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CASE REPORT

X-linked spliceosome gene *GPKOW* mutation associated with small head, small size, and mild speech delay in a 4 year old child

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ABSTRACT

The protein product of the X-linked gene *GPKOW* is a fundamental element of pre-mRNA splicing within a cellular structure called the spliceosome, important in the synthesis of the body's proteins. A single case report linked a *GPKOW* defect with fetal demise and severe small head size in 2017. And recently, 3 severely affected cases have been described. Here we describe a young boy with a likely pathologic *GPKOW* genetic variant and an apparently normal head size *in utero*, a small head size at birth, and a progressively smaller head size with time. He had a mildly small body size at birth and fell below the normal growth curve by 2 months of age. An early motor delay was mild and resolved after a year of age, and his attention span, sociability, and early language skills did not indicate developmental delay. His mother, a carrier of the gene, has a small head size, mild short stature, and absent any neurologic issues. *GPKOW* is an important gene for accessory protein synthesis and brain and body growth, and male patients with less impactful variants in the gene may thrive and demonstrate mild and not severe outcomes.

Introduction

The *GPKOW* gene (OMIM # 301003) is one of many elements of the spliceosome, which trims the pre-mRNA molecule to an effector mRNA molecule leading to cellular protein synthesis[1]. Encoded on the X chromosome at Xp11.23, the *GPKOW* protein functions as an essential activator of the major spliceosome by directly interacting with the RNA helicase *DHX16* and promoting catalytic activation of stalled spliceosomal complexes. Pathogenic variants in *GPKOW* are rare; to date, reported cases have been limited to severe or lethal presentations. A case of an infant child with a c.331+5G>A *GPKOW* gene defect was described associated with a severe X-linked recessive male-lethal disorder with extreme microcephaly, intrauterine growth restriction (IUGR), and lack of fetal movement on prenatal ultrasound and death *in utero* or stillbirth[2]. This variant disrupts normal splicing of its pre-mRNAs[1].

A recent study of 3 males from 2 unrelated families with intrauterine growth restriction, microcephaly/microencephaly, and eye, brain, skin, and skeletal abnormalities showed hemizygous pathologic frameshift variants p.(Arg441SerfsTer30) and p.(Ser444GlufsTer28) affecting the last exon of *GPKOW*[3]. In vivo studies in Drosophila melanogaster targeting the sole *GPKOW* fly ortholog, CG10324 were used to confirm pathogenicity. Heterozygote carrier females presented with short stature, microcephaly, and vision problems[3]. Several *GPKOW* variants of unknown significance (VUS) cases are reported in ClinVar and Franklin databases (Fig 1)[4]. We describe a phenotypically mild case with a likely pathologic *GPKOW* mutation and suggest that *GPKOW* gene defects with a lesser impact may be associated with early small head and slow growth after birth.

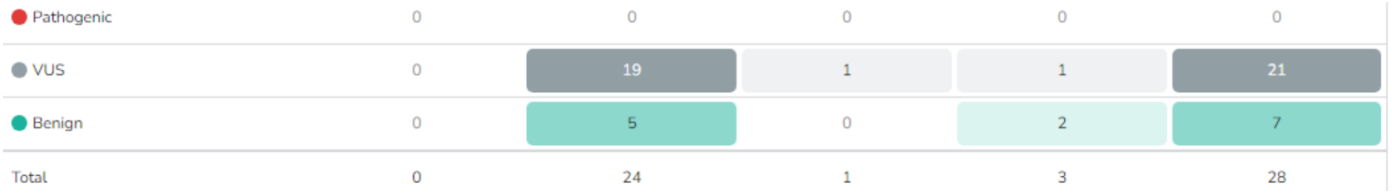

Figure 1: Missense variants in the gene *GPKOW* (from Franklin)

Case Report:

An infant male was seen in Genetic Clinic for small head size that began in the neonatal period. Prenatal ultrasound at 38 weeks gestation, one week before delivery was not alarming. However, head circumference was 30.5 cm. at birth and stayed well below normal with time (Figure 2A). Birth length was at 10 percentile and fell below 1 percentile at 2 months of life and stayed well below normal with time (Figure 2B). Head ultrasound after birth showed a questionable "tiny" left grade 1 germinal matrix hemorrhage. A head MRI at 2 weeks of life and a CT a month later were normal and the CT did not show premature closing of sutures. Viral studies including cytomegalovirus (CMV) PCR urine and IgM, Zika PCR, and Rubella IgM were

Mild gross motor delay was noted at 6 months of age with poor ability to roll over back to front. He made good eye contact, was interactive, able to sit and stand with support, and had fair head control at 6-7 months of age. By age 8 months, he was still only rolling over front to back, babbling, sitting independently, turning to sounds or in response to his name, giggling, and eating solids. He was able to reach but not transfer. Motor skills improved, and he began walking at 14 months of age.

By age 15 months, he began babbling and spoke a few words such as "Mama and Dada" not in context. He was able to reach and transfer. By age 17 months, he did not hold a bottle well and lacked a pincer grasp. But by 18 months he could run and climb up a few stairs. Motor ability was normal at 22 months. His speech was mildly delayed, as he could only say a few more words at 3 yrs., however, he could communicate well and actively used sign language to interact and indicate his needs and understands "everything." He knows colors and shapes, animals, and some letters, and is socially interactive and has a normal personality. The parents felt that physical and occupational therapies were helpful.

Physical Examination showed a small child for age and a small head with a mild prominent forehead. No other dysmorphic features were found (Figure

1). Genetic testing with Chromosome microarray and karyotype were normal. Whole exome sequence (WES) testing showed a hemizygous variant c.1244 C>T (p.(P415L) in the X-linked gene *GPKOW* gene inherited from the mother. In-silico tools of this mutation were "evaluated on a research basis by GeneDx, and showed a high likelihood of pathogenicity."[5] His mother has normal intelligence, mild short stature, a small head that measured 51.5 cm.(< 2 percentile) and no other findings.

Figure 2: Child Head Circumference & Length

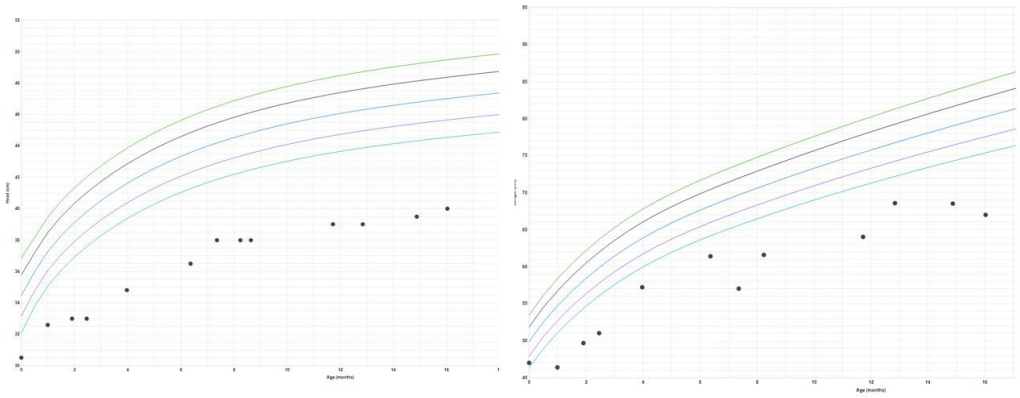

Figure 2: HEAD CIRCUMFERENCE AND LENGTH IN THE FIRST 1.5 YEARS OF LIFE. Head Circumference and length were small at an early age, and continued to be small.

Figure 3: CHILD AT AGE 18 MONTHS. He has a mildly prominent forehead and the size of a one year old child.

Discussion:

The expression of a gene starts with the transcription of the gene coding sequence on chromosomal DNA to a pre-RNA product within the spliceosome and the nucleus, followed by the conversion of the pre-RNA product to mRNA, and the export of the mRNA to the ribosome in the cytoplasm[6]. The spliceosome is a large RNA-protein complex that facilitates pre-mRNA splicing and the removal of introns from the transcript, ligating flanking exons to generate a mature coding mRNA[7]. Splicing is carried out by one of two multi-megadalton ribonucleoprotein complexes called the major and the minor spliceosomes. The major spliceosome, consists of five small nuclear ribonucleoprotein complexes (snRNPs): U1, U2, U4, U5 and U6, and hundreds of proteins[8], potentially leading to large, and not small room for genetic errors.

The *GPKOW* gene plays a role in pre-mRNA splicing as a component of the spliceosome[1]. Pathogenic splice variant have been reported in a male fetus and newborn infants with severe microcephaly and intrauterine growth restriction[1,3] and there are a few more milder cases in ClinVar and Franklin (Figure 1). The missense variant in our case, c.1244 C>T (p.P415L) in the *GPKOW* gene was not found at significant frequency in large populations but was likely pathogenic when tested on a research basis by GeneDx[5]. *GPKOW* is found in the human spliceosome and reacts directly with the *DHX16/hPRP2* in GPKOW immune-depleted nuclear extracts, activating an inactive spliceosome[1]. Here the small head and short stature in the *GPKOW* carrier mother of the mutation also suggests pathogenicity. Like the lethal case and severely affected cases and affected carrier mothers described[1,3], our variant is likely pathogenic too, with the substitution of proline (with absent charge) to leucine (with negative charge) at an early, not late coding segment of the gene potentially the reason for less adverse effect on the splicing mechanisms and synthesis of the translated protein. Interestingly, other specific same gene p.P415L mutations are pathogenic in other genetic disorders, including pathogenic p.P415L mutation in X(+)-linked chronic granulomatous disease with defective NADPH oxidase activity[9], a pathogenic p.P415L mutation in Mucopolysaccharidosis type VII (MPS VII, Sly syndrome) associated with a deficiency in beta-glucuronidase activity[10,11], a *SPRED* P415A human mutation and the same mutation generated in mice that abolish its membrane localization and form a Neurofibromatosis-like Legius syndrome in humans but neuro-degeneration cerebellar ataxia and Purkinje cell loss in mice[12], and a pathogenic p.P415L mutation *SMAD6* variant with significantly ($P < 0.05$) lower activity than wild-type *SMAD6* inhibiting BMP signaling in a transcriptional reporter assay and causally associated with a form of congenital heart disease[13]. With so many p.P415L mutations causing so many pathologic proteins & disorders, in so many disparate genetic disorders, we suggest the p.P415L *GPKOW* mutation in our case causes mild dysfunction in head and body growth in our patient. This patient was suggested for study prior to the recent report of *GPKOW* mutations in other patients[3], but the absence of P415L triplet in the *GPKOW* Drosophila gene precluded inclusion of our patient in that study[14].

Analysis of the presentation of the child and his carrier mother, mild findings specifically in line with findings found in patients with a more severe presentation, is consistent with an interpretation of adding to "accurate interpretation fo the pathogenicity of variants, bringing a consistent clinical value[15]. There are many genetic defects of protein synthesis and spliceosome function, including *CWC27*-related spliceosomapathy associated with a spectrum of overlapping phenotypes, including retinal degeneration, skeletal anomalies, short stature, neurological defects, and hypergonadotropic hypogonadism, hypoplastic/agenesic teeth, and cataracts[16]. Spliceosome genetic defects usually include an element of developmental delay, such as pathogenic variants in *U2AF2* and *PRPF19*, encoding spliceosome subunits in neurodevelopmental disorders[17], *U SNAPC4* variants including *SNAPC4* that encodes one of the five SNAPc subunits critical for DNA binding and mutations led to individuals who present with delayed motor development and developmental regression after the first year of life and progressive spasticity that led to gait alterations, paraparesis, and oromotor dysfunction[18]. Other spliceosome defects include *Smad* nuclear-interacting proteins in neurodegenerative disorder[19], *RNU4ATAc* mutations in Taybi Linter group of developmental

delay disorders[20], *WBP4* defects associated with a neurodevelopmental delay syndrome[21], *SART 3* mutation defects, associated with global delay in both sexes and gonadal dysgenesis in males[22], *THUMPD2* mutations, associated with retinal degeneration[23], *SNRPA* defects, associated with developmental delay, short stature and hand anomalies[24], and *SF3B4* spliceosomal gene, associated with Nager Syndrome[25]. All disorders above are associated with some degree of neurologic dysfunction; here, we have primarily growth dysfunction. The most common reason for small heads in an individual is that their brains show slow growth, but many persons with small heads have normal intelligence[26]. Our case shows that a *GPKOW* mutation and small size and small head can be associated with a relatively normal neuronal function. Our case has a dramatic absence of the many clinical findings of other spliceosome genetic defects, but it can now be said that a slowed neuronal and somatic cell growth with a potential decreased volume of cells can be less impactful effect than the more severe expressing mutations in previous cases.

Conclusion:

This case report expands the clinical spectrum of *GPKOW*-related disease by demonstrating that hemizygous missense variants in this X-linked spliceosome gene can result in a milder phenotype than previously reported. The presenting features in this child—microcephaly, short stature, and mild speech delay with preservation of cognitive and social function—together with small head size and mild short stature in his carrier mother, are consistent with a pathogenic role for the c.1244 C>T (p.P415L) variant. *GPKOW* is an important gene for brain and body growth, and clinicians should consider *GPKOW* variants in male patients presenting with microcephaly and growth restriction even in the absence of severe neurologic impairment. Female carriers may exhibit subclinical features including mild microcephaly and short stature. Identification of milder *GPKOW* presentations, such as the one described here, will refine our understanding of genotype-phenotype correlations in spliceosome disorders and may facilitate earlier diagnosis and targeted clinical management.

Conflict of Interest Statement:

None.

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