatory potentials were preserved. Transient full-field photopic ERGs revealed a broad a-wave and narrow b-wave, and the photopic 30-Hz flicker ERG had a sawtooth waveform. The scotopic b-waves of both cases were attenuated to the fifth percentile, whereas scotopic a-wave amplitudes were at the 50th to 75th percentile, giving a reduced a/b ratio. The scotopic a-wave waveform was well defined to bright flash luminance. The number of scotopic oscillatory potentials was preserved, although amplitudes were smaller than average. Scotopic 15-Hz flicker ERGs were evident to a range of flash luminances and showed an expected phase cancellation between −1.5 and −1.0 log scotopic td (troland) s, but phase increased only for the fast rod pathway.

Conclusions: We find, for the first time to our knowledge, an association of PMM2-CDG with a selective on-pathway dysfunction in the retina. This ERG phenotype localizes the site of retinal dysfunction to the on-bipolar synapse with photoreceptors. Modeling the unusual combination of ERG findings helps our understanding of the role of N-glycosylation at this synapse and provides a focus for future studies of potential intervention.


CONGENITAL DISORDERS OF glycosylation (CDGs) comprise a genetically heterogeneous group of multisystem disorders that result from enzymatic defects in the glycosylation of proteins and lipids.1-3 Congenital disorder of glycosylation due to phosphomannomutase deficiency (PMM2-CDG) (previously known as congenital disorder of glycosylation type 1a) is by far the most common diagnosis (Online Mendelian Inheritance in Man #212065). It is associated with a deficiency of phosphomannomutase encoded by the PMM2 gene located on chromosome 16p13.3,4 This enzyme is located in the cytosol and converts mannose-6-phosphate to mannose-1-phosphate.2 This enzyme has an essential role early in the N-glycosylation process2 and in the synthesis of glycosylphosphatidylinositol,3,4 which is used to anchor proteins to the cell membrane. PMM2-CDG is a multisystem disease with a broad clinical spectrum that ranges from mild to severe. Infants may come to us because of a failure to thrive, developmental delay, or muscle hypotonia; the childhood mortality rate due to infection or organ failure is approximately 20%.5,6 Characteristics that may be identified in the infantile period are inverted nipples, abnormal distribution of fat pads, and cerebellar hypoplasia, although not all patients manifest these signs.2 Older children frequently present with convergent squint and myopia. Retinitis pigmentosa (RP) has been described as another common ocular association.7 Our study of 2 mildly affected siblings provides new insight into the mechanism of retinal dysfunction in PMM2-CDG.

METHODS

Two male siblings (16 years old [patient 1] and 14 years old [patient 2]) of nonconsanguineous white parents diagnosed as having PMM2-CDG were referred for investigation of any signs of RP. Patient 1 failed to thrive in the early new-
The scotopic ERG waveforms for each patient are shown in Figure 2. No interocular difference was found in scotopic ERG amplitudes in patient 1 after a longer duration of dark adaptation of one eye (6 hours compared with 20 minutes). Amplitudes and time to peaks were compared with fifth and 95th percentile normative data. The scotopic a-waves fall within the reference range, but the scotopic b-wave amplitudes fall at and below the fifth percentile. This gives a reduced a:b amplitude ratio of 1 or less, a so-called negative ERG waveform. The b-wave time to peaks are within the reference range. Fourier analysis of the scotopic 15-Hz flicker waveform revealed significant data. The magnitude profiles of both patients were similar to normal profiles, albeit with smaller amplitude in patient 2. Data for complete and incomplete congenital stationary blindness (CSNB) are shown for comparison. Although both patients' data show an expected phase cancellation at midflash strength, an increase in phase with increasing flash strength was noted only for the second limb, the fast pathway (Figure 3). The scotopic OPs are present at normal time to peaks but are small amplitude.

Photopic ERG waveforms have broad a-waves of normal amplitude, followed by sharply defined, narrow b-waves. The photopic 30-Hz flicker ERGs have a sawtooth waveform (Figure 4). Photopic OPs to transient flashes are normal for patient 1 but small for patient 2 (Figure 5). Photopic prolonged on-off ERGs show a large, well-defined off-response, but the photopic on-response b-wave is absent (Figure 6). These on-off waveforms were filtered, revealing preserved on- and off-
pathway OPs. These responses are displayed with comparison data from a control participant and a patient with CSNB type 1 (CSNB1). To 4-millisecond single flashes, a single photopic OP is seen in CSNB1, but the prolonged on-response b-wave and prolonged on-pathway OPs are absent.

Mutational analysis of the PMM2 gene revealed that each case was a compound heterozygote for 2 point mutations: P69S c.205C>G and R141H c.422G>A. Mutational screening of the parents revealed that the father carried P69S and the mother carried R141H. No mutations were found in the tested array of 9 congenital stationary night blindness genes: RHO, GNAT1, PDE6B, SAG, NYX, CACNA1F, GRM6, CABP4, and CACNA2DA (Asper Biotech).

**COMMENT**

To our knowledge, our data are the first description of a negative ERG caused by an on-pathway deficit in PMM2-CDG, a congenital disorder of N-glycosylation. N-linked glycans are present in all layers of the retina, but the lack of photopic on-response b-wave in our pa-

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**Figure 2.** Scotopic electroretinogram waveforms for patients 1 and 2. The b-wave amplitudes are reduced compared with the normal profile, and an a:b ratio of 1 is seen to scotopic 3 flashes. Gray area shows the control data.
tients places the site of retinal dysfunction in PMM2-CDG at the cone photoreceptor synapse with the on-bipolar cell. The ERG b-wave is generated from depolarizing rod and cone on-bipolar cells. To model our ERG findings, the N-glycosylation defect, directly or indirectly, must prevent depolarization of cone on-bipolar cells, yet must allow signal transfer to spiking neurons to preserve the photopic on-oscillatory potentials. Depolarization of rod on-bipolar cells has to occur to account for the preserved rod-driven b-waves.

Figure 3. Scotopic 15-Hz flicker magnitude and phase for patients 1 and 2 compared with 2 healthy controls and patients with incomplete congenital stationary blindness (iCSNB) and complete congenital stationary blindness (cCSNB). The magnitudes show the normal profile and lower amplitude in patient 2. The phase data show a lack of phase change to low flash strength in patients 1 and 2 compared with control data. Connecting lines to phase data are drawn starting with the first significant data point. Open circles indicate not significant; closed circles, \( P < .05 \).

Figure 4. Photopic electroretinograms for patients 1 and 2 compared with control data and complete congenital stationary blindness (cCSNB) traces. The broad a-wave and narrow b-wave are emphasized to photopic 10 flashes.
Only a few clinical reports of patients with PMM2-CDG display ERG waveforms, but most describe reduced scotopic ERG amplitudes, indicating a generalized rod system defect. Cone involvement has been reported in patients as young as 18 months\(^1\) and more frequently as patients get older, providing evidence that PMM2-CDG is associated with a progressive rod cone dystrophy.\(^7,12-16\) A postmortem study\(^17\) found degeneration and loss of photoreceptors in the outer nuclear layer, and Andréasson et al\(^18\) hypothesized that RP in PMM2-CDG could be due to a glycosylation defect that involved the photoreceptor glycoprotein opsin and interreceptor binding protein. Few conditions to date have been associated with interreceptor binding protein, although recently autosomal recessive RP was described with mutations of \(\text{IMPG2}\), which encodes the interphotoreceptor matrix proteoglycan.\(^19\) Negative ERGs with a:b-wave amplitude ratios of 1 or less have been reported in RP, but both the b-wave and the a-wave amplitudes are subnormal.\(^20,21\) In addition, both the on- and off-responses of the photopic prolonged-flash ERG are affected in RP.\(^21\) In contrast, our patients have normal rod photoreceptor function, evidenced by normal a-wave amplitudes after standard dark adaptation times, and the cone off-response is normal.

Retinal arteries were slightly narrowed in patient 1, but pigmentary changes, typically seen in RP, were not evident. A closer examination of magnified fundus photographs revealed subtle, fine, yellow dots at the macula of each case, although autofluorescent imaging findings were unremarkable. A combination of reduced a:b ratio and white dots has been reported in retinoschisis.\(^22,23\) Also, a predominant on-pathway deficit has been described,\(^22,24\) but the cone photoreceptor a-wave is reduced in retinoschisis.\(^24,25\) In our patients, the cone a-wave amplitude is normal, and we did not observe any schitic areas.

To look for candidate N-linked glycoproteins responsible for the ERG features in our cases of PMM2-CDG, we reviewed the photoreceptor-bipolar signaling cascades using the UniProt database (www.uniprot.org). Our current knowledge of the bipolar synapse derives mostly from recent gene knockout and ERG studies on animal models.\(^26-28\) It is known that depolarization of the on-bipolar cells involves metabotropic synapses with photoreceptor cells, whereas off-bipolar cells use ionotropic synapses. All of the rod and half of the cone bipolar cells are on-bipolar cells that receive direct glutamatergic input from photoreceptor cells in the dark.\(^29\) The mGluR6 receptor is the primary postsynaptic glutamate receptor on the invaginating on-bipolar synapse. The N-glycosylated proteins nycotalopin and mGluR6 work together at this synapse to trigger a second messenger cascade involving G\(_{\alpha}\)o.\(^30\) Nycotalopin is tethered to the cell membrane via a glycosylphosphatidylinositol anchor, but it is the presence of nycotalopin at the cell membrane that is essential for function rather than its anchor.\(^31\) Other G proteins and regulators of G-protein signaling (RGS) colocalize with G\(_{\alpha}\)o. These G\(_{\alpha}\)o-interacting proteins, G\(_{\beta}\)5-RGS7 and G\(_{\beta}\)5-RGS11 complexes, determine bipolar cell kinetics.\(^32\) All these proteins are located in the dendritic tips of rod and cone on-bipolar cells.

The decrease in glutamate released by photoreceptors in response to light rapidly inactivates the mGluR6-
coupled Geo. This process removes a negative signal from its downstream target, the transient receptor potential cation channel subfamily M member 1 (TRPM1), also known as melastatin 1, and opens the channels to a cation current that depolarizes the on-bipolar cells. Knockout mice models show negative ERGs when any part of this cascade is disrupted (e.g., there is a complete absence of scotopic b-wave and absent scotopic and photopic OPs if there are defects in proteins mGluR6, Geo, G85, or Trpml). In humans, mutations in the NYX (nycalopin), GRM6 (mGluR6), or TRPM1 (TRPML1) genes are associated with on-pathway dysfunction, which clinically manifest as X-linked and autosomal recessive CSNB1 (complete CSNB). The CSNB1 phenotype shows complete absence of photopic on-response b-wave, preservation of the off-response, a sawtooth waveform 30-Hz flicker ERG, and absence of scotopic b-waves. The OPs in the transient flash ERG are reduced in number in CSNB1 and GRM6. Zeitz et al describe a remnant OP at 33 milliseconds that has been attributed to the off-pathway. This finding concurs with the prolonged on-off OP data shown in CSNB1 in Figure 5. Therefore, the ERGs associated with dysfunction of N-linked proteins mGluR6 and nycalopin share a number of features with our patients, but there are some distinct differences. Our patients have a full complement of photopic OPs to both transient and prolonged on-off stimulation, have scotopic OPs, and retain scotopic ERG b-waves.

Scotopic 15-Hz flicker is thought to distinguish 2 of 5 rod pathways: a slow pathway at low flash strengths and a fast pathway at higher flash strengths. The slow pathway reflects a network connecting rods via rod bipolar and AII amacrine to cone on-bipolar cells, whereas the fast rod pathway uses gap junctions between rod and cone pedicles to reach the cone on-bipolar cells. Our 15-Hz magnitude data suggest both fast and slow pathways are functioning in a similar proportion to normal pathways (Figure 3), which can explain why scotopic OPs are preserved; however, the slow pathway phase distinction does not increase in the same way as normal pathways, which suggests that, although still active, it is compromised more than the fast pathway.

A closer link between our ERG findings in PMM2-CDG and the reported RP phenotype might be made if the PMM2 mutation has greater effect at the presynaptic photoreceptor terminals that control glutamate release. Slowing calcium influx reduces glutamate release. L-voltage-dependent calcium channel heteromultimeric protein subunits are essential for Ca2+ channel assembly and function. Mutations in CACNA1F, the voltage-dependent L-type channel subunit 1F (Ca1.4), which is expressed presynaptically, causes X-linked CSNB2 (incomplete CSNB). Patients with this mutation have some preservation of scotopic rod-driven ERG b-waves. CACNA1F has a potential N-linked site, and interestingly, patients with CSNB2 have preserved but delayed OPs. AII subunit α1F occurs in rods and cones and α1D only in cones. This explains why both cone- and rod-driven ERGs are affected in CSNB2 and why both on- and off-responses of the photopic ERGs are reduced. No human disease has been described yet with mutations in CACNA1D. Our patients are similar to the CSNB2 phenotype because the cone pathway is affected more than the rod pathway, but in contrast to CSNB2, our patients have profound electronegative, photopic, prolonged-flash on-responses; preserved off-responses and OPs are not delayed. The possibility that our patients had dual conditions is unlikely because no known mutations were found in 9 genes associated with X-linked, autosomal recessive, and autosomal dominant CSNB.

The ERGs of mouse models of presynaptic no-b-wave mutants share some characteristics with other mutants that show disrupted control of glutamate release, including mutations of the glycoprotein dystrophin. Dystrophin and b-dystroglycan, a member of the dystrophin-associated glycoprotein complex, colocalize at the invaginated synaptic complex of photoreceptors. Mutations in dystrophin affecting the retinal isoform are known to cause negative scotopic ERGs in Duchenne muscular dystrophy, and there is a concomitant loss of OPs with increasing severity of on-pathway dysfunction. Dysglycosylation of dystroglycan to date has been associated only with defects in O-linked glycosylation, recently referred to as dystroglycanopathies. Five conditions belonging to that group have mutations in proven or putative glycosyltransferases, leading to a disruption of the heavily O-glycosylated part of the dystroglycan complex. Examples are POMT1/POMT2-CDG, POMTGN1-CDG, FKTN-CDG, FKR-CDG, and LARGE-CDG.

To explain the greater photopic ERG alteration in our cases, it may be necessary to postulate a glycoprotein receptor or subunit with greater expression in the cone, rather than rod, pathway. For example, the Na/Ca-K exchanger (N-linked NCKX) plays a critical role in Ca2+ homeostasis in retinal rod and cone photoreceptors. The NCKX1 isoform is found in rods, whereas the NCKX2 isoform is found in cones.

Although we have considered direct influences of N-glycosylation at the on-bipolar synapse, it is possible that glycosylation defects influence other proteins and indirectly affect the ERG. For example, at gap junctions, connexin 43 itself is not glycosylated, yet inhibition of glycosylation causes connexin 43 cell-to-cell channels to open. Deletion of the amacrine AII localized connexin 36 and the on-bipolar localized connexin 45 can reduce scotopic b-wave amplitude by 40% to 50%. More recently, N-glycosylated pannexins have been described that colocalize with connexins. They are plentiful in the retina and could alter gap junction cell-to-cell communication. Gap junctions between cone bipolar to AII and AII-to-AII are bidirectional over a wide range of light intensities.

The sporadic evidence that some retinal diseases have a greater effect on the on-pathway of cones rather than rods has intrigued observers for some time. Sieving described a hyperpolarizing pattern of ERG in which the photopic on-response was affected, but the scotopic b-wave was near normal. In these cases, the photopic, prolonged-flash off-responses were preserved, even enhanced. He advanced 2 hypotheses. The first was that the 2-amino-4-phosphonobutyrate-sensitive glutamate receptors on the rod on-bipolar and cone on-bipolar cells have some unknown subtle differences. The second drew...
on the demonstration that a timing delay at the on-synapse of as little as 2.5 milliseconds could mimic the negative on-response waveform recorded in CSNB1 (this assumed the b-wave is the result of subtractive interference of depolarizing and hyperpolarizing bipolar cells). Changes in membrane resting potential and reduced dynamic range have been described in presynaptic no b-wave models, which could potentially manifest such a timing effect. In this instance, on-pathway signaling may proceed to the inner retina, but the slight delay would alter the epiphenomenon of the ERG b-wave (ie, markedly attenuate amplitude). This explanation requires the OP latencies to be unaffected by this delay because timing in our patients was normal. A dissociation of OPs from a- and b-wave amplitudes has been noted recently in a rabbit model of retinal degeneration caused by a rhodopsin mutation. This finding opens the possibility that changes in inner retinal neuronal networks, secondary to photoreceptor dysfunction, can be associated with diminished b-wave but preserved, even enhanced, OPs. 

We have shown, for the first time to our knowledge, a negative ERG waveform in association with PMM2-CDG. This places the site of dysfunction predominantly at the cone on-bipolar synapse with the photoreceptors. Studies of N-glycosylation disruption at this site may help identify a potential therapeutic intervention to prevent progressive retinal dysfunction. Our findings also provide a clinical example of differential resilience of OPs and an insight of retinal networks in disease.

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