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Multiple Symmetrical Lipomatosis – A Mitochondrial Disorder of Brown Fat

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ABSTRACT

Multiple Symmetrical Lipomatosis (MSL) is an unusual disorder characterized by the development of axial lipomas in adulthood. The pathoetiology of lipoma tissue in MSL remains unresolved. Seven patients with MSL were followed for a mean period of 12 years (8-20 years). All patients had cervical lipomas ranging from subtle lesions to disfiguring masses; six patients had peripheral neuropathy and five had proximal myopathy. Myoclonus, cerebellar ataxia and additional lipomas were variably present. All patients showed clinical progression. Muscle histopathology was consistent with mitochondrial disease. Five patients were positive for mtDNA point mutation m.8344A>G, three of whom underwent lipoma resection – all samples were positive for uncoupling protein-1 mRNA (unique to brown fat). Lipoma from one case stained positive for adipocyte fatty-acid protein-2 (unique to brown fat and immature adipocytes). This long-term study hallmarks the phenotypic heterogeneity of MSL's associated clinical features. The clinical, genetic and molecular findings substantiate the hypothesis that lipomas in MSL are due to a mitochondrial disorder of brown fat.

Keywords: Multiple Symmetrical Lipomatosis, Madelung's Disease, Brown Fat, Uncoupling protein-1 mRNA, Adipocyte fatty-acid protein-2.

1. Introduction

Multiple Symmetrical Lipomatosis (MSL) is an unusual disorder characterized by the presence of non-encapsulated axial lipomas.(Berkovic et al., 1991a). Since Brodie's original phenotypic description of the condition in 1846,(Brodie, 1846) it has been variously labeled as Benign Symmetrical Lipomatosis,(Gonzalez-Garcia et al., 2004) Ekbom's Syndrome,(Ekbom, 1975) Madelung's Disease,(Madelung, 1888) and Launois-Bensaude Syndrome(Launois and Bensaude, 1898) – but the pathogenesis of lipoma formation in MSL remains unclear. A leading hypothesis is that MSL is fundamentally a mitochondrial disorder.(Berkovic et al., 1991a). Three lines of evidence support this: i) Phenotypic – the midline location of the lipomas (neck, mediastinum, axillae, periaortic, perirenal, suprapubic) mirrors the distribution of brown fat, a tissue known to be rich in mitochondria;(Kodish et al., 1974; Nechad, 1986) ii) Genetic – MSL has a well documented clinico-pathologic association with known mitochondrial cytopathies, such as myoclonic epilepsy and ragged red fibers (MERRF),(Austin et al., 1998; Berkovic et al., 1991a; Berkovic et al., 1991b; Gamez et al., 1998; Klopstock et al., 1994; Naumann et al., 1997) and mitochondrial mutations have been isolated from lipoma tissue in MSL;(Nisoli et al., 2002; 1995; Vila et al., 2000) iii) Molecular – uncoupling protein 1 (UCP-1), a mitochondrial carrier protein unique to brown fat, (Ricquier et al., 1991) has been detected in MSL lipomas.(Nisoli et al., 2002; Ricquier et al., 1991; Vila et al., 2000)

There are two riders here: i) Alcohol appears to be a risk factor for MSL,(Berkovic et al., 1991a; Haller and Knochel, 1984)and ii) Some investigators have failed to identify mitochondrial mutations in patients with MSL.(Heckmann et al., 2000; Klopstock et al., 1997; Matthews et al., 1995)

This long-term study has two aims – 1) to examine the clinical outcome in MSL, 2) to re-examine the twin hypotheses that MSL is a mitochondrial disorder and that MSL lipomas derive from vestigial brown fat.

2. Material and Methods

Seven cases with Multiple Symmetrical Lipomatosis (MSL) were followed for a mean period of 12 years (8-20 years) by clinical history, serial examination and investigation. All were of Australasian heritage except Patient 4, who was born in India. Mean interval from symptom-onset to diagnosis was 11.5 years (2-30 years). Investigations included magnetic resonance imaging (MRI) brain, electroencephalography (EEG), nerve conduction studies (NCS), electromyography (EMG), muscle biopsy, mitochondrial deoxyribonucleic acid (mtDNA) sequencing (blood, lipoma, muscle), uncoupling protein-1 (UCP-1) messenger RNA (mRNA) analysis (lipoma), and adipocyte fatty-acid protein-2 (aP2) immunohistochemistry (lipoma). One patient underwent post mortem examination. Muscle biopsy specimens were snap-frozen with liquid nitrogen. Cryostat sections were stained with hematoxylin and eosin (H&E), Sudan IV, gomori-trichrome, nicotinamide adenine dinucleotide (NADH), adenosine triphosphatase (ATPase) preincubated at pH 9.4, 4.6 and 4.3, succinate dehydrogenase (SDH), cytochrome c oxidase (COX), and periodic acid-Schiff (PAS) with and without diastase. Samples of post mortem muscle and sural nerve were fixed in glutaraldehyde for electron microscopy. All other tissues were fixed in buffered formalin for paraffin embedding. Paraffin sections were stained with H&E. Standard PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) and sequencing were used to screen for mitochondrial DNA mutations m.8344A>G and m.8363G>A – if absent, a Southern analysis was performed to screen for multiple deletions.(Marotta et al., 2004) – if multiple deletions were not detected, full mtDNA sequencing was then performed. TRIzol Reagent (Invitrogen) was used for RNA extraction from lipoma tissue and from 100 mg

control white fat (subcutaneous). 0.5 μg RNA was reverse transcribed using Sensiscript and Omniscript (Qiagen) to synthesize complementary DNA (cDNA). Phusion Hot Start DNA Polymerase (Finnzymes) and gene specific primers to human UCP-1 were used for cDNA amplification (UCP-1 Forward 5'-TAGGTATAAAGGTGTCCTGG - 3'; UCP-1 Reverse 5'-CACTTTTGTACTGTCCTGGTGG-3'). Human cyclophilin A cDNA (hCyp A) was amplified (to act as an internal control and to verify the integrity of RNA templates) using primers hCyp A Forward 5'-ATGGTCAACCCCACCGTG- 3', hCyp A Reverse 5'-TGCAATCCAGCTAGGCATG -3'.(Vila et al., 2000) For immunohistochemistry, 5μm paraffin-embedded sections of the formalin-fixed lipoma samples from Patients 5 and 6 were mounted onto glass slides and stained with an anti-aP2 polyclonal antibody raised against a synthetic peptide corresponding to the amino acid sequence of residues 121 to 132 of the aP2 protein.(Bennett et al., 1995) **(**The aP2 protein plays a role in lipid metabolism and is preferentially expressed in immature adipocytes, particularly brown fat cells and lipoblasts).(Joyner et al., 1999; Yang et al., 2001) Informed written consent for genetic and molecular studies was obtained from all patients. (Ethics board approval was not otherwise obtained because all other aspects of the study fell within the bounds of routine clinical patient work-up). Patient 1 gave permission for use of an identifiable image.

3. Results

Table 1 summarizes the clinical data.

Patient 1: (Figure 1) a female, presented at age 53 years with multiple axial lipomas, proximal myopathy and peripheral neuropathy. Past history included hypertension, diabetes mellitus (DM) type 2 and hyperlipidaemia. A posterior neck mass was first noted 10 years earlier and progressive enlargement caused neck and shoulder pain. Lipoma tissue was confirmed on post-resection histology. Within two years to presentation the neck mass had recurred and bilateral axillary masses had developed. By this time proximal limb strength had deteriorated and she struggled to climb a flight of stairs. Distal lower limb paraesthesia had developed over the preceding six months. Three years following presentation, proximal limb strength and sensory symptoms had worsened. A third resection of the cervical lipoma was performed but the mass had fully returned by 12 months. Co-enzyme Q 100 mg daily was tried for four years without clear benefit. Lower limb electromyography (EMG) and nerve conduction studies (NCS) at presentation revealed myopathic changes and a peripheral axonal sensory neuropathy; repeat studies eight years later confirmed disease progression with evidence of more widespread myopathic change and axonal degeneration. Muscle biopsy (vastus lateralis) showed atrophic fibers, cytochrome oxidase (COX)-negative fibers (though no ragged red fibers), and abnormal mitochondrial clumping around increased numbers of cytoplasmic lipid-containing vacuoles. Her 25 year old son presented with a two year history of proximal muscle weakness (but no lipomas) – his muscle biopsy (deltoid) showed ragged red fibers. Both mother and son were negative for mtDNA deletions and point mutations m.8344A>G, m.8363G>A (blood, muscle). Subsequent full mtDNA sequencing in Patient 1 did not reveal low level deletions or genomic variations of known pathological significance.

Patient 2: a female, presented at age 45 years with bilateral ptosis, facial weakness, dysphagia and proximal myopathy. Distal upper and lower limb dysesthesia began two years later and a posterior neck mass arose over the ensuing 10 years. Past history included DM type 2, psoriasis, hypothyroidism and hyperlipidaemia. Bilateral tarsorrhaphy partially relieved the ptosis. Coenzyme Q 200 mg daily subjectively improved her limb strength. At 20 years follow up, her dysphagia had progressed (she was unable to swallow dry solids) and she was having falls (from worsening myopathy and ataxia). EMG and NCS showed myopathic changes and a peripheral sensory axonal neuropathy. Muscle biopsy (deltoid) revealed COX-negative ragged red fibers. No mtDNA deletions or point mutations (m.8344A>G, m.8363G>A) were found (blood, muscle). Subsequent full mtDNA sequencing did not reveal variations of recognized pathological significance.

Patient 3: a male, presented at age 45 years with a 15 year history of recurrent, massive cervical lipomas, myoclonus, generalized seizures, deafness and gout. There was a two year history of worsening gait ataxia, cognitive impairment, dysarthria, dysphagia and proximal limb weakness. Cervical lipoma surgery was performed three times with each excision netting over one kilogram of lipomatous tissue. At 11 years follow-up, his increasing ataxia and myoclonus led to wheelchair dependence. Audiometry was consistent with severe mixed hearing loss bilaterally, maximal for higher frequencies. Lower limb EMG and NCS reflected a myopathic process associated with mild neuropathic changes. Muscle biopsy (vastus lateralis) revealed focal necrotizing myositis and fiber atrophy with COX-negative ragged red fibers. MRI brain showed mild generalized cerebral atrophy. EEG showed polyspike wave discharges and theta background slowing. Mitochondrial DNA analysis was positive for the tRNA Lys MERRF mutation (m.8344A>G) blood (78% heteroplasmy), lipoma (84%). UCP-1 analysis of lipoma tissue was positive for UCP-1 mRNA(Figure 3).

Patient 4: a female, presented at age 36 years with a 10 year history of neck and interscapular lipomas. Myoclonus and generalized seizures had been present for 15 years. Past history included multiple spontaneous abortions and hypothyroidism. Her youngest son had progressive myoclonic epilepsy, proximal myopathy, but no lipomas; her other son and a daughter were well. Her eldest brother had midline lipomas; her sister had a large cervical lipoma and her son had myoclonus and seizures (Figure 2A). The patient deteriorated over the subsequent 12 years with worsening myoclonus, proximal myopathy, gait ataxia, and a peripheral sensory neuropathy. Her EEG showed generalized polyspike wave on a normal background rhythm. The cervical lipoma had been resected twice but quickly recurred on each occasion. Blood mtDNA analysis was positive for the m.8344A>G point mutation (88%). Her children were also mtDNA m.8344A>G positive (both sons 86%, daughter 84%).

Patient 5: a female, presented at age 37 years with multiple cervical lipomas – the largest was resected 10 years previously but soon recurred. There was a nine year history of progressive gait ataxia and a two year history of cerebellar dysarthria, deafness, upper limb inco-ordination and myoclonus. The clinical course was marked by worsening ataxia and myoclonus such that, at 12 years follow-up, she was wheelchair dependent. At presentation, audiometry confirmed bilateral high frequency sensorineural hearing loss. Lower limb NCS reflected a sensory axonal neuropathy. EEG showed frequent epileptiform discharges. MRI showed cerebellar atrophy. Muscle biopsy (quadriceps) revealed an excess of COX-negative fibers; mtDNA analysis confirmed the m.8344A>G point mutation (99% muscle, 99% lipoma). UCP-1 analysis of lipoma tissue was positive for UCP-1 mRNA (Figure 3). Histology showed variation in fat cell size with a few brown fat cells and numerous small lipoblast-like fat cells which expressed aP2 (Figure 4A).

Patient 6: a female (mother of Patient 5), presented at age 63 years with a 30 year history of deafness and recurrent cervical lipomas. While the subsequent 12 years saw a worsening of

her deafness, no further neurological symptoms developed. Blood and lipoma mtDNA analyses were positive for the m.8344A>G point mutation (75% blood, 96% lipoma); UCP-1 analysis of lipoma tissue was positive for UCP-1 mRNA (Figure 3). Histopathology of the lipoma showed mature fat cells with little variation in size; no lipoblasts or brown fat cells were identified and there was no staining for aP2 (Figure 4B).

Patient 7: a female, presented at age 41 years with optic atrophy, cervical lipomas, and deafness. Her mother had myoclonus and deafness (but no lipomas); a sister had midline lipomas and deafness (Figure 2B). The patient re-presented three years later with myoclonus complicated by falls. EEG showed paroxysmal epileptiform discharges. EMG of the lower limbs revealed changes consistent with a mild myopathic process. By 10 years, the lipomas had enlarged (despite earlier resections), her deafness and myoclonus had worsened, and she was experiencing dysphagia and progressive respiratory muscle weakness. Invasive mechanical ventilation was offered but she declined. She ultimately died from type 2 respiratory failure. Post-mortem examination confirmed non-encapsulated lipoma tissue at the posterior neck. The diaphragm showed marked fiber atrophy and COX-negative ragged red fibers (Figure 5A). On electron microscopy (Figure 5B), mitochondria were enlarged with paracrystalline inclusions and reduced cristae (skeletal muscle, sural nerve). Skeletal muscle mtDNA analysis was positive for the m.8344A>G point mutation (96%).

4. Discussion

4.1 MSL clinical features and disease course

The range of clinical features seen across the cohort (Table 1) underscores the phenotypic heterogeneity of this disorder for both clinical presentation and disease course. Diagnostic delay was common – while the mean age at presentation was 45.7 years (36-63 years), symptoms had been present for some time (2-30 years). All patients had cervical lipomas seated at the posterior neck. These varied from subtle midline lesions to large disfiguring

masses. Without exception, lipoma resection (for neck pain or cosmesis) was followed by recurrence, often rapid (an experience in line with earlier reports).(Gabriel et al., 2001) Most (five patients) had proximal myopathy in combination with a predominantly axonal peripheral sensory neuropathy. Myoclonus, cerebellar ataxia, deafness, dysarthria, dysphagia, optic atrophy, and additional midline lipomas were variably present. Such phenotypic heterogeneity is a common feature of the mitochondrial disorders as a group and it is thought to reflect 1) the percentage variation in mutant to wild-type mitochondrial DNA as a function of tissue type (heteroplasmy) and 2) tissue-specific vulnerability to defects in mitochondrial-driven oxidative metabolism (threshold effect).(DiMauro and Schon, 2003)

All patients evidenced disease progression of varying severity with the most extreme case (Patient 7) succumbing to type 2 respiratory failure from end-stage respiratory muscle weakness. While this phenomenon has been cited in MERRF,(Hammans et al., 1993) few deaths have been described in MSL,(Enzi et al., 2002) (though most studies are retrospective case reports blinded to clinical outcome). While not the case here, cardio-respiratory function can also be compromised in MSL when large lipomatous deposits crowd the mediastinum.(Enzi, 1984) We agree with Enzi et al that the label "Benign Symmetrical Lipomatosis" be abandoned. (Enzi et al., 2002)

4.2 MSL as a mitochondrial cytopathy

All patients met the diagnosis of 'clinically definite' mitochondrial disease based on the revised classification system by Bernier and colleagues.(Bernier et al., 2002) The concept of MSL as a mitochondrial disorder was first raised 20 years ago in a four-patient case series,(Berkovic et al., 1991a) (independent of the present series), in which all patients had cervical lipomas and all had genetic and histological evidence of a concurrent mitochondrial cytopathy. As also reflected by our series, the most common mtDNA defect linked to MSL is the m.8344A>G point mutation (known to occur in MERRF);(Austin et al., 1998; Berkovic et

al., 1991a; Gamez et al., 1998; Naumann et al., 1997) but single large scale deletions,(Campos et al., 1996) multiple deletions,(Klopstock et al., 1994) and the m.8363G>A point mutation(Casali et al., 1999) have also been reported. Several investigators, however, have failed to detect abnormalities of mtDNA in MSL,(Heckmann et al., 2000; Klopstock et al., 1997; Matthews et al., 1995) and two patients tested negative for mtDNA mutations in our study. We would argue that this does not exclude MSL as a mitochondrial cytopathy because complete nuclear DNA (nDNA) sequencing was not performed – it is estimated that > 95% of mitochondrial disorders arise from errors in the nDNA programming of mitochondrial function.(Finsterer, 2005) While there was no history to suggest Mendelian inheritance in our two patients, we could not rule out the possibility of spontaneous nDNA mutations. Tissue-specific heteroplasmy will also influence laboratory detection of mitochondrial defects – some tissues are more likely to declare a mitochondrial mutation than others – in MSL, mutant mtDNA loads tend to be highest in lipoma tissue versus muscle or blood.(1995; Vila et al., 2000) Fresh lipoma tissue was unavailable in our two patients whose blood and muscle samples tested negative for a mitochondrial mutation, though both patients had histological changes on muscle biopsy favoring a mitochondrial cytopathy. Other investigators have failed to show such changes and cite this as further evidence against the mitochondrial hypothesis in MSL.(Matthews et al., 1995) The problem here is that mtDNA mutations do not necessarily translate as structural abnormalities in muscle and, when they do, muscle denervation and atrophy can limit their detection.(Berkovic et al., 1991a) When these factors are taken into account, we contend that the available histological and genetic evidence (from the present study and from previous work) weighs in favor of MSL as a mitochondrial cytopathy.

4.3 MSL lipomas arise from brown fat

Three patients underwent lipoma resection during the course of the study and all lipoma samples were positive for UCP-1 mRNA expression. We could not test lipomas in the other four patients as either lipoma tissue was never resected (Patient 2 had a small cervical lipoma) or the UCP-1 assay was not available at the time of lipoma resection (Patients 1, 4, 7) – the stored histological samples were unsatisfactory for subsequent UCP-1 analysis. Vila and colleagues(Vila et al., 2000) tested lipoma samples from two patients with mtDNA mutations but the results were contradictory. Kazumi and colleagues(Kazumi et al., 1994) tested one patient and the result was negative – arguably because they used Northern blots (a less sensitive detector of weakly expressed UCP-1 mRNA).(Nisoli et al., 2002) Nisoli's group(Nisoli et al., 2002) tested ten patients with 'characteristics' of MSL (no other clinical data were given) and while all lipoma tissue samples were positive for UCP-1, only three patients had an identifiable mutation (4977-bp mtDNA deletion). UCP-1 is unique to brown fat – the high mitochondrial load gives it this hue –but lipomas are macroscopically white on resection because lipid storage in inactive cells renders brown fat white on appearance. Why might lipomas of brown fat origin arise in patients with mtDNA mutations? Brown fat is used by mammals for thermoregulation, a process that relies on UCP-1 acting as a carrier protein to 'short circuit'(Nisoli et al., 2002) the oxidation phosphorylation pathway –heat is generated at the cost of reduced ATP synthesis – the cascade is triggered by noradrenaline (NA), which stimulates the brown fat beta-3 adrenoceptor. Because the pathway is coupled to normal lipolysis in brown fat, defective mitochondrial respiration, with reduced uptake of free fatty acids, might be expected to lead to lipid accumulation and lipoma formation.(Berkovic et al., 1991a) (Defective mitochondrial respiration from the m.8344A>G mutation in MERRF is associated with a decrease in the synthesis of complex IV/cytochrome c oxidase,(Antonicka et al., 1999) which potentially leads to decreased beta-oxidation.) An alternative hypothesis – that reduced lipolysis arises from a defect at the level of the beta-3

adrenoceptor – did not gain support from Nisoli's group(Nisoli et al., 2002) who saw no effect of beta-3 adrenoceptor gene polymorphism on NA signaling.

While defective mitochondrial respiration might lead to lipoma formation in MSL, what of the observation by investigators that UCP-1 mRNA expression is *reduced* in brown fat harboring mitochondrial mutations.(Nisoli et al., 2002; Puigserver et al., 1992) We would argue that our positive UCP-1 results remain compatible with a mitochondrial hypothesis for MSL because UCP-1 mRNA expression is not necessarily lost, but reduced *relative* to 'normal' brown fat (infantile brown fat, hibernoma). Without access to normal brown fat to act as a 'strongly' positive control, we could not quantify UCP-1 expression in our patients, though we would anticipate lower UCP-1 levels against normal brown fat.(Manieri et al., 2010) Partial UCP-1 mRNA expression in mitochondrial disease might stem from a threshold effect or a failure of a mitochondrial defect to completely inactivate NA-induction. This might partly explain negative UCP-1 results in MSL lipomas when a less sensitive testing method is used.(Kazumi et al., 1994)

4.4 MSL lipomas may contain immature adipocytes

UCP-1 was expressed in lipomas with high mutant loads in more uniform, fairly mature (aP2 negative) inactive-appearing adipocytes (Patient 6, 96% load), as well as in lipoma tissue with a combination of mature and immature (aP2 positive) adipocytes (Patient 5, 99% load). Because the UCP-1 and aP2 studies were performed in different centers, we cannot comment on the relative degrees of UCP-1 expression by the different cell subtypes within the mixed histology lipoma (Patient 5); interestingly though, a recent UCP-1 immunohistochemistry study of a rare hibernoma showed variable expression of UCP-1 between the more typical histologically immature brown adipocytes (strongly UCP-1 positive) and the more inactive white adipose-like cells (weakly UCP-1 positive or UCP-1 negative).(Manieri et al., 2010) It seems plausible then, that the more immature aP2 positive lipoblasts resembling brown fat

cells in the lipoma sample from Patient 5 would have higher UCP-1 expression than the sample's white adipose-like components. The positive aP2 staining for Patient 5 might also flag the presence of some 'active' brown fat cells or a greater rate of lipoma proliferation, which would be in keeping with the rapid regrowth post-resection seen in this patient – the histology and aP2 staining in Patient 5 certainly differed from a typical benign lipoma (in which aP2 is usually negative). Further work is needed to clarify the link between UCP-1 and aP2 expression in MSL.

4.5 Lipoma formation in MSL may be a multifactorial process

Despite the above argument for a brown fat origin of lipomas in MSL, proof will only come when the full pathogenic sequence is unraveled at both the genetic and molecular levels. This proof should also satisfy the obverse question – Why don't all patients with proven mitochondrial mutations develop midline lipomas? Tissue heteroplasmy and the threshold effect noted earlier are two potential factors, but other (non-genetic) factors, such as alcohol, seem to influence disease expression. The highest incidence of MSL reported is amongst alcoholic Mediterranean males (Italy 1/25000).(Enzi et al., 1985) Alcohol is a mitochondrial toxin and it promotes liponeogenesis by reducing beta oxidation at the level of the beta adrenoceptor.(Banerjee et al., 1978) We disagree though with Ampollini and Carbognani(Ampollini and Carbognani, 2011) who recently implied that alcohol is a dominant factor in MSL – this is not representative of the MSL literature (indeed, none of our patients were alcoholics).

4.6 Therapeutic options

Future work on the interplay between the genetic and environmental determinants of brown fat metabolism will hopefully solve the riddle of lipoma pathogenesis in MSL. The goal is for targeted medical therapy to treat what is often a painful, disfiguring, and potentially fatal

disease. Surgery – the only therapeutic option these patients have at present – is generally unsatisfactory (masses inevitably recur) and re-resection is not without risk.

5. Conclusion

This study on a cohort of seven patients with Multiple Symmetrical Lipomatosis with long-term follow-up underscores the phenotypic heterogeneity of the disorder. Beyond the development of midline lipomas, affected patients can carry an array of associated clinical features including myoclonic epilepsy, proximal myopathy, peripheral neuropathy and cerebellar ataxia. Disease progression is the rule. Our clinical, histological, and genetic findings support the hypothesis that MSL is a mitochondrial disorder, while the UCP-1 mRNA and aP2 immunohistochemistry findings support the hypothesis that lipomas in MSL originate from brown fat. While the exact pathogenic mechanism of lipoma formation in MSL remains elusive, we suspect that defective mitochondrial respiration interferes with normal lipolysis in brown fat and this potentiates the development of midline lipomas. The immediate implications for clinical practice are that midline lipomas should serve to flag the presence of mitochondrial disease in patients with otherwise puzzling neurological symptoms and signs, and that such patients should be offered genetic counseling.

Table 1 Clinical data for the seven Multiple Symmetrical Lipomatosis (MSL) patients

Pt	Lipoma	Clinical Findings	Electrophysiology	Histopathology	Genetic, Molecular
1	cervical, axillary, occipital, suprapubic interscapular (large)	myopathy, neuropathy	NCS sensory neuropathy EMG myopathic	COX -ve fibers (M)	mtDNA (B/M) -ve
2	cervical (small)	myopathy, neuropathy, bulbar weakness, ptosis	NCS sensory neuropathy EMG myopathic	RRF (M)	mtDNA (B/M) -ve
3	cervical (massive)	myoclonus, myopathy, neuropathy, deafness ataxia, bulbar weakness	EEG Polyspike-wave NCS sensory neuropathy EMG myopathic	RRF (M)	mtDNA (B/M/L) m.8344A>G+ve UCP-1mRNA (L) +ve
4	cervical, interscapular (large)	myoclonus, myopathy, neuropathy, ataxia	EEG Polyspike-wave	not performed (M)	mtDNA (B) m.8344A>G+ve
5	cervical (large)	myoclonus, ataxia bulbar weakness neuropathy	EEG Polyspike-wave NCS sensory neuropathy	COX-ve fibers (M) Mixed fat cells, some aP2 +ve lipoblasts (L)	mtDNA (M/L) m.8344A >G +ve UCP-1mRNA (L) +ve
6	cervical (large)	deafness	not performed	Mature fat cells, aP2 -ve (L)	mtDNA (B/L) m.8344A>G+ve UCP-1mRNA (L) +ve
7	cervical, occipital (large)	myoclonus, myopathy, neuropathy, ataxia ataxia, optic atrophy	EEG Polyspike-wave EMG myopathic	RRF, COX -ve (M) abnormal mitochondria (N,M)	mtDNA (B) m.8344A>G+ve

Abbreviations: Pt (Patient), NCS (nerve conduction study), EMG (electromyography), EEG (electroencephalography), M (skeletal muscle), N (nerve), mtDNA (mitochondrial deoxyribonucleic acid), B (blood), L (lipoma), RRF (ragged red fibers), COX (cytochrome oxidase), UCP-1 mRNA (uncoupling protein-1 messenger ribonucleic acid), m.8344A>G (mtDNA point mutation adenosine to guanine, position 8344), aP2 (adipocyte fatty-acid protein 2), +ve (positive), -ve (negative) .

Competing Interests: Nil

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Contributorship: As corresponding author, I confirm that all Authors listed met the ICMJE criteria for authorship.

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Figure Captions

Figure 1 Patient 1 at presentation

Multiple axial lipomas are visible at the central abdominal and suprapubic regions, at both axillae (left), and at the posterior neck (right).

Figure 2 Pedigrees

Pedigree data for Patient 4 (A) and Patient 7 (B). Note the phenotypic variation within and between the two pedigrees for the same mtDNA point mutation (m.8344A>G), a feature that typifies mitochondrial disease.

Abbreviations: A (ataxia), D (premature onset deafness), L (midline lipoma), M (myoclonus), MP (myopathy), S (seizures).

Figure 3 Lipoma UCP-1 expression

A. UCP-1 was first detected in the proband lipoma (P5L). This served as the UCP-1 positive control. Non-lipoma adipose tissue (white control fat, CF) was negative for UCP-1 and

served as the negative control. Two lipoma samples from Patient 6 ($P6L^1$, $P6L^2$) were then run; both were UCP-1 positive against the P5L reverse transcript (RT) control. Vertical line delineates DNAse treated RNA with no reverse transcriptase (NRT) UCP-1 PCR and DNAse treated RNA reverse transcriptase (RT) UCP-1 PCR.

B. Expression of UCP-1 in lipoma tissue of Patient 5 (P5L) and Patient 3 (P3L). Human cyclophilin A (hCypA) expression is shown to indicate RNA integrity in white control fat and lipoma samples. Result reproducibility is evidenced by Patient 5 lipoma (P5L) remaining positive for UCP-1 ten years after initial testing (2A); serving again as the positive control, it flagged UCP-1 expression in P3L.

Abbreviations: M (marker), H (water control), P3L (Patient 3 lipoma), P5L (proband patient 5 lipoma), P6L (family member patient 6 lipoma), CF (non-lipoma white control fat), [1] First RNA extraction [2] Repeat RNA extraction, PH (PCR water control), hCypA (Human cyclophilin A), UCP-1 (uncoupling protein-1).

Figure 4 Lipoma aP2 immunohistochemistry

A. Indirect immunoperoxidase staining for aP2 of lipomatous tissue from Patient 5 showing variation in fat cell size with numerous small (lipoblast-like) and brown fat cells which react for aP2 (two examples arrowed).

B. Indirect immunoperoxidase staining for aP2 of lipomatous tissue from Patient 6 showing mature fat cells which show little variation in size and are negative for aP2.

Figure 5 Diaphragmatic muscle biopsy (Patient 7)

A. Diaphragmatic muscle succinate dehydrogenase (SDH) stain (dark blue) from Patient 7 showing sub-sarcolemmal aggregates of abnormal mitochondria (asterisked) in "ragged red fiber" distribution (x 200).

B. Diaphragmatic muscle high powered electron micrograph (EM) from Patient 7 showing paracrystalline inclusions (asterisked) in abnormal mitochondria. (x 44,100)

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Figure 1. Patient 1at presentation

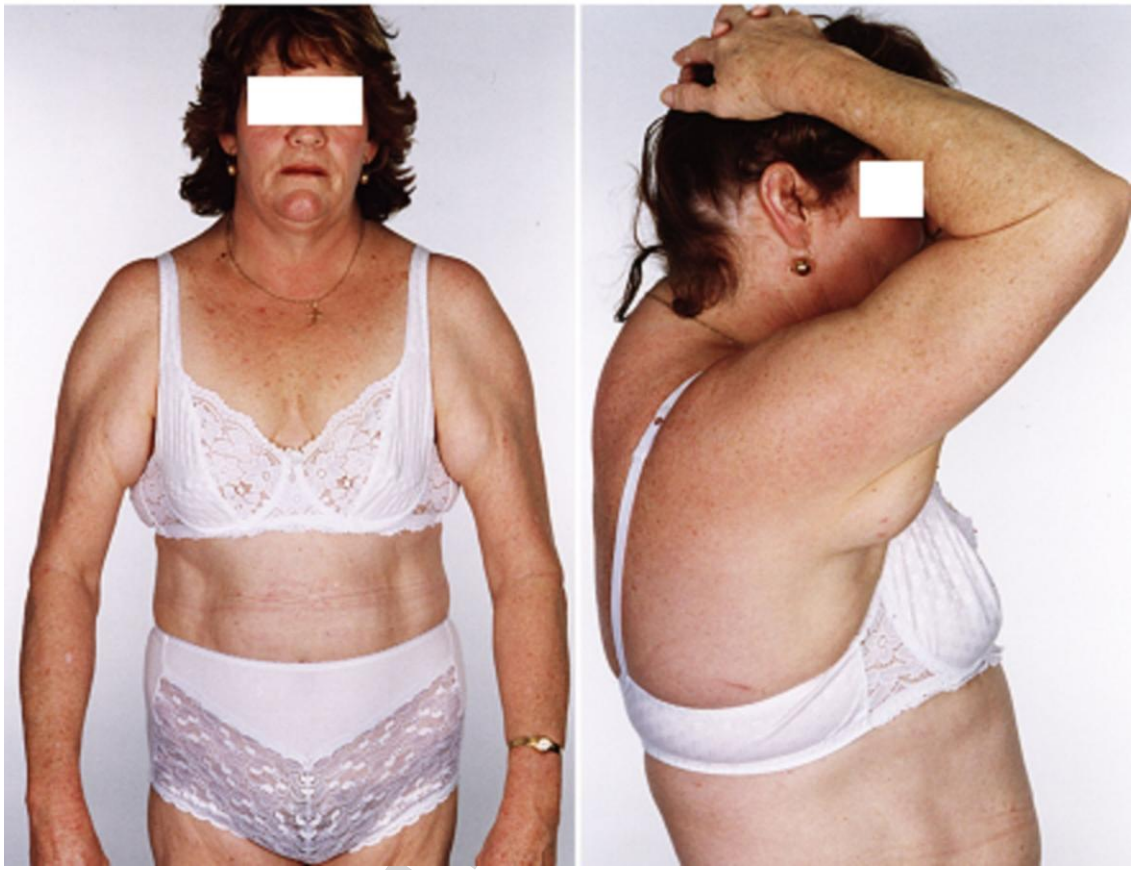
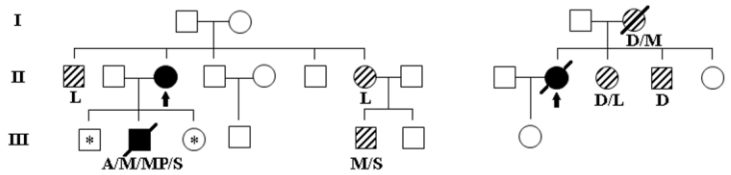


Figure 2. Pedigrees



A. Patient 4 **B. Patient 7**

index patient

symptoms/signs of mitochondrial disorder in presence of mitochondrial mutation

symptoms/signs of mitochondrial disorder in absence of genetic testing

no symptoms/signs of mitochondrial disorder in presence of mitochondrial mutation

no symptoms/signs of mitochondrial disorder

Figure 3. Lipoma UCP-1 expression

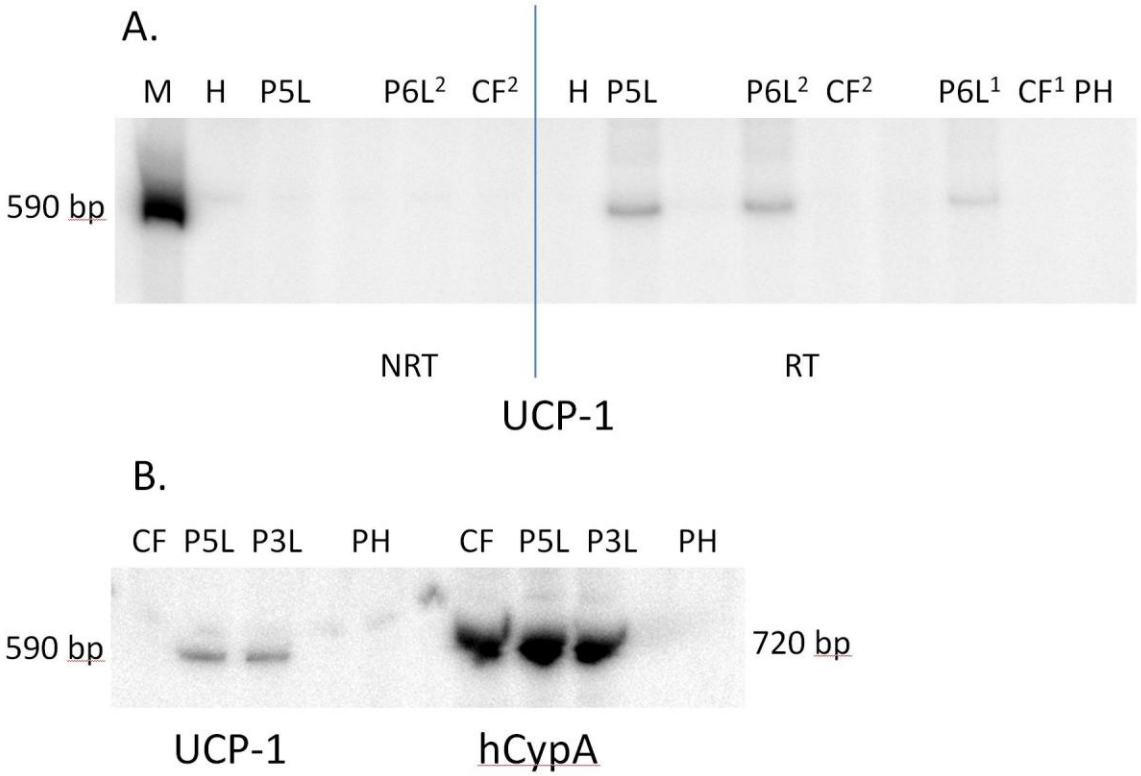
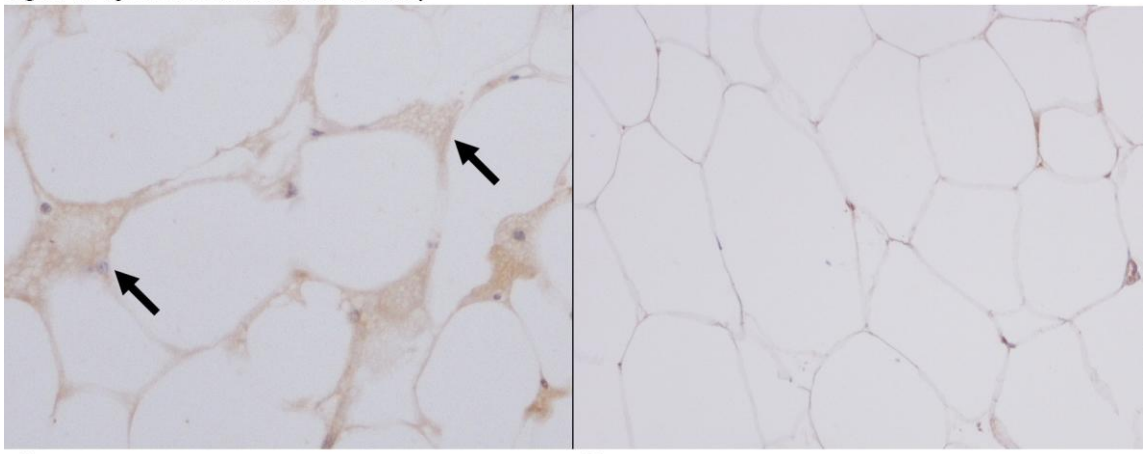
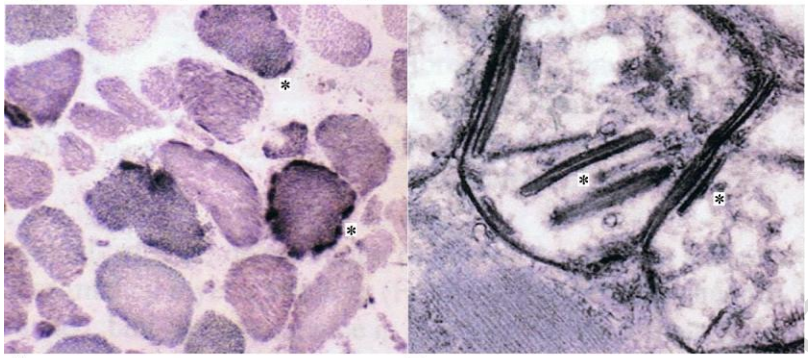


Figure 4. Lipoma aP2 immunohistochemistry



A. B.

Figure 5. Diaphragmatic muscle biopsy (Patient 7)



A. B.

Highlights: "Multiple Symmetrical Lipomatosis – A Mitochondrial Disorder of Brown Fat"

1. This is a long-term (8-20 year) follow up of seven patients with Multiple Symmetrical Lipomatosis (MSL).

2. The clinical, electrophysiological, histological, and genetic evidence substantiates the hypothesis that MSL is a mitochondrial cytopathy

3. The molecular and immuno-histochemical evidence strengthens the hypothesis that MSL lipomas are derived from vestigial brown fat.