各論VII

2. Familial X-linked myalgia and cramps

2 Familial X-linked myalgia and cramps

☑ 疾患の要点

・X 連鎖性の遺伝性疾患で，筋痛や有痛性筋攣縮を特徴とし，筋力低下がみられないことが特徴である
・血清のクレアチンキナーゼの上昇，時にミオグロビン尿症を呈する
・筋病理では筋原性の変化を呈することが多い
・遺伝子解析ではジストロフィン遺伝子の欠失を示す
・ジストロフィン遺伝子欠損部位から，ベッカー型筋ジストロフィーの mild type か variant であると推定されている

■遺伝情報：*dystrophin*　染色体部位 Xp21.1
■遺伝形式：X 連鎖性劣性遺伝
■OMIM #300376

代謝の病態

本疾患は，明らかな筋エネルギー代謝系の異常がなく，筋力低下を伴わず，運動あるいは平常活動によって誘発される筋痛と有痛性筋れん縮を特徴とする X 連鎖性劣性の遺伝性疾患として，1989 年に Gospe らが提唱した疾患である[1]．ジストロフィン解析ではジストロフィン遺伝子の欠失やジストロフィンサイズの変化が報告されており，ジストロフィン異常症と考えられている．

ジストロフィン遺伝子は X 染色体の p21 に存在し 79 個のエクソンからなり，筋細胞膜を裏打ちする細胞骨格タンパクであるジストロフィンタンパクをコードしている．ジストロフィンタンパクは筋肉の細胞膜の内側に存在し，細胞膜を支えるのに不可欠な蛋白で，4 つの領域に分類されており，N 末端，ロッド，システインリッチ，C 末端がある．

ジストロフィン遺伝子変異が疾患を引き起こす過程には，①遺伝子レベル：ジストロフィン遺伝子の変異が生じる．②タンパクレベル：機能が異常なタンパク質ができる，あるいは蛋白質が作られない．③細胞レベル：細胞の正常な機能が保てない(ジストロフィン異常で細胞膜が弱くなる)．④組織レベル：筋肉の壊死や線維化，脂肪化が生じる．⑤機能レベル：筋痛や有痛性筋れん縮といった症状から，筋力低下による運動機能や嚥下機能，呼吸機能，心機能の障害を引き起こすことがあり，重症度はその遺伝子変異に影響される．

本疾患もこの機序により起こると考えられるが，臨床症状としてはいずれも軽症で，筋以外の症状は示さないとされている．

臨床症状，病型

運動あるいは平常活動で誘発される筋痛，有痛性筋れん縮を特徴とし，発症時期は様々だが，幼児期から思春期の発症が多い．発達は正常で，身体診察では腓腹筋の仮性肥大を認めるが，筋力低下や神経学的異常所見は認めない．発熱時や運動時に褐色尿(ミオグロビン尿)を認めることがある．進行性の経過はまれで，運動不耐性を有する場合と有さない場合がある．これまでの報告においては心筋罹患の報告はない．

前述の Gospe らの報告後も孤発例の報告が散見され，病因としてジストロフィン遺伝子の欠失が報告されている(表 1)．症例について概説すると，Figarella-Branger ら[3]は，運動不耐性を有し，免疫組織学的な異常を示した男児 5 例を報告し，DNA 解析で 5 例中の 2 例でジストロフィン遺伝子の欠失(エクソン 45 ～ 52，エクソン 13 ～ 19)を認めた．Ishigaki ら[4]は，生後 28 か月に筋痛，

表1　ジストロフィン遺伝子の欠失が同定された症例まとめ

参考文献	遺伝子欠失	発症年齢(歳)	診断年齢(歳)	筋痛	有痛性筋れん縮	腓腹筋肥大	筋力低下	心症状	血清CK上昇(IU/L)	ミオグロビン尿症
1)	ｴｸｿﾝ 10～22	5	33	+	+	+	−	−	+，(1,458)	−
	10～22	nd	26	+	+	+	−	−	+，(2,200)	nd
	10～22	nd	22	+	+	+	−	−	+，(2,000)	nd
	10～22	2	12	+	+	nd	−	−	+，(2,213)	nd
	10～22	幼児期	16	+	+	+	−	−	+，(4,030)	−
	10～22	nd	10	+	+	+	−	−	+，(7,990)	nd
	10～22	nd	6	+	+	+	−	−	+，(12,270)	+
	10～22	nd	4	+	+	+	−	−	+，(11,750)	nd
2)	ｴｸｿﾝ 45～52	4	49	+	+	+	−	−	+，(2,560)	+
	45～52	小児期	76*	−	+	+	−	−	+，(384)	−
	45～52	<10	45	+	+	+	−	−	+，(1,250)	−
	45～52	<10	40	+	+	+	−	−	+，(953)	−
	45～52	小児期	52*	−	+	+	−	−	+，(327)	−
	45～52	4	17	+	+	+	−	−	+，(4,000)	+
	45～52	3	7	+	+	nd	−	−	+，(3,212)	+
3)	ｴｸｿﾝ 45～52	nd	19	+	+	−	−	−	+	−
	13～19	nd	30	+	+	−	−	−	+	−
4)	ｴｸｿﾝ 13～18	2	4	+	+	−	−	−	+，(2,202)	+
5)	ｴｸｿﾝ 45～51	nd	8	+	−	+	−	−	+，(>1,000)	nd
6)	ｴｸｿﾝ 45～48	nd	9	+	−	+	−	−	+	+

nd: no data，*：女性例

有痛性筋れん縮を発症した4歳男児の症例を報告した．身体所見や筋生検では異常所見はみられなかったが，安静時血清クレアチンキナーゼ(creatin kinase：CK)の上昇がみられ，DNA解析ではジストロフィン遺伝子のエクソン13-18に欠失がみられた．Kleinsteuberら[5]は，筋痛，腓腹筋肥大がみられた8歳男児例でジストロフィン遺伝子のエクソン45～51に欠失を報告した．Doriguzziら[6]は，運動時の筋痛とミオグロビン尿がみられる9歳男児例で，ジストロフィン遺伝子のエクソン45～48の欠失を報告した．家系の報告では2005年にSánchez-Arjona MBら[2]が，筋痛，有痛性筋攣縮を示しジストロフィン遺伝子のエクソン45～52の欠失を示したスペインの1家系を報告しており，家系内ではジストロフィン欠失を示した女性例でも筋痛，有痛性筋れん縮を示し，血清CKの上昇を認めた例を報告している．

臨床検査，診断，鑑別診断

理学所見，家族歴，血清CK値および筋生検，遺伝子解析の組合せにより診断される．X連鎖性劣性遺伝で．主に男児に発症するが，時に保因者女性でも同様の症状を呈することがある[2)8)]

血清CKが安静時でも上昇している事や反復性のミオグロビン尿症は本疾患を疑う所見である．筋生検では多くは筋原性変化(筋線維の大小不同，局所的な壊死・再生，硝子様変化など)がみられるが，異常がない例もみられる．筋の免疫組織化学的検索やイムノブロット法ではジストロフィン異常の所見が確認される．筋電図は神経性原性疾患と筋原性疾患を鑑別するために有用であるが，本疾患では異常がみられないことも多い．確定診断にはジストロフィン遺伝子の解析が必要であり，ジストロフィン遺伝子の欠失が多数報告されている．既報告ではロッド領域の欠失(rod：proximal and central rod domain(エクソン9～44)の異常，distal rod：distal rod domain(エクソン45～60))であり，機能的に重要ではない部分であるため，軽症にとどまると推定されている．

鑑別疾患としては，ジストロフィン異常症の代表的な疾患であるデュシェンヌ型筋ジストロフィー(Duchenne Muscular Dystrophy: DMD)，ベッカー型筋ジストロフィー(Becker Muscular Dystrophy: BMD)があるが，筋力低下や進行性の経過を示さないこと，心筋罹患がないこと，平常活動でも有痛性筋攣縮がある点がDMDやBMD典型例との相違点である．近年は本疾患でみられるジス

トロフィン遺伝子の欠失部位がBMDでみられる欠失の"hot spot"と称される部位と類似しており，BMDの軽症型あるいは亜型ではないかと推測されている[2]．

また，運動誘発性の筋痛や有痛性筋れん縮，血清CKの上昇，ミオグロビン尿症がみられることから，運動不耐型の代謝性ミオパチーの鑑別が必要である．

治療，予後

特異的な治療法はないが，症状の程度に応じて，過度の運度を避ける．運動を始めるときには十分な準備運動を行うなどの生活指導が必要である．通常は進行性の経過を示さず，予後は良好であり，治療を必要としないことが多い．

文献（* 重要文献）

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1 横紋筋融解症／ミオグロビン尿症

横紋筋融解症とミオグロビン尿症は基本的には同義であるが，後者は診断名というより検査所見による状況を示しているのに反し，前者は病態を示す診断名として取り扱われているので前者を用いる方が良い．本症の記載は古くは出エジプト記に，多くのユダヤ人がHamlock(ソクラテスが服毒した)を食べたウズラを摂取したことでけいれん，横紋筋融解症を起こしたことが記載されている．横紋筋融解症は何らかの原因で(代謝性基盤，物理的な筋への挫滅，脱水，薬物など)一度に大量の筋崩壊が起こった際に，筋細胞内の逸脱酵素，Ca^{2+}，K，タンパクなどが漏出し血流に放出され，ミオグロビン尿が検出され，腎不全を呈するものと定義される．ミオグロビンは筋細胞内のヘム蛋白で大量に尿中に排泄され，その結果尿色が褐色となる．ミオグロビンが尿中に検出される時は，通常起こりえない大量の筋崩壊(横紋筋融解症)が短時間に起こり，大量のミオグロビン分子を中心とした筋細胞内の逸脱酵素，物質が血中に排泄されていることを示唆している．最も注意すべき合併症は腎合併症で，早期に適切な治療が必要である[1]．

1 ミオグロビンについて

ミオグロビンはヘモグロビンと同様の呼吸蛋白で骨格筋，心筋に広く分布している．臨床現場でよく利用される骨格筋崩壊のマーカーであるクレアチンキナーゼ(CK)の分子量は，82,000で，骨格筋・心筋に同様に分布するaspartic aminotransferase (AST)，lactic dehydrogenase (LDH)の血中での上昇が遅れるのは，それぞれの分子量が約95,000，140,000で，CKのそれより大きいためであると考えられている．

一方ミオグロビンの分子量は17,800と，ヘモグロビンの約1/4で，CK，AST, LDHに比較して小さな分子量であるため筋崩壊の際はCKなどより早く血中で上昇がみられる．ミオグロビンは大部分が腎臓，肝臓で代謝され，通常の尿中への排泄量はわずかである．したがって筋崩壊が軽微である場合はCKが上昇し，ミオグロビンが血流に放出されても，体内で代謝されるため尿中にミオグロビンが排出されることはほとんどない．しかし大量の筋崩壊が短時間に起こった場合には尿中へ大量に排泄され，100 mg/dLを超えると褐色尿を呈する[2]．

2 一般検尿検査で見るミオグロビン尿の鑑別（表1）

検尿で見られる特徴は，潜血反応が強陽性でありながら，沈渣で赤血球の増加がない点である．ヘモグロビン尿との鑑別が重要であるが，血清CKが高値な点，ハプトグロブリンが低下していない点，あるいは臨床的に，筋痛，筋力低下などの筋症状を合併していることで判断できる．ミオグロビン尿の特徴と鑑別を表1に示す[3]．

3 横紋筋融解症の症状，原因と病態[4)〜7)]

1．原因

大量の筋崩壊は様々な原因で起こる．表2に主な原因を示した．骨格筋はBody Massの約40%を占めるためたとえ局所の筋細胞障害でも，大きな影響を与える．

表1　褐色尿の鑑別

診断	尿潜血反応	尿沈渣	血液生化学
血尿	陽性	赤血球増加	−
ミオグロビン尿	陽性	正常	ハプトグロブリン正常 CK上昇
ヘモグロビン尿	陽性	正常	ハプトグロブリン低下 ビリルビン上昇 CK正常
ポルフィリン尿	陰性	正常	−

表２　横紋筋融解症の原因

原因となる状況	具体的事例
運動負荷（生理的非生理的）	マラソン，けいれん，筋トーヌスの異常（痙性，ジストニア，アテトーゼ），長時間の心肺蘇生，気管支ぜんそく重積など
筋への圧迫，挫滅	Crush syndrome，強制姿位，電気ショック
低酸素（虚血）	血管の圧迫，コンパートメント症候群
代謝異常と筋疾患（先天性，後天性）	・先天性 代謝性ミオパチー 筋型糖原病 脂肪酸代謝異常症 ミトコンドリア病 プリン代謝異常症 ジストロフィノパチー Duchenne 型，Becker 型 ・後天性 電解質異常，アシドーシス 内分泌異常（糖尿病性ケトアシドーシス，副腎不全，甲状腺機能低下症，高アルドステロン症）
感染症	ウイルス感染症 細菌感染症など
炎症性筋疾患	多発性筋炎，皮膚筋炎
薬剤性，毒素	麻酔薬，向精神薬，脂質異常症（高脂血症）治療薬 蛇毒，スズメバチ毒など
体温異常	熱中症，低体温，火傷

2．病態（図１）

筋細胞の崩壊に伴って筋細胞内の種々の蛋白，酵素(CK，LDH，AST，アルドラーゼ)，リン，尿酸が逸脱し血中に流出する．時に播種性血管内凝固(DIC)や多臓器不全，呼吸筋力低下による呼吸障害に進展することもある．また高カリウム血症，高リン血症，低カルシウム血症も見られる．ミオグロビン尿に起因する合併症で重要なのは急性腎障害である．ミオグロビンは，分子量 17,800 のヘム蛋白で，糸球体で濾過されエンドサイトーシスで尿細管の上皮細胞に入り代謝される．筋細胞の急激で大量の壊死が起こることによりオーバーフローしたミオグロビンが，腎の域値である 0.5 ～ 1.5 mg/dL を超えると尿中に排泄され，いわゆる褐色尿を呈する．ミオグロビンによる腎障害の起序は十分解明されていないが，図に示すように(a)腎血管の収縮による虚血，(b)近位尿細管の虚血，および直接作用，(c)尿細管内に生じるミオグロビン円柱による遠位尿細管の閉塞等が関わっていると思われる．腎血管の虚血に関してはミオグロビンによる NO のキレート作用が腎血管収縮を増強しているといわれている．

3．症状

古典的な 3 主徴は①筋痛，②筋力低下，③褐色尿である．しかし約半数は筋痛，筋力低下があまり目立たない場合がある．筋は固く，また腫脹していることもある．全身症状としては発熱，全身倦怠感，頻脈，嘔気嘔吐がみられる．その後ミオグロビンの大量放出により，腎不全，DIC などへ進行する．

4．検査所見

血清 CK が高値で上記症状を認めるときは本症を疑う．CK が 5,000 IU 以上である場合は，急性腎不全が今後続発する可能性も考える必要がある．CK は筋崩壊が起こってから 12 時間以内に上昇し始め 1 ～ 2 日でピークとなる．CK の半減期は 1.5 日であるので，その後 1 週間ほどで基礎値に復帰する．ただし CK が極端に高値の場合は 2 週間ほど正常化するのに要する場合がある．一方ミオグロビンは CK より分子量が小さく早期に上昇するが，半減期も短く(2 ～ 4 時間)，24 時間以内には正常化するので注意が必要である．他の横紋筋融解症のマーカーとして，アルドラーゼや carbonic anhydrase III などがあるが，CK が横紋筋融解症のモニターとして“Gold Standard”である．血清カリウム，リンの上昇も崩壊の程度と腎不全のマーカーである．血清カルシウムの低下は障害を受けた筋への沈着に起因する．腎不全発症の危険性の目安として，Watanabe らは急性腎不全に移行した群と，移行しなかった群で血清ミオグロビン値に差があることを報告しているが[8]，ミオグロビン直接の腎毒性に加え，脱水，虚血，代謝

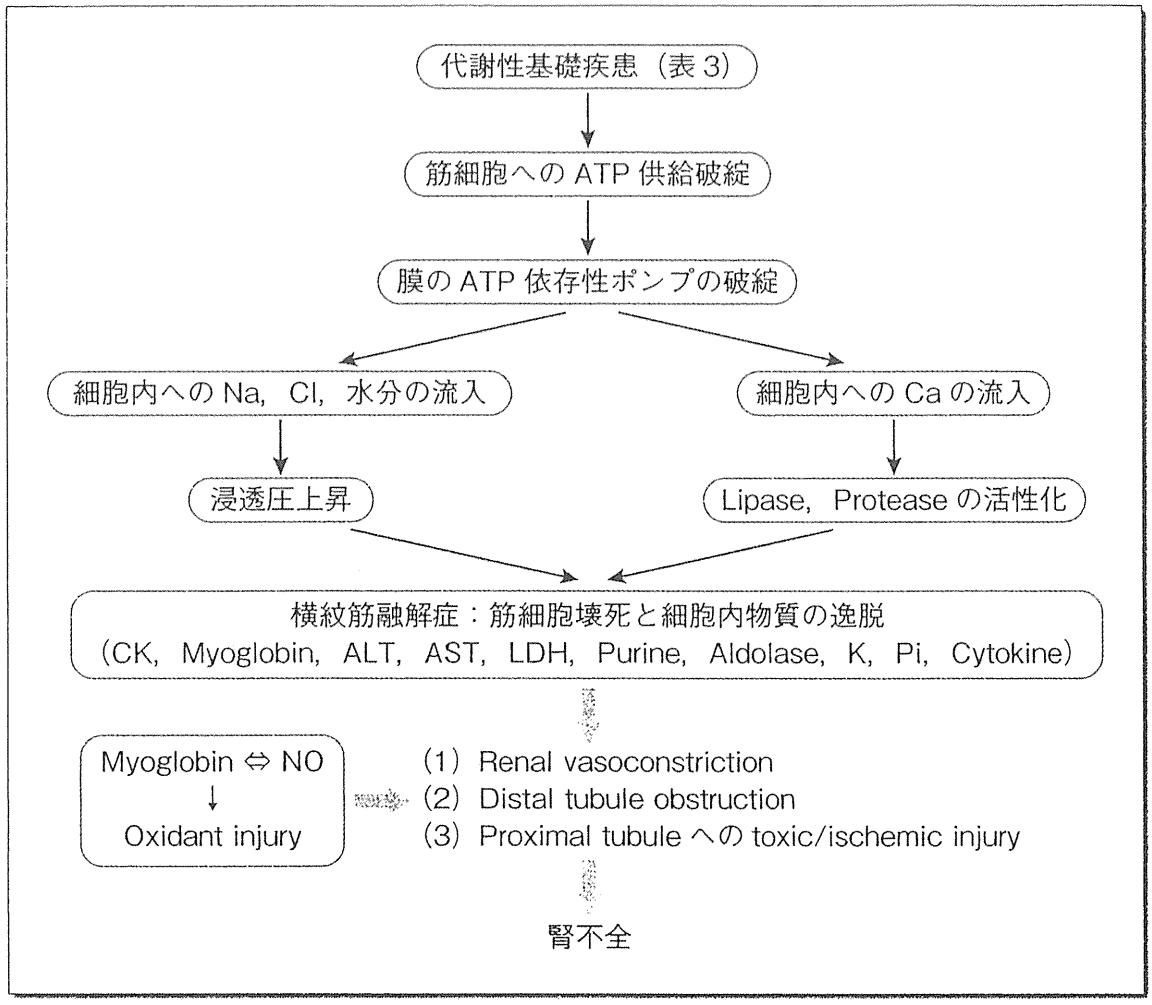

図1　横紋筋融解症と腎障害の機序

性アシドーシスなどによる影響も重要である．また尿中のneutrophil gelatinase associated lipocalin (N-GAL)が注目されている．これは腎虚血あるいは腎毒性に暴露された際にいち早く放出される蛋白である．血中尿中のN-GALは腎虚血が起こって2時間以降に上昇するので，これが上昇している場合は，腎不全が続発してくるリスクが高いと考えてよい．またMRI画像で横紋筋融解の範囲程度を調べることが可能である．特にT2強調像やSTIR画像が有力である[9]

5．治療

現在のところ十分なエビデンスに基づいた横紋筋融解症の治療方針はない．唯一推奨されているのは最初の24時間に十分な生食での輸液で脱水を矯正することである．尿のアルカリ化療法は表3に示すように尿pHが酸性に傾くほどミオグロビンの析出が多くなることから行われているが，尿のアルカリ化のための重炭酸とマニトール，フロセミドの併用については臨床的なエビデンスには乏しい．高カリウム血症あるいは腎不全が進行した場合は透析が必要である．

表3　尿pHとミオグロビンの析出

尿pH	析出するミオグロビン%
8.5	7.5
6.5	4
5.5	23
5.0	46
<5.0	73

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REPORT

Mutations in *GTPBP3* Cause a Mitochondrial Translation Defect Associated with Hypertrophic Cardiomyopathy, Lactic Acidosis, and Encephalopathy

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Respiratory chain deficiencies exhibit a wide variety of clinical phenotypes resulting from defective mitochondrial energy production through oxidative phosphorylation. These defects can be caused by either mutations in the mtDNA or mutations in nuclear genes coding for mitochondrial proteins. The underlying pathomechanisms can affect numerous pathways involved in mitochondrial physiology. By whole-exome and candidate gene sequencing, we identified 11 individuals from 9 families carrying compound heterozygous or homozygous mutations in *GTPBP3*, encoding the mitochondrial GTP-binding protein 3. Affected individuals from eight out of nine families presented with combined respiratory chain complex deficiencies in skeletal muscle. Mutations in *GTPBP3* are associated with a severe mitochondrial translation defect, consistent with the predicted function of the protein in catalyzing the formation of 5-taurinomethyluridine (τm^5U) in the anticodon wobble position of five mitochondrial tRNAs. All case subjects presented with lactic acidosis and nine developed hypertrophic cardiomyopathy. In contrast to individuals with mutations in *MTO1*, the protein product of which is predicted to participate in the generation of the same modification, most individuals with *GTPBP3* mutations developed neurological symptoms and MRI involvement of thalamus, putamen, and brainstem resembling Leigh syndrome. Our study of a mitochondrial translation disorder points toward the importance of posttranscriptional modification of mitochondrial tRNAs for proper mitochondrial function.

Defects of the mitochondrial respiratory chain underlie a diverse group of human disorders characterized by impaired oxidative phosphorylation (OXPHOS). The generation of a functional respiratory chain requires the coordinated expression of both the nuclear genome and mitochondrial DNA (mtDNA). Defective translation of mtDNA-encoded proteins, caused by mutations in either the mitochondrial or nuclear genomes, represents a rapidly expanding group of human disorders, which often manifest as severe infantile combined OXPHOS deficiencies.[1]

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The mitochondrial genome contains a total of 37 genes, 13 of which encode protein subunits of the respiratory chain complexes and the ATP synthase. Translation of these genes is achieved by the organelle's own protein synthesis machinery, of which only the RNA components (rRNAs and tRNAs) are encoded by mtDNA. All protein factors required for mitochondrial translation are encoded in the nucleus and must be imported after their synthesis in the cytoplasm. Mitochondrial (mt-) tRNAs require extensive posttranscriptional modifications before achieving translation competency. Modifications to tRNAs might contribute to their proper folding, stability, or decoding capacity. In mitochondria a minimal set of 22 different tRNAs is used to translate all codons.[2] Modifications to the wobble position of the anticodon loop of mt-tRNAs play an important role in ensuring correct mRNA-tRNA interactions. In ten mt-tRNA species, all of which correspond to two codon sets, four different types of modified nucleotides have been identified at the wobble position.[3,4] One of these modifications is 5-taurinomethyluridine (τm^5U), found at position 34 (U34) of mt-tRNAsLeu^{UUR}, Trp, Gln, Lys, and Glu, which has been suggested to be synthesized cooperatively by GTPBP3 and MTO1.[5] In addition to τm^5U, mt-tRNAs Gln, Lys, and Glu also contain a 2-thiouridine modification at U34 (s^2U), introduced by TRMU (also known as MTU1). This results in a 5-taurinomethyl-2-thiouridine ($\tau m^5 s^2 U$) modification in these mt-tRNA molecules. Modifications of U34 have been proposed to modulate either the accuracy or the efficiency of translation.[6,7] Three types of mutations affecting U34 have been associated with human mitochondrial disease: (1) mutations in the mt-tRNAs;[8] (2) mutations in *TRMU* (MIM 610230) affecting U34 2-thiouridylation and leading to acute infantile liver failure resulting from combined OXPHOS deficiency;[9] and (3) more recently, mutations in *MTO1* (MIM 614667) found to underlie cases of hypertrophic cardiomyopathy and lactic acidosis, associated with impaired mitochondrial translation rate and reduced respiratory chain activities.[10,11]

Whole-exome sequencing (WES) of 790 individuals with suspected mitochondriopathy in five centers identified eight index case subjects (plus two affected siblings) with homozygous or two heterozygous rare variants (minor allele frequency < 0.1%) in *GTPBP3* (MIM 608536), with no such case being found in 11,295 control subjects. This presents a genome-wide significant enrichment in *GTPBP3* (RefSeq accession number NM_032620.3) mutation load in samples from individuals with the clinical diagnosis "mitochondrial disease" ($p < 3.2 \times 10^{-10}$, Fisher exact test) in comparison to nonmitochondrial disorder samples. In addition, when filtering for genes coding for mitochondrial proteins,[12] in several individuals *GTPBP3* was the only gene with two mutations. Further evidence for the pathogenic role of *GTPBP3* mutations was derived from follow-up candidate gene sequencing of 18 individuals with similar phenotypes, which identified two more index cases. Collectively, mutations in *GTPBP3* were detected in 12 individuals from 10 families. However, segregation analysis of a single affected individual (#66654) revealed that the two identified heterozygous mutations in *GTPBP3* affected the same allele, leaving genetic evidence about 11 individuals from 9 families (Figure 1).

Written informed consent was obtained from all individuals investigated or their guardians, and the ethics committee of the Technische Universität München approved the study.

Individual #49665 (family F1, Figure 1A) is a boy born to consanguineous parents from the UAE. He presented at the age of 10 years with mild intellectual disability, fatigability, mild hypertrophic cardiomyopathy, and visual impairment. At presentation he measured 134 cm with a body weight of 25 kg. Clinical examination revealed slight dyspnea when climbing stairs and mild intellectual disability. Plasma lactate was consistently elevated (3.0 to 7.2 mmol/l, reference < 2.1 mmol/l). Electroencephalogram, hearing test, and visual-evoked potentials showed no abnormalities. Electrocardiography (ECG) revealed signs of left ventricular hypertrophy confirmed by echocardiography. There was no obstruction of the left ventricular outflow tract. He had a pale optic disc on both sides but visual acuity and visual field could not be examined. Brain MRI was normal, but MR spectroscopy revealed lactate peaks in the parietal and precentral cortex. Respiratory chain (RC) measurement in muscle revealed a significant reduction of complex I and IV activities. He was substituted with CoQ_{10} (200 mg/day), riboflavin (400 mg/day), carnitine (1 g/day), and a fat-rich diet (60% of daily caloric intake). A follow-up examination 1 year after the initial presentation showed no significant changes of his clinical signs/symptoms.

His 17-year-old elder brother, individual #36349 (family F1, Figure 1A), had a very similar clinical picture.

Individual #66143 (family F2, Figure 1A), a boy, is the second child of healthy unrelated parents of Arab-Moslem origin from Israel. He presented at the age of 2 years with sudden respiratory failure. Heart ultrasonography indicated a hypertrophic cardiomyopathy and congestive heart failure. His cardiac symptoms improved on treatment with furosemide, spironolactone, carvedilole, and digoxin. In addition, a high-dose vitamin treatment (100 mg/day riboflavin, 100 mg/day vitamin B1, and 60 mg/day CoQ_{10}) was initiated. RC enzyme measurement in muscle revealed a significant reduction of complex I and IV activities. On follow-up examinations (over 3 years), the child's psychomotor development is normal and his parents reported that he is active like his peers. Digoxin and spironolacton treatment was stopped and his recent echocardiography revealed a stable condition of the heart including normal global function of left ventricle with no further hypertrophy of interventricular septum and no pulmonary hypertension.

Individual #72425 (family F3, Figure 1A) was a girl born to unrelated parents. At 3 months of age, she had feeding difficulties and failure to thrive. At the age of 7 months,

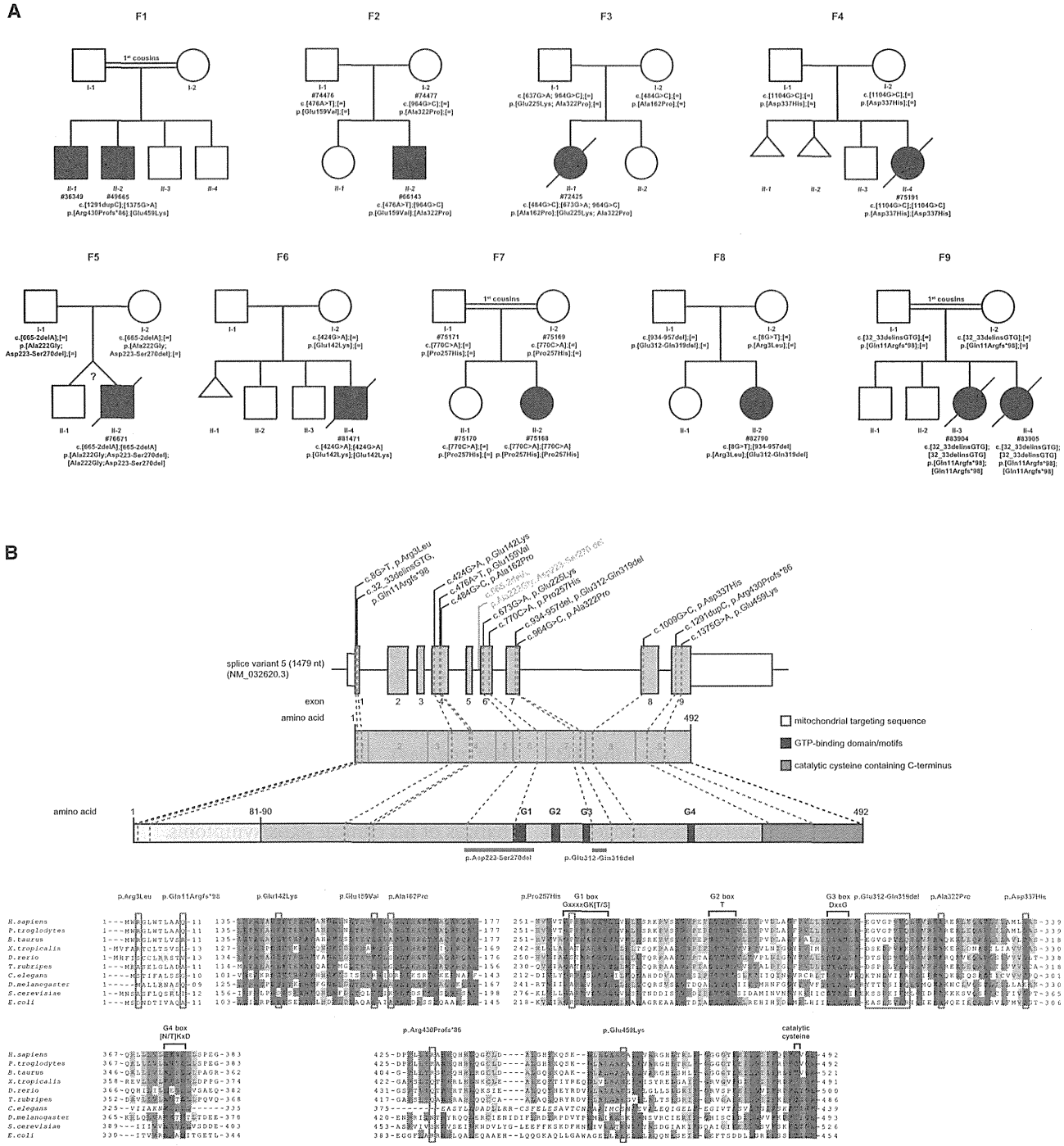

Figure 1. ***GTPBP3*** **Mutation Status and Gene Structure**
(A) Pedigrees of nine families with mutations in *GTPBP3*.
(B) Gene structure of *GTPBP3* with known protein domains of the gene product and localization and conservation of amino acid residues affected by mutations. Black and orange text indicate exonic and intronic variants. Intronic regions are not drawn to scale. Coloring in the sequence alignment represents the identity of amino acid residues.

she developed recurrent cough and fever and was admitted to the emergency room with severe fatigue, pallor, and progressive malaise. Blood exams showed leukocytosis, and 2 days later her general condition worsened, showing cyanosis and hyporeactivity. Echocardiography showed severe dilated cardiomyopathy with an ejection fraction of 20% that was unresponsive to therapy. She had severe refractory hyperlactatemia (23.3 mmol/l, reference range 0.5–2.3 mmol/l). Histochemical and spectrophotometric analysis of the muscle biopsy showed a severe complex IV deficiency. She died 10 days after admission from cardiac failure.

Individual #75191 (family F4, Figure 1A), a girl, was born to nonconsanguineous parents after an uneventful pregnancy of 40 weeks. The mother had had two miscarriages at 6 and 8 weeks and had a healthy son aged 16 months. In the first hours after birth, individual #75191 developed mild stridor and dyspnea which rapidly worsened. She fed poorly and became less responsive, and a Kussmaul breathing pattern was seen. She was transferred to a specialist center and was found to be severely hypotonic, moving very little, either spontaneously or after stimulation. She had hyperlactatemia (23 mmol/l), hypoglycemia (18 mg/dl), hyperammonemia (135 μmol/l, control value 11–48 μmol/l), and hyperlactaturia. She progressively developed respiratory insufficiency and bradycardia. Cardiac ultrasound showed apical right ventricular hypertrophy and an open duct of Botalli with minor shunting. Fractional shortening was 28% (mildly decreased). Cerebral ultrasound showed a minimal grade I bleeding, and the cerebral matter appeared mildly hyperechogenic. She died of asystolia at day 1. A muscle biopsy performed immediately after death showed decreased activities of RC complexes I and IV.

Individual #76671 (family F5, Figure 1A) was the second boy of nonconsanguineous parents. The infant was born at 41 weeks of gestation from a twin pregnancy. Generalized hypotonia and difficulty in suction was noted since birth and he rapidly developed failure to thrive. He acquired head control at the age of 7 months but parents reported normal cognitive skills. At the age of 9 months he was admitted to the intensive care unit for acute aspiration pneumonia that required intubation. Laboratory test revealed a metabolic acidosis with hyperlactatemia (5.2 mmol/l) and brain MRI showed bilateral thalamic T2-weighted hyperintense abnormalities with low diffusion. Analysis of a muscle biopsy revaled a clear reduction in histochemical cytochrome *c* oxidase activity and decreased complex I and IV enzyme activities. The cardiological examination disclosed hypertrophic cardiomyopathy and a Wolff-Parkinson-White pre-excitation syndrome (MIM 194200). The baby died after 15 days of hospitalization with clinical signs of heart failure.

Individual #81471 (family F6, Figure 1A) was a boy born to nonconsanguineous Romanian parents at 34 weeks gestation (birth weight 2.18 kg). His mother had premature and prolonged (85 hr) rupture of membranes before delivery, and the baby was treated with i.v. antibiotics before being discharged home on day 7. He was readmitted to hospital on day 25 with weight loss (2.23 kg). He was hypothermic and jaundiced and initial blood analysis showed profound metabolic acidosis. He was treated with i.v. antibiotics for presumed sepsis. The acidosis did not resolve, and serum lactate was elevated (11.0 mmol/l). ECG was abnormal and echocardiography showed concentric left ventricular hypertrophy. CSF lactate was 12.4 mmol/l (normal range 0.9–2.4 mmol/l) prompting bicarbonate treatment. Brain MRI showed abnormal diffusion of the subthalamic nuclei extending down to the brain stem. There was abnormal T2 signal in the midbrain and basal ganglia bilaterally. On examination he was thin but not dysmorphic. He was mildly jaundiced and had puffy feet. There was little spontaneous movement but normal muscle bulk and he was distinctly hypotonic. Feeding through a nasogastric tube was established but he did not become responsive despite high caloric intake. He developed recurrent apnea and died aged 5 weeks. Biochemical analysis performed in muscle revealed a significant decrease of RC complexes I and IV.

Individual #75168 (family F7, Figure 1A) is the second girl of first-cousin parents from India. She was first seen at the age of 2 years with development delay. She was able to walk but she couldn't speak. She received occupational and speech therapy. During a febrile illness when she was 3 years old, she had an acute metabolic failure with hyperlactatemia and hyperlactatorachia. She recovered but had epileptic seizures and more severe intellectual disability. Brain MRI showed pronounced bilateral hyperintensities affecting the whole thalamus and extending to the mesencephalon. Hyperlactatemia (>10 mmol/l) and hyperlactatorachia (6 mmol/l) were noticed. RC activity in muscle was normal as well as PDH complex tested by immunoblot. The girl was treated with qa carnitine 3 × 350 mg/day, CoQ_{10} 3 × 50 mg/day, vitamins B1 3 × 50 mg/day and B6 3 × 50 mg/day, and bicarbonate 4 × 1 g. Epilepsy was in good control with levetiracetame 40 mg/kg/day and a high-fat diet. The girl is in a special school for children with developmental delay. Her general condition is good. She is always in a good temper. Development is delayed about 1.5 years. She has continual hyperlactatemia (8–10 mmol/l).

Individual #82790 (family F8, Figure 1A) is a girl born at 40 weeks of gestation with normal birth weight to nonconsanguineous Japanese parents. At the age of 1 year, she developed frequent epileptic seizures, and she was medicated with phenobarbital. Severe developmental delay was noted and at the age of 15 months she was admitted to children's hospital. Her weight gain (9.25 kg, −0.06 SD) is within the normal range, but she developed severe muscle hypotonia. There is no cardiac involvement by ECG and echocardiogram. Hyperlactatemia was noted (5.72–6.49 mmol/l) whereas metabolic profiling of amino acids, urinary organic acids, and acylcarnitine was normal. RC analysis in muscle showed a significant decrease in complexes I and IV activities. Brain MRI showed bilateral hyperintensities in the putamen and weakly also in the anterior thalamus. A lactate peak was detected on $[H^+]$-MR spectroscopy. She is now 2 years of age and still presents with a severe global developmental delay.

Individual #83904 (family F9, Figure 1A) was the second child of consanguineous, healthy parents of Turkish origin. She was born at 39 weeks of gestational age (birth weight 2,740 g, length 49 cm, head circumference 32 cm). Shortly after birth, she presented with Wolff-Parkinson-White syndrome. Cardiac ultrasound was normal. Treatment was started with amiodarone and she

Table 1. Genetic and Clinical Findings in Individuals with *GTPBP3* Mutations

		GTPBP3 Mutations	OXPHOS Activities in Skeletal Muscle				Clinical Features				
ID	Sex	cDNA (NM_032620.3) and Protein (NP_116009.2)	RCC	% of Lower Control Range	Absolute Values	Reference Range	AO	Course	HCM	Histochemical COX Defect	Other Features
#49665[a,b]	male	c.[1291dupC; 1375G>A], p.[Pro430Argfs*86; Glu459Lys]	I	15%	0.025	0.17–0.56	10 years	alive 14 years	yes	ND	consanguineous parents (1st cousins), mild intellectual disability, fatigability, limited vision, lactic acidosis
			II	ND	ND	ND					
			II+III	normal	0.201	0.08–0.48					
			IV	24%	0.267	1.1–5.0					
#36349[b]	male	c.[1291dupC; 1375G>A], p.[Pro430Argfs*86; Glu459Lys]	I	no data	no data	no data	no data	alive 17 years	no data	no data	sibling of #49665 with similar clinical symptoms
			II								
			II+III								
			IV								
#66143[a]	male	c.[476A>T; 964G>C], p.[Glu159Val; Ala322Pro]	I	7%	0.01	0.19–0.48	2 years	alive 5 y ears	yes	ND	unrelated parents, sudden respiratory failure, lactic acidosis
			II	normal	0.10	0.07–0.12					
			II+III	normal	0.12	0.09–0.22					
			IV	28%	0.12	0.44–0.92					
#72425[a]	female	c.[484G>C; 673G>A; 964G>C], p.[Ala162Pro; Glu225Lys; Ala322Pro]	I	14%	0.015	0.11–0.30	3.5 months	died 8 months	DCM	yes	unrelated parents, cyanosis, hyporeactivity, DCM with residual ejection fraction of 20%, lactic acidosis
			II	normal	0.21	0.12–0.25					
			II+III	normal	0.06	0.006–0.14					
			IV	45%	0.76	1.7–4.0					
#75191[a]	female	c.[1009G>C; 1009G>C], p.[Asp337His; Asp337His]	I	31%	0.03	0.10–0.25	birth	died 1 day	yes	yes	unrelated parents, Kussmaul breathing, stridor, hypotonic, hyporeactivity, RVH, lactid acidosis
			II	normal	0.16	0.14–0.25					
			II+III	normal	0.12	0.13–0.25					
			IV	15%	0.09	0.60–1.48					
#76671	male	c.[665−2delA; 665−2delA], p.[Ala222Gly; Asp223_Ser270del; Ala222Gly; Asp223_Ser270del]	I	45%	0.05	0.11–0.30	birth	died 10 months	yes	yes	unrelated parents, hypotonia from birth, RVH, WPW, lactic acidosis
			II	normal	0.16	0.12–0.25					
			II+III	ND	ND	0.06–0.14					
			IV	17%	0.29	1.7–4.0					
#81471[a]	male	c.[424G>A; 424G>A], p.[Glu142Lys; Glu142Lys]	I	12%	0.012	0.104 ± 0.036	4 weeks	died 5 weeks	yes	yes	consanguineous parents, two healthy siblings, one miscarriage, FTT, poor weight gain and feeding, concentric LVH, lactic acidosis
			II	normal	0.098	0.145 ± 0.047					
			II+III	normal	0.850	0.544 ± 0.345					
			IV	17%	0.127	1.124 ± 0.511					

(*Continued on next page*)

Table 1. *Continued*

		GTPBP3 Mutations	OXPHOS Activities in Skeletal Muscle				Clinical Features				
ID	Sex	cDNA (NM_032620.3) and Protein (NP_116009.2)	RCC	% of Lower Control Range	Absolute Values	Reference Range	AO	Course	HCM	Histochemical COX Defect	Other Features
#75168[a]	female	c.[770C>A; 770C>A], p.[Pro257His; Pro257His]	I	normal	no data	no data	2 years	alive 5 years	no	ND	consanguineous parents (1st cousins), developmental delay, epileptic seizures, intellectual disability, MRI hyperintense lesions of basal ganglia typical to Leigh syndrome, lactic acidosis
			II	normal							
			II+III	normal							
			IV	normal							
#82790[a]	female	c.[8G>T; 934_957del], p.[Arg3Leu; Gly312_Val319del]	I	36%	0.107	0.301 ± 0.05	1 year	alive 2 years	no	ND	unrelated parents, seizures, severe hypotonia, developmental delay, lactic acidosis
			II	normal	0.424	0.272 ± 0.05					
			II+III	normal	0.21	0.25 ± 0.093					
			IV	21%	0.008	0.035 ± 0.011					
#83904[a,c]	female	c.[32_33delinsGTG; 32_33delinsGTG], p.[Gln11Argfs*98; Gln11Argfs*98]	I	64%	4.2	6.5–17	1 week	died 9 months	yes	ND	consanguineous parents (1st cousins), lactic acidosis, WPW
			II	normal	16.1	13.6–45.7					
			II+III	normal	5.8	4.3–13.2					
			IV	25%	9.9	74–294					
#83905[a,c]	female	c.[32_33delinsGTG; 32_33delinsGTG], p.[Gln11Argfs*98; Gln11Argfs*98]	I	no data	no data	no data	birth	died 10 days	yes	ND	consanguineous parents (1st cousins), lactic acidosis, WPW
			II								
			II+III								
			IV								
#66654[a]	female	c.[673G>A; 964G>A]; [=] p.[Glu255Lys; Ala322Pro]; [=]	I	64%	0.09	0.14–0.35	1.5 months	alive	no	ND	intrauterine growth retardation, lactic acidosis, leukodystrophy, generalized hypotonia
			II	normal	0.19	0.18–0.41					
			II+III	90%	0.27	0.30–0.67					
			IV	normal	1.42	0.42–1.26					

Abbreviations are as follows: AO, age of onset; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; FTT, failure to thrive; LVH/RVH, left/right ventricular hypertrophy; ND, not determined; WPW, Wolff-Parkinson-White syndrome.
Mitochondrial respiratory chain complexes (RCC) in muscle: I, NADH:CoQ-oxidoreductase; II, succinate:CoQ-oxidoreductase; II+III, succinate:cytochrome c reductase; IV, cytochrome c oxidase (COX).
Enzyme activities were determined in muscle biopsies and normalized to citrate synthase (CS). Absolute values and reference ranges are given in [mU / mU CS].
[a]Investigated by exome sequencing.
[b,c]These individuals are siblings.

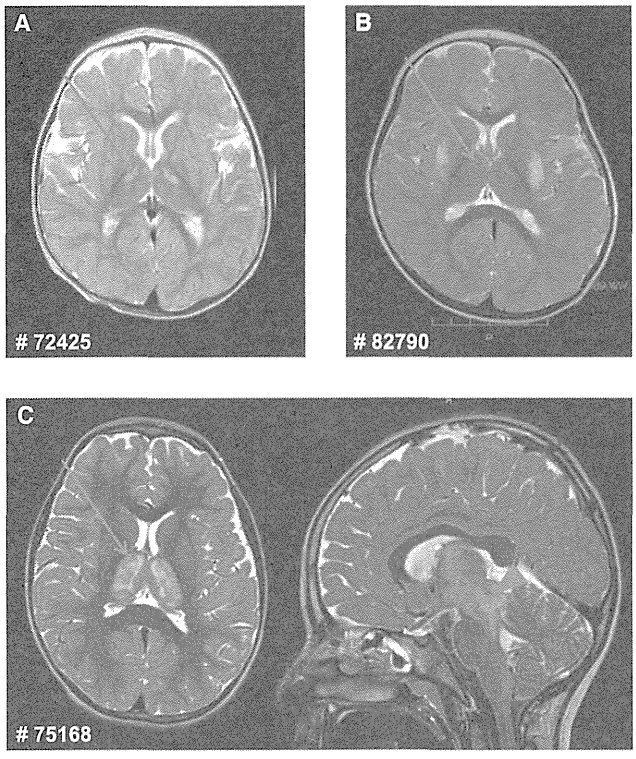

Figure 2. Brain MRI of Affected Individuals #72425, #82790, and #75168

(A) MRI of individual #72425 shows small T2 hyperintensities in the anterior thalamus bilaterally (arrow).

(B) In individual #82790, T2-weighted MRI shows bilateral hyperintensities in the putamen (arrowhead) and weakly also in the anterior thalamus (arrow).

(C) T2-weighted MRI of individual #75168 shows pronounced bilateral hyperintensities affecting the whole thalamus (arrow, axial view at the left) and extending to the mesencephalon (arrowhead, sagittal view at the right).

remained stable, without cardiac symptoms or arrhythmia. At 7 months of age, she had cardiogenic shock and metabolic acidosis. Heart ultrasound detected dilated cardiomyopathy and decreased contractility (ejection fraction 35%). She presented hyperlactatemia (20 mmol/l), hyperalaninemia (1,175 μmol/l; normal range, 190–450 μmol/l), and an increased lactate-to-pyruvate ratio (47; normal range, 10–20). Her disorder progressed despite intensive medication for heart failure. She died at the age of 9 months of cardiac insufficiency with arrhythmia.

Her younger sister, individual #83905 (family F9, Figure 1A), had a very similar clinical picture. She died at 6 months of age of cardiac insufficiency unresponsive to resuscitation procedures.

Genetic, biochemical, and clinical findings are summarized in Table 1. Pedigrees of the families we studied are shown in Figure 1A. The location of the identified mutations within the gene and the conservation of the affected amino acid (aa) residues are shown in Figure 1B. Individual #49665 (F1: II-2) was found to carry a frame shift and one missense variant. The next generation sequencing (NGS) data demonstrated a compound heterozygous status of the two variants (Figure S1 available online). Individual #76671 (F5: II-2) was homozygous for an intronic single base pair deletion, c.665−2delA, which is predicted to cause the loss of a splice acceptor site. Analysis of cDNA from fibroblasts revealed a shorter transcript, and sequencing found that in more than 95% of transcripts, the downstream acceptor of exon 7 was used for splicing, resulting in the skipping of exon 6 including the conserved G1-box guanine nucleotide-binding signature motif (Figure S2). Individual #82790 (F8: II-2) was found to be compound heterozygous for a missense mutation c.8G>T (p.Arg3Leu) and a 24 bp deletion c.934_957del (p.Gly312_Val319del). The 24 bp deletion is predicted to cause the deletion of 8 amino acids containing conserved residues. The p.Arg3Leu substitution at the very N terminus of the protein is scored as a predicted polymorphism but causes a loss of a positively charged residue, which is predicted to interfere with mitochondrial targeting (Predotar, PsortII). The two missense variants found in individual #66654, c.[673G>A; 964G>C], p.[Glu225Lys; Ala322Pro], were identical to the variants found on the paternal allele of individual #72425 (F3: II-1). Analysis of parental DNA revealed that both variants were also located on the same allele in individual #66654, meaning that only one allele is affected. Because of this observation, combined with the absence of a heart phenotype and because this individual is the only one exhibiting an isolated complex I defect, we consider the mutations found in *GTPBP3* not to be causative in subject #66654.

In summary, the identification of 13 different alleles in 11 individuals with suspected mitochondrial disease from 9 families provides strong evidence for the pathological role of mutant *GTPBP3* in the investigated families. It links *GTPBP3* mutations to combined respiratory chain complex deficiency (9/11), cardiomyopathy (9/11), lactic acidosis (11/11), and encephalopathy (4/11).

Brain MRI was performed in three individuals (Figure 2). It showed bilateral T2 hyperintensities in the thalamus, ranging from weak (#82790) or small (#72425) changes in the anterior thalamus to very pronounced hyperintensities affecting the whole thalamus in individual #75168. In addition, T2 hyperintensities affected the putamen bilaterally in individual #82790 and extended markedly to the mesencephalon in individual #75168. Taken together, the MRI involvement of basal ganglia and brainstem resembles the (MRI) findings in Leigh syndrome (which is, however, an ill-defined entity).

Skin fibroblast cell lines were available from seven individuals for functional studies. We first analyzed the cellular oxygen consumption rate (OCR^{13}) by microscale respirometry with the XF96 extracellular flux analyzer (Seahorse Bioscience). When cells of individuals from families F1 to F5 were cultured in glucose-containing medium, only cell lines from individuals #75191 (F4: II-4) and #76671 (F5: II-2) showed a decreased OCR (of 59% and 58%, respectively) indicating defective oxidative phosphorylation

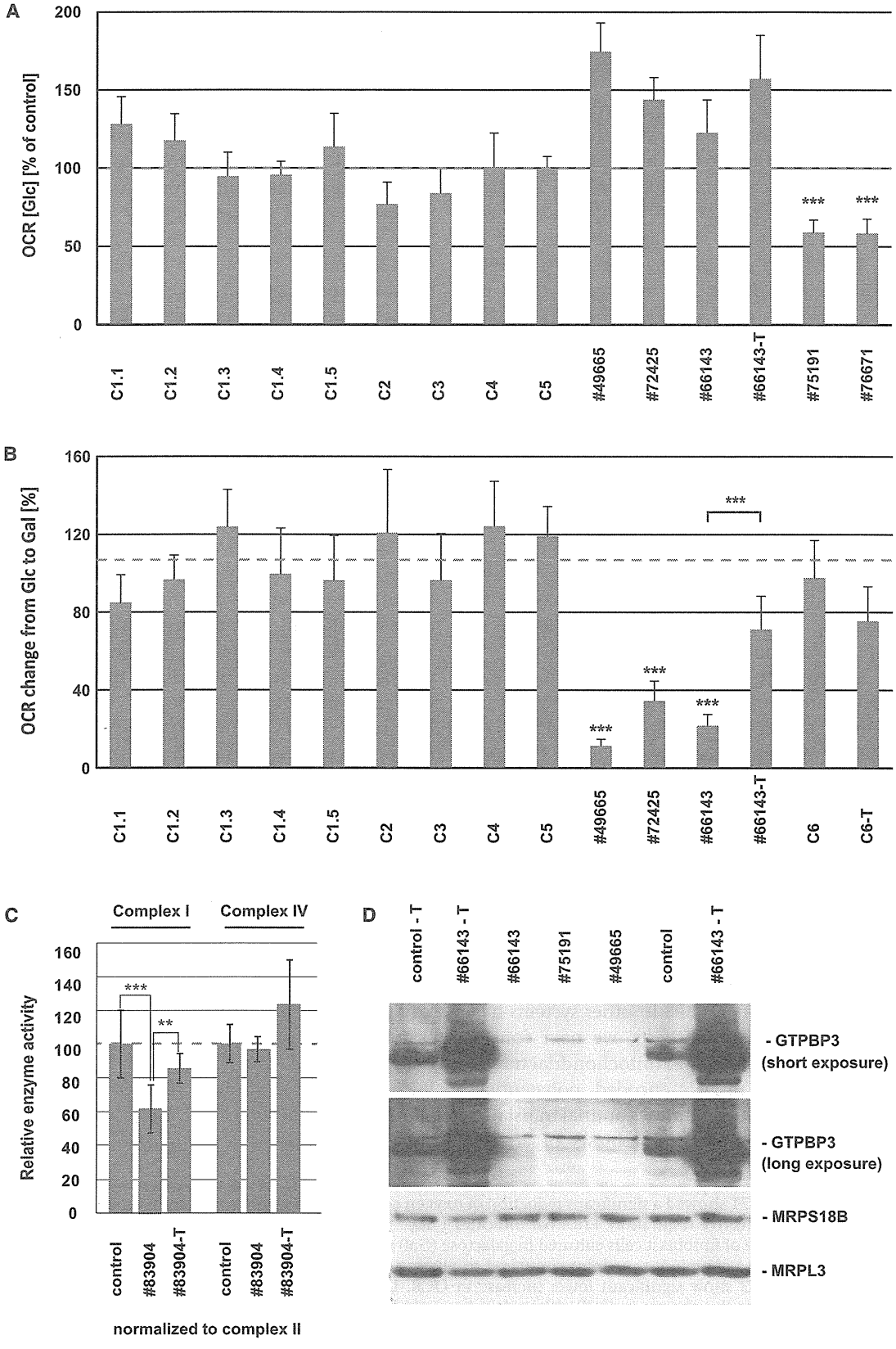

Figure 3. Analysis of Respiration Defects and GTPBP3 Protein Levels in Fibroblast Cell Lines

(A) Oxygen consumption rate (OCR) of fibroblast cell lines from five affected individuals and five control subjects cultured in high-glucose (Glc) medium. Each analysis was performed in more than 15 replicates. Control one (C1) was measured five time at different passage numbers (C1.1–1.5, NHDFneo, Lonza). OCR was expressed as percentage relative to the average of all controls. Cells from

(legend continued on next page)

(Figure 3A). When cells were cultured with galactose as the primary carbon source, rather than glucose, cells are forced to rely on oxidative phosphorylation rather than glycolysis in order to meet the energy demand.[14,15] Accordingly, in control cells an increase in OCR of approximately 2-fold was observed when galactose was substituted as the primary carbon source. This increase in OCR was impaired in fibroblasts from affected individuals #49665 (F1: II-2), #66143 (F2: II-2), and #72425 (F3: II-1), which showed OCR increases of only 11%, 35%, and 22%, respectively (Figure 3B). In order to confirm that defects in GTPBP3 are the cause of this defect, we transduced three cell lines with a wild-type copy of *GTPBP3* cDNA (RefSeq NM_32620.3) by using a lentiviral vector (pLenti 6.3/V5 TOPO, Life Technologies) as previously described.[16,17] Fibroblasts from individuals #49665 and #66143 were used for the rescue experiment, with fibroblasts from #66654 (subject with only one affected allele) being included as a control (C6). Unfortunately, we were unable to recover any viable cells from subject #49665 after the transduction procedure. Although the transduction had no noticeable effect on the control cell line (C6-T), transduced fibroblasts from #66143 (#66143-T) displayed a significant improvement of OCR in galactose-containing medium (Figure 3B). Furthermore, we detected an isolated respiratory chain complex (RCC) I deficiency in a fibroblast cell line from family 9. Cotransfection of individual #83904 fibroblasts with two putative *GTPBP3* isoforms amplified by RT-PCR, RefSeq NM_32620.3 and NM_0128855.2 (missing 63 base pairs of exon 8), significantly improved enzyme activities of RCC I (pIRES2-EGFP, Clontech) (Figure 3C). Analysis of the protein levels of GTPBP3 in five fibroblast cell lines demonstrated reduced or undetectable amounts in individuals #49665, #75191, #66143, #83904, and #83905, although they showed a clear increase after transduction or transfection (Figures S4 and 3D). In conclusion, our data demonstrate a causal role for *GTPBP3* mutations in the oxidative metabolism deficiency in these individuals.

Given that homologs of GTPBP3 in other systems have been implicated in protein synthesis, we next concentrated on the analysis of GTPBP3 in mitochondrial translation. The synthesis of mtDNA-encoded polypeptides, investigated by pulse-labeling of mitochondrial translation products via [^{35}S]methionine in fibroblasts of affected individuals (for methods see Haack et al.[18]) was severely and uniformly decreased to 20%–30% of control levels in individuals #49665, #66143, and #75191 (Figures 4A and 4B). There was no detectable defect in fibroblasts from individual #72425, which might be explained by the relatively low conservation of the mutated residue in this individual (Figure 1B). In order to exclude possible defects of mitochondrial transcription or precursor RNA processing, we analyzed all mitochondrially encoded rRNAs and mRNAs in fibroblasts of individuals #49665, #66143, #72425, and #75191 by RNA blotting and by RNA-seq in fibroblasts of individual #49665. We found no differences in the expression levels of the mt-RNAs between case and control subjects. On average, the mt-RNA expression levels were only 6% lower in individual #49665 as compared to control individuals (data not shown). We did not observe any appreciable reduction in steady-state levels of mature RNAs, nor was there any accumulation of precursor RNAs (Figure S3A). Next, we analyzed the steady-state levels of mt-tRNAs, including those five species for which the τm^5 U modification has been reported in mammals (Gln, Glu, Lys, Leu^{UUR}, and Trp).[4] We again observed no appreciable changes in their steady-state levels (Figure S3B). In order to further corroborate a direct role of GTPBP3 in mitochondrial translation, we downregulated its expression via RNA interference in HeLa cells (Figure 4C). Reduction of GTPBP3 protein levels upon RNAi treatment of HeLa cells was comparable to the reduction of its level in *GTPBP3* mutant fibroblasts (Figure 4D). Downregulation of *GTPBP3* expression resulted in a general mitochondrial translation defect, similarly to the reduction observed in subject fibroblasts (Figure 4D). In conclusion, the reduced translation efficiency observed in three out of four *GTPBP3* mutant cell lines, as well as in human cells treated with *GTPBP3* RNAi, confirmed an important function for GTPBP3 in efficient mitochondrial protein synthesis.

In order to test the consequences of this reduced translation rate upon the protein levels of OXPHOS complexes in mutant fibroblast cell lines, we analyzed the steady-state levels of several nuclear-encoded subunits of the OXPHOS system by immunoblotting. In fibroblasts from individuals #72425, #75191, and #76671 (F3: II-1, F4: II-4, and F5: II-2), we observed strongly reduced amounts of RCC IV. Fibroblasts from subjects #72425, #75191, and #49665 also showed reduced levels of RCC I, whereas the levels

individuals #75191 and #76671 showed a significant reduction of oxygen consumption whereas cells from individuals #49665, #72425, and #66143 presented no defective respiration. Error bar indicates 1 SD; ***$p < 0.001$.

(B) Oxygen consumption rate of fibroblast cells cultured in galactose (Gal) growth medium. The average increase of OCR from five control cells cultured in galactose-containing medium compared to glucose-containing medium was 107%. Cell lines from individuals #49665, #72425, and #66143 show significant lower increase in OCR. Lentiviral expression of wt*GTPBP3* in cells from individual #66143 significantly increases the change in OCR although it has only little effect in control cells (C6-T). Error bar indicates 1 SD; ***$p < 0.001$.

(C) Activities of respiratory chain complexes I and IV (expressed as ratio to CII activity) are decreased in individual #83904 cells transfected by electroporation with empty vector (pIRES2-EGFP) according to the manufacturer's protocol (LONZA) but are improved upon expression of *GTPBP3* cDNAs from the same plasmid. Measurements were performed as previously described.[29,30] Error bar indicates 1 SD. Activity in controls was set as 100%. **$p < 0.01$, **$p < 0.001$.

(D) Levels of GTPBP3 were reduced in cells from individuals #49665, #75191, and #66143 and elevated after transduction with wt*GTPBP3* cDNA. MRPS18B and MRPL3 served as mitochondrial loading controls.

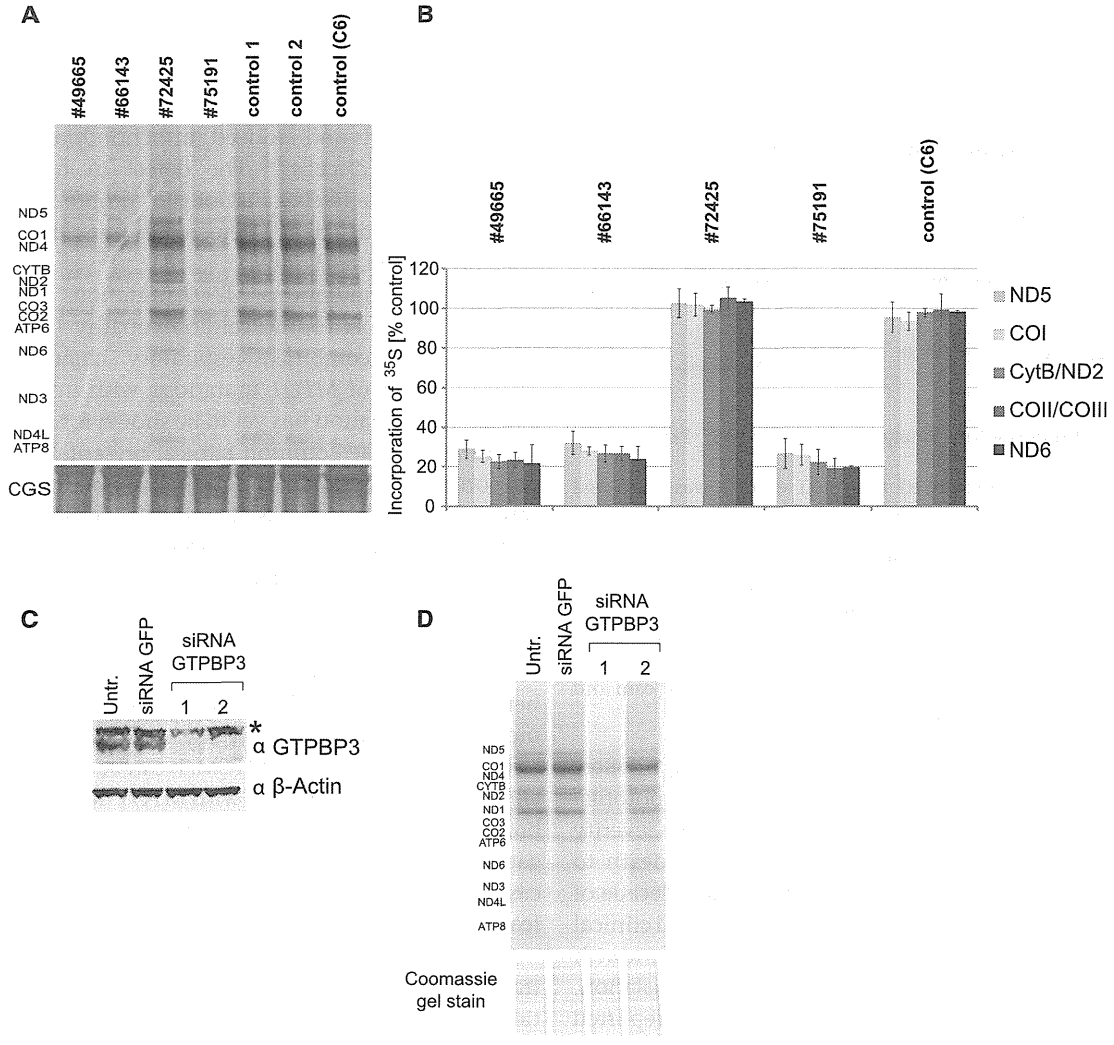

Figure 4. Analysis of Mitochondrial Protein Synthesis in Primary Fibroblasts and in HeLa Cells Treated with RNAi against GTPBP3
(A) [^{35}S]methionine metabolic labeling of mitochondrial proteins in fibroblasts. Products of mitochondrial translation were labeled with [^{35}S]methionine for 30 min, separated by a 4%–12% gradient SDS-PAGE, and visualized by autoradiography. To validate equal protein loading, a small section of the gel was stained with Coomassie (CGS). Fibroblasts from individuals #49665, #66143, and #75191 demonstrate significant inhibition of mitochondrial protein synthesis although translation in cells from individual #72425 is not affected.
(B) Quantification of radiolabelled products of mitochondrial translation. Incorporation of [^{35}S] as in (A) was quantified by ImageQuant software after exposure to a PhosphorImager screen from three independent experiments. Error bar indicates 1 SD.
(C) Downregulation of GTPBP3 in HeLa cells via RNA interference. Immunoblot analysis of total HeLa cell lysate transfected with two different siRNA to GTPBP3 show decreased level of GTPBP3 upon RNAi treatment for 6 days. siRNA to GFP was used as transfection control. Asterisk indicates nonspecific band recognized by anti-GTPBP3 antibody in HeLa cells. β-actin serves as a loading control. Two different siRNA duplexes targeting GTPBP3 were used, 1 and 2.
(D) Mitochondrial translation in HeLa cells upon GTPBP3 downregulation. HeLa cells were transfected for 6 days with siRNA against GTPBP3 and subjected to [^{35}S]methionine metabolic labeling. Inactivation of GTPBP3 leads to the reduced efficiency of mitochondrial translation. Two different siRNA duplexes targeting GTPBP3 were used, 1 and 2.

of RCC II and V remained normal in all cell lines (Figure 5). The diminished steady-state levels of respiratory chain complexes I and IV in fibroblast cell lines are in agreement with the impaired mitochondrial de novo translation rates in these cells and match the enzymatic defects identified in muscle biopsies of the same individuals.

Within an international cooperation between European (Germany, UK, Italy, France, and Belgium), Israeli, and Japanese Centers for mitochondrial disorders, we provide statistically convincing evidence for *GTPBP3* mutations leading to mitochondrial disease. To further support collaborative studies, the global mitochondrial disease community has established a Mitochondrial Disease Sequence Data Resource (MSeqDR) for common genomic data deposition and mining.

The genotype-driven analysis performed here was independent from the clinical presentation. Nevertheless, we identified common clinical features of the affected individuals that include lactic acidosis (11/11), cardiomyopathy (9/11), and neurological symptoms (6/11). The latter

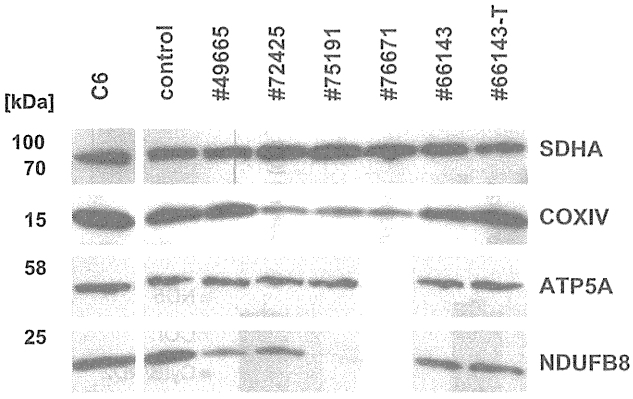

Figure 5. Immunoblot Analysis of OXPHOS Proteins in Fibroblasts
10 μg of detergent-solubilized total cell extract was subjected to immunoblot analysis of OXPHOS components. Amounts of SDHA (complex II) and ATP5A (ATPase) were unchanged in all individuals. In cells from individuals #72425, #75191, and #76671, a reduction of COXIV (complex IV) was observed. Cells from individuals #49665, #72425, and #75191 showed decreased levels of NDUFB8 (complex I). Antibodies used: mouse antibodies against SDHA (ab14715), NDUFB8 (ab110242), ATP5A (ab14748), and rabbit antibodies against COXIV (ab16056) from Abcam and rabbit anti GTPBP3 (HPA042158) from SIGMA Aldrich.

comprised symptoms such as development delay, intellectual disability, feeding difficulties, muscle hypotonia, fatigue, visual impairment, and epileptic seizures. Severity of the disease ranged from neonatal onset and death to late-infantile onset and survival into the second decade of life. Most affected individuals, however, manifested clinical symptoms before their first birthday. This is consistent with the normal cellular respiration, in organello translation, and normal levels of respiratory chain complexes reported in individuals less severely affected and the significantly reduced mitochondrial translation, respiration, and low levels of complex I and IV in those severely affected.

Modifications of the tRNA "wobble-base" in the anticodon loop are required for accurate and efficient codon recognition. The modification of position 5 (xm^5) of the U34 wobble-base of certain tRNAs is evolutionarily well conserved, although different modified side chains have been identified in different species. In mammals, mitochondria 5-taurinomethyluridine (τm^5U34) is found at the wobble-base position.[19] Based upon studies in bacteria and yeast mitochondria, GTPBP3 and MTO1 have been proposed to generate this modification in mammalian mitochondria. Although this prediction awaits direct biochemical validation, the proposed functional conservation of GTPBP3 and MTO1 has been supported by the mitochondrial localization of these proteins in human cells and by complementation of the respiratory-deficient phenotype in yeast by their mammalian homolog cDNAs.[20,21] Functional deficiency of homologs of GTPBP3 and MTO1 in bacteria and yeast mitochondria has been associated with abnormal U34 modification and consequently a reduced efficiency of translation.[21–23] Our data support an analogous activity of GTPBP3 in human mitochondria since we identified a reduced efficiency of translation in three cell lines with *GTPBP3* mutations and in cells with RNAi-mediated downregulation of GTPBP3 expression. Other groups have also reported impaired protein synthesis and reduced mitochondrial function in GTPBP3-depleted cells.[24] The defect in mitochondrial translation was a likely cause of the combined respiratory chain complex deficiency detected in muscle tissues of all but one affected individual.

Like *GTPBP3* mutations, *MTO1* mutations are also associated with hypertrophic cardiomyopathy (HCM), lactic acidosis, and combined respiratory chain deficiency. An association of *MTO1* mutations with impaired mitochondrial translation has yet to be shown for human mitochondria, but the common clinical presentation provides support for a common pathomechanism in the U34 modification for both diseases. So far, all individuals with *MTO1* mutations presented a HCM. However, nearly all of them have been specifically screened for *MTO1* mutations based on the clinical presentation of a HCM. Clinical and MRI signs of brain involvement are found for both *GTPBP3* and *MTO1* cases. The genotype-driven investigation presented here identified individuals with lactic acidosis, developmental delay, and MRI involvement of thalamus, putamen, and brainstem but without HCM. It can be expected that the clinical spectrum associated with MTO1 deficiency will also broaden, with more subjects being genome-wide investigated. In a very recent study, Taylor et al. indeed reported a case subject with *MTO1* mutations and central neurological features who did not have a cardiomyopathy.[25]

Our study highlights that defects in mitochondrial translation, probably owing to incorrect posttranscriptional modification of mt-tRNAs, are an important contributory factor to the spectrum of human mitochondrial disease. Recent data have suggested that more than 7% of all mt-tRNA residues undergo posttranscriptional modification, with close to 30 different modifications so far described.[4] Therefore, it is expected that future WES analyses of individuals clinically diagnosed with mitochondriopathy will reveal further mutations within genes coding for mt-tRNA modifiers. Indeed, in addition to the aforementioned mutations in *MTO1* and *TRMU*, mutations in *PUS1* (MIM 608109) (which introduces pseudouridine [Ψ] at base positions 27, 28, and 29 in several mt-tRNAs) have been reported in subjects affected with mitochondrial myopathy and sideroblastic anemia (MLASA)[26] (MIM 600462) and very recent studies have identified mutations in *TRIT1* (which is responsible for i^6A37 modification of a subset of mt-tRNAs) in individuals with severe combined mitochondrial respiratory chain defects.[27] Furthermore, mtDNA mutations in mt-tRNA genes, which are a very frequent cause of human respiratory chain deficiencies (MITOMAP), might also affect mt-tRNA modification. Related to the present study, it has been reported that τm^5U34 is not present in mt-tRNALeu^{UUR} harboring the m.3243A>G mutation (or other pathological mutations) responsible for mitochondrial encephalopathy, lactic

acidosis, and stroke-like episodes (MELAS) (MIM 540000). The absence of τm^5U34 has been suggested to be responsible for the mitochondrial translation defect in these subjects.[28] These results imply that deficiency of mt-tRNA modification plays a critical role in the molecular pathogenesis of human respiratory chain disease. Further studies of these pathways, such as analysis of tissue-specific regulation of mt-tRNA-modifying enzymes, might help to explain the clinical heterogeneity observed for mitochondrial diseases caused by mutations in mt-tRNA genes.

In conclusion, this study shows a mitochondrial translation disorder with a broad spectrum of clinical presentations, which emphasizes the importance of post-transcriptional modification of mitochondrial tRNAs for proper mitochondrial function.

Supplemental Data

Supplemental Data include four figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.10.017.

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Web Resources

The URLs for data presented herein are as follows:

MITOMAP, http://www.mitomap.org/MITOMAP
MSeqDR, https://mseqdr.org/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
Predotar, https://urgi.versailles.inra.fr/predotar/predotar.html
PSORTII Prediction, http://psort.hgc.jp/form2.html
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq

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