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Macrocephaly as a Clinical Indicator of Genetic Subtypes in Autism

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Abstract

An association between autism and large head size has been previously described. Historically a subset of these cases have been correlated with mutations in the gene phosphatase and tensin homolog (PTEN). However, for the majority of cases the etiology is not known. We have studied 33 patients with autism and large head size. Within this group, we confirm the association of PTEN mutations and extreme head size and identify mutations in 22% of cases, including three novel PTEN mutations. In addition we define three novel phenotypic subgroups: (1) cases associated with somatic overgrowth (2) those with disproportionate macrocephaly and (3) those with relative macrocephaly. Members of these subgroups lack changes in the PTEN gene and furthermore we report two novel copy number changes in these patients.

An association between autism and macrocephaly has been previously described. A subset of cases with extreme macrocephaly (>3SD, 99.7th %ile) have been correlated to mutations in the gene phosphatase and tensin homolog (PTEN). However, the phenotypic and genetic characterization of the remaining cases remains unclear. We report the phenotypic classification and genetic testing evaluation of a cohort of 33 patients with autism and macrocephaly. Within our cohort, we confirm the association of PTEN mutations and extreme macrocephaly (> 3SD, 99.7th %ile) and identify mutations in 22% of cases, including three novel PTEN mutations. In addition we define three phenotypic subgroups: (1) those cases associated with somatic overgrowth, (2) those with disproportionate macrocephaly, and (3) those with relative macrocephaly. We have devised a novel way to segregate patients into these subgroups that will aid in the stratification of autism macrocephaly cases. Within these subgroups, we further expand the genetic etiologies for autism cases with macrocephaly by describing two novel suspected pathogenic Copy Number Variants (CNVs) located at 6q23.2 and 10q24.32. These findings demonstrate the phenotypic heterogeneity of autism cases associated with macrocephaly and their genetic etiologies. The clinical yield from PTEN mutation analysis is 22% and 9% from Chromosomal Microarray (CMA) testing within this cohort. The identification of three distinct phenotypic subgroups within macrocephaly autism patients may allow for the identification of their respective distinct genetic etiologies which to date have remained elusive.

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Keywords

Autism; Macrocephaly; PTEN; Overgrowth; Hypotonia

INTRODUCTION

Autism was first reported by Kanner, in 1943 [Kanner 1943], as a developmental disorder in which patients present with severely affected expressive and receptive language skills, stereotypical behaviors, and diminished social interactive skills [Kanner 1943] [Kanner 1943]. The incidence of this condition appears to be as high as 1:100 children. Its etiology is unclear but a number of studies have demonstrated strong support for a genetic basis for the pathogenesis of autism [Bruining and others 2010; Iurov and others 2010], both from twin and familial studies [Muhle and others 2004; Spence 2004]. In addition, there are numerous single gene disorders (Fragile X, OMIM: 300624; Bannayan–Riley–Ruvalcaba, OMIM: 153480; and Rett Syndrome, OMIM: 312750) and chromosome abnormalities (for example, 15q and 1q21.1 duplications) that are associated with autistic behaviors. A few single genes have been identified that cause autism, including CNTNAP2 [Whitehouse and others 2011] and Neuroligins [Etherton and others 2011]. Cumulatively these genetic etiologies account for less than 10% of cases [Brunetti-Pierri and others 2008], and the exact cause of the remaining 90% remains elusive.

There has been a long-standing association between macrocephaly and autism that was first reported by Kanner [Kanner 1943][Kanner 1943; Kanner 1968], and this has been confirmed by many studies (Courchesne, 2004; Courchesne and others 2003). Interestingly, cases of extreme macrocephaly (>3SD) have been correlated to mutations in the gene phosphatase and tensin homolog (PTEN) [Brunetti-Pierri and others 2008; Buxbaum and others 2007; Herman and others 2007a; McBride and others 2010]. This has made PTEN testing the standard of care for patients who present with autism and macrocephaly [Herman and others 2007b]. A number of recent studies have demonstrated the association of copy number variations (CNVs) with autism [Rosenfeld and others 2010]. The high diagnostic yield of chromosomal microarray analysis (CMA) testing has prompted standard practice guidelines from the American College of Medical Genetics (ACMG), which recommends this testing modality as part of an autism evaluation [Manning and Hudgins 2010].

In this study we report the phenotypic classification, PTEN status and genetic testing results from a cohort of 33 cases of autism and macrocephaly and describe novel phenotypic subgroups.

MATERIALS AND METHODS

This study was approved by the David Geffen School of Medicine Institutional Review Board. A retrospective chart review was carried out for all patients evaluated at the UCLA Medical Genetics clinic from 2008-2011 who presented with autism and macrocephaly, defined as a head circumference measurement at least at the 90th %ile (~1.5 standard deviations) above the mean for age or head circumference above 85th%ile with height below the 50th%ile. Somatic overgrowth was defined as a height and weight above the 95th%ile (around 1.8 standard deviations above the mean for age). Disproportionate macrocephaly refers to a height %ile/head circumference %ile ratio of <0.7. Relative macrocephaly refers to a height %ile/head circumference %ile >0.7. All growth parameter percentiles were calculated using the 2010 CDC growth charts [Rollins and others 2010]. Patients were referred to the clinic by a neurologist or a psychiatrist who evaluated the patients based on the DSM IV criteria for the diagnosis, including instruments such as ADOS, PL-ADOS,

CHAT and STAT. Head circumference was obtained by two independent measurements and plotted on standard growth charts by two dysmorphologists. The chromosomal microarray platform was developed by Affymetrix and its performance characteristics determined by UCLA clinical laboratory as required by the CLIA '88 regulations. The assay compared the patient's DNA to 270 HapMap normal controls, using the genome-wide SNP array 6.0. This array platform contains 1.8 million markers for copy number variant detection chosen at ~ 696 bp spacing throughout the human genome. Oligonucleotide probe information is based on the 36 build of the Human Genome (UCSC Genome Browser, hg18, March 2006). Non-diagnostic copy number changes are referenced to the Database of Genomic Variants (<http://projects.tcag.ca/variation>).

RESULTS

We report on a group of 33 patients evaluated in a Medical Genetics Clinic for autism and macrocephaly, their phenotypic findings, and sub-groupings (Table 1)

Confirmed PTEN Mutations

Within our cohort five patients had confirmed PTEN mutations. Careful phenotypic analysis revealed that (4/5) 80% of those positive for mutations in PTEN presented with "extreme macrocephaly" or head circumference that was greater than or equal to 3SD (99.7th %ile) above the mean. This extent of macrocephaly was present in a range of ages, from 31 months to 15 years old. The one patient (Patient 5) whose head circumference was 1-2 SD (92nd %ile) above the mean also carries a novel missense mutation in PTEN, V255A. This mutation is in a well-conserved residue in many species, and is reported to be "possibly damaging" by Polyphen software (PP1). Additionally, patients 1 and 3 presented with novel PTEN mutations, P38H and Y68N. The clinical findings for patient 1 were recurrent otitis media, lipomas, and pigmented glans of the penis. This mutation (P38H) is predicted to be "probably damaging" by Polyphen software (PP1) and also the amino acid shows conservation across numerous species. . Patient 3 presented with a flattened nasal bridge and plagiocephaly. The mutation (Y68N) is predicted to be "possibly damaging" by Poly phen software (PP1) and the amino acid is well conserved . Additional clinical findings of these patients can be found in Figure 1.

Autism with Disproportionate Macrocephaly (ADM)

Among patients with macrocephaly, we identified a novel phenotype consisting of macrocephaly that was disproportionate to height. There were 10 patients with this phenotype; 9/10 (90%) of these patients had normal PTEN testing. Interestingly 8/10 (80%) of the patients in this group presented with hypotonia and 5/10 (50%) of these patients presented with flattened nasal bridges. A complete list of the physical findings of this group can be found in Figure 1. Chromosomal microarray analysis (CMA) revealed a copy number variation (CNV) in one case. Patient 7 had a 767 kb duplication of an interstitial region at the cytogenetic position 6q23.2 and also presented with the clinical findings of bilateral epicanthal folds and a flattened nasal bridge.

Autism with Relative Macrocephaly

In our cohort we identified a group (n=12) with "relative macrocephaly". Of these, 5/12 (42%) presented with bilateral clinodactyly. In comparison to the ADM subgroup only 2/12 (17%) had *de novo* subgroup only 2/12 (17%) had hypotonia and 1/12 (8%) had a flattened nasal bridge. CMA testing revealed a *de novo* 324 kb deletion of 10q24.32 in Patient 26. Additional clinical findings in this patient included flattened nasal bridge and bilateral clinodactyly.

Autism Macrocephaly with Somatic Overgrowth (AMSO)

An additional subgroup of patients presented with somatic overgrowth (n=6) in addition to macrocephaly. The additional physical findings of this group can be found in Figure 1, including normal bone ages.

DISCUSSION

Autism is a complex disease, which has highly variable severity and associated physical findings. The evidence for a genetic basis of the disease is compelling, although the large number of genome-wide association studies (GWAS) performed points to many genetic alterations in non-overlapping associated regions of the genome [Hu and others 2011]. While this is suggestive of a multifactorial etiology, it is also likely that lumping of all autism cases confounds what may be a heterogeneous group of phenotypes and clouds the ability to demonstrate true causality. Approaches at identifying “endophenotypes” offer the ability to parse out possible distinct genetic etiologies and enhance genotype-phenotype correlations.

We have described a cohort of patients with autism and macrocephaly associated with PTEN mutations, some previously unreported which add to those already described in the literature. All but one of our cases with documented PTEN mutations exhibited “Extreme Macrocephaly,” defined as 3SD (>99.7 %ile) above the mean. Interestingly, we identified a patient with a PTEN mutation and a head circumference that is between 1-2SD (92nd %ile) above the mean (Patient 5). This extent of macrocephaly is less than what has been reported previously in cases of PTEN-associated macrocephaly autism syndrome [Butler and others 2005; McBride and others 2010; Mester and others 2011]. In that case, the mutation identified, V255A, is novel and may reflect a hypomorphic allele, which manifests with a lesser degree of macrocephaly. However further causative studies are needed to confirm that this mutation is in fact pathogenic. We identified two additional novel PTEN mutations, P38H (Patient 1) and Y68N (Patient 3) that presented with unique clinical findings including brachycephaly, nevi, and plagiocephaly (Figure 1). The pigmented macules on the glans of the penis along with reported lipomas suggest that patient 1 has Bannayan-Riley-Ruvalcaba-Syndrome (OMIM 15348) [Hobert and Eng 2009].

We report a clinical yield of 22% (5/23) for PTEN testing in this cohort, which is larger than the previously reported value of 10% reported by Herman et al in 2007, perhaps due to our small sample size. We suggest that disproportionate macrocephaly may very well have a genetic etiology different from PTEN, which is evidenced by the lack of PTEN mutations in that cohort. Future genome analysis of this group may uncover a common genetics etiology which would allow for a clinical determinant of the need for PTEN testing.

In our analysis we found a group (N=6) with not only macrocephaly but also generalized somatic overgrowth. We contend that this represents a distinct subgroup of macrocephaly autism cases. This subgroup will be a cohort amenable for future genomic studies that may contribute to the understanding of autism as well as the etiology of somatic overgrowth. Autistic findings have been described in overgrowth syndromes [Cohen 2003; Kent and others 2008], and this subgroup of macrocephaly autism cases with somatic overgrowth may share genetic defects in a common signaling pathway related to PTEN.

In our remaining patients without somatic overgrowth we have herein used a novel clinical calculation to separate disproportionate from relative macrocephaly. We contest that the Autism Disproportionate Macrocephaly (ADM) Subgroup is a distinct cohort whose genetic etiology for autism and/or macrocephaly may be different from the PTEN-positive and somatic overgrowth subtypes. In particular, hypotonia (90%) and a flattened nasal bridge

(50%) are the most common clinical findings that distinguish this group and may, in the future, help to further subdivide these cases into subphenotypes. Recent reports have identified gene mutations that can be associated with autism and disproportionate macrocephaly, including the small GTPase RAB39B [Giannandrea and others 2010], and GlialCAM [Lopez-Hernandez and others 2011]; mild mutations in these genes may be associated with this subset of patients who present with autism-macrocephaly in the absence of somatic overgrowth and mutations in PTEN.

We stress the importance of CMA testing in all cases of macrocephaly autism. A significant number of chromosomal CNVs, both duplications and deletions, have been associated with autistic manifestations [Vorstman and others 2006]. Two separate reports have previously demonstrated that both duplications and deletions can result in autism and macrocephaly [Brunetti-Pierri and others 2008; Moreno-De-Luca and others 2010; Naqvi and others 2000]. We have observed two CNVs in our cohort: a novel duplication (767kB) at 6q23, and an additional *de novo* variant deletion located at 10q24.32 similar to a previously reported overlapping deletion at 104251636-104668797 (genome assembly hg18). This patient presented with severe mental retardation, autistic behavior, hypotonia, macrocephaly, cerebellar vermis agenesis and partial sight [Jaillard and others 2010]. The duplicated genomic region at 6q23 (132,777,055-133,543,604) was reported as a “copy number change of unknown significance” however it contains over 10 genes some of which have roles in neurological development. A search of copy number variants did not identify any report of this duplication in the general population, indicating that it is clinically relevant.

We report a 22% yield for PTEN testing, a 7% yield with CMA, and 0% with fragile X in cases with macrocephaly. These yields are significantly higher than previously reported which may be due to the small sample size of this study, [Herman and others 2007b; Shen and others 2010] suggesting that the use of head circumference as a clinical indicator will increase overall yield in identifying a genetic etiology.

We contend that there is a subgroup of patients with autism and macrocephaly who do not have PTEN mutations and that represent a distinct subgroup of phenotypes amenable for gene association studies. Furthermore, there is a cohort of these patients who present with somatic overgrowth (AMSO), which may represent a distinct genetic etiology. Clinical features, such as macrocephaly, that are associated with autism, allow for subgroups to be established and facilitate genetic association studies, significantly enhancing testing yield.

In conclusion, we report on a group of patients with autism and macrocephaly. We recommend that two distinct subgroups be recognized: (1) autism with disproportionate macrocephaly (ADM), and (2) autism macrocephaly with somatic overgrowth (AMSO), both distinct from Autism with relative macrocephaly (ARM) and can be segregated based on clinical measurements. These will be amenable for genetic characterization in future studies.

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Phenotypic Findings with PTEN Mutations	Phenotypic Findings with ADM	Phenotypic Findings with ARM	Phenotypic Findings with AMSO
Pigmented glands of penis Recurrent otitis media Lipomas Plagiocephaly High broad forehead Nevus Torticollis Flat nasal bridge Brachycephaly Colon Polyps Micrognathia Pectus Excavatum	Malar hypoplasia Epicanthal Folds Two Hair Whorls Coarse Facial Features Single Palmar Crease Hemangioma Pes Planus Prominent Finger Pads Café au lait spots Advanced bone age Cupped Pinna Hemangioma Abnormal Gait	Malar hypoplasia Epicanthal Folds Advanced Bone Age Two Hair Whorls Everted Lower Eyelids Strabismus Crowded Dentition Stiff Gait Café-au lait spot Bilateral 2,3 Toe Syndactyly Pectus Excavatum Macroducty	Hemangiomas Palatal pits Café-au lait spot Superumerary Nipple Hypotonia Brachycephaly Ligamentous Laxity Ear Clefing Pes Planus Tremor Down-slanting Palpebral Fissures Abnormal pinnae Celiac Disease

Figure 1.
Phenotypic features of individuals with Autism Macrocephaly subtypes

Table One

Phenotypic features of individuals with Autism and Macrocephaly

Individuals with confirmed PTEN Mutations					ADM (Autism with disproportionate Macrocephaly)										
#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gender	M	M	M	M	M	M	M	M	M	F	M	M	F	M	M
Age	15y	3y	4y	2 y 6m	4y 4m	18y 11m	17y	5y 4m	3y	3y 2m	6y 11m	17y 6m	7y	2y 8m	2y 9m
Height cm (%ile)	169 (50)	102 (>95)	125.6 (>95)	99 (>95)	106.8 (75)	158 (<5)	168.3 (15)	106.5 (20)	92.5 (25)	91.8 (25)	117.5 (25)	174.5 (48)	123 (53)	92 (50)	95 (60)
Weight kg (%ile)	62.6 (74)	17.4 (95)	24.8 (>95)	13.2 (50)	18.7 (80)	91.8 (93)	73.6 (76)	17.3 (25)	14.3 (50)	14.2 (50)	35.8 (>95)	61.9 (30)	26.5 (80)	14.5 (73)	16.6 (95)
HC cm (%ile)	58.5 (>99.7)	56 (>99.7)	56 (>99.7)	55.5 (>99.7)	53 (92)	62.5 (>99.7)	57.3 (85)	53 (87)	53 (>97)	51 (92)	53.5 (85)	58 (91)	54 (>97)	51 (85)	53 (>97)
HC SD	> 3	> 3	> 3	> 3	1-3	>3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3
HT/HC %ile	.5	.98	.98	.96	.82	.05	.18	.23	.25	.27	.29	.53	.55	.59	.62
Hypotonia	-	+	-	-	-	+	+	+	+	+	+	-	+	-	+
Flat Nasal Bridge	-	-	-	-	-	+	+	+	-	-	+	-	+	-	-
Clinodactyly	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
CMA	?	?	?	?	-	?	dup6q23.2	-	-	-	-	-	-	-	?
PTEN	P38H	R130X	Y68N	R130L	V255A	-	-	-	-	-	-	-	-	-	?

ARM (Autism with Relative Macrocephaly)										AMSO (Autism Macrocephaly with Somatic Overgrowth)									
#	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
Gender	F	F	M	F	M	F	M	M	M	M	M	M	M	F	M	M	M	M	
Age	7y 10m	2y 7m	3y 1m	4y 7m	3y 10m	2y 7m	7y	3y 8m	4y 8m	6y	1y 6m	3y 2m	3y	3y 6m	14y 4m	3y 1m	4y 1m	6y 11m	
Height cm (%ile)	129 (74)	94 (75)	98 (73)	107.5 (74)	104 (76)	94.6 (85)	126.7 (80)	104.5 (89)	112 (89)	120 (80)	87.5 (95)	102 (93)	113 (>95)	109.8 (>95)	181.3 (>95)	104.8 (>95)	116.7 (>95)	140.3 (>95)	
Weight kg (%ile)	32.3 (91)	17.6 (>95)	16.8 (91)	17.5 (50)	17.2 (80)	17.5 (>95)	24.9 (74)	18.4 (92)	21 (90)	23.3 (80)	13.1 (80)	17.1 (90)	35 (>95)	20.3 (>95)	70.3 (92)	19 (>95)	22.2 (>95)	56.7 (>95)	
HC cm (%ile)	55.5 (>97)	53 (>97)	52 (93)	52.5 (95)	53 (96)	55.5 (>99.7)	54 (90)	54 (>97)	54 (>97)	53.5 (90)	52.5 (>99.7)	52.5 (96)	55 (>99.7)	52 (>97)	57.5 (96)	53 (>97)	54.5 (>97)	56 (>97)	
HC 1-3SD	+	+	+	+	+	>3SD	+	+	+	+	>3SD	+	>3SD	+	+	+	+	+	
HT/HC %ile	.76	.77	.79	.79	.79	.85	.89	.91	.91	.89	.95	.97	.98	.98	.98	.98	.98	.98	

ARM (Autism with Relative Macrocephaly)												AMSO (Autism Macrocephaly with Somatic Overgrowth)						
#	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Gender	F	F	M	F	M	F	M	M	M	M	M	M	M	F	M	M	M	M
Age	7y 10m	2y 7m	3y 1m	4y 7m	3y 10m	2y 7m	7y	3y 8m	4y 8m	6y	1y 6m	3y 2m	3y	3y 6m	14y 4m	3y 1m	4y 1m	6y 11m
Hypotonia	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	+	—	—
Flat Nasal Bridge	+	—	—	+	—	—	—	—	—	—	—	—	+	—	—	—	+	—
Clinodactyly	—	—	—	—	+	—	—	+	—	+	+	+	+	—	—	—	—	—
CMA	?	—	?	—	—	?	—	—	—	?	del10q24.32	—	?	—	—	—	—	—
PTEN	—	?	?	?	—	?	—	—	?	?	?	?	?	—	?	?	—	—

CMA: Chromosomal Microarray; HC: Head Circumference; HT: Height; %ile: percentile; ?: unknown; +: feature present; - : normal or not present; SD: standard deviation

Table 2

CNVs in patients with macrocephaly autism

Study	CNVs	Subgroup	Clinical Findings	Parental Origin
Klein, 2012	Duplication of 6q23.2 (132777055- 133543604)	Autism Disproportionate Macrocephaly (ADM)	Bilateral epicanthal folds Flattened nasal bridge Strabismus Hyperpigmented lesion	Unknown
Klein, 2012	Deletion of 10q24.32 (104345460- 104670581)	Autism Relative Macrocephaly (ARM)	Flattened nasal bridge Clinodactyly of 4 th and 5 th finger and 5 th toe bilaterally Plagiocephaly	<i>De Novo</i>