



Case Report

De novo ATP1A3 and compound heterozygous NLRP3 mutations in a child with autism spectrum disorder, episodic fatigue and somnolence, and muckle-wells syndrome



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ABSTRACT

Complex phenotypes may represent novel syndromes that are the composite interaction of several genetic and environmental factors. We describe an 9-year old male with high functioning autism spectrum disorder and Muckle-Wells syndrome who at age 5 years of age manifested perseverations that interfered with his functioning at home and at school. After age 6, he developed intermittent episodes of fatigue and somnolence lasting from hours to weeks that evolved over the course of months to more chronic hypersomnia. Whole exome sequencing showed three mutations in genes potentially involved in his clinical phenotype. The patient has a predicted pathogenic *de novo* heterozygous p.Ala681Thr mutation in the *ATP1A3* gene (chr19:42480621C > T, GRCh37/hg19). Mutations in this gene are known to cause Alternating Hemiplegia of Childhood, Rapid Onset Dystonia Parkinsonism, and CAPOS syndrome, sometimes accompanied by autistic features. The patient also has compound heterozygosity for p.Arg490Lys/p.Val200Met mutations in the *NLRP3* gene (chr1:247588214G > A and chr1:247587343G > A, respectively). *NLRP3* mutations are associated in an autosomal dominant manner with clinically overlapping auto-inflammatory conditions including Muckle-Wells syndrome. The p.Arg490Lys is a known pathogenic mutation inherited from the patient's father. The p.Val200Met mutation, inherited from his mother, is a variant of unknown significance (VUS). Whether the *de novo* *ATP1A3* mutation is responsible for or

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plays a role in the patient's episodes of fatigue and somnolence remains to be determined. The unprecedented combination of two NLRP3 mutations may be responsible for other aspects of his complex phenotype.

1. Introduction

Gene mutations associated with impairing behavioral disorders that present in childhood may hold the key to discovering highly penetrant mutations and actionable genetic mechanisms that lead to these disorders. We describe a child with autism spectrum disorder, as well as worsening fatigue and somnolence. We present the analysis performed in the de-convolution of the genetic origins of this patient's complex genetic disease.

1.1. Patient description

The proband is a 9 year-old male with a history of motor and speech developmental delays, autism spectrum disorder, low vision, and strabismus. He has fixed interest in superheroes consistent with his autism spectrum disorder. At 5 years of age, he manifested severe perseveration, which interfered with functioning in the school and home. This perseveration improved with risperidone and later with aripiprazole treatment. At age 6 years, his parents and teachers noted intermittent fatigue and excessive daytime sleepiness with longer nocturnal sleep times (13–16 h/night) than expected for age [1]. He was difficult to arouse in the morning from this long sleep. These symptoms would last 2–3 days and occur approximately every two weeks. No other mood, behavior or eating changes were reported with these hypersomnia episodes. In between episodes, sleep patterns were normal and daytime sleepiness less severe. Over the course of months, sleepiness and fatigue with long sleep times became more of a chronic, daily issue. The patient

was falling asleep through the day and began to rely on brief scheduled naps through the school day to manage excessive daytime sleepiness. The patient denied symptoms of narcolepsy including sleep paralysis, hypnagogic/hypnopompic hallucinations and cataplexy. Physical examinations have consistently revealed his weight and height to be in the 60th percentile, and head circumference between the 15th and 25th percentile. He has no dysmorphic features or neurocutaneous stigmata. His hair is blonde and thin. He can follow commands and speak fluently but he is talkative and impulsive. He had significantly decreased visual acuity, decreased visual field bilaterally, bilateral nystagmus and limitation of abduction of the left eye (status post-operative procedure for strabismus), and a form of dyspraxia of horizontal movements on lateral gaze. Motor exam showed generalized hypotonia but no spasticity, dystonia, or tremor. He has full strength. Reflexes were 1+ and symmetric without evident clonus. He had occasional overflow movements with stressed gait testing. Plantar response was bilaterally flexor. His coordination when running was mildly impaired but his cerebellar and gait examinations were otherwise normal.

1.2. Neurological evaluation

Brain MRIs demonstrated small nonspecific white matter hyperintensities in the bilateral centrum semiovale and right corona radiata (Supplemental Fig. 1) stable over multiple exams. There was no edema, atrophy, or other parenchymal signal alteration. MRI of the temporal bones demonstrated a hypoplastic right posterior semicircular canal, dysmorphic left posterior semicircular canal with no bone island (arrowhead), and a globular left vestibule (“v”). The cochlea (*), lateral semicircular canals (arrows), and superior semicircular canals (dashed arrows) appear normal bilaterally.

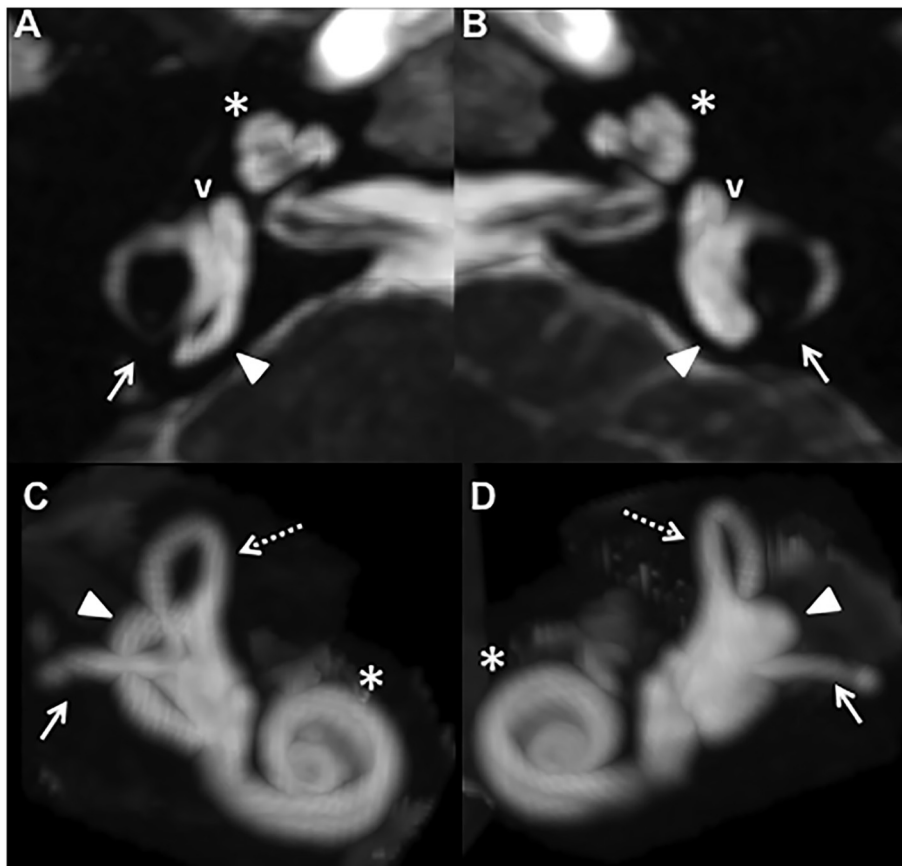


Fig. 1. Temporal bone MRI obtained at 7 years of age. Segmented axial 3D FIESTA (fast imaging employing steady-state acquisition) images (A,B) and MIP (maximum intensity projection) images (C, D) of the right (A,C) and left (B, D) temporal bones demonstrate hypoplastic appearance of the right posterior semicircular canal (arrowhead), dysmorphic left posterior semicircular canal with no bone island (arrowhead), and a globular left vestibule (“v”). The cochlea (*), lateral semicircular canals (arrows), and superior semicircular canals (dashed arrows) appear normal bilaterally.

island), and globular left vestibule. The cochlea, superior/lateral semicircular canals, and cochlear nerves were normal (Fig. 1). Despite abnormal bone structure, a hearing test indicated normal hearing.

An electroencephalogram (EEG) showed rare frontal sharp waves and intermittent slowing. He had one episode of staring and unresponsiveness that could have been a seizure when he was 3 years old.

He had long-term video EEG monitoring during one of the 2-week episodes of increased somnolence to determine if seizure activity could be contributing to his periods of altered sensorium. These periods included excessive somnolence for up to 16 h at a time from which he was difficult to arouse. No seizure activity was detected that could explain the episodes. During the periods of increased somnolence, the patient experienced stomach pain, fatigue, and sleepiness. Polysomnography was normal during the night but was not performed during daytime somnolent episodes. Multiple sleep latency test (MSLT) testing did not reveal rapid onset REM activity.

1.3. Sleep studies

A nocturnal polysomnogram and daytime multiple sleep latency test was conducted at age 8 years for evaluation of excessive daytime sleepiness. The patient was on REM-suppressing medications of aripiprazole and guanfacine at time of testing. The patient slept 8.2 h and had a sleep efficiency of 89% (within normal range) on the nocturnal polysomnography. The study revealed a mildly increased apnea-hypopnea index (AHI) of 1 h (< 1/h is normal) [2]. Because this AHI was mild, obstructive sleep apnea was not felt to be causal to his severe daytime sleepiness. The next day after the polysomnogram, he had the multiple sleep latency test that confirmed objective hypersomnia. During the testing, he fell asleep during all 5 nap periods and his mean sleep latency was 3.9 min (< 8 min is consistent with clinical criteria for hypersomnia disorders) [2].

The sleep studies were repeated at 9 years of age and patient was on aripiprazole and guanfacine. The overnight polysomnogram was reported as normal. Again, the multiple sleep latency test confirmed objective hypersomnia with a mean sleep latency of 3.4 min and no sleep onset REM periods. It should be noted that the diagnosis of narcolepsy, a disorder of daytime sleepiness and abnormal REM sleep intrusions [2], could not be excluded based on sleep study testing because of the patient's use of REM-suppressing medications (aripiprazole and guanfacine).

The patient was negative for the HLA DQB1*0602 found in 85–95% of patients with narcolepsy with cataplexy but positive for a less sensitive marker associated with this condition (HLA DRB1*15) [3]. Hypocretin CSF testing results are pending.

His past medical history shows he was delivered by emergency cesarean at 31 weeks due to severe maternal preeclampsia. Birth weight was 3 pounds 14 oz, and length was 16 in.; head circumference was normal. Apgar scores were 3 at 1 min and 7 at 5 min. He required resuscitation at birth and was ventilated for the first 24 h of life. He received continuous positive airway pressure (CPAP) for 48 h. Neonatal jaundice was treated with light therapy and apnea of prematurity with caffeine. He stayed in the NICU for 36 days and was discharged when the apnea resolved. He went home without home apnea monitoring. Cranial ultrasound showed left grade I intraventricular hemorrhage. Since birth, his mother noted that he had difficulty opening his left eye. At about 3 months of age, his mother noted impaired ability to fix and track. In addition, he had left exotropia measuring about 35–40 prism diopters at distance and near, and was also esotropic on occasion. He had bilateral horizontal nystagmus. He also had hypopigmented hair and fundus with blunted macular and foveal reflexes. He underwent strabismus surgery on his left eye. He continues to have cortical visual impairment and is legally blind with glasses for corrected vision.

Motor development was delayed. He did not walk until he was 2.5 years old and even then was very clumsy and had frequent falls. An extensive evaluation for ataxia was negative. His apparent ataxia

improved over time. Occasional subtle posturing of either leg occurs while walking fast or running.

Speech development was delayed. He began to use single words at age 2 and phrases at 2.5 years. At 4 years of age, he had reduced eye contact, deficits in social reciprocity, a rigid adherence to preferred routines, and repetitive vocalizations and was diagnosed with autism spectrum disorder. His social reciprocity, flexibility, and repetitive vocalizations improved over time, although he continues to be inflexible.

Administration of the Wechsler Intelligence Scales for Children, 4th Edition at age 6.75 years revealed that verbal comprehension (standard score 100, 50%) and perceptual reasoning (standard score 92, 44%) were average. Processing speed was borderline (standard score 75, 5%) and working memory (standard score 84, 13%) was below average. He had limited endurance for tasks that require sustained attention and detail. Academically he is functioning at a grade level consistent with his age.

At 5 years of age, longstanding fixed interests in superheroes increased in intensity and pervasiveness. He was immersed in perseverative behavior throughout so much of the day that it interfered with learning at school. An evaluation was performed at age 7 due to concerns with worsening perseveration. After a month of treatment with risperidone at 1 mg per day, the perseverative behavior lessened. He was also able to avert his focus from this play and onto other activities including his studies.

The patient was treated for attention deficit hyperactivity disorder (ADHD) before the escalation of his behavioral problems. He became more aggressive when treated with mixed amphetamine salts (Adderall) so this treatment was discontinued. A trial of methylphenidate was terminated because he spoke nonstop and had markedly decreased sleep for 48 h after the first dose. At 7.5 years of age, guanfacine was initiated to treat his hyperactivity. The dose was gradually increased to 1.25 mg in the morning. The guanfacine decreased his hyperactivity. He was treated with risperidone at 0.5 mg twice per day when he was 7 years and 6 months of age. He was later switched to aripiprazole due to excessive weight gain on risperidone. Moving drug dosing to bedtime only did not improve his daytime sleepiness.

Temperature-dependent hives, daily fevers, abdominal discomfort, chronic constipation, and joint pain remitted with initiation at age 6 years of injections with the human anti-Interleukin-1 β monoclonal antibody, canakinumab. Canakinumab neutralizes Interleukin-1 β signaling, resulting in suppression of inflammation in patients with disorders of autoinflammatory origin. His eczema has improved after starting this medication.

1.4. Family history

Both parents are Caucasian. The father has photosensitivity. The mother has mild neurosensory hearing loss, cold sensitivity, rashes, joint pain, abdominal discomfort, and headache. In addition, she was treated for anxiety in the past but has not required treatment in years. There is no history of hypersomnia in the family. The patient does not have any siblings and parents deny consanguinity.

2. Methods

Written, informed consent was obtained at The Manton Center for Orphan Disease Research, Gene Discovery Core and at the NIH Clinical Center. Research protocols were approved by the Institutional Review Boards of Boston Children's Hospital and the National Human Genome Research Institute. Whole Exome Sequencing (WES) was performed at GeneDX. Using genomic DNA from the submitted individuals, the Agilent SureSelect XT2 All Exon V4 kit was used to target the exonic regions of the genome. These targeted regions were sequenced using the Illumina HiSeq 2000 sequencing system with 100 bp paired-end reads. The DNA sequence was mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. Tile

targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. The XomeAnalyzer was used to evaluate sequence changes in this individual compared to other sequenced family members. Selected sequence variants in the proband and relatives were confirmed by conventional di-deoxy DNA sequence analysis. Patient data were also run through the Codified Genomics Pipeline (proprietary algorithm, Houston TX). The Exome Variant Server, 1000 Genomes, ExAc and ClinVar databases were checked on June 21, 2016.

For the chromosomal microarray, genomic DNA was examined by array-based comparative genomic hybridization (aCGH) using the current version of ExonArrayDx. The array contains DNA oligonucleotide probes in or flanking most exons of the evaluated genes. The array is designed to detect most single-exon deletions and duplications. Probe sequences and locations are based on Genome Reference Consortium build 37 (GRCh37)/UCSC hg19. Data analysis was performed with Agilent Genomic Workbench software, using gene-specific filtering by Cartagenia BENCH software. Structural modeling was created by MODELLER [4].

3. Results

Chromosomal microarray was normal. WES revealed a novel *de novo* mutation at c.2041G > A, p.Ala681Thr in exon 15 in the *ATPIA3* gene (GRCh37/hg19, chr19:42480621C > T, ENST00000302102.5, NM_152296.4) (Fig. 2), which encodes an isoform of the alpha subunit of the ATP-dependent transmembrane sodium/potassium pump that helps maintain electrochemical gradients across the plasma membrane of neurons [5, 6]. WES also revealed two mutations in *NLRP3* (chr1:247588214G > A, ENST00000336119.3:c.1469G > A, p.Arg490Lys, and chr1:247587343G > A, c.598G > A, p.Val200Met). *NLRP3* encodes a component of the inflammasome and is associated with Muckle-Wells syndrome (OMIM 191900) [7].

Case molecular details have been referenced in DECIPHER database for chromosomal aberrations: # 328583 [8].

Although the patient had light pigmentation and a somewhat blond fundus on ophthalmic examination, a definitive diagnosis of albinism was lacking. In particular, iris transillumination could not be documented. Because mild hypopigmentation can occur with some subtypes of Hermansky-Pudlak syndrome (HPS), this diagnosis was pursued. However, whole mount electron microscopy of platelets showed a normal contingent of dense bodies, and western blotting for HPS-1, HPS-3, HPS-4, HPS-5, and HPS-6 proteins showed normal amounts, ruling out the disorder. WES did not reveal any plausible disease-causing variants in the genes associated with HPS (*HPS1*, *AP3B1*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBP1*, *BLOC1S3*, *PLDN*, and *AP3D1*). Another possible diagnosis related to hypopigmentation would be Griscelli syndrome; in fact, the patient had a paternally inherited VUS in *MYO5A* associated with Griscelli syndrome type 1 (chr 15:52667641G > A, ENST00000399231.3:c.2437C > T, p.Arg813Cys, scored as damaging by SIFT and Polyphen2, MutationTaster scored as “Disease Causing”).

However, a second mutation was not found, and the patient's hair did not manifest the typical findings for Griscelli type 1. Hence, we conclude that the patient's hypopigmentation was not a significant pathological finding.

3.1. ADOS-2

In order to carefully ascertain to what extent he continued to meet criteria for an autism spectrum disorder while treated with aripiprazole at age 8 years, he was administered Module 3 of the Autism Diagnostic Observation Schedule-2 (ADOS-2) by a research reliable administrator in a clinical setting. The ADOS-2 is a series of structured and semi-structured tasks designed to elicit behaviors indicative of a diagnosis of autism spectrum disorder. Module 3 is intended for verbally fluent children and younger adolescents [9]. Throughout the assessment, he used complex language and engaged in reciprocal conversations; however, these conversations were almost exclusively about his own restricted interests. His speech had irregular rhythm and flat intonation. His Total Score exceeded the cutoff of 9 for an Autism classification. Taking into account his age and the ADOS module administered, his Total Score maps to an ADOS-2 Comparison Score of 8 indicating a high level of autism spectrum-related symptoms. In addition to the DSM-5 criteria, he met criteria on the ADOS-2 for autism [10–12].

4. Discussion

This report describes a mutation in *ATPIA3* and compound heterozygous mutations in *NLRP3* in a 9-year old boy with autism spectrum disorder with extreme perseverations and fatigue and hypersomnia. He also has structural abnormalities of the vestibule/posterior semicircular canal bilaterally with absence of a normal posterior semicircular canal on the left side.

4.1. *ATPIA3*

The patient has a *de novo* p.Ala681Thr (GCC > ACC): c.2041 G > A in exon 15 in the *ATPIA3* gene (NM_152296.4). *ATPIA3* mutations have been reported to encompass an expanding array of phenotypes ranging from alternating hemiplegia of childhood (AHC) to rapid-onset dystonia-parkinsonism (RDP) to a variety of more complex phenotypes. Patients with AHC manifest a complex neurodevelopmental phenotype with a wide variety of acquired and episodic neurologic features. However, emergence of behavioral phenotypes in early childhood is frequently reported, accompanied by hypersensitivity to a variety of environmental stimuli, and need for prolonged periods of sleep to abort episodes of neurologic dysfunction [13]. In addition, a subset of patients with *ATPIA3* mutations resulting in cerebellar ataxia, areflexia, pes cavus, optic atrophy, sensorineural hearing loss (CAPOS syndrome) are reported to have autistic symptoms [14].

To our knowledge, the *de novo* p.Ala681Thr mutation in *ATPIA3* has not been reported previously as a disease-causing mutation or a

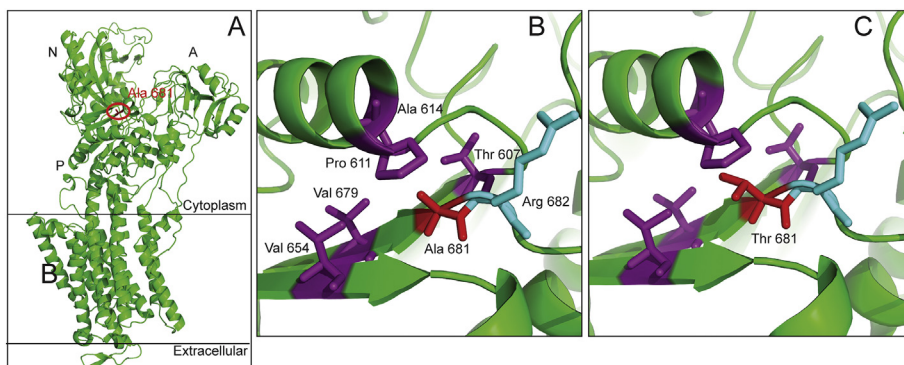


Fig. 2. Model of the p.Ala681Thr mutation in *ATPIA3*. The structural model of *ATPIA3* (A), and residue 681 (B–C) made by PYMOL. Residue Ala681 (B) and mutant Thr681 (C) are colored in red, and the hydrophobic core around residue 681 is colored in purple, while the adjacent residue Arg682 is colored in cyan. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

benign polymorphism. The p.Ala681Thr mutation was not observed in approximately 6500 individuals of European and African American ancestry in the Exome Sequencing Project, nor was the variant seen in the Exome Aggregation Consortium (ExAc) database, indicating that it is not a common benign variant in the populations. The change is predicted to be damaging by Polyphen2 and SIFT (scored 0.0), and was predicted to be “Disease Causing” by MutationTaster.

A structural model of ATP1A3 was created by MODELLER [4] based on the structure of a sodium - potassium pump (PDB code: 2ZXE), which had high homology in the Protein Data Bank (PDB) (Fig. 2) [15]. Residue Ala681 is located at the phosphorylation (P) domain in the cytoplasm, other domains being A and N (Fig. 2A). The residue Ala681 is buried in a hydrophobic core, which is composed of several hydrophobic residues, including Thr607, Pro611, Ala614, Val654, and Val679 (Fig. 2B). These residues are within 5 Å distance from the residue Ala681 calculated by Swiss PDB viewer V4.1. Moreover, there is a complex network of hydrogen bonds and polar interactions close to Ala681, including the adjacent Arg682, that transiently link the P, A, and N domains and are important for stabilizing sodium-bound and potassium-bound conformations during ion transport. The mutation p.Ala681Thr introduces a polar side chain that looks likely to disturb these networks and either reduce activity or reduce affinity for sodium, which are the sequelae of many ATP1A3 mutations [16].

This substitution occurs at an amino acid position that is well conserved across vertebrates (Fig. 3). A missense mutation in a nearby residue (S684F) has been reported in the Human Gene Mutation Database in association with RDP, supporting the functional importance of this region of the ATP1A3. The p.Ala681Thr may be a disease-causing mutation. While prematurity and intraventricular hemorrhage could in part contribute to his developmental deficits, the absence of long tract signs such as increased muscle tone (spasticity), increased reflexes or clonus, or focal motor weakness preclude a diagnosis of cerebral palsy. Based on strong similarities to features frequently observed in children with ATP1A3 mutations, including nystagmus, speech delay, developmental delay, hypotonia, limb posturing, apparent ataxia, EEG slowing, and autistic features, it is possible that his hypersomnia is attributable, at least in part, to his ATP1A3 variant [13].

4.2. NLRP3

The patient is also compound heterozygous for the p.Arg490Lys and p.Val200Met variants in NLRP3. Mutations in the NLRP3 gene are associated with a group of autosomal dominant, systemic inflammatory disorders collectively referred to as the cryopyrin-associated periodic syndromes and comprised of Muckle-Wells syndrome, Familial Cold Autoinflammatory Syndrome (FCAS), and Neonatal Onset Multisystem

Inflammatory Disease (NOMID), also known as Infantile Neurologic Cutaneous and Articular (CINCA) syndrome, and recently reviewed in Aksentijevich et al. [17]. These disorders are characterized by intermittent episodes of fever (or persistent fever in severe forms such as NOMID), urticarial rash, arthritis and abdominal pain. In FCAS, the symptoms are precipitated by exposure to cold. In both Muckle-Wells syndrome and CINCA/NOMID, progressive sensorineural hearing loss is common, while chronic meningitis, uveitis, optic disc edema, developmental delay and a characteristic deforming arthropathy (bony overgrowth) is observed only in CINCA/NOMID. Renal and systemic amyloidosis is a major cause of morbidity especially in Muckle-Wells syndrome. Treatment with IL-1 receptor antagonist, Anakinra, results in marked improvement of symptoms. In the largest cohort of patients reported to date, mutations in NLRP3 were identified in approximately 89% of patients with FCAS, 75% of patients with Muckle-Wells syndrome, and 51% of patients with CINCA/NOMID. Almost all reported mutations are missense mutations in exon 3 that are expected to affect the secondary structure, function, or protein-protein interaction of NLRP3.

Both p.Arg490Lys and p.Val200Met are in exon 3 in NLRP3 (NM_004895.4) and likely explain the patient's diagnosis of Muckle-Wells syndrome. This individual's father is heterozygous for the p.Arg490Lys mutation and his mother is heterozygous for the p.Val200Met variant, and both parents also have some symptoms of Muckle-Wells syndrome though neither have had the typical fevers or rash. The paternally inherited p.Arg490Lys mutation is located in a functional domain of the protein and has previously been reported in the literature, as p.Arg488Lys (NM_001243133.1:c.1463G > A), in association with cryopyrin-associated disorders [17, 18]. However, it should be noted that unaffected family members in the Aksentijevich et al. study were also found to harbor the p.Arg490Lys mutation, indicating that p.Arg490Lys may be associated with incomplete penetrance of clinical features. The NHLBI Exome Sequencing Project reports p.Arg490Lys was observed at a frequency of 0.17% (15/8600 alleles from individuals of European ancestry), indicating it may be a rare variant in this population. The ExAc database found this variant at a frequency of 72/121824 (0.059% ± 0.015%). The variant has been reported in 186/275688 alleles in gnomAD, and as “conflicting interpretations of pathogenicity” in ClinVar. SIFT predicted this change as damaging (0.02), MutationTaster scored this variant as a polymorphism, and Polyphen2 varied its score from Benign to Damaging depending on transcript.

The maternally inherited p.Val200Met variant has been reported previously in association with Muckle-Wells syndrome and FCAS and was observed in 2 of 50 individuals with uncharacterized periodic fevers; however, it was also identified in 1 of 130 UK Caucasian and 2 of

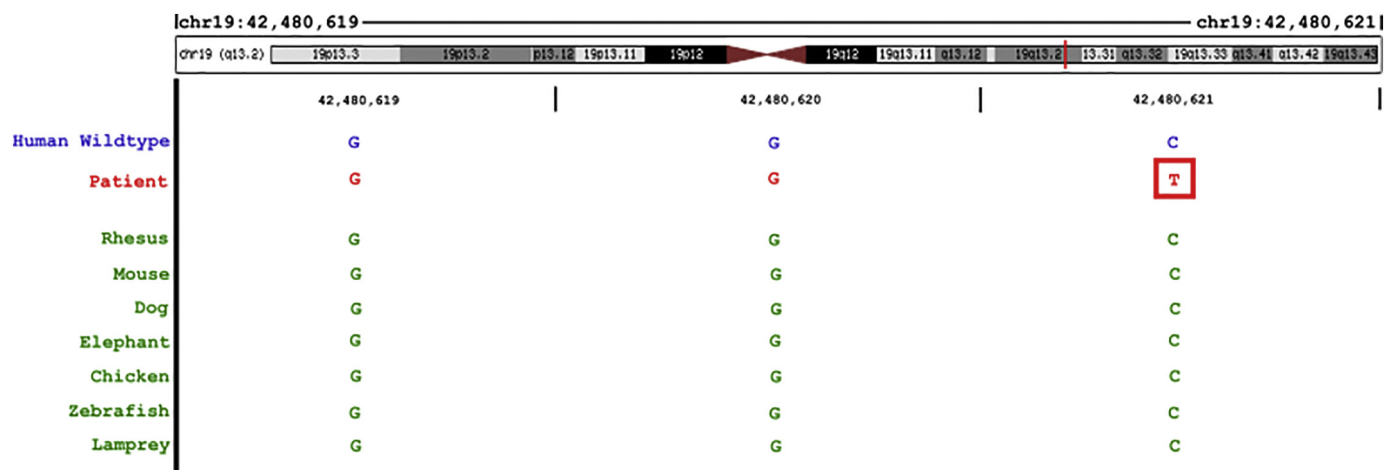


Fig. 3. Conservation of the p.Ala681Thr mutation at chr19:42480621C > T GRCh37/hg19 (ENST00000302102.5:c.2041G > A).

48 Asian Indian healthy controls. Additionally, p.Val200Met was identified in 2 of 742 healthy North American Caucasian controls [17]. This variant is a substitution of non-polar amino acids and *in silico* analysis predicts this variant likely has a benign effect on the protein structure/function. This variant is predicted as benign by SIFT (0.16) and Polyphen2, but “Disease Causing Automatic” by MutationTaster.

The p.Val200Met variant was observed at a frequency of 0.9%, 82/8600 alleles, in individuals of European ancestry by the NHLBI Exome Sequencing Project. The ExAc database found this variant at a frequency of 1010/122416 (0.825% ± 0.052%). The variant has been reported in 2343/276948 alleles in gnomAD and as “conflicting interpretations of pathogenicity” in ClinVar. It is currently unclear whether p.Val200Met is a reduced penetrance mutation with variable expressivity or a benign polymorphism. It has, however, been reported to elevate IL-1 β secretion in one patient [19]. It is possible that this variant (and elevated cytokines) confer an unknown advantage, which is why this variant is tolerated in the population.

4.3. MYO5A

The patient also has a predicted pathogenic p.R813C mutation in *MYO5A*, inherited from his father (15:52667641G > A, NM_000259, seen at a frequency of 35/276818(0.013% ± 0.005%) in gnomAD). Patients with two pathogenic mutations in *MYO5A* have Griscelli syndrome, type 1 (also called Elejalde syndrome) [20]. The phenotype of type 1 Griscelli syndrome includes skin pigment dilution, silver hair, and variable immunodeficiency. Some patients with type 1 Griscelli syndrome also present with developmental delay [20]. However, a second mutation was not found in the proband, and deletion/duplication analysis showed no deletion of the other allele. A deep intronic mutation contributing to the phenotype cannot be excluded, as exome sequencing does not capture that type of change. However, examination of the patient's hair sample ruled out the findings typically seen in the hair of patients with type 1 Griscelli.

In summary, several lines of evidence suggest that the complex phenotype described in the patient may be the result of a combination of several genetic factors as described above. While loss of heterozygosity and mosaicism cannot be ruled out, these findings suggest that the compound heterozygous mutations in *NLRP3* contribute to the proband's features that are seen in Muckle-Wells syndrome. Whether the other features are due to the *de novo* mutation in the *ATPIA3* gene remain to be determined. It is possible that the proband's *de novo* mutation in *ATPIA3* contributes to his hypersomnia, ptosis, inner ear abnormalities, worsening nystagmus and eye flutter, as well as autistic behaviors and other indications seen in *ATPIA3* patients.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmgmr.2018.06.001>.

Declarations

Written, informed consent was obtained at The Manton Center for Orphan Disease Research, Gene Discovery Core and at the NIH Clinical Center. Research protocols were approved by the Institutional Review Boards of Boston Children's Hospital and the National Human Genome Research Institute.

Competing interests

C. Brownstein is a consultant for WuXi Nextcode. In the past 3 years, Dr. Gonzalez-Heydrich has received grant support from the Tommy Fuss Fund and the Al Rashed Family. He has equity in and is founding head of the scientific advisory board for Neuro/motion, Inc., a company that is developing technology based games to foster development of emotional regulation skills. Robin Kleiman has been a consultant for Ironwood Pharmaceuticals and for En Vivo Pharmaceuticals in the past. She was formerly an employee of Pfizer for 12 years, and Selventa for

1 year. She currently works at Biogen. K Swoboda is an unpaid member of the scientific advisory board for the Alternating Hemiplegia of Childhood Foundation. Fatma Dedeoglu is a member of an advisory board for Novartis, manufactures of Canakinumab. Dr. Maski has received grant support from Jazz Pharmaceuticals and has served on advisory boards for Jazz Pharmaceuticals, Harmony Biosciences and Roche Pharmaceuticals.

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Authors' contributions

A. Torres contributed to the clinical evaluations of the proband since he was an infant to the present day, the interpretation of the genetic data, and wrote the manuscript, and C. Brownstein directed the genetic investigation including sequencing and data interpretation, and wrote the manuscript. S Temblekar analyzed the data, researched and interpreted the literature relevant to mutation and helped write the manuscript. K. Graber reviewed the clinical history and revised the manuscript. C. Genetti worked with the patient and family and helped gather patient data. R. Kleiman helped revise the manuscript. K. Sweadner helped research the structure and literature relevant to the mutation and revise the manuscript. K. Liu helped research and interpret the literature relevant to the mutation, C Mavros and N. Smedemark-Margulies and J Shi helped interpret the data and literature relevant to the mutation and performed the modeling for the figures. P. Agrawal and A. Beggs helped analyze and interpret the data. E. D'Angelo, S. Lincon, D. Carrol, F. Dedeoglu, W. Gahl, C. Biggs, and K. Swoboda treated the patient and contributed to the clinical understanding of the case and reviewed and revised the manuscript. G. T. Berry treated the patient, contributed to the clinical understanding of the case and helped write the manuscript. J Gonzalez-Heydrich oversaw the project, treated the patient, led the clinical interpretation of the data and literature, and helped write the manuscript.

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