
THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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Abstract

**Purpose:** To assess the frequency of *PTEN* pathogenic variants in a cohort of pediatric subjects with autism spectrum disorder (ASD), intellectual disabilities (ID), and/or global developmental delays (GDD), in the presence of macrocephaly.

**Methods:** Retrospective chart review of subjects who were evaluated through the Nationwide Children’s Hospital Child Development Center between January 1, 2015 and June 30, 2016 and received a diagnosis of ASD, ID, and/or GDD, and had macrocephaly. Medical record was abstracted to determine whether *PTEN* gene analysis was ordered, if testing was completed, and the results of testing.

**Results:** One hundred eight subjects who met the study criteria had *PTEN* genetic testing ordered during this period. Fifty-eight subjects completed *PTEN* genetic testing. No *PTEN* pathogenic variants were identified (0/58; 95% CI: 0.0-6.3).

**Discussion/Conclusion:** This data revealed that the frequency of *PTEN* pathogenic variants in subjects with a diagnosis of ASD, ID, and/or GDD in the presence of macrocephaly is less than the previously reported estimate of 10% in the literature.
Dedication

This document is dedicated to my family and friends who have shaped me into the person I am today.
Acknowledgments

A special thanks to The Ohio State University Genetic Counseling Program directors, faculty, staff, fellow students, and alumni for their guidance, support, and encouragement; the Nationwide Children’s Hospital faculty and staff for their expertise, guidance, and support; to my family for their encouragement, love, and support; to my friends for their patience and support.
Vita

May 2014 .................................................. B.S. Human Biology, Indiana University
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Fields of Study

Major Field: Genetic Counseling
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Chapter 1: Background and Study Aims

Background

Autism Spectrum Disorder (ASD)

Autism spectrum disorder (ASD) consists of a group of heterogeneous neurodevelopmental disorders. Previously, the diagnosis of ASD was divided into subtypes including early infantile autism, childhood autism, Kanner’s autism, high-functioning autism, atypical autism, pervasive developmental disorder not otherwise specified (PDD-NOS), childhood disintegrative disorder, and Asperger’s disorder. However, the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) combines all of the subtypes into one diagnosis, with a range of characteristics and severity. The key characteristics of an ASD diagnosis, as defined by the DSM-5, are persistent impairment in reciprocal social communication and social interaction along with restricted, repetitive patterns of behavior, interests, or activities. These symptoms present in early childhood typically between 12-24 months of age or earlier, and lead to limitations or impairment of day-to-day functioning. Symptoms of ASD can present differently depending on the individual’s age, intellectual level, and language ability, and can be influenced by past treatment and support services.
In addition to these cardinal features, children with ASD also display a higher incidence of other traits, such as seizures/epilepsy, insomnia, constipation, anxiety, oppositional defiant disorder (ODD), attention deficit hyperactive disorder (ADHD), and/or depression.\textsuperscript{2,3,4,5} The prevalence of seizures/epilepsy in children with ASD is estimated to be between 22-25%.\textsuperscript{4,6} Insomnia, along with other abnormal sleep patterns, has been reported in approximately 60% of children with ASD and constipation has been reported in approximately 45%.\textsuperscript{4} A study conducted by Simonoff and colleagues found that in a cohort of 112 children with ASD, 70% had at least one psychiatric disorder diagnosed: 41% had anxiety or phobic disorder (including OCD), 30% had oppositional or conduct disorder, 28% had ADHD, and 1% had depressive disorder.\textsuperscript{7} Children with ASD may also display some degree of hypersensitivity or hyposensitivity to either a specific sound or physical stimuli.\textsuperscript{4}

Before 1990, the prevalence of autism was estimated to be 1/2,000 to 1/2,500 children and increased from between 1/323 and 1/476 in the 1990’s to 1/88 by 2008.\textsuperscript{4,8,9,10,11} As of 2016, the Centers for Disease Control and Prevention (CDC) Autism and Developmental Disabilities Monitoring (ADDM) Network estimated the prevalence of autism to be 1/68.\textsuperscript{8,11,12,13} The cause for this increase in prevalence may be due to the broadening of diagnostic criteria, incorporation of similar disorders into one ASD diagnosis, earlier recognition of symptoms, and/or increased awareness by both the public and clinicians. There is also some evidence which suggests that the actual rate of autism itself may be increasing, although probably not as rapidly as prevalence estimates have indicated.\textsuperscript{4}
addition, the prevalence of ASD also differs between genders depending on whether the ASD is syndromic or non-syndromic. Non-syndromic ASD is used to describe cases of ASD that are not associated with or caused by a Mendelian disorder. Non-syndromic ASD shows a sex bias between males and females with a ratio of 4:1. Syndromic ASD is used to describe cases of ASD that are associated with or caused by a Mendelian disorder. In contrast to non-syndromic ASD, syndromic ASD shows no sex bias between males and females with a ratio of 1:1.

Intellectual Disabilities (ID)

Intellectual disability (ID), as defined by the World Health Organization (WHO), is an intelligence quotient (IQ) of less than 70. It is typically present in infancy or early childhood although some cases may not be diagnosed until a child is over the age of five and able to complete more reliable standardized measures of developmental skill. Intellectual disability is characterized by significantly limited cognitive functioning, coupled with limitations in adaptive skills in at least two of the following domains: social skills, community living, communication, home living, health, self-direction, work, and leisure. Intellectual disability is also divided into four subcategories based on severity. Those with mild ID are defined as having an IQ of 50-70, moderate as having an IQ of 35-50, severe as having an IQ of 20-35, and profound as having an IQ of less than 20. Individuals with ID can also display a variety of other diagnoses/features, such as congenital anomalies, seizures/epilepsy, dysmorphic features, and other
health/mental issues, in addition to ASD and/or global developmental delays (GDD).\textsuperscript{16,17} Similar to ASD, ID can also be categorized as non-syndromic or syndromic. Non-syndromic ID is used to describe cases of ID that are not associated with or caused by a Mendelian disorder.\textsuperscript{1,14,15} Syndromic ID is used to describe cases of ID that are associated with or caused by a Mendelian disorder.\textsuperscript{1,14,14} Prevalence estimates of these diagnoses/features in individuals with ID have not been well documented, including those individuals with syndromic ID. The prevalence of ID in the general population, is estimated to be approximately 1-3% with mild ID occurring 7-10 times more frequently than moderate or severe.\textsuperscript{14,15}

\textit{Global Developmental Delays (GDD)}

Global developmental delays (GDD) describe significant deficits in two or more of the following areas: cognition, speech/language, gross/fine motor skills, social/personal skills, and daily living.\textsuperscript{1,14,15} Global developmental delays are apparent as age-specific deficits in learning skills and adaptation in comparison with chronological peers.\textsuperscript{1,14,15} The diagnosis of GDD is generally reserved for children ages 5 years or younger, prior to the age at which IQ testing can be applicable and reliably measured.\textsuperscript{1,14,15} Global developmental delays can also be associated with other diagnoses/features including, ASD, ID, congenital anomalies, seizures/epilepsy, and other health/mental issues.\textsuperscript{16} A study conducted by Koul and colleagues on 110 children with GDD found that 76.4% had abnormal neurological findings, 47.3% had dysmorphic features, and 42.7% had a
history of seizures.\(^{18}\) The prevalence of GDD in the general population is estimated between 1-3\%.\(^{1,14,15}\)

**ASD, ID, GDD and Macrocephaly**

Macrocephaly, defined as an occipitofrontal head circumference (OFC) larger than 2 standard deviations above the mean for a given age and gender, is seen at a higher frequency in individuals with ASD.\(^{11,13,19}\) In Kanner’s initial description of autism published in 1943, he reported that 5 of 11 (45\%) children had unexpectedly large heads.\(^{20,21,22}\) A study by Fombonne and colleagues which examined the frequency of macrocephaly in children with ASD, found that macrocephaly was not correlated with gender, developmental level, severity of ASD, or the presence or absence of medical disorders, or epilepsy.\(^{20}\) Based on this data and the data of previous studies, current estimates suggest that approximately 20\% of children with ASD have macrocephaly.\(^{12,13,19,20}\) Studies examining the frequency of macrocephaly among children with GDD have reported a much lower frequency of macrocephaly as compared to those with ASD. A study by Nevo and colleagues on 4,309 children with developmental delays found that only 1\% had macrocephaly.\(^{23}\) In addition, a study by Koul and colleagues on 110 children with GDD found that 5.5\% had macrocephaly.\(^{18}\) While the frequency of macrocephaly in children with ASD and GDD has been previously estimated, the frequency of macrocephaly among children with idiopathic or syndromic ID has not been assessed.
Genetic Etiology of ASD, ID, and GDD

While the underlying etiology of most ASD, ID, and GDD cases is currently unknown, chromosomal abnormalities, copy number variants, and single gene disorders have been shown to be associated with an increased incidence of ASD and the underlying cause of some ID and GDD.

Chromosomal aneuploidies are present in approximately 2-5% of children with ASD and 15-40% of children with ID and/or GDD. For example, all individuals with Down syndrome will show some degree of intellectual and developmental disabilities. In addition, among the Down syndrome population there is an incidence of ASD in 5-7%.

Furthermore, research on individuals with sex chromosome abnormalities, such as Turner syndrome, 47,XXX, 47,XXY, and 47,XYY, also have shown an increased incidence of ASD.

Copy number variants (CNVs) are present in approximately 1% of cases of ASD and greater than 10% of cases of ID/GDD. Examples of such CNVs include maternally derived 15q11-q13 duplications and 16p11.2 deletions and duplications, both of which are associated with an increased risk for ASD. In addition, 7q11.23 duplications of the William syndrome region have also been found to convey an increased risk for ASD as well as ID and/or GDD. Other CNVs such as 22q11.2 microdeletion syndrome
(DiGeorge syndrome) and 17p11.2 (Smith-Magenis syndrome) also convey an increased risk for varying degrees of ID and/or GDD.\textsuperscript{4,16} With exclusion of the 7q11.23, 22q11.2, and 17p11.2 microdeletion/duplication syndromes, many CNVs, while conveying an increased risk for ASD, ID, and/or GDD, are themselves not necessarily causative, since they can also be inherited from a parent with little to no symptoms.\textsuperscript{4}

Single gene disorders are present in approximately 5\% of cases of ASD and 13-35\% of cases of ID/GDD.\textsuperscript{24,25} There are also several single gene disorders, such as Fragile X syndrome, Tuberous Sclerosis Complex (TSC), and PTEN Hamartomatous Tumor syndrome, which are associated with ID, GDD, and an increased risk for the development of ASD.\textsuperscript{4,13,21,27} It is estimated that approximately 25-30\% of individuals with Fragile X syndrome will develop ASD or autistic-like features and among individuals with TSC, approximately 25-50\% will have features of ASD.\textsuperscript{29} Individuals with PTEN Hamartomatous Tumor Syndrome also have an increased risk for ASD and are the focus of this study, as will be discussed below.\textsuperscript{10,11,30}

Given that ASD, ID, and GDD can be due to underlying chromosomal and molecular causes, the American Academy of Pediatrics and the American College of Medical Genetics and Genomics, recommend the use of chromosomal microarray and Fragile X as a first tier test in the diagnostic work-up of patients with these conditions and/or multiple congenital anomalies.\textsuperscript{14} In addition, the American College of Medical Genetics and Genomics recommends \textit{PTEN} gene analysis as a second tier test for the diagnostic
work-up of patients with ASD and a head circumference >2.5 standard deviations above the mean.\textsuperscript{14,31} The American College of Medical Genetics and Genomics also recommends \textit{MECP2} gene analysis as a second tier test for the diagnostic work up of females with ASD and duplication analysis for males if indicators are present.\textsuperscript{14,31}

\textit{PTEN Hamartomatous Tumor Syndrome (PHTS)}

The phosphatase and tensin homologue (\textit{PTEN}) gene was first identified in 1997.\textsuperscript{32} Located on chromosome 10q23, the \textit{PTEN} gene contains nine exons and encodes for a 403 amino acid protein also known as PTEN.\textsuperscript{32} The PTEN protein functions as a dual-specificity phosphatase, which is active in numerous pathways involved with cellular growth and tumor suppression.\textsuperscript{12,13,33,34} Clinically, loss of \textit{PTEN} has been identified as a driver in the development of many sporadic cancers as well as macrocephaly.\textsuperscript{35} Heterozygous germline pathogenic variants in the \textit{PTEN} gene are implicated in a rare family of related tumor syndromes collectively referred to as PTEN hamartomatous-tumor syndrome (PHTS).\textsuperscript{33,34,36} Individually, the syndromes that make-up PHTS are Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS), Proteus-like syndrome, and ASD with macrocephaly (when a pathogenic \textit{PTEN} variant is present).\textsuperscript{3,4,12,13,33,34}

PHTS is an autosomal dominant genetic condition characterized by multiple features with variable expressivity among patients, and even between families and patients harboring
the same pathogenic variant. Features of PHTS can include benign hamartomas, an increased risk for multiple cancers (female breast, thyroid, colon, renal, and endometrial cancer), extreme macrocephaly (>3 standard deviations above the mean), Lhermitte-Duclose disease, dermatological findings, penile freckling, and an increased risk for ASD, ID, and GDD. While many features of PHTS do not present until adulthood, thyroid disease, dermatologic findings, macrocephaly, and ID/ASD may be seen in childhood. Thirty to sixty-eight percent of adults and 2-14% of children with PHTS exhibit thyroid abnormalities and dysfunction including, thyroid nodules, multinodular goiter, Hashimoto’s disease, and thyroid adenoma. One study examining children with PHTS reports the average age of diagnosis for benign thyroid pathology was between 6 to 12 years of age. The estimated lifetime risk for thyroid cancer in individuals with PHTS is believed to be between 3-17% compared to the general population lifetime risk of 1-2%. Although there is limited data on the frequency of intellectual disabilities/developmental delay in children with PHTS, current reports have suggested that the frequency is between 15-20%. The presence of macrocephaly in both children and adults with PHTS was thought to be between 40-50%; however, more recent studies have found the frequency of macrocephaly in CS patients to be between 80-100%.

The first report of a PTEN pathogenic variant in the presence of ASD and macrocephaly was by Zori and colleagues who reported on a family in which the mother had manifestations of Cowden syndrome (CS) and her son had Bannayan-Riley-Ruvalcaba
syndrome (BRRS) with macrocephaly and “autistic behavior”. Other case reports describing children with ASD and macrocephaly who had PTEN pathogenic variants followed. However, the first study to clearly link germline pathogenic variants in the PTEN gene to ASD and macrocephaly was published by Butler and colleagues in 2005. This study reported that 3 out of 18 (17%) children with autism and macrocephaly who underwent PTEN mutation analysis tested positive for a PTEN pathogenic variant. Since then, several studies have examined the frequency of pathogenic variants in the PTEN gene in children with ASD. Buxbaum and colleagues examined 88 subjects with ASD and macrocephaly and found that 1.1% had a germline PTEN pathogenic variant and in another study of 16 subjects with autism and macrocephaly 8% were found to have PTEN pathogenic variants. Two other cohort studies reported that 22% (2/23) and a 27% (6/12) of subjects with ASD and macrocephaly had PTEN pathogenic variants.

Other studies expanded the cohorts to include children with macrocephaly, ASD, and/or intellectual disabilities/developmental delays. Varga’s group reported that among subjects with macrocephaly and ASD, 8.3% (5/60) had an identifiable PTEN pathogenic variant and in those subjects with ID/DD, 12.2% (6/49) were found to have a pathogenic PTEN variant. Whereas, McBride and colleagues found 5.1% (2/39) of macrocephalic subjects with ASD and 3.9% (2/51) of subjects with ID/DD had PTEN pathogenic variants. Overall the frequency of PTEN pathogenic variants in these cohorts was 10% and 4.4%, respectively.
Based on the studies to date, the frequency of pathogenic *PTEN* variants in children with ASD, ID, and/or GDD, in the presence of macrocephaly ranges from 1-27%. Many of these studies have examined small cohort sizes; the largest being a cohort of 109 subjects which found that 10% had *PTEN* pathogenic variants.\textsuperscript{34} To reconcile the varying reports from these studies, an assessment of a larger cohort is necessary to adequately delineate the frequency of *PTEN* pathogenic variants in children with ASD, ID, and/or GDD in the presence of macrocephaly. Understanding the true frequency of *PTEN* pathogenic variants in these children is important for many reasons. First, discerning the true prevalence of *PTEN* pathogenic variants in these patients will allow for more accurate genetic counseling (e.g. what are the odds an at-risk patient will test positive for a *PTEN* pathogenic variant). Second, for those children who test positive, screening recommendations would be based on the National Comprehensive Cancer Network (NCCN) screening and management guidelines. These guidelines recommend that children with PHTS undergo annual thyroid ultrasounds and annual physical examinations with particular focus on dermatologic manifestations. In addition, symptom-based management should be carried out. Third, for those children with positive *PTEN* results, genetic testing and accurate recurrence risk estimates can be provided for the parents, child, and other at-risk relatives. The current study aims to better refine the estimated frequency of *PTEN* pathogenic variants utilizing a large cohort of children with ASD, ID, and/or GDD in the presence of macrocephaly.
Study Aims

The overall aim of this study is to assess the frequency of *PTEN* pathogenic variants in a large cohort study of children with autism spectrum disorder (ASD), intellectual disabilities (ID), and global developmental delays (GDD), in the presence of macrocephaly as compared to what has been previously published in the literature.

The specific aims of this study are to determine:

- **Aim 1:** Determine how many subjects evaluated through the Child Development Center Interdisciplinary Diagnostic Assessment met criteria for *PTEN* gene analysis.
- **Aim 2:** Delineate how many of these subjects completed *PTEN* gene analysis.
- **Aim 3:** Calculate how many of these subjects tested positive for *PTEN* pathogenic variants.
Chapter 2: Methodology

The Ohio State University Institutional Review Board (IRB) ceded authority to the Nationwide Children’s Hospital (NCH) IRB, which subsequently approved this study (IRB16-00942).

Eligibility for the study was as follows:

- Patient evaluated by the NCH Child Development Center/Autism Center between the dates of January 1, 2015 to June 30, 2016.
- Subjects must have received a diagnosis of ASD, ID, and/or GDD.
- Subjects must have received a diagnosis of macrocephaly (as defined as an OFC ≥2.0 standard deviations above the mean)
- Subjects must have had PTEN genetic testing completed

After obtaining IRB approval, the electronic medical record was queried for subjects who met the eligibility criteria. In addition, the following information was obtained: medical record number, subject name, date of birth, if PTEN gene analysis was ordered, and the outcome of genetic testing. Medical and family history information for those subjects who had PTEN testing ordered was extracted from the medical records and analyzed to identify subjects who may have had an increased likelihood to test positive for a
pathogenic variant in the *PTEN* gene. Therefore, subject’s medical records were also assessed for a documented family history of: ASD, ID/GDD, macrocephaly, cancer, and thyroid problems/dysfunction.

For those reported to have undergone genetic analysis of the *PTEN* gene and/or had macrocephaly documented in the problem list, occipitofrontal circumference (OFC) standard deviation (by age and sex) was calculated based on Centers for Disease Control growth charts for ages 2-18 years of age.\(^{47}\) For the purpose of data analysis, subjects were categorized as having ASD alone, ID alone, GDD alone, and mixed diagnoses (ASD/ID, ID/GDD, ASD/ID/GDD) based on recorded diagnosis in the medical record. Subjects who did not have a documented diagnosis of ASD, ID, and/or GDD were excluded from the data analysis. In order to assess the possibility of testing biases, subjects who had *PTEN* genetic testing ordered but not completed were assessed with the same methods and compared to those who completed testing. Statistical analysis was completed using Stata version 14.1.
Chapter 3: Results

A total of 1,650 subjects who received a diagnosis of ASD, ID, and/or GDD through the Interdisciplinary Diagnostic Assessment (IDA) process at the Nationwide Children’s Hospital Child Development Center between the dates of January 1, 2015 to June 30, 2016 were identified as eligible for the retrospective chart review. Among these eligible subjects, 131 (7.9%, 95% confidence interval (CI): 6.7-9.3) had PTEN genetic testing ordered. Twenty-two subjects who completed PTEN genetic testing were omitted from our data analysis due to the absence of macrocephaly (OFC <2.0 standard deviations). In addition, a single subject was excluded from data analysis because they had PTEN testing completed prior to their IDA assessment. This individual had a diagnosis of both ASD and GDD along with an OFC Z-score of 4.4 standard deviations above the mean and a positive PTEN result, but did not have any other clinical findings of PHTS on exam. The subject did have a family history of learning/intellectual disabilities and breast cancer. In total, 23 subjects (n=23) were omitted from final data analysis, leaving 108 subjects with confirmed macrocephaly and PTEN genetic testing ordered for analysis. See Figure 1 for assessment of potentially eligible subjects for study.
Out of the 108 subjects eligible for study, 54% (n=58) had *PTEN* genetic testing completed. All 58 subjects who completed testing were negative for variants in the *PTEN* gene (95% CI: 0.0-6.3). Additional characteristics of these 58 subjects can be found in Table 1.
<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 (78%)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (22%)</td>
</tr>
<tr>
<td>Macrocephaly Z-score (Standard Deviation)</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>40 (69%)</td>
</tr>
<tr>
<td>3-4</td>
<td>12 (21%)</td>
</tr>
<tr>
<td>4-5</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>5-6</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>6-7</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Table 1: Characteristics of Eligible Subjects Who Completed *PTEN* Genetic Testing

Of the 58 subjects who completed *PTEN* genetic testing, 62% (n=36) had a diagnosis of ASD alone, 3% (n=2) had a diagnosis of ID alone, 3% (n=2) had a diagnosis of GDD alone, 12% (n=7) had a diagnosis of both ASD and ID, 18% (n=10) had a diagnosis of both ASD and GDD, and 2% (n=1) had a diagnosis of ASD, ID, and GDD. Diagnoses of the 58 subjects can be seen in Figure 2.
Figure 2: Diagnoses of Subjects Who Completed *PTEN* Genetic Testing

Five of the 58 subjects (9%) had documented dermatological findings in their chart. Of those 5 subjects, 5% (n=3) had documentation of café-au-lait spots, 2% (n=1) had a documented Mongolian spot, and 2% (n=1) had documented acanthosis nigricans. None of the 45 males who completed *PTEN* genetic testing, were documented as having penile freckling. Chart documented family history revealed that of the subjects who completed *PTEN* genetic testing, 12% (n=7) had a documented family history of ASD, 34% (n=20) had a family history of ID and/or GDD, and 24% (n=14) had a family history of macrocephaly. Additionally, 12% (n=7) had a documented family history of a PHTS related cancer and 17% (n=10) had unspecified thyroid problems documented in the
family history. The dermatologic and family history of these subjects is summarized in Table 2.

<table>
<thead>
<tr>
<th>Dermatological Findings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Penile Freckling</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Café-au-lait Spots</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Acanthosis Nigricans</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Mongolian Spot</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family History</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>ID/GDD</td>
<td>20 (34%)</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>14 (26%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Thyroid Problems</td>
<td>10 (17%)</td>
</tr>
</tbody>
</table>

Table 2: Dermatological and Family History Findings of the 58 Subjects Who Completed PTEN Genetic Testing

As noted in the methodology, these features were also assessed for those subjects who had PTEN genetic testing ordered but not completed in order to check for possible sources of testing biases. Of the 108 subjects eligible for study, 46% (n=50) had PTEN
genetic testing ordered but not completed: subjects had either no results at time of chart review (n=1), testing was cancelled (n=4), or the subject had not had blood drawn for analysis (n=45). Additional characteristics of these 50 subjects can be found in Table 3.

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39 (78%)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Macrocephaly Z-score (Standard Deviation)</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>31 (62%)</td>
</tr>
<tr>
<td>3-4</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>4-5</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>5-6</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>6-7</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Table 3: Characteristics of Subjects Who Did Not Complete PTEN Genetic Testing

Among the subjects who did not complete PTEN genetic testing, 78% (n=39) had a diagnosis of ASD alone, 12% (n=6) had a diagnosis of ASD and ID, and 10% (n=5) had a diagnosis of ASD and GDD. Two subjects (4%) had documented dermatological findings. Of those 2 subjects, one had a documented café-au-lait spot and the other
subject had documented acanthosis nigricans. None of the 39 males who did not complete \textit{PTEN} genetic testing, were documented as having penile freckling. Twenty-eight percent (n=14) had a family history of ASD, 22\% (n=11) had a family history of ID and/or GDD, and 16\% (n=8) had a family history of macrocephaly. Additionally, 10\% (n=6) had a family history of a PHTS related cancer and 20\% (n=10) had a family history of unspecified thyroid problems.
We report on a cohort of 58 subjects diagnosed with ASD, ID, and/or GDD in the presence of macrocephaly for which PTEN genetic testing was completed. We found that, of those who completed testing, none (0/58; 0%) were positive for pathogenic variants in the PTEN gene. This data suggests that the frequency of PTEN pathogenic variants in a cohort of subjects with a diagnosis of ASD, ID, and/or GDD, in the presence of macrocephaly, is less than the previously reported estimates of approximately 10% (1.7%-17%).\textsuperscript{12,13,34} In contrast to these studies, we did not find any PTEN pathogenic variants in subjects with a diagnosis of ASD, ID, and/or GDD in the presence of macrocephaly. It is important to note, that the methods used to obtain our subject population, differed from those in prior studies. For example, Butler and colleagues accrued subjects with only a diagnosis of ASD and/or PDD-NOS in the presence of macrocephaly, whereas our study population included subjects with ASD, as well as those with a diagnosis of ID and/or GDD in the presence of macrocephaly.\textsuperscript{12} Our subject population also differed with regards to the clinic in which they were evaluated. Our subjects were referred for evaluation due to concerns for ASD, ID, and/or GDD only. This differed from Butler, Buxbaum, and Varga and colleagues as these subjects were
evaluated by a geneticist in a genetics clinic and may have had medical and/or family histories that were more suggestive of PHTS. In addition, we defined macrocephaly as an OFC ≥2.0 standard deviations above the mean which is in contrast with their definition of macrocephaly as an OFC>2.5 standard deviations above the mean. While Buxbaum and colleagues, definition of macrocephaly was identical to ours, our study methods differed in that our subject population included those with a diagnosis of ASD, ID, and/or GDD whereas their study only included subjects with ASD in the presence of macrocephaly. In addition, both of these groups accrued subjects prospectively and ordered PTEN genetic testing for all accrued subjects based on study design, where as we retrospectively examined subjects with a diagnosis of ASD, ID, and/or GDD in the presence of macrocephaly and assessed those who had PTEN genetic testing ordered by a provider and completed testing. Our methods also differ from the study done by Varga and colleagues who accrued subjects who had completed PTEN genetic testing through their institutional protocol and retrospectively assessed for the presence of ASD and/or ID/GDD along with macrocephaly. While the definition of macrocephaly in this cohort was identical to ours, Varga and colleagues did acknowledge a possible ascertainment bias, as their cohort included subjects for whom there was a high index of suspicion for PTEN pathogenic variants.

Our data also did not show an increase in the frequency of PTEN pathogenic variants in subjects who had macrocephaly greater than 4.0 (OFC>4.0) standard deviations above the mean. Previous studies such as, Butler and colleagues found that 33% (n=6) had
OFCs greater than 4.0 standard deviations above the mean. Of those 6 subjects, 50% (n=3) had pathogenic variants in the PTEN gene. Similarly, in our cohort of 58 subjects who completed PTEN genetic testing, 10% (n=6) had OFCs greater than 4.0 standard deviations above the mean. However, unlike Butler and colleagues, we did not identify any PTEN pathogenic variants in this group. In addition, the frequency of macrocephaly within our subject population was approximately 7% (108/1,649). While this differs from the reported prevalence of 20% by Fombonne and colleagues, their study population consisted of 109 subjects whereas our study population consisted of 1,649 subjects. This may suggest that the frequency of macrocephaly within this subject population is lower than previously estimated. Future studies are needed to assess if there is a correlation between the degree of macrocephaly and positive PTEN genetic testing results as well as the possible predictive value this may hold for subjects and clinicians.

Within our cohort, only 5 subjects (9%) had documented dermatological findings and none of these subjects were positive for pathogenic variants in the PTEN gene. In addition, none of the male subjects within our cohort had penile freckling documented in their electronic medical record. However, it is unclear if providers uniformly assessed dermatological findings across all subjects with PTEN genetic testing ordered. Documented family history, taken by either a genetic counselor or a non-genetics clinician, also demonstrated that, among subjects with a positive family history of ASD, ID, GDD, macrocephaly, cancer, and/or thyroid issues, PTEN genetic testing results were negative. These results indicate that those subjects, for whom personal and family
histories were more suggestive of PHTS, also tested negative for pathogenic variants in the *PTEN* gene. Future studies with particular focus on the documentation and uniform assessment of dermatologic findings and family history may provide better insight into the possible contribution these factors have on positive *PTEN* predicative value.

As noted in the methodology, the features of those 50 subjects who did not complete *PTEN* genetic testing was assessed to determine whether there was any testing bias between this group and the 58 subjects who completed testing. The results indicated that there was minimal difference between these subject groups. The male to female ratio of 3:1, although slightly below the 4:1 prevalence typically seen in the ASD population, remained the same across both groups. Although the number of subjects with macrocephaly between 2-3 standard deviations differed slightly between groups (40 completed testing and 31 did not), macrocephaly between 3-7 standard deviations was roughly the same (18 completed testing and 19 did not). In addition, family history of PHTS related cancers also showed little difference (7 completed testing and 6 did not). The largest difference between those who completed testing and those who did not complete testing was family history. Fourteen subjects who did not complete testing had a family history of ASD whereas only 7 subjects who completed testing had a family history of ASD. In contrast, family history of ID/GDD and macrocephaly, both individually and in summation, was greater in those individuals who completed testing in comparison to those who did not (46 completed testing and 24 did not). While the difference in a small subset of documented family history and degree of macrocephaly
may represent a small testing bias, the lack of significant difference in other facets between our populations who completed and did not complete PTEN genetic testing, we believe, reflects a low testing bias.

Study Limitations

A limitation to this study is the number of subjects who had PTEN genetic testing ordered, but did not complete testing. There may be PTEN pathogenic variants in the untested subject population, which may have contributed to the reason we did not find any pathogenic variants in our cohort. However, as noted above, there was no evidence of testing bias between the tested and untested cohorts. Another limitation was the retrospective chart review design of the study, causing the need to rely on documented physical exams to assess for physical features of PHTS. It is possible that some features a subject may have had may not have been accurately recorded and that some physical features seen in PHTS was not expressly assessed. Many features of PHTS are also age depended and may not be present in young children. It is also unclear as to whether specific dermatological findings, such penile freckling, were assessed uniformly across all subjects. This could be said for the family history documentation as well. It is also possible that subjects with phenotypic features or family histories suggestive of PHTS did not present to IDA clinic and were instead directly referred for assessment by a pediatric geneticist. Thus, they were not included in this cohort, reducing the number of subjects with possible pathogenic PTEN variants in our study.
Conclusion

Our data suggest the frequency of $PTEN$ pathogenic variants among subjects with ASD, ID, and/or GDD in the presence of macrocephaly is lower than the estimated frequency of 10% previously reported in the literature. Future studies are needed to better delineate the frequency of pathogenic variants in the $PTEN$ gene in these individuals and whether medical and family histories suggestive of PHTS contribute to a higher detection rate. In addition, future studies are needed to assess possible correlations between the degree of macrocephaly and positive $PTEN$ predictive value as this may impact testing recommendations. The results of this study may have clinical implications, particularly for genetic testing and counseling along with counseling regarding the benefits/limitations of testing in addition to the possible results of genetic testing.
References


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