Loss of PTEN phosphorylation via single point mutation alters cortical connectivity and behaviour

This scientific commentary refers to ‘The impact of phosphorylated PTEN at threonine 366 on cortical connectivity and behaviour’ by Ledderose et al. (doi:10.1093/awac188).

The PTEN (phosphatase and tensin homologue) gene on human chromosome 10q23.3 was originally identified as a bona fide tumour suppressor gene, sparking interest in its cellular function. PTEN is a dual-specificity lipid and protein phosphatase that acts as a gatekeeper of the phosphoinositide 3-kinase (PI3K) pathway (via dephosphorylation of PIP3 to PIP2) and thus represses downstream signals that control cell proliferation, survival, and protein synthesis. In 2015, germline mutations in PTEN were discovered as a cause of autism spectrum disorder (ASD) in children with macrocephaly.1 Almost all individuals with inherited or de novo mutations in a single copy of PTEN show symptoms of macrocephaly and a spectrum of behavioural abnormalities, cognitive disabilities, and epilepsy,2 with ~25% of individuals with PTEN mutations meeting the criteria for ASD.3 As a result, many studies have examined the role of Pten in brain development and neurocognitive functions using transgenic mice with Pten haploinsufficiency or conditional homozygous deletion in forebrain neurons. Although these latter studies have provided a wealth of information on the impact of decreased PTEN levels on brain development, genetic variation in Pten results not only in decreased PTEN levels, but may also affect its phosphorylation, specifically at site T366.4 Phosphorylation at this site is important for controlling PTEN activity and stability in a cell-dependent manner,5 but its role in brain...
development and behaviour is unknown. To address this knowledge gap, Ledderose and colleagues generated transgenic mice with a specific mutation (threonine 366 substitution with alanine) that prevents T366 phosphorylation.

Homozygous Pten\textsuperscript{T366A} mice were found to be viable and fertile. Their brains displayed normal levels of PTEN and PI3K-downstream signals (phosphorylated AKT and phosphorylated S6, an mTOR readout) at P7 to P14. However, when examined at the single cell level rather than in forebrain homogenates, phosphorylated S6 was selectively increased in cortical pyramidal neurons, suggesting cellular specificity for the role of T366. In addition, further specificity was reported based on the layer positioning of pyramidal neurons. Layer (L) 2/3 and L4, but not L5, neurons displayed altered PI3K signalling. Layer-specific pyramidal neuron regulation has also been reported in heterozygous and conditional homozygous knockout mice. In light of these molecular changes, the authors performed a series of behavioural tests. They found no changes in levels of anxiety or locomotion, but alterations in cognitive processes, decreased repetitive behaviour (grooming), and a striking decrease in tactile sensory processing suggestive of hyposensory responsiveness. Alterations in sensory processing are a characteristic feature of ASD, including PTEN individuals with ASD, and remain understudied.

To address how a single mutation could lead to such a prominent sensory abnormality in mice, the authors next used a battery of approaches to examine cell proliferation, morphology, positioning, and circuit connectivity in the primary somatosensory cortex (S1), which are known to be regulated by PTEN and may contribute to ASD traits. The investigators found no change in neural progenitor cell proliferation (examined at P1 and P8 following bromodeoxyuridine...
(BrdU) injections in pregnant mice) or cortical layering (using immunostaining for the different layer-specific pyramidal neurons and BrdU). Nevertheless, they identified a series of morphological alterations, including increased cell size and overgrown dendrites (using GFP-tagged AAV injections in P0 mice), that are conserved across Pten transgenic mice. Consistent with an increase in phosphorylated S6 in select pyramidal neuron populations, changes in soma size were pyramidal neuron (or layer) and age specific. Soma size was increased in L2/3 and L4 neurons at P8 and P14 but was unchanged on P21 and P42. Intriguingly soma size was not increased in cultured cortical neurons. Dendrites were analyzed for L2/3 pyramidal neurons and L4 interneurons and showed increased arborization from P8 onwards (analyzed up to P42). These data suggest an intriguing uncoupling between soma size and dendrite growth.

Finally, the investigators examined axonal connectivity, which is recognized as being abnormal in individuals with ASD and which has been shown to contribute to ASD traits in Pten heterozygous mice. They focused on cortico-cortical and thalamo-cortical connections. Specifically, they used several viruses and two injection sites; the first injection was in the thalamus and contained a Cre-encoding anterograde transsynaptic virus (AAV-Cre, expressing Cre in thalamic neurons and postsynaptic target neurons in S1), whereas the second injection was in S1 cortex and contained a helper Flex AAV expressing the EnvA receptor (TVA) and EGFP upon Cre expression together with a retrograde monosynaptic rabies virus that only infects cells expressing TVA receptors and encodes mCherry. Thus, only S1 cells that receive inputs from thalamic neurons infected with AAV-Cre will express TVA and EGFP, allowing them to be infected by the rabies virus expressing mCherry. These GFP+/mCherry+ cells are starter neurons. Neurons from the thalamus and other cortical brain regions that make a monosynaptic
connection (i.e., presynaptic neurons) to these starter neurons will express mCherry (due to the retrograde transport of the rabies virus). The investigators counted the number of starter neurons and mCherry+ neurons in S1 cortex and other cortical regions and in the thalamus to deduce the percentage of short versus long range cortical inputs. They found an imbalance of short versus long range connections onto S1 neurons. They also examined connections in S1 neurons without AAV-Cre expression and found no difference in the weight of local versus long range inputs to S1, suggesting that only cortical connectivity to S1 neurons receiving inputs from the thalamus was affected.

This study raises many further questions: would heterozygous mice also display deficits? Are dendritic spines altered? Spines are altered in ASD: is the abnormal connectivity responsible for the behavioural deficits? The next step is to identify molecular dysfunctions that can be targeted in rescue experiments. Many other abnormalities, even those that are transient during development, could contribute to long lasting behavioural deficits, including but not limited to abnormal calcium activity (the form of communication of immature neurons) or cell intrinsic excitability due to altered ion channel expression.¹⁰

Collectively, this study identified a specific role of Pten T366 in cortical development and more specifically in cortico-cortical and thalamocortical connectivity. Mutation in T366 led to specific cellular and connectivity abnormalities associated with a spectrum of neurocognitive deficits and tactile hypo-responsiveness. These data clearly emphasize that different Pten variants will have different impacts on brain development and function that are expected to contribute to the large spectrum of behavioural abnormalities in individuals with Pten mutations despite every
individual displaying macrocephaly. There is thus a need to understand the function of specific mutations and perform screening to identify gene variants.

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