

## ORIGINAL ARTICLE

# French Guidelines of the AchroPuce Network for the Interpretation and Reporting of Constitutional Copy Number Variants

Céline Pebrel-Richard<sup>1</sup> | Paul Kuentz<sup>2</sup> | Anne-Claude Tabet<sup>3,4</sup> | Jean-Michel Dupont<sup>5</sup> | Chantal Missirian<sup>6</sup> | Serge Romana<sup>7,8</sup> | Detlef Trost<sup>9</sup> | Caroline Rooryck<sup>10</sup> | Valérie Malan<sup>7,8</sup> | Matthieu Egloff<sup>11,12</sup>

<sup>1</sup>Service de Cytogénétique Médicale, UIC CYTMRR, CHU Clermont-Ferrand, Clermont-Ferrand, France | <sup>2</sup>Université de Franche-Comté, CHU Besançon, Oncobiologie Génétique Bioinformatique, Besançon, France | <sup>3</sup>Département de Génétique, Hôpital Robert Debré, AP-HP, Unité fonctionnelle de Cytogénétique, Paris, France | <sup>4</sup>Human Genetics and Cognitive Functions, Institut Pasteur, UMR3571 CNRS, Université de Paris, Paris, France | <sup>5</sup>Fédération de Génétique et de Médecine Génomique, Service de Médecine Génomique des Maladies de Système et d'Organe, APHP, Centre – Université Paris Cité, Paris, France | <sup>6</sup>Service Biologique de Génétique Médicale, Hôpital Timone Adultes, M2GM, AP-HM, Marseille, France | <sup>7</sup>Hôpital Necker-Enfants Malades, APHP, Service de Médecine Génomique Des Maladies Rares, Paris, France | <sup>8</sup>INSERM UMR1163, Imagine Institute, Developmental Brain Disorders Laboratory, Université Paris Cité, Paris, France | <sup>9</sup>Laboratoire CERBA, zac des Epineaux, Frepillon, France | <sup>10</sup>CHU de Bordeaux, Service de Génétique Médicale, INSERM, U1211, Bordeaux, France | <sup>11</sup>Université de Poitiers, INSERM 1084, LNEC, Poitiers, France | <sup>12</sup>CHU de Poitiers, Service de Génétique, Poitiers, France

**Correspondence:** Céline Pebrel-Richard ([cpebrel@chu-clermontferrand.fr](mailto:cpebrel@chu-clermontferrand.fr))

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## ABSTRACT

Over the past 15 years, molecular methods for human genome analysis have evolved significantly, becoming integral to routine genetic diagnostics. Among various genomic alterations, copy-number variations (CNVs) are particularly important as sources of both benign and pathogenic variants. Accurate assessment of these variants' clinical implications is critical, especially for rare, non-recurrent CNVs and for susceptibility loci linked to neurodevelopmental disorders (NDDs). To address these challenges, the French AchroPuce CNV Interpretation Working Group proposes a novel classification termed “PIEV,” referring to CNVs associated with NDDs characterized by incomplete penetrance and variable expressivity. This category complements the existing five-tier ACMG classification system, supporting genetic professionals in harmonizing practice through standardized French national guidelines, thereby enhancing genetic counseling and clinical interpretation precision. Distinguishing clearly pathogenic variants from those with incomplete penetrance is crucial, and the consistent classification of these CNVs independently of the clinical context is essential. Clinical significance assessments should entail collaboration between biologists and multidisciplinary clinical teams, especially in prenatal diagnostics. The working group maintains an annually reviewed curated list of recurrent neurodevelopmental CNVs with reduced penetrance and provides consensus recommendations with a customized interpretation tool to enhance national consistency in CNVs reporting.

Valérie Malan and Matthieu Egloff contributed equally as last authors.

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## 1 | Introduction

Chromosomal microarray analysis (CMA) has demonstrated efficacy in identifying pathogenic copy-number variations (CNVs) across numerous diseases. CNVs impact phenotypes by disrupting genes, altering gene dosage, uncovering recessive variants, or affecting gene expression via position effects [1–8]. Since its nationwide implementation in France in 2010, CMA has become the first-line diagnostic method for neurodevelopmental disorders, congenital anomalies, and prenatal ultrasound abnormalities. In this context, the AchroPuce Network was created to coordinate laboratory practices across France, facilitating knowledge sharing and consistency (<https://acpa-achropuce.com>).

Despite significant expertise gained over the past decade, interpreting CNVs remains challenging, often resulting in discrepancies between laboratories [9], thus complicating clinical decision-making. To address these issues, the American College of Medical Genetics and Genomics (ACMG) developed and later expanded a tiered classification system [10], incorporating rigorous semi-quantitative standards in collaboration with The Clinical Genome Resources (ClinGen) [11]. However, variability in practices between countries, particularly concerning CNVs associated with incomplete penetrance and/or variable expressivity, highlights the need for standardized national guidelines, adapted to specific healthcare, ethical, and legal contexts [12]. The AchroPuce Network introduces French-specific recommendations with clear interpretation tools and proposes a new classification category for CNVs associated with NDDs as risk factors with incomplete penetrance and variable expressivity (PIEV), in addition to the five internationally recognized CNV classes.

## 2 | Incomplete-Penetrant and Variable Expressivity CNVs: PIEV

While most CNVs identified in laboratories fit within the ACMG guidelines' five-tier system (classes 1 to 5), a subset of recurrent CNVs presents specific challenges. Recurrent CNVs occur in defined genomic regions susceptible to deletions and duplications resulting from non-allelic homologous recombination (NAHR) between low-copy repeats (LCRs) [13, 14]. Some recurrent CNVs are associated with susceptibility to neurodevelopmental disorders (NDDs), such as intellectual disability, developmental delay, autism spectrum disorders, epilepsy, and psychiatric or behavioral disorders. These CNVs often demonstrate reduced penetrance, are frequently inherited from apparently unaffected parents, are more likely identified in case cohorts, and display variable expressivity, resulting in a broad spectrum of clinical presentations [15, 16].

Recent guidelines acknowledge specific considerations for classifying reduced-penetrance CNVs, although these guidelines primarily address high-penetrance Mendelian variants. Currently, no standardized quantitative criteria exist to define penetrance or risk thresholds clearly distinguishing low-penetrance variants from risk alleles or highly penetrant variants. Thus, classifications remain qualitative due to limited data and variability across diseases. Recently reported expert consensus estimates

low-penetrance variants to confer an absolute risk between 10% and 20% at the lower bound and up to 50% at the upper bound [17].

Management of CNVs with reduced penetrance and variable expressivity remains contentious and varies among laboratories and countries, particularly in the prenatal setting [18–22]. For example, Belgium established a national consensus reporting a limited list of CNVs based on clinical spectrum, severity of expected neurodevelopmental and psychiatric disorders, odds ratios or penetrance values, and considering foetal and parental phenotypes [18]. Similarly, Canadian guidelines recommend reporting only CNVs with high risk of neurodevelopmental abnormalities or congenital malformations, aligning closely with the Belgian approach [19]. The UK guidelines classify incomplete-penetrance and variable expressivity CNVs as pathogenic, but provide curated lists categorizing CNVs as reportable, non-reportable, or context-specific [20, 21]. In Israel, standardized consent forms allow patients or parents to opt out of receiving results for specific CNVs characterized by penetrance below 10% and population frequency exceeding 0.1% [22].

Thus, there is a growing need to introduce a new category specifically for neurodevelopmental disorders susceptibility CNVs. In France, for this reason, the AchroPuce CNV Interpretation Working Group proposes a separate category called “PIEV” (Penetrance: Incomplete; Expressivity: Variable). Classification as PIEV aims for consistency across diverse clinical contexts, independently of referral reason, patient demographics (e.g., sex, age), or clinical background. We also suggest modulating this class with a penetrance criterion based on literature data.

## 3 | French Guidelines for CNVs' Classification

The Working Group has developed national consensual guidelines that undergo constant evaluation and refinement to facilitate and standardize clinical CNV interpretation and customize an interpretation tool based on a combination of major and minor criteria of evidence (Table 1).

### 3.1 | Class 5: Pathogenic CNVs

CNVs are deemed pathogenic when supported by strong clinical evidence from multiple studies and databases. This applies to CNVs: (1) associated with a recurrent disorder with a defined phenotype listed in OMIM or ClinGen Curated Pathogenic (excluding PIEV); or (2) not listed but either (a) enriched in cases and reported in  $\geq 3$  unrelated patients with a concordant phenotype, (b) fully overlapping a known pathogenic CNV (excluding PIEV), or (c) encompassing multiple genes including at least one dosage-sensitive or candidate gene linked to a known syndrome (excluding PIEV).

### 3.2 | Class 4: Likely Pathogenic CNVs

Class 4 encompasses CNVs that often exhibit strong evidence for disease causality, but require additional proofs to confirm their

**TABLE 1** | French guidelines by the AchroPuce Network for the interpretation and reporting of constitutional Copy Number Variants.

**Class 5: Pathogenic CNVs**

Necessary and sufficient evidence: the CNV is well documented as clinically significant in multiple peer-reviewed publications and patients databases

**Class 4: Likely pathogenic CNV**

Two major evidences or one major and two minor evidences must be provided to belong to class 4

**Major evidences:**

- Medical literature:
  - In case of non-specific phenotype (e.g., intellectual disease (ID), autism spectrum disease (ASD)): CNV is described in the literature in at least one patient with a concordant phenotype
  - In case of specific phenotype: CNV is described in the literature in at least one patient with only a few common phenotypic criteria with the carrier patient

**Minor evidences:**

- Epidemiology:
  - In ID: CNV is identified more frequently in ID patients than in controls in Coe et al. study [23]
  - CNV is absent from control databases
- Gene content:
  - CNV partially overlaps with CNVs identified as pathogenic in OMIM or ClinGen Curated Pathogenic without a clearly identified candidate gene
- Size:
  - > 1 Mb

• Gene content:

- The genes contained in the CNV have been associated with a concordant phenotype in the literature or there are strong functional data: studies in cells and/or animal models which may provide relevant information about its potential pathogenicity
- The CNV corresponds to a region close to a candidate gene for the disease characterized by a specific phenotype

- The CNV is a deletion involving the 5' region with additional coding sequence of an established haploinsufficient gene

• Nature:

- CNV is a homozygous deletion
- CNV corresponds to a triplication or amplification (four copies at least or more) not referenced in curated databases as a common polymorphism

**Class 3: VUS**

Necessary and sufficient evidence: the CNV does not have sufficient criteria to belong to another class

(Continues)

TABLE 1 | (Continued)

**Class 2: likely benign**

Two major evidences or one major and two minor evidences must be provided to belong to class 2.

**Major evidences:**

- Epidemiology:
  - CNV is often reported in healthy population but not enough to be considered as a polymorphism (< 1%)
  - CNV is reported at least once in the GnomAD and/or DGV-gold polymorphic variant databases with an overlap of 80%.
- Inheritance:
  - CNV is inherited from a healthy parent or does not segregate with the phenotype within family
- Gene content:
  - CNV contains no genes or contains only repeats/pseudogenes/segmental duplications
  - CNV contains only genes without argument for a role in human pathology (unknown function, patterns of expression, low pLI and high LOEUF...)

**Minor evidences:**

- Epidemiology:
  - In ID: CNV is identified with the same or lower frequency in ID patients compared to controls
  - CNV is reported at least once in the ExAC/DGV-gold polymorphic variant databases but with a threshold of overlap between 50% and 80%
- Gene content:
  - No gene contained in the CNV has been associated with the same phenotype in the literature or there is no argument for the role of one or more genes included in the CNV in the pathology presented by the patient
- Size:
  - Not exceeding 500 kb

**Class 1: Benign CNV**

Necessary and sufficient evidence: There are sufficient evidences in multiple peer-reviewed publications or in curated databases to consider them as benign variants

pathogenicity. To be categorized in this class, the CNV must present either two major or one major and two minor pieces of evidence.

### 3.2.1 | Major Evidences

**3.2.1.1 | Medical Literature.** In cases of non-specific phenotypes, such as intellectual disability (ID) or autism spectrum disorders (ASD), the CNV is documented in the literature in at least one patient who exhibits a concordant phenotype. In cases of specific phenotypes, the CNV is described in the literature in at least one patient who shares a few common phenotypic criteria with the carrier patient.

**3.2.1.2 | Parental Inheritance.** The CNV arises *de novo*, is inherited from an affected parent with the same phenotype, or is inherited from a healthy or mildly affected parent carrying the CNV in a mosaic state.

**3.2.1.3 | Gene Content.** The gene(s) within the CNV have been associated with a matching phenotype in the literature, or there are robust functional data, including cellular studies and/or animal models, offering relevant insights into its potential pathogenicity or the CNV corresponds to a region in close proximity to a candidate gene associated with a disease characterized by a specific phenotype (position effect) or the CNV is a deletion involving the 5' region and additional coding sequences of an established haploinsufficient gene.

**3.2.1.4 | Nature.** The CNV is a homozygous deletion or a triplication or amplification (four copies at least or more) not referenced in curated databases as a common polymorphism [23].

### 3.2.2 | Minor Evidence

**3.2.2.1 | Epidemiology.** The CNV is significantly more frequent in patients with NDD than in controls, as observed in the study by Coe et al. [24] or the CNV is absent from control databases, such as GnomAD v3.1 (<https://gnomad.broadinstitute.org/news/2020-10-gnomad-v3-1/>) and/or DGV-Gold polymorphic variant databases (<https://dgv.tcag.ca/dgv/app/home>).

**3.2.2.2 | Gene Content.** The CNV partially overlaps (> 50%) with CNVs identified as pathogenic in OMIM or ClinGen Curated Pathogenic without a clearly identified candidate gene.

**3.2.2.3 | Size.** The CNV exceeds 500 kb in size.

### 3.3 | Class 3: VUS

A CNV is considered to be of unknown or uncertain significance if it does not meet the criteria to belong to any other class.

They represent a category of CNVs that have not been reported and/or lack sufficient evidence or have conflicting evidence to be classified as pathogenic/likely pathogenic or benign/likely benign CNVs. CNVs initially classified as VUS may be reclassified as a new clinical or scientific data become available [25].

This may include: (1) CNV present in the healthy population, but not at a high enough frequency to be considered a polymorphism (<1%); (2) CNV containing a small number of genes, for which haploinsufficiency or triplosensitivity is not clearly established; (3) CNV described in publications and/or databases with conflicting interpretations about its pathogenicity; (4) CNV corresponding to an intragenic deletion or duplication whose effect on transcription is not clearly established.

It should be noted that the quantitative scoring framework formulated by ACMG-ClinGen guidelines can be useful for refining the interpretation of a CNV that does not meet the criteria for other classes according to the AChroPuce classification [11]. Although a follow-up evaluation by a biologist may be necessary, online variant annotation tools (CNV Explorer—<https://cnvexplorer.com/>, CNV Hub—<https://cnvhub.net/>, Franklin Genoox—<https://franklin.genoox.com/>, ...) can significantly facilitate the process by evaluating the variant according to ACMG/AMP guidelines and assessing its clinical significance.

### 3.4 | Class 2: Likely Benign CNVs

Likely benign CNVs are unlikely to be causative of a genetic disorder, yet further evidence is required to confirm their benign nature. To be classified into Class 2, two major pieces of evidence or one major and two minor pieces of evidence must be provided.

#### 3.4.1 | Major Evidence

**3.4.1.1 | Epidemiology.** The CNV is frequently reported in the healthy population but does not reach the threshold for consideration as a polymorphism (<1%) or the CNV is documented at least once in databases such as GnomAD v3.1 (<https://gnomad.broadinstitute.org/news/2020-10-gnomad-v3-1/>) and/or DGV-Gold polymorphic variant databases (<https://dgv.tcag.ca/dgv/app/home>), with an overlap of 80%.

**3.4.1.2 | Inheritance.** The CNV is inherited from a healthy parent or does not segregate with the phenotype within the family.

**3.4.1.3 | Gene Content.** The CNV does not contain genes or it only contains repeats, pseudogenes, or segmental duplications. Or, the genes within the CNV lack compelling arguments for their involvement in human pathology, exhibiting characteristics like unknown function, expression patterns in tissues not affected by the patient's phenotype, low probability of loss of function intolerance (pLI), and high LOEUF (Loss-of-Function observed/Expected Upper bound Fraction) according to gnomAD v3.1 in case of deletion.

#### 3.4.2 | Minor Evidence

**3.4.2.1 | Epidemiology.** The CNV is identified with a similar or lower frequency in patients with NDD when compared

to controls [24] or the CNV is found in at least one instance in the DGV-gold polymorphic variant databases but with an overlap between 50% and 80%.

**3.4.2.2 | Gene Content.** None of the genes within the CNV is associated with the same phenotype in the literature, or there is insufficient evidence to support the involvement of one or more of the genes in the patient's phenotype.

**3.4.2.3 | Size.** CNV does not exceed 500 kb, taking into account that 90%–95% of benign CNVs are <500 kb in size according to Itsara et al. [26].

### 3.5 | Class 1: Benign CNVs

There are sufficient evidences in multiple peer-reviewed publications or in curated databases to consider them as benign CNVs.

This class may include: (1) CNV enriched in the normal population (> 1% in the healthy population) or (2) CNV referenced in curated databases as a common polymorphism [23].

### 3.6 | Class PIEV: CNVs With Incomplete Penetrance and Variable Expressivity

The Working Group maintains a curated list of recurrent CNVs, each assigned a consensus-based classification (Table 2). The available evidence concerning these CNVs is reassessed annually, allowing guidelines to be updated and refined as necessary.

As of the most recent update on February 2025, the AchroPuce CNVs Interpretation working group has designated the following neurodevelopmental disorders susceptibility CNVs as PIEVs and their gene candidate:

- Distal 1q21.1 deletion and duplication (*GJA5, GJA8*)
- 2q13 deletion (*BUB1*)
- 3q29 deletion (*DLG1, BDH1*)
- 10q11.21q11.2 deletion (*CHAT, SLC18A3*)
- 15q13.3 BP4-BP5 deletion (*CHRNA7, OTUD7A*)
- 15q13.3 deletion CHRNA7-LCR-BP5 (*CHRNA7*)
- 16p13.11 deletion (*NDE1, MYH11*)
- 16p12.2 deletion (*EEF2K, POLR3E*)
- 16p11.2 distal deletion (*SH2B1*)
- 16p11.2 proximal deletion and duplication (*TBX6, KCTD13*)
- 17q12 deletion (*HNF1B*)
- 22q11.2 proximal duplication (*TBX1*)
- 22q11.21 central deletion (*SCARF2, SNAP29*)
- 22q11.21 distal type I deletion (*MAPK1*)
- 22q11.21 distal type III deletion (*SMARCB1*)

Within the PIEV category, a distinction is made between low-penetrance PIEVs and high-penetrance PIEVs, as discussed below (Table 2).

## 4 | Reporting Considerations

The AchroPuce Network recommends reporting pathogenic (P) and likely pathogenic (LP) CNVs that are relevant to the reason for the patient's referral. According to international recommendations, likely benign (LB) and benign (B) CNVs should generally not be reported [11]. Variants of unknown significance (VUS) are systematically reported postnatally. Conversely, prenatal reporting of VUS is generally not recommended unless there is strong suspicion of pathogenicity without fully meeting LP criteria. In such cases, parental segregation may help biologists in interpreting the CNV and determining whether or not it should be reported.

Reporting strategies for PIEVs depend on the clinical context. Postnatally, the AchroPuce Network recommends systematic PIEV reporting, allowing the biologist, in consultation with clinicians, to assess whether the PIEV contributes to the patient's phenotype. In prenatal diagnosis, PIEVs are not routinely reported. Reporting decisions must consider the ultrasound findings, the family history, and the specific PIEV involved, with particular attention to those associated with high penetrance [27]. Prenatal PIEV reporting decisions typically require consultation with a multidisciplinary team, including biologists, clinicians, and the local multidisciplinary prenatal diagnosis committee.

## 5 | Discussion

Recent guidelines significantly contributed to the standardization of CNVs classification and interpretation, particularly for high-penetrance variants [11]. They also acknowledge the specific challenges posed by reduced-penetrance CNVs regarding reporting and genetic counselling. To reduce inter-laboratory variability, the AchroPuce Network proposes the "PIEV" category to complement ACMG's existing five-tier system [11]. Indeed, we observed that laboratories used to classify these CNVs variably as VUS, LP, P, or LB, with interpretations often adjusted based on clinical context. This inconsistency in CNVs classification can create confusion and complicate genetic counselling, particularly when multiple individuals within the same family carry an identical CNV that is classified differently depending on clinical presentation. One frequently proposed solution is to systematically report these CNVs as pathogenic [11]. However, this approach may mislead patients and medical professionals into incorrectly assuming that these variants inevitably result in neurodevelopmental disorders. Thus, clearly distinguishing pathogenic CNVs from PIEVs is crucial to avoid misinterpretation potentially affecting clinical decisions, such as unwarranted pregnancy terminations or premature cessation of diagnostic investigations. Indeed, in severe or atypical phenotypes, whole genome sequencing is recommended to identify a second genetic event that may independently cause or amplify the effects of the PIEV (second-hit) [28].

TABLE 2 | Classification of recurrent CNVs.

CNV	Genomic coordinates	Candidate genes	Variation	Classification
Proximal 1q21.1 (BP2-BP3)	GRCCh37: chr1:145386507-145748064	RBM8A	Del	Class 3: VOUS
	GRCCh38: chr1:145686999-146048495		Dup	Class 3: VOUS
Distal 1q21.1 (BP3-BP4)	GRCCh37: chr1:146577486-147394506	GJA5, GJA8	Del	PIEV (high penetrance)
	GRCCh38: chr1:147105904-147917509		Dup	PIEV (low penetrance)
2q11.2	GRCCh37: chr2:96739012-97671429	ARID5A, KANSL3,	Del	Class 3: VOUS
	GRCCh38: chr2:96073264-97005692	TMEM127	Dup	Class 3: VOUS
2q13	GRCCh37: chr2:111392193-113104742	BUB1, BCL2L11	Del	PIEV (low penetrance)
	GRCCh38: chr2:110634616-112347165		Dup	Class 3: VOUS
3q29	GRCCh37: chr3:195756054-197344662	DLG1, BDHI	Del	PIEV (high penetrance)
	GRCCh38: chr3:196029183-197617791		Dup	Class 3: VOUS
10q11.21q11.23 (LCRC-D)	GRCCh37: chr10:49389703-51053583	CHAT, SLC18A3	Del	PIEV (low penetrance)
	GRCCh38: chr10:48181660-49845537		Dup	Class 3: VOUS
15q11.2 (BP1-BP2)	GRCCh37: chr15:22832519-23090897	NIPAL, NIPA2	Del	Class 3: VOUS
	GRCCh38: chr15:22782170-23040134		Dup	Class 1: Benign
15q13.3 (BP4-BP5)	GRCCh37: chr15:31192889-32445405	CHRNA7, TRPM1, OTUD7A	Del	PIEV (high penetrance)
	GRCCh38: chr15:30900686-32153204		Dup	Class 3: VOUS
15q13.3 (D-CHRNA7 to BP5)	GRCCh37: chr15:3201962132445405	CHRNA7, OTUD7A	Del	PIEV (high penetrance)
	GRCCh38: chr15:31727418-32153204		Dup	Class 1: Benign
16p13.11	GRCCh37: chr16:15511711-16292265	NDEL, MYH11	Del	PIEV (low penetrance)
	GRCCh38: chr16:15417854-16198408		Dup	Class 3: VOUS
16p12.2	GRCCh37: chr16:21948445-22430804	EEF2K, POLR3E, CDR2	Del	PIEV (low penetrance)
	GRCCh38: chr16:2193712422419483		Dup	Class 3: VOUS
Distal 16p11.2 (BP2-BP3)	GRCCh37: chr16:28822635-29046499	SH2B1	Del	PIEV (high penetrance)
	GRCCh38: chr16:28811314-29035178		Del	Class 5: Pathogenic in case of obesity
Proximal 16p11.2 (BP4-BP5)	GRCCh37: chr16:29649997-30199852	TBX6, KCTD13, PRRT2	Dup	Class 3: VOUS
	GRCCh38: chr16:29638676-30188531		Dup	PIEV (high penetrance)

(Continues)

TABLE 2 | (Continued)

CNV	Genomic coordinates	Candidate genes	Variation	Classification
17q12	GRCh37: chr17:34815072-36192489 GRCh38: chr17:36458167-37854616	<i>HNF1B</i>	<b>Del</b>	<b>PIEV (low penetrance)</b> <b>Class 5: Pathogenic</b> in case of renal and pancreatic damages
Proximal 22q11.2 (DGS/VCFS)	<i>LCR22-A-D</i> GRCh37: chr22:18912231-21465672 GRCh38: chr22:18924718-21111383	<i>TBX1</i>	<b>Dup</b> <b>Dup</b>	<b>Class 3: VOUS</b> <b>PIEV (low penetrance)</b>
(LCR22-A-D ou LCR22-A-B)	<i>LCR22-A-B</i> GRCh37: chr22:18912231-20287208 GRCh38: chr22:18924718-20299685			
Central 22q11.21 (LCR22-B/C-D)	GRCh37: chr22:20731986-21465672 GRCh38: chr22:20377696-21111383	<i>CRKL, SCARF2, SNAP29</i>	<b>Del</b> <b>Dup</b>	<b>PIEV (low penetrance)</b> <b>Class 3: VOUS</b>
Distal 22q11.21	<i>Type I</i> GRCh37: chr22:21090000-23650000 GRCh38: chr22:20735712-23307813	<i>BCR, TOP3B, MAPK1</i>	<b>Del type I</b>	<b>PIEV (low penetrance)</b>
Type I: LCR22-C/D-E/F	<i>Type II</i> GRCh37: chr22:23119414-23649111 GRCh38: chr22:22776924-23306924		<b>Del type II</b>	<b>Class 3: VOUS</b>
Type II: LCR22-E-F	<i>Type III</i> GRCh37: chr22:21917117- 24994433 GRCh38: chr22:21562828- 24598466		<b>Del type III</b>	<b>PIEV (low penetrance)</b>
Type III: LCR22-D/E/F-H			<b>Dup</b>	<b>Class 3: VOUS</b>
Xp22.3	GRCh37: chrX:6455812-8124954 GRCh38: chrX:6537771-8156913	<i>STS, VCX3</i>	<b>Del</b> <b>Dup</b>	♂: <b>class 3: VOUS</b> ♀: <b>class 2: Likely Benign</b> ♂: <b>class 2: Likely Benign</b> ♀: <b>class 1: Benign</b>

Note: Classification - red for pathogenic CNV (class 5), yellow for VUS (class 3), purple for PIEV class, blue for Likely benign (class 2) and green for benign CNV (class 1).

Establishing robust, clinically meaningful penetrance estimates for recurrent CNVs is critical yet challenging [27, 29]. Several factors can lead to inaccurate penetrance estimations, including the rarity of certain CNVs, the neurodevelopmental phenotype heterogeneity of affected individuals in patient cohorts, and the composition of control cohorts. A significant source of bias arises when affected individuals are inadvertently included in control cohorts, especially in cases of mosaic CNVs, mild clinical presentations, or presymptomatic phases of a late-onset disorder. This issue is also relevant for CNVs associated with both neurodevelopmental and non-neurodevelopmental phenotypes, such as 17q12 [HNF1B] rearrangements or distal 16p11.2 deletions [SH2B1], which are fully penetrant for renal dysfunction or obesity, respectively, but exhibit partial penetrance for neurodevelopmental features. These CNVs may be classified inconsistently across control and patient cohorts, resulting in potential over- or underestimation of penetrance.

Yet, defining strict penetrance thresholds for CNVs classification also remains challenging, due to the limited availability of robust quantitative data, apart from the recent pooled analysis by Goh et al. [27]. In line with the recommendations of the ClinGen Low Penetrance Working Group [17], we chose to provide broad interpretive ranges rather than rigid cutoffs. In brief, CNVs with penetrance exceeding 25% according to Goh et al. [27] have generally been categorized as PIEVs, specifying whether the penetrance appears to be low (typically < 50%) or high (typically > 50%). In contrast, CNVs with less than 10% penetrance have usually been classified as variants of uncertain significance (VUS) or likely benign variants. For CNVs exhibiting intermediate penetrance (10%–25%), in cases where classification varies significantly across studies—VUS, PP, LB, or PIEV—where a normal or subnormal phenotype remains the most predominant presentation and when the CNV is frequently identified in control cohorts (e.g., proximal 1q21.1 deletions [RBM8A], distal 1q21.1 duplications [GJA5, GJA8], 16p13.11 deletions [MYH11], 16p12.2 deletions [EEF2K, POLR3E], distal 16p11.2 duplications [SH2B1], 17q12 duplications [HNF1B] or 22q11.2 central duplications [SCARF2, SNAP29]), a consensus-based classification was established using available scientific evidence including peer-reviewed literature, prevalence data, ClinGen curation efforts [30] and the expertise of Working Group members. Our conclusions are reported in Table 2. In the absence of sufficient and robust scientific evidence, it is generally recommended to classify and report recurrent CNVs as VUS until additional data support a more definitive interpretation.

Beyond adding the “PIEVs class” into the ACMG’s five-tier classification system [11], the AchroPuce Network updates national consensus guidelines for interpretation and reporting of CNVs by providing a yearly curated registry of recurrent CNVs, including curated literature data, all available at <https://acpa-achropuce.com/diagnostic-postnatal/>.

## 6 | Conclusion

Determining the pathogenicity of genetic variants is crucial to ensure patients receive accurate diagnoses and appropriate medical care. The complexity caused by the incomplete penetrance and variable expressivity of certain CNVs presents significant challenges.

The French AchroPuce Network proposes introducing the PIEV class to the existing five-tier classification system established by the ACMG. This initiative aims to distinguish these CNVs from those that are always leading to a neurodevelopmental phenotype. We also emphasize that the clinical impact of a PIEV should be evaluated subsequently. Systematic PIEV reporting is recommended postnatally, whereas prenatal reporting remains cautious, aligning with France’s ethical and legislative context, favoring individualized multidisciplinary evaluations [31–34].

As we move forward, the classification and reporting of genetic variants must remain dynamic, continually adapting to our expanding knowledge of genetic mechanisms and variant effects by embracing the complexity of genetic variation and consistently refining our approaches.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

## Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.70027>.

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