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Identification of *STAC3* variants in non-Native American families with overlapping features of Carey-Fineman-Ziter syndrome and Moebius syndrome

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Abstract

Horstick et al. (2013) previously reported a homozygous p.Trp284Ser variant in *STAC3* as the cause of Native American myopathy (NAM) in 5 Lumbee Native American families with congenital hypotonia and weakness, cleft palate, short stature, ptosis, kyphoscoliosis, talipes deformities, and susceptibility to malignant hyperthermia (MH). Here we present two non-Native

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American families, who were found to have *STAC3* pathogenic variants. The first proband and her affected older sister are from a consanguineous Qatari family with a suspected clinical diagnosis of Carey-Fineman-Ziter syndrome (CFZS) based on features of hypotonia, myopathic facies with generalized weakness, ptosis, normal extraocular movements, cleft palate, growth delay, and kyphoscoliosis. We identified the homozygous c.851G>C;p.Trp284Ser variant in *STAC3* in both sisters. The second proband and his affected sister are from a non-consanguineous, Puerto Rican family who was evaluated for a possible diagnosis of Moebius syndrome (MBS). His features included facial and generalized weakness, minimal limitation of horizontal gaze, cleft palate, and hypotonia, and he has a history of MH. The siblings were identified to be compound heterozygous for *STAC3* variants c.851G>C;p.Trp284Ser and c.763_766delCTCT;p.Leu255IlefsX58. Given the phenotypic overlap of individuals with CFZS, MBS, and NAM, we screened *STAC3* in 12 individuals diagnosed with CFZS and in 50 individuals diagnosed with MBS or a congenital facial weakness disorder. We did not identify any rare coding variants in *STAC3*. NAM should be considered in patients presenting with facial and generalized weakness, normal or mildly abnormal extraocular movement, hypotonia, cleft palate, and scoliosis, particularly if there is a history of MH.

Keywords

Myopathy; cleft palate; Native American Myopathy; Carey-Fineman-Ziter syndrome; Moebius syndrome; p.Trp284Ser variant; Qatar; Puerto Rican

Introduction

Congenital myopathies are a group of clinically and genetically heterogeneous conditions, and many still have an unknown molecular etiology. A homozygous missense variant (c.851G>C;p.Trp284Ser) in *STAC3*, a novel component of excitation-contraction (EC) coupling in skeletal muscles, segregated with Native American myopathy (NAM) (MIM 255995) in 5 Lumbee Native American families with 5 affected individuals thereby expanding the molecular basis for congenital myopathies [Horstick et al., 2013]. Also, compound heterozygous *STAC3* variants, (c.862A>T;p.Lys288* and c.432+4A>T), were recently reported in an individual with NAM of Turkish descent [Grzybowski et al., 2017]. NAM is an autosomal recessive condition characterized by congenital weakness, hypotonia, myopathic facies, ptosis, cleft palate, short stature, kyphoscoliosis, talipes deformities, and susceptibility to malignant hyperthermia (MH) provoked by anesthesia [Horstick et al., 2013; Stamm et al., 2008a; Stamm et al., 2008b]. Moebius syndrome (MBS) (MIM 157900) and Carey-Fineman-Ziter syndrome (CFZS) (MIM 254940) are congenital syndromes presenting predominantly with characteristic facial weakness. MBS is defined clinically as the presence of congenital, nonprogressive facial weakness with limited abduction of one or both eyes [Miller 2007; Rucker et al., 2014; Webb et al., 2012]. An additional criteria of full vertical motility has also been proposed [MacKinnon et al., 2014]. *De novo* rare variants in *PLXND1* and *REV3L* were suggested to cause a variety of phenotypes including MBS, congenital facial weakness disorders, and congenital fibrosis of the extraocular muscles in a limited number of cases [Tomas-Roca et al., 2015]. The first case report of autosomal recessive CFZS was in two affected siblings with marked bilateral facial weakness, mild

limitation of horizontal gaze, Pierre Robin sequence, hypotonia with generalized muscle hypoplasia, scoliosis, delayed motor milestones, failure to thrive, and normal intelligence [Carey 2004; Carey et al., 1982]. Additional cases have subsequently been reported with the suggested diagnoses of CFZS [Baraitser and Reardon 1994; Carey 2004; Maheshwari et al., 2004; Ryan et al., 1999; Schimke et al., 1993; Verloes et al., 2004]. The molecular etiology of CFZS has not yet been determined, and the clinical spectrum of this disorder will likely be better elucidated after identification of the causative gene. Here we report two sets of sibling pairs, one from Qatari and one from Puerto Rican ancestry, presenting with suspected clinical diagnoses of CFZS or MBS, who were diagnosed with NAM using whole exome sequencing (WES).

Clinical Report

The proband in Family 1 (Figure 1; Figure 3A) was an 8-year-old female who had been diagnosed clinically with CFZS and who presented for a genetic evaluation prior to a planned scoliosis surgery. Her older sister carried the same diagnosis and had a history of MH during a prior scoliosis surgery (Figure 3A). The sisters shared features of growth delay, cleft palate, kyphoscoliosis, hypotonia, ptosis and myopathic facies. The proband was born to a G5P4 36-year-old mother via vaginal delivery at full term but small for gestational age (<2 kg). Cleft palate, hypotonia and limited body movement were noted at birth, and she also required syringe feeding. She had early gross and fine motor delays, which were attributed to weakness and hypotonia. Due to her cleft palate and hypotonia she had speech delay and swallowing difficulties, but no overt cognitive deficits. She was fluent in 3 languages, comprehensible over 90 percent of the time, and able to participate in low to moderate intensity age-appropriate physical activity. She tolerated the surgical closure of her palate and dental procedures with general anesthesia.

On exam, at 8 years of age, she was symmetrically small for age with height and weight at the 50^{th} centile for 4-1/2-year-old female and head circumference at −2SD for age. She had a long face with dolichocephaly and downslanting palpebral fissures with severe ptosis. The mouth was held in an open position with a tented upper lip, downturned corners of the mouth and a surgically intact palate. There was pectus excavatum, short trunk and S-shaped scoliosis of the thoracic and lumbar regions with asymmetric hip position while standing. There were decreased skin creases over distal interphalangeal joints (DIPs), wrists, palms, and plantar surface of feet, likely due to decreased fetal movements (Figure 1). Her neurologic exam was significant for axial and appendicular hypotonia plus mild weakness (4/5) of the upper and lower extremities throughout. There was bilateral ptosis and limited ability to raise the lids or furrow the brow, but intact extraocular movements. She had a very weak smile and was unable to puff her cheeks by closing her mouth completely. She sensed touch, temperature, and pain appropriately and ambulated with a wide gait. Additional evaluations included mild to moderate conductive hearing loss, but normal echocardiogram, EKG, and pulmonary function tests. A skeletal survey confirmed the thoracic and lumbar scoliosis as well as lateral subluxations of bilateral tibiae and generalized osteopenia but normal C-spine stability. A SNP microarray was normal with 4.8 percent absence of heterozygosity reported throughout the genome. There was no CK level or muscle biopsy performed.

The proband's 18-year-old sister was not available for clinical evaluation, but medical history was provided by her father and by review of medical records. She was the couple's first child and was born with hypotonia, cleft palate and ptosis. Over time she demonstrated poor growth and hearing loss that was treated with hearing aids, but normal cognition. Her abnormal eye/lid movement and facial diplegia was initially diagnosed as MBS and subsequently, she was given the diagnosis of CFZS. Severe scoliosis and kyphoscoliosis evolved over her early teens. When undergoing anesthesia induction with inhaled agents for spine surgery at 15 years of age, she experienced body stiffening that was considered malignant hyperthermia which was treated in the OR. The surgery proceeded and was uneventful until an episode of prolonged hypotension due to blood loss at the end of the procedure. She was in a coma for 15 days and sustained severe neurologic injury including loss of ambulation and expressive language. After intensive and prolonged therapy, she walked with assistive equipment, though was largely wheelchair bound and had some verbal communication which was difficult to understand.

The proband in Family 2 (Figure 2A, B, C, D; Figure 3B) is a 21-year-old male who presented with a possible diagnosis of MBS. He was the first child of non-consanguineous Puerto Rican parents. His mother and father were 27 and 26 years old, respectively, at the time of conception. The pregnancy was conceived naturally, and the mother received routine prenatal care and denied prenatal exposures to infections, medications, and drugs. The proband was born via Cesarean section at 36 weeks of gestation due to a nuchal cord. Birth weight was 2.637 kg. At delivery, facial weakness, ptosis, hypotonia, cleft palate, weak cry, and respiratory distress were noticed. The proband was hospitalized in the neonatal intensive care unit for approximately 1 month for respiratory support including intubation, poor feeding, and failure to thrive. During this time, a diagnosis of MBS was made.

Past medical history was notable for conductive hearing loss diagnosed at age 7 years; he wears hearing aids. Pulmonary function tests revealed respiratory insufficiency. Past surgical history was notable for a first episode of MH at 9 months of age during the first attempt of cleft palate correction. Cleft palate repair and bilateral tympanostomy tubes placement was completed at age 4 years without complications. At age 7 years bilateral tympanostomy tubes were placed again without complications. Posterior spinal fusion for scoliosis was completed at age 12 years, which was complicated by a second episode of MH. During this hospitalization, a tracheostomy was placed and was removed after 3 months. Early motor milestones were delayed. Intelligence was normal. He is currently attending college.

On exam, at 21 years 4 months, weight was at the 25th centile and height was <3rd centile (50th centile for a 10-year-old). Head was normocephalic (25–50th centile). He had a long face with bitemporal narrowing, midface hypoplasia, downslanting palpebral fissures, bilateral epicanthal folds, and ptosis. He had normal distribution of hair with normal anterior and posterior hairlines. Ears were low-set and posteriorly rotated. He had a smooth philtrum and full lips. He had a surgically intact, but high-arched palate. Neck was short and notable for a well-healed tracheostomy scar. Chest was well-formed and without pectus deformity. Cardiovascular and pulmonary exams were normal, and abdomen was benign. He had a short trunk with scoliosis of the thoracic region, which had been surgically treated. Hands and feet were small (total hand length <<3rd centile bilaterally, 50th centile for a 10-year-old;

foot length <<3rd centile bilaterally, 50th centile for a 11-year-old). Camptodactyly was noted at the DIP joint of both fifth fingers. Decreased skin creases were also noted at the DIP joint of all digits on both hands (Figure 2A, B, C, D). Neurologic exam was significant for bilateral congenital upper and lower facial weakness and oral hypotonia. Speech was notable for dysarthria. Extraocular movements were notable for very mild limitation of abduction bilaterally. He had axial and appendicular hypotonia with generalized weakness of upper and lower extremities. The distal muscle groups were more affected than the proximal groups. He had decreased deep tendon reflexes in all extremities. Gait was normal. *RYR1* gene sequencing was normal (Prevention Genetics). CK levels obtained shortly after birth and at 4 years of age were normal.

The proband's 16-year-old sister (Figure 2E, F, G, H; Figure 3B) was born at 36 weeks by Cesarean section due to fetal tachycardia. The pregnancy was complicated by premature contractions, urinary tract infection, and hyperemesis. The mother was treated with terbutaline, folic acid, and antibiotics. Birth weight was 2.3 kg and length was 45.72 cm. At delivery, respiratory distress, hypotonia, and cleft palate were noticed. She was admitted to the neonatal intensive care unit for 5 months, after which time she was discharged on mechanical ventilation with a gastrostomy tube.

Past medical history was notable for multiple episodes of pneumonia and respiratory distress. Pulmonary function tests have revealed respiratory insufficiency. Hearing was normal. Past surgical history was notable for tracheostomy and gastrostomy placement at 1 month of age. Cleft palate correction was performed at age two years. Gold weights were placed bilaterally for treatment of lagophthalmos at age 5 years. Scoliosis surgery was performed at age twelve years. Currently she receives ventilatory support with oxygen supplementation at night. She had no history of MH.

On exam, at 16 years 5 months, head circumference was at the 3rd–10th centile, weight was <3rd centile (50th centile for a 12-year-old), and length was <<3rd centile (50th centile for a 10-year-old). Her head was brachycephalic. She had a long face with bitemporal narrowing, midface hypoplasia, downslanting palpebral fissures, bilateral epicanthal folds, and ptosis. Posterior hairline was low. Ears were large (>2SD above mean), but normal in position and rotation. Nasal tip was deviated to the right. She had a surgically intact, but high-arched palate. Neck was short and notable for torticollis and limited range of motion. She had a tracheostomy. Chest was without pectus deformity. Cardiovascular exam was normal, and pulmonary exam revealed transmitted upper airway rhonchi. Abdominal exam was notable for a G-tube. She had a short trunk with scoliosis of the thoracolumbar region. Hands and feet were small (total hand length <<3rd centile bilaterally, 50th centile for a 6- year-old; foot length <<3rd centile bilaterally, 50th centile for a 6-year-old). Feet were notable for brachydactyly and hypoplasia of metatarsals of digits 2 through 5 bilaterally. Overlapping toes were also noted (Figure 2E, F, G, H). Neurologic exam was significant for normal intelligence and full extraocular movements. She had bilateral congenital upper and lower facial weakness and oral hypotonia. Speech was notable for dysarthria. She had axial and appendicular hypotonia with muscle wasting and generalized weakness of upper and lower extremities. She had decreased deep tendon reflexes in all extremities and was wheelchair-bound due to generalized weakness.

Methods and Results

The proband in Family 1 was identified and recruited from the Johns Hopkins Hospital Genetics Clinic. Informed consent was obtained through a Johns Hopkins Medical Institutions IRB approved protocol for all recruited family members (Fig. 2A). All the recruited family members were submitted to the Baylor–Hopkins Center for Mendelian Genomics (BHCMG) study through the PhenoDB (www.mendeliangenomics.org) online submission portal for consideration of WES [Hamosh et al., 2013; Sobreira et al., 2015]. The proband in Family 2 was referred for participation in our research study by the Moebius Syndrome Foundation. The proband, affected sister, and both parents were enrolled in an IRB-approved protocol for the genetic study of MBS and related disorders at the Icahn School of Medicine at Mount Sinai (Fig. 2B). Written informed consent was obtained for all participating family members.

The samples received included whole blood for the proband in Family 1 as well as whole blood from all participating family members in Family 2. Saliva samples were obtained from family members of proband 1 including the parents, the affected 18-year-old sister, and three unaffected brothers. Genomic DNA was purified from fresh whole blood using the Gentra Puregene Kit (Qiagen Sciences, Germantown, MD, USA). Genomic DNA from saliva samples was purified using the Gentra Puregene kit (Qiagen Sciences, Germantown, MD, USA). Whole genome SNP genotyping was performed using the Affymetrix Genome Wide Human SNP Nsp/Sty 6.0 array (Affymetrix, Inc., Santa Clara, CA) for Family 1 members.

For Family 1, we performed WES on DNA from the proband, her 18-year-old affected sister and one of their unaffected brothers using the Agilent SureSelect Human All Exon 50Mb Kit (Agilent Technologies, Santa Clara, CA) and paired end 100 bp reads using the Illumina HiSeq2000 platform (Illumina, Inc. San Diego, CA). Read alignment to the reference genome (NCBI human genome assembly build 36; Ensembl core database release 50_361) [Hubbard et al., 2009] was performed using the Burrows-Wheeler Alignment (BWA) tool [Li and Durbin 2009], and single nucleotide variants (SNVs) and small insertion/deletions (indels) were identified using SAMtools [Li et al., 2009]. PCR duplicates were removed using the Picard software. We also performed local realignment and base call quality recalibration using GATK [McKenna et al., 2010].

Using the PhenoDB Variant Analysis Tool [Sobreira et al., 2015], we analyzed the WES data by applying a filter designed to prioritize rare (Minor Allele Frequency <1 percent) functional variants (missense, nonsense, splice site variants, and indels) that were homozygous or compound heterozygous in the proband and the affected sister but absent or heterozygous in the unaffected brother. We excluded variants found in dbSNP 126, 129 or 131, and variants with a MAF >1 percent in the Exome Variant Server and the 1000 Genome Project. We identified the p.Trp284Ser variant (c.851G>C) in exon 10 of *STAC3* (NM_145064, rs140291094) that was homozygous in the proband and her affected sister, but was heterozygous in the unaffected brother. By Sanger sequencing we validated and confirmed the segregation of the p.Trp284Ser variant in the proband, her affected sister, unaffected parents and 3 unaffected brothers (Fig. 2A).

For Family 2, DNAs from the two affected siblings and both parents were processed for WES in the Mount Sinai Genomics Core Facility. Genomic libraries were selectively enriched using the SureSelect V5 library (Agilent Technologies, Santa Clara, CA). Individual samples were barcoded, and samples were multiplexed for sequencing on a HiSeq 2500 instrument (Illumina, San Diego, CA) with a 100-bp paired-end protocol. Alignment and variant calling was completed using an in-house GATK-based pipeline [Linderman et al., 2014].

Called variants were filtered with Ingenuity Variant Analysis (Qiagen, Redwood City, CA), http://www.ingenuity.com). A total of 151,802 total variants in 17,817 genes were identified in the four samples. Variants were filtered based on confidence (call quality of ≥20, passed upstream pipeline filtering, and outside top 3 percent most exonically variable 100 base windows and/or 3 percent most exonically variable genes in healthy public genomes included), frequency (variants excluded if frequency was above 1.0 percent in the 1000 Genomes Project, NHLBI ESP exomes, or Exome Aggregation Consortium (ExAC)), predicted deleteriousness (frameshift, in-frame indel, start/stop codon changes, missense changes, splice site loss up to 6 bases into intron or as predicted by MaxEntScan, and variants listed in HGMD were included), and genetic analysis (variants selected if they were homozygous or compound heterozygous in 2 of 2 case samples consistent with autosomal recessive inheritance). This filtering strategy led to the identification of 23 variants in 11 genes. We identified the proband and his affected sister to be compound heterozygous for two *STAC3* variants, including the previously reported *STAC3* c.851G>C; p.Trp284Ser variant and a novel frameshift variant c.763_766delCTCT;p.Leu255IlefsX58 (NM_145064.2). The mother was heterozygous for the *STAC3* c.851G>C; p.Trp284Ser variant, and the father was heterozygous for the *STAC3* c.763_766delCTCT;p.Leu255IlefsX58 variant, consistent with an autosomal recessive mode of inheritance (Fig. 2B). PCR and Sanger sequencing was completed and confirmed the *STAC3* variants for each family members.

We screened the *STAC3* coding regions by WES or Sanger sequencing in 50 individuals with a clinical diagnosis of MBS or a congenital facial weakness disorder and 12 individuals from 11 families with a diagnosis of CFZS (8 unpublished individuals and 4 individuals previously reported by Carey et al. (1982), Ryan et al. (1999) and Dufke et al. (2004)), and we did not identify any additional rare coding variants.

Discussion

Here we report molecularly confirmed NAM in individuals who are not known to have Lumbee tribe Native American ancestry. The first proband is a female patient from Qatar who presented with a clinical diagnosis of CFZS based on her features of facial diplegia, ptosis, hypotonia, poor growth, cleft palate, and scoliosis and her similarly affected sister who suffered an episode of MH after exposure to inhaled anesthesia. Both females have normal extraocular movements. The second proband is a Puerto Rican male with symptoms of facial weakness, mild abduction deficits, hypotonia, cleft palate, and a history of MH. His sister is also affected and has more pronounced muscle weakness and muscle wasting, but full ocular motility and no history of MH. Given the different ethnic backgrounds and

clinical presentation prior to the report of a single non-Native American case with congenital muscle weakness, short stature, scoliosis, and early-onset ventilatory failure and *STAC3* mutations [Grzybowski et al., 2017], NAM was not considered in the differential diagnoses for either family. Instead, CFZS and MBS, syndromes with overlapping features, were considered, until WES confirmed *STAC3* variants and established the diagnosis of NAM.

Upon review of the literature, we note that MH is extremely rare in the setting of MBS and has not been associated with CFZS. Fernandes et al. (2013) report a single case of a child diagnosed with MBS who experienced MH after exposure to sevoflurane and succinylcholine during attempted clubfoot repair [Fernandes et al., 2013], but the limited clinical and family history details provided do not allow comparisons with the clinical findings in NAM or MBS and no molecular testing was performed to confirm the diagnosis. Patients with NAM are at relatively high risk for MH if exposed to triggering anesthesia and have a mortality rate of 21 percent during the newborn period and an overall 36 percent mortality rate during adulthood, largely attributable to MH [Stamm et al., 2008a]. Individuals with NAM have normal CK levels [Stamm et al., 2008a; Stewart et al., 1988], but there is conflicting data about their EMGs and muscle biopsy findings. Stewart et al. (1988) showed that EMGs in five of their NAM patients revealed evidence of myopathy, whereas muscle biopsy revealed either nonspecific myopathy or was normal [Stewart et al., 1988]. However, Stamm et al. (2008a) reported normal EMGs in 3 patients with NAM and widespread myopathy in a fourth. The proband in Family 1 has not had a muscle biopsy and unfortunately reports from her older sister in early childhood are not available. Neither had an EMG or CK level. The proband and affected sister in family 2 have had normal CK levels; neither affected sibling has had an EMG.

The SH3 domain and cysteine rich (C1) domain 3 (*STAC3*) gene is the third member of the *STAC* gene family. SH3 domain and the C1 functional domains are frequently components of signaling proteins [Reinholt et al., 2013] and *STAC3* encodes a novel component of excitation-contraction (EC) coupling in skeletal muscle. The affected sisters in family 1 harbor the *STAC3* homozygous c.851G>C (p.Trp284Ser) missense variant that was also reported as the genetic cause of NAM in 5 Lumbee Native American families [Horstick et al., 2013]. This p.Trp284Ser variant is in the first SH3 (Src homology three) domain of STAC3 and the tryptophan in this position is highly conserved across SH3 domains. It is present in the ExAC database as heterozygous in 13 alleles out of 121350 alleles (12 from African population and 1 from Latino population); no homozygotes are described. The affected siblings in Family 2 were compound heterozygous for the c.851G>C variant and a novel c.763_766delCTCT (p.Leu255IlefsX58) loss of function variant not found in dbSNP, but described in ExAC as 2 heterozygous in 2 alleles out of 121242 alleles (2 from African population) and in no homozygotes.

Muscle contraction requires a series of events known as excitation–contraction (EC) coupling to regulate Ca2+ release from internal stores to initiate muscle contraction. *STAC3* product participates in EC coupling machinery [Horstick et al., 2013; Nelson et al., 2013]. EC coupling involves a complex of proteins that include dihydropyridine receptor (DHPR) and ryanodine receptor 1 (RyR1), two Ca^{2+} channels among many other components. Although STAC3 is required for voltage-induced Ca^{2+} release in skeletal muscle, there may

still be unknown proteins that are required for this process with which STAC3 might interact [Nelson et al., 2013].

Recently several animal models were employed to ascertain the role of Stac3. Though different mechanisms are proposed, all agree Stac3 is essential for functional skeletal muscle. Bower et al. (2012) used morpholino knockdown studies in zebrafish embryos and RNAi in cultured C2C12 mouse myoblasts and reported that Stac3 is required for myotube formation and differentiation of skeletal myoblasts in zebrafish [Bower et al., 2012]. Reinholt et al. (2013) reported that the homozygous deletion of Stac3 in mice produced either a lethal skeletal muscle phenotype or mice which had an abnormal body curvature and dropping forelimbs and did not respond to prodding. They found Stac3 was expressed only in the skeletal muscle but was not expressed or expressed at very low levels in brain, heart, and the smooth muscle-containing stomach. Reinholt et al. (2013) hypothesized that Stac3 gene pathogenic variants disrupted myofiber maturation and may be involved in the translocation of myonuclei during skeletal muscle development [Reinholt et al., 2013].

In contrast, Nelson et al. (2013) report that although myofibers from Stac3 knockout mice are dysmorphic and have abnormalities in the subcellular structure, these results are secondarily from the complete absence of muscle contractility and primarily from lack of Ca2+ transients, rather than from a primary role of STAC3 in regulating myogenesis. The authors report that STAC3, a skeletal muscle protein, localizes to T tubules and is essential for coupling membrane depolarization to Ca^{2+} release from the sarcoplasmic reticulum (SR). They proposed that STAC3 may promote coupling between or possibly link the DHPR voltage sensor and the RyR Ca2+ release channel [Nelson et al., 2013].

Additionally, Horstick et al. (2013) used a forward genetic screen in the zebrafish model to analyze their muscles in vivo with electrophysiology and live imaging in order to understand EC coupling [Horstick et al., 2013]. The authors identified Stac3 as the gene responsible for defective EC coupling in zebrafish. The loss of Stac3 resulted in a progressive breakdown of myofibers during larval stages with swollen SR observed by 7 days post fertilization [Horstick et al., 2013]. Finally, Cong et al. (2016) used RT-qPCR and western blotting analyses on the limb muscles of 4-week-old mice after tamoxifen injection to demonstrate that Stac3 had an important role in postnatal skeletal muscle growth, fiber hypertrophy and muscle strength, muscle composition, and muscle contraction [Cong et al., 2016].

In summary, our report of molecularly confirmed NAM in families from ancestries other than the Lumbee tribe of Native Americans, together with the reported Turkish case [Grzybowski et al. 2017], highlights the need to consider NAM in the differential diagnosis of congenital myopathy in patients of all ethnicities. Of note, the Qatari sisters were noted to be homozygous for the same variant, *STAC3* p.Trp284Ser, identified in the Lumbee tribe, and the Puerto Rican siblings were compound heterozygous for *STAC3* variants, with one variant also being this same change. But our data shows that this is not a common founder case since the p.Trp284Ser variant in these 2 families is not in the same haplotype (data not shown). Despite the clinical overlap between NAM, CFZS, and MBS, they have important differences, and *STAC3* mutations were not identified in a large cohort of MBS or CFZS. Ophthalmological examination should distinguish the ptosis without significant

ophthalmoplegia found in NAM and possibly CFZS from the abduction deficit that together with facial weakness defines MBS (Table 1). Electrophysiology, brain and muscle imaging studies can help exclude a primary defect in cranial nerve development typical of MBS and narrow the diagnosis. Moreover, the presence of MH is one distinctive feature that supports a diagnosis of NAM, and *STAC3* gene sequencing should be recommended to confirm this diagnosis. Additionally, early diagnosis of NAM is especially helpful for anticipatory management of MH.

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Appendix

Membership of the Moebius Syndrome Research Consortium

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Figure 1.

Informed consent for photograph use was obtained. A) Proband is symmetrically small for age with scoliosis and asymmetric hip position. B) C) and D) long face with dolichocephaly, myotonic facies, downslanting palpebral fissures with severe ptosis, tented upper lip, downturned corners of the mouth. E) decreased skin creases over distal interphalangeal joints and wrists

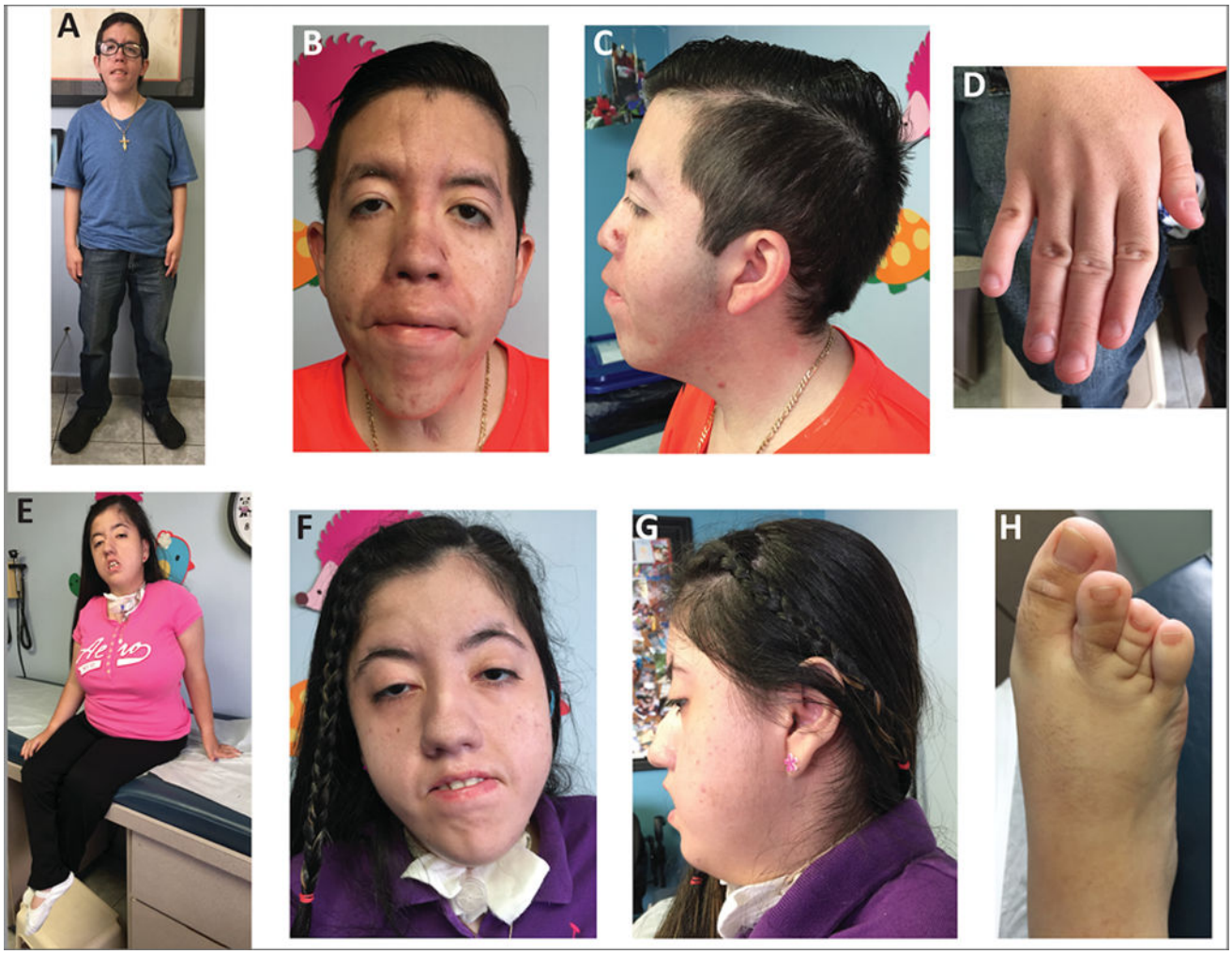

Figure 2.

Informed consent for photographs was obtained. A) The proband is noted to have significant short stature. Head is normocephalic. B,C) The proband has a long face with bitemporal narrowing, midface hypoplasia, downslanting palpebral fissures, bilateral epicanthal folds, ptosis, low-set and posteriorly rotated ears, and hypoplastic nasolabial folds. D) Camptodactyly is noted at the DIP joint of the 5th digit. Decreased skin creases are noted at the DIP joint of all digits. E) The proband's sister has significant short stature and a tracheostomy. F–G) Head is brachycephalic. She has a long face with bitemporal narrowing, midface hypoplasia, downslanting palpebral fissures, bilateral epicanthal folds, and ptosis. H) Feet are notable for brachydactyly, overlapping toes, and hypoplasia of metatarsals 2 through 5.

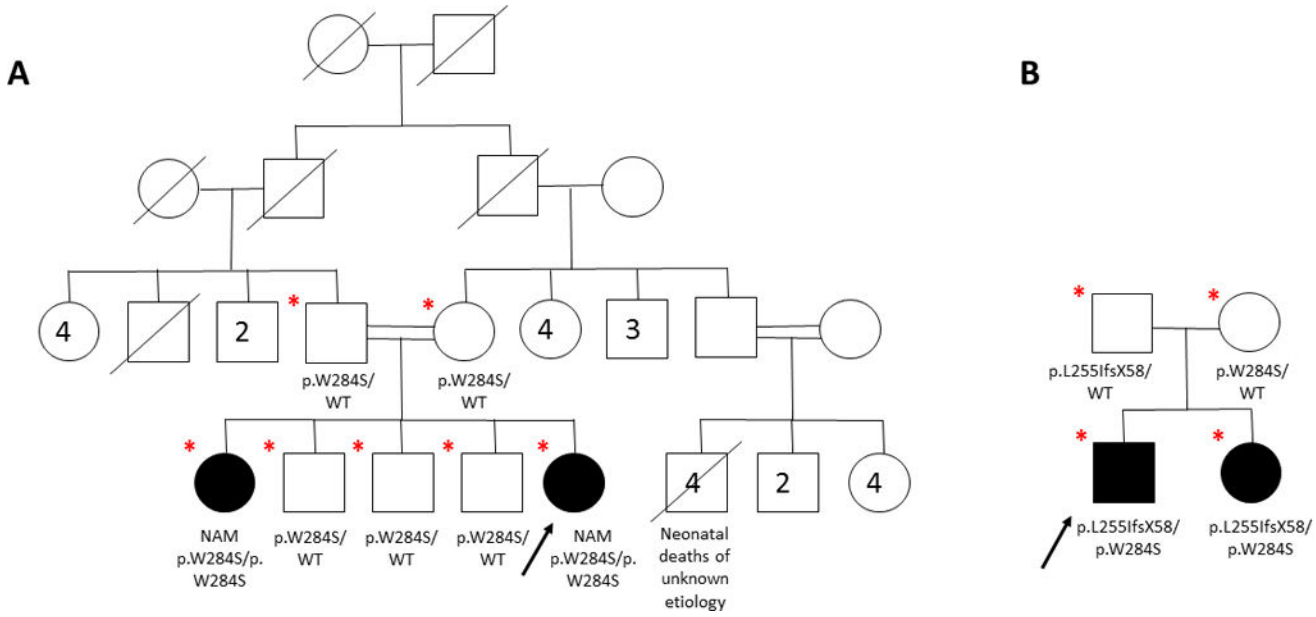

Figure 3.

A. Family history is significant for 18-year-old similarly affected sister and parental consanguinity in a family form Qatar. A maternal uncle had 4 male children with neonatal deaths of unknown etiology from a consanguineous union. The same maternal uncle had 6 healthy children. Note, the presence of the star indicates that DNA samples of these individuals were available for testing. The black circles represent the clinically affected individuals with Native American Myopathy (NAM). Genotypes of the family members indicate biallelic variants in the two affected sisters with NAM, and the heterozygous state of the parents and the unaffected brothers. B. Family history is notable for two affected siblings born from a noncosanguineous union. Maternal and paternal lineages are of Puerto Rican ancestry. Genotypes of the family members indicate biallelic variants in the two affected siblings with NAM.

Table 1

Clinical Features of Native American Myopathy, Carey Fineman Ziter syndrome, and Moebius syndrome.

	Native American Myopathy	Carey Fineman Ziter syndrome	Moebius syndrome
Facial Weakness	+	+	+ (Must be present *)
Impairment in Ocular Abduction	−	+/−	+ (Must be present *)
Full eye movements	+/−	+/−	−
Other Cranial Nerve Abnormalities	−	−	+/−
Short stature	+/−	+	+/−
Cleft palate	+/−	+/−	+/−
Poland anomaly	−	+/−	+/−
Scoliosis	+/−	+/−	+/−
Limb reduction defects	−	−	+/−
Talipes equinovarus	+/−	+/−	+/−
Joint contractures	+/−	+/−	+/−
Hypotonia	+	+	+/−
Intellectual Disability	Rare	Rare	+/−
History of Malignant Hyperthermia	+	−	Rare **

* For a diagnosis of Moebius syndrome, patients must have facial weakness (unilateral or bilateral) and the inability to abduct one or both eyes.

** Fernandes et at. reports one case of a child with Moebius syndrome experiencing malignant hyperthermia during clubfoot repair, with limited clinical description.