Atypical phenotype? The answer's in the genotype: AGS caused by a novel *RNASEH2C* variant combined with XLA caused by a BTK deficiency.

Running title: Combined *RNASEH2C* and *BTK* causal variants.

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Key message: Strategic application of WES in cases of atypical presentation can be a powerful diagnostic tool.

Dear Editor,

The feasibility of testing multiple genes at once using Next Generation Sequencing (NGS), particularly Whole Exome Sequencing (WES), has expanded the phenotypic spectrum associated with many disease genes. Distinguishing atypical presentation from combined gene effects and incidental from causal findings can however be challenging, particularly when composite phenotypes are themselves extremely rare. Here, we describe a child with Aicardi-Goutières Syndrome (AGS) associated with a novel *RNASEH2C* variant (1, 2) identified by WES. What was initially thought to be a highly unusual and severe history of infections secondary to the neurological deficits of AGS, was deciphered to be symptomatic of a distinct disorder, first suggested by blood tests and then confirmed by the WES data: X-linked agammaglobulinemia (XLA) caused by a second novel mutation, in the *BTK* gene. This demonstrates the unique power of WES as a cost-effective, efficient tool in the pediatric rheumatology setting (3-5). It also underlines the importance of multi-modal testing and close, multi-disciplinary interactions: between specialists in different rare diseases, clinical biologists, and data-analysts.

A nine-year old boy born to consanguineous parents (**Supp Fig. 1**) from Conakry, Guinea, presented with neurological delay and irritability from the first months of life (**Supp Fig. 2A**). Suspicion of early onset encephalopathy was confirmed by hypomyelination and suggestion of microcalcifications on cerebral MRI. Interferon alpha (IFN-α) activity level was elevated to 75UI/ml in cerebro-spinal fluid (CSF) (**Supp Fig. 2B**), with a six-gene Type I IFN-response signature elevated in blood cells (**Supp Fig. 2C**) (6). Consistent with these features, WES performed on the trio provided a molecular diagnosis of AGS3 (MIM 610329) caused by a novel missense substitution in *RNASEH2C* (*NM_032193.3*:

c.248T>C, p.Val83Ala), homozygous in the affected child and heterozygous in both unaffected parents (**Table 1)**. The variant is absent in large public sequence databases including the Genome Aggregation database (gnomAD), UK10K, and Genome of the Nethrelands (GoNL), and predicted to be pathogenic by 7 out of 8 *in silico* prediction tools.

The patient was noted to have a history of symptoms atypical of AGS: three episodes of pyelonephritis and frequent bouts of broncho-pneumonia, four of which resulted in pleural effusion and required treatment with intravenous antibiotics. Surprisingly, the same pneumococcal serotype (15A) was identified in two separate infectious episodes. While the neurological symptoms of AGS (e.g., problems with airway clearance or swallowing disorder) can result in increased respiratory tract infections, this unusual and severe history prompted us to assess for adaptive immune defects. loodwork showed an absence of B cells and extremely low immunoglobulin titers: typical features of XLA (MIM 300755; **Supp Fig. 2B**) and WES data revealed that, in addition to the *RNASEH2C* variant, the patient carried a hemizygous nonsense mutation in *BTK* (*NM_000061.2*: c.763C>T, p.Arg255*; **Table 1**), absent in public sequence databases, inherited from his heterozygous mother. Fourteen *RNASEH2C* variants have been identified in AGS, a rare auto-inflammatory disorder (1-5/10,000) of the Type 1-Interferonopathy spectrum, in 35 families world-wide(2). An Arg69Trp founder-mutation accounts for a large proportion in the South Asian population (**Supp Fig. 3A**) (1). Similar to previously reported AGS3 variants, the variant identified here is localized near the complex-stabilizing interface between the three subunits of the heterotrimeric RNASE H2 enzyme complex (**Supp Fig. 3B**) (7): the catalytic RNASE H2A, and the auxiliary H2B and H2C, encoded by separate genes; over 50% of AGS patients carry biallelic mutations in one of the three (1). The

enzyme degrades RNA strands from RNA/DNA hybrids, removes ribonucleotides from double-stranded DNA (8), and is essential for the maintenance of genome integrity (9). AGS-associated variants decrease enzyme function, thought to lead to the triggering of nucleotide sensors by endogenous nucleic acids. In an *Rnaseh2b* knock-out mouse model, Mackenzie et al. (10) demonstrated breakdown of the envelope of cytoplasmic micronuclei leading to rapid cGAS accumulation, providing a link between DNA damage and aberrant immune activation.

The identification of two coexisting rare disorders have real implications for the clinical management of this child and his family. For one, the profound XLA-associated defect in antibody-based immunity could be quickly and effectively improved by treatment with substitutive immunoglobulin. Moreover, it is possible that the recurrent viral infections associated with defective humoral immunity had an exacerbating effect on the IFN-induced AGS phenotype. Up to 10% of males with pathogenic *BTK* variants are not recognized to have immunodeficiency until after ten years of age (if not adulthood). WES not only confirms and complements clinical and biological tests, it allows for (indeed compels) appropriate genetic testing and counselling, particularly urgent in the case of female carriers of the *BTK* mutation.

The diagnostic yield of WES makes it an attractive tool in clinical practice: in pediatric neurology, it is estimated to identify disease-causative genes in 16-45% of patients (3). WES also lends itself to periodic re-analysis, of particular value in the rapidly evolving field of rare disease genetics. In high-volume settings such as large hospitals, WES is most effective when deployed strategically rather than indiscriminately: for patients with severe, complex or atypical, unusually early-onset, or familial forms of disease. WES has been

key in expanding the notion of a one-to-one association of disease with gene, to that of disease-gene networks, giving impetus to the growth of network medicine. It is however critical to distinguish between correlation and causation, incidental and disease-relevant findings when populating these knowledge-bases (4, 5). As our study exemplifies, it is also critical to distinguish between true phenotypic heterogeneity, and the co-occurrence of distinct rare diseases.

Gene symbol	Chromosome	Position (hg19/GRCh37)	hgvs_DNA	hgvs_Protein	gnomAD	In-house database	Prediction of pathogenicity	ACMG Classification
RNASEH2C	11	65,487,813	c.248T>C	p.Val83Ala	Absent	Absent	7	Pathogenic
BTK	X	100,615,569	c.763C>T	p.Arg255*	Absent	Absent	N/A (Stop)	Likely pathogenic

Table 1. Variant details. hgvs: Human Genome Variation Society nomenclature for nucleotide (DNA) and amino acid (Protein) change. gnomAD: frequency in Genome Aggregation Database v2.1.1 (125,748 WES and 15,708 WGS); In-house WES database: 2,300 WES and 20 WGS. Prediction of pathogenicity: Number of tools (out of 8) that predict a missense variant to affect protein function. N/A: Not applicable to nonsense variant (Stop). ACMG Classification: based on American College of Medical Genetics and Genomics guidelines.

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