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Molecular Case Studies

Somatic mosaicism detected by genome-wide sequencing in 500 parent–child trios with suspected genetic disease: clinical and genetic counseling implications

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Abstract Identifying genetic mosaicism is important in establishing a diagnosis, assessing recurrence risk, and providing accurate genetic counseling. Next-generation sequencing has allowed for the identification of mosaicism at levels below those detectable by conventional Sanger sequencing or chromosomal microarray analysis. The CAUSES Clinic was a pediatric translational trio-based genome-wide (exome or genome) sequencing study of 500 families (531 children) with suspected genetic disease at BC Children's and Women's Hospitals. Here we present 12 cases of apparent mosaicism identified in the CAUSES cohort: nine cases of parental mosaicism for a disease-causing variant found in a child and three cases of mosaicism in the proband for a de novo variant. In six of these cases, there was no evidence of mosaicism on Sanger sequencing—the variant was not detected on Sanger sequencing in three cases, and it appeared to be heterozygous in three others. These cases are examples of six clinical manifestations of mosaicism: a proband with classical clinical features of mosaicism (e.g., segmental abnormalities of skin pigmentation or asymmetrical growth of bilateral body parts), a proband with unusually mild manifestations of a disease, a mosaic proband who is clinically indistinguishable from the constitutive phenotype, a mosaic parent with no clinical features of the disease, a mosaic parent with mild manifestations of the disease, and a family in which both parents are unaffected and two siblings have the same disease-causing constitutional mutation. Our data demonstrate the importance of considering the possibility of mosaicism whenever exome or genome sequencing is performed and that its detection via genome-wide sequencing can permit more accurate genetic counseling.

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INTRODUCTION

Mosaicism, a biological phenomenon in which an individual has two or more major populations of genetically distinct cells as a result of a postzygotic mutation, may have important

clinical implications. The most frequent serious clinical manifestation by far is cancer, which is usually the result of a series of mutations occurring within a line of somatic cells (Watson et al. 2013). Mosaicism may also present as a genetic disease, which can be indistinguishable from that caused by the equivalent constitutive mutation or be milder and more variable, depending on the proportion of affected cells and tissues involved (Bartsch et al. 2010; Acuna-Hidalgo et al. 2015; Donkervoort et al. 2015; Kiritsi et al. 2016).

When postzygotic mosaicism for a variant predisposing to the disease in question originates in a proband, the parents of the affected proband are not at an increased risk of having another similarly affected child, as the disease-causing mutation detected in the child is a sporadic event. In contrast, recurrence risk assessments increase if mosaicism is discovered in a clinically unaffected or mildly affected parent of a child with a constitutive dominant genetic disease (Jónsson et al. 2018). If a mosaic parent has transmitted the variant to her/his child, there must be gonadal mosaicism. Theoretically, the risk of recurrence can be as high as 50% if the variant arose in the primordia of all the germ cells or as low as 1%–2% if it arose in a single primordial germ cell (McLaren 1999); this is a marked increase above the 1% empirical risk of having a second affected child that is often cited for parents of a child with a de novo dominant condition (Gardner et al. 2012).

Next-generation sequencing (NGS) has been shown to have greater sensitivity in detecting low-level mosaicism as compared to Sanger sequencing (Qin et al. 2016; Yang et al. 2017; Cao et al. 2019). Sanger sequencing is unable to detect mosaic alleles below a threshold of 15%–20% (Rohlin et al. 2009) because both alleles of an autosomal locus are sequenced concurrently and are displayed as an analog electropherogram. The ratio of two alleles is not precisely quantifiable by Sanger sequencing because the allele peak sizes are a continuous variable, the baseline (no alternate allele present) at any position is often not quite "0," and heterozygotes frequently have allelic ratios different from 1.0 (Gomes and Korf 2018). In contrast, NGS technologies have a digital output—multiple individually discernible reads across an area of interest, permitting a quantitative approach to assessing mosaicism. Accurate interpretation of genetic testing results with regards to the possibility of mosaicism is essential for providing accurate genetic counseling for families.

The CAUSES (Clinical Assessment of the Utility of Sequencing and Evaluation as a Service) Study at Children's and Women's Health Center of British Columbia performed trio-based genome-wide (exome or genome) sequencing (GWS) of 500 families with suspected genetic disorders. GWS was done on a research basis through the CAUSES study, but clinical Sanger sequencing was used to confirm variants that were deemed possibly or definitely causal of a participant's phenotype. Participants were usually counseled on the basis of their clinical Sanger sequencing results. Here we present 12 families enrolled in the CAUSES study in whom clinically relevant somatic mosaicism was found in either the proband or in a parent by GWS.

RESULTS

Mosaicism was found in either the proband or a parent in 12 families, 4.6% of the 261 CAUSES families in whom a genetic disease was diagnosed.

Clinical Presentation and Family History
Family 1

The proband is a male who was first referred for genetic evaluation at 5 yr of age for microcephaly, dysmorphic features (short nose with anteverted nares, smooth philtrum, thin upper lip, fifth finger clinodactyly), bilateral cataracts, retinal dystrophy, obesity, motor delay, mild

intellectual disability (ID), hypogonadism, and hypertension. His growth parameters were within normal range. He was born to healthy nonconsanguineous parents of European and Indigenous ancestry. He has an older maternal half-brother who is unaffected, and there is no family history of congenital anomalies or ID. His head magnetic resonance imaging (MRI) and computed tomography (CT) of the abdomen were normal. A chromosomal micro-array analysis (CMA) identified a paternally inherited 428-kb duplication of 7q22.3 that was not thought to contribute to the proband's phenotype.

The family was referred to the CAUSES study when the proband was 11 yr old. Trio exome sequencing identified a hemizygous missense variant in the proband in *SMC1A* (Table 1). Possible mosaicism for this variant was also identified in the mother (Table 2). Sanger sequencing confirmed the presence of the variant, which was classified as a variant of uncertain significance (VUS), in the proband. Sanger sequencing detected heterozygosity for the variant in the mother (it did not appear to be mosaic). The family was counseled that these results were consistent with a diagnosis of Cornelia de Lange syndrome (OMIM#300590) in the proband and that the mother, who was unaffected, was heterozygous for the variant. Genetic testing was offered to the maternal grandparents to determine whether the variant had occurred de novo in the proband's mother; however, the family declined this additional testing.

Family 2

The proband is a male who was referred for genetic evaluation because of hypotonia in infancy, gross motor delay, and attentional and behavioral difficulties. When assessed by a medical geneticist, the proband was noted to have mild facial dysmorphisms and cerebellar findings including difficulty with the finger-to-nose test and an intention tremor.

The proband's sister is similarly affected, with hypotonia in infancy, delayed gross motor skills, mild facial dysmorphisms, and similar cerebellar findings. These children were born to healthy nonconsanguineous parents of South-Asian descent. There is no family history of congenital anomalies or ID.

When the proband was 13 and his sister was 8 yr of age, exome sequencing identified a heterozygous *EBF3* variant in both affected siblings (Table 1). Apparent mosaicism for the *EBF3* variant was noted in one of the parents on exome sequencing; however, Sanger sequencing did not detect the *EBF3* variant in either parent's blood (Table 2). The variant was classified as pathogenic. The *EBF3* gene has been implicated in a neurodevelopmental disorder characterized by moderate-to-severe ID, cerebellar ataxia, and subtle facial dysmorphisms (OMIM#607407). This family was included in a publication characterizing this syndrome (Sleven et al. 2017). The family was counseled that recurrence risk could be as high as 50%.

Family 3

The proband is a female referred for genetic evaluation at 3 yr of age for global developmental delay and dysmorphic features. She was the first child of healthy nonconsanguineous parents with no learning or developmental concerns. The mother is of French–Canadian descent and the father of European and Scandinavian descent. A maternal aunt had miscarried a fetus with multiple congenital anomalies, but no specific diagnosis was made in the fetus.

The proband has one sibling with mild speech and language delay but no other family history of congenital anomalies or ID. Her parents first noticed a head tilt and began to be concerned about her speech development at 12 mo of age. Radiographic and CT examination of the spine revealed an os odontoideum. At 3 yr of age, she was noted to have asymmetry of the face, frontal bossing, hypertelorism, down-slanting palpebral fissures, long

Table 1. Variants found in probands and parents with apparent mosaicism

Family	Gene	HGVS DNA nomenclature	HGVS protein nomenclature	Variant type	Predicted effect	Proband genotype on GWS	Clinico-molecular interpretation	ACMG variant classification	Database (accession number)
1[a]	SMC1A	NM_006306.4:c.2945A>T	NP_006297.2: p.Asp982Val	Missense	Substitution	Hemizygous	Probably disease-causing	VUS	Did not consent to public release of genomic sequence data
2[a]	EBF3	NM_001375380.1:c.616C>T	NP_001362309.1: p.Arg206Ter	Stop gain	Premature protein truncation	Heterozygous	Definitely disease-causing	Pathogenic	ClinVar (SCV000599257.1)
3[a]	USP9X	NM_001039591.3:c.5595delT	NP_001034680.2: p.Val1866TrpfsTer40	Frameshift	Premature protein truncation	Heterozygous	Probably disease-causing	Likely pathogenic	ClinVar (SCV000599286.1)
4[a]	RAI1	NM_030665.4:c.4673dup	NP_109590.3: p.Arg1559AlafsTer16	Frameshift	Premature protein truncation	Heterozygous	Definitely disease-causing	Pathogenic	ClinVar (SCV000680108.1)
5[a]	EHMT1	NM_024757.5:c.2214_2230dup	NP_079033.4: p.Phe744SerfsTer7	Frameshift	Premature protein truncation	Heterozygous	Definitely disease-causing	Pathogenic	ClinVar (SCV000599258.1)
6	CASK	NM_001367721.1:c.2040-1G>A		Splice acceptor	Aberrant splicing	Mosaic (hemizygous)	Definitely disease-causing	Pathogenic	ClinVar (SCV000803701.1)
7	TRIO	NM_007118.4:c.4283G>A	NP_009049.2: p.Arg1428Gln	Missense	Substitution	Mosaic (heterozygous)	Definitely disease-causing	Pathogenic	ClinVar (SCV000803691.1)
8	SLC6A8	NM_005629.4:c.743_745del	NP_005620.1: p.Phe248del	Deletion	Deletion	Mosaic (hemizygous)	Definitely disease-causing	VUS	DECIPHER (452745)
9[a]	GNAO1	NM_020988.3:c.451G>A	NP_066268.1: (p.Asp151Asn)	Missense	Substitution	Heterozygous	Probably disease-causing	VUS	DECIPHER (452747)
10[a]	BCL11B	NM_138576.4:c.726_727insCG CAGCAC	NP_612808.1: p.Thr243ArgfsTer41	Frameshift	Premature protein truncation	Heterozygous	Definitely disease-causing	Likely pathogenic	DECIPHER (452749
11[a]	TRIP12	NM_001348323.3:c.5230T>G	NP_001335252.1: p.Tyr1744Asp	Missense	Substitution	Heterozygous	Probably disease-causing	VUS	DECIPHER (452750)
12[a]	SPG7	NM_003119.3: c.1226A>C	NP_003110.1: p.Glu409Ala	Missense	Substitution	Heterozygous	Probably disease-causing	Likely pathogenic	Did not consent to public release of genomic sequence data

(HGVS) Human Genome Variation Society, (ACMG) American College of Medical Genetics and Genomics, (VUS) variant of uncertain significance.
[a]Families where a parent was mosaic for the variant.

Table 2. Clinical phenotype and sequencing results of apparently mosaic individuals

Family	Gene	Genetic diagnosis in proband	Individual in whom mosaicism was identified	Phenotype of mosaic individual	Variant/ total reads	Exome or genome sequencing	Sanger results in mosaic individual
1	SMC1A	Cornelia de Lange syndrome 2	Proband's mother	No clinical features noted	15/81 (18%)	Exome	Heterozygous for variant
2	EBF3	Hypotonia, ataxia, and delayed development syndrome (two affected sibs)	Parent of proband	No clinical features noted	2/127 (1.6%)	Exome	Variant not detected
3	USP9X	X-linked syndromic mental retardation 99, female-restricted (female)	Proband's mother	Dysmorphic facial feature similar to, but milder than proband; no intellectual disability	17/104 (16%)	Exome	Heterozygous for variant
4	RAI1	Smith–Magenis syndrome	Proband's mother	No clinical features noted	14/105 (13%)	Exome	Mosaic
5	EHMT1	Kleefstra syndrome	Proband's mother	No clinical features noted	23/161 (14.3%)	Exome	Mosaic
6	CASK	Mental retardation and microcephaly with pontine cerebellar hypoplasia (male)	Proband	Asymmetry of legs (mosaicism suspected clinically); phenotype milder than expected for hemizygous CASK variant	7/15 (46%)	Genome	Mosaic
7	TRIO	Autosomal dominant mental retardation 44	Proband	Consistent with constitutional phenotype	5/38 (13%)	Exome	Heterozygous for variant
8	SLC6A8	Cerebral creatine deficiency syndrome 1 (male)	Proband	Consistent with constitutional phenotype	9/10 (90%)	Genome	Mosaic
9	GNAO1	Early infantile epileptic encephalopathy 17	Proband's mother	No clinical features noted	6/82 (7.3%)	Exome	Variant not detected
10	BCL11B	Intellectual developmental disorder with dysmorphic facies, speech delay, and T-cell abnormalities	Proband's father	No clinical features noted	5/79 (6.3%)	Exome	Mosaic
11	TRIP12	Autosomal dominant mental retardation 49 (two affected sibs)	Proband's father	No clinical features noted	8/70 (11%)	Exome	Mosaic
12	SPG7	Autosomal recessive spastic paraplegia 7	Proband's mother	No clinical features noted	4/53 (7.5%)	Genome	Variant not detected

bulbous nose, anteverted nares, long columella, low-set posteriorly rotated ears, asymmetry of the chest and back, and prominent fingertip pads. Genetic workup included a normal chromosome analysis (46, XX), a normal CMA, and normal fragile X testing.

The proband was referred to CAUSES at 8 yr of age. Exome trio analysis identified a heterozygous variant in the *USP9X* gene (Table 1) and maternal mosaicism for the variant (Table 2). Sanger sequencing confirmed the presence of the variant in the proband and in the mother but showed no evidence of allelic imbalance indicative of mosaicism in the mother. The

proband was diagnosed with "female-restricted X-linked syndromic mental retardation 99" (OMIM #300968). Her dysmorphisms (facial features, hands, fingers, and toes) were consistent with the diagnosis. The family was counseled on the variability of the condition and that some individuals present with almost no features. It was noted that the mother had dysmorphic features consistent with the condition, although much milder than the proband, and that she does not have ID.

Family 4

The proband is a 5-yr-old female referred for genetic evaluation for proportional overgrowth, insatiable appetite, sleep disturbance, and hypotonia. She has a younger sibling who is healthy and no family history of congenital anomalies or ID. The proband spent 2 wk in the neonatal intensive care unit (NICU) at birth because of difficulty feeding. She had been born at 35-wk gestational age (GA) following an otherwise-uncomplicated pregnancy; birth weight was 2950 g (92%ile for GA). Pediatric exam confirmed physical characteristics consistent with 35-wk GA. Hypotonia was not mentioned in the birth record. Breastfeeding was difficult. Eventual feeds with expressed breast milk did not seem to produce satiety. Sleep was unusually difficult, with 4–6 wakings at night, difficulty settling back to sleep, and resistance to naps during the day. Genetic workup included normal female CMA and normal Prader–Willi syndrome methylation testing.

The child was referred to CAUSES at 5 yr of age. Trio exome analysis identified a heterozygous frameshift variant in *RAI1* (Table 1) in the proband and maternal mosaicism for the same variant (Table 2). Sanger sequencing confirmed the presence of the variant in the proband and was consistent with low-level mosaicism in the mother. A diagnosis of Smith–Magenis syndrome (OMIM#182290) was made in the proband, and the family was informed that there may be a recurrence risk because the mutation was inherited from a parent who had mosaicism for the mutation.

Family 5

The female proband was born to healthy nonconsanguineous parents; there was no family history of congenital anomalies or ID. Fetal bradycardia was noted prior to delivery. She was born at 41 wk 5 d gestation, with vacuum assist, and a birth weight of 3884 g (74%ile for GA). As a newborn the proband required oxygen for the first 7 h and had low blood glucose concentration, which was managed with frequent feeds after initial feeding problems. She did not make eye contact from a young age, did not cry often, and was quite lethargic until age 1 yr.

At 2 yr of age, she was referred to medical genetics for facial dysmorphisms, autism, developmental delay, hypotonia, alternating exotropia, hyperopia, and sleep difficulties. Genetic workup included normal chromosome analysis (46, XX), CMA, Angelman syndrome methylation testing, and fluorescence in situ hybridization (FISH) for tetrasomy 12p. Additional investigations included normal cardiology evaluation, head ultrasound exam, and chest X-ray examination.

She developed focal motor seizures with impaired awareness, currently controlled on lamotrigine, at age 6 yr. Electroencephalogram (EEG) revealed dysrhythmic background with very rare right frontal sharp waves in sleep. Head MRI at 2 yr 9 mo showed small area of focal gliosis in left posterior periventricular white matter, reduced volume of pons, and incidental cavum velum interpositum cyst.

Trio-based exome sequencing identified a heterozygous *EHMT1* variant in the proband (Table 1) and mosaicism for this variant in her mother (Table 2). Sanger sequencing confirmed the presence of the variant in the proband and was consistent with low-level mosaicism in the mother. Kleefstra syndrome (OMIM#610253) was diagnosed in the proband, but

no features of this condition were apparent in the mother. The family was counseled that the mother was mosaic for the *EHMT1* variant and that she had an increased risk for having another child with Kleefstra syndrome. The risk was uncertain but could range from 1% to 2% to as high as 50%.

Family 6

The male proband was born to healthy nonconsanguineous parents with no family history of congenital anomalies or ID. At 2 wk of age his parents first noted that his right leg was larger than the left. At birth he had a relatively large head circumference but progressively developed microcephaly. Head circumference at birth (41 wk GA) was 36.5 cm (91%ile), at 2 mo was 38.0 cm (16%ile), at 4 mo was 40 cm (9%ile) at 17 mo was 44.0 cm (1%ile), at 27 mo was 45.4 cm (1%ile), and at 3 yr, 9 mo was 46.0 cm (<1%ile). He was evaluated in medical genetics for Beckwith–Wiedemann syndrome at 17 mo of age but did not meet clinical diagnostic criteria. By 3 yr of age the proband experienced developmental regression in speech—he had initially been able to say "hi," used a few words nonspecifically, and knew some letters, but was nonverbal by 3 yr of age. Karyotype, CMA, fragile X testing, MRI of the brain, and spine and abdominal ultrasound examination were all normal.

He was referred to CAUSES at 4 yr of age; trio genome sequencing identified a de novo mosaic *CASK* variant (Table 1). Sanger sequencing was consistent with hemizygous mosaicism, and the child was diagnosed with "mental retardation and microcephaly with pontine cerebellar hypoplasia" (OMIM#300749) (Table 2). The family was informed that they were not at increased risk of having another affected child.

Family 7

The proband is a male referred for genetic evaluation at 2 yr of age for microcephaly, global developmental delay, mild dysmorphic features (hypotonic face, dolichocephaly, mild fifth finger clinodactyly bilaterally), bicuspid aortic valve, and delayed myelination. He was the third-born child to healthy nonconsanguineous parents. His two siblings are healthy with no learning or developmental concerns. There is no family history of congenital anomalies or ID.

The pregnancy was complicated by bleeding in the first trimester. He was born by induction at 35 wk GA weighing 2268 g (19%ile for GA); head measurements are not available, but he was noted to have microcephaly at birth. He spent 3.5 wk after birth in the NICU having episodes of apnea and bradycardia. This resolved with time, and he was doing well at discharge. Cardiac evaluation identified a bicuspid aortic valve that did not require intervention.

At 2 yr of age, he was referred for genetic evaluation. At that time his motor skills were quite delayed, and he was noted to be hypotonic and hypermobile and to have mild gastroesophageal reflux disease. Head circumference was measured to be 44.5 cm (1%ile). Brain MRI showed delayed myelination of the posterior corpus callosum and a slightly small pons. CMA was normal.

He was referred to the CAUSES study at 2 yr of age; trio exome sequencing identified a de novo, apparently mosaic *TRIO* variant in the proband (Table 1). Sanger sequencing was consistent with heterozygosity for the de novo variant and showed no evidence of allelic imbalance indicative of mosaicism (Table 2). The child was diagnosed with autosomal dominant intellectual developmental disorder-44 with microcephaly (OMIM#617061). This variant was not found in either parent. The family was counseled that this was a de novo variant in the proband.

Family 8

The proband, a male, is the second-born child to healthy nonconsanguineous parents of European descent. His sibling is healthy with no learning or developmental concerns. There is no relevant family history. The proband's speech and fine motor development were delayed, and he has anxiety as well as obsessive and aggressive behaviors. At 4 yr of age, he was diagnosed with moderate ID and a complex neurodevelopmental disorder. He began having grand mal seizures when he was 15 yr old. He has no major malformations and is not generally dysmorphic; his growth and pubertal development have been normal. CMA, head MRI, head CT scan, and fragile X testing results were all normal.

He was referred to the CAUSES study at age 16 yr. Trio genome sequencing identified an apparently mosaic hemizygous variant in *SLC6A8* (Table 1). Sanger sequencing was consistent with mosaicism in the proband and found no evidence for the variant in either parent (Table 2). The family was informed of the mosaicism and the low risk for recurrence. Although the variant was classified by the clinical laboratory as a VUS, subsequent electrophysiological studies using whole-cell patch clamp recording suggested that the variant produces a dysfunctional SLC6A8 transporter that is unable to uptake cellular creatine. On the basis of these functional studies, the *SLC6A8* variant was interpreted as definitely disease-causing, and the child was diagnosed with X-linked recessive cerebral creatine deficiency syndrome-1 (OMIM#300352).

Family 9

The proband is a female referred for genetic evaluation for global developmental delay, seizures, and mild dysmorphic features (hypertelorism and epicanthal folds). She was born to healthy nonconsanguineous parents of Central American and European descent. The proband has two maternal half-siblings; one half-sister has mild ID. At age 5 the proband developed seizures, which are currently controlled with a moderate dose of valproic acid. Her karyotype was normal, and CMA showed a 312.2-kb copy loss at 3p14.2 that was inherited from her mother and classified as a VUS.

The proband was referred to CAUSES at age 6 yr. Trio exome sequencing identified a heterozygous variant in *GNAO1* in the proband (Table 1) and maternal mosaicism for the same variant (Table 2). Sanger sequencing confirmed the variant in the proband, but the *GNAO1* variant was not detected in the mother. The family was counseled that the proband was heterozygous for a de novo variant that was contributing to the proband's condition. The child was diagnosed with autosomal dominant early infantile epileptic encephalopathy-17 (OMIM#615473).

Family 10

This male proband was born to a healthy nonconsanguineous couple. He had a maternal half-sister with unilateral microtia but no other family history of congenital anomalies or ID. The proband was very delayed developmentally and had failure to thrive and hypotonia as an infant. He was subsequently found to have profound ID with no expressive speech, dysmorphic features (hypertelorism, broad nasal root, prominent columella, long well-grooved philtrum, thin upper lip, small mouth, bilateral epicanthal folds, brachycephaly), mild foot deformities, and mild scoliosis. Investigations included normal karyotype, normal Prader–Willi methylation studies, and a CMA that identified a 333-kb maternally inherited deletion containing no OMIM genes at 13q21.1 that was considered to be benign. Brain MRI revealed two foci of hyperintense T2 signal and mild prominence of ventricular system and extra-axial spaces.

He was referred to the CAUSES study at age 7 yr. Trio exome sequencing identified a heterozygous *BCL11B* variant in the proband (Table 1) and mosaicism for the same variant in the father (Table 2). Sanger sequencing confirmed the presence of the variant in the proband and was consistent with mosaicism in the father. Intellectual developmental disorder with speech delay, dysmorphic facies, and T-cell abnormalities (OMIM#618092) was diagnosed in the child. The child's dysmorphic features were consistent with the genetic diagnosis. The family was informed that the father was mosaic for the variant, that the mosaicism was expected to be of no clinical significance, and that there was a chance of recurrence.

Family 11

The proband is a male who was referred for genetic evaluation because of borderline ID (IQ of 70), markedly delayed speech, macrosomia, nail dystrophy, and an excessively friendly personality. Head MRI was normal.

The proband's sister is similarly affected, with ID and an excessively friendly personality. These children were born to nonconsanguineous parents. The proband exhibited early feeding intolerance, followed by frequent vomiting and dysphagia for 4 yr, along with hypotonia and poor coordination. He had widespread eczema and drooling until age 2 yr.

The proband has two brothers who did not speak until age 3 yr but exhibited normal speech development thereafter. Their mother had a significant learning disability, and several of her relatives were also reported to have problems with learning. The father was healthy with no learning problems.

When the proband was 13 yr old and his sister was 7, exome sequencing identified a heterozygous *TRIP12* variant in both affected siblings (Table 1), and both were diagnosed with "autosomal dominant mental retardation-49" (OMIM#617752). Mosaicism for the *TRIP12* variant was noted in the father on exome sequencing and confirmed by Sanger sequencing (Table 2). The family was informed that father was mosaic for the variant although clinically unaffected.

Family 12

A 10-yr-old female was referred for genetic evaluation for ocular motor apraxia, unsteady gait, increased tone in lower legs, Achilles' tendon contractures, and optic atrophy.

The proband had an older sister who passed away at 2 yr of age with hypotonia, abnormal ocular movements, and a seizure disorder. No specific diagnosis was made in this sibling, but the parents felt that she had the same condition as the proband. There was no other family history of congenital anomalies or ID.

Brain MRI was normal in infancy, but at 4 yr of age showed atrophy of the optic chiasm and at 10 yr also showed cerebellar atrophy. Genetic investigations included normal CMA, mitochondrial DNA (mtDNA) testing, and *OPA1* testing.

When the proband was 10 yr of age, trio whole-genome sequencing identified a heterozygous variant in *SPG7* (Table 1) and maternal mosaicism for the same variant. Sanger sequencing confirmed the variant in the proband, but the *SPG7* variant was not detected in the mother (Table 2). The variant was classified as likely pathogenic and was considered to be the probable cause of some, but not all, of the phenotypic features in the proband. Although the condition diagnosed is called autosomal recessive spastic paraplegia 7 (OMIM#607259), unequivocal dominant transmission with apparent heterozygous expression has been seen in some families (Sánchez-Ferrero et al. 2013). The family was informed that there was a question of whether the mother was mosaic for this *SPG7* variant, as mosaicism was seen on the research genomic sequencing, but was not detected by the clinical lab on Sanger sequencing.

DISCUSSION

Mosaic disease-causing mutations may present clinically in six different ways:

1. In a proband with a phenotype that includes classical clinical features of mosaicism, such as segmental abnormalities of skin pigmentation or asymmetrical growth of bilateral body parts;

2. In a proband with unusually mild manifestations of a de novo dominant genetic disease (or X-linked recessive disease in a male);

3. In a proband with typical features of a de novo dominant genetic disease (or X-linked recessive disease in a male) who is found on genetic testing to have substantially <50% of the variant disease-causing allele (or both normal and variant alleles for an X-linked recessive disease in a hemizygous male);

4. In a family in which the proband has a disease-causing constitutional mutation and typical features of a de novo dominant genetic disease (or X-linked recessive disease in a male) and a parent who has mild manifestations of the disease and is found to carry the same disease-causing mutation in mosaic form;

5. In a family in which the proband has a disease-causing constitutional mutation and typical features of a de novo dominant genetic disease (or X-linked recessive disease in a male) and a parent who has no clinical features of the disease but is found to carry the same disease-causing mutation in mosaic form; or

6. In a family in which two or more sibs have the same disease-causing constitutional mutation and typical features of a dominant genetic disease (or X-linked recessive disease in males) and a clinically unaffected or only mildly affected parent is found to carry the same disease-causing mutation in mosaic form.

In this study of 12 instances of mosaicism found among 500 families who underwent GWS in the CAUSES study, we saw examples of all six presentations.

Mosaicism was suspected clinically in the proband of Family 6, owing to the lower limb hemihypotrophy (the right leg was appropriately grown; the left was small). Individuals with constitutional *CASK* variants often display growth retardation (Burglen et al. 2012); therefore, somatic mosaicism for the pathogenic *CASK* variant may account for the limb size discrepancy in this proband. This proband also had a milder phenotype than other males who have been reported with splice site variants in *CASK* (Burglen et al. 2012; Moog et al. 2015). The other probands in our cohort with mosaicism (Family 7 and Family 8) had phenotypes that were clinically indistinguishable from the constitutive counterparts of their respective disorders.

Although low-level mosaicism for a disease-causing mutation in the blood can be associated with clinical manifestations in many genetic disorders (Moyhuddin et al. 2003), somatic and/or germline mosaicism may also be found in the clinically unaffected parent of a proband with a genetic disease (Biesecker and Spinner 2013). Careful clinical examination of seemingly unaffected parents is important as subtle clinical features may be initially missed (Ngai et al. 2010). In our cohort, only one of the mosaic parents of a child with the same disease-causing mutations (Family 3) presented with apparent clinical features. This parent had facial dysmorphisms consistent with the condition, although much milder than the proband, but did not present with ID or learning challenges. Parents were usually asked if they might have had subtle learning disabilities or challenges in school, but none was reported. Similar rates of parental mosaicism to that found in our cohort have been reported in other studies (Acuna-Hidalgo et al. 2015; Rahbari et al. 2016; Stosser et al. 2018; Cao et al. 2019).

An additional consideration when a female child presents with an X-linked condition and the mother is more mildly affected is the possibility of skewed X-inactivation (Villard et al. 2001; Huppke et al. 2006). For Family 3, the detection of mosaicism in the mother provided a more likely explanation of her milder phenotype. Had the mother's mosaicism not been detected, we might have invoked skewed X-inactivation as an explanation and considered additional studies to clarify the situation. Thus, detection of mosaicism for an X-linked condition may prevent unnecessary follow-up testing in some families.

When genetic evaluation is performed in a family in which the parents are healthy and two or more children are affected with a similar disorder, the most likely cause is an inherited autosomal recessive condition. Alternatively, the disease could result from segregation of an unbalanced product of a balanced chromosomal rearrangement from one parent or, if the affected children are all males, from an X-linked recessive condition inherited from a heterozygous carrier mother. However, if the affected children have clinical features of an autosomal dominant or X-linked dominant disease, the possibility that one parent carries the same disease-causing mutation in mosaic form, as was the case for Family 2 and Family 11, must be considered (Kunishima et al. 2009; Elalaoui et al. 2010; Jónsson et al. 2018). In Family 2, the two similarly affected siblings were found to carry the same disease-causing variant and, although neither parent showed evidence of the alternative allele on Sanger sequencing, low levels of the variant on exome sequencing suggested mosaicism in one parent.

As is demonstrated in our cohort and in numerous other studies (Rohlin et al. 2009; Jamuar et al. 2014; Yang et al. 2017; Cao et al. 2019), Sanger sequencing is not as sensitive as NGS technologies in detecting mosaicism. The allele peaks produced by Sanger sequencing represent a continuous variable (Gomes and Korf 2018), whereas NGS produces a digital result (numbers of variant and reference reads). More sensitive detection and more precise quantitation of mosaicism, especially at low levels, are possible with techniques such as digital droplet polymerase chain reaction (PCR) (Zhou et al. 2018). NGS has been shown to identify mosaicism at levels of 1%–10%, whereas digital droplet PCR can detect mosaicism at levels as low as 0.01%–0.001% (Keppler-Noreuil et al. 2015). Such methods would be more appropriate than Sanger sequencing for confirming suspected mosaicism if they were routinely available clinically.

In our cohort, of the 12 individuals who were identified as being mosaic on either exome or genome sequencing, only six were confirmed to be mosaic by Sanger sequencing. Three others (Family 1, Family 3, and Family 7) appeared heterozygous on Sanger sequencing, with no evidence of allelic imbalance for the disease-causing variant, and in three (Family 2, Family 9, and Family 12) only the reference sequence was seen on Sanger sequencing of both parents, with no evidence of the disease-causing allele found in one parent and the child on GWS. The 95% confidence interval of the expected heterozygous allele ratio (0.5) has been estimated to be 0.24–0.76 (Heinrich et al. 2012). According to these parameters, the three individuals in our cohort (Family 1, Family 3, and Family 7) who appeared heterozygous on Sanger sequencing are likely to be true mosaics because their allele ratios on exome sequencing were 0.18, 0.16, and 0.13, respectively.

The parents in Family 9 and Family 12 also likely represent cases of true mosaicism. As NGS is known to exhibit background noise, a low–allele frequency variant could be a false positive caused by background noise. Brewer et al. (2020) aimed to quantify the sequencing noise in control samples at target sites that harbored low–allele frequency variants. They found that most often, control samples did not carry the target variant in any reads; occasionally it appeared in a single control read, but never in >1% of control reads for a given sample. In contrast, the variant alleles for the mosaic individuals in Family 9 and Family 12 were present in 7.3% and 7.5% of reads, respectively (Table 2). The variant allele was present in only 1.6% of reads in the parent in Family 2, but the evidence of transmission of exactly the same variant allele to two affected children makes parental mosaicism virtually certain in this family.

In our cohort, all GWS and Sanger sequencing were performed only on blood samples. The mutations that produce somatic mosaicism can occur at any developmental stage, and their distribution may vary greatly in different tissues (Gambin et al. 2020). Mosaic variants in the blood may be over- or underrepresented in comparison to other tissues as a result of clonal expansion of blood cells (Shlush 2018), particularly if the variant affects cellular survival or proliferation (Gambin et al. 2020). Levels of mosaicism in blood have also been shown to vary over time in the same individual (Qin et al. 2016), further emphasizing that the degree of mosaicism in blood is an unreliable basis for estimating the risk of transmission of the variant to offspring. Alternate tissues such as hair follicles may correlate better with mosaic allele ratios in other somatic tissues (Gambin et al. 2020), but further studies are needed to determine the correlation of mosaic ratios across different tissues and to determine if any relationship exists between variant allele ratios in somatic tissues and the germline.

Clinically relevant mosaicism is not limited to variations at the single-nucleotide level. Mosaic structural variants may be pathogenic causes of ~1% of developmental disorders (King et al. 2015) and have been shown to predispose adults to hematological malignancies (Jacobs et al. 2012; Laurie et al. 2012). Structural variants have conventionally been assessed using cytogenetic testing. Single-nucleotide polymorphism (SNP) microarray technology is well-suited for detecting genomic copy-number mosaicism, as probe density is high (King et al. 2017), but CMA cannot detect balanced structural variants or copy-number variants of <100,000 bp or so. NGS technologies can detect mosaicism for smaller copy-number variants (King et al. 2015, 2017; Balachandran and Beck 2020), and newer research technologies like long-read sequencing show great promise for detecting mosaicism involving small, balanced, and complex structural variants as well (Fujimoto et al. 2021; Roberts et al. 2021).

GWS through the CAUSES study was done on a research basis with clinical confirmation by Sanger sequencing of any variants deemed possibly or definitely causal of a participant's phenotype. Participants were usually counseled on the basis of their Sanger results. The mosaicism detected by NGS in our study was not confirmed by alternate methods such as digital PCR, which shows great promise for quantifying low-level somatic mosaicism (Zhou et al. 2018).

Genetic Counseling Implications

Accurate genetic counseling and estimates of recurrence risk for genetic disease are dependent on the ability to interpret genetic testing results regarding low levels of mosaicism. Recurrence risks for de novo mutations depend upon whether mosaicism is detected in a parent. Jónsson and colleagues studied 251 families with multiple offspring and generated sex- and age-specific recurrence risks for de novo mutations (Jónsson et al. 2018). When maternal somatic mosaicism was identified, the recurrence risk was predicted to be 21.4% (95%, CI 18.2–25.1) and 14.7% (95%, CI 13.3–16.3) when the somatic mosaicism was paternal in origin. When somatic mosaicism was not detected, predicted probabilities for recurrence depended on parental age and sex and whether there was a previously affected child. The range of estimated recurrence probabilities was 0.011%–28.5% (with the upper range represented by a previously affected sibling and maternal mosaicism), estimates higher than previously proposed. In three of the families reported in our series, parental somatic mosaicism identified by GWS (Family 2, Family 9, and Family 12) was not detected by Sanger sequencing. Lack of detection on a clinical report can result in lower than actual recurrence risks being communicated to a family. However, for Family 2, the GWS findings and the presence of the affected sibling influenced the counseling, and for Family 12, the physician was aware of the mosaicism detected by GWS, and the family was counseled accordingly. Conversely, for two families (Family 1 and Family 3), the maternal somatic mosaicism identified by GWS was reported as heterozygous by Sanger sequencing, thereby inflating

recurrence risks. These families reflect the importance of communication of GWS results to referring clinicians so that accurate genetic counseling can take place. In the research setting, having the research genetic counselor from the GWS study participate in the result sessions can mitigate these issues.

In summary, the cases presented here highlight several key principles regarding the clinical assessment of mosaicism. Mosaicism may be suspected when an individual exhibits asymmetry, a segmental abnormality, or characteristic alterations of skin pigmentation or presents with a milder form of a recognizable phenotype. Clinically unaffected parents of an affected proband may have detectable levels of somatic mosaicism. Careful clinical examination of seemingly unaffected parents is important as subtle clinical features may be missed. Two siblings affected with the same autosomal or X-linked dominant condition with unaffected parents are highly suggestive of parental mosaicism. Advances in NGS technologies have allowed for better detection of mosaicism, but such mosaicism is not always detected on Sanger sequencing. Conversely, a mosaic parent may have a variant that appears heterozygous with no evidence of allelic imbalance on Sanger sequencing.

These concepts have important implications for accurate genetic counseling. Even when mosaicism can be detected reliably, providing precise recurrence-risk estimates is often difficult or impossible. Regardless of how accurately mosaic ratios in blood samples are estimated, this information alone cannot be used to estimate risk of transmission precisely.

METHODS

The clinical workflow for the CAUSES study has been previously described (Elliott et al. 2018; Dragojlovic et al. 2020). After receiving pretest genetic counseling and providing consent, probands and their parents provided blood samples. DNA was extracted by the BC Children's Hospital clinical laboratory and sent for research-based sequencing, either exome or genome, by an external laboratory that provided raw data as FASTQ or BAM files to the study's bioinformatics team. A genome analyst generated a candidate variant list using a custom bioinformatics pipeline that focused on known disease genes initially but was expanded to include all variants if the initial analysis was uninformative (Tarailo-Graovac et al. 2016; Myers et al. 2017; Sleven et al. 2017). Variants determined to contribute to a definite or probable genetic diagnosis, by the CAUSES team together with the referring physician, were clinically confirmed by Sanger sequencing on the trio's blood samples and interpreted by the Genome Diagnostics Laboratory at BC Children's and Women's Hospitals according to American College of Medical Genetics and Genomics criteria (Richards et al. 2015). Clinical data were abstracted from patient charts; details regarding how the families were counseled were summarized from the referring physicians' consult letters.

ADDITIONAL INFORMATION

Data Deposition and Access

All variants that were classified as likely pathogenic or pathogenic by the Genome Diagnostics Laboratory at BC Children's and Women's Hospitals were submitted to ClinVar by the Molecular Genetics Laboratory. For Family 10, the variant was originally classified as a VUS but subsequently reclassified as likely pathogenic, therefore this variant has not yet been deposited into ClinVar. All CAUSES participants consented to have genomic sequence data deposited into DECIPHER. Relevant accession numbers for ClinVar and DECIPHER can be seen in Table 1. Family 1 and Family 12 did not consent to public release of their genomic sequence data from the CAUSES study.

Ethics Statement

Acknowledgments

Author Contributions

C.B.C., J.M.F., and A.M.E. drafted the manuscript and L.A., C.F.B., C.d.S., M.K.D., W.T.G., and M.S.P. revised the manuscript. L.A., C.F.B., L.A.C., C.d.S., M.K.D., W.T.G., H.G., E.L., M.S.P., K.S., and Z.A.-S. were the referring physicians or were involved in the care of the proband and contributed phenotypic data. All authors read and approved the final manuscript.

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