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# Beyond the molecular diagnosis: clinically relevant findings from genomic reassessment in patients with Prader-Willi syndrome

**Elizabeth Worthey**

lworthey@uab.edu

University of Alabama Birmingham

**Manavalan Gajapathy**

https://orcid.org/0000-0002-8606-0113

**Brandon Wilk**

**Donna Brown**

**Caroline Vrana-Diaz**

**Gurpreet Kaur**

https://orcid.org/0000-0001-8426-7666

**Tarun Mamidi**

**Deeptha Srirangam**

**Jessica Bohonowych**

https://orcid.org/0009-0006-1021-9956

**Jaimie Richards**

**Shaurita Hutchins**

**Bitota Lukusa-Sawalena**

**Theresa Strong**

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Table 1 to 3 are available in the Supplementary Files section.

**Title:**

# Beyond the molecular diagnosis: clinically relevant findings from genomic reassessment in patients with Prader-Willi syndrome

**Author names:**

Manavalan Gajapathy[1*], Brandon M. Wilk[1*], Donna M Brown[1], Caroline Vrana-Diaz[2], Gurpreet Kaur[1], Tarun Karthik Kumar Mamidi[1], Deeptha Srirangam[1], Jessica Bohonowych[2], Jaimie L. Richards[1,3], Shaurita D. Hutchins[1], Bitota Lukusa-Sawalena[1], Theresa V. Strong[1,2], Elizabeth A. Worthey[1#]

**Author affiliations:**

[1]Department of Genetics, Marnix E. Heersink School of Medicine, University of Alabama Birmingham, Birmingham, AL, USA

[2]Foundation for Prader-Willi Research, Covina, CA, USA

[3]Division of General Internal Medicine and Population Science, Marnix E. Heersink School of Medicine, University of Alabama Birmingham, Birmingham, AL, USA

[*]These authors contributed equally to this work.

[#]**Corresponding Author:**

Elizabeth A. Worthey, lworthey@uab.edu, (205) 934-3496

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**ABSTRACT**

Prader-Willi syndrome (PWS) results from loss of paternal gene expression at chromosome 15q11.2-q13. Conventional testing reliably establishes the diagnosis and defines the molecular class but may not resolve complex chromosome 15 architecture or detect genome-wide variation relevant to medication response and other clinical risks. Historical results generally remain the basis for lifelong classification without re-evaluation as technologies advance.

We evaluated whether genome sequencing (GS) could add clinically useful information beyond molecular confirmation of PWS. Fifty individuals underwent GS through a fully remote, registry-enabled study integrating structural, copy-number, small-variant, pharmacogenomic, phenotype, and medication-history analyses with multidisciplinary interpretation, genetic counseling, and return of selected findings.

Overall, 34/50 participants (68%) had at least one finding beyond that provided by conventional PWS testing. GS revealed previously unresolved or more complex chromosome 15 architecture in 13/50 participants (26%), refined or resolved molecular subtype assignments in 8/50 (16%), identified guideline-supported pharmacogenomic recommendations in 20/48 (41.7%), detected ACMG secondary findings in 2/47 (4.3%), and identified additional variants for clinically contextualized return in 12/47 (25.5%); categories were not mutually exclusive.

In the largest GS PWS cohort reported to date, GS improved resolution of the disease-causing locus while identifying additional findings relevant to prescribing, surveillance, and individualized clinical evaluation. These results support genomic reassessment as a means of refining longstanding molecular classifications and identifying clinically relevant variation beyond the primary diagnosis, whilst demonstrating the feasibility of comprehensive genomic interpretation and responsible return of results through a fully remote framework.

**KEYWORDS**

53 Prader-Willi syndrome; genome sequencing; pharmacogenomics; genomic reassessment;
54 structural variation
55

## INTRODUCTION

Prader-Willi syndrome (PWS; OMIM 176270) is a rare genetic disorder affecting approximately 1 in 10,000-30,000 live births [1]. Neonatal hypotonia and feeding difficulty are followed by hyperphagia, endocrine dysfunction, developmental disability, and characteristic behavioral manifestations, including impulsivity, temper outbursts, cognitive rigidity, obsessive-compulsive symptoms, and self-injury [2]. Management commonly requires endocrine, nutritional, behavioral, psychiatric, cardiac, respiratory, and orthopedic care [3].

PWS results from loss of expression of paternally derived genes within 15q11.2-q13, most commonly through paternal deletion (~60%), maternal uniparental disomy (UPD; ~35%), or an imprinting defect [4]. Deletions are commonly categorized as Type I, spanning BP1-BP3, or Type II, spanning BP2-BP3. Molecular diagnosis emerged through chromosome 15 deletion and parental-origin studies in the 1980s and was refined by methylation testing in the early 1990s. Parent-specific methylation analysis now detects >99% of cases, with copy-number, parental-origin, and imprinting-center studies used to define the underlying mechanism [5]. However, depending on the testing performed, these approaches may not fully resolve breakpoint architecture, complex rearrangements, clinically significant regions of homozygosity, or genome-wide variation relevant to medication response and other clinical risks. Clinical features overlap across PWS subtypes, but the frequency of some manifestations, particularly psychiatric disorders, differs by molecular subtype [6].

Conventional testing reliably establishes PWS, but historical results may remain the basis for lifelong molecular classification without re-evaluation as technologies advance. Individuals diagnosed using earlier technologies may therefore have little reason or opportunity to benefit from subsequent advances in genome-wide analysis. We asked what contemporary genomic evaluation might add once the primary molecular diagnosis is already established, applying comprehensive GS to determine whether reassessment could refine PWS molecular architecture and identify clinically relevant variation beyond the primary diagnosis.

## MATERIALS AND METHODS

### Participants

Fifty individuals with molecularly confirmed PWS were recruited through the Global PWS Registry, managed by the Foundation for Prader-Willi Research (FPWR). The study was approved by the North Star Non-Profit Research Ethics Review Board (protocol NB100013), with remote identity verification and sample chain-of-custody documentation. Recruitment and consent are described elsewhere [7]. All study procedures were conducted remotely. Participants or legally authorized representatives provided consent by videoconference and selected return-of-results preferences. Registry patient or caregiver-reported surveys gathered demographics, PWS diagnosis methods and results, anthropometric data, congenital anomalies, sleep, seizures, endocrine history, orthopedics, vision, thrombosis, behavior/mental health, hyperphagia/food behaviors measured by the Hyperphagia Questionnaire for Clinical Trials (HQ-CT) [8], anxiousness as measured by the PWS Anxiousness and Distress Questionnaire (PADQ) [9], and medications.

### Genome sequencing and quality control

DNA was extracted from dried blood spots and sequenced using PCR-free 2 x 150-bp libraries on an Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA), targeting at least 30× mean coverage. Reads were aligned to GRCh38 using BWA-MEM. QuaC was used for sequencing quality control [10]. Coverage ranged from 29-66x (Supplementary Table I). Software versions, detailed criteria, and workflow parameters are provided in Supplementary Methods.

### Structural variant, copy-number, and homozygosity analysis

Structural and copy-number variants were detected using Manta and CNVpytor and manually reviewed after annotation and population filtering. Exomiser supported phenotype-

based prioritization. Regions of homozygosity (ROH) were identified using AutoMap and filtered to exclude genomic gaps, coverage artifacts, heterozygous deletions, and low-confidence regions; detailed thresholds are provided in the Supplementary Methods.

**GS-based PWS molecular subtyping and concordance analysis**

GS-based subtype classification was performed without reference to prior labels (Figure 1). Prior methylation or parental-origin results were reviewed to confirm consistency with GS findings and where GS could not distinguish heterodisomic UPD from an imprinting defect due to epimutation (Figure 1). Deletions were categorized using GRCh38 breakpoint coordinates (Table I) as Type I (BP1–BP3), Type II (BP2–BP3), imprinting-center, or atypical, and described as classic or complex according to contiguous versus noncontiguous copy-loss profiles. Prior methylation or parental-origin results were consulted when GS alone could not distinguish heterodisomic UPD from epimutation-related imprinting defects.

Complete or near-complete chromosome 15q ROH was interpreted as isodisomy and cumulative ROH >5 Mb as segmental isodisomy; cases with additional CNVs in the imprinted region were classified as atypical UPD. GS-based PWS subtype assignments were compared with participant-provided classifications to assess concordance and were used for all downstream analyses. In participants without a deletion or extensive chromosome 15 ROH, GS alone could not distinguish heterodisomic UPD from an epimutation-related imprinting defect; where available, prior parental-origin or methylation results were used to resolve this distinction.

**Genotype-phenotype analyses**

FPWR Registry derived phenotypes were mapped to Human Phenotype Ontology terms [11] using a custom Python workflow and manual review. Hyperphagia and anxiousness were defined as HQ-CT ≥4 and PADQ ≥15. Phenotypic similarity was calculated using *hpo3* (v1.3.1) with average-linkage clustering; 31 parent phenotype categories were visualized using *plotly*

(v6.3.0). Quantitative fields and missing responses were excluded from similarity calculations. Subtype frequencies were compared using Fisher's exact tests with Benjamini–Hochberg correction [12].

**Small-variant analysis**

Small variants were called using GATK HaplotypeCaller and annotated using clinical, population, and prediction resources described in the Supplementary Methods. Interpretation considered allele frequency, molecular consequence, conservation, transcript relevance, gene-disease validity, inheritance, phenotype concordance, and published evidence. Variants were classified according to ACMG/AMP guidelines and evaluated separately for participant return or research aggregation (Supplementary Table III).

**Pharmacogenomic analysis**

PGx star alleles, diplotypes, and copy-number states were generated using Aldy and interpreted using PharmCAT calls. Metabolizer phenotypes and gene-drug recommendations were assigned using CPIC, DPWG, and FDA guidance. Rare medication-safety variants outside standard star-allele models were reviewed separately. Participant-reported medications were matched to pharmacogenes, and findings were considered actionable when supported by prescribing guidance specifying dose modification, monitoring, or avoidance, as described in the Supplementary Methods.

Cohort metabolizer frequencies were compared with a previous PWS cohort and ancestry-specific reference populations using exact binomial and multinomial goodness-of-fit tests, with Benjamini-Hochberg correction. A review of commonly prescribed medications in PWS was performed (Supplementary File 1).

## Confirmation and reporting

All findings considered for return were reviewed by a multidisciplinary committee comprising clinical variant analysts, geneticists, computational biologists, and PWS experts. Return categories included: 1) ACMG SF v3.0 findings, 2) guideline-supported pharmacogenomic findings, and 3) selected non-ACMG findings for which additional evaluation, surveillance, medication review, or specialist consultation could reasonably be considered [13,14]. Non-ACMG findings were returned as contextualized observations rather than diagnoses and only when multidisciplinary review determined that they could reasonably inform low-risk clinical evaluation or interpretation of an existing phenotype.

Return decisions considered gene-disease validity, inheritance, variant classification, phenotype concordance, penetrance, availability of confirmatory evaluation, potential risks, and clinical consequences of non-disclosure. Heterozygous findings in recessive genes were considered only when published evidence supported a possible carrier phenotype or the finding could inform interpretation of PWS-relevant clinical testing, and low-risk clinical evaluation could clarify its relevance.

Variants selected for return were orthogonally confirmed by Sanger sequencing, with long-range PCR as needed to avoid pseudogene interference. GS-derived PGx calls were cross-validated using a commercial Kailos (Huntsville, AL) panel from independently collected buccal samples. Concordance was assessed for loci covered by both approaches.

# RESULTS

GS demonstrated substantially greater molecular diversity within conventional PWS molecular classes than was captured by historical subtype labels. This included refinement of the primary PWS molecular architecture as well as pharmacogenomic and secondary findings elsewhere in the genome.

## Participants

Participants resided in 27 U.S. states. Twenty-eight of 50 (56%) were female. Age ranged from 10 to 47 years (mean 20.0, SD 9.7), including 28 pediatric participants. Mean BMI was 25.3 kg/m² (range 15.8 to 46.0, SD 7.5). Prior classifications included 29 deletions, 17 UPDs, three imprinting-center defects, and one unknown subtype; deletion subtypes were incompletely specified in 14 cases. These frequencies were consistent with prior reports [15].

All participants completed the study remotely and elected primary findings; 49 elected PGx analysis, 48 completed orthogonal PGx testing, and 47 elected secondary/additional findings. Twenty-five (50%) reported ≥7 medications, over-the-counter products, or supplements, and 35 (70%) reported a prescription change in the preceding two years because of lack of effectiveness (36%), new symptoms (34%), side effects (24%), or no longer needing the medication (14%).

## Refinement of chromosome 15 architecture

GS revealed additional architectural complexity in 13 participants, whereas this information changed or resolved the clinically recorded molecular subtype in eight.

## Deletions

Large heterozygous deletions involving the PWS critical region were identified in 29 participants: nine Type I, 17 Type II, and three atypical deletions with noncanonical boundaries (Figure 2). Read-depth and B-allele-frequency profiles reliably detected copy loss, although precise breakpoint resolution was limited by the region's dense segmental duplications and pericentromeric architecture. Deletions were subclassified as classic (contiguous) or complex (noncontiguous) based on architecture (Supplementary Figures 1-3).

Most Type I deletions were complex (8/9), whereas most Type II deletions were classic (15/17). Complex Type I endpoints varied across the BP1-BP3 *GOLGA6*/*GOLGA8* and 7SL

RNA pseudogene-rich repetitive regions. Some apparent interruptions may reflect short-read mapping limitations, although this is unlikely to explain all observed complexity. Overall, conventional Type I and Type II labels encompassed substantially greater structural diversity than their categorical designations imply. Of three atypical deletions, two terminated proximal to BP3 and one extended from BP1 beyond BP5 (Figure 2, Supplementary Figure 4). Two cases showed apparent noncontiguous copy loss, including a spared interval also observed in Type II profiles. No large chromosome 15 ROH (>5 Mb cumulative) were present in participants with Type I, Type II, or atypical deletions.

Three participants carried BP4-BP5 duplications encompassing *CHRNA7*, the α7 nicotinic acetylcholine receptor subunit. This region is flanked by highly homologous *GOLGA8*-containing segmental duplications that mediate recurrent 15q13.3 microdeletions and microduplications associated with neurodevelopmental, behavioral, psychiatric, and seizure phenotypes [16,17]. These manifestations were present in carriers, although no independent effect could be inferred given the small number and overlap with PWS symptomology.

All Type II deletions spared the BP1-BP2 interval containing *NIPA1*, *NIPA2*, *CYFIP1*, and *TUBGCP5*, although additional structural variability was observed. One atypical deletion terminated within *GABRG3*, preserving *OCA2* and *HERC2* (Figure 2), potentially preserving pigmentation-related effects associated with *OCA2* and *HERC2*; the clinical consequences of disruption near *GABRG3* remain unclear [18].

GS recharacterized one imprinting-center deletion as a 185-kb event encompassing the *SNURF-SNRPN* promoter/PWS imprinting center, *PWAR5*, and proximal *SNORD* genes while sparing *SNORD116* (Supplementary Figure 4). Although *SNORD116* remained intact, loss of the *SNURF-SNRPN* promoter/PWS IC would disrupt transcription of the downstream paternal host transcript, providing a mechanism for PWS [19].

## Uniparental disomy

Among 21 participants without a PWS-region deletion, GS identified two with near-complete chromosome 15 isodisomy, 11 with segmental isodisomy, and one with atypical UPD accompanied by a duplication CNV within the imprinted domain (Figure 3; Supplementary Figure 5). Six participants lacked extensive chromosome 15 ROH and could not be distinguished by GS alone as heterodisomic UPD versus imprinting defect due to epimutation. This distribution was consistent with expectations [20]. Three participants with UPD carried the same short BP1-proximal 7SL pseudogene/golgin region duplication noted above.

Segmental-isodisomy profiles varied in number, size, and location of ROHs, consistent with differing recombination patterns after trisomy rescue [21]. Transitions between isodisomic and heterodisomic regions likely mark crossover boundaries, although some shorter apparent gaps may reflect low variant density. Isodisomic segments variably included the PWS region or distal 15q, including q23-q26. Their size and location determine which maternally inherited recessive alleles become homozygous, potentially unmasking additional disorders.

## Subtype refinement

Our GS subtype classifications were concordant with previously reported assignments in 42/50 participants (84%) and provided refinement or resolution in the remaining eight (16%) (Figure 4). Refinements included previously unrecognized isodisomy, reclassification among all deletion subtypes, and additional CNV complexity (Figures 3-4; Supplementary Figures 4-5). A previously reported distal 15q26 deletion was not detected. In another with an unspecified deletion and t(15;20)(q14;q13.3), GS could not fully resolve the rearrangement but identified an atypical deletion extending from BP2 beyond BP5 to the *GOLGA8* array near *DPH6*. A participant previously classified with segmental isodisomy was reclassified as atypical UPD after identification of an additional ~310-kb duplication and concurrent deletion within 15q11.2.

GS could not independently distinguish heterodisomic UPD from an imprinting defect by epimutation in six participants without extensive chromosome 15 ROH, due to absence of parental GS and methylation data. Overall, GS improved structural resolution beyond standard diagnostic testing but complemented rather than replaced methylation or parental-origin studies, which remained necessary to resolve these cases.

**Phenotypic heterogeneity across molecular subtypes**

HPO-based profiling showed phenotypic heterogeneity within and across molecular subtypes (Supplementary Figure 6). Core PWS manifestations, including behavioral disturbance, sleep-related breathing disorder, vertebral and growth-hormone abnormalities, daytime somnolence, and hyperphagia, occurred in every molecular subtype, but were not uniformly reported in all individuals. Type I deletions showed nominally higher frequencies of hyperphagia, hip dysplasia, fractures, and inflexibility, whilst psychotic features were more frequent in UPD and imprinting-defect groups. Age, BMI, HQ-CT, and PADQ distributions overlapped broadly, and HPO-based clustering did not produce clear subtype-specific groups. No subtype-associated phenotype remained significant after multiple-testing correction, consistent with substantial clinical heterogeneity within conventional molecular classes [6,22].

**Pharmacogenomic findings relevant to medication management**

Guideline-supported gene–drug recommendations were identified in 20/48 participants (41.7%), including four with multiple recommendations (Table II). Metabolizer frequencies did not differ from ancestry-specific references and more closely resembled population expectations than reported in a previous PWS cohort [23-25.] For example, CYP2D6 poor metabolizers comprised 2.4% of our cohort versus 2.2% in reference populations, while CYP2C19 intermediate metabolizers comprised 23.8% versus 21.4%, respectively. We found no evidence for PWS-specific enrichment; differences from the earlier cohort may reflect ascertainment,

ancestry, assay coverage, or noted enrichment for complex behavioral/psychiatric concerns and prior medication failure. Findings with guideline-supported prescribing implications were returned to inform future medication selection, dosing, and monitoring.

Six participants were *CYP2C19* poor metabolizers, for whom guidelines recommend dose reduction or alternative therapy for citalopram/escitalopram because of increased exposure and QT-prolongation risk [26]. These agents are often prescribed for anxiety, rigidity, and mood symptoms and may compound behavioral or cardiac risk [27]. Four with moderate-to-severe anxiety had discontinued these drugs because of worsening symptoms (n=3) or lack of efficacy (n=1). Worsening was biologically consistent with reduced clearance, although incomplete dosing and temporal data precluded causal attribution.

Seven participants were *CYP2C19* rapid metabolizers, a phenotype associated with reduced citalopram/escitalopram exposure and potentially reduced probability of response [23,24,26]. Three were taking one of these agents and reported benefit, consistent with the fact that this phenotype does not preclude clinical response.

Drug-specific guidance applies to several psychotropic and stimulant medications used in PWS [26,28]. One participant was a *CYP2D6* poor metabolizer, 12 were intermediate metabolizers, and two were ultrarapid metabolizers. *CYP2D6* guidance exists for several psychotropic and stimulant medications, including venlafaxine, aripiprazole, risperidone, and selected amphetamine formulations [26]. For poor metabolizers, guidance may support alternative therapy, dose reduction, or enhanced monitoring [26,28]. Three intermediate metabolizers reported use of *CYP2D6*-substrate psychotropics or stimulants; whilst the poor metabolizer had not received these agents. One ultrarapid metabolizer had discontinued a *CYP2D6*-metabolized medication after worsening symptoms, although causality could not be established. This phenotype is associated with reduced exposure to selected drugs, and increased conversion of codeine or tramadol to active metabolites [28].

Beyond common diplotype-based metabolizer phenotypes, GS identified rare

medication-safety variants in three participants that would not typically be captured by standard pharmacogenomic panels. One participant carried a hemizygous pathogenic *G6PD* variant associated with reduced enzyme activity and susceptibility to oxidant-induced hemolysis during infection, after fava bean ingestion, or following exposure to certain medications, supporting targeted avoidance and clinical vigilance [29,30]. This is relevant in PWS, where recurrent infections and polypharmacy may increase the likelihood of exposure to precipitating factors [31].

Two participants carried rare variants in *POLG* or *POLG2* genes essential for mitochondrial DNA replication and maintenance [32,33]. The *POLG* variant was classified as likely pathogenic (ClinVar ID: 1957072). Evidence supporting pathogenicity included pathogenic variation affecting the same residue and functional evidence of a deleterious effect, although the available evidence remained insufficient to classify the variant categorically as pathogenic. The *POLG2* splice-site variant was also classified as likely pathogenic (ClinVar ID: 1318948), supported by its occurrence in individuals with progressive external ophthalmoplegia and mitochondrial DNA deletions and reported segregation with disease in affected relatives. Because pathogenic variation in these genes can increase susceptibility to valproate-associated hepatotoxicity, both findings were returned with explicit uncertainty and a recommendation for clinical reassessment before valproate exposure when relevant [32,33].

**Established guideline ACMG findings**

ACMG secondary findings were identified in 2/47 (4.3%) who opted in for this return, consistent with reported yields in other studies (Table III) [34]. One participant carried a pathogenic *BRCA2* frameshift associated with hereditary breast, ovarian, pancreatic, and prostate cancer risk, supporting clinical testing and guideline-based surveillance [35]. In PWS, where comorbidities may delay diagnosis and complicate care, identifying hereditary cancer predisposition may support proactive surveillance [36]. A second participant carried a pathogenic *CACNA1S* nonsense variant associated with malignant hyperthermia susceptibility and

hypokalemic periodic paralysis, with direct implications for anesthesia planning and evaluation of episodic weakness [37]. This finding is particularly relevant in PWS where anesthesia exposure is common and episodic weakness may compound baseline hypotonia and motor instability [38].

**Additional clinically contextualized observations**

Twelve participants (25.5%) received additional research findings that, following multidisciplinary review, were considered sufficient to warrant focused evaluation, surveillance, medication review, or specialist consultation (Table III). Return required a plausible gene-phenotype relationship and a specific, low-risk clinical evaluation that could inform interpretation, with explicit qualification regarding carrier status, reduced penetrance, and clinical relevance.

**Metabolic and endocrine findings returned**

Returned metabolic/endocrine findings included pathogenic or likely pathogenic variants in *MT-ND6, ACADS, DUOX2,* and *CYP17A1*. The *MT-ND6* variant was present at 78% heteroplasmy and has been associated with Leber hereditary optic neuropathy and broader neurologic manifestations, including neurologic features, weakness, exercise intolerance, and chronic fatigue [39]. Heterozygous *DUOX2* variants have been associated with thyroid dysfunction, making this finding relevant when assessment is being performed in patients with PWS, where thyroid abnormalities are common [40,41]. Heterozygous *ACADS* and *CYP17A1* findings were returned cautiously because they may influence interpretation of metabolic, endocrine, or adrenal testing prompted by relevant phenotypes observed in PWS [42,43].

**Hematologic findings returned**

Returned hematologic findings included four participants with thromboembolism and thrombophilia-associated *F2* and *F5* variants and two with *F12* loss-of-function variants affecting

coagulation testing [44,45]. These findings were returned in the context of the existing elevated baseline thrombotic risk and testing in PWS [46,47]. Two participants were heterozygous for the low-penetrance *HFE* C282Y allele, potentially relevant to interpretation of iron or liver abnormalities in PWS, where hepatic and metabolic abnormalities are common [48,49]. One participant was heterozygous for a pathogenic *PKLR* truncating variant. Heterozygous carriers are typically asymptomatic, but may show intermediate pyruvate kinase activity that could inform evaluation of unexplained hematologic findings [50].

**Cardiac and skeletal findings returned**

A likely pathogenic *CACNA1C* splice variant was returned to support cardiac evaluation as it is associated with variably expressive autosomal-dominant arrhythmia syndromes relevant in PWS, where cardiometabolic and arrhythmia risks are increased [51]. A likely pathogenic *ALPL* frameshift was returned because heterozygous loss-of-function variants can cause hypophosphatasia, relevant to the low bone density and scoliosis common in PWS [52]. A cysteine-introducing *FN1* variant was similarly returned with explicit uncertainty because pathogenic *FN1* variants can cause dominant skeletal and renal disease, including scoliosis and fractures [53].

**Research only findings**

Beyond findings considered appropriate for participant return, GS identified additional rare small variants and SVs retained as a resource for future studies of phenotypic variability in PWS (Supplementary Tables II-III). These included 14 deletions (1.3-779.4 kb) and six duplications (27.9-389.9 kb), each unique to one participant, as well as rare heterozygous small variants in genes with potential relevance to metabolic and other clinical phenotypes. None had sufficient evidence for clinical return.

## DISCUSSION

This study demonstrates that an established molecular diagnosis need not represent the endpoint of molecular evaluation. In the largest genome-sequenced PWS cohort reported to date, GS revealed substantially greater diversity in the disease-causing chromosome 15 locus than captured by historical classifications, while also identifying genome-wide findings relevant to prescribing, surveillance, and individualized clinical evaluation. Thus, genomic reassessment can both refine the known molecular diagnosis and identify independently relevant variation. These findings also illustrate the value of interpreting genome-wide variation in the context of phenotype, medication exposure, patient preferences, and established disease biology, rather than viewing GS solely as a diagnostic test.

Subtype assignments were refined in 16% of participants, guideline-supported pharmacogenomic recommendations were identified in 42%, ACMG secondary findings in 4%, and additional variants were returned for clinically contextualized evaluation in 25.5%. Overall, 68% received at least one finding beyond conventional PWS testing.

Pharmacogenomic findings provided the most immediate opportunities to inform prescribing. Their relevance lay not in PWS-specific enrichment but in identifying genotypes that could influence drug choice, dosing, or monitoring, including rare medication-safety variants outside conventional PGx panels. Genetically influenced treatment efficacy or adverse effects may also contribute to apparent phenotypic heterogeneity. ACMG secondary findings had implications for surveillance, anesthesia planning, or family testing, whereas other findings supported focused clinical evaluation. These results indicate that genomic reassessment can add clinically relevant information for prescribing, surveillance, risk assessment, and interpretation of existing manifestations.

Refinement of PWS-locus deletion and UPD configurations improved etiologic classification and showed that conventional Type I and Type II labels simplify a broader spectrum of breakpoint and recombination diversity. This greater resolution may strengthen

future genotype–phenotype and modifier studies that currently group individuals into broad deletion, UPD, or imprinting-defect categories despite substantial genomic diversity within them.

GS nevertheless complemented rather than replaced established testing because methylation and parental-origin studies remained necessary to distinguish heterodisomic UPD from imprinting defects in some cases; long-read, methylation-aware sequencing may ultimately resolve these features within a single assay.

Several limitations should be considered. The modest cohort size limited genotype-phenotype analyses and assessment of rare findings, while short-read GS may not fully resolve complex rearrangements in repetitive regions of 15q11.2-q13. Limited parental data prevented definitive classification in some participants, and registry and caregiver-reported phenotypic and medication histories limited causal interpretation of pharmacogenomic associations. The study evaluated genomic findings and returnability rather than downstream changes in management or clinical outcomes; prospective follow-up will therefore be required to establish clinical utility.

Finally, this study demonstrates that sequencing, multidisciplinary interpretation, orthogonal confirmation, genetic counseling, and return of results can be delivered through a fully remote, registry-enabled framework. This model may be particularly valuable for rare disorders in which patients are geographically dispersed and where behavioral, developmental, medical, or caregiver burdens limit access to specialized testing.

In conclusion, an established molecular diagnosis need not mark the endpoint of molecular evaluation in rare disorders. In PWS, GS refined the primary disease-causing architecture while identifying additional findings relevant to prescribing, surveillance, and individualized evaluation. More broadly, these results support the value of genomic reassessment for individuals whose original evaluation preceded contemporary genomic technologies. As sequencing and interpretation continue to advance, patients with longstanding molecular diagnoses should not necessarily be excluded from those advances simply because their original diagnostic question has already been answered. The role of GS may therefore

extend from ending the diagnostic odyssey to revisiting diagnoses already considered molecularly resolved.

## DATA AVAILABILITY STATEMENT

Participant-level data are subject to privacy and ethical restrictions that preclude deposit in external repositories but have been, and will continue to be, made available to qualified researchers from the corresponding author under an approved data-use agreement.

## CODE AVAILABILITY

Analyses were performed using established software and computational workflows as described in the Methods. Analysis code is released under the GNU General Public License v3 at https://github.com/uab-cgds-worthey/pws_wgs_remote_study, and archived on Zenodo at https://doi.org/10.5281/zenodo.19074972. The repository contains versioned workflows, software requirements, and documentation for reproducing the computational analyses with appropriately authorized input data.

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610

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615

616 **AUTHOR CONTRIBUTION STATEMENT**

617 Conceptualization: E.A.W., T.V.S.; Funding acquisition: E.A.W.; Investigation: T.V.S.,
618 D.M.B., C.V.D., J.B.; Methodology: E.A.W., M.G., B.M.W., D.S., D.M.B., J.L.R.; Project
619 administration: M.G., B.M.W., D.M.B., E.A.W.; Resources: T.V.S., C.V.D., D.M.B., J.L.R.; Data
620 curation: M.G., B.M.W., D.S.; Formal analysis: M.G., B.M.W., D.M.B., D.S., G.K., T.K.K.M.,
621 S.D.H., B.L.S., E.A.W.; Software: M.G., B.M.W., D.S.; Supervision: E.A.W., T.V.S.; Validation:

T.V.S., J.B., C.V.D.; Visualization: M.G., B.M.W.; Writing-original draft: E.A.W., M.G., B.M.W.; Writing-review & editing: All authors.


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## ETHICAL APPROVAL

The study was approved by the North Star Non-Profit Research Ethics Review Board (protocol NB100013) and conducted in accordance with the Declaration of Helsinki and applicable regulations. Written informed consent was obtained from participants or their legally authorized representatives. Data used for analysis were deidentified.

## COMPETING INTERESTS

The authors declare no competing interests.

## FIGURE LEGENDS

**Figure 1. GS-based classification of molecular subtypes in participants with PWS.** This flowchart shows the decision framework used to assign molecular subtypes within our PWS study cohort. Because only proband GS was performed, UPD was presumed to be maternal, consistent with participants' prior PWS diagnoses. Of the six cases classified as either UPD by heterodisomy or imprinting defect by epimutation, consideration of the prior clinical molecular diagnostic testing results allowed us to resolve one case as an imprinting defect by epimutation and two cases as UPD resulting from heterodisomy.

**Figure 2. CNV and ROH called in the BP1-BP5 region from PWS participants with deletions.** PWS-region breakpoint (BP) coordinates on GRCh38 (Table I) were defined relative to known genes in the 15q11.2–q13 region and used to classify deletions. Type I deletions spanned BP1–BP3 (~6 Mb), Type II deletions BP2–BP3 (~5–5.5 Mb), and imprinting center (IC) deletions were confined to the PWS imprinting region; other deletion patterns were categorized as atypical. For descriptive analyses, deletion profiles were classified as **classic** when copy loss appeared contiguous from BP1 or BP2 through BP3 and **complex** when read-depth/BAF profiles suggested noncontiguous loss or secondary CNVs across the interval. The genes panel shows selected landmark genes (black), genes within the imprinted domain (cyan), and GOLGA6/A8 genes present at the breakpoints (red); other genes are omitted for visual clarity. Block color indicates published expression pattern: blue, paternal; gray, biallelic; brown, maternal. GS-derived CNVs (orange, duplication; blue, deletion) and ROH (dark green, pipeline-called; light green, manually verified) are shown for each participant, grouped by deletion type. No ROH were identified within the region shown. Architectural heterogeneity, including complex profiles, was observed among participants with both Type I and Type II deletions.

**Figure 3: Figure 3: CNV and ROH in chromosome 15 in participants with UPD.** Genes panel shows landmark genes within the BP1-BP5 region only; others are omitted for visual clarity. Block colors represent expected expression patterns for these genes. Brown: Maternal expression. Gray: Biallelic. Blue: Paternal. CNVs (Blue: Deletion, Orange: Duplication) and ROH (Dark green: ROH called by pipeline, Light green: manually verified ROH; missed by pipeline due to low SNV density) are shown in the cases panels, sorted by UPD type; three (1.01 Mb and 2.9 Mb in participant 39 and 2.6 Mb in participant 21) were ROH that Automap failed to call due to low SNV density. Isodisomy cases have ROH across all/most of the chr15 q-arm, segmental isodisomy cases have total ROH >5 Mb in chr15, and atypical cases have duplication CNVs in the imprinted domain region and total ROH >5 Mb. Six cases are classified by the process of exclusion, as UPD by heterodisomy or IC epimutation. UPD in these cases are presumed maternal, as they are singleton and do not have parental GS data. ROH were not called in the p-arm of acrocentric chr15 due to its highly repetitive and segmentally duplicated sequence architecture, which precludes reliable read alignment and variant detection. ROH patterns vary across cases; many show long ROH tracts interleaved with heterozygous intervals and multiple crossovers, consistent with trisomy-15 rescue after maternal meiosis I nondisjunction. Most UPD cases had segmental isodisomy.

**Figure 4. Reclassification of PWS molecular subtype based on GS findings.** Band width in the Sankey diagram is proportional to the number of participants (in parentheses). Participant-reported subtypes from the Global PWS Registry are shown on the left, GS-derived classifications in the center, and consolidated molecular classes on the right. Most prior diagnoses were confirmed, while GS added value by resolving unspecified deletions, refining conventional deletion classes, and identifying atypical structural or UPD configurations. Type I deletions span BP1-BP3 (~6 Mb) and Type II BP2-BP3 (~5-5.5 Mb); deletions were described as complex when multiple noncontiguous copy-loss segments were present and as atypical

when breakpoints or rearrangements were noncanonical. The atypical deletion marked with an asterisk extended beyond BP5 and was associated with a chromosome 15 translocation. UPD was classified as heterodisomy, isodisomy, or segmental isodisomy. Imprinting-center defects include epimutations and microdeletions affecting paternal IC methylation or copy number. GS resolved all 14 previously unspecified deletions as Type I or Type II, identified previously unrecognized deletion complexity in nine participants, reclassified one Type I deletion as Type II, two participant-reported Type II deletions as complex Type I, and one UPD case as atypical UPD with an additional 15q11.2 duplication and deletion. Deletion subtypes were refined more frequently than UPD or imprinting-defect categories.

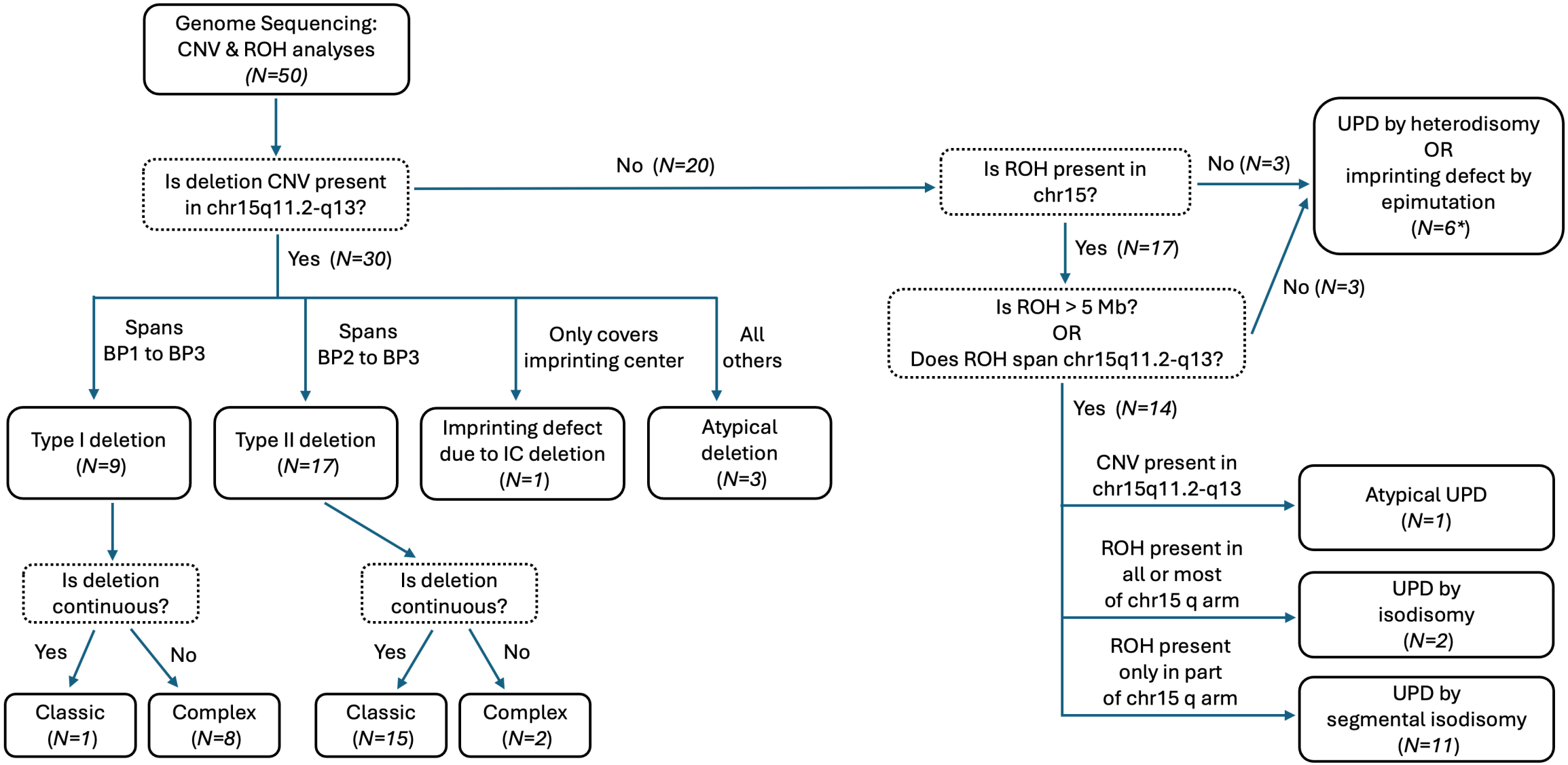
## TABLE LEGENDS

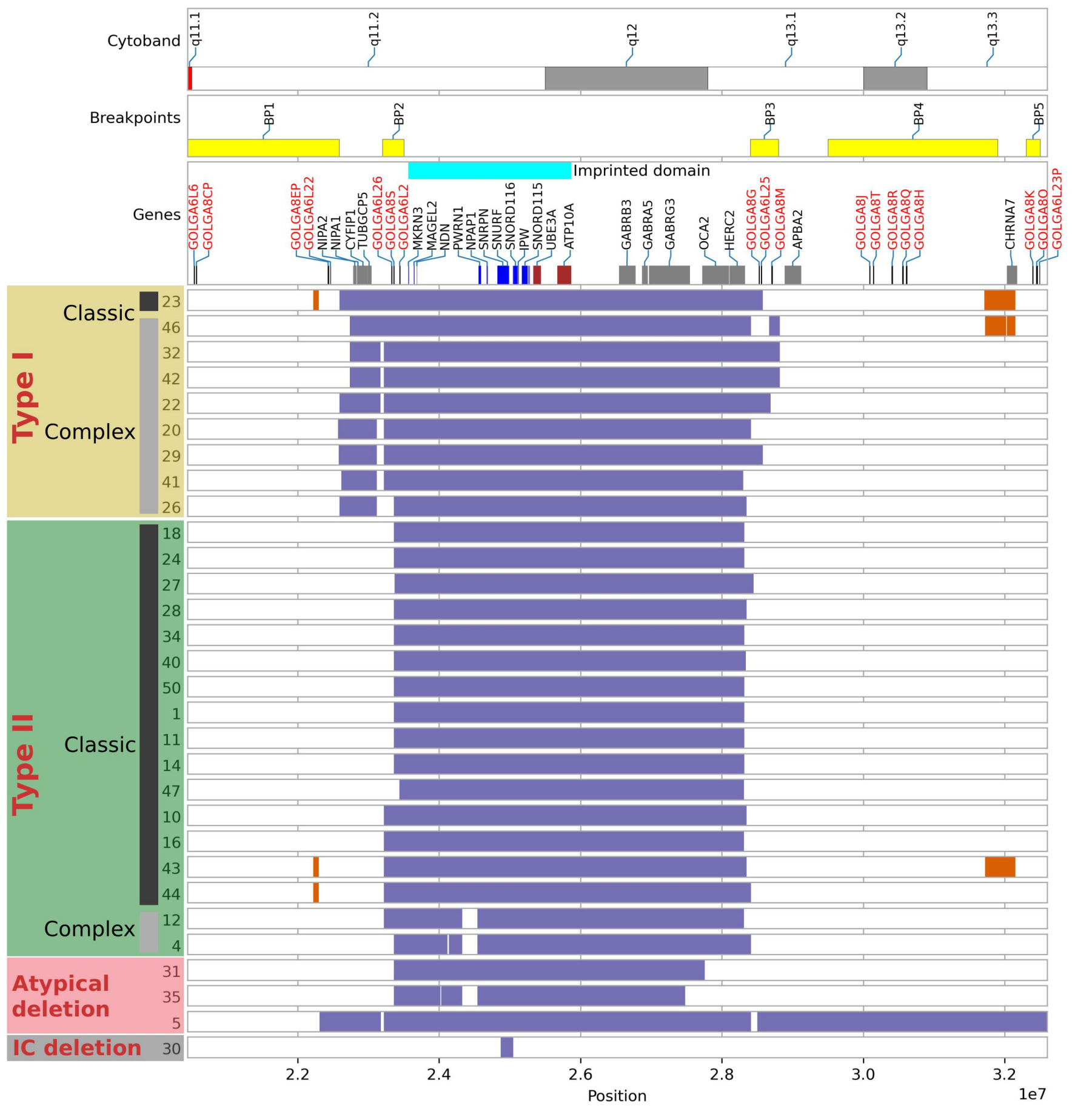
**Table I. PWS breakpoints in chromosome 15.** Canonical PWS deletions result from non-allelic homologous recombination between low-copy repeats in 15q11.2-q13, typically with proximal breakpoints at BP1 or BP2 and a distal breakpoint at BP3. Additional repeat clusters at BP4 and BP5 contribute to atypical and complex rearrangements. Coordinates are reported on GRCh38.

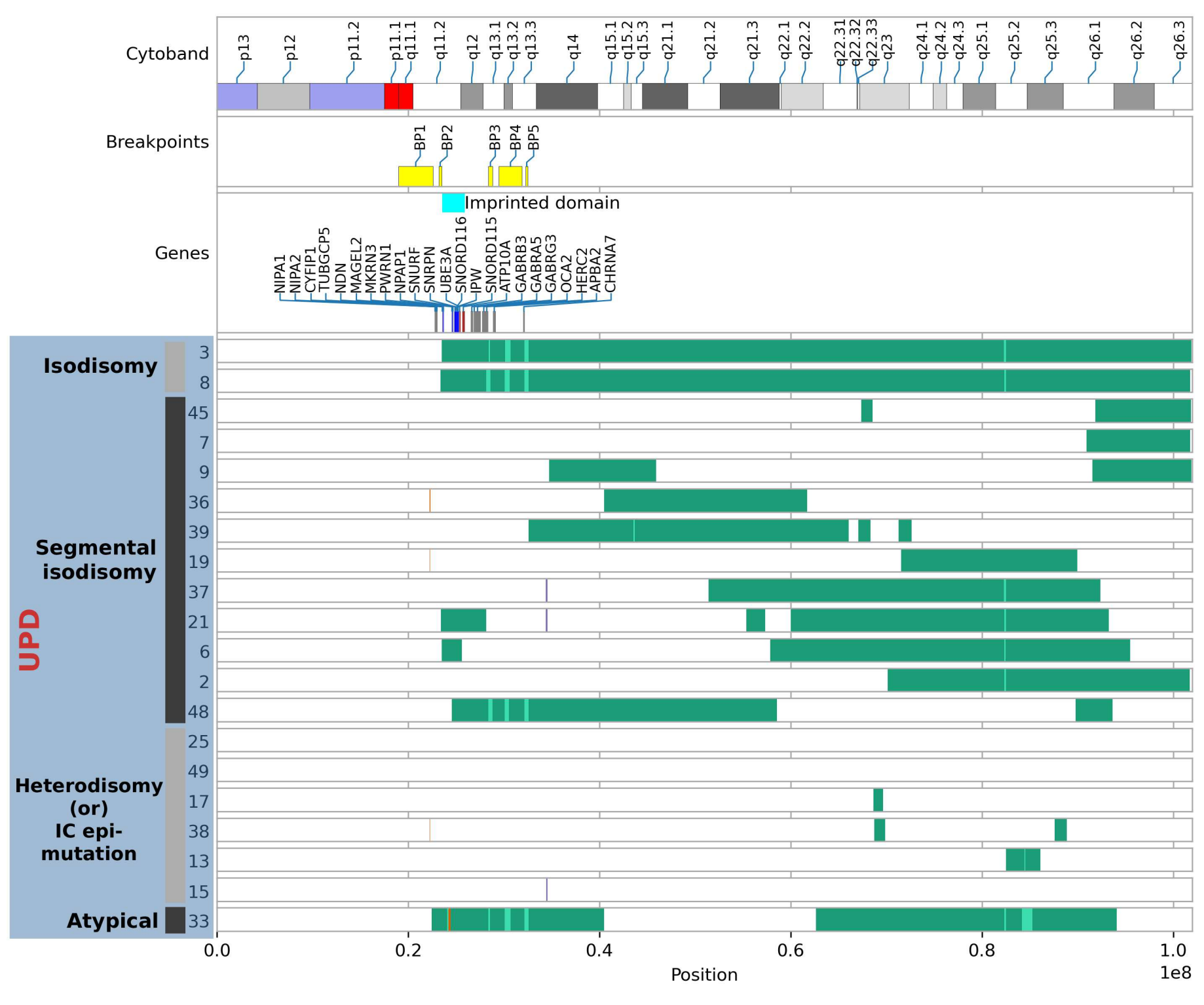
**Table II. Pharmacogenomic findings and gene-drug guidance.** Pharmacogenomic findings were derived from GS using Aldy and PharmCAT and, where represented on the commercial panel, confirmed using an independently collected buccal sample. *G6PD*, *POLG*, and *POLG2* variants were not covered by the commercial panel and were confirmed separately where applicable. Listed medications are examples; recommendations depend on drug-specific CPIC, DPWG, or FDA guidance.Pathogenic/likely pathogenic POLG/POLG2 variants may increase valproate-associated hepatotoxicity risk; the findings were returned with explicit qualification regarding the strength of evidence and recommendation for clinical reassessment before

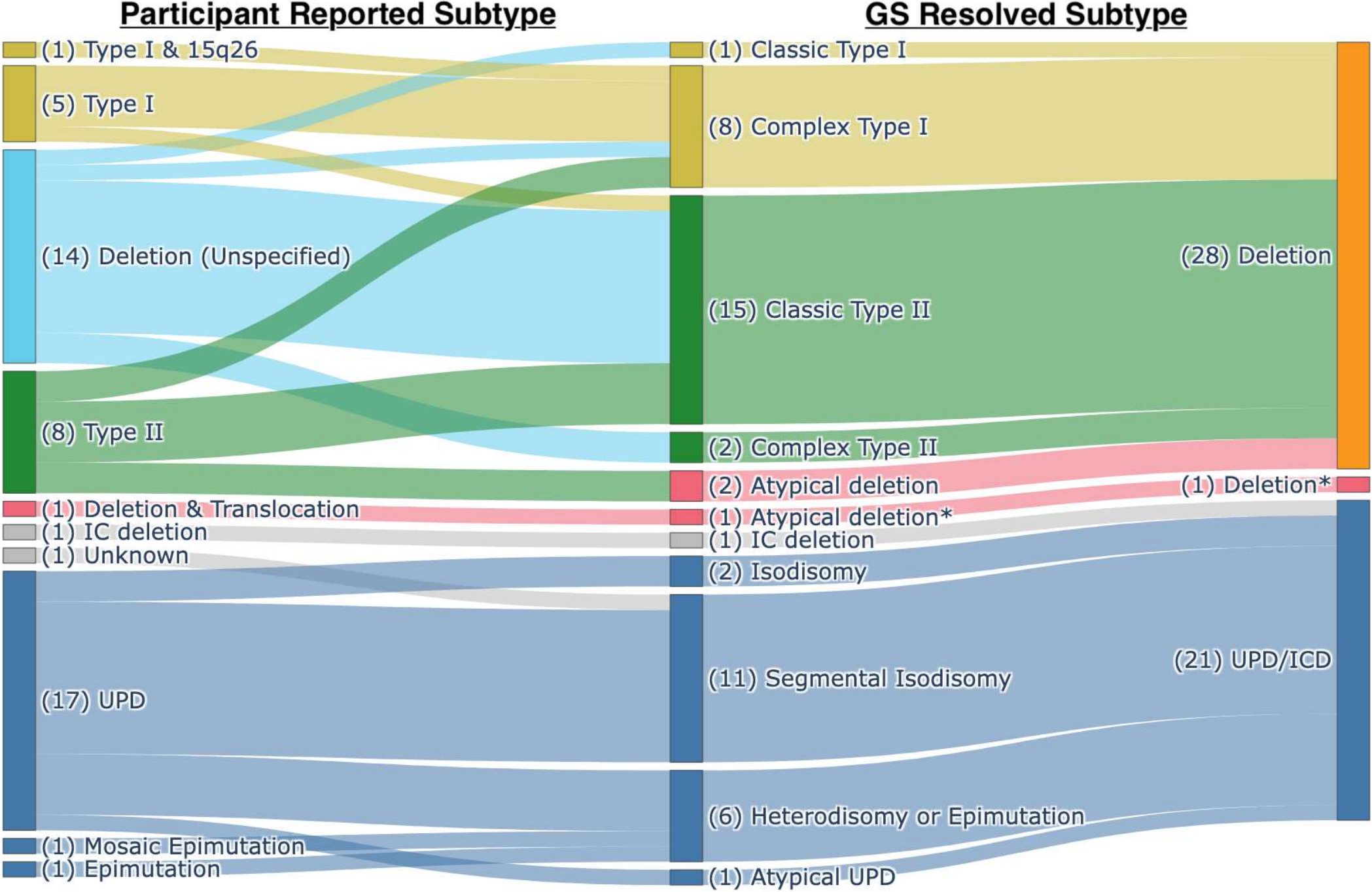
valproate exposure when relevant. In all cases GS PGx calls were fully concordant with the orthogonal Kailos panel. Abbreviations: PGx, pharmacogenomics; GS, genome sequencing; LOF, loss-of-function.

**Table III. ACMG secondary findings and additional variants returned for clinically contextualized evaluation.** Shown are ACMG secondary findings and selected non-ACMG variants returned to prompt clinically contextualized evaluation. ACMG classification, zygosity or heteroplasmy, GnomAD allele frequency (v4.1; "Not seen" indicates absence), and number of affected participants are shown. For heterozygous variants in recessive genes, return did not establish a diagnosis; results were communicated to support targeted evaluation where clinically appropriate. All returned findings underwent orthogonal confirmation using an assay appropriate to the variant type. Coordinates are reported on GRCh38.


Genome Sequencing:
CNV & ROH analyses
(N=50)
Is deletion CNV present
in chr15q11.2-q13?
No (N=20)
Is ROH present in
chr15?
No (N=3)
UPD by heterodisomy
OR
imprinting defect by
epimutation
(N=6*)
Yes (N=17)
Is ROH > 5 Mb?
OR
Does ROH span chr15q11.2-q13?
No (N=3)
Yes (N=30)
Spans
BP1 to BP3
Spans
BP2 to BP3
Only covers
imprinting center
All
others
Type I deletion
(N=9)
Type II deletion
(N=17)
Imprinting defect
due to IC deletion
(N=1)
Atypical
deletion
(N=3)
Yes (N=14)
CNV present in
chr15q11.2-q13
Atypical UPD
(N=1)
ROH present in
all or most
of chr15 q arm
UPD by
isodisomy
(N=2)
ROH present
only in part
of chr15 q arm
UPD by
segmental isodisomy
(N=11)
Is deletion
continuous?
Is deletion
continuous?
Yes
No
Yes
No
Classic
(N=1)
Complex
(N=8)
Classic
(N=15)
Complex
(N=2)


Cytoband
q11.1
q11.2
q12
q13.1
q13.2
q13.3
Breakpoints
BP1
BP2
BP3
BP4
BP5
Genes
Imprinted domain
GOLGA6L6
GOLGA8CP
GOLGA8EP
GOLGA6L22
NIPA2
NIPA1
CYFIP1
TUBGCP5
GOLGA6L26
GOLGA8S
GOLGA6L2
MKRN3
MAGEL2
NDN
PWRN1
NPAP1
SNRPN
SNURF
SNORD116
IPW
SNORD115
UBE3A
ATP10A
GABRB3
GABRA5
GABRG3
OCA2
HERC2
GOLGA8G
GOLGA6L25
GOLGA8M
APBA2
GOLGA8J
GOLGA8T
GOLGA8R
GOLGA8Q
GOLGA8H
CHRNA7
GOLGA8K
GOLGA8O
GOLGA6L23P
Type I
Classic
23
Complex
46
32
42
22
20
29
41
26
Type II
Classic
18
24
27
28
34
40
50
1
11
14
47
10
16
43
44
Complex
12
4
Atypical deletion
31
35
5
IC deletion
30
2.2
2.4
2.6
2.8
3.0
3.2
Position
1e7


Cytoband
p13
p12
p11.2
p11.1
q11.1
q11.2
q12
q13.1
q13.2
q13.3
q14
q15.1
q15.2
q15.3
q21.1
q21.2
q21.3
q22.1
q22.2
q22.31
q22.32
q22.33
q23
q24.1
q24.2
q24.3
q25.1
q25.2
q25.3
q26.1
q26.2
q26.3
Breakpoints
BP1
BP2
BP3
BP4
BP5
Imprinted domain
Genes
NIPA1
NIPA2
CYFIP1
TUBGCP5
NDN
MAGEL2
MKRN3
PWRN1
NPAP1
SNURF
SNRPN
UBE3A
SNORD116
IPW
SNORD115
ATP10A
GABRB3
GABRA5
GABRG3
OCA2
HERC2
APBA2
CHRNA7
UPD
Isodisomy
3
8
Segmental isodisomy
45
7
9
36
39
19
37
21
6
2
48
Heterodisomy (or) IC epi-mutation
25
49
17
38
13
15
Atypical
33
0.0
0.2
0.4
0.6
0.8
1.0
Position
1e8


Participant Reported Subtype
GS Resolved Subtype
(1) Type I & 15q26
(5) Type I
(14) Deletion (Unspecified)
(8) Type II
(1) Deletion & Translocation
(1) IC deletion
(1) Unknown
(17) UPD
(1) Mosaic Epimutation
(1) Epimutation
(1) Classic Type I
(8) Complex Type I
(15) Classic Type II
(2) Complex Type II
(2) Atypical deletion
(1) Atypical deletion*
(1) IC deletion
(2) Isodisomy
(11) Segmental Isodisomy
(6) Heterodisomy or Epimutation
(1) Atypical UPD
(28) Deletion
(1) Deletion*
(21) UPD/ICD

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3.xlsx
- SupplementalTableIII.Rarestructuralandcopynumbervariationoutsideofchromosome15.xlsx
- SupplementalTableII.Hypothesisgeneratingsmallvariants.xlsx
- Table1.xlsx
- Table2.xlsx
- SupplementalTableI.GenomesequencingQCmetrics.xlsx