PNKP

Patient Description:
The patient is a 3-year old male with *PNKP* related microcephaly, seizures and developmental delay. He was born full-term to non-consanguineous Ashkenazi Jewish parents who had compatable carrier screening for common Ashkenazi connections. The pregnancy was notable for microcephaly at 32 weeks gestation. After birth, he was noted to have type 3 tracheoesophageal fistula and severe microcephaly. Evaluation for VACTERL association identified a left pelvic kidney. At two months of age, the proband developed seizures and respiratory decompensation during those episodes. After the TEF repair, he obtained a G-tube through which he relies on for intake. The proband continues to experience difficulties in gaining weight (<0.01%ile). He has been diagnosed with cortical blindness and significant motor and language delays. At three years old, the proband is unable to sit unsupported or walk, he does not have any language but is able to verbalize sounds and rollover.

First-tier testing included karyotype, chromosomal microarray, and DEB breakage studies which were all normal. Whole exome sequencing was performed shortly after birth and identified a likely pathogenic homozygous missense variant in exon 15 of *PKNP*. This variant was previously reported in a patient with microcephaly, primordial dwarfism, and infantile epilepsy.

Disease/Syndrome Features:
Biallelic pathogenic variants in *PKNP* are associated with multiple, distinct phenotypes with strikingly different prognoses. patient's phenotype is consistent with *M*icro*c*ephaly, *S*eizures, and developmental delay (MCSZ). Mutations in this condition cause loss of protein function, with truncating mutations being reported with a more protracted phenotype. The cells of patients with MCSZ display an inability to repair hydrogen peroxide-induced free radical DNA damage. Despite having shown involvement in DNA repair pathways, it has not been associated with features of immunodeficiency or early-onset cancer, as is commonly seen in other conditions that impair these pathways.[1.]

PKNP is also associated with other clinical phenotypes which include Ataxia-oculomotor apraxia type 4 (AOA4, MIM:616267) and Charcot-Marie-Tooth Syndrome type 2B2 (CMT2B2, MIM: 605589). AOA4 is a rapidly progressive neurological condition associated with ataxia and dystonia. Symptoms typically appear in the first decade with ataxia and dystonia which leaves most patients wheelchair-bound by the second or third decade.[2.]

CMT2B2 shows some overlap with AOA4, with impaired gait although it tends to present later in life and tends to be much more variable in its presentation. Sensorineural axonal peripheral neuropathy is responsible for the symptoms which mostly affect lower limbs, with upper limbs sometimes reported. The condition tends to be slowly progressive and can include a variety of findings such as cerebellar atrophy, dysarthria, and abnormal eye movements[3]

Protein/Pathway:
PNKP located on Chromosome 19q13.33 is responsible for producing a polypeptide of 521 amino acids, a dual-action polynucleotide kinase 3'-phosphatase (PNKP) which is an essential enzyme for nuclear and mitochondrial DNA repair. [4]

The protein contains three domains; an N-terminal fork-head-associated (FHA) domain, a DNA phosphatase domain, and a DNA kinase domain. The FHA domain mediates interactions with the X-Ray Repair Cross complementing 1 protein (XRCC1) to assist in single-strand break (SSB) repair. This FHA domain also interacts with XRCC4, as part of the non-homologous end joining (NHEJ) repair pathway necessary for double-stranded break (DSB) repair.[3] The enzyme is also thought to be involved in an alternative NHEJ pathway that involves other genes proteins including PARP1 and an XRCC1/LIG III complex.[5]

Neurons are particularly prone to degradation by oxygen reactive series, radiation, and other environmental exposures and therefore, highly dependent on different DNA repair pathways at different stages of the cell cycle. Reductions of phosphatase and kinase levels due to PNKP deficiency impair multiple DNA repair pathways. Double-stranded DNA breaks occur frequently during neurogenesis, where, if not repaired, lead to cell death and is the main etiology of neurodevelopmental disease. Single-stranded breaks commonly caused by oxygen reactive series, pose a significant risk to the genomic stability of post-mitotic cells and can interfere with transcription.[6]

While these pathways may provide some insight into the neurodegenerative versus neurodevelopmental phenotype, a genotype-phenotype correlation has not yet been established. One possible theory involves PNKP's interaction with mitochondria. The kinase domain of PNKP contains a mitochondrial signaling target (MTS) region which allows it to enter the mitochondria. Reduced levels of PNKP have been seen with dysfunctional mitochondria. Understanding PNKP's role in mitochondrial homeostasis may provide alternative treatment options for patients with *PNKP*-related disease[5,6].

Publications:

Bermúdez-Guzmán, L., & Leal, A. (n.d.). DNA repair deficiency in neuropathogenesis: when all roads lead to mitochondria. https://doi.org/10.1186/s40035-019-0156-x

Bras, J., Alonso, I., Barbot, C., Costa, M. M., Darwent, L., Orme, T., Sequeiros, J., Hardy, J., Coutinho, P., & Guerreiro, R. (2015). Mutations in *PNKP* cause recessive ataxia with oculomotor apraxia type 4. American journal of human genetics, 96(3), 474–479. https://doi.org/10.1016/j.ajhg.2015.01.005

Dumitrache, L.C., McKinnon P.J. (2017) Polynucleotide kinase-phosphatase (PNKP) mutations and neurological disease. Mechanisms of Ageing and Development 161:121-129.

Jilani, A., Ramotar, D., Slack, C., Ong, C., Yang, X. M., Scherer, S. W., & Lasko, D. D. (1999). Molecular Cloning of the Human Gene, PNKP, Encoding a Polynucleotide Kinase 3′-Phosphatase and Evidence for Its Role in Repair of DNA Strand Breaks

Caused by Oxidative Damage. Journal of Biological Chemistry, 274(34), 24176–24186. https://doi.org/10.1074/jbc.274.34.24176
Leal A, Bogantes-Ledezma S, Ekici AB, et al. The polynucleotide kinase 3'-phosphatase gene (PNKP) is involved in Charcot-Marie-Tooth disease (CMT2B2) previously related to MED25. Neurogenetics. 2018;19(4):215-225. doi:10.1007/s10048-018-0555-7
McKinnon P.J. (2013). Maintaining genome stability in the nervous system. Nature neuroscience, 16(11), 1523–1529. https://doi.org/10.1038/nn.3537
Shen, J., Gilmore, E. C., Marshall, C. A., Haddadin, M., Reynolds, J. J., Eyaid, W., Bodell, A., Barry, B., Gleason, D., Allen, K., Ganesh, V. S., Chang, B. S., Grix, A., Hill, R. S., Topcu, M., Caldecott, K. W., Barkovich, A. J., & Walsh, C. A. (2010). Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. Nature genetics, 42(3), 245–249. https://doi.org/10.1038/ng.526

Support Groups and Information:

PNKP, children with the gene disorder *PNKP* - Private Facebook Group
facebook.com/groups/2243536475656637