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Possible incomplete penetrance of Xq28 int22h-1/int22h-2 duplication

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Abstract

Xq28 int22h-1/int22h-2 duplication is the result of non-allelic homologous recombination between int22h-1/int22h-2 repeats separated by 0.5 Mb. It is responsible for a syndromic form of intellectual disability (ID), with recurrent infections and atopic diseases. Minor defects, nonspecific facial dysmorphic features, and overweight have also been described. Half of female carriers have been reported with ID, whereas all reported evaluated born males present mild to moderate ID, suggesting complete penetrance. We collected data on 15 families from eight university hospitals. Among them, 40 patients, 21 females (one fetus), and 19 males (two fetuses), were carriers of typical or atypical Xq28 int22h-1/int22h-2 duplication. Twenty-one individuals were considered asymptomatic (16 females and 5 males), without significantly higher rate of recurrent infections, atopia, overweight, or facial dysmorphism. Approximately 67% live-born males and 23% live-born female carriers of the typical duplication did not have obvious signs of intellectual disability, suggesting previously undescribed incomplete penetrance or low expression in certain carriers. The possibility of a second-hit or modifying factors to this possible susceptibility locus is yet to be studied but a possible observational bias should be considered in assessing such challenging X-chromosome copy number gains. Additional segregation studies should help to quantify this newly described incomplete penetrance.

For affiliations refer to page 244

KEYWORDS

CLIC2, incomplete penetrance, *RAB39B*, X-linked intellectual disability, Xq28 duplication, Xq28 int22h-1/int22h-2 duplication

1 | INTRODUCTION

X-linked intellectual disability (XLID) is a genetically heterogeneous disorder that affects 1/1000 males[1,2] associated with more than 140 genes.[3] Various X-chromosome abnormalities have been described,[4] many inherited from a healthy heterozygous carrier mother. Pathogenic microduplications and functional disomies have long been described within the Xq28 gene-dense region.[5,6] The presence of several low-copy repeats (LCRs) in the region predisposes it to recurrent chromosomal rearrangements by non-allelic homologous recombination (NAHR).[7–10] One of the most commonly reported variants is the Xq28 duplication that includes the dosage sensitive *MECP2* gene, responsible for Lubs X-linked intellectual developmental disorder syndrome (OMIM # 300260).[11,12]

More distally from the *MECP2* gene, El-Hattab et al. first reported a recurrent ~0.5 Mb duplication between LCRs int22h-1 and int22h-2 as being pathogenic in 2011 and then in 2015.[13,14]

To date, 35 cases (19 males and 16 females) with typical int22h-1/int22h-2-mediated duplication have been reported in the literature.[15,16] It is considered to be causal for syndromic XLID, with a number of inconstant distinguishing behavioral, facial, anthropometric, and immune features in males. A minority of female carriers are symptomatic, presenting with milder ID than males.[17–20]

The duplication includes three disease-causing genes: *F8*, *RAB39B*, and *CLIC2*. The gene *F8* encodes coagulation factor VIII, deficient in hemophilia type A (HA) (OMIM 306700).[21] *RAB39B* and *CLIC2* are two major candidate genes for neurodevelopmental disorders observed in cases of duplication at this locus. Indeed, deleterious *CLIC2* sequence variants have been proposed to be causative for X-linked syndromic ID in a single family,[22] whereas loss-of-function variants of *RAB39B* lead to Waisman syndrome (OMIM 311510),[23,24] a form of early onset parkinsonism with ID. However, Vanmarsenille et al.[20] reported an inconstant increase in *CLIC2* mRNA expression in intellectually disabled male carriers of recurrent int22h-1/int22h-2-mediated duplications and concluded that *CLIC2* "diplosensitivity" is unlikely. The same study showed increased expression of *RAB39B* in male individuals with ID and recurrent int22h-1/int22h-2-mediated duplications. It is presumed that *RAB39B* is dose sensitive. The authors concluded that the involvement of *RAB39B* in neurodevelopmental disorders in Xq28 int22h-1/int22h-2 duplication is still unclear but plausible.

Of note, healthy male carriers of atypical Xq28 duplications overlapping the int22h1-int22h2 interval have been described.[25–27] Following an initial puzzling observation of an asymptomatic male carrier with the typical int22h-1/int22h-2-mediated duplication and given the males reported to carry the atypical Xq28 duplication without ID, we decided to study the penetrance of this syndrome in males.

In this retrospective study, we describe a cohort of 15 unrelated families with typical (10 families) and atypical (5 families) Xq28 int22h-1 and int22h-2 duplication.

2 | FAMILIES

Through the French Array Comparative Genomics Hybridization (CGH) network AChro-Puce, we collected data from 15 unrelated families in 8 French university hospitals (written informed consents were received from the patients). The pedigrees are presented in Figure 1. Ten families with typical Xq28 int22h-1/int22h-2-mediated duplication carriers (Families 1, 2, 3, 5, 6, 7, 8, 10, 11, 12) and five families with atypical duplication carriers (Families 4, 9, 13, 14, 15), presented in Figure 2. Here, we describe the four families in which an unaffected male carrier was identified and Family 15, containing a symptomatic male individual without ID. Other families are described in Figures 1, 2, and Table 1.

2.1 | Family 1

The proband (II-5) showed early childhood language regression, had no motor delay, and no dysmorphic features. His clinical examination was normal, except for large tonsils associated with probable recurrent infections. His metabolic blood tests were also normal. He was overweight, of large stature and had an enlarged head circumference. A familial study showed that the duplication was maternally inherited and that it was present in three of six sons. Brother II-1 was an asymptomatic carrier. He went to school and earned a vocational diploma. Brother II-3, also a carrier, required specialized education but experienced neonatal anoxia, a potential confounding factor. The asymptomatic mother (I-2) was a carrier of the typical Xq28 duplication and was unemployed. She was not tested for X-inactivation bias. The non-carrier brothers II-2, II-4, and II-6 had no particular problems or phenotype to report.

2.2 | Family 3

The proband IV-1 was a male fetus (mother III-2). Prenatal array-CGH analysis was performed at 27 weeks of gestation after identification of bilateral talipes equinovarus. This phenotype has not been reported for patients with typical Xq28 duplications and was considered to be an incidental finding. Based on reported complete penetrance in males of Xq28 duplication, the parents opted for termination of the pregnancy. For the second male fetus, IV-2 (asymptomatic mother III-4),

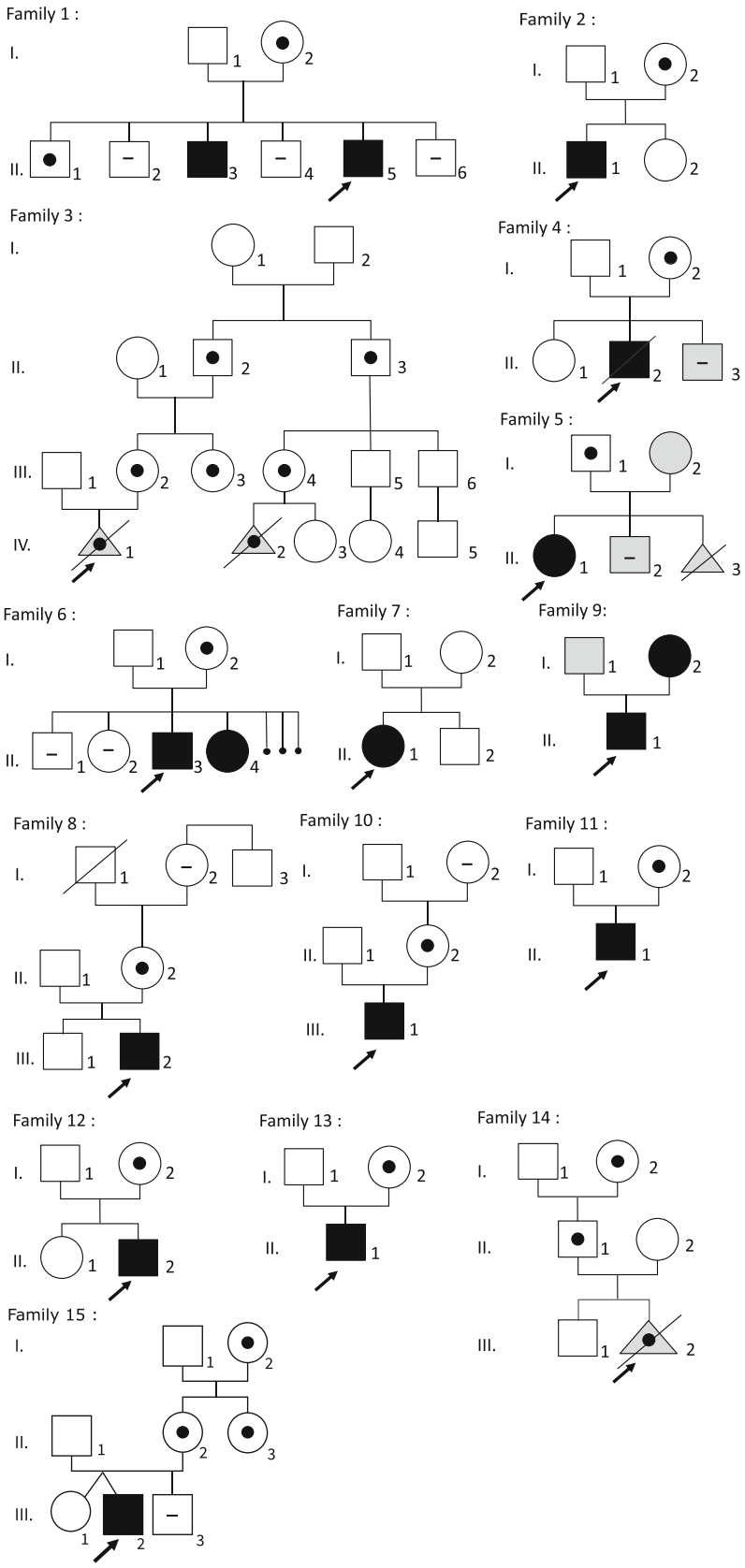

FIGURE 1 Pedigrees. The squares (males) and circles (females) are filled with black (symptomatic carrier), white (asymptomatic or status unknown), and gray (noticeable phenotype not related to Xq28 syndrome); black dot (asymptomatic carrier); minus sign (tested non-carrier). Arrows indicate the probands. The status concerning intellectual disability (ID) for Family 6 I-2 is unknown. Family 3 II-2 was not tested but was a compulsory carrier. Family 3 IV-1 had bilateral talipes equinovarus and Family IV-2 intrauterine fetal growth restriction and duplex kidneys. Family 4 II-3 had the same phenotype as his brother. Family 5 I-2 has seizures treated by sodium valproate, Family 5 II-1 and II-2 have features of fetal valproate syndrome, and Family 5 II-3 had spina bifida. Family 9 I-1 has West syndrome and mild ID. Siblings of Family 12 I.1 are not represented but there is a history of depressive disorder (sister), schizophrenia (another sister), and dyslexia (brother's niece). Family 14 III-2 had myelomeningocele. Family 15 III-2 has anterior balanic hypospadias and hemophilia due to the recurrent pathogenic intron 22 inversion of *F8* (II-2, II-3, and I-2 are also carriers of this inversion); III-3 was a tested non-carrier for F8 int22 inversion and considered to be non-carrier of the familial xq28 duplication (these two elements considered to be linked).

ultrasound showed intrauterine growth retardation and duplex kidneys. Array-CGH identified the Xq28 duplication. The parents opted for termination of the pregnancy. Further familial investigations showed that individual II-3 carried the duplication. He was a craftsman and considered to be asymptomatic. Individual II-2 could not be genetically tested for the duplication but is considered to be an

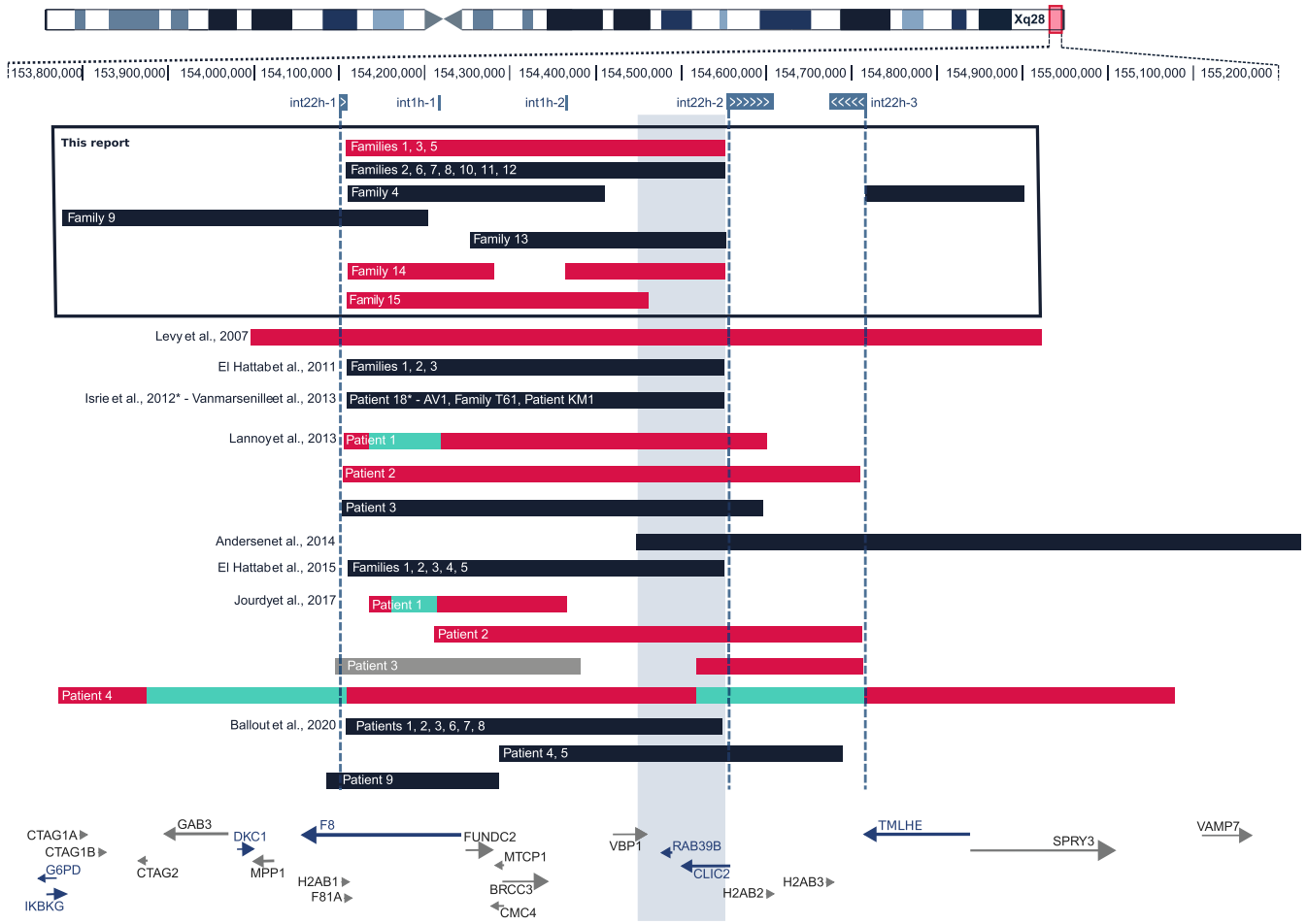

FIGURE 2 Xq28 int22h-1/int22h-2-mediated duplications in our cohort and in the literature sorted by date. CNV are represented as dark blue boxes (male duplication carriers with intellectual disability [ID]), red boxes (male duplication carriers without ID, or families with at least one male carrier without ID), green boxes (triplication), or a light gray box (deletion). The light gray-blue box is the minimum overlap area of duplication with OMIM "morbid" genes in patients with ID (except for the *F8* region, considered to be non-causal for ID). The blue arrows represent OMIM "morbid" genes and the light gray arrows OMIM "non-morbid" genes. The direction of the arrows indicates the direction of transcription. Patient 1 from the study of Lannoy et al. was reported to have moderate HA; Patient 2, severe HA with intron 22 inversion of *F8*; and Patient 3, ID and no HA. Patient 1 from the study of Jourdy et al. was reported to have HA and intron 1 inversion of *F8* and Patients 2, 3, and 4, HA and intron 22 inversion. The four carriers of Family 15 also have the *F8* intron 22 inversion (1 male patient with HA and 3 female patients without HA). The direction of the LCR is represented in accordance with the literature.[35] *Patient 18 from the study of Isrie et al. is the same as Patient AV1 from that of Vanmarsenille et al.

obligatory carrier (Figure 1). We have no information about his professional activity or study path, but he was not reported to have an ID. He had two asymptomatic carrier daughters, III-3 and III-2. No female carrier was tested for X-inactivation bias in this family. No history of intellectual impairment was reported in the rest of the family.

2.3 | Family 5

In this family, the asymptomatic father (I-1) and his daughter (II-1) were carriers of the typical Xq28 duplication. His son (II-2) did not carry the duplication.

The mother was treated with sodium valproate for seizures (treatment continued during pregnancy). Her two children showed behavioral problems that may fit the spectrum of valproate fetopathy. The daughter (II-1) had a specific learning disability without ID and anxiety-depressive disorder. For this study, she was considered to be symptomatic, as involvement of Xq28 duplication could not be ruled out. Her brother (patient II-2) had oral language disorders and a working memory defect.

2.4 | Family 14

In Family 14, female fetus III-2 was prenatally affected by myelomeningocele with a cerebral impact for which amniocentesis was performed. Array-CGH showed an atypical Xq28 duplication (cf. Table 1 and Figure 2) inherited from the asymptomatic father (II-1), who had

TABLE 1 Characteristics of CNVs (minimal genomic coordinates, Hg19) with the patients' phenotype.

Family	Family 1				Family 2		Family 3						
CNV genomic coordinates (hg19)	chrX:154,118,619-154,560,375				chrX:154,120,738-154,560,375		chrX:154,118,619-154,560,375						
Typical duplication	+				+		+						
Atypical duplication	−				−		−						
Length	441 kb				439,6 kb		441 kb						
Individual	**II-5**	I-2	II-1	II-3	**II-1**	I-2	**IV-1**	III-2	III-3	II-2	IV-2	III-4	II-3
Gender	M	F	M	M	M	F	M	F	F	M	M	F	M
Age at screening	6	37	18	13	5		29GW				in utero		
Skewed X inactivation		NP				NP							
Phenotype													
Symptomatic	+	−	−	+	+	−	NA	−	−	−	+	−	−
Unaffected	−	+	+	−	−	+	NA	+	+	+	−	+	+
Intellectual disability	+	−	−	+	+	−	NA	−	−	−	NA	−	−
Motor delay	−						NA				NA		
Language delay	+				+		NA				NA		
Hypotonia	−						NA				NA		
Behavioral abnormalities	+						NA				NA		
Sleep disturbance	−				Nocturnal enuresis		NA				NA		
Psychiatric signs	−						NA				NA		
Recurrent infections	Tonsillectomy						NA				NA		
Atopia	−						NA				NA		
Overweight	+			−			NA				NA		
Dysmorphic features	−				Strabismus Low-settled ears Imperforate anus		Bilateral talipes equinovarus				NA		
Other	Encopresis			Perinatal anoxia	Encopresis		Medical termination of pregnancy	Polycystic ovaries			IUGR Duplex kidneys Medical termination of pregnancy		
Study level/schooling	NA	Stopped at middle school	Vocational baccalaureate	special needs educational division	NA		NA				NA		

TABLE 1 (Continued)

Family	**Family 1**					**Family 2**	**Family 3**			
Profession	NA		Unemployed	NA	NA	NA	NA		NA	Craftsman
Family	**Family 4**		**Family 5**		**Family 6**				**Family 7**	
CNV genomic coordinates (hg19)	chrX:154,120,738-154,417,044		chrX:154,118,619-154,560,375		chrX:154,120,738-154,560,375				chrX:154,120,738-154,560,375	
Typical duplication	−		+		+				+	
Atypical duplication	+		−		−				−	
Length	296,3 kb		441 kb		440 kb				440 kb	
Individual	**II-2**	I-2	**II-1**	I-1	**II-3**	I-2	II-4		**II-1**	
Gender	M	F	F	M	M	F	F		F	
Age at screening	2	36			10				28	
Skewed X inactivation		NP				93/07	−		90/10	
Phenotype										
Symptomatic	+	−	+	−	+	−	+		+	
Unaffected	−	+	−	+	−	+	−		−	
Intellectual disability	+ (severe)	−	+	−	+	−	+ (mild)		+	
Motor delay	+ (severe)				+		−		UN	
Language delay	+ (severe)				+		+ (deafness)		UN	
Hypotonia	+ (neonatal)				−		−		UN	
Behavioral abnormalities	UN		Anxio-depressive disorder		ADHD OCD		ADD		Self-harming	
Sleep disturbance	UN						Sleep apnea			
Psychiatric signs	−						−		+	
Recurrent infections	UN (VZV septicemia)				−		UN			
Atopia	Eczema				−		−			
Overweight	−				−	−			−	
Dysmorphic features	Microcephaly (−2.5 SD) Large fontanelles		+		−		Macroglossia (neonatal) Hemihypertrophy		Facial dysmorphia Long breakable teeth High-arched palate	
Other	Convulsive encephalopathy Cerebral MRI anomalies Died at 2,years		Fetal valproate syndrome Deciduous teeth persistency		Long QT (*KCNE1* mutation) Ventricular septal defect	Syringomyelia Protein C deficiency (thrombosis)	Hydramnios Precocious puberty Pyelonephritis Negative Beckwith-Wiedemann syndrome testing		Lymphoedema	
Study level/schooling	NA					Vocational baccalaureate			Vocational qualification	

(Continues)

TABLE 1 (Continued)

Family	**Family 4**	**Family 5**	**Family 6**				**Family 7**	
Profession	NA	NA	NA	Housewife	NA			
Family	**Family 8**		**Family 9**		**Family 10**		**Family 11**	
CNV genomic coordinates (hg19)	chrX:154,120,738-154,560,375		chrX:153,786,432-154,213,569		chrX:154,118,619-154,560,375		chrX:154,118,619-154,560,375	
Typical duplication	+		−		+		+	
Atypical duplication	−		+		−		−	
Length	440 kb		427 kb		441.757 kb		442 kb	
Individual	**III-2**	II-2	**II-1**	I-2	**III-1**	II-2	**II-1**	I-2
Gender	M	F	M	F	M	F	M	F
Age at screening	14	38	3		11	41	16	46
Skewed X inactivation		84/16		98/02		NP		NP
Phenotype								
Symptomatic	+	−	+	+	+	−	+	−
Unaffected	−	+	−	−	−	+	−	+
Intellectual disability	+	−	+ (severe)	+	+ (mild)	−	+ (moderate)	−
Motor delay	+		+		−		−	
Language delay	+		+		−		+	
Hypotonia	+ (axial)/peripheral hypertonia		+ (generalized)		−		−	
Behavioral abnormalities	Aggressiveness Intolerance to frustration Tantrum		ADHD Stereotypy		−		ADD Anxiety Impulsiveness Self-harm	
Sleep disturbance			+ (Night awakenings)				Enuresis	
Psychiatric signs			−		+		+	
Recurrent infections	−		−				+ (otitis)	
Atopia	−		−					
Overweight	−		+		+		+	
Dysmorphic features	Facial asymmetry Triangular facies Long eyelashes Large ears Thin superior lip High arqued palate Retrognathism Unilateral ptosis Scoliosis		Micro-plagiocephaly Hypertrophy of the nasal bones Middle face hypoplasia Blepharophimosis Low set ears Pectus excavatum Single transverse palmar fold Long thing fingers		−		Thick eyebrows Index fingers clinodactyly	

TABLE 1 (Continued)

Family	Family 8	Family 9	Family 10	Family 11
Other	Bilateral cryptorchidia MRI white matter intensities Hypermetropia astigmatism Constipation	Balanic hypospadias Gynecomastia Epilepsy Ventricular septal defect Prematurity (33GW)	Constipation Hypermetropic strabismus	Pes planus
Study level/schooling	Needs specialized educational care		National Diploma	Medical educational institute
Profession	NA — Secretary	NA — Sheltered workshop	Temporary worker	NA — Office worker

Family	Family 12		Family 13		Family 14			Family 15				Total = 15	
CNV genomic coordinates (hg19)	chrX:154,118,619-154,560,375		chrX:154,261,914-154,560,375		chrX:154,120,432-154,290,166 // chrX:154,375,294-154,560,375			chrX:154,120,738-154,464,663					
Typical duplication	+		−		−			−				14,M	13F
Atypical duplication	−		+		+			+				5,M	8F
Length	441.757 kb		298.462 kb		200 kb // 80 kb			345 kb					
Individual	**II-2**	I-2	**II-1**	I-2	**III-2**	II-1	I-2	**III-2**	II-2	II-3	I-1	Total = 40	
Gender	M	F	M	F	F	M	F	M	F	F	F	19,M	21F
Age at screening	10	35	14	46	in utero	34		4					
Skewed X inactivation		NP		NP	NP				NP	NP	NP		
Phenotype													
Symptomatic	+	−	+	−	NA	−	−	+	−	−	−	12,M	4F
Unaffected	−	+	−	+	NA	+	+	−	+	+	+	5,M	16F
Intellectual disability	+ (mild, WISCV)	−	+ (mild)	−	NA	−	−	−	−	−	−	11,M	4F
Motor delay	−		−		NA			−					
Language delay	+		+		NA			−					
Hypotonia	−		−		NA			−					
Behavioral abnormalities	+ Anxiety		Flapping Swinging		NA			−					
Sleep disturbance	Difficulties in falling asleep				NA			−					
Psychiatric signs	+				NA			−					
Recurrent infections	−				NA			−					

(Continues)

TABLE 1 (Continued)

Family	Family 12	Family 13	Family 14	Family 15			Total = 15
Atopia	–		NA	–			
Overweight	+	+	NA	–			
Dysmorphic features	–	–	NA	–			
Other	Astigmatism Normal cerebral IRM	Gynecomastia Pes planus	Myelomeningocele	Anterior balanic hypospadias Severe early onset hemophilia (*F8* int22 inversion)	*F8* int22 inversion carrier, no hemophilia	*F8* int22 inversion carrier, no hemophilia	*F8* int22 inversion carrier, no hemophilia
Study level/ schooling	Needs specialized educational care		Baccalaureate	Needs specialized educational care	School certificate	NA	Advanced
technician's certificate	No difficulties						
Profession	NA	Cashier	NA	Cleaner	NA	Technician	NA

Note: Probands are shown in bold. Empty cells correspond to data not provided. Fetus family 3 IV-1 and IV-2 and fetus family 14 III-2, with bilateral talipes equinovarus, intrauterine fetal growth restriction and duplex kidneys, and myelomeningocele, respectively, were considered to be neither symptomatic nor asymptomatic because the antenatal signs were too nonspecific and have not been reported for xq28 duplication in the literature.
Abbreviations: ADD, attention deficit disorder; ADHD, attention-deficit/hyperactivity disorder; F, female; GW, gestational week; IUGR, intrauterine fetal growth restriction; M, male; NA, not applicable; NP, not-performed; OCD, obsessive-compulsive disorder; UN, unknown.

an advanced technician's certificate and did not report academic difficulties. These duplications were considered to be incidental findings in the fetus but because of the severity of the myelomeningocele, the parents opted to terminate the pregnancy at 23 weeks gestation (fetal autopsy was rejected). The paternal grandmother was a heterozygous carrier.

We considered this duplication to be atypical because there were normal array-CGH probes within the copy number variation (CNV) (Figure 2). Both duplications have been proven to be in *cis*. We were unable to further characterize this chromosomal rearrangement.

2.5 | Family 15

The proband of this family was a male (III-1) diagnosed with hypospadias at birth and severe hemophilia at 1 year of age due to multiple episodes of ecchymosis. He carried an atypical Xq28 duplication that did not span *RAB39B* nor *CLIC2*, associated with the recurrent pathogenic intron 22 inversion of *F8* responsible for hemophilia A (HA). He showed normal psychomotor development. His mother (II-2), an aunt (II-3), and his grandmother (I-2) were asymptomatic carriers. For this study, we considered patient III-1 to be symptomatic because of hypospadias (already described in the Xq28 duplication syndromic spectrum) but he did not show a neurodevelopmental disorder.

2.6 | Overall cohort

Our cohort included 40 patients, 21 females (one fetus) and 19 males (two fetuses). Twenty-one individuals were considered to be asymptomatic (16 females and 5 males). Probands had inherited the Xq28 duplication from an unaffected carrier parent in 13 cases (11 mothers, 2 fathers) and inheritance was not known in one case (Family 7). Asymptomatic carrier mothers possibly inherited the duplication from their fathers in Families 8 and 10, in which segregation was impossible for the father and mothers were proven non-carriers (Figure 1). The CNV of all probands was confirmed by targeted analysis, such as qPCR, MAQ, MLPA, or FISH (not available for Family 5). For two of the symptomatic carriers, environmental factors could explain part of their phenotype (Family 1, individual II.3: perinatal anoxia; Family 5, individual II-1: fetal valproate syndrome; see Table 1 and Figure 1). No identical formal investigation of ID was performed on our patients.

The cohort included three carrier fetuses. Their neurodevelopmental status is unknown (terminated pregnancies, impossible to carry out a childhood evaluation).

The Xq28 chromosomal microduplications from our cohort are presented in Figure 2 and compared to those reported in the literature. Typical recurrent Xq28 int22h-1/In22h-2-mediated duplication was present in Families 1, 2, 3,5, 6, 7, 8, 10, 11, and 12. Atypical Xq28 duplications were present in Families 4, 9, 13, 14, and 15 (Figure 2).

In Family 4, the proband and his asymptomatic mother were also carriers of another Xq28 duplication, classified as a variant of unknown significance (arr[GRCh37] Xq28(154722370_154908471)x2 mat).

Although recurrent infections, atopia, overweight or obesity, and facial dysmorphism have been described in the literature, none of these elements were clearly apparent in our cohort. Patient II-1 of Family 11 was the only one reported with recurrent infections (otitis). Patient II-2 from Family 4 died from varicella zoster virus septicemia at 2 years of age, which can be considered as a sign of immune dysfunction, but without certitude. He was also the only patient in our cohort with atopia. Finally, patient II-5 from Family 1 had a tonsillectomy, which could be a sign of recurrent infections, but his medical record did not explicitly mention it. Six of our patients were overweight or obese and seven were not (status unknown for 24, nonapplicable for the three fetuses). We found no recurrent or distinctive facial dysmorphism in our cohort.

3 | DISCUSSION

Our study is the first to report unaffected male carriers of the typical int22h-1/int22h-2 duplication (Families 1, 3, 5). This finding suggests incomplete penetrance of this CNV in male hemizygous carriers and should change genetic counseling with respect to neurodevelopment when this variant is identified. Among the 27 patients with a typical int22h-1/int22h-2 Xq28 duplication in our cohort, 8 of 12 (~67%) live-born male carriers are intellectually disabled (including Patient II-3 from Family 1, who experienced perinatal cerebral anoxia). Patient I-1 from Family 10 and I-1 from Family 8 are likely male carriers, but they were not tested and therefore their symptomatic/asymptomatic status is unknown. Three of 13 (~23%) live-born female carriers of a typical Xq28 duplication are intellectually disabled (including a probable case of valproate fetal syndrome for Patient II-1, Family 5). Taking into account the typical and atypical duplication carriers, 11/17 (~65%) live-born male carriers are intellectually disabled (~59% excluding questionable Patient II-3 from Family 1) and 4/20 (20%) of live-born female carriers are intellectually disabled (15% excluding questionable Patient II-1, Family 5). Interestingly, the variant is present 13 times in gnomAD-SV v4.0 including three cases of male hemizygous carriers.

These results are inconsistent with data from the most recent review of the literature in which 100% of assessable males and 50% of assessable females with Xq28 duplications were reported to be intellectually disabled.[17] In Family 4, the Xq28 duplication is shorter than the typical duplication, and spare the two ID candidate genes *RAB39B* and *CLIC2* (Figure 2). The proband (II-2) and his mother (I-2) are also carriers of another small Xq28 duplication, considered to be a variant of unknown significance (arr[GRCh37] Xq28 (154722370_154908471)x2 mat) that affects the *TMLHE* gene. A complex chromosomal rearrangement with a duplication of *TMLHE* has already been reported for an individual with severe HA without ID[28] in which the proband's brother had the same phenotype but was not a carrier of the *TMLHE* duplication. This may suggest that this CNV is not causative, but no further studies were performed. In Family 9 of our cohort, the duplication only partially overlapped with the classical Xq28 int22h-1/int22h-2-mediated duplication (Figure 2), excluding *RAB39B* and *CLIC2*. The familial history suggests a

neurological condition for the father (seizures) and ID for the mother. Genetic investigations could be pursued. In Family 14, we found two monoallelic duplications. These duplications appear to spare the *BRCC3* gene but candidate genes *RAB39B* and *CLIC2* are included (see Figure 2). Ballout et al. reported an atypical Xq28 duplication associated with ID in males (patient 9)[15,16] that spares *RAB39B* and *CLIC2*.

There are several possible explanations for our observations. A two-hit model, as discussed before for 16p12.1 and 16p13.11 deletions,[29,30] could explain the observed incomplete penetrance in males and Xq28 int22h-1/int22h-2-mediated duplication could be a risk factor for ID. In our study, there was no exome or genome sequencing data to investigate this possibility, except for Patient II-2 of Family 4 (no additional variant reported). Polygenic etiology or disruption of regulatory elements could also be involved. Another as yet unexplored possibility is the existence of environmental modifying factors (exogenic agents in pregnancy, educational environment, etc.).

In females, incomplete penetrance could result from skewed X-inactivation but no correlation between affected females and X-inactivation has been yet described.[31–34] In our study, six females were tested for skewed X-inactivation. Two asymptomatic carrier mothers (Family 6, Patient I-2; Family 8, Patient II-2) and two symptomatic carrier probands (Family 7, Patient II-1; Family 9, Patient I-2) show skewed X-inactivation. However, one symptomatic carrier female does not show skewed X-inactivation (Family 6, Patient II-4). Thus, there is no clear correlation between skewed X-inactivation and the phenotype.

The last possibility is that of an observational/reporting bias. Xq28 int22h-1/int22h-2-mediated duplication could be a rare CNV incidentally identified in affected patients referred for testing in a setting in which array-CGH are used as a first-tier test. In our four families with asymptomatic male carriers of Xq28 duplications, family testing was required after discovery of the CNV in fetuses, with array-CGH indicated for common ultrasound signs. Asymptomatic male carriers of atypical duplications encompassing the int22h-1/int22h-2 interval and the candidate genes for ID have been reported (Levy et al.[25]; Lannoy et al.[26]; Jourdy et al.[27]; gnomAD DUP_X_54663 and DUP_X_54668) but are poorly discussed in the reports that describe the affected patients. To date and to our knowledge, no benign or likely benign typical Xq28 int22h-1/int22h-2 duplication are described in publicly available databases (Decipher, Clinvar). The variant has been observed 13 times in gnomAD-SV v4 population database with equivalent allele frequencies for females ($n = 10$, 0.015%) and males ($n = 3$, 0.01%).

Our retrospective study is based on data collection by multiple teams in multiple French university hospitals. Some of the collected data from the medical records may be imprecise. The method used to evaluate ID was not specified in our data, but array-CGH testing of probands was requested by specialists accustomed to determining ID. To overcome the absence of formal neuropsychological evaluation of unaffected carriers, we used educational attainment to approximate the autonomy of carriers and therefore the absence of ID.

Our study sheds new light on the possible incomplete penetrance of Xq28 int22h-1/int22h-2-mediated duplication. This has an important impact on genetic counseling, in particular, in situations of prenatal diagnosis. In our cohort, three fetuses had inherited the duplication from their mother. In all cases, the CNV was considered to be an incidental finding with respect to the reason for the referral. Several couples opted for termination of the pregnancy, assuming complete penetrance.

Further characterization of the penetrance of Xq28 duplication will require extensive segregation studies to increase the number of male carrier cases and thus clarify genetic counseling. If incomplete penetrance is confirmed, this would justify the pursuit of genetic investigations beyond array-CGH to rule out the presence of another pathogenic variant before genetic counseling.

Our cohort study allowed us to assert incomplete penetrance for ID in males carrying the 0.5 Mb typical Xq28 int22h-1/int22h-2-mediated duplication, as we report four unaffected male carriers. Further studies with extensive segregation analysis are required to better determine and quantify penetrance and expressivity so that genetic counseling can be adapted accordingly.

AUTHOR CONTRIBUTIONS

Nicolas Chatron co-written the paper with Alexis Billes. Alexis Billes made the figures. Mathilde Pujalte helped to collect data on families, to review the manuscript and she created the framework for the Table 1. Gilles Morin made feedback on the manuscript. Audrey Putoux, Nicolas Chatron, Mathilde Pujalte, Damien Sanlaville, Caroline Schluth Bolard and Marianne Till from the regional universitary hospital Hospices Civils de Lyon and James Lespinasse from Centre Hospitalier de Chambéry provided information on families 1 and 2. Alexis Billes, Gilles Morin, Guillaume Jedraszak and Florence Amram from the regional universitary hospital of Amiens Picardie provided information on family 3. Klaus Dieterich, Gaëlle Vieville and Charles Coutton from the regional universitary hospital of Grenoble-Alpes provided information on family 4. Patrick Callier and Benoît Mazel from the regional universitary hospital of Dijon Bourgogne provided information on family 5. Perrine Pennamen and Caroline Rooryck from the regional universitary hospital of Bordeaux provided information on families 6, 7, 8 and 9. Elise Brischoux-Boucher, Daniel Amsallem, Paul Kuentz, Juliette Piard, Virginie Roze-Guillaumey, Chloé Trouve and Anne-Laude Avice Denizet from regional universitary hospital of Besançon provided information on families 10, 11, 12 and 13. Odile Boute, Sonia Bouquillon, Elise Boudry-Labis and Mélanie Rama from the regional universitary hospital of Lille provided information on family 14. Marlène Rio from the Necker–Enfants Malades Hospital provided information on family 15.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Written informed consent was received from the patients. The authors adhere to the Declaration of Helsinki Principles. This study was approved by the local institutional Ethics Committee (CNIL Register Number 22_5867).

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