

**PHENOTYPICAL DESCRIPTION OF A SUBSET OF INDIVIDUALS WITH *PTEN*
GERMLINE MUTATIONS, AUTISM SPECTRUM DISORDER AND MACROCEPHALY**

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Abstract

Introduction. Autism Spectrum Disorder is a challenging neurodevelopmental disorder, with a profound multifactorial origin and a complex inheritance. Studying proven susceptibility genes and working on clinical endophenotypes are needed to define more valid genotype-phenotype correlations. *PTEN* is a tumour suppressor gene located on chromosome 10q23.3. Inactivation of *PTEN* results in upregulation of the PI3K/AKT signalling pathway, which affects multiple cellular processes. Besides, *PTEN* has an essential role in brain development, and thus in normal social behaviour, where tight control of the PI3K/AKT/mTOR pathway is of great importance. Multiple studies have emphasized a linkage between *PTEN* mutations and children presenting macrocephaly together with ASD, intellectual disability or neurodevelopmental delay. For that reason, *PTEN* mutation testing is a major consideration in cases of ASD and/or neurodevelopmental delay with macrocephaly. In the present study we aim to report a subset of three individuals with a *PTEN* germline mutation and both ASD and macrocephaly.

Patients and Methods. We report a sample of three caucasian patients, with ASD and macrocephaly ranging from +3SD to +4SD. ASD was diagnosed based on a positive score for both ADI-R and ADOS, and fulfilment of DSM-5 criteria. Furthermore, all underwent an extensive clinical evaluation, including an intellectual and functional evaluations with Griffiths Mental Development Scale and Vineland Adaptive Behaviour Scale. Besides *PTEN* molecular analysis, laboratory tests to rule out medical causes were performed.

Results. Our findings revealed significant phenotypical heterogeneity. Three *PTEN* mutations were found: a missense variant c.737C>T (p.Pro246Leu) heterozygous in exon 7, a *de novo* duplication in exon 6 c.493-?_634+?(2), and a missense mutation c.359C>A (p.Ala120Glu) heterozygous in exon 5. MRI showed several abnormalities.

Discussion. We encountered a *de novo* duplication in exon 6, which to our knowledge has never been described in ASD or PHTS patients. Clinical evidence strongly points to the pathogenicity of this variant. Our results reinforce the multifactorial complexity of ASD, and the necessity to identify biological markers and specific endophenotypes. MRI can also be a helpful instrument in the investigation of these patients. A multidisciplinary cancer surveillance regimen extended to adulthood is of great importance in all cases of *PTEN* mutation. Additional research in ASD patients with known *PTEN* mutation aetiology is necessary to disclose the full potential of target therapeutics in this neurobehavioural syndrome.

Conclusion. This case report is concordant with contemporaneous investigation on the aetiology of neurodevelopmental disorders and adds a *de novo* mutation with clinical value, not yet described.

Keywords

PTEN mutation, PTEN Phosphatase; Macrocephaly; Autism Spectrum Disorder; Genotype-phenotype correlation; Missense Mutation; Genomic Segmental Duplication.

Abbreviations

ADI-R – Autism Diagnostic Interview-Revised

ADOS – Autism Diagnostic Observation Schedule

AKT – Protein Kinase B

ASD – Autism Spectrum Disorder

DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

DQ – Developmental quotient

EEG - Electroencephalogram

MAPK – Mitogen Activated Protein Kinases

MRI – Magnetic Resonance Imaging

mTORC1 – Mammalian Target of Rapamycin Complex 1

PI3K - Phosphatidylinositol-3-kinase

PIP3 - Phosphatidylinositol-(3,4,5)-triphosphate

PHTS – PTEN Hamartoma Tumour Syndromes

PTEN - Phosphatase and Tensin homologue deleted on chromosome Ten

SD – Standard Deviation

TKI – mTOR kinase inhibitor

TPdI – mTOR/PI3K dual inhibitor

TSC – Tuberous Sclerosis Complex

VABS – Vineland Adaptive Behaviour Scale

Introduction

Autism spectrum disorder (ASD) is a challenging neurodevelopmental disorder, with an estimated prevalence of 10 per 10 000 children in Portugal.¹ Currently the diagnosis of ASD is purely based on clinical features,² comprising persistent impairment in reciprocal social communication and interaction, and restricted, repetitive patterns of behaviour, interests, or activities, typically present from early childhood.³

Given the profound multifactorial origin and complex inheritance of ASD,⁴ studying proven susceptibility genes and working on clinical endophenotypes might help to define more specific genotype-phenotype correlations.⁵ Therefore, efforts must be made to identify biological markers that can able clinicians to reach earlier clinical and aetiological diagnosis, as well as to predict clinical prognosis and treatment response.⁶

Phosphatase and Tensin homologue deleted on chromosome Ten (PTEN) was first identified as a putative tumour suppressor gene in 1997.⁷ Cytogenetically located on chromosome 10q23.3, it encodes a 403-amino-acid protein containing an amino-terminal region with a dual-specificity phosphatase domain (Figure 1).⁸ *PTEN* gene is ubiquitously expressed in the human body and displays great complexity in both its functions and regulation.⁹

Biochemical studies disclosed that *PTEN* acts antagonistically to phosphatidylinositol-3-kinase (PI3K), dephosphorylating the second messenger phosphatidylinositol-(3,4,5)-triphosphate (PIP3), thus functioning as a negative regulator of the PI3K/Protein kinase B (AKT) signalling pathway (Figure 2).^{8,10-12} Consequently, inactivation of *PTEN* leads to constitutive and unregulated activation of AKT,¹³ which affects multiple cellular processes, including cell growth, survival, and proliferation, as well as cell metabolism, polarity and movement.^{9,11,12,14}

A. *PTEN*



B. *PTEN*

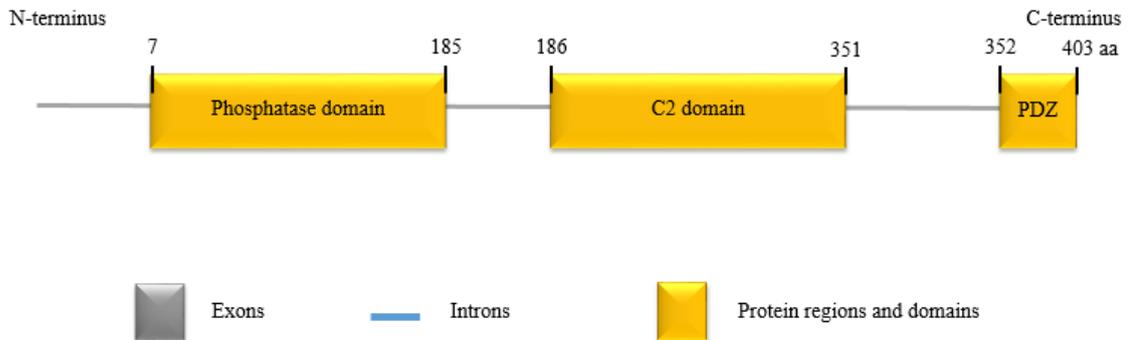


Figure 1. *PTEN*: gene (A) and protein (B) structure. *PTEN* gene is formed by 9 exons which encode a ubiquitously expressed tumour suppressor dual-specificity phosphatase.⁸ ATG is the start codon in the *PTEN* genomic sequence. The *PTEN* structure reveals two main domains. The phosphatase domain (aa 7-185) in the N-terminal region, where *PTEN*'s active site that dephosphorylates lipid substrates is located. The C2 domain (aa 186-351) in the C-terminal region binds phospholipids and positions the catalytic domain on the membrane, being important for *PTEN* stability and enzymatic activity.⁹

Besides, *PTEN* protein has also PI3K pathway independent functions, namely PIP3-independent mechanisms to inhibit cell migration and to regulate genomic stability, and takes part in the regulation of the mitogen-activated protein kinase (MAPK) signalling pathways.^{10,14}

Mutations coursing with loss of *PTEN* phosphatase functions are then responsible for several disturbances in cell and organism physiology, typically linked to increases in cell growth and proliferation.⁹ Clinically *PTEN* loss has been identified as a driven event in the development of many sporadic cancers, associated with a lifetime cancer risk of over 80%.⁹ Female breast cancer, thyroid, kidney and endometrial cancers are some of the most frequent malignancies described.^{9,14,15}

Although *PTEN* gene is better known for its tumour suppressor functions, its action is not limited to that field, playing also an important role in brain development.¹¹ *PTEN*'s functions in the brain are not only cell-type specific, but also within the same cell type *PTEN* can regulate multiple aspects of cell activity. In addition, it is an important regulator of neural connectivity and plasticity.¹⁶ Consequently, reducing *PTEN* function in neurons has profound effects on neuronal morphology and circuitries^{11,12} which ultimately may lead to a series of neurological disorders, including macrocephaly, epilepsy, intellectual disability, global developmental delay, and ASD.¹²

The major PI3K/AKT pathway downstream effector mediating the behavioural changes observed in *PTEN* mutants is the TSC/mTORC1 pathway (Figure 2).¹² It has indeed been described that a tight regulation of the PI3K/AKT/mTOR pathway is essential for normal social behaviour.¹⁷

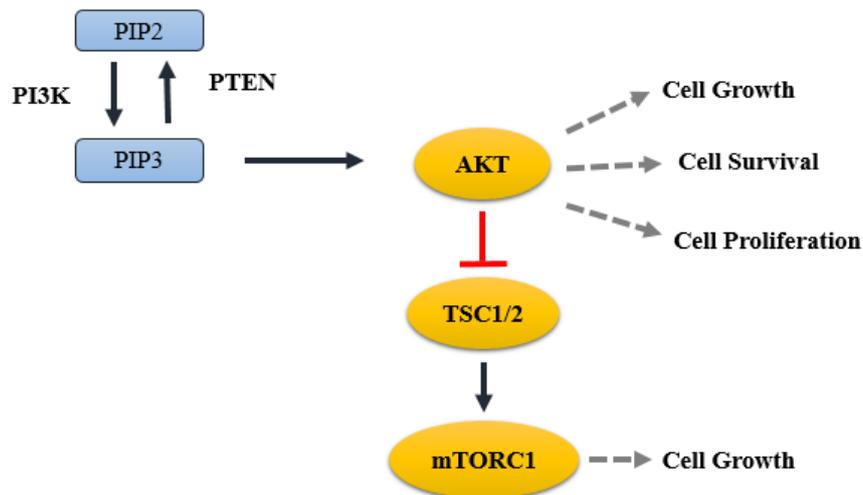


Figure 2. PTEN impact in the PI3K/AKT/mTOR pathway.⁸⁻¹²

Multiple lines of epidemiologic evidence emphasize a linkage between *PTEN* gene mutations and children presenting macrocephaly together with ASD, intellectual disability or global developmental delay.^{4,15,18}

In fact, *PTEN* has been validated as a susceptibility gene for ASD.¹⁷ *PTEN* germline mutations are estimated to be present in approximately 5% of those tested with ASD and concomitant macrocephaly.¹⁹

Macrocephaly is one of the most consistently encountered endophenotypes amongst ASD, and is estimated to be present in 15,7% of ASD patients according to a systematic review and meta-analysis updated to November 2014.^{6,20}

For that reason, *PTEN* mutation testing is a major consideration in cases of ASD with macrocephaly.^{4,5,12,15,17,18,21,22} Recently, it has been proposed the extending of the indications of *PTEN* mutation screening.²¹⁻²³ Hence, in case of macrocephaly not only coexistence of ASD should motivate *PTEN* investigation, but also existence of other clinical features such as developmental delay, dermatological features (including lipomas, trichillemomas, oral papillomas, and penile freckling), vascular features (arteriovenous malformations or hemangiomas), and gastrointestinal polyps.²¹⁻²³

Strengthening the importance of the subject, in the present study we aim to report a subset of three individuals with a *PTEN* germline mutation and both ASD and macrocephaly.

Patients and Methods

We report three caucasian patients, two male and one female, with current ages between 5 and 13 years old. The subjects were first diagnosed and followed in our Autism and Neurodevelopmental Unit, national reference for autism in a tertiary Paediatric Hospital in the centre region of Portugal.

All subjects presented with ASD and macrocephaly (i.e., occipital-frontal circumference more than 2 standard deviations (SD) above the mean for one's height, gender, and ethnicity) ranging from +3SD to +4SD.

ASD was diagnosed based on a positive score for both *Autism Diagnostic Interview-Revised* (ADI-R)²⁴ and *Autism Diagnostic Observation Schedule* (ADOS),²⁵ and according to the *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* (DSM-5) criteria.³ For a brief tools description see *Supplementary Material* (Appendix A).

Furthermore, ASD was appraised through an extensive clinical evaluation conducted by experienced neurodevelopmental paediatricians working daily in a multidisciplinary team. A comprehensive clinical history was collected, including data from the pre- and perinatal periods. Likewise, in all subjects a physical and neurological examination was conducted. Developmental quotient (DQ) and functional development were evaluated with *Griffiths Mental Development Scale*²⁶ and *Vineland Adaptive Behaviour Scale* (VABS),²⁷ respectively. Cerebral magnetic resonance imaging (MRI) was also carried out. Additionally, an electroencephalogram (EEG) was performed on Patient 2.

Medical conditions such as neurocutaneous syndromes, fragile X syndrome, and acquired conditions were excluded. The laboratory tests used to rule out associated medical conditions encompassed an array-comparative genomic hybridization (array-CGH), fragile X PCR-based testing, and metabolic screening (complete blood count, serum metabolic profile, and serum amino acid and urine screening for glycosaminoglycans), according to standard procedures.¹⁹ At last, *PTEN* screening was performed in all patients and their parents. *PTEN* molecular genetic analysis was implemented on genomic DNA extracted from peripheral blood leukocytes, followed by real-time quantitative multiplex PCR analysis and a sequence analysis of *PTEN* exons.²⁸ Images of analysed sequence data, commonly referred as electropherogram, are represented on Figures 4 and 5.

All patients were subjected to routine cervical and abdominal ultrasounds, within the cancer risk surveillance.

Written informed consent was obtained from all individual participants included in the study.

Patient 1

Patient 1 is a 13-year-old boy, the second child of healthy unrelated parents. The pregnancy was well monitored and held without complications. He was born at 37 weeks gestation with 3650g (50th percentile), 49cm (25th percentile) and a head circumference of 35,5cm (between 25th-50th percentiles).

He was considered a healthy child until the age of 5, when he was referred by his paediatrician to our specialized autism appointment, for significant global developmental delay, with special impairment in speech and social interaction areas.

By then detailed clinical observation showed deficits in social communication and social interaction, mainly manifested by absence of verbal communication, trouble in behaviour adjustment, sudden periods of aggressiveness, difficulties with environmental transitions, and highly preoccupation with tidiness. He had macrocephaly (+3SD) without facial dysmorphisms, and hearing impairment. It was visible an achromatic stripe on the thorax region.

ADI-R (social interaction >10; communication >7; repetitive behaviours >3), ADOS (total communication and social interaction >12), and DSM-5 criteria supported the diagnosis of ASD with accompanying severe intellectual disability. DQ was significantly lower than the mean for his age (56%). VABS suggested a functional age lower than the expected for his age (-3SD), corresponding to the 60th percentile for the population of children with ASD.

By the age of 13 years old he maintained absence of verbal communication, and social impairment was intensified. Regular medication included Risperidone, Chlorpromazine, and recently Paliperidone.

Cerebral MRI showed prominence of the perivascular space in the white matter of both cerebral hemispheres.

The molecular analysis found a pathogenic missense mutation on *PTEN* c.737C>T (p.Pro246Leu) heterozygous in exon 7. The same germline mutation was found on his sister,

father, and grandmother, but none of them exhibited macrocephaly nor other pathological condition. This variant results in the change of an aminoacid Proline to Leucine (CCG>CTG) in exon 7, producing a different protein sequence, but whit length preserved. Electropherogram is displayed on Figure 4.

Cervical ultrasound described a thyroid gland with normal morphology, though a diffuse heterogenic parenchyma with bilateral microcalcifications was found. Abdominal ultrasound showed no alterations.

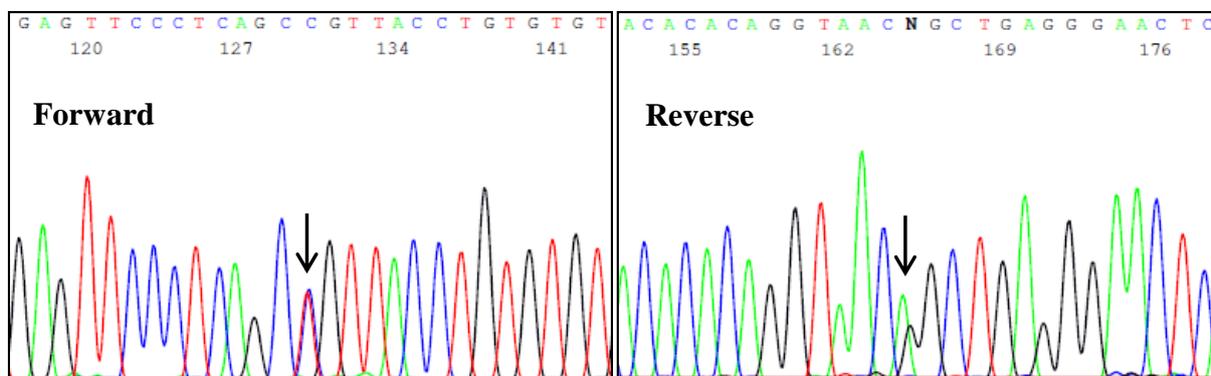


Figure 4. Electropherogram from Patient 1 showing an overlapping peak identifying a putative single base pair mutation on *PTEN* c.737C>T (p.Pro246Leu) heterozygous in exon 7.

Patient 2

Patient 2 is a 5-year-old girl, the second child of a non-consanguine couple. The pregnancy was effectively monitored, and the ultrasounds suggested the diagnosis of prenatal macrosomia and macrocephaly. She was delivered by caesarean section at 38 weeks gestation and had a birth weight of 4195g (90th percentile), height of 52cm (80th percentile), and head circumference of 39cm (over the 97th percentile). Study of prenatal macrocephaly by ultrasound suggested no other anatomic pathological alterations.

Concerning family history, her mother had had an abortion caused by Trisomy 21 and had history of depression controlled with Alprazolam during pregnancy. Her father had no known diseases, but presented with macrocephaly (+2SD). Her older sister had rheumatoid arthritis.

At the age of 3 years old she presented to our unit with suspicion of ASD together with global developmental delay. She exhibited macrosomia, and macrocephaly (+4SD) with craniofacial dysmorphisms: dolichocephalism and protruding forehead. Other features included persistent deficits in social interaction and social communication, manifested by abnormalities in eye contact, unadjusted behaviour, no interest in general tasks, failure to follow simple orders, and lack of verbal communication. Stereotypies using the hands and arms were evident. Further clinical investigation showed frequent blinking, and a clumsy but non-pathologic neurological examination revealing tiptoe walking. There were no other associated medical conditions.

ADI-R (social interaction >10; communication >7; repetitive behaviours >3), ADOS (total communication and social interaction >12), and DSM-5 criteria supported the diagnosis of ASD with accompanying severe global developmental delay. DQ was lower than the mean for her age. VABS suggested a functional age lower than the expected for her age (-3SD).

Cerebral MRI showed no endocranial lesions and a normal myelinisation pattern. It was possible to distinguish a mild enlargement of the frontal-parietal subarachnoid space, with no pathological meaning giving macrocephaly. EEG results were normal.

The genetic investigation discovered a *PTEN* mutation not yet described: a *de novo* duplication c.493-?_634+?(2) in exon 6.

Cervical and abdominal ultrasound did not find any pathologic alterations.

Patient 3

Patient 3 is a 7-year-old boy, the second child of healthy unrelated parents. The pregnancy was adequately monitored and ultrasounds described foetal macrosomia and macrocephaly. He was born with 38 weeks gestation in an assisted delivery, and had a birth weight of 4295g (90th percentile), a height of 52,5cm (75th percentile), and a head circumference of 39,5cm (over the 97th percentile). Imaging investigation of macrocephaly at 3 months of age showed no alterations in MRI. The two parents had macrocephaly.

Healthy until the age of 3, he was then referred to our unit for investigation of speech delay and social impairment. Other clinical features included diminished interest in peers, deficits in understanding and use of gestures, and mild motor stereotypies. He had no cognitive impairment. Besides macrosomia and macrocephaly (+4SD), the patient exhibited several facial dysmorphisms: dolichocephalism, protruding forehead, mild bilateral ptosis, low nose base, and broad nasal bridge with anteverted nostrils. Further clinical examination found hypotonia, and motor tics. Hypochromatic skin patches were visible in the low back region.

ADI-R (social interaction >10; communication >8; repetitive behaviours >3), ADOS (total communication and social interaction >12), and DSM-5 criteria supported the diagnosis of ASD. DQ was not significantly lower than the mean for his age (99%). VABS suggested a functional age lower than the expected for his age (-2SD), corresponding to the 70th percentile for the population of children with ASD.

Cerebral MRI showed a small (3,8 mm diameter) hyperintense focus in the middle portion of the white matter in the right semioval center on T2W and FLAIR images and isointense on T1W, non-specific but suggestive of focal gliosis or hamartoma.

PTEN molecular analysis found a pathogenic missense mutation on *PTEN* c.359C>A (p.Ala120Glu) heterozygous in exon 5. This variant was not found on neither parents nor sister. This missense mutation results in the change of an aminoacid Alanine to Glutamine

(GCA>GAA) in exon 5, producing a different protein sequence, but whit length preserved.

Electropherogram is displayed on Figure 5.

Cervical and abdominal ultrasound showed nodular lesions in the thyroid and pancreatic glands, respectively, without pathological features.

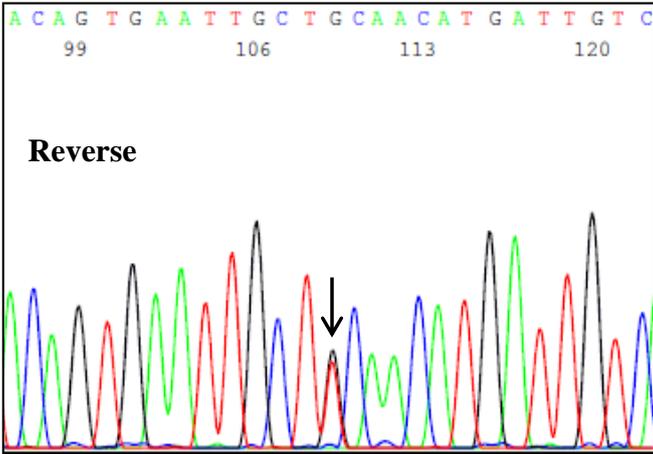


Figure 5. Electropherogram from Patient 3 showing an overlapping peak identifying a putative single base pair mutation on *PTEN* c.359C>A (p.Ala120Glu) heterozygous in exon 5.

Table 1. Main phenotypical characteristics of the subset of patients with *PTEN* mutations.

	Patient 1	Patient 2	Patient 3
Major findings			
Gender	M	F	M
HC at birth (percentile)	25th-50th	>97th	>97th
Reason for referral	GDD	GDD	GDD
Age of diagnosis (years)	5	3	3
Clinical features			
Macrocephaly	+3SD	+4SD	+4SD
Macrosomia	-	+	+
Craniofacial dysmorphism	-	+	+
Skin patches	+	+	-
Hearing impairment	+	+	-
Motor delay	+	+	+
Speech delay	+	+	+
ASD	+	+	+
Neurological examination	normal	clumsy	clumsy
PTEN screening	Inherited missense c.737C>T (p.Pro246Leu), exon 7	De novo duplication c.493-?_634+?(2), exon 6	Missense c.359C>A (p.Ala120Glu), exon 5

Legend: M: male; F: female; HC: head circumference; GDD: global developmental delay; ASD: autism spectrum disorder; SD: standard deviation.

Discussion

After the discovery of *PTEN* as a tumour suppressor gene, studies have been conducted corroborating that its absence is directly related to a great number of cancers. More recently, *PTEN* was proven to also have an important role in brain development⁴ and studies have reliably associated *PTEN* mutations and ASD, more often in the simultaneous presence of macrocephaly.^{4,15,17,19}

Likewise, the present case reports children diagnosed with ASD and having a positive mutation screening for *PTEN*, as well as an increased occipital-frontal circumference.

The germline mutations found are localized on exons 5, 6 and 7, what comes to an agreement with other studies that determined the existence of hotspots in the same exons.^{17,20-22}

Patient 1 inherited a heterozygous mutation of *PTEN* (c.737C>T) in exon 7, identified at codon 246, that had already been observed in individuals with Bannayan-Riley-Ruvalcaba syndrome or who met criteria for Cowden syndrome.^{22,28,29} Our data supports the pathogenicity of the variant, but also suggests that the phenotype may not be predictable on the basis of molecular analysis. No clear phenotype-genotype correlation can be made, since the variant was inherited from his father, which was an asymptomatic carrier, as well as the subject's sister and grandmother. Besides, this reinforces the multifactorial complexity of ASD, which involves multiple genes but is among the most heritable neurodevelopmental disorders, with a concordance rate in monozygotic twins of 90% and siblings with an approximately 50-fold increased risk. Moreover, many of the genetic variants found in ASD show incomplete penetrance and variable phenotypic expression, and only 30% of the cases have an identified aetiology.^{19,30}

In Patient 2 we encountered a *de novo* *PTEN* mutation, a duplication within exon 6, which to our knowledge has never been described in ASD or *PTEN* hamartoma tumour syndromes (PHTS) patients. Clinical evidence strongly points to the pathogenicity of this variant. Further

molecular and functional studies would be necessary to accurately understand the functional implication of this duplication regarding protein sequence and expression levels.

Concerning this topic it should be mentioned that the methodology of *PTEN* molecular screening is currently accomplished by sequencing genomic DNA isolated from the patient's blood samples or saliva, and is mostly limited to the coding sequence, which could mean an underestimation of *PTEN* mutation frequency, as mutations in non-coding regions might also perturb *PTEN* protein levels.¹² There are only a few studies examining the promoter and exon-flanking regions.^{4,13} Similarly, most studies exclude a possible reduction in *PTEN* levels that may occur either by mutational or epigenetic mechanisms somatically within specific brain regions that would not be detectable in peripheral blood sampling.^{11,12} An association of the genome sequencing procedure with a transcriptome sequencing could somehow surpass some of these obstacles, as blood transcriptomic data could reveal functional correlations of genetic variants, including changings in splicing, expression levels, and allelic expression, along with novel candidates with allelic imbalance.³⁰

At last, Patient 3 had a heterozygous missense mutation of *PTEN* (c.359C>A) in exon 5, previously described as pathogenic.²² Exon 5 is considered as a hotspot, which encodes the N-terminal phosphatase catalytic domain, essential for *PTEN*'s correct functioning.²¹

Remarkably, while Patient 2 and 3 had foetal macrocephaly, Patient 1 was born with a head circumference between 25th-50th percentiles, and only later macrocephaly was identified. This corroborates other study that already stated that neonates later developing ASD and macrocephaly apparently display normal occipital-frontal circumferences at birth. That is, during the first year of life head growth rates begin accelerating, continue approximately until 4 years of age, and then again slow down undertaking a premature arrest.⁶

Cerebral MRI can be a helpful instrument in the investigation of these patients. According to a recent study, the existence of multifocal static white matter abnormalities and dilated

perivascular spaces should suggest the possibility of a *PTEN* spectrum disorder in a patient with macrocephaly and global developmental delay and/or ASD.³¹ As to our results, Patient 1 showed prominence of the perivascular space in the white matter of both cerebral hemispheres, which comes to an agreement with the study above.

Noteworthy, all patients have already initiated a cancer risk surveillance, which demonstrated several nodular lesions in the thyroid and pancreatic glands, without accompanying symptoms, but in need of close follow-up.

Further investigation is required to accurately evaluate the cancer risk of children with ASD and *PTEN* mutations, distinguishing it from the risk of those having other PHTS. A consensus cancer surveillance protocol in this situation has not been formally instituted.²¹ Nonetheless, given *PTEN* gene association with increased risk for malignancy, notwithstanding ASD status, identification of a mutation in this gene should prompt the beginning of a multidisciplinary cancer vigilance regimen extended to adulthood.¹⁷ In children, monitoring should be annual, including complete clinical examination, specific dermatologic examination, and psychomotor skills assessment. Also, latest studies recommend a thyroid ultrasound at the time of genetic diagnosis, since thyroid cancer can appear earlier than 10 years old, and it should be repeated annually. In adults, yearly cancer screenings should be expanded to include mammograms and/or breast MRI, as well as endometrial surveillance for women, colonoscopy, and a skin examination. The latter is important because dermatologic features, even if subtle, are highly predictive of a germline *PTEN* mutation, and there is also an increased risk for melanoma. Kidney surveillance begins at the age of 40 years, continuing every other year.^{17,20}

Since a tight regulation of the PI3K/AKT/mTOR signalling pathway is essential for normal development of neurological areas implicated in social behaviour,¹⁷ and given the association of this pathway with ASD, it would be important to investigate target therapeutics with efficacy in this patients. The inhibition of both mTOR complexes with mTOR kinase inhibitors (TKIs),

which concomitantly minimize the feedback activation of PI3K/AKT, have demonstrated a favourable response in some cancers, but not in cases of *PTEN* mutation.³² Furthermore, synergistic inhibition with mTOR/PI3K dual inhibitors (TPdIs) that completely suppress PI3K/AKT signalling pathway is currently on clinical trial, and have demonstrated encouraging results in some cancers.³² Additional research in ASD patients with known *PTEN* mutation aetiology is necessary to disclose the full potential of target therapeutics in this neurodevelopmental syndrome.

Conclusions

This case report is concordant with contemporaneous investigation on the aetiology of neurodevelopmental disorders and adds a *de novo* duplication with clinical value, not yet described.

Our results reinforce the phenotype-genotype heterogeneity and multifactorial complexity of ASD, acknowledging the importance of exploiting susceptibility genes and biological markers so solid clinical and aetiological correlations can be made.

Moreover, we highlight the need for the formal institution of a consensual cancer surveillance protocol, given the increased risk of malignancy from early age associated with *PTEN* mutations.

Larger surveys should also be encouraged to consolidate the role of cerebral MRI in the study of these patients.

At last, target therapeutic to pathways involved in ASD aetiology should instigate intensive research while an increasing number of studies gives clues to the understanding of these signalling pathways and their role in neural or glial development and ASD.

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Appendix A - Supplementary Material

Autism Diagnostic Interview-Revised (ADI-R)²⁴ - By means of a careful interview with the parents, ADI-R assess with good reliability and validity an individual's early childhood and current social-communication development, as well as stereotyped, repetitive behaviours and interests. A positive score for autism is defined as a score above 7 for nonverbal subjects and above 8 for subjects with verbal communication, simultaneously with a score of 10 in the social interaction area and of 3 in the repetitive behaviours and stereotyped patterns area.

Autism Diagnostic Observation Schedule (ADOS)²⁵ - ADOS consists of a semi-structured interactive observation session that includes games, activities and an interview for older-verbal subjects, organized in 4 modules according to age and verbal activity. Results corresponding to the minimum cut-off for ASD for all modules are: Module 1- total cut-off for communication and social interaction = 7; Module 2 – total cut-off for communication and social interaction = 8; Module 3 - total cut-off for communication and social interaction = 7; and Module 4 - total cut-off for communication and social interaction = 7.

Griffiths Mental Development Scale (2-8 years old)²⁶ – Griffiths assesses the mental development of children from 2 to 8 years old. It comprises six subscales: locomotor, personal-social, hearing and speech, eye and hand coordination, performance, and reasoning. This subscales yield standardized scores for each domain and a composite general quotient. For each subscale, a standardized score over 2SD below the mean indicates severe impairment. The evaluation of mental development through appraisal of different developmental areas allows results to demonstrate the relative level of development of each area.

*Vineland Adaptive Behaviour Scale (VABS)*²⁷ - VABS is a standardized, although semi-structured, interview for children with autism that measures their adaptive abilities. It comprises three main domains (communication, daily living skills, and socialization) and two optional domains (motor skills for children under 6 years old and maladaptive behaviour for those over 5 years old). Each domain contains several subdomains that enquire a specific developmental area. VABS total score, the Adaptive Behaviour Composite, is calculated as the sum of the raw scores from the domains measured.