

WHY GENOTYPE ADOLESCENT PROGRESSIVE MYOCLONIC EPILEPSY (PME)

Genotyping Adolescent PME will separate Lafora disease from Unverricht-Lundborg PME and from Juvenile Myoclonic Epilepsy. In their early stages, Lafora disease, Unverricht-Lundborg PME and JME are clinically similar (see Table 1). Genotyping patients with PME will identify mutations in one of three known genes, namely, EMP2A/Laforin or EMP2B/Malin, which cause the majority of Lafora progressive myoclonus epilepsy or cystatin B which cause almost all of Unverricht Lundborg PME (Delgado-Escueta et al., 2007). A second reason for genotyping adolescent PME is to identify nonsense mutations which can be treated with premature stopcodon readthrough drugs, such as gentamicin.

Mutations in Lafora disease genes (Laforin and Malin) and in Unverricht Lundborg PME genes (cystatin B) are inherited in an autosomal recessive fashion. The gene EPM2A makes an enzyme called dual specificity protein phosphatase or Laforin and the gene EPM2B makes another enzyme named ubiquitin E3 ligase or Malin. Thus, majority of cases of Lafora PMEs are enzymopathies, are classified as lysosomal diseases and produce a system wide glycogen storage disorder. A minority of cases of Lafora disease are caused by as yet unidentified genes. Cystatin B is a cysteine protease inhibitor and its deficiency causes abnormal activation of cathepsin S. G1qB-chain of complement, beta2-microglobulin, glial fibrillary acidic protein, apolipoprotein D, fibronectin 1 and metallothionein II, which are factors involved in proteolysis, apoptosis and glial activation (Leiuallen et al., 2001). The lysosome-associated functions of cystatin B are also associated with the pathogenic mutations suggesting Unverricht-Lundborg PME may also be a lysosomal disease (Alakurtti et al., 2005).

Most commonly Lafora PME, Unverricht Lundborg PME and JME, start as myoclonic, tonic clonic grand mal and absence seizures at late childhood or adolescence. Rarely, Lafora PME begins in 5 to 6 year old children as a learning disorder.

Can early Lafora PME and Unverricht Lundborg PME be clinically distinguished from JME? Yes, a clinician experienced in PME can look for subtle differences between these epilepsies, but genotyping solidifies and clarifies diagnosis. Unlike JME and Unverricht-Lundborg PME, Lafora PME usually has visual occipital seizures in addition to myoclonias, grand mal and absences at the start of illness. Myoclonias can be present in facial muscles in both Unverricht-Lundborg PME and in Lafora PME and are never present in facial muscles in JME. Myoclonias are stimuli sensitive in both Lafora and Unverricht-Lundborg PME and merely tapping the knee jerk or shaking hands can be enough to induce a large myoclonia. JME is usually not stimuli sensitive except for the few that respond to photic stimulation. Of course when a sibling or first cousin has passed away from "biopsy proven" Lafora disease, the diagnosis is made easy for the community practitioner. Taking a good family history is therefore crucial. There is a higher incidence of Lafora disease in children and adolescents with ancestral origins from the Middle East, Mediterranean or Southern Europe (Spain, France and Italy), South Asia (India and Pakistan) and North Africa. In these countries consanguineous marriages are not uncommon. Unverricht-Lundborg PME is most common in patients of Northern European origin, e.g., the Baltic region and Scandinavia, and is found worldwide because of human migration. While lafora PME and Unverricht Lundborg PME are rare diseases, Juvenile myoclonic epilepsy is a common epilepsy which is present in all continents and racial-ethnic groups.

Laforin deficient PME is more rapidly fatal than malin deficient PME. In Laforin deficient PME, cognitive decline and poor school performance usually starts with seizures or follows seizures in 1 to 2 years. Ataxia and spasticity is present by 14 to 16 years, and dementia and mutism by 17 to 20 years. Anorexia and swallowing difficulties compel gastrostomy by 18 to 22 years. Respiratory assist is necessary usually by 20 to 25 years. In malin deficient PME, mild cognitive decline doesn't start until 16 to 27 years, ataxia and spasticity at 16 to 27 years, dementia and mutism at 26 to 32 years and gastrostomy and respiratory assist between 26 to 37 years (see table 1).

Because of its progressive and invariably fatal nature, the community neurological practitioner should genotype Lafora PME in its early stages, when it is indistinguishable from JME and Unverricht Lundborg PME, before independent daily living activities are lost and certainly before the need for gastrostomy is realized. If genotyping does not show mutations in laforin or malin, a skin biopsy should be performed. Biopsy of sweat glands in the axilla should show the disease causing inclusion bodies that stain with periodic acid Schiff (PAS) inside eccrine sweat duct cells or apocrine myoepithelial cells. These inclusion bodies are made of abnormally branched glycogen called polyglucosan that are present in excessive amounts in various major organs including brain. If PAS + inclusion bodies are present in the skin biopsy but mutations are absent in laforin or malin, a rarer form of Lafora PME is present caused by the as yet unidentified third or fourth gene for Lafora PME.

“Boosting protein synthesis from <1% to as little as 5% of normal levels reduces severity of disease phenotype” (Kerem, 2004; Ramalho et al., 2002)

Genotyping in Lafora PME will show if deletions, frameshifts, missense or nonsense mutations are present in laforin or malin. If nonsense mutations are present, the practitioner should work with a specialist in PME and consider using intravenous gentamicin, a premature stopcodon readthrough drug that is clinically justified for “compassionate use” in a fatal disorder (Barton-Davis, et al., 1999; Clancy, 2001; Politano, 2003; Wagner et al., 2001; Wilschanski et al., 2003; Welch et al., 2007, Brooks et al., 2006). PTC 124, a new orally bioavailable nontoxic, small molecule that selectively induces ribosomal readthrough of premature but not terminal codons and superior in potency to gentamicin is not yet commercially available. PTC124 does not penetrate the BBB.

Because Lafora PME is a lysosomal disease and presently has no therapeutic options, high concentrations of intravenous gentamicin can be justified for compassionate use especially before the needs for gastrostomy and respiratory assist ensue. Lafora PME accumulates polyglucosan bodies in liver, retina, cardiac muscles, esophageal skeletal muscles, and diaphragm muscles producing anorexia, malaise, visual problems, cardiac arrhythmias, swallowing and breathing difficulties. Boosting protein synthesis of laforin and malin which function to purge polyglucosan inclusion bodies from cells should slow the disease and alleviate the peripheral pathologies responsible for anorexia, malaise swallowing and breathing difficulties. High concentrations of intravenous gentamicin can also be expected to cross the blood-brain barrier because of continuing and frequent seizures and because endothelium and neuronal cell death break open the blood-brain barrier. Selective and specific readthrough of disease causing premature termination codons in endothelium and neurons should boost protein synthesis of laforin and malin which purge the neurons of Lafora inclusion bodies and alleviate the central pathology of Lafora PME. Potential renal and otic toxicities produced by high concentrations of intravenous gentamicin limit their clinical use to invariably fatal diseases like Lafora PME.

GENOTYPING CYSTATIN B IN UNVERRICHT-LUNDBORG PME

Clinicians have known for the past 25 years ,at least since the report of Elridge et al. (1983) that phenytoin aggravates the ataxia and dementia of Unverricht Lundborg type of PME. More recent experience has shown that carbamazepine, gabapentin, vigabatrin and lamotrigine also aggravate the ataxia and dementia of Unverricht-Lundborg PME (Genton et al., 2005). In March 2007, during an International Workshop on Progressive Myoclonus Epilepsies in Sarlat, France, Tassinari (Italy) and Genton et al (France) independently validated the experience of clinicians that dementia and ataxia in Unverricht Lundborg PME are both iatrogenic and caused by specific antiepileptic drugs. They showed cohorts of Unverricht-Lundborg type of PME who had expansions of CCCCGCCCCGCG dodecamer in the 5'untranslated region of cystatin B. None of these patients received phenytoin or carbamazepine or oxcarbazepine or lamotrigine. Treatment relied on valproate plus a benzodiazepine like clonazepam or clobazam or plus topiramate or zonisamide. Patients also received high doses of piracetam or levetiracetam or combined piracetam

and levetiracetam and most recently brexiracetam (Koskiniemi et al., 1998; Genton and Gelisse, 2000; Genton, Guerrini Remy, 1999; Genton and van Vleyman, 2000; Genton et al., 2006; Magaouda et al., 2004; Crest et al., 2004; Kinrions et al., 2003). None had dementia and ataxia was at most minimal. Both Tassinari and Genton (2007) claim that these patients were clinically indistinguishable from juvenile myoclonic epilepsy because they had no dementia and ataxia was minimal. For this clinical reason, both Tassinari and Genton suggest that patients with adolescent onset myoclonias and grand mal seizures should be genotyped for cystatin B mutations to separate Unverricht Lundborg type PME from JME (see table 1). A second acceptable reason for genotyping in Unverricht Lundborg PME would be to look for rare nonsense mutations that can then be treated with premature termination codon readthrough drugs. A third reason for obtaining an accurate diagnosis of cystatin B mutations is N-acetylcysteine. Some families swear to the ameliorating effects of the long term use of this anti-oxidant in Unverricht Lundborg PME (Hurd et al, 1996).

GENOTYPING JME GENES

When genotyping for Lafora PME and Unverricht Lundborg PME gives negative results and parents are still wary of a progressive myoclonic epilepsy because of the presence of ataxia, it helps to genotype for JME and show its mutation causing gene. Such findings then allay the fear of a PME and justify genotyping for JME.

Of 13 chromosome loci genetically linked to JME, three mendelian genes (α 1-subunit of the GABA_A receptor [GABRA1], chloride channel 2 gene [CLCN2], and Myoclonin1/EFHC1) and two SNP-susceptibility alleles of putative JME genes in epistases (bromodomain-containing protein 2 [BRD2] and connexin [Cx]-36) have been identified, so far. Because none of these genes so far mandate a choice in antiepileptic drugs for treatment, or a change in management, genotyping is presently not justified for routine use in JME. The use of stop codon readthrough drugs cannot be justified for "compassionate use" to correct nonsense mutations in JME because JME is not a debilitating or fatal syndrome. When we obtain stop codon readthrough drugs which cross the blood brain barrier and whose adverse effects are equal to or less than antiepileptic drugs, then perhaps such drugs can be used to treat nonsense mutations in JME. When such happens, routine genotyping for JME will be justified.

GENOTYPING HELPS COUNSELING, PSYCHOLOGICAL AND SOCIAL SUPPORT

Genotyping not only ensures accurate diagnosis and helps treatment but it also leads to more accurate genetic counseling, psychological and social support for patients and families. Genotyping will direct families to organizations that have support groups and similar empathetic families who are educated about the PMEs or Dravet syndrome and who are devoted to finding cures for their specific epilepsy diseases. Social and psychological help is of critical importance in coping with depression and the debilitating neurologic deficits of PME or the cognitive problems of children in Dravet syndrome.

REFERENCES

- Alakurtti K, Weber E, Rinne R, et al. (2005). Loss of lysosomal association of cystatin B proteins representing progressive myoclonus epilepsy, EPM1, mutations. *Eur J Hum Genet* 13: 208-215.
- Barton-Davis ER, Cordier L, Shoturma DI, Leland SE, Sweeney HL (1999). Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice, *J Clin Invest* 104, 375-381.
- Brooks DA, Muller VJ, Hopwood JJ (2006). Stop-codon read-through for patients affected by a lysosomal storage disorder, *Trends in Mol Med*, 12(8): 367-373.

- Clancy JP et al. (2001). Evidence that systemic gentamicin suppresses premature stop mutations in patients with cystic fibrosis, *Am J Respir Crit Care Med*, 163, 1683-1692.
- Delgado-Escueta (2007). Advances in Lafora Progressive Myoclonus Epilepsy *Current Neurol Neurosci Rep*. 2007, Sept. 7(5):428-433,
- Kerem E (2004). Pharmacologic therapy for stop mutations: how much CFTR activity is enough? *Curr Opin Pulm Med* 10, 547-552.
- Leiuallen K, Pennacchio LA, Park M, Myers RM, Lennon GG (2001). Cystatin B-deficient mice have increased expression of apoptosis and glial activation genes. *Hum Mol Genet* 10: 1867-1871.
- Politano L et al. (2003). Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results, *Acta Myol.* 22, 15-21.
- Ramalho AS et al (2002). Five percent of normal cystic fibrosis transmembrane conductance regulator mRNA ameliorates the severity of pulmonary disease in cystic fibrosis. *Am J Respir Cell Mol Biol*, 27: 619-627.
- Wagner KR et al. (2001). Gentamicin treatment in Duchenne and Becker muscular dystrophy due to nonsense mutations. *Ann Neurol* 49, 706-711.
- Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, Wilde RG, Karp G, Takasugi J, Chen G, Jones S, Ren H, Moon Y-C, Corson D, Turpoff AA, Campbell JA, Conn MM, Khan A, Almstead NG, Hedrick J, Mollin A, Risher N, Weetall M, Yeh S, Branstrom AA, Colacino JM, Babiak J, Ju WD, Hirawat S, Northcutt VJ, Miller LL, Spatrick P, He F, Kawana M, Feng H, Jacobson A, Peltz SW, Sweeney HL (2007). PTC124 targets genetic disorders caused by nonsense mutations, *Nature*, 447, 88-93.
- Wilschanski M et al. (2003). Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N Engl J Med* 349: 1433-1441.

Table 1. Evolution of Some Myoclonic Epilepsies

| Epilepsies | Seizures | | Age (yrs) at onset of neurological deterioration | | | | | |
|--|--|--------------------|--|--------------------|--|---|---|-----------------------------------|
| | Age (yrs) at onset of Grand Mal, Myoclonic and Absence | Occipital Seizures | <i>Mild cognitive decline</i> | <i>Mild ataxia</i> | <i>Severe ataxia dementia, preserved daily living activities</i> | <i>Impaired/loss of independent daily living activities</i> | <i>Anorexia, swallowing difficulties, gastrostomy</i> | <i>Respiration Assist; Mutism</i> |
| (a) Laforin-deficient | 6-19 years (80% at 10-12 yrs) | Yes | 10 to 14 yrs | 10 to 14 yrs | 17 to 20 yrs | 16 to 20 yrs | 18 to 20 yrs | 20 to 25 yrs |
| (b) Malin-deficient | 7-15 yrs | Yes | 16 to 27 yrs | 16 to 27 yrs | 26 to 32 yrs | 26 to 32 yrs | 26 to 37 yrs | 26 to 37 yrs |
| Unverricht-Lundborg PME (cystatinB deficient)* | 7 to 16 yrs (86% at 9-13 yrs) | None | None | Yes | None | None | None | None |
| Juvenile Myoclonic Epilepsy (JME) * (Herpin-Rabot-Janzy type) | 8 to 26 yrs (75% at 12-18 yrs); (Mean age onset 14 yrs✓) | None | None | None | None | None | None | None |

* Some cases with myoclonus only

✓ Usually triggered by sleep deprivation, menses, stress, fatigue, alcohol