

# The prevalence of *PTEN* mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly

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**Purpose:** To define the prevalence of *PTEN* mutations in a clinical cohort of pediatric subjects with autism spectrum disorders (ASDs), developmental delay/mental retardation (DD/MR), and/or macrocephaly and to assess genotype–phenotype correlations. **Methods:** Medical records of patients who had clinical *PTEN* gene sequencing ordered through our institution between January 1, 2005 and December 31, 2007 were abstracted to confirm genetic test results and medical diagnoses. Phenotypic information related to the diagnoses, prenatal history, early developmental milestones, physical characteristics, and family history for those with a confirmed *PTEN* mutation was also recorded. **Results:** One hundred fourteen patients were tested during this time period for indications of ASDs ( $N = 60$ ), DD/MR ( $N = 49$ ), or macrocephaly only ( $N = 5$ ). Eleven mutations were identified: five in patients with ASDs and six in those with DD/MR, resulting in a prevalence of 8.3% and 12.2% in these respective clinical populations. All individuals with a *PTEN* mutation had significant macrocephaly ( $>2.0$  SD). **Conclusions:** These data illustrate that *PTEN* gene sequencing has a high diagnostic yield when performed in a selected population of individuals with ASDs or DD/MR and macrocephaly. Germline mutations in *PTEN* are an important, identifiable etiology among these patients. *Genet Med* 2009; 11(2):000–000.

**Key Words:** *PTEN*, autism, macrocephaly, developmental delay

The phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*) gene (MIM 601728) is a tumor suppressor gene that functions as a dual-specificity phosphatase active in numerous pathways involved with cellular growth.<sup>1,2</sup> It functions primarily as a lipid phosphatase acting to down-regulate the phosphoinositol 3-kinase/AKT pathway. This pathway is involved in cell proliferation, and its dysregulation has been associated with overgrowth syndromes.<sup>3</sup> Heterozygous germline mutations in *PTEN* have been identified in a family of related disorders, including Cowden syndrome (CS—MIM 158350), Bannayan-Riley-Ruvalcaba syndrome (BRRS—MIM 153480), Proteus and Proteus-like syndromes. Individuals with CS typically develop multiple benign hamartomas and have an increased risk of certain cancers, particularly of the breast, uterus, and thyroid. BRRS is characterized by macrocephaly, hamartomas (including lipomas, hemangiomas, or intestinal

polyps), penile freckling in males, and often developmental delay with childhood onset. Approximately 80% of individuals who meet diagnostic criteria for CS and 60% who meet clinical criteria for BRRS have detectable *PTEN* mutations.<sup>4</sup> Although genotype–phenotype correlations have been sought, it seems that CS and BRRS may actually be one condition with variable expressivity and presentation at different ages.<sup>5</sup> Because of phenotypic overlap among these disorders, and with Proteus-like syndromes, they have collectively been termed the *PTEN* hamartoma tumor syndromes.<sup>6</sup>

Although *PTEN* may be most recognized for its role in carcinogenesis, several recent reports have identified heterozygous germline *PTEN* mutations in children with autism spectrum disorders (ASDs) and macrocephaly where a parent had previously been diagnosed with CS.<sup>7–9</sup> On the basis of these findings, Butler et al.<sup>10</sup> screened 17 children with an ASD and macrocephaly with no family history of CS and identified *PTEN* mutations in three of them. Subsequently, we reported additional *PTEN* mutations in two unrelated children with significant macrocephaly and autism.<sup>11</sup> Finally, Buxbaum et al.<sup>12</sup> screened 88 patients with an ASD and macrocephaly and discovered a de novo missense mutation (D326N) in one subject.

Although these findings have led the authors to conclude that *PTEN* sequencing is warranted in patients with ASDs and significant macrocephaly, the prevalence of pathogenic *PTEN* mutations within this population remains unknown. We sought to define the detection rate of *PTEN* mutations in a cohort of pediatric patients undergoing clinical gene sequencing between January 1, 2005 and December 31, 2007. Concurrently, we performed a retrospective chart review to confirm the diagnosis of autism, significant developmental delay/mental retardation (DD/MR), and macrocephaly, and recorded the *PTEN* mutation status. Phenotypic information related to the prenatal history, early developmental milestones, diagnosis of ASD, physical characteristics, and family history for those with and without *PTEN* mutations was also recorded, with a goal of further delineating possible genotype–phenotype correlations.

## MATERIALS AND METHODS

After institutional review board approval, we obtained a list of patients who were tested clinically for *PTEN* mutations through our institution between January 1, 2005 and December 31, 2007. We developed a guideline for genetic testing of children with ASD at our hospital in January 2005, which included *PTEN* testing for those individuals with ASD and macrocephaly.<sup>13</sup> This guideline is used in virtually all children with ASD presenting to our institution.

ICD-9 diagnosis codes and testing information were obtained for each patient. Clinical records were requested from ordering

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providers, most commonly from the Divisions of Molecular and Human Genetics and Neurology and the Developmental Disabilities/Autism Clinics at Nationwide Children’s Hospital. Records were abstracted by two board-certified genetic counselors (E.A.V. and M.P.). Prenatal history, current and past medical diagnoses; findings on physical exam, such as weight, height, and occipital-frontal circumference (OFC); criteria for autism diagnoses (if applicable); family history including cancers, psychiatric diseases, and/or ASDs; and indication for testing and genetic test results were recorded. Information abstracted from medical records was collected and stored in a password-protected Microsoft Access database. OFC Z scores (by age and sex) were calculated using the Abase Anthropometry Database version 1.2,<sup>14</sup> which is based on Centers for Disease Control growth charts for ages 0–2 years and Canadian normative data for ages 3–18 years.<sup>15,16</sup> For the purpose of data analysis, subjects were categorized as having an ASD, DD/MR, and/or macrocephaly. Patients who did not have a diagnosis of ASD, DD/MR, or macrocephaly, and those who were subsequently found to have an alternate genetic diagnosis to explain their findings, were excluded from data analysis.

All *PTEN* gene sequencing was performed by the Clinical Laboratory Improvement Amendments (CLIA) certified Molecular Pathology Laboratory at The Ohio State University. Both strands of the *PTEN* coding region and the intron–exon junctions were sequenced. The two strands of the polymerase chain reaction (PCR) products were sequenced with the BigDye terminator cycle sequencing kit v1 (Applied Biosystems, Foster City, CA) and analyzed on capillary electrophoresis on an ABI Prism 3100 sequencer (Applied Biosystems). Alterations from the wild-type sequence were identified using the software program Mutation Surveyor (SoftGenetics, State College, PA). The *PTEN* promoter was not examined. Sequence data on 800 normal control alleles has been previously collated by this clinical laboratory.

We searched the literature and the Human Mutation Database for the *PTEN* gene ([http://www.hgmd.cf.ac.uk/ac/gene.php?gene = PTEN](http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PTEN)) to determine if the mutations identified in our subjects had been previously described. Novel mutations were analyzed in POLYPHEN<sup>17</sup> (<http://genetics.bwh.harvard.edu/pph/>) to determine possible pathogenic significance. Cross species comparison for sequence conservation was performed by CLUSTALW alignment of protein sequences downloaded from the Ensembl website database (<http://www.ensembl.org/index.html>—March 2008, release 49).

One subject (Patient 5) was studied in more detail for possible splice alterations in *PTEN*. Primary white blood cells and Epstein-Barr virus–derived lymphoblastoid cell lines were used for analysis in the subject. Lymphoblastoid cell lines for both parents and one normal control were also used. Total RNA was obtained with the RNeasy Mini Kit (Qiagen, Valencia, CA). Genomic DNA was removed by DNase, and cDNA was prepared using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Reverse transcriptase PCR products covering from exons 4 to 9 were amplified with the previously described primers 4F and 9R.<sup>17</sup>

We used Stata version 10 (Stata Corp, College Station, TX) for all descriptive and comparative statistical analysis. Categorical variables were compared for differences by  $\chi^2$  or Fisher exact test. For  $\chi^2$  analysis, one degree of freedom was used. Quantitative variables were first assessed to ensure the group variances were equal by the Bartlett test of homogeneity, and then analyzed by *t* test. Significance was set at *P* < 0.05.

RESULTS

Subject characteristics

*PTEN* gene sequencing for germline mutations was performed on 136 subjects in our Molecular Pathology Laboratory during the 3-year period analyzed (see Methods section). Eight of these patients did not have a diagnosis of macrocephaly, DD/MR, or an ASD and were excluded from data analysis. Genetic testing revealed an alternate genetic diagnosis in another 12 patients: two patients with 46,XY,dup(15)(q11.2q13); and one patient each with: mitochondrial complex II deficiency; duplication of MeCP2; 45X,dic(Y;22)(q12;q12;p11.2); de novo del 9q22.32; de novo del 22q11.2; dup(22)(q11.2)mat; del(1)(q21.1)mat; del(3)(p26.3)mat; del(X)(p22.22)mat; and dup(13)(q32.3q33.1)mat. Finally, two subjects tested were first-degree relatives of a proband; one subject was tested after a *PTEN* mutation was identified in his identical twin and another was tested at the same time as her mother who had DD/MR and macrocephaly. This resulted in 22 individuals being excluded from our sample, leaving 114 for analysis.

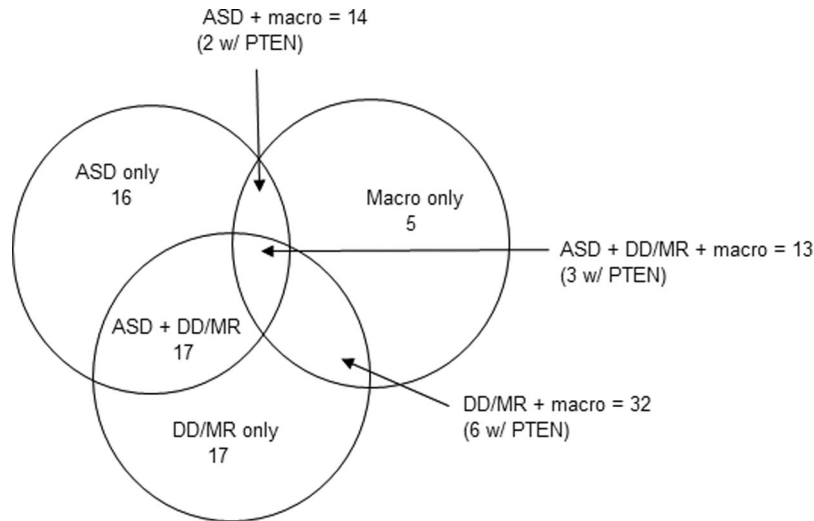
Characteristics of the 114 subjects are summarized in Table 1. Patients ranged in age from 3 months to 35 years (mean 5.8 years; median 4.3 years). Eighty-five were male and 29 were female (ratio 3.4:1); this ratio is reflective of the increased prevalence of ASDs in males.<sup>18</sup> A majority of the subjects were white (82%).

Sixty subjects had a diagnosis of an ASD based on DSM-IV criteria. Twenty-five of the subjects were diagnosed with an

Table 1 Subject characteristics (N = 114)

Age range	3 mo–35 yr
Gender	
Male	85
Female	29
Ethnicity	
White	93
African American	7
Somali	6
Hispanic/mixed race	4
Other/unknown	4
Testing physician	
Geneticist	67
Development or general pediatrician	35
Neurologist	7
Other/unknown	5
Diagnosis <sup>a</sup>	
ASD (total)	60
ASD and macrocephaly	27
DD/MR without ASD (total)	49
DD/MR and macrocephaly	32
Macrocephaly without ASD/DD/MR	5

<sup>a</sup>On the basis of diagnoses that were verified through chart review. ASD, autism spectrum disorder; DD/MR, developmental delay/mental retardation.



**Fig. 1.** Diagnoses of study population: Sixteen subjects had an autism spectrum disorder (ASD) without developmental delay/mental retardation (DD/MR) or an occipital-frontal circumference (OFC)  $> 2.0$  (macro). Seventeen subjects had DD/MR only. Seventeen subjects had ASD and DD/MR without macro. Fourteen subjects had ASD and macro, two of whom were found to have a *PTEN* mutation (*PTEN*). Thirty-two subjects had DD/MR and macro, six of whom were found to have *PTEN*. Thirteen subjects had ASD, DD/MR, and macrocephaly, three of whom were found to have *PTEN*. Five subjects had macrocephaly only; none of these subjects were found to have *PTEN*.

ASD by a developmental pediatrician, eight were diagnosed through a multidisciplinary evaluation (including a psychologist and developmental pediatrician at a minimum), three by a neurologist, and two by a psychiatrist, with the remaining being diagnosed by other physicians. The diagnosis of an ASD was confirmed in 13 subjects using the Autism Diagnostic Observation Scale.<sup>19</sup> As depicted in Figure 1, 30 of 60 (50%) subjects with ASD also had DD/MR. Twenty-seven (45%) of subjects with ASD also had macrocephaly. Forty-nine subjects without ASD had a diagnosis of DD (age  $< 5$  years) or MR (age  $> 5$  years). Of these, 32 (65%) were macrocephalic. The remaining five subjects in our cohort were evaluated for an indication of macrocephaly without ASD, DD, or MR.

*PTEN* gene sequencing was most commonly ordered by clinical geneticists ( $N = 67$ ), whereas developmental and general pediatricians performed testing in 35 cases. In addition to *PTEN* sequencing, additional diagnostic testing was performed on most of the subjects. Peripheral blood chromosomal analysis (at least 550 band metaphase spread) was performed in 97 subjects; DNA testing for fragile X syndrome was obtained in 88; and targeted BAC microarray (including Spectral 2600 and Constitutional, Signature V.3.0 and V4.0 and Baylor College of Medicine's chromosomal microarray) was completed in 73 subjects. Two subjects had abnormalities identified on chromosome analysis that were present in one parent, one of which is a known chromosomal variant. Two had abnormalities on microarray that were also present in one of the parents and are known copy number variants in the general population (as determined by accessing the Database of Genomic Variants, <http://projects.tcag.ca/variation/>) and are thus considered normal variants.

#### Prevalence and description of *PTEN* mutations in our subject population

Eleven *PTEN* mutations were identified in our clinical cohort, resulting in a prevalence of 9.6%. Clinical summaries for Patients 1 and 5 have been reported previously.<sup>11</sup> Clinical case

reports for the other nine patients are provided in the online Supplementary material with relevant data (see **Supplemental Digital Content 1**, <http://links.lww.com/A687>), including mutation descriptions, summarized in Tables 2 and 3.

Five subjects with a DSM-IV diagnosis of an ASD had a *PTEN* mutation, resulting in a prevalence of 5 of 60 (8.3%) in this subgroup (Table 2). Six *PTEN*-positive subjects had a diagnosis of DD and macrocephaly without ASD resulting in a prevalence of 6 of 49 (12.2%) in the DD/MR subgroup.

The distribution of OFC  $Z$  scores in our cohort and among those with *PTEN* mutations is illustrated in Figure 2. Subjects with a *PTEN* mutation had an average OFC  $Z$  score that was higher than those without a mutation ( $4.3 \pm 1.3$  vs.  $2.3 \pm 1.6$ ;  $P < 0.0001$ ). The percentage of subjects with a mutation increased with increasing head size. Mutation rates for OFC  $Z$  scores from 2.0 to 3.9 was 5 of 43 (11.6%), whereas rates were 6 of 26 (23.1%) for subjects with OFC  $Z$  scores  $\geq 4.0$ . This finding concurs with results from previous studies demonstrating a higher yield of *PTEN* sequencing in those with marked ( $Z$  score  $\geq 4.0$ ) macrocephaly.<sup>10</sup> However, the largest  $Z$  score of 9.4 was observed in a subject with DD without a *PTEN* mutation. Although none of the subjects with a *PTEN* mutation had an OFC  $Z$  score under 2.0, the identical twin of Subject 2 had a diagnosis of ASD, carried the same E157G change, and had an OFC  $Z$  score of 0.99.

Comparisons of problems reported in the medical history or findings on physical exam were made between those subjects with a *PTEN* mutation and those without a mutation. No differences were noted between the groups for the presence of birth defects or reported history of problems in the visual, central nervous system, respiratory, gastrointestinal, genitourinary, musculoskeletal, dermatologic, or immune systems.

The spectrum of *PTEN* mutations identified included four missense, three nonsense, three splice-site, and one frameshift mutation. Three of the mutations were paternally inherited, one was maternally inherited, and four were de novo. In three

**Table 2** Germline *PTEN* mutations in subjects with ASD

Pt	Mutation	Inherited	Sex	OFC	Age	Diagnosis	Medical Hx./ Physical exam	Family history
1	R130X <sup>a</sup> (exon 5)	Paternal	M	55.5 cm; +3.1 SD	4 yr	DD, SpD—18 mo; Autism—4 yr		Father: previous ADHD
2	E157G (exon 5)	No	M	55.5 cm; +2.9 SD	3 yr 8 mo	PDD-NOS—3 yr 5 mo		Identical twin: ASD and same mutation
3	L139X (exon 5)	Paternal	M	57.5 cm; +3.9 SD	6 yr 8 mo	Autism—6 yr 6 mo		Father: normal. Paternal great grandmother: <i>uterine cancer (50s)</i>
4	520insT <sup>a</sup> (exon 6)	No	F	54 cm; +5.8 SD	16 mo	DD—16 mo; Autism— 25 mo	Macrocephalic at birth (OFC = 39 cm)	
5	IVS6-3C>G	No	M	58 cm; +4.2 SD	6 yr, 11 mo	PDD-NOS—3 yr 11 mo	<i>Small nevi: right chest (1), left shoulder (1) and back (2) Dolichocephaly, midface hypoplasia, long face, bilateral epicanthal folds, almond-shaped eyes, mild hypertelorism</i>	

Age is age at initial assessment. Words in italics signify features that may be consistent with BRRS/Cowden syndrome.

<sup>a</sup>Case previously reported in Ref. 11.

ADHD, attention deficit-hyperactivity disorder; BRRS, Bannayan-Riley-Ruvalcaba syndrome; CALM, Café-au-lait macule; DD, developmental delay; GI, gastrointestinal; Hx, history; LD, learning disability; ND, not determined; OFC, occipito-frontal circumference; PDD-NOS, pervasive developmental delay not otherwise specified; Pt, patient number; SD, standard deviation; SpD, speech delay.

subjects, the inheritance was unknown because parental testing was not performed.

## DISCUSSION

We report on a cohort of 114 patients selected for ASD, DD/MR, and/or macrocephaly that had clinical *PTEN* gene sequencing. We identified mutations in 11 patients (9.6%). We found that 5 of 60 (8.3%) patients with a confirmed ASD were *PTEN* mutation positive. Further, we found that *PTEN* mutations were identified in 6 of 49 (12.2%) of patients sequenced for an indication of DD/MR without ASD. Taken together, these data illustrate that *PTEN* sequencing has a high diagnostic yield when performed in carefully selected populations.

Similar to others, we did not observe a genotype-phenotype correlation in this study. But, like Lachlan et al.<sup>5</sup> macrocephaly was a consistent finding. We noted that no *PTEN* mutations were found in subjects with a normal OFC, with the exception of an identical twin with a diagnosis of autism who had relative, but not absolute, macrocephaly. Although our findings suggest that *PTEN* testing should be considered in cases of autism and macrocephaly, other syndromes, including fragile X, Sotos syndrome, neurofibromatosis Type 1, and 22q13 deletions may also account for some cases, and multiple causes of brain overgrowth may predispose to autism.<sup>12</sup>

Various phenotypes, including macrocephaly or macrosomia and DD (without other features of BRRS), VATER-like and Proteus-like phenotypes, have been associated with *PTEN* mutations.<sup>5</sup> The clinical features in our subjects were quite variable and presentations differed significantly even within families. Although we observed unusual skin findings in our patient sample, including café au lait spots, hemangiomas, and a verrucous lesion, these are not the traditional hamartomatous lesions most commonly described in CS/BRRS. None of the

subjects with a *PTEN* mutation fulfilled diagnostic criteria for a *PTEN*-related syndrome before testing. In some, such as Patients 7 and 11, this may be because they were evaluated at a very young age before clinical features of CS or BRRS became apparent.

Interestingly, in two of our patients with known pathogenic *PTEN* mutations (L139X, R130X) the mutation was also identified in phenotypically normal fathers. This emphasizes the need for careful examination of relatives to detect features that may be subtle, or those that are not evident from physical examination alone (such as gastrointestinal hamartomas). Others have observed that a milder phenotype may occur in non-probands with the same mutation.<sup>5</sup>

Several of the mutations in our cohort have been reported previously, including three nonsense mutations (R130X, L139X, and R233X), which have been observed in CS, BRRS, and CS/BRRS overlap families and are known to be pathogenic.<sup>20</sup> L139X has also been observed in a patient with CS and Sjogren syndrome.<sup>21</sup> The novel 520insT frameshift mutation in exon 6 results in premature termination in Patient 4 with autism and macrocephaly<sup>22</sup> and is also considered pathogenic.

We identified three splice-site mutations in this clinical sample. The IVS6-1G>A mutation (Patient 9 with DD) results in an alteration of a splice acceptor site as does IVS8-2A>G (Patient 11). Both are considered pathogenic. The latter mutation was also found in the father of Patient 11. He exhibited features of BRRS, including macrocephaly, learning disability, a history of gastrointestinal bleeding, and freckling of the glans penis. IVS8-2A>G has also been previously observed as a somatic mutation in glioblastoma multiforme.<sup>23</sup> Patient 5, who possesses an IVS6-3C>G, immediately adjacent to the consensus acceptor site, was investigated further. RT-PCR products of the 4F-9R *PTEN* primer pairs from Patient 5 contained



**Table 3** Germline *PTEN* mutations identified in subjects without ASD

Pt	Mutation	Inherited	Sex	OFC	Age	Diagnosis	Medical Hx./ physical exam	Family history
6	G44D (exon 2)	ND	F	54.5 cm; +5.0 SD	2 yr 3 mo	DD and SpD	Reflux and bilateral hernia repair at 1 mo. <i>Large CALM spot on chest and abdomen</i>	Brother: SpD, Mother: ? depression or bipolar disorder
7	R173H (exon 6)	Maternal	M	50.8 cm; +4.4 SD	9 mo	DD		Maternal 1/2 sister: "Large head." Mother: "Large head," mild MR, epilepsy, tremors. Maternal grandmother: "Large head," frontal bossing, tremors. Maternal aunt: tremors
8	T202I (exon 6)	No	M	57 cm; +6.5 SD	20 mo	DD	<i>Hemangioma of glabella and nape of neck. Right calf smaller than left. Three, 1 cm CALM (back, R chest, R knee); axillary freckling</i>	Brother: "Large head at birth"
9	IVS6-1G>A	ND	F	59.2 cm; +5.6 SD	8 yr, 3 mo	DD; obesity	Frontal bossing, small hands and feet, deep set nails, <i>verrucous-like lesions on left arm</i>	
10	R233X (exon 8)	Father negative; Mother ND	M	56.5 cm; +2.8 SD	8 yr 6 mo	Expressive SpD	Multiple freckles. Mild myopia	Mother: <i>Macrocephaly, LD</i> . Brother and sister: ADHD and behavior problems
11	IVS8-2A>G	Paternal	M	49.5 cm; +3.5 SD	9 mo	DD	Born 29 weeks gestation. Alcohol exposure first trimester. History of apnea, reflux, and eczema. Notched ears, right ear larger than left, facial asymmetry	Father: <i>macrocephaly, LD, GI bleeding, and freckling on the glans penis; diagnosed with BRRS</i>

Words in italics signify features that may be consistent with BRRS/Cowden syndrome.

ADHD, attention deficit-hyperactivity disorder; BRRS, Bannayan-Riley-Ruvalcaba syndrome; CALM, Café-au-lait macule; DD, developmental delay; GI, gastrointestinal; Hx, history; LD, learning disability; MR, mental retardation; ND, not determined; OFC, occipito-frontal circumference; Pt, patient number; SpD, speech delay.

only a single band of the expected ~1000 bp size, identical to the control. To ensure there was no altered splicing event, we sequenced products from 15 clones. All were identical to the reference cDNA sequence, with no evidence of altered splice site variants. The significance of this variant is, therefore, unclear.

We identified four missense mutations, one of which has been previously reported as a somatic mutation. The R173H mutation in exon 6 (Patient 7) is one of the most commonly mutated residues in individuals with *PTEN*-related tumors.<sup>24</sup> The missense mutation E157G, observed in Patient 2, results in a change in amino acid charge. The region is also highly conserved in species analyzed, including the frog, chicken, rat, mouse, Rhesus monkey, Chimpanzee, and humans, and it was

predicted to be "possibly damaging" using the PolyPhen program. Furthermore, the same mutation was found in the proband's symptomatic identical twin who has a diagnosis of an ASD. The G44D mutation, identified in Patient 6, has not been previously reported. This mutation results in a change in amino acid charge and would be expected to alter the protein's secondary structure. It is also conserved in all species analyzed, suggesting it is pathogenic. Finally, for the T202I alteration found in Patient 8, it is unclear whether or not this change would result in deleterious effects. The Polyphen analysis predicted that this would be a benign variation, yet this region was conserved in all species analyzed. Further, this variant was not observed in 800 control alleles analyzed in our laboratory. More data are required to determine if this variant is pathogenic.

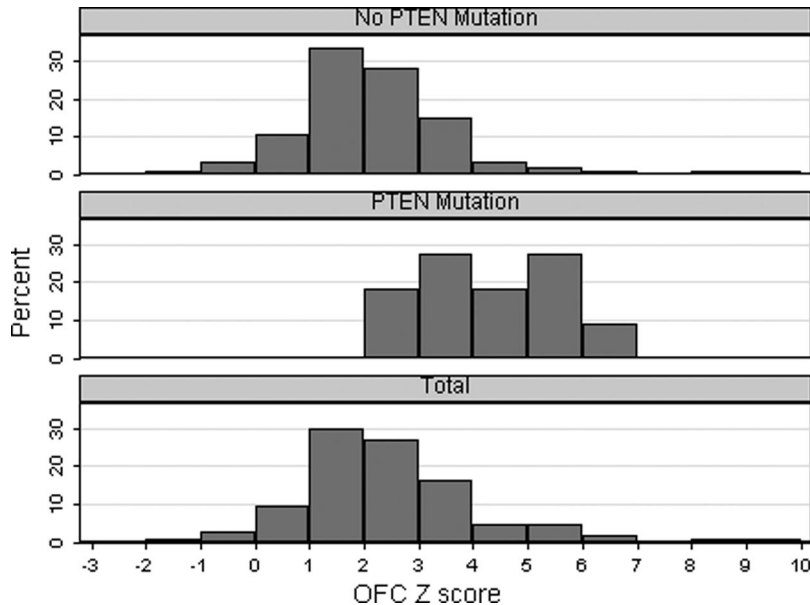


Fig. 2. Distribution of occipital-frontal circumference (OFC) Z scores among subjects. Top, distribution in PTEN-negative subjects; Middle, distribution in PTEN-positive subjects; Bottom, distribution among all subjects.

The strength of this study lies in the complete capture of all individuals tested for *PTEN* mutations at our institution. All testing for mutations is performed in our laboratory, and can be tracked through our laboratory information system. A primary limitation of this study includes ascertainment bias. Our cohort includes patients in whom there was a high index of suspicion for *PTEN* mutations, thereby limiting the generalizability of our findings. Nonetheless, our study provides clues as to the prevalence of *PTEN* mutations in highly selected cohorts, such as those referred to genetics, developmental pediatric and neurology clinics for evaluation of ASDs or DD/MR and/or macrocephaly.

Another limitation includes the retrospective accumulation of clinical data. Some clinical details were not recorded or provided in sufficient detail. Furthermore, diagnoses may evolve over time. Four of our DD/MR subjects with *PTEN* mutations were younger than 2.5 years at the time of study, and may develop an ASD in the future. Therefore, a prospective study to further delineate genotype-phenotype correlations will be needed.

In summary, we have identified a high prevalence of *PTEN* mutations in a selected population of patients with ASDs, DD/MR, and/or macrocephaly. Several of the mutations that we report are newly identified, adding to the body of literature on this topic. As evidence mounts to support the occurrence of *PTEN* mutations in the population of children with ASDs and macrocephaly, large prospective studies will be needed to elucidate the true prevalence of these mutations. Our findings suggest that *PTEN* sequencing is warranted in patients with macrocephaly (>2.0 SD), ASDs, and/or DD. Identification of *PTEN* mutations has implications for genetic counseling of relatives, and future surveillance of patients and family members.

Patient 5 (mutation IVS6-3C>G) has been subsequently reevaluated in clinic at age 8 years 6 months. He now demonstrates freckling of the glans penis suggesting a diagnosis of BRRS, providing additional evidence the *PTEN* mutation he carries is pathogenic.

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