Six at Sixty. Commentary on identification of the *PTEN* gene as a major contributor to autism spectrum disorder

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In 2005, the genetic aetiology of autism was considered elusive. Occasionally individuals with overgrowth disorders and hamartomas including Bannayan-Riley-Ruvalcaba and Proteus or Proteus-like and/or cancer such as Cowden syndrome, an autosomal dominant disorder with a high risk of breast, thyroid and endometrial cancer, were noted to have neuro-behavioural features resembling autism, learning problems and macrocephaly. There was growing evidence that these disorders may have *PTEN* gene mutations.[1] Based on earlier observations of children presenting with overgrowth including macrocephaly, tumours, developmental delay and autistic features in the clinical setting, we undertook *PTEN* gene mutation analysis in 18 subjects with autism spectrum disorders (ASD) and macrocephaly. These comprised 13 males and five females, prospectively ascertained with an age range of 3–18 years and head circumferences of 2.5–8 SD above the mean. Three of the males with the largest head circumferences were found to have novel germline *PTEN* mutations.[2]

The *PTEN* (phosphatase and tensin homolog) gene located on chromosome 10q23.31 acts as a dual-specificity protein phosphatase ubiquitously expressed tumour suppressor that antagonises the PI3K signalling pathway and negatively regulates MAPK (mitogen-activated protein kinase 1) pathway through its protein phosphatase activity.[3] These findings suggested that *PTEN* gene testing should be considered for patients with autistic behaviour and macrocephaly, which would impact recurrence risks and medical management.[2]

To further understand the role of the *PTEN* gene, the STRING database (www.string-db.org) was used to identify predicted protein–protein associations, functional interactions and biological network analysis.[4] Our current analysis found 10 nodes for PTEN protein with each representing all proteins produced including isoforms and 30 edges, which indicate both direct and predicted functional and physical protein–protein associations with interactions for each gene (see figure 1). Functional enrichments related to the *PTEN* gene involved the top biological processes, molecular functions, cellular components, pathways and disease-gene associations as shown in table 1. The top 10 significantly associated proteins or predicted functional partners found for PTEN using the STRING database are described in table 2. These include TP53, MAGI2, PIK3R1, DLG1, PIK3CA, PTK2, MAST2, PREX2, SPOP and AKT1. The proposed functions of these 10 genes with their encoded proteins and interactions lend strong evidence of the role of *PTEN* and involvement in autism, overgrowth and cancer.

The *PTEN* gene and autism report in 2005 was recently recognised as one of the most cited research articles published in the *Journal of Medical Genetics* since inception in the past 60 years. As a medical geneticist devoted to the study of genetic disorders over my academic medical career covering five decades, particularly in autism, our research expanded following this report and influenced by more genetic testing options, greater awareness of autism and the role of genetics. In looking back over the past two decades since reporting the *PTEN* and autism study in 2005, my interest in genetics of autism was increased and stimulated by earlier observations or experiences in the clinical setting while evaluating patients presenting for genetic services. Particularly, a young male patient comes to mind who I evaluated in the mid-1990s with overgrowth, macrocephaly, hemangiomas and gastrointestinal bleeding with a history of neurodevelopmental problems. His father with a large size and asymmetric growth had similar clinical findings. The overgrowth disorders such as Bannayan-Riley-Ruvalcaba and/or Cowden syndrome with clinical findings were described after the evaluation of this male patient. When examining a young child years later in a separate clinic setting, illustrated in the *PTEN* gene and autism report in 2005, I recalled the experience evaluating the young male patient and his father previously. With *PTEN* gene mutations in overgrowth disorders being reported in the literature at about that time, we investigated laboratories interested in analysing *PTEN* gene mutations clinically and reached out to Dr Charis Eng. Her research laboratory was conducting *PTEN* gene testing in clinical cohorts, which led to our study identifying one in five patients presenting with macrocephaly and learning-behaviour problems with autism and having a *PTEN* gene defect. Hence, this led to the identification of the *PTEN* gene as a major contributor to ASD.

Our studies on the genetics of autism have generated 79 peer-reviewed publications cited in the literature as a coauthor and will be briefly discussed below. These studies involved clinical and genetic characterisation of autism with examples of syndromic autism with causes, the role of associated and related genes, their functions and variants along with development of testing options and protocols for use in the clinical setting. For

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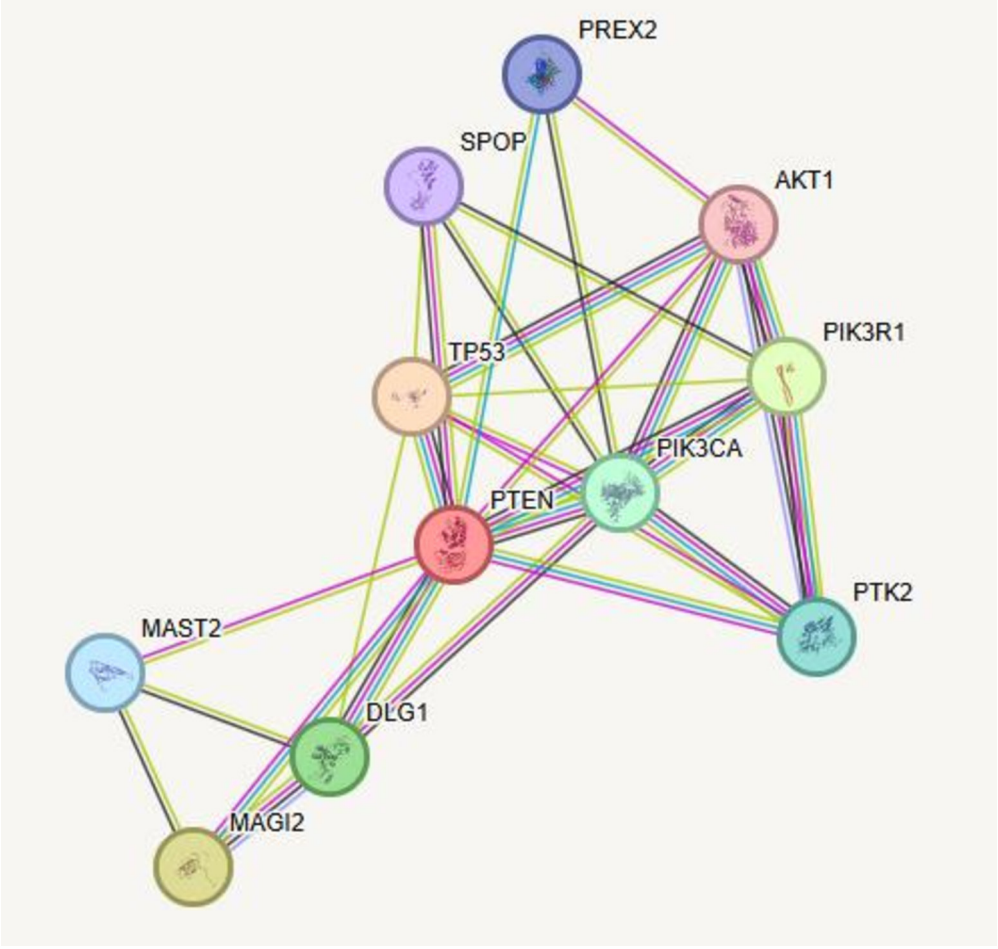

Figure 1 STRING protein–protein interaction network for the *PTEN* gene with functional interactions involving 10 associated nodes and 30 edges with their predicted functional interactions including biological processes and molecular functions (https://string-db.org) (STRING Consortium 2023). Edges represent protein–protein associations, which are considered specific and meaningful such as proteins that jointly contribute to a shared function. Network nodes represent proteins with splice isoforms or having post-translational modifications and collapsed into each node for all proteins produced by a single protein-coding gene such as *PTEN*.

example, we undertook early whole exome sequencing in understudied females with autism and found 5 of the 30 unrelated females with functional variants of X-linked genes and cadherin, protocadherin and ankyrin repeat gene families, most commonly altered.[5] We also reported early microRNA analysis in individuals with autism and found feasibility for studying microRNAs in lymphoblastoid cells in this population[6] and later applied in silico functional analysis of candidate genes for autism.[7]

When searching the literature in 2015, we found 792 genes playing a role in autism and organised their locations on chromosome ideograms[8] stimulating additional research. The number and location of the recognised genes from the literature for other psychiatric and behavioural disorders besides the genes for autism were also reported and compared including for bipolar disorder (n=290 genes) and schizophrenia (n=560 genes) using GeneAnalytics, an analysis pathway enrichment tool, with existing gene datasets to identify shared pathways, mechanisms and phenotypes. We applied this tool to bipolar disorder and schizophrenia and found 23 genes in common with all three disorders mapped to nine biological super pathways controlling circadian rhythm with overlapping genes impacting dopamine and serotonin homeostasis and signal transduction

Table 1 STRING: predicted functions for *PTEN* gene

Biological process (gene ontology)	*Molecular function (gene ontology)*	*Cellular component (gene ontology)*	*KEGG pathways*	*Reactome pathways*	*Disease–gene association*
Phosphatidylinositol 3-kinase signalling Protein kinase B signalling Regulation of protein kinase B signalling Response to growth hormone Negative regulation of macroautophagy	Phosphatase binding Insulin receptor substrate binding Protein kinase binding Kinase activity Enzyme binding	Phosphatidylinositol 3-kinase complex, class IA Cytoplasmic side of plasma membrane Cell–cell junction Extrinsic component of membrane Myelin sheath	Small cell lung cancer Endometrial cancer Central carbon metabolism in cancer Glioma Melanoma	Extra-nuclear oestrogen signalling CD28 dependent PI3K/Akt signalling Nephrin family interaction PIP3 activates AKT signal VEGFA-VEGFR2 pathway	Head and neck cancer Proteus syndrome Endometrial cancer Cowden syndrome PTEN hamartoma tumour syndrome

Table 2 Predicted functions of top 10 significantly associated proteins with *PTEN**

Protein symbol	*Description*
TP53	A tumour suppressor in many types of tumours by inducing growth arrest or apoptosis depending on the physiological circumstances and cell cycle by controlling a set of genes required for this process.
MAGI2	Generates scaffold molecules at synaptic junctions by assembling neurotransmitter receptors and cell adhesion proteins and plays a role in nerve growth factor recruitment.
PIK3R1	Binds phosphorylated-Tyr kinases and as an adapter, mediates the association of the p110 catalytic unit to plasma membranes necessary for insulin-stimulated increase in glucose uptake and glycogen synthesis playing an important role in signalling of several fibroblast growth factor receptors and modulating cellular response to stress.
DLG1	An essential multi-domain scaffolding protein required for normal development by recruiting channels, receptors and signalling molecules to plasma membrane domains in polarised cells. It plays a role in adherence, cell proliferation, synaptogenesis and lymphocyte activation as well as regulation of excitability of cardiac myocytes.
PIK3CA	Generates cellular phosphorylation and activation of signalling cascades involved in cell growth, survival, proliferation, motility and morphology. It affects several disorders such as breast, lung, ovary, colorectal, liver and gastric cancer, Cowden syndrome, cerebral cavernous malformations, overgrowth, body and facial asymmetry, macrodactyly, skin changes, vascular malformations, lipomatous tumours, megalencephaly, CLOVE and CLAPO syndromes.
PTK2	Essential for regulating cell migration, adhesion, spreading, reorganisation of the cytoskeleton required for early embryonic development and the placenta. It regulates embryonic angiogenesis, cardiomyocyte migration and proliferation with axon growth and neuronal cell migration, branching and synapse formation.
MAST2	Linked to the dystrophin/utrophin network with microtubule filaments via syntrophins affecting muscle and plays a role in spermatid maturation as a member of the protein kinase superfamily.
PREX2	Functions as a RAC1 guanine nucleotide exchange factor (GEF) activating Rac proteins by exchanging bound GTP to free GTP and may be an important mediator of Rac signalling downstream of both G protein-coupled receptors and phosphoinositide 3-kinase.
SPOP	Functions in the ubiquitin ligase complex pathway involved in proteasomal degradation of targeted proteins involved in cell cycle regulation. It may play a role in global development, variable behavioural problems, head size and dysmorphic facial features.
AKT1	One of three closely related serine/threonine kinases (AKT1, AK2 and AKT3) that generate specific inositol lipids implicated in the regulation of cell growth, proliferation, survival, differentiation and cytoskeletal changes impacting cancer and overgrowth of cells with implication in glucose transport.

*STRING database (www.string-db.org).

pathways, along with impact on mood, behaviour and physical activity. This supported a core aetiological relationship between neuropsychiatric illnesses and sleep disruption with hypoxia and central brain stem dysfunction.[9] Similarly, a combined list of autism (n=792) and malignancy (n=3500) genes were studied by Gabrielli *et al*[10] and 138 overlapping genes (17% of all autism genes) were found and profiled with the GeneAnalytics programme. Interestingly, seven significantly associated diseases were implicated with shared pathology for ASD and cancer. These findings included pathway disturbances for ERK (extracellular signal-regulated kinase 1/2) and major cell-signalling factors and metabolism, biological processes involving gene transcription, protein binding and colorectal cancer. Hence, these risk factors for cancer and overgrowth with autism showed disturbed overlapping genes that impact cell growth, gene–gene or protein interactions, altered pathways and biological functions with shared mechanisms, leading to identification of a common pathology requiring more research. A better understanding of causation with potential treatment options to lessen the severity of ASD-related symptoms and relationship with malignancy in those affected through new investigations may be attainable.

About 50% of individuals with ASD have common behavioural and other problems due to chromosomal abnormalities, single gene variants and/or recognised syndromic autism. Functional analyses and research of genetic defects associated with autism have led to at least three functional pathways including chromatin modelling, Wnt, Notch and other signalling pathways and metabolic disturbances involving neuronal growth and dendritic spine profiles as recently reviewed by Genovese and Butler[11] in 2023. We also summarised the behaviour and genetics of 12 classic syndromic autism disorders such as Phelan-McDermid, Williams, tuberous sclerosis complex, Rett, Down, PKU, de Lange, DiGeorge, fragile X and chromosome 15q disorders including Prader-Willi (PWS), Angelman (AS), Burnside-Butler and 15q duplications. Several of these disorders were further studied to identify, characterise and review clinical manifestations and their genetic findings to include unique *FMR1* gene defects in fragile X syndrome[12] and potential nationwide newborn screening options for chromosome 15 imprinting disorders using an *SNRPN* methylation analysis approach.[13]

Our numerous clinical, genetic and natural history reviews reported in PWS and AS syndromes have led to further characterisation of chromosome 15 defects and impaired imprinting status impacting gene expression patterns, clinical presentations and outcomes, natural history and genetic diagnostic approaches.[14–19] These reports may stimulate additional research to improve treatment options and care with more accurate counselling for at-risk family members depending on the observed molecular genetic classes. For PWS, the typical 15q11-q13 deletion is seen in about 60% of cases, maternal disomy 15 in about 35% and the remaining patients has imprinting centre defects or another chromosome 15 abnormality.[14–17] Those with imprinting defects due to microdeletions only of the imprinting centre, which controls activity of genes in the 15q11-q13 region may have a recurrence risk of 50%.[14–19] Individuals with PWS and typical 15q11-q13 deletions have more severe problems such as lower academic achievement along with more self-injury, behavioural problems and hypopigmentation. Those with non-deletion status or maternal disomy 15 are more atypical in their clinical presentation, leading to a delay in recognition and diagnosis and are more prone to autism in childhood with psychosis in early adulthood.[14 15 17]

Gene expression patterns and relationships between *UBE3A* and *SNORD116*, both imprinted and with a role in AS and PWS syndromes, respectively, were studied.[20 21] Both syndromes are at risk for autism. We reported that *UBE3A* and *SNORD116* messenger RNA levels were positively correlated with all developmental functioning and autism scores in a deletion AS cohort and autism features in a non-deletion PWS group. The findings suggested the presence of novel interactions between the expression of *UBE3A* and *SNORD116* in peripheral blood monocytes and brain-specific processes underlying motor and language

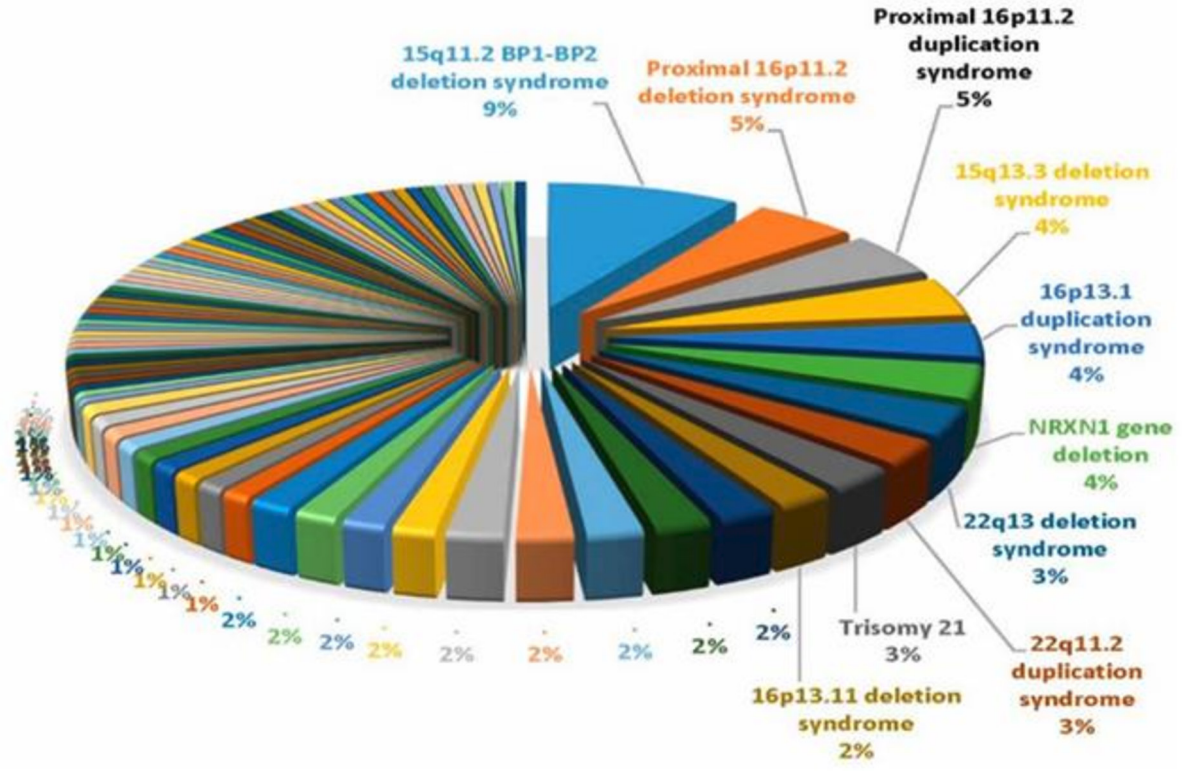

Figure 2 The top 10 genetic findings shown in the pie chart were identified by using ultra-high resolution chromosomal microarray analysis from a patient cohort of over 10 000 consecutive individuals with neurodevelopmental disorders including autism spectrum and/or developmental/intellectual disabilities from data summarised by Ho *et al* (reprinted with permission from Genovese and Butler).

impairments and autism in these two genomic imprinting disorders.[21] We also used whole genome microarray analysis and gene expression of males with PWS and showed alterations in expression of serotonin receptor genes (eg, *HTR2B*) and other genes involved in eating behaviour and obesity (eg, *ADIPOR2, MC2R, HCRT, OXTR*). As expected, the 15q11-q13 genes showed reduced expression when compared with controls.[20]

Atypical and emerging microdeletion syndromes associated with autism[22] were studied by delineating and characterising the clinical phenotype such as the 15q11.2 BP1-BP2 deletion (Burnside-Butler) syndrome.[14 16 17 23 24] This emerging disorder involving four genes in the region is characterised by developmental delay, neurobehavioral problems and/or autism in 75% of cases followed by speech and motor delay, poor coordination and white matter disease along with attention deficit hyperactivity.[17 23] This chromosome 15 finding has been observed as the most common genetic defect identified with ultra-high chromosome microarray analysis reported by Ho *et al*[24] and undertaken on 10 351 patients with neurodevelopmental disorders presenting for genetic services and laboratory testing. The most often referring physicians were neurologists followed by developmental paediatricians, paediatricians and medical geneticists. In this patient cohort, 4657 had no indication of ASD, while 5694 individuals had an ASD diagnosis with or without other features such as congenital anomalies, seizures, developmental delay and/or intellectual disability. For those patients presenting with a non-ASD diagnosis, the most common defect was a 22q11.2 deletion followed by 15q11.2 BP1-BP2 deletion and 16p11.2 deletion. In the combined ASD group, the most common genetic finding was the 15q11.2 BP1-BP2 deletion, followed by 16p11.2 duplication and *NRXN1* gene deletion. Figure 2 shows a pie chart with the top 10 findings out of 85 genetic defects observed from the ultra-high-resolution chromosomal microarray approach from a large patient cohort of consecutive patients presenting for genetic services.

Continued advances in genomic testing with bioinformatics, access to expanding gene variant databases and computational biology will improve the diagnostic yield from next-generation exome or whole genome sequencing in identifying disease-causing gene defects or variants. These studies will further impact our understanding of both protein coding genes and their encoded structural and regulatory proteins along with incorporation of proteomics and epigenetics in the future. The identification of molecular signatures of novel genes and their disturbed expression patterns with variations in relationship to disease states using total RNA or single-cell RNA sequencing of available tissue (eg, existing brain samples from affected individuals) will identify disease-specific profiles and patterns for a better understanding of causation in autism and level of severity, important for medical care in the future. Interconnected gene pathways are recognised that impact neurobehavioral phenotypes and should hold promise for the study and treatment of autism and the use of pharmacological agents based on DNA changes of candidate genes. The role of pharmacogenomics testing will further impact care in medication selection and management. Currently, over 150 genes are recognised as causative for ASD and available commercially for testing in those patients presenting for genetic services and evaluation.

Study of the qualitative and quantitative effects of non-messenger RNA such as micro-RNAs and sno-RNAs may lead to new areas of investigation with the potential to impact medical therapies for human diseases. RNA analysis should be considered in the future for diagnostic evaluations of ASD as we learn more about genetics, gene functions and biological pathways

in autism in either those with sporadic occurrences or with a positive family history. The development, validation and streamlining of laboratory testing[25] for syndromic or non-syndromic autism will benefit earlier diagnosis, improved treatment and more accurate counselling for at-risk family members.

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