***DPAGT1* Deficiency with Encephalopathy (DPAGT1-CDG): Clinical and Genetic Description of 11 New Patients**

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Abstract Pathogenic mutations in *DPAGT1* cause a rare type of a congenital disorder of glycosylation termed DPAGT1-CDG or, alternatively, a milder version with only myasthenia known as DPAGT1-CMS. Fourteen disease-causing mutations in 28 patients from 10 families have previously been reported to cause the systemic form, DPAGT1-CDG. We here report on another 11 patients from 8 families and add 10 new mutations. Most patients have a very severe disease course, where common findings

are pronounced muscular hypotonia, intractable epilepsy, global developmental delay/intellectual disability, and early death. We also present data on three affected females that are young adults and have a somewhat milder, stable disease. Our findings expand both the molecular and clinical knowledge of previously published data but also widen the phenotypic spectrum of DPAGT1-CDG.

Abbreviations

CDG	Congenital disorder of glycosylation
CMS	Congenital myasthenic syndrome
DPAGT1	Dolichyl-phosphate GlcNAc

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	phosphotransferase 1
EEG	Electroencephalogram
EOEE	Early-onset epileptic encephalopathy
GlcNAc	<i>N</i> -acetyl glucosamine
IEF	Isoelectric focusing
LC/MS	Liquid chromatography/mass spectrometry
MRI	Magnetic resonance imaging
N-linked	Asparagine linked
PMM2	Phosphomannomutase 2
TF	Transferrin

Background

Protein glycosylation is the most frequent posttranslational protein modification, showing an immense variation (Eklund and Freeze 2005). It can occur at different amino acids [for instance, asparagine (*N*-linked) (Stanley and Taniguchi 2015) or serine/threonine (*O*-linked)], using several different monosaccharides as the linkage sugar [e.g., glucose, mannose, or *N*-acetylglucosamine (GlcNAc)] (Seeberger 2015), and the final carbohydrate decoration may be everything from a monosaccharide to a large polysaccharide (Prestegard et al. 2015). A few percent of the genome is dedicated to these processes (Henrissat et al. 2015), and mutations in these genes constitute the basis for the congenital disorders of glycosylation (CDG). The number of genetically confirmed CDG types has increased exponentially since the first one was described in 1980 (Jaeken et al. 1980) and today includes almost 130 different entities. Almost 70 of these involve deficient *N*-glycosylation, where phosphomannomutase 2 deficiency (PMM2-CDG) is the most common type (Freeze et al. 2015).

Dolichyl-phosphate GlcNAc phosphotransferase 1 (DPAGT1) catalyzes the first step in the biosynthetic pathway of the precursor oligosaccharide for *N*-linked glycosylation: the transfer of GlcNAc-1-P from UDP-GlcNAc to dolichol phosphate, resulting in the formation of dolichol pyrophosphate GlcNAc (Wu et al. 2003). The first patient identified with mutations in *DPAGT1* was described in 2003 (Wu et al. 2003), and thereafter over 40 patients have been described in publications (Jaeken et al. 2015). Interestingly, a recently released manuscript on the preprint server for biology “bioRxiv” investigates in great detail known mutations in *DPAGT1*, using crystal structures of the enzyme together with either the substrate UDP-GlcNAc or the inhibitor tunicamycin (<https://doi.org/10.1101/291278>). Two clinically distinguishable phenotypes are seen with the first being a systemic disease with encephalopathy [DPAGT1-CDG (formerly CDG-Ij)] and the second a congenital myasthenic syndrome (DPAGT1-CMS) with tubular aggregates seen in muscle biopsies (Jaeken et al. 2015). The DPAGT1-CDG phenotype is most

often a severe disease, where more than 80% of the reported patients died before 5 years of age. However, 18 of the previously reported 29 patients were from the same consanguineous family (Imtiaz et al. 2012), thus the very high mortality rate may be skewed by one specific mutation. Few patients with a milder (but systemic) disease have been described, for instance, two siblings in their 30s (Iqbal et al. 2013). All patients have shown moderate to severe intellectual disability. Epilepsy is a common feature and often debuts early. A number of symptoms typical for CDG, such as feeding difficulties, cataracts, hypertrichosis, hyporeflexia, joint contractures, elevated liver enzymes, and abnormal brain magnetic resonance imaging (MRI), have been described also in this subtype. However, findings otherwise common in CDG, such as night blindness, inverted nipples, and lipodystrophy, have only rarely been reported (Jaeken et al. 2015).

In this report, we present the genetic and clinical data available for an additional 11 patients with the systemic form of this disease, most of whom presented with a very severe phenotype.

Methods

Genetic Characterization

Exome sequencing was performed as previously described (Simon et al. 2017). Sanger sequencing was performed using standard PCR methods targeting the nine coding exons for human *DPAGT1* (NM_001382.3). Primer sequences are available upon request.

Results

Clinical Characterization

The inclusion criteria for this study was a biochemical test showing underglycosylation of transferrin (TF) and/or exome sequencing data showing likely damaging homozygous or compound heterozygous mutations in the *DPAGT1* gene.

Table 1 is a summary of mutations as well as clinical symptoms of our 11 patients from 8 families and also 13 patients previously published (Wu et al. 2003; Imtiaz et al. 2012; Iqbal et al. 2013; Yuste-Checa et al. 2017; Vuillaumier-Barrot 2005; Wurde et al. 2012; Carrera et al. 2012; Timal et al. 2012; Ganetzky et al. 2015). Another 16 patients from 1 consanguineous family were affected, all died in early infancy and showed a similar clinical phenotype, but they are not included in the following calculations as they were not formally tested and the individual clinical descriptions are lacking (Imtiaz et al. 2012). There seems to be no gender predilection for

Table 1 Patient data

Reference	Mutation 1 (protein consequence)	Mutation 2 (protein consequence)	gnomAD (no of het, homo)	Tf glycosylation	Gender	Ethnicity	BW (g/GL wks + days)	Percentiles (weight)	Deceased	MRI	EEG
This report; P1	c.27>C (p.Met1?)	c.341C>G (p.Ala114Gly)	5,0/1,0	Type 1 pattern	F	Armenian	—/—	—	—	Normal	—
P2	c.509A>G (p.Tyr170Cys)	c.584G>C (p.Ala195Gly)	4,0/0,0	Type 1 pattern	M	Native Am./Caucasian	3,390/38+0	75	—	—	—
P3	c.509A>G (p.Tyr170Cys)	c.584G>C (p.Ala195Gly) (assumed, never tested sib)	4,0/0,0	Normal (8 weeks)	M	Native Am./Caucasian	—/—	—	5 months	—	+
P4	c.117C>G (p.Pro373Ala)	c.1197T>A (p.Tyr399*)	0,0/0,0	Type 1 pattern	M	Caucasian	1,875/36 + 0	<1	—	Normal	—
P5	c.116..117delinsAA (p.Ala39Lys)	c.380..395dup16 (p.Ser133Alafs*64)	0,0/2,0	Type 1 pattern	F	Caucasian	3,064/term	25	3 months	Normal	—
P6	c.419A>G (p.Tyr140Cys)	c.419A>G (p.Tyr140Cys)	1,0/1,0	Type 1 pattern	M	Arabic	3,350/41 + 2	40	10 months	Thin corpus callosum	Hyps
P7	c.380..395dup16 (p.Ser133Alafs*64)	c.739C>T (p.Arg247Trp)	2,0/3,0	Type 1 pattern	M	—	3,239/term	40	Alive at 7 years	Increased space below cerebellum	—
P8	c.380..395dup16 (p.Ser133Alafs*64)	c.739C>T (p.Arg247Trp) (assumed, never tested sib)	2,0/3,0	Never performed	F	—	2,670/term	10	4 years	General atrophy	General slowing
P9	c.488T>C (p.Leu163Pro)	Unidentified deletion	4,0/—	Type 1 pattern	F	Caucasian	—/—	—	Alive at 18 years	Normal	Hyps
P10	c.488T>C (p.Leu163Pro)	Unidentified deletion	4,0/—	Type 1 pattern	F	Caucasian	—/—	—	Alive at 16 years	Normal	+
P11	c.26dupT (p.Met91Leu80*)	c.739C>T (p.Arg247Trp)	8,0/3,0	Type 1 pattern	F	Caucasian	2,400/38 + 0	5	Alive at 17 years	Normal	—
Wu et al. (2003)	c.509A>G (p.Tyr170Cys)	Unknown (splice —> message decay)	4,0/—	Type 1 pattern	F	—	—/41 + 3	—	Alive at 21 years	Normal	Hyps
Vuillaumier-Barrot (2005)	c.890A>T (p.Ile297Phe)	c.162-8G>A (splice)	6,0/0,0	Type 1 pattern	—	—	—/—	—	—	—	—
	c.890A>T (p.Ile297Phe)	c.162-8G>A (splice)	6,0/0,0	Type 1 pattern	—	—	—/—	—	—	—	—
Imtiaz et al. (2012) ^a	c.902G>A (p.Arg301His)	c.902G>A (p.Arg301His)	5,0/5,0	Type 1 pattern	M	Arabic	—/term	—	5 years	Less myelin	+
	c.902G>A (p.Arg301His)	c.902G>A (p.Arg301His)	5,0/5,0	Type 1 pattern	M	Arabic	—/—	—	—	—	—
Wurde et al. (2012)	c.341C>G (p.Ala114Gly)	c.341C>G (p.Ala114Gly)	1,0/1,0	Type 1 pattern	F	Turkish	2,610/38 + 3	10	8 months	Global atrophy	Hyps
	c.341C>G (p.Ala114Gly)	c.341C>G (p.Ala114Gly)	1,0/1,0	Type 1 pattern	F	Turkish	2,600/38+6	5	1 year	—	+
Carrera et al. (2012)	c.901C>T (p.Arg301Cys)	c.1054T>G (p.Leu385Arg)	4,0/7,0	Type 1 pattern	M	Spanish	3,095/term	25	6 weeks	Normal	—
Timal et al. (2012)/Adamowicz et al. (2011) (abstr)	c.206T>A (p.Ile69Asn)	c.161+5G>A (splice)	1,0/2,0	Type 1 pattern	M	—	1,410/—	<1	2.5 years	—	+
Iqbal et al. (2013)	c.85A>T (p.Ile29Phe)	c.503T>C (p.Leu168Pro)	26,0/0,0	Type 1 pattern	F	Pakistani	—/—	—	Alive at 34 years	—	—
	c.85A>T (p.Ile29Phe)	c.503T>C (p.Leu168Pro)	26,0/0,0	Type 1 pattern	M	Pakistani	—/—	—	Alive at 32 years	—	—
Ganetzky et al. (2015)	- (p.Leu118Val)	- (p.Leu118Val)	0,0/0,0	Type 1 pattern	F	—	5th centile/40 + 3	5	1.5 months	Cerebellar hypoplasia	—
Yuste-Checa et al. (2017)	c.329T>C (p.Phe110Ser)	c.902G>A (p.Arg301His)	0,0/5,0	—	—	—	—	—	—	—	—

ID/DD	Epilepsy	Other neurological	Eye/retina	Skin	Liver	Heart	Muscles	Skeletal	Other
-	-	Microcephaly	Strabismus	Hypertrichosis, lipodystrophy	Elevated transaminases	Long QT	Axial hypotonia, extremity hypertonia	-	Low AT-III, PLE
-	-	Intracranial hemorrhage	-	-	-	-	Congenital hypertonia	Contractures	FTT
++	+	Intracranial hemorrhage	-	-	-	-	-	-	FTT
-	-	Arthrogryposis	Congenital cataract	Anasarca	Normal transaminases	Normal	Fetal and newborn akinesia	Contractures	Lung hypoplasia
++	No	Microcephaly	-	Loose (due to underweight)	Elevated transaminases	-	Hypotonia	-	FTT, retained umb cord
-	ES ->EE	Tremor	Congenital cataract	Hypertrichosis	Enlarged, elevated transaminases	Normal	Hypotonia	No dysplasia	FTT, low prot S/AT-III
++	+	Non-ambulatory, non-verbal	Strabismus	Normal	Elevated transaminases	Long QT	Hypotonia, exotropia	Normal	FTT, repeat pneumonias
++	+	Rocking, head banging	Optic nerve atrophy, nystagmus	Normal	Elevated transaminases	Normal	Hypotonia, fetal akinesia	Normal	-
++	ES ->ME	Stroke-like episodes, autism	Visual range 2 m, strabismus	Normal	Normal transaminases	Normal	Hypotonia, areflexia	Scoliosis, osteoporosis	High protein S, spont menarche
++	Focal ep	Autism	Strabismus	Normal	Normal transaminases	Normal	Hypotonia, areflexia	Scoliosis	High protein S, spont menarche
++	No	Microcephaly, ataxia	RP, optic nerve atrophy	Normal	Normal transaminases	Normal	Hypotonia	Severe scoliosis	Induced puberty
++	ES ->EE->no sz	Microcephaly	Exotropia	Dimples	Normal transaminases	Normal	Hypotonia	Clinodactyly	Spont menarche
-	-	-	-	Normal	Normal transaminases	-	-	NK	-
-	-	-	-	Normal	Normal transaminases	-	-	NK	-
++	+	-	-	-	Elevated transaminases	-	Hypotonia	-	Repeat aspirations
-	-	Fetal hypokinesia	-	-	-	-	-	-	-
++	ES ->EE	Hyperexcitability, microcephaly	Congenital cataract, nystagmus	Hypertrichosis, inverted nipples	Hepatomegaly	-	Hypotonia, strabismus	-	-
++	EE	Hyperexcitability, microcephaly	Congenital cataract	-	-	-	-	-	-
-	-	Akinesia	Papillar atrophy	Thick skin, hypertrichosis	-	-	Hypotonia	Campodactyly, contractures	-
-	Tonic seizures	-	Congenital cataract	-	Elevated transaminases	-	Hypertonia	Joint contractures	FTT, low AT-III, chronic anemia
+	+	Aggressiveness, speech delay	Night blindness	-	-	-	Hypotonia	-	-
+	+	Aggressiveness, speech delay	-	-	-	-	Hypotonia	-	-
-	-	Arthrogryposis, fetal akinesia	Congenital cataract	Normal	-	-	Hypotonia	Arachnodactyly	-
+	-	Hypocacusia	-	-	-	-	Hypotonia, weakness	-	-

- = not known/performed or not reported, no = not present/negated, + = pathological finding not further specified, ++ = severe finding not further specified, gnomAD = number of hetero- and homozygotes reported in the database per allele

BW birth weight, *GL* gestational length, *hyps* hypsarrhythmia, *ID/DD* intellectual disability/developmental delay, *ES* epileptic spasms (West syndrome), *EE* epileptic encephalopathy, *ME* myoclonic epilepsy, *FTT* failure to thrive, *RP* retinitis pigmentosa, *AT-III* antithrombin III

^a The paper indicates 16 additional members of the family being affected, all died in early infancy, similar clinical phenotype

DPAGT1-CDG, out of the previously published patients where the gender is reported, 5/10 patients were female, and in our cohort 6/11 are female. The lengths of the pregnancies were reported in 13/24 of the cases, and in these, delivery was at term in 12/13. However, the babies generally have a birth weight in the lower spectrum, ranging from <1 to 40th percentile in all but one case. The phenotype is usually very severe, and including our patients, 10 patients have been confirmed deceased before the age of 5 years (6 died at or before 12 months of age; data is missing on 7). Neurologically these patients are often severely afflicted: All patients eligible for testing have shown signs of developmental delay/cognitive impairment of at least moderate severity. 13/24 patients were reported to have epilepsy, mostly of early-onset types, where four had West syndrome with hypsarrhythmia on electroencephalogram (EEG) and clinical spasms at debut. So far, only 3/24 described patients have been negated to have epilepsy. Sixteen patients were described as hypotonic, whereas another three were described as hypertonic. Other common neurological findings include microcephaly (6/24), arthrogryposis/fetal akinesia (5/24), and strabismus/squint (6/24) (Table 1). Ten patients have had an MRI scan of their brains reported, but no consistent pathological findings were seen. In 6/10 patients, the scans were considered as normal; others had findings such as global atrophy, cerebellar hypoplasia, and thin corpus callosum. Cataract is a common finding in this CDG type, described in 7/24 patients, and 4/24 patients have a confirmed retinal pathology. Hypertrichosis has been noted in 4/20 patients. Lab investigations available from 22 out of 24 patients show that all but one patient had a type I TF pattern using isoelectric focusing (IEF) or liquid chromatography/mass spectrometry (LC/MS) analysis. 6/14 patients reported an elevation in their liver transaminases (data missing on 10 patients). Five patients have a reported deficiency in protein S and/or antithrombin III.

Mutational Spectrum

We combined exome sequencing and Sanger sequencing to identify 12 different *DPAGT1* mutations in 11 patients, of which only 2 [c.341C>G; p.(Ala114Gly); c.509A>G p.(Tyr170Cys)] were previously reported to cause DPAGT1-CDG (Wu et al. 2003; Wurde et al. 2012) (Table 1). We then searched the gnomAD (Genome Aggregation Database) (gnomad.broadinstitute.org) [gnomADr2.0.2, accessed 03.01.2018], containing data from 123,136 exome sequences and 15,496 whole-genome sequences of unrelated individuals, and found that 15 of the mutations were found at low frequency in heterozygous carriers, whereas 8 were previously unreported. None of the mutations occurred in a homozygous state (Table 1).

The most prevalent heterozygous mutation was c.85A>T; p.(Ile29Phe), with a total of 26 reported carriers and a carrier frequency of 0.00084 in a South Asian population.

In the previously published patients, 13 different pathological alleles have been described: 2 affecting a splice site and the rest being missense mutations (Table 1). We add one stop mutation, two frameshift mutations, six missense mutations, and one mutation of the initiating methionine. In two siblings the amino acid exchange p.(Leu163Pro) seems to be heterozygous based on genomic DNA analysis, but homozygous based on cDNA, suggesting an undetected deletion. Only one parent (the father) was a carrier. The pathogenicity of the mutations were tested in silico, and based on our previous experience (Ng et al. 2016), we used the in silico pathogenicity determining program Combined Annotation Dependent Depletion (CADD) (<http://cadd.gs.washington.edu/>).

Discussion

We present data on 11 unreported patients with DPAGT1-CDG, increasing the known patient population by one third. Most of the mutations are missense, and no new splice mutations were found. Combining the novel mutations and the already published ones, they occur throughout the gene without locus-specific accumulation (Fig. 1). However, 14/20 missense mutations are within or very proximal to a membrane-spanning section of the protein (Fig. 1). The full-blown phenotype of this CDG type is very severe, showing early-onset epileptic encephalopathy (EOEE), pronounced muscular hypotonia, severely delayed development, and early death (Jaeken et al. 2015). In our cohort this was confirmed as 30% of the cases died before the age of one, and only three lived to be teenagers. This makes DPAGT1-CDG one of the most severe CDG types. Mortality in, for instance, the most common type, PMM2-CDG, is estimated to 20% during the first year in the severe cases, after which it stabilizes. Epilepsy is a common finding in patients with glycosylation deficiencies, with a wide spectrum of severity and semiology (Freeze et al. 2015). Severe epilepsy, often beginning as Ohtahara syndrome (early infantile epileptic encephalopathy) or West syndrome (hypsarrhythmia, clinical spasms, and developmental arrest) and sometimes developing into multifocal hard-to-treat epilepsy, has been reported for many CDG types, including ALG1-CDG (Fiumara et al. 2016; Barba et al. 2016), ALG3-CDG (Fiumara et al. 2016; Barba et al. 2016), ALG6-CDG (Fiumara et al. 2016), ALG13-CDG (Hamici et al. 2017), ALG14-CDG (Schorling et al. 2017), DPM2-CDG (Fiumara et al. 2016), DOLK-CDG (Helander et al. 2013), RFT1-CDG (Barba et al. 2016), SLC35A2-CDG (Ng et al. 2013), and SLC35A3-CDG (Marini et al. 2017). In the published cohort of DPAGT1-CDG patients

Fig. 1 A depiction of the predicted structure of the DPAGT1 protein. There are ten membrane-spanning domains with the N- and C-termini both on the luminal side. All known missense mutations, a stop codon-introducing mutation, a mutation of the initiating methionine, and two frameshift mutations are indicated with boxes. Gray circles

indicate amino acids not affected in DPAGT1-CDG so far, white circles depict amino acids that have previously been published to be exchanged in DPAGT1-CDG, and black circles indicate amino acids changes described for the first time in this paper

including this report, 4/24 patients have had the diagnosis West syndrome, and 9 more have a definitive diagnosis of epilepsy, whereas only 3 were negated to have epilepsy. This strengthens the notion that one should consider CDG as a cause of EOEE, when its etiology is unknown. Why incorrect glycosylation causes epilepsy is not fully known, but it must be multifactorial since deficiencies in many different glycosylation pathways cause epilepsy (Freeze et al. 2012, 2015). Many N- and O-glycoproteins are involved in CNS development and function, including NCAMs, voltage-gated channels, and neurexins (UniProt Consortium 2015). Deficient glycosylation of these types of proteins may well cause epilepsy, but there is very little literature on the subject readily available. A recent report (Izquierdo-Serra et al. 2018) showed nicely that hypoglycosylation of the $\text{Ca}_v2.1$ channel (a neuronal pore-forming voltage-gated calcium channel) might be the molecular explanation to a phenomenon known as stroke-like episodes, a feared complication in CDG. These episodes involve confusion, hemiparesis, and sometimes seizures and may last several days. There is no standard, widely accepted, treatment; however, antiepileptic drugs seem to have a place in the treatment arsenal (Izquierdo-Serra et al. 2018). Also, deletions in the glycoprotein neurexin 1 (NRXN1) gene cause epilepsy (Perez-Palma et al. 2017) and autistic features (Kasem et al. 2018), both common symptoms in many CDG types.

In many CDG types, female teenagers do not enter puberty spontaneously, and puberty therefore needs to be induced using estrogens and progesterone (de Zegher and Jaeken 1995; Miller and Freeze 2003). In our cohort, two young women have gone through puberty without the aid of exogenous hormones and continue to menstruate regularly, whereas puberty in the third girl was recently

induced with hormonal therapy. Furthermore, the clinical data on the first DPAGT1-CDG patient published (Wu et al. 2003) was updated during the finalization of this paper (Table 1), and she is also regularly menstruating without the aid of hormones (Freeze, HH, pers. comm.). In the only previously published description of an adult female with DPAGT1-CDG, there was no information on her sexual development (Iqbal et al. 2013). Thus, females with DPAGT1-CDG seem to have a fair chance of developing normal menstruations, and it may be clinically relevant to await spontaneous menarche.

The general suggestion in the literature is to avoid surgery in CDG patients due to coagulation abnormalities (Linssen et al. 2013). A problem noted in all our adult patients, however, was severe scoliosis, having an immense impact on their quality of life. We therefore decided to perform spinal surgery on three of the adolescent patients (P9-11), closely monitoring the coagulation parameters. The procedures were well tolerated and radically improved their quality of life.

In conclusion we describe 11 new patients with DPAGT1-CDG and confirm the previous notion that this is generally a severe subtype. However, attenuated forms exist. Here, patients survive into the adulthood presenting a rather static encephalopathic phenotype with other problem such as scoliosis. With the more general introduction of massive parallel sequencing in the diagnostic arsenal of cognitive impairment and epilepsy, we certainly expect more patients with attenuated types of CDG to be presented in the literature within the near future.

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Synopsis

A thorough analysis of the genetic and clinical features of 11 novel DPAGT1-CDG patients.

Details of the Contributions of Individual Authors

BGN, HHF, and EAE designed the study. HRU, LP, XZ, CAS, KDJ, and EAE provided and analyzed clinical information. BGN, JMR, SG, AM, MK, DAN, KJB, JS, and MJB provided and analyzed genetic data. PB provided and analyzed biochemical data. EAE wrote the initial manuscript; all authors revised the manuscript and approved the final version.

Competing Interest Statement

Bobby G. Ng, Hunter R. Underhill, Lars Palm, Per Bengtson, Jean-Michel Rozet, Sylvie Gerber, Arnold Munnich, Xavier Zanlonghi, Cathy A. Stevens, Martin Kircher, Deborah A. Nickerson, Kati J. Buckingham, Kevin D Josephson, Jay Shendure, Michael J. Bamshad, Hudson H. Freeze, and Erik A. Eklund declare that they have no conflict of interest.

Compliance with Ethic Guidelines

The research was performed under a Sanford Burnham Prebys Medical Discovery Institute IRB protocol. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Patient Consent Statement

Families included in this research study provided written informed consent. Proof that informed consent was obtained is available upon request.

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