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## 3q29 duplications: a cohort of 46 patients and a literature review

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### **Conflicts of interest**

The authors declare no conflict of interest.

### **Data availability statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### **Editorial Policies and Ethical Considerations**

All clinical geneticists obtained informed consent from the patient or their legal guardian, in accordance with French legislation.

**Abstract:**

Duplications of the 3q29 cytoband are rare chromosomal copy number variations (CNVs) (overlapping or recurrent ~1.6 Mb 3q29 duplications). They have been associated with highly variable neurodevelopmental disorders (NDD) with various associated features, or reported as a susceptibility factor to the development of learning disabilities and neuropsychiatric disorders. The smallest region of overlap and the phenotype of 3q29 duplications remain uncertain. We here report a French cohort of 31 families with a 3q29 duplication identified by chromosomal microarray analysis (CMA), including 14 recurrent 1.6 Mb duplications, eight overlapping duplications (>1Mb), and nine small duplications (<1Mb). Additional genetic findings that may be involved in the phenotype were identified in 11 patients. Focusing on apparently isolated 3q29 duplications, patients present mainly mild NDD as suggested by a high rate of learning disabilities in contrast to a low proportion of patients with intellectual disabilities. Although some are *de novo*, most of the 3q29 duplications are inherited from a parent with a similar mild phenotype. Besides, the study of small 3q29 duplications doesn't provide evidence for any critical region. Our data suggest that the overlapping and recurrent 3q29 duplications seem to lead to mild NDD, and that a severe or syndromic clinical presentation should warrant further genetic analyses.

**Keywords:** 3q29 duplication, chromosomal microarray analysis, copy number variation, genomic disorder, neurodevelopmental disorders, multiple molecular diagnoses.

**Introduction**

The advent of chromosomal microarray analysis (CMA) has allowed the discovery of reciprocal recurrent copy number variations (CNVs), which are flanked by segmental duplication (SD) and mediated by non-allelic homologous recombination (NAHR). For most of these reciprocal CNVs, the duplications' pathogenicity was assessed later than for deletions and the phenotype may be less severe. At the 3q29 cytoband, a ~1.6 Mb recurrent reciprocal CNV is found from 195,6 to 197,3 Mb

(GRCh37/Hg19) flanked by SD [Yilmaz et al., 2023]. This region contains 22 protein-coding genes currently without strong evidence for candidate critical genes. The recurrent 3q29 deletion is found with a frequency of 0.002% in the UK biobank population [Crawford et al., 2019]. It has been established to display incomplete penetrance and variable expressivity, characterized by developmental delay (DD), mild to moderate intellectual disability (ID), neuropsychiatric disorders (NPD) such as attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), anxiety, and schizophrenia, pediatric feeding problems, cardiac and cerebral malformations, ocular and dental abnormalities [Sanchez Russo et al., 2021].

The reciprocal 3q29 duplication is less frequent. In the UK biobank population, the estimated frequency is 0.001% [Crawford et al., 2019]. In the DGV and gnomAD SV databases, the recurrent duplication has not been reported [Collins et al., 2020]. 3q29 duplications have been associated with highly variable neurodevelopmental disorders (NDD) ranging from mild to severe ID, learning difficulties, neuropsychiatric disorders, and associated to miscellaneous clinical features such as cardiac, cerebral, or ocular malformations. Patients described had heterogeneous phenotype, and some are reported as asymptomatic [Holinski-Feder et al., 2000; Ballif et al., 2008; Lisi et al., 2008; Goobie et al., 2008; Aleixandre Blanquer et al., 2011; Schilter et al., 2013; Fernández-Jaén et al., 2014; Quintela García et al., 2015; Kessi et al., 2018; Tassano et al., 2018; Streata et al., 2020; Coyan and Dyer, 2020; Bauleo et al., 2022]. Furthermore, a case-control study suggested the 3q29 duplication might be a susceptibility locus for NDD (mainly learning disability and neuropsychiatric disorders such as ASD) [Pollak et al., 2020].

In large NDD cohorts (DD, ID and/or ASD), the 3q29 duplication prevalence is not significantly increased compared to controls [Moreno-De-Luca et al., 2013; Kushima et al., 2018; Stefansson et al., 2014; Coe et al., 2014; Zarrei et al., 2019]. However, meta-analysis of rare CNV in nearly one million individuals identified an association between 3q29 duplication and neurodevelopmental phenotype ( $p=10^{-4}$ ) [Collins et al., 2022].

To date, the impact of the 3q29 duplication remains unclear. The hypothesis of multiple molecular diagnoses has been raised in recent reports to explain the variable expressivity [Coyan and Dyer, 2020; Bauleo et al., 2022]. To improve phenotype delineation and consequently the interpretation of this CNV, we here present a cytogenetic and clinical description of a French cohort with a duplication in the 3q29 region (chr3: 194803300-198810000, GRCh37/Hg19). We also investigate the implication of additional CNVs in our cohort, as well as SNVs as identified by exome sequencing.

### **Methods**

Our multicenter retrospective study was performed from December 2018 to October 2022. Patients with a duplication in the chr3:194800000-198810000 (GRCh37/hg19) region were recruited from 14 French genetic centers, thanks to a national collaboration within the ACLF network (association of French speaking cytogeneticists). Clinical features and genetic data were collected using a questionnaire completed by clinical geneticists and cytogeneticists. The collected data included: age at diagnosis, age at last follow-up, medical history, clinical signs, para-clinical exams, the chromosomal and molecular genetic analyses performed, the characteristics of 3q29 duplication (size, location, inheritance), and the presence of additional genetic variations (CNVs and SNVs).

Sixty-four patients were recruited, including 3q29 duplication carrier relatives. Eighteen patients were excluded from our cohort due to lack of clinical information or due to the cytogenetic data (location of the duplication found in very low proportions in the cohort) (figure 1).

CNVs were identified by CMA analysis and confirmed by qPCR, FISH or qMPSF. Exome sequencing (ES) or a targeted gene sequencing of 286 genes involved in intellectual disability (ID genes panel) were performed in 10 families. Trio analyses were prioritized when parent samples were available. For one family, symptomatic siblings were also analyzed. Genomic mapping was performed on the Human GRCh37/hg19 assembly, with a custom track tool of University of California Santa Cruz (UCSC) Genome Browser custom track tool.

Editorial Policies and Ethical Considerations

All clinical geneticists obtained informed consent from the patient or their legal guardian, in accordance with French legislation.

## Results

Forty-six patients from 31 families were included in the total cohort (figure 1): 23 males and 24 females, with ages ranging from 1 year to adulthood, and three fetuses. We observed two groups according to CNV size and genomic location that we described separately (cohort A and cohort B, figure 1 and 2). Cohort A includes recurrent interstitial duplications and >1 Mb duplications overlapping more than 80% of the recurrent locus (“overlapping duplications”). Cohort B includes small duplications <1 Mb located distally to the recurrent locus (figure 1 and 2).

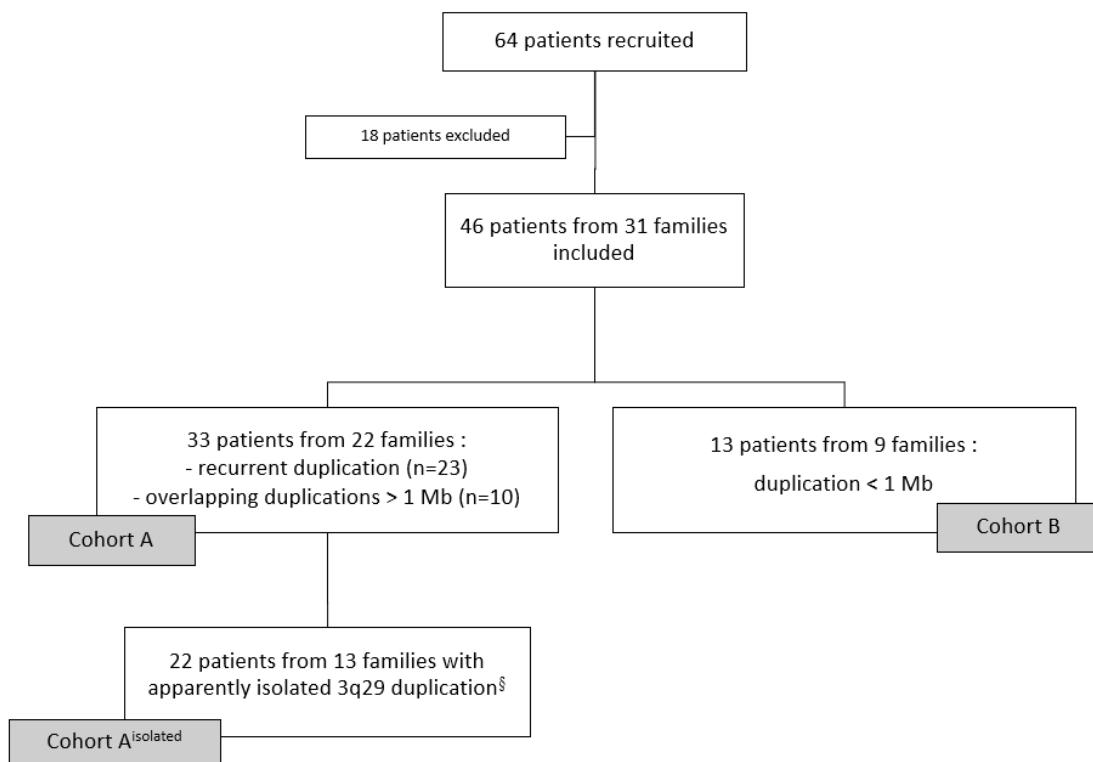


Figure 1: Flowchart summarizing patients' distribution in sub-cohorts according to the duplication location and presence of additional genetic finding. § Five families with exome sequencing or ID genes panel, and eight families without further additional genetic analyses.

## 1- Cohort A results

## a. 3q29 duplication cytogenetic data



Figure 2: Mapping of duplications identified in the families of the cohort (dark bars), segmental duplications (Segmental Dups) and OMIM genes in the region Chr3: 194000000-198022430, using UCSC genome browser (build GRCh37/hg19). Cohort A includes recurrent and overlapping duplications >1 Mb and § represents families with apparently isolated 3q29 duplication (Cohort A<sup>isolated</sup>); Cohort B includes duplications < 1 Mb

Thirty-three individuals from 22 families have a duplication included in chr3: 194803300-198810000 (GRCh37/Hg19) region (figure 2). Twenty-three patients from 14 families have the recurrent ~1.6 Mb duplication located between 195.6 Mb and 197.3 Mb of the chromosome 3. Ten patients from eight families have overlapping duplications with sizes ranging from 1.76 Mb to 3.03 Mb, six of which occurred through unbalanced translocation. The inheritance data are available for 17 patients from 14 families (table 1). Three duplications are *de novo*, maternally inherited in seven families and paternally in two families (including one family with four siblings). For two families, duplications are inherited from a paternal balanced translocation.

#### b. Additional genetic findings

Eleven patients from nine families have an additional genetic variation, including eight patients with an additional CNV and three with an additional single nucleotide variant (SNV), as summarized in table 1. One patient has a recurrent 15q11.2 BP1-BP2 deletion. Four patients have an unbalanced chromosomal translocation, with pathogenic deletions: two 11 Mb 11q24.1q25 deletions (P32 and P33), a 3.4 Mb 12p13.33p13.32 deletion (P24), and a 3.4 Mb 2q37 deletion (patient P30 previously published [Leroy et al., 2013]). Three patients carry an unbalanced translocation with uncertain significance deletion: a 0.8 Mb 17p13.3 deletion (P30) and a 3.2 Mb 1q44 deletion (P25 and P26), both of which do not include the critical region of the known associated syndrome (17p13.3 deletion syndrome (OMIM #247200) and 1q44 syndrome (OMIM #612337) respectively). Exome sequencing was performed for seven families (solo analysis for family 8, trio analysis for families 3, 9, 10, 11 and 16, and quintet analysis for family 12), with negative result for five of them (table 1). One patient and his symptomatic mother (family 3) have a missense variant of uncertain significance (VUS) in the *TRRAP* gene. A female patient has a splice acceptor VUS in the X-linked *PHF6* gene without segregation data available.

#### c. Clinical presentation

We describe the clinical presentation of cohort A and of “the cohort A<sup>isolated</sup>” a subset of 13 families with apparently isolated 3q29 duplication (table 1). In the subset “cohort A<sup>isolated</sup>”, five families had negative exome or ID genes sequencing (without any pathogenic variant, likely pathogenic variant, or VUS) and further genetic analyses were not performed for eight families. Clinical features are summarized in the table 1.

##### a.i. Cohort A phenotype

Developmental delay is reported in 67% patients (18/27), learning disabilities requiring special education in 64% patients (16/25) and 20% (5/25) have an intellectual disability (mild-to-severe severity). 43% (9/21) present neuropsychiatric disorders, with ASD for two patients. Cardiac

malformations are present in four patients with septal defects, a deviation of conal septum and a bicuspid aortic valve, and one fetus presented a complex cardiopathy with left heart hypoplasia, hypoplasia of the aortic arch, mitral and aortic dysplasia and a VSD (associated with bilateral nephromegaly). Other reported features include neonatal hypotonia (n=4), unilateral deafness (n=3) among which two were unilateral inner ear malformations, obesity (n=3), seizures (n=3), macrocephaly (n=1), bilateral pelvicalyceal dilatation (n=1), clubfeet (n=1) present in the fetus P1 with a late intrauterine death. Two individuals (P8 and P23) are known as asymptomatic, tested for familial segregation.

a.ii. Cohort A<sup>isolated</sup> phenotype

Focusing on the 22 patients of 13 families without known additional genetic variation, 69% (13/19) exhibit DD, 56% (9/16) have learning disabilities, 19% (3/16) present with mild ID, and 29% (4/14) have NPD, two of which present ASD. The remaining associated features are cardiopathies (n=2), neonatal hypotonia (n=3), unilateral deafness (n=3), obesity (n=2), seizures (n=2), macrocephaly (n=1), and club feet (n=1). The asymptomatic patients (P8 and P23, table 1) are among this subset. The affected patients do not share any dysmorphic facial features (data not shown).

Table 1 : Summary of genetic and clinical data in the cohort A

| Family  | Patient      | § | 3q29 duplication coordinates (GRCh37, Hg19) | size    | Sexe | Age   | DD               | ID               | Learning disabilities | NPD                        | Malformation  | Others features                                      | Inheritance                     | Additional genetic data (GRCh37, Hg19)                                   |  |
|---|--------------|---|---|---------|------|-------|------------------|------------------|-----------------------|----------------------------|---|--|---------------------------------|--|--|
| Family 1  | P1           | § | chr3:195623619-197317244                    | 1.69 Mb | M    | 14y   | mild global      | mild             | -                     | +                          | -   | neonatal hypotonia and obesity                       | na                              | negative ID NGS panel (286 genes)  |  |
| Family 2  | P2           | § | chr3:195645536-197339329                    | 1.69 Mb | M    | fetus | na               | na               | na                    | na                         | club feet   | IUFD   | na                              | na   |  |
| Family 3  | P3 (Child)   | § | chr3:195602582-197385966                    | 1.70 Mb | M    | 16y   | global           | mild to moderate | -                     | ADHD                       | -   | -  | Mat                             | NM_003496.4(TRRAP):c.2675A>G (p.Y892C) ‡                                 |  |
|   | P4 (Mother)  | § |   |         | F    | adult | +                | -                | +                     | na                         | -   | -  | na                              | NM_003496.4(TRRAP):c.2675A>G (p.Y892C) ‡                                 |  |
| Family 4  | P5           | § | chr3:195738406-197332150                    | 1.59 Mb | M    | 14y   | -                | -                | -                     | ASD                        | -   | one epileptic seizure                                | De novo                         | no other genetic analyses performed                                      |  |
| Family 5  | P6           | § | chr3:195740357-197310451                    | 1.57 Mb | M    | 5y    | +                | -                | +                     | -                          | -   | unilateral deafness and obesity                      | Mat                             | no other genetic analyses performed                                      |  |
| Family 6  | P7           | § | chr3:195740357-197310451                    | 1.57 Mb | F    | 8y    | -                | -                | +                     | -                          | left stapes aplasia   | deafness   | De novo                         | no other genetic analyses performed                                      |  |
| Family 7  | P8 (Child)   | § | chr3:195740357-197317103                    | 1.57 Mb | F    | 24y   | -                | -                | -                     | -                          | -   | -  | Pat                             | na   |  |
|   | P9 (Father)  | § |   |         | M    | adult | motor delay      | -                | +                     | na                         | na  | -  | na                              | na   |  |
| Family 8  | P10          | § | chr3:195740357-197324783                    | 1.86 Mb | F    | na    | -                | -                | +                     | na                         | -   | epilepsy and obesity                                 | na                              | NM_032335.3(PHFG):c.139-2A>G ‡   |  |
| Family 9  | P11          | § | chr3:195747856-197339329                    | 1.59 Mb | F    | 3y    | mild motor delay | na               | na                    | na                         | atrial septal defect  | prematurity (28 WG)                                  | De novo                         | Negative ES  |  |
| Family 10                                       | P12 (Child)  | § | chr3:195747856-197398813                    | 1.69 Mb | F    | 1y    | global           | na               | na                    | na                         | -   | neonatal hypotonia                                   | Mat                             | Negative ES  |  |
|   | P13 (Mother) | § |   |         | F    | adult | +                | -                | +                     | -                          | -   | -  | na                              | Negative ES  |  |
|   | P14          | § |   |         | F    | 6y    | global           | -                | +                     | ASD                        | -   | -  | Mat                             | Negative ES  |  |
| Family 11                                       | P15 (Mother) | § | chr3:195747856-197339329                    | 1.59 Mb | F    | adult | na               | -                | major                 | na                         | -   | -  | na                              | Negative ES  |  |
|   | P16 (Child)  | § |   |         | F    | 13y   | global           | mild             | -                     | -                          | -   | -  | Pat                             | Negative ES  |  |
|   | P17 (Child)  | § |   |         | M    | 12y   | language delay   | -                | +                     | -                          | -   | -  | Pat                             | Negative ES  |  |
| Family 12                                       | P18 (Child)  | § | chr3:195747856-197339329                    | 1.59 Mb | F    | 5y    | mild global      | na               | na                    | -                          | -   | -  | Pat                             | Negative ES  |  |
|   | P19 (Child)  | § |   |         | F    | 3y    | mild global      | na               | na                    | -                          | -   | neonatal hypotonia                                   | Pat                             | Negative ES  |  |
|   | P20 (Father) | § |   |         | M    | adult | +                | -                | +                     | anxiety                    | -   | -  | na                              | Negative ES  |  |
| Family 13                                       | P21          | § | chr3:195747856-197387258                    | 1.63 Mb | M    | 16y   | language delay   | -                | +                     | ADHD                       | -   | -  | na                              | arr[hg19]15q11.2(22765628_23208901)x1 ¶                                  |  |
| Family 14                                       | P22 (Child)  | § | chr3:195908864-197310451                    | 1.40 Mb | M    | 4y    | -                | -                | -                     | -                          | external auditory canal agenesis and malleus hypoplasia   | macrocephaly   | Mat                             | no other genetic analyses performed                                      |  |
|   | P23 (Mother) | § |   |         | F    | adult | -                | -                | -                     | -                          | -   | -  | na                              | no other genetic analyses performed                                      |  |
| Family 15                                       | P24          | § | chr3:194803317-197837049                    | 3.03 Mb | M    | 8y    | +                | severe           | -                     | intolerance to frustration | -   | -  | na                              | der(12)t(3;12)(q29;p13.32) arr[hg19]12p13.32p13.33(194249_3607170)x1 ¶   |  |
| Family 16                                       | P25 (Child)  | § | chr3:194828987-197837049                    | 3.00 Mb | F    | 10y   | -                | -                | +                     | ADHD                       | atrial septal defect  | -  | Mat                             | der(1)t(1;3)(q44;q29) arr[hg19]1q44(245986076_249212668)x1 ‡ negative ES |  |
|   | P26 (Mother) | § |   |         | F    | adult | na               | -                | +                     | -                          | na  | -  | na                              | arr[hg19]1q44(245986076_249212668)x1 ‡ negative ES                       |  |
| Family 17                                       | P27 (Child)  | § | chr3:195425875-197608392                    | 2.18 Mb | F    | 3y    | -                | na               | na                    | na                         | VSD with deviation of conal septum and bicuspid aortic valve  | -  | Mat                             | no other genetic analyses performed                                      |  |
|   | P28 (Mother) | § |   |         | M    | 27y   | na               | -                | +                     | na                         | na  | -  | na                              | no other genetic analyses performed                                      |  |
| Family 18                                       | P29          | § | chr3:195456487-197310451                    | 1.85 Mb | F    | 35y   | global           | mild             | -                     | na                         | -   | two seizures   | na                              | no other genetic analyses performed                                      |  |
| Family 19                                       | P30          | § | chr3:195565159-197645254                    | 2.28 Mb | M    | adult | na               | -                | +                     | -                          | na  | -  | na                              | der(17)t(3;17)(q29;p13.3) arr[hg19]17p13.3(51885_853962)x1 ‡             |  |
| Family 20                                       | P31          | § | chr3:195907302-197837049                    | 1.76 Mb | M    | adult | +                | -                | +                     | aggressive behavior        | -   | -  | na                              | der(2)t(2;3)(q37.3;q29) arr[hg19]2q37.3(238104834_242690037)x1 ¶         |  |
| Family 21                                       | P32          | § | chr3:196007825-197837049                    | 1.82 Mb | M    | fetus | na               | na               | na                    | na                         | left heart hypoplasia, hypoplasia of the aortic arch, mitral and aortic dysplasia, VSD nephromegaly | -  | balanced paternal translocation | der(11)t(3;11)(q29;q24.1) arr[hg19]11q24.1q25(123386794_134927114)x1 ¶   |  |
| Family 22                                       | P33          | § | chr3:196042935-197840339                    | 1.79 Mb | M    | 1y    | -                | na               | na                    | na                         | VSD   | neonatal hypotonia bilateral pelvic/colic dilatation | balanced paternal translocation | der(11)t(3;11)(q29;q24.1) arr[hg19]11q24.1q25(123414357_134868407)x1 ¶   |  |
| Total Cohort A                                  |              |   |   |         |      |       | 18/27 (67%)      | 5/25 (20%)       | 16/25 (64%)           | 9/21 (43%)                 | 8/29 (28%)  |  |                                 |  |  |
| Total cohort A <sup>isolated</sup> (Patients 5) |              |   |   |         |      |       | 13/19 (69%)      | 3/16 (19%)       | 9/16 (56%)            | 4/14 (29%)                 | 5/20 (25%)  |  |                                 |  |  |

P, patient; M, male; F, female; y, years; DD, developmental delay; ID, intellectual disability; NPD, neuropsychiatric disorders; ; na, not available; ADHD, attentional deficit/hyperactivity disorder; ASD, autistic spectrum disorder; VSD, ventricular septal defect; IUFD, intra-uterine fetal death; WG, week of gestation; NGS, next generation sequencing; ES, exome sequencing; Pat, paternal; Mat, Maternal, +/- presence or absence of clinical features; § patient in cohort A<sup>isolated</sup>; ¶ pathogenic variation; + variation of uncertain significance

## 2- Cohort B results

Thirteen patients from nine families have <1 Mb duplications (sizes ranging from 129 kb to 857 kb), located in the chr3:196639900-197597300 (GRCh37/Hg19) region (figure 2). Among the five families with inheritance data provided, all duplications are inherited, three of which from an asymptomatic parent. Patients from this cohort present DD (n=3/9), ID (severe for one) (n=3/9), learning disabilities (n=2/9) and NPD (n=6/9) with ASD (n=3), ADHD (n=2), and aggressive behavior (n=1) (table S1). Further clinical features are detailed in table S1.

## Discussion

This study reports a large cohort of 3q29 duplication carriers with familial data and additional genetic events identified by CMA and exome sequencing recruited among French laboratories.

3q29 duplications are rare CNVs with much lower frequency than other recurrent CNVs associated with NDD of variable expressivity, such as 16p11.2 BP4-BP5 duplication, 16p12.2 deletion or 15q11.2 deletion [D'Angelo et al., 2016; Girirajan et al., 2010; Crawford et al., 2019; Jønch et al., 2019]

Previously, 86 patients with 3q29 duplications have been reported in the literature [Holinski-Feder et al., 2000; Ballif et al., 2008; Lisi et al., 2008; Goobie et al., 2008; Aleixandre Blanquer et al., 2011; Schilter et al., 2013; Fernández-Jaén et al., 2014; Quintela García et al., 2015; Kessi et al., 2018; Tassano et al., 2018; Streata et al., 2020; Coyan and Dyer, 2020; Pollak et al., 2020; Bauleo et al., 2022]. To discuss patients reported in the literature with our cohort, we separated individuals from the literature with >1 Mb 3q29 duplication overlapping the recurrent locus and those with smaller duplication (figure S1 and S2). Similarly, we focused on the reported individuals with apparently isolated duplication for clinical presentation.

### **Recurrent and overlapping 3q29 duplications: Cohort A and literature review**

Seventy-seven individuals have been previously reported with duplication encompassing the region: chr3:195345000-197745100 (GRCh37/Hg19) (figure S1 and S2). Among them, 46 patients from 23 families were described in case reports or small cohorts [Holinski-Feder et al., 2000; Ballif et al., 2008; Lisi et al., 2008; Goobie et al., 2008; Aleixandre Blanquer et al., 2011; Schilter et al., 2013; Fernández-Jaén et al., 2014; Quintela García et al., 2015; Streata et al., 2020; Coyan and Dyer, 2020]. Additionally, Pollak et al. compared 31 patients with 3q29 duplication with 64 controls without duplication and with 117 patients with 3q29 deletion [Pollak et al., 2020]. In this study, the 3q29 duplications' location and size were not specified.

In cohort A, we mostly found recurrent interstitial ~1.6 Mb duplications mediated by NAHR [Carvalho and Lupski, 2016; Yilmaz et al., 2023]. The recurrent duplication was previously reported in 17 patients (figure S2) [Lisi et al., 2008; Ballif et al., 2008; Aleixandre Blanquer et al., 2011; Fernández-

Jaén et al., 2014; Quintela García et al., 2015; Cohan and Dyer, 2020]. A derivative chromosome from an unbalanced reciprocal translocation can lead to a 3q29 terminal duplication with an associated variable deletion, but only one large family has yet been reported with a chromosomal translocation [Holinski-Feder et al., 2000]. Other duplications reported in our cohort (n=2) and the literature are the result of different mechanisms leading to nonrecurrent CNV (figure S2) [Goobie et al., 2008; Schilter et al., 2013; Streat et al., 2020; Carvalho and Lupski, 2016].

In cohort A, around a third of patients (n=11/33) have additional genetic findings (CNV or SNV). Among them, three unrelated patients have pathogenic deletions related to well-described syndromes: the terminal 11q24.1q25 deletion (Jacobsen syndrome, OMIM #147791), the 2q37 deletion (OMIM #600430), and the 12p13.33p13.32 deletion [Han and Park, 2021]. These deletions unequivocally participate to the phenotype. One patient carries the recurrent 15q11.2 deletion BP1-BP2 known as a susceptibility locus for NDD (OMIM #615656), and four families (3, 8, 16 and 19) have a chromosomal deletion or a SNV of uncertain significance, for which we cannot exclude a contribution to the phenotype.

In the literature review, few 3q29 duplication carriers appear to have undergone exome sequencing. In some reports, additional CNVs were reported. A large family was reported with an unbalanced translocation  $t(3;16)(q29;p13.3)$  in 10 siblings, leading to a 16p13.3 deletion associated with Rubinstein-Taybi syndrome [Holinski-Feder et al., 2000]. Two reports include a patient with an additional CNV of uncertain significance [Goobie et al., 2008; Cohan and Dyer, 2020]. Environmental factors such as fetal alcohol exposure have also been mentioned in two families [Cohan and Dyer, 2020]. In the case-control study, patients with an additional genetic diagnosis were excluded [Pollak et al., 2020].

Additional genetic events are an important factor to consider in 3q29 duplication interpretation. Multiple molecular diagnoses have already been well-established to explain phenotype expansions or phenotypic heterogeneity [Karaca et al., 2018; Posey et al., 2017] and notably with pathogenic CNVs

“of mild effect size” and variable expressivity like the recurrent 16p11.2 BP4-BP5 duplications, 16p12.2 deletions or 15q11.2 BP1-BP2 deletions [D’Angelo et al., 2016; Girirajan et al., 2010; Pizzo et al., 2019; Jønych et al., 2019]

As a reminder, in the subset cohort A<sup>isolated</sup>, five families have negative genetic sequencing, and further genetic analyses were not performed for eight families. High rates of developmental delay and learning disabilities are observed, while low rates of neuropsychiatric disorders and intellectual disability are observed. These proportions are similar in the cohort A but we note that ID is exclusively of mild severity when we exclude the patients with an additional genetic finding. Other features are found in low proportions in the cohort A and even lower in the cohort A<sup>isolated</sup>, supporting that these features can be imputed to the additional genetic finding.

For the literature review, we discussed the phenotype of the patients with an apparently isolated duplication (table 2), keeping in mind that genetic sequencing analyses have not been detailed or have not been conducted [Lisi et al., 2008; Ballif et al., 2008; Goobie et al., 2008; Alexandre Blanquer et al., 2011; Schilter et al., 2013; Quintela García et al., 2015; Fernández-Jaén et al., 2014; Tassano et al., 2018; Streata et al., 2020; Pollak et al., 2020]. The cohort reported by Coyan *et al.* was not included into the review because the phenotype was not detailed for each family.

Table 2 : Summary of clinical features of patients with apparently isolated 3q29 duplication larger than 1Mb in the previous studies and in our cohort

|                                     | Case reports                      |                           |   |                                 |                                 |   |  |                                      |                                       |
|-------------------------------------|-----------------------------------|---------------------------|---|---------------------------------|---------------------------------|---|--|--------------------------------------|---------------------------------------|
|                                     | Lisi et al. 2008                  | Ballif et al. 2008        | Goobie et al. 2008  | Blanquez et al. 2011            | Schilter et al. 2013            | Fernandez et al. 2014                       | Quintela et al. 2015                     | Tassano et al. 2018                  | Streata et al.                        |
| No. of individuals                  | 5                                 | 3                         | 9<br>(3 probands and 6 relatives)   | 2<br>(1 proband and 1 relative) | 1                               | 1   | 1  | 1                                    | 1                                     |
| No. of families                     | 1                                 | 3                         | 3   | 1                               | 1                               | 1   | 1  | 1                                    | 1                                     |
| Inherited 3q29dup                   | 4 (1 family)                      | na                        | 2/3   | 1                               | na                              | <i>de novo</i>                              | na                                       | na                                   | na                                    |
| <b>Neurodevelopmental disorders</b> |                                   |                           |   |                                 |                                 |   |  |                                      |                                       |
| Developmental delay                 | 3/4                               | 3/3                       | 3/9   | 1/2<br>language delay           | -                               | global                                      | global                                   | global                               | -                                     |
| Intellectual disability             | 3/3<br>(mild to moderate)         | 3/3<br>(mild to moderate) | 3/8<br>(moderate to severe)   | -                               | -                               | severe                                      | /  | mild                                 | severe                                |
| Learning disabilities               | /                                 | /                         | 2/8   | 1/2                             | -                               | /   | +  | /                                    | /                                     |
| Behavioral disorders                | na                                | na                        | 0/9   | -                               | na                              | na  | ADHD + ODD                               | ODD + ASD                            | +                                     |
| <b>Associated features</b>          |                                   |                           |   |                                 |                                 |   |  |                                      |                                       |
| Neonatal hypotonia                  | na                                | na                        | 2/9   | -                               | na                              | +   | na                                       | -                                    | -                                     |
| Feeding problems                    | na                                | na                        | 1/3   | na                              | na                              | na  | na                                       | na                                   | -                                     |
| Obesity                             | 3/4                               | -                         | 1/9   | na                              | na                              | mild truncal obesity                        | -  | -                                    | na                                    |
| Seizure                             | na                                | na                        | 0/9   | -                               | na                              | focal then generalized seizures             | na                                       | mild focal EEG abnormalities         | -                                     |
| Ear abnormality                     | na                                | na                        | 1/9<br>- conduction deafness  | na                              | na                              | -   | na                                       | -                                    | -                                     |
| Cardiac abnormality                 | 1/5<br>bicommissural aortic valve | na                        | 2/9<br>- AVSD<br>- AVSD and atrial septal defect  | 1/2<br>VSD                      | na                              | atrial septal defect                        | -  | -                                    | na                                    |
| Cerebral abnormality                | na                                | na                        | 2/9<br>- Dandy-Walker malformation<br>- cerebellar vermis hypoplasia  | -                               | na                              | -   | -  | periventricular nodular heterotopias | cortical an<br>cerebellar atr         |
| Ophthalmologic abnormality          | na                                | na                        | 2/9<br>- bilateral microphthalmia, unilateral iris coloboma and unilateral cataract<br>- unilateral iris coloboma | na                              | bilateral severe microphthalmia | -   | -  | strabismus                           | -                                     |
| Musculoskeletal abnormality         | na                                | na                        | 1/3<br>- bilateral radioulnar synostosis  | -                               | na                              | na  | -  | -                                    | -                                     |
| Microcephaly                        | 4/5                               | 1/3                       | 1/9   | -                               | na                              | -   | -  | -                                    | na                                    |
| Macrocephaly                        | 1/5                               | 1/4                       | 0/3   | 1/2                             | na                              | -   | -  | -                                    | na                                    |
| Other features                      |                                   |                           | toe syndactyly (2/3)<br>cleft palate (1/3)  | na                              |                                 | distal spasticity<br>height <3rd percentile | fetal distress<br>bilateral hydrophrosis |                                      | severe cogni<br>regression from<br>10 |
| Asymptomatic                        | -                                 | -                         | 3/9   | 1                               | -                               | -   | -  | -                                    | -                                     |

Notes: <sup>1</sup> Only global DD ; <sup>2</sup> In the first year of life; <sup>3</sup> Recurrent infections, ear pain, dizziness, or tinnitus; <sup>4</sup> unilateral deafness; <sup>5</sup> Strabismus and/or optical refractive disorder.  
Abbreviations : No, number; na, not available; ADHD, attention deficit/hyperactivity disorder; ODD, oppositional defiant disorder; ASD, autistic spectrum disorder; AVSD, Atrioventricular septal defect

The case-control study of Pollak *et al.* found significantly higher rates of neonatal hypotonia, learning disabilities and neuropsychiatric disorders such as autism spectrum disorder, anxiety disorders, and attention deficit hyperactivity disorder in 31 patients with 3q29 duplication compared to 64 controls (table 2). Psychomotor development was delayed in all skills, especially communication milestones. However, the majority of children did achieve developmental milestones and the rate of intellectual disability was not significantly higher than in the control group. The mild neurodevelopmental phenotype reported in our cohort A<sup>isolated</sup> is consistent with the result of Pollak's study, except regarding neuropsychiatric disorders that seem to be more frequent than in our cohort (58% and 29% respectively, table 2) [Pollak *et al.*, 2020].

In the other previously published patients with apparently isolated 3q29 duplication (n=28, table 2), a highly variable neurodevelopmental phenotype has been reported: developmental delay (13/17), mild to severe intellectual disability (12/21), learning difficulties (4/21), and neuropsychiatric disorders (2/8). Severity of NDD in these case reports is more variable than in our cohort A<sup>isolated</sup> (table 2) [Lisi et al., 2008; Ballif et al., 2008; Goobie et al., 2008; Alexandre Blanquer et al., 2011; Fernández-Jaén et al., 2014; Quintela García et al., 2015; Schilter et al., 2013; Tassano et al., 2018; Streata et al., 2020].

Whether in our cohort or in the literature review, multiple clinical features are described in very low and variable proportions. We note that cardiac abnormalities are mainly cardiac defects (table 2) [Lisi et al., 2008; Ballif et al., 2008; Goobie et al., 2008; Alexandre Blanquer et al., 2011; Schilter et al., 2013; Fernández-Jaén et al., 2014; Quintela García et al., 2015; Tassano et al., 2018; Streata et al., 2020; Pollak et al., 2020]. Asymptomatic patients were reported, 2/22 in our cohort and 4/24 patients in the literature, suggesting incomplete penetrance [Goobie et al., 2008; Alexandre Blanquer et al., 2011].

Consistently with the literature's data regarding the inheritance pattern [Lisi et al., 2008; Goobie et al., 2008; Alexandre Blanquer et al., 2011; Fernández-Jaén et al., 2014], 3q29 duplications are more often a familial variation in our cohort A<sup>isolated</sup> (Table 2). The CNV segregates with the symptomatic phenotype, except in two families where it was found in an asymptomatic relative. Intra-familial phenotypes seem homogenous (table 1), as illustrated by family 12, where the father and his four children have the same mild NDD, without additional genetic variation found. A high transmission may consistent with the concept of inherited genetic background in mild NDD [Reichenberg et al., 2016; Kurki et al., 2019]. The above-mentioned recurrent CNVs, the 16p11.2 BP4-BP5 duplications, 16p12.2 deletions and 15q11.2 BP1-BP2 deletions exhibit a similar inheritance pattern [Girirajan et al., 2012]. Furthermore, we observed one-quarter *de novo* duplications which are, interestingly, only found in cohort A<sup>isolated</sup>.

### **3q29 genes**

The identification of critical phenotype driving genes is a challenge in the study of pathologic CNVs. The recurrent 3q29 CNV encompasses 22 protein-coding genes (3q29 genes), including 19 OMIM genes, currently without strong evidence for one or more candidate critical genes. Some genes have been more often discussed such as *DLG1*, *PAK2*, *NCBP2*, *BDH1* but with inconclusive functional data or without a clearly established link with NDD [Carroll et al., 2011; Rutkowski et al., 2021; Antonarakis et al., 2022; Singh et al., 2020]. Triplosensitivity (i.e. sensitivity to increased DNA dosage) of 3q29 genes could be a guiding element towards the identification of phenotype-driving genes. Among the 1,559 triplosensitive genes predicted by Collins et al., two genes *UBXN7* and *PAK2* are in the recurrent 3q29 locus [Collins et al., 2022]. Furthermore, the impact assessment of 3q29 deletion on the human neural transcriptome prioritized nine genes involved in different biological processes : *BDH1*, *CEP19*, *DLG1*, *FBXO45*, *PIGZ*, *RNF168*, *SENP5*, *WDR53*, and in particular *UBXN7* as major driver genes in 3q29 deletion phenotype [Sefik et al., 2021]. A recent functional study on human cortical organoids and a mouse model suggests that the 3q29 deletion can lead to mitochondrial dysfunction. This dysfunction was partially reproduced by *PAK2* knockout, suggesting a partial role for *PAK2* in 3q29 deletion phenotype [Purcell et al., 2023]. Thus, these studies suggest a probable complex impact of 3q29 deletion on several biological processes, involving several 3q29 genes, which may also be the case for duplication.

### **Smaller than 1 Mb duplications: Cohort B and literature review**

We also considered small 3q29 duplications in a distal region of the recurrent duplication between 196,6 and 197,6 Mb of chromosome 3 (cohort B, figure 2) previously reported in the literature [Tassano et al., 2018; Coyan and Dyer, 2020; Bauleo et al., 2022] Nine individuals from four families have been reported with similar duplications (figure S2). Among which, three patients had an associated pathogenic genetic variation and one had a variation of uncertain significance. Four relatives were asymptomatic (three parents and one sister). One patient had a syndromic mild ID,

and two had isolated neuropsychiatric disorders [Tassano et al., 2018; Coyan and Dyer, 2020; Bauleo et al., 2022]. In cohort B, patients have NDD of variable severity (Table S1). We observe a high proportion of neuropsychiatric disorders, to be considered with caution due to the small cohort size. In all patients with available inheritance data (n=3/9 families), the duplication is inherited from an asymptomatic parent (Table S1). However, no additional genetic finding was identified in these patients, particularly in three families with negative exome sequencing.

In the DGV gold database and gnomAD database, 3q29 duplications in this region were reported (4/13759 exomes and 7/21694 respectively) [Coe et al., 2014; Collins et al., 2020]. ACMG classification of these small duplications differ in the DECIPHER database [Firth et al., 2009] and are mostly considered as benign or likely benign by the French cytogenetic laboratory network. The encompassed *BDH1* gene in almost all duplications and *DLG1* gene for some of duplications cannot explain the phenotype on their own, as previously discussed. Thus, our data does not provide evidence in support for a possible critical region.

### **Conclusion**

Individuals in our cohort and previously reported carriers of 3q29 duplications are heterogeneous regarding their clinical features and the duplications' size and location. However, after harmonization, overlapping or recurrent ~1.6 Mb 3q29 duplication could lead to a mild neurodevelopmental phenotype with variable expressivity and incomplete penetrance, supported by several observations: the clinical presentation of patient with isolated 3q29 duplication (cohort A<sup>isolated</sup>), the inheritance pattern, the consistent segregation in mild NDD families, and the proportions of additional genetic events. This highlights the need for further genetic investigations when patients present a severe or syndromic phenotype.

### **Author contributions**

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### **Supplemental Information**

Table S1: Summary of genetic and clinical data in the cohort B.

Figure S1: Flowchart summarizing previously reported patients' distribution, according to the duplication location and presence of additional genetic finding.

Figure S2: Mapping of duplications identified in our families' cohort (dark bars), the duplications of families previously reported (grey bars), segmental duplications (segmental Dups) and OMIM genes in the region Chr3: 194000000-198022430, using UCSC genome browser (build GRCh37/hg19).

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