Supplementary Data

Title: Hemizygous Mutations in SNAP29 Unmask Autosomal Recessive Conditions and Contribute to Atypical Findings in Patients with 22q11.2DS

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Supplementary notes on clinical description of patients

Patient 1 is a now 5 year 9 month old male who was the former 2835 gram (60%) product of a 36 week pregnancy born via induced vaginal delivery to a then 31 year old primigravida at an outside institution. Parents are non-consanguineous of Cuban descent. The pregnancy was complicated by polyhydramnios and detection of a dilated kidney. At delivery, Apgar scores were 8 at 1 minute and 9 at 5 minutes but he was transferred to the neonatal intensive care unit with labored breathing, temperature instability, poor feeding and hypotonia. A cardiology consult revealed a small patent ductus arteriosus and a brain MRI was suggestive of polymicrogyria. A repeat study at 7 months confirmed polymicrogyria. At 9 months of age, he was evaluated in Genetics at The Children's Hospital of Philadelphia and based on his constellation of findings including gastroesophageal reflux disease, FTT, chronic infection, and dysmorphic features was diagnosed with a *de novo* 22q11.2 deletion by FISH. A subsequent whole genome-wide SNP array confirmed a standard 22q11.2 A-D deletion.

Review of systems is significant for: optic nerve hypoplasia, congenital nystagmus, and esotropia - status post repair; bilateral sensorineural hearing loss, posterior laryngomalacia, diffuse tracheomalacia and obstructive sleep apnea requiring CPAP - status post tonsillectomy and adenoidectomy; submucosal cleft palate; significant feeding difficulties and recurrent choking episodes requiring G-tube placement, GERD, constipation, and failure to thrive; chronic infection leading to multiple hospitalizations secondary to episodes of respiratory syncytial virus, adenovirus, bronchiolitis, pneumonia, and rotavirus – resulting in the use of intravenous immunoglobulin therapy beginning at age 3 years; IgM deficiency currently treated with Hizentra; chronic bilateral peribronchial thickening with lung hyperinflation; idiopathic nephrocalcinosis and hypercalciuria; inguinal hernia and left undescended testis – status post repair.

His skin is notable in that it was normal in appearance at birth, however, an ichthyosiform dermatitis has been present since early infancy, characterized by generalized xerosis with fine white scaling as well as superficial desquamation concentrated on the forearm areas. Beginning between 1 to 2 years of age, a palmoplantar keratoderma became evident defined by a diffuse and waxy thickening of the palms and soles that has become more prominent over time. Over the past 2 months, use of topical tretinoin 0.025% nightly to the palms has resulted in significant clinical improvement of the palmoplantar keratoderma. In addition, he also has features of atopic dermatitis, with patches of erythema and scaling on flexural areas on the extremities that have responded in part to use of topical corticosteroid. In warm weather, he has trouble with heat and has a history of hypohidrosis. He has a normal complement of teeth, but has had a

history of enamel hypoplasia, as has been seen in other children with 22q11.2DS. His hair density is normal except for a focal area of frictional alopecia on the vertex caused by repeated rubbing of the scalp at this site during ambulation. Fingernails and toenails are normal in appearance. No other features are present to suggest an ectodermal dysplasia. He had a Spitz nevus on the left knee that was removed previously; on histologic evaluation, atypia was not otherwise noted.

From an immune perspective, this child was seen in infancy, prior to his diagnosis of chromosome 22q11.2DS, for recurrent infections and was known to have low immunoglobulin levels. After the diagnosis was established, a more thorough evaluation was performed. At two years of age, he had poor T cell proliferative responses, but normal CD4+ and CD8+ T cells counts. His IgG was 541 mg/dl and his IgA was 135mg/dl (both normal). His IgM was low at 29mg/dl. He had a low normal response to the tetanus vaccine. He was seen again as a four year old and found to have absent responses to vaccines for tetanus, diphtheria, and pneumococcus. He received a booster DTaP and Prevnar but failed to respond to the vaccines. At that time, his IgG and IgA were normal and his IgM was low for age. He was begun on immunoglobulin and has had a markedly improved infection pattern. The constellation of poor antibody responses with normal T cell counts is unusual in chromosome 22q11.2 deletion syndrome.
Pertinent negatives include: no evidence of hypocalcemia, hypoparathyroidism, hypothyroidism, hyperthyroidism or seizures.

Developmentally, he began to walk with assistance at age 4 years and concurrently began to vocalize using single syllables. He ambulates primarily by using a commando crawl now but previously scooted on his back using the vertex of his scalp for leverage. He smiles, laughs and is able to distinguish his parents from his teachers and other care givers.

On physical exam, his height is 102.2 cm (5%); weight is 16.6 kg (10%); OFC is 45.5 cm (<2%, 50% for 8 months); interpupillary distance is 4.8 cm (30% for age but >97% for head circumference); and chest to nipple ratio is .31 (>97%). Facial features are significant for a short forehead, epicanthal folds, hooding of the eyelids; overfolded and squared off helices and asymmetric crying facies. In addition, he is generally hirsute.

Patient 2 is a now 5 year 8 month old male who was the former 3317 gram (50%) product of a 41 week pregnancy born via C-section secondary to failure to progress and breech presentation to a then 36 year old G2P1SAB1 mother at an outside institution. Parents are non-consanguineous of French, Italian and Irish descent. The pregnancy was conceived via *in vitro* fertilization due to maternal endometriosis and

was complicated by a prenatal diagnosis of dilated ventricles (L>R). The child was discharged to home on time but referred for outpatient evaluation to Plastic Surgery and Genetics at The Children's Hospital of Philadelphia due to micrognathia, poor feeding, a failed newborn hearing screen and microcephaly. He was evaluated by both services concurrently on day of life 6 and admitted to the hospital due to poor weight gain and significant feeding difficulties. Physical examination at the time was notable for significant failure to thrive, microcephaly, micrognathia, a left preauricular ear tag, and a sacral dimple. Inpatient evaluation included a brain MRI which revealed diffuse polymicrogyria, bilateral abnormal configuration of the Sylvian fissures, dysmorphic lateral ventricles and choroid plexus cysts. Comparative genomic hybridization identified an atypical nested *de novo* 22q11.2 B-D deletion.

Review of systems is significant for: bilateral optic nerve hypoplasia, amblyopia, intermittent esotropia, cortical visual impairment; bilateral sensorineural hearing loss; feeding difficulties requiring NG tube feedings initially followed by G-tube placement at 4 months of age, chronic constipation; tracheomalacia requiring oxygen supplementation until 6 months of age; diabetes mellitus with onset at age 3 years, currently managed via an insulin pump; a mild right hemiparesis; and a right 19% thoracolumbar scoliosis.

Skin findings are notable in that he has had dry skin since birth characterized by a powdery white scale that is generalized but more prominent on the arms and legs. Between 1 to 2 years of age, he developed thickened areas of skin on the palms overlying the hypothenar areas bilaterally as well as the heels of the feet, with extension of the palmoplantar keratoderma onto the palmar aspects of the fingers. Use of urea 40% cream has provided some benefit with softening of the affected areas. The keratoderma manifests a yellow-orange coloration, likely a result of increased intake of pureed vegetables resulting in incidental carotenoderma. He has also had features of atopic dermatitis, with patches of erythema and scaling on flexural areas on the extremities that have responded in part to use of topical corticosteroid. In warm weather, he has trouble with heat and has a history of hypohidrosis. Fingernails and toenails are normal in appearance. No other features are present to suggest an ectodermal dysplasia.

Pertinent negatives include: no cardiac disease, palatal anomalies, hypocalcemia, hypoparathyroidism, thyroid disease, or seizures, and his immune system is unremarkable.

Developmentally, he began to crawl at age 2 – 3 years and walk with assistance at age 5 years. He has no words but utilizes a few signs.

On physical exam his height is 100.6 cm (<2%, 50% for 4 years); weight is 17.5 kg (15%). OFC is 48 cm (<2%, 50% for 15 months); IPD is 75% for OFC; and chest to nipple ratio is .25 (80%). Additional findings include: relative hirsutism; flat occiput; left preauricular ear tag; bulbous nasal tip with hypoplastic alae nasi and a nasal crease; micrognathia; a deep sacral dimple; and mild hypospadias.

Patient 3 is a now 14 year old female who was the former 2608 gram (25%) product of a 37 week pregnancy born via emergency C-section secondary to prolonged bradycardia, to a then 27 year old G3P1→2 mother at an outside institution. Parents are non-consanguineous of Vietnamese descent. The pregnancy was unremarkable with a reportedly normal triple screen. At delivery, a sacral level myelomeningocele was noted and she was transferred to The Children's Hospital of Philadelphia where she underwent surgical repair on day of life 1. Genetics was consulted on day 2. Physical examination at that time was notable for epicanthal folds; crumpled and overfolded helices bilaterally; a very flat, wide nasal bridge with hypoplastic alae nasi and a bulbous nasal tip; asymmetric crying facies; cleft palate; and 5th finger clinodactyly. Further evaluation revealed hypocalcemia and tetralogy of Fallot with pulmonary atresia and major aortopulmonary collateral arteries. FISH studies revealed a 22q11.2 deletion. Maternal FISH studies were normal; paternal studies have not been performed to date.

Review of systems is significant for: microcephaly; hydrocephalus requiring VP shunt placement on day of life 11 with shunt revision at 5 years; astigmatism; bilateral sensorineural hearing loss; subglottic stenosis status post tracheostomy and later decannulation at age 7 years; cleft palate status post repair; asthma and allergies; gastroesophageal reflux disease, significant feeding difficulties requiring G-tube placement, chronic constipation; tetralogy of Fallot with pulmonary atresia and multiple pulmonary collaterals status post unifocalization and recent homograft enlargement; neurogenic bladder, hydronephrosis, recurrent urinary tract infections; and tight heel cords.

Developmentally, she sat at 2 years 6 months old; walked at 3 years; and has no verbal speech, but a vocabulary of around 75 signs. She has significant behavioral issues.

On physical exam, her height is 146.3 cm (5%); weight is 43.8 kg (40%), and head circumference is 50 cm (<2%, 50% for 5 years%); she has epicanthal folds and upslanting palpebral fissures; relative hypertelorism with an interpupillary distance of 5.5 (50%); small ears with attached lobes and thick crumpled and overfolded helices; a bulbous nasal tip and hypoplastic alae nasi with a nasal crease and slit like nares; and long slender fingers.

Patient 4 is a now 9 year 3 month old female who was the former 3195 gram (75%) product of a 37 week pregnancy born via repeat C-section to a then 34 year old G2P2 mother at an outside institution. Parents are non-consanguineous of Irish, English, German and Dutch descent. At birth, a bilateral cleft lip and palate was noted. The infant required suctioning and chest physiotherapy for copious secretions and was administered oxygen by mask in the delivery room. She was noted to be in moderate respiratory distress with increased work of breathing and low saturations requiring intubation without difficulty. On day of life 2, extubation was attempted but she became bradycardic, apneic and was subsequently reintubated. She was then transferred to the NICU at The Children's Hospital of Philadelphia for further evaluation and treatment. Inpatient evaluation included an echocardiogram, which revealed an atrial septal defect (ASD); a normal renal ultrasound; and a normal brain MRI. She was also noted to have hypocalcemia. A Genetics consultation was performed on day of life 3. Physical examination at the time was notable for overriding sutures; small palpebral fissures with a question of microphthalmia; squared and overfolded helices; bilateral cleft lip (R>L) and cleft palate; retrognathia; and long slender digits. FISH studies to rule out 22q11.2DS were performed and later confirmed a *de novo* 22q11.2 deletion.

Review of systems is significant for: pseudoexotropia secondary to hypertelorism; bilateral mild to moderate conductive hearing loss; bilateral cleft lip and palate, status post three surgical procedures; mild dental crowding with a Class I occlusion; an ASD v. PFO without surgical intervention; and hyperextensible joints.

Pertinent negatives include: no evidence of immunodeficiency, hypoparathyroidism, hypothyroidism, hyperthyroidism or seizures.

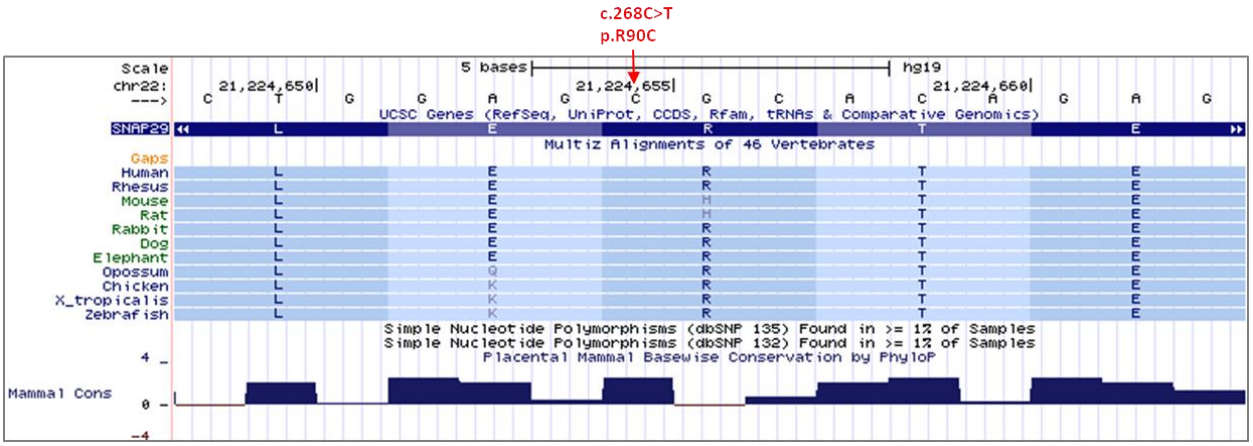
Developmentally, she sat at 7 months and walked at 13 months. She recently completed third grade in a mainstream class setting with an individualized educational plan.

On physical exam, her height is 127.8 cm (40%); weight is 28.7 kg (60%); and OFC is 54.5 cm (98%, 50% for a 15 year old). She is hyperteloric with an interpupillary distance of 6.25 cm (>97%, 75% for a 15 year old) and chest to nipple ratio is .25 (75%). Facial features are significant for malar flatness, hooding of the eyelids, upslanting palpebral fissures, overfolded helices with small attached lobes, a bulbous nasal tip with hypoplastic alae nasi, a nasal crease, a small mouth, and micrognathia.

Supplementary Figures

Supplementary Fig 1: Sanger sequencing verification of SNAP29 in patient 2's family revealed that the father carries a heterozygous mutation (c.317A>G) within exon2 resulting in Glutamine to Arginine changes (shown in red).

Supplementary Fig 2: Multiple protein sequence alignments of SNAP29 at the genomic position chr22:21,224,655 (made by UCSC) showed that the residue is highly conserved across species except in rodent (with Arginine to Histidine changes). The Arginine to Cysteine substitution (p.R90C) is predicted to be possibly damaging (score= 0.89) by PolyPhen2. The mutation position is shown by red arrow.



Supplementary Tables

Supplementary Table 1: Summary of the whole exome sequencing data analysis in each patient

Exome sequenced patients	Patient 1 (ID:11771)	Patient 5 (ID: 11134)	Patient 6 (ID: 1168)	Patient 7 (ID: 11745)
Total number of reads	150M	164M	140M	193M
Total number of high quality aligned reads	147M (98%)	161M (98%)	137M (98%)	189M (98%)
Bases covered at >20X	88%	90%	89%	89%
Mean target coverage	66X	91X	78X	76X
Number of all called variants	299K	286K	265K	269K
Number of filters passed variants	539	405	470	1444
Number of homozygous variants	20	13	17	45
Homozygous variants on chromosome 22	2	0	0	0