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Title:

**Microarray diagnosis of Autoimmune PolyEndocrinopathy-Candidiasis-Ectodermal
Dystrophy (APECED) caused by a novel homozygous intragenic *AIRE* deletion**

Type of Manuscript:

Instructive Case

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/jpc.15124](https://doi.org/10.1111/jpc.15124)

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Acknowledgements:

The authors have no funding sources to declare.

Conflict of interest:

The authors declare that they have no real or perceived conflicts of interest to disclose.

Learning Points:

- Massively parallel sequencing, including exome sequencing, does not detect all types of genetic variants.
- When mutational mechanisms for suspected diagnoses include haploinsufficiency, chromosomal microarray should be considered prior to exome sequencing to detect

pathogenic copy number changes, and/or to identify regions of homozygosity which may unmask autosomal recessive disease caused by homozygous pathogenic variants.

Introduction

Genomic sequencing is revolutionising the diagnosis of monogenic conditions. Exciting applications of genomic sequencing include children with undiagnosed syndromes,(1) and rapid genomic diagnosis programs in paediatric acute care.(2) With testing costs decreasing and availability more widespread, genomic sequencing is increasingly offered earlier in the diagnostic pathway. Exome sequencing, which targets coding exons, is generally considered to be more accessible than whole genome sequencing due to its lower cost and more straightforward bioinformatic analysis.(3) Current technology limits the utility of exome sequencing to detect some types of genetic variants, including copy number changes, intronic variants, methylation abnormalities, repeat expansions, and mitochondrial variants (Table 1).

With these limitations, exome sequencing should be complemented by other appropriate genetic tests. We present a clinical case that remained undiagnosed following exome sequencing. Following clinical re-evaluation, a genetic diagnosis was confirmed by chromosomal microarray.

Case report

The proband (V:2 in the pedigree) is a 15-year-old male born to consanguineous parents (first cousins once-removed) of Lebanese ethnicity. He is the oldest of 5 siblings (Figure 1). There was no significant family history. The proband initially presented to an overseas centre in early childhood with hypoparathyroidism complicated by hypocalcemia, seizures, cataracts, and treatment-emergent nephrocalcinosis, and recurrent mucocutaneous candidiasis.

Investigations included exome sequencing in 2015 which identified a homozygous *ALMS1* variant (NM_015120.4(*ALMS1*): c.1570-1571insCTC). *ALMS1* variants cause Alstrom syndrome (MIM# 203800), which was thought to be a partial phenotypic match, although the proband did not have typical truncal obesity. Based on this result, in planning their next pregnancy, his parents elected to undertake preimplantation genetic diagnosis to avoid transmission of this variant, resulting in the birth of healthy female twins.

The proband presented to our centre at age 15 years with the additional feature of hepatitis with elevated transaminases and decreased synthetic function resulting in coagulopathy. Screening autoimmune antibodies were negative and he underwent liver biopsy which showed non-specific hepatitis with bridging necrosis. Close examination of the proband's nails identified dystrophic halluces and 4th fingernails, with normal skin and teeth. He did not currently have mucocutaneous candidiasis. Following multidisciplinary input from gastroenterology, endocrinology, and infectious diseases, a clinical diagnosis of APECED (MIM# 240300) was considered possible but not secure. He was referred for clinical genetics assessment and the previous overseas exome sequencing report was reanalysed. The *ALMS1* variant (NM_015120.4(*ALMS1*): c.1570-1571insCTC) was noted to be very common in

population databases (gnomAD v2.1.1, available at <https://gnomad.broadinstitute.org>; 178,443 heterozygotes, 58,196 homozygotes; allele frequency 0.6364) and was discounted at our centre as a benign population variant.

As part of his diagnostic work-up, the proband underwent chromosomal microarray. The cytogeneticist was informed of the clinical suspicion of APECED, with *AIRE* a gene of interest. Cytogenetic analysis identified long continuous stretches of homozygosity representing 2.2% of the genome, consistent with known consanguinity. A small novel homozygous intragenic *AIRE* deletion, incorporating exons 2 and 3, was identified in a region of homozygosity (arr[hg19] 21q22.3(45,706,476-45,707,016)x0; 7 probes deleted) (Figure 2). This was predicted to result in a frameshift and a truncated protein (NG_009556.1(NM_000383.4): c.(132+1_133-1)_(463+1_464-1)del; NP_000374.1(*AIRE*): p.(Glu45Alafs*3)) likely to undergo nonsense-mediated decay, with no expected residual protein function. Based on the spectrum of pathogenic variants in the disease database ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>), loss of function is a recognised mutational mechanism for *AIRE*, with biallelic (homozygous or compound heterozygous) variants causing autosomal recessive APECED.

The proband's clinical diagnosis of APECED was confirmed and he was commenced on intravenous corticosteroids for immunosuppression. Initial testing of adrenal function was equivocal, and further assessment was confounded by immunosuppressive glucocorticoid therapy. Following development of odynophagia, endoscopy demonstrated oesophageal

candidiasis. He was commenced on azathioprine for autoimmune hepatitis and remains in complete biochemical remission.

Segregation testing in the proband's siblings identified his brother (V:1) and sister (V:4) as unaffected carriers, while his younger sister (V:5), previously considered to be clinically unaffected, was also homozygous for the deletion. In retrospect, her parents report a previous unexplained urticarial rash, which has been reported as a pre-symptomatic disease manifestation.⁽⁴⁾ She is currently undergoing full clinical assessment to determine whether she has any further manifestations of APECED. The parents expressed disappointment that one of their daughters, conceived using preimplantation genetic diagnosis, has the same condition as the proband.

Discussion

This case report describes an adolescent male with a complex multisystem presentation who received a rare disease diagnosis through cytogenetic analysis (chromosomal microarray) following non-diagnostic exome sequencing. The case was interesting for the diagnostic dilemma posed by an unusual combination of clinical features and an unexpected mutational mechanism.

A monogenic disorder was considered at an overseas centre. Genomic sequencing such as exome sequencing is often considered early in the investigation of suspected monogenic disorders, particularly in the context of consanguinity, where homozygous sequence variants

are suspected as a potential cause of disease. However, as this case demonstrates, copy number changes not detectable by exome sequencing are also a potential cause of monogenic conditions in the context of consanguinity. The frequency with which homozygous deletions are detected by microarray in recessive conditions has not been systemically studied. At a fraction of the cost of exome sequencing, a microarray may provide a diagnostic answer when the mutational mechanism is amenable to cytogenetics. Array platform selection depends on probe coverage over the genes of interest, which requires good communication of the clinical presentation and suspected diagnoses between clinicians and cytogeneticists.

Emerging techniques for copy number analysis on genomic sequencing data may improve detection of deletions and duplications in the future. Copy number variant detection is superior in whole genome sequencing compared to exome sequencing, and in the future, this platform may provide sufficient copy number analysis alongside sequence variant analysis for many cases (5). Copy number variant detection for exome sequencing is more technically difficult (5) and in current practice, copy number variant detection on either exome or genome sequencing does not yet replace chromosomal microarray, particular newer array platforms that offer high-resolution copy number analysis.

A clinical diagnosis of APECED was suspected in this patient. Although liver manifestations are uncommon, a variable autoimmune hepatitis phenotype is reported in 5-20% of individuals with APECED.(4) An interesting antibody target profile with autoantibodies specific for liver-expressed cytochromes CYP1A2 and CYP2A6 differs from isolated

autoimmune hepatitis where ANA, anti-smooth muscle, anti-liver cytosol, anti-soluble liver protein/liver pancreas, and anti-P450 2D6 autoantibodies may be detected.⁽⁶⁾ Whilst usually self-limiting, a fulminant course has been reported and thus recommendations include immediate immunosuppression.⁽⁶⁾ The proband in this case demonstrated elevated transaminases, decreased synthetic function with coagulopathy, and a liver biopsy that showed non-specific hepatitis with bridging necrosis, which in retrospect was thought to be atypical for but consistent with autoimmune hepatitis in the context of APECED.

With the recent Australian government funding for exome sequencing in children with intellectual disability, this case provides a timely reminder of the limitations of exome sequencing. Copy number changes, intronic variants, presence of pseudogenes, methylation changes, repeat expansions, and mitochondrial variants are all examples of variant types not well covered by exome sequencing, although advances in bioinformatics may allow some of these variants to be more readily detected in the future. This case also highlights the importance of good communication between clinicians and cytogeneticists, which facilitated detection of a very small pathogenic copy number change in *AIRE*. In the absence of the provision of phenotypic information, small copy number changes of unclear significance may be overlooked.

While a discussion of ethical issues is beyond the scope of this article, this case also highlights the need for diagnostic certainty prior to utilisation of variants for reproductive management of individuals and families with inherited conditions.

In summary, we have demonstrated a novel homozygous intragenic deletion in *AIRE*, diagnosing APECED, detected by cytogenetic analysis but not exome sequencing. This case provides an example of the limitations of exome sequencing in detecting copy number changes and serves as a timely reminder of the role of complementary genetic tests including cytogenetic analysis in the genomic era.

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Tables

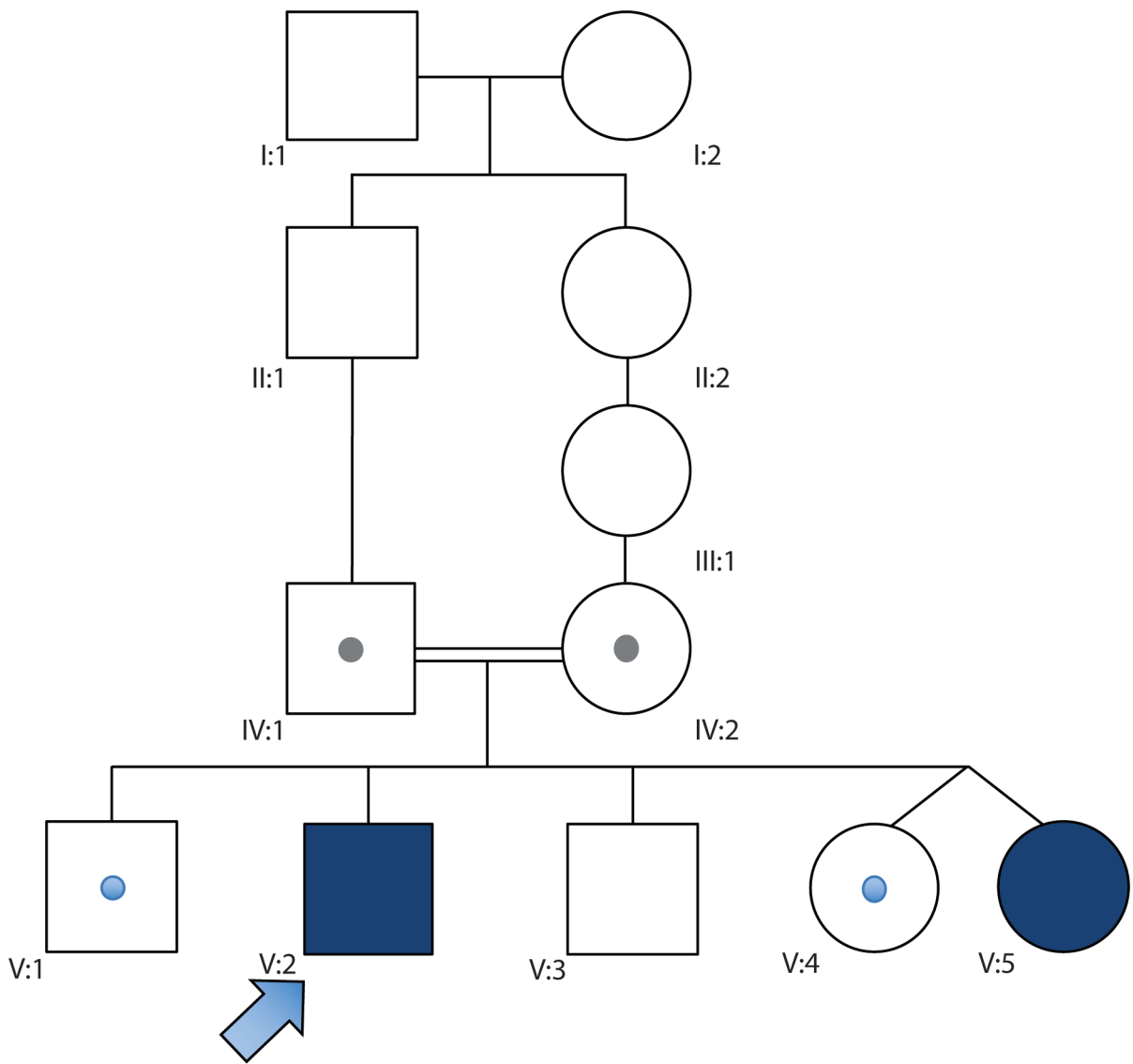
Table 1. Variant types difficult to detect by exome sequencing

Variant type	Examples (see GeneReviews)(7)
<i>Copy number changes:</i>	
- Multigenic deletions	Williams syndrome (7q11.23 deletion)
- Single gene deletions	Neurofibromatosis type 1 (<i>NFI</i>)
- Intragenic deletions	Spinal muscular atrophy (<i>SMN1</i> , exon 7)
<i>Methylation changes</i>	Angelman syndrome (15q11.2q13)
<i>Intronic variants</i>	Fukuyama congenital muscular dystrophy (deep intronic)
<i>Repeat expansions:</i>	
- Triplet repeat	Fragile X syndrome (<i>FMRI</i> , CGG repeat)
- Polyalanine repeat	Congenital central hypoventilation syndrome (<i>PHOX2B</i>)
<i>Mitochondrial variants</i>	Pearson syndrome (mtDNA deletion)
<i>Presence of pseudogenes</i>	Polycystic kidney disease (<i>PKDI</i>)

Figure Legends

Figure 1. Pedigree with affected siblings shown in dark blue, confirmed carrier siblings with a blue central circle, and presumed carrier parents with grey central circle. The proband is indicated by the arrow.

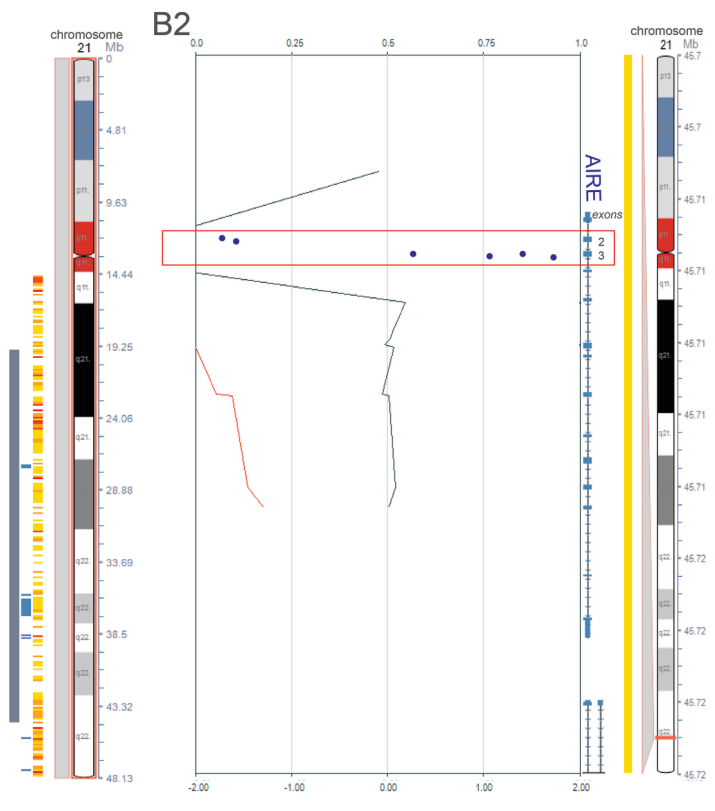
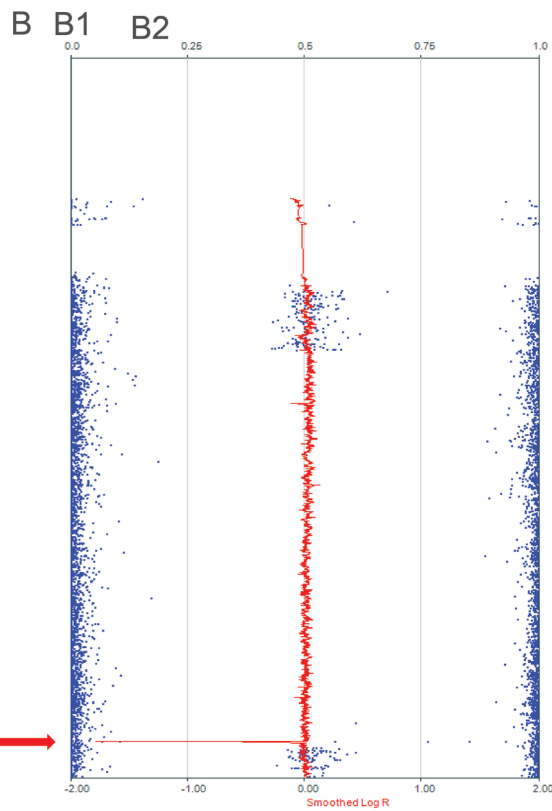
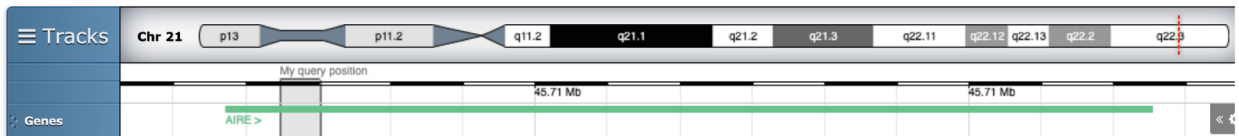
Figure 2. Novel homozygous intragenic *AIRE* deletion. A) DECIPHER genome browser(8) showing deleted intragenic region. B) Chromosomal microarray showing B1. Loss of signal within a region of homozygosity on chromosome 21q22.3 (red arrow); B2. Localisation of the loss of signal (7 probes) to exons 2 and 3 of *AIRE* (red box). This figure has been digitally altered to improve visibility of the SNP array probes.



JPC_15124_Figure 1.tif

A Genome Browser 21:45,706,476-45,707,016

Jump to position, gene or band



JPC_15124_Figure 2 digitally enhanced-01.tif