Clinical/Scientific Notes

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NOVEL *FHL1* MUTATIONS IN FATAL AND BENIGN REDUCING BODY MYOPATHY

Reducing body myopathy (RBM) is a rare disorder characterized pathologically by the presence of intracytoplasmic inclusions strongly stained by menadione-NBT (nitroblue tetrazolium) staining in the absence of the substrate α-glycerophosphate. The causative gene for RBM was recently identified as *FHL1* on chromosome Xq27 encoding four and a half LIM domains 1.[1] FHL1 is a 32 kDa protein, composed of four LIM domains preceded by a single N-terminal zinc finger. FHL1 is highly expressed in skeletal muscle and heart. Here, we searched for *FHL1* mutations in three sporadic cases[2-4] and one familial case[5] of RBM we previously reported.

Methods. All clinical materials used in this study were obtained for diagnostic purpose with informed consent. Patient 1 and patient 2 have fatal infantile form,[2,3] and patient 3 has adult-onset form.[4] Patients 4 (son) and 5 (his mother) had familial cases.[5] We directly sequenced all exons and their flanking intronic regions of *FHL1* in the five RBM patients and 250 Japanese controls. Frozen muscle specimens were examined by immunohistochemistry and immunoblotting using standard technique.

Results. We identified four novel mutations in *FHL1*: a heterozygous missense mutation of c.449G>A (p.C150Y) in patient 1 and c.302G>T (p.C101F) in patient 2, an in-frame 9 bp deletion at c.304-312delAAGGGGTGC (p.102-104delKFC) in patient 3, and a hemizygous mutation c.310T>C (p.C104R) in patient 4. The mother (patient 5) had the same mutation in heterozygous mode. All mutations we identified are located in the second LIM domain of FHL1 (figure e-1 on the *Neurology*® Web site at www.neurology.org).

Immunohistochemical analysis of patients' muscles showed strong immunoreactive depositions of FHL1, α5-integrin, myosin heavy chain-slow (MyHC-slow), ribosomal proteins, and nucleolar protein coilin (figure). Protein amount of FHL1 was significantly reduced in patients 2 and 4 with less reduction in patient 5 after normalization to actin level. In contrast, patient 3 showed mild increase in FHL1 (figure).

Supplemental data at www.neurology.org

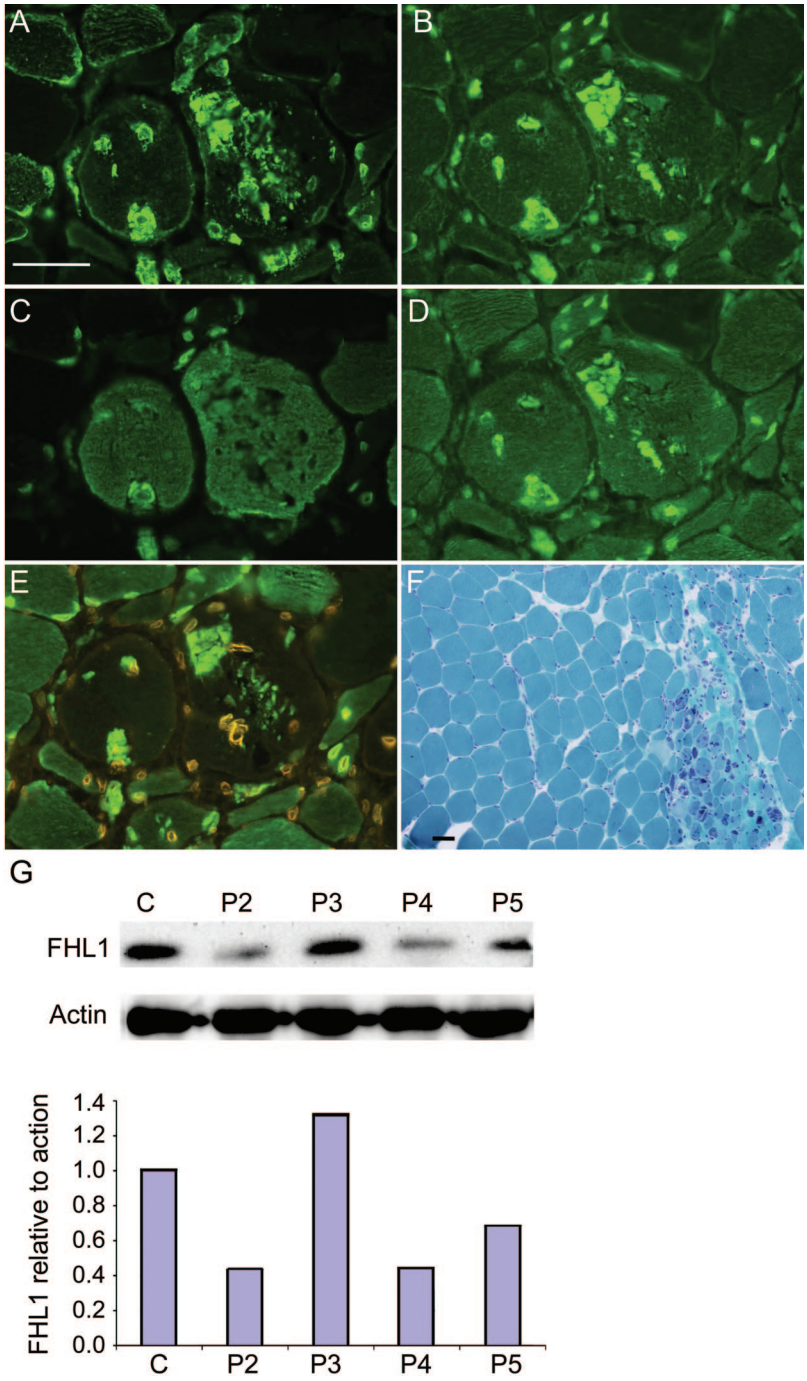
Discussion. All our RBM patients, with a wide range of clinical phenotypes, fatal infantile (patient 1 and 2), benign childhood (patient 4), and adult-onset (patients 3 and 5), had novel *FHL1* mutations, confirming the recent report that *FHL1* is the causative gene for RBM.[1] All the mutations identified in RBM patients affects the cysteine or histidine residues located within the second LIM domain of FHL1, indicating their irreplaceable role in stabilizing FHL1 (figure e-1). Phenotypic severity may depend on how the altered residue affects the zinc binding sites and resulting disruption of the structure and function of the LIM domain.

In this study, clinical severity is correlated with the amount of the FHL1 protein. Nevertheless, previously reported fatal RBM patients show increased FHL1 amount.[1] Since RBM shows asymmetric muscle involvement and focal pathologic changes in the same muscle specimen (figure), the decrease or increase of FHL1 amount may depend on the degree of affection of the biopsied part of the muscle. We should also consider the degree of protein degradation/turnover.

Here we showed that MyHC-slow is aggregated in patient muscles. It was reported that both overexpression and underexpression of FHL1 were associated with the failure of myosin to assemble into thick filaments. Aggregation of myosin was also noted in FHL1 knockdown cells. In RBM muscles, mislocalization of myosin filaments and the sarcomeric disassembly may be caused by FHL1 dysfunction. Surprisingly, α5-integrin was also highly aggregated in RBM patients although normally α5-integrin is expressed in myoblasts and during primary myogenesis, and is downregulated in mature muscle. FHL1 was reported to induce α5β1-integrin-dependent myocyte elongation. Whether or not there is a correlation between α5-integrin aggregation and the suggested role of FHL1 in integrin signaling and regulation of cytoskeletal dynamics during muscle differentiation is not clear.

To date, only 6 families and 16 sporadic patients with RBM have been reported. However, RBM patients may be overlooked and underestimated, since reducing bodies can be observed in selective parts of the muscle, as shown in the figure. Furthermore, menadione-

Figure **Immunohistochemical and immunoblotting analyses**



(A–E) Immunohistochemical analysis of patient 3 was performed using antibodies against FHL1 (AVIVA), α5-integrin (Chemicon), slow myosin heavy chain (MyHC-slow; Novocastra), ribosomal protein L28 (Santa Cruz), coilin (Sigma), and lamin C (see reference e-1 at www.neurology.org). Abnormal accumulation of FHL1 (A), α5-integrin (B), MyHC-slow (C), and ribosomal proteins (D) are seen. Double immunostaining of coilin (green) and lamin C (orange) revealed intracytoplasmic and perinuclear accumulation of coilin (E). These findings may be characteristic for reducing body myopathy (RBM) as it was observed in patients 2, 4, and 5 (fatal and benign RBM) but not seen in muscle specimens from a healthy control or diseased controls. Because of the limited amounts of the specimens, we could not examine in patient 1. Bar = 50 μm. (F) Modified Gomori-trichrome staining from patient 3 shows focal involvement in the muscle section. Bar = 50 μm. (G) Immunoblotting analysis of FHL1 in muscle specimens from patients 2, 3, 4, and 5 show variable amount of FHL1. Patients 2, 4, and 5 show significant reduction in FHL1 amount. Patient 4 (son) shows more reduction in FHL1 amount than patient 5 (his mother). Patient 3 shows slight increase in FHL1. Relative amount of FHL1 was calculated and normalized to actin (Nichirei).

NBT staining without substrate is not performed unless RBM is suspected. *FHL1* mutations have also been reported as the cause of X-linked scapuloperoneal myopathy (SPM)[6] and X-linked myopathy with postural atrophy (XMPMA).[7] Certainly, RBM, SPM, and XPMPA share common clinicopathologic features such as scapuloperoneal dominant muscle involvement, asymmetric muscle weakness, rigid spine, myofibers with core-like appearance on NADH, and rimmed vacuoles, and this finding raises a possibility that they may be a single entity. In addition, reducing bodies detected in a SPM patient strengthens this idea (unpublished data).

Further studies together with the identification of more RBM patients may help refine the diagnostic criteria for RBM and may explain the pathomechanism underlying the formation of reducing bodies which is unclear.

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ELEMENTAL MERCURY NEUROTOXICITY FROM SELF-INJECTION

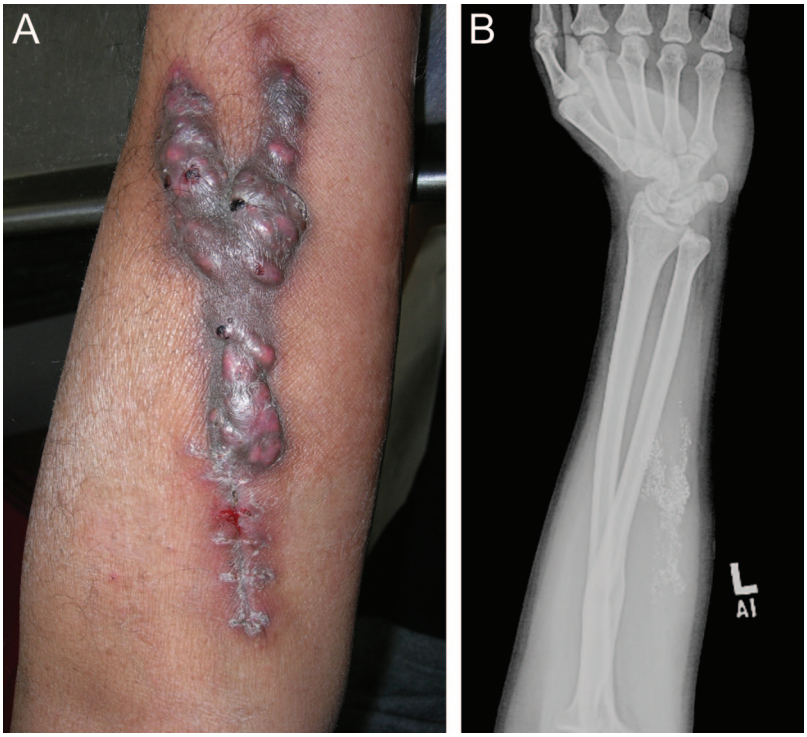
We describe a Guyanese diabetic man who developed an elevation in body burdens of mercury following repeated self-injection of elemental mercury. His early signs of neurotoxicity responded to excision of the injection sites and chelation therapy.

Case report. A 61-year-old diabetic man presented with burning pain in the feet and tremor of the hands. During his 20 years of insulin-dependent diabetes, his most common insulin injection sites were the forearms and abdominal wall. About 4 to 6 years earlier, he first experienced burning discomfort in the feet, attributed initially to his diabetes. This intensified, and a tremor appeared shortly thereafter, which had gradually become disabling. His handwriting steadily progressed to illegibility, and it was difficult for him to drink from a glass without spillage. In the past 4 years he also noticed the appearance of raised black protuberant scars over his volar forearms and abdomen at the usual injection sites.

Examination disclosed numerous raised, firm keloids over both forearms (figure, A). Mental status examination revealed normal cognitive function. There was a mild fine rapid tremor of the fingers at rest that became intense on movement. There was no rigidity, bradykinesia, or cogwheeling. Tendon reflexes were 2/4 in the upper limbs, absent in the lowers. Sensory abnormalities were confined to the distal lower limbs and were consistent with diabetic symmetric neuropathy. Nerve conduction studies disclosed absent sural, radial, median, and ulnar responses and reduced motor amplitudes in peroneal and tibial nerves. Psychiatric consultation revealed no psychiatric or cognitive disorders. A polar biopsy of one of the forearm lesions was unexpectedly consistent with a mercury granuloma. Subsequent radiographs of the forearm (figure, B) and abdomen radiograph demonstrated radiopaque densities consistent with mercury deposition.

A 24-hour urine mercury and whole blood mercury concentration (WBMC) were 321 μg/L (normal <20 μg/L) and 230 μg/L (normal <10 μg/L); whole blood lead was undetectable. IM dimercaprol (British anti-Lewisite) was administered pre- and post- surgical excision of the forearm and abdominal lesions. Oral succimer (2,3-dimercaptosuccinic acid) was provided postoperatively for 19 days. Further therapy was declined. At a 2-week follow-up examination, his tremor was diminished, his handwriting legible, and WBMC had fallen to 92 μg/L. Nine months later, the tremor had disappeared, the sensory (diabetic?) neuropathy was unchanged, and WBMC was 7 μg/L.

Figure Preoperative images from the left forearm



(A) Photograph of keloid following polar biopsy (before radical excision). (B) X-ray showing radiopaque subcutaneous deposition of mercury.

Discussion. Upper limb tremor is commonly the heralding symptom of elemental mercury neurotoxicity and improves pari passu with significant reduction in body burden.[1] Elemental mercury intoxication of sufficient magnitude to affect the adult CNS is usually associated with occupational inhalation of vapor.[2] While chelation removes very little mercury deposited in tissue, BAL followed by succimer was administered in this case due to the potential for extensive intraoperative mobilization.[3]

Self-administration of elemental mercury is usually associated with ointments, accidents, suicide attempts,[4] alternative medicine therapy, or tattoo augmentation. There is also a widespread belief in Far Eastern and Central and South American countries that mercury injection will increase limb and penile strength or ward off evil.[5,6] Elemental mercury is sold in urban religious stores (botanicas) for use in Esperitismo, Santeria, and voodoo magicoreligious practices, as well as for self-medication. A 1996 survey found 38 of 41 surveyed New York City botanicas sold capsules or vials of elemental mercury.[7] Despite warnings from public health advocates, pediatricians, and toxicologists, there is still no ban on the sale of elemental mercury. This should be pursued as clearly consequential misuse continues.

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LINEZOLID INDUCING COMPLEX PARTIAL STATUS EPILEPTICUS IN A PATIENT WITH EPILEPSY

Many antibiotics can worsen seizures in patients with epilepsy and provoke seizures in patients without epilepsy. The agents most commonly associated with this adverse effect include penicillin and other β-lactams, imipenem-cilastatin, and quinolones.[1] The mechanism by which antibiotics can induce seizures can be related to lowering seizure threshold by affecting neurotransmitters or by decreasing the efficacy of antiepileptic drugs (AEDs).[2,3]

Linezolid (Zyvox) is the first agent in a new class of antibiotics known as oxazolidinones. Oxazolidinones act primarily against Gram-positive bacteria by inhibiting protein synthesis. The most common side effects from linezolid are nausea, headache, and diarrhea.[4] Serious adverse effects such as reversible myelosuppression have been reported.[4,5] Linezolid may increase the risk of serotonin syndrome when taken with other serotonergic agents.[4,6] Seizures have been reported as side effects in the package insert in children[4]; however, no case reports of linezolid-induced seizures exist in the medical literature. We report a patient with epilepsy who experienced complex partial status epilepticus (CPSE) upon receiving linezolid.

Case report. The patient is a 45-year-old left-handed woman with a history of epilepsy since childhood. She has been followed in the Epilepsy Clinic at The Ohio State University (OSU) for more than 10 years. She has rare tonic-clonic seizures, frequent (3 per day) simple partial (right-sided dystonic posture for a few seconds), and frequent (2 per day) complex partial seizures (extension of both legs and clonic movements of right side lasting for less than a minute). Her seizures have been intractable despite trying all approved AEDs, vagus nerve stimulator, and two resective brain surgeries (left frontal lobe) that resulted in right-sided weakness. Her past medical history is significant for postictal psychosis and depression. Her medications included zonisamide, clonazepam, acetazolamide, aripiprazole, and lorazepam as needed. She had no history of unusual reaction to any medication.

On April 10, 2008, she underwent an excision of a painful wart-like growth on her right knee. Her wound did not heal well and a wound infection was suspected. Wound cultures on April 22, 2008, were consistent with nonpathologic skin flora. On April 24, 2008, she underwent debridement of her wound and was placed on IV vancomycin, then switched to IV linezolid on April 25, 2008, receiving one dose. She was discharged home on April 26, 2008, on oral linezolid. Two doses were taken. In the evening of April 26, 2008, her habitual complex partial seizures became more frequent and longer in duration. Oral lorazepam did not help. The seizures became almost constant. She was admitted to OSU Medical Center with a diagnosis of CPSE. She was intubated, placed on a propofol drip, and given IV levetiracetam in addition to her home AEDs. IV vancomycin was started in place of linezolid. Continuous EEG monitoring showed initially frequent seizures of left fronto-central onset that subsided with treatment. On the morning of April 28, 2008, propofol was stopped and she was successfully extubated. Her seizures were back to baseline (few daily seizures). Her workup ruled out any acute systemic or neurologic process. She was febrile at presentation; however, no source of infection was found. On April 29, 2008, all IV medications were switched to oral formulations with plans to complete a 7-day course of antibiotics. Oral linezolid was restarted on April 30, 2008. Late that morning, she started to have prolonged and frequent seizures. She received benzodiazepines and her levetiracetam was increased. Seizures became more frequent. On the morning of May 1, 2008, linezolid was stopped after a total of three doses. She was loaded with IV fosphenytoin. Within 18 hours, the

patient's seizure frequency improved dramatically and she seemed to be at her baseline. She was discharged home on May 2, 2008.

Discussion. Our patient developed CPSE and had worsening of her seizures. The patient had no acute neurologic or systemic illness to explain the worsening of her seizures. She has no history of status epilepticus in the past. Historically, she is a very compliant patient. Although we cannot be definite about the exact cause, the chronological correlation suggests that linezolid was the cause. Our patient was given linezolid on two separate occasions. On each occasion, within less than 24 hours of starting linezolid, she had significant worsening of her seizures. Seizures improved dramatically within 24 hours of stopping linezolid.

The mechanism by which linezolid worsened our patient's seizures is not clear. A review of the patient's concomitant medication list did not reveal any possible drug–drug interactions with linezolid. Unfortunately, the levels of zonisamide and clonazepam were not checked to determine if linezolid affected either one. Linezolid should be administered cautiously in patients with a history of epilepsy.

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