BRIEF COMMUNICATION

22q11.2 rearrangements found in women with low ovarian reserve and premature ovarian insufficiency

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Abstract

Ovarian reserve represents the number of available follicles/oocytes within ovaries and it can be assessed by follicle stimulating hormone levels, anti-Müllerian hormone levels, and/or antral follicle count determined by ultrasounds. A low ovarian reserve is defined by an abnormal ovarian reserve test. This condition can be considered premature if it occurs before the age of 40, leading to premature ovarian insufficiency. Despite the growing knowledge concerning the genetic basis of ovarian deficiency, the majority of cases remain without a genetic diagnosis. Although 22q11.2 deletions and duplications have been associated with genitourinary malformations, ovarian deficiency is not a commonly reported feature. We report here four patients bearing a 22q11.2 rearrangement, identified during the clinical assessment of their low ovarian reserve or premature ovarian insufficiency, and discuss the molecular basis of the ovarian defects.

Background

The 22q11.2 region is enriched with low-copy repeats (LCR22-A to LCR22-H). The misalignment of these LCRs during meiosis leads to non-allelic homologous recombination and is an important mechanism underlying chromosomal 22q11.2 microdeletions or microduplications [1]. Although different-sized 22q11.2 deletions have been identified, most of the patients (~90%) have a common ~3 Mb deletion (LCR22-A/LCR22-D), whereas most other patients (~7%) have a smaller ~1.5 Mb deletion (LCR22-A/LCR22-B). A small percentage of affected individuals have shorter deletions [1]. These 22q11.2 rearrangements are known to be responsible for multiple congenital anomalies. The 22q11.2 deletion syndrome (OMIM 188400, OMIM 192430) is the second most common cause of developmental delay and congenital heart defect after Down syndrome, with a prevalence estimated to be 1 in 4000 live births, and is a de novo event in 90% of cases [2]. Individuals with 22q11.2 deletion syndrome can show a broad spectrum of phenotypic abnormalities, with a significant variability of expression and in the penetrance of each feature: conotruncal congenital heart malformations, palatal and velopharyngeal defects, facial dysmorphism, hypocalcemia, immunodeficiency, learning disabilities, psychiatric disorders, genitourinary (GU) anomalies, amongst others. The comparison between individuals with common and atypical 22q11.2 deletions did not demonstrate in most cases a correlation between the size and the location of the deletion and the clinical features of the subjects [3]. The 22q11.2 duplication syndrome (OMIM 608363) is considered as a different clinical entity from 22q11.2 deletion syndrome. The 22q11.2 duplication frequency is estimated to be half of the 22q11.2 deletion

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Table 1 Clinical and molecular features of patients 1 to 4 of the study, DECIPHER, and published literature patients

Patient	Clinical features	Familial features	Karyotype	Genes candidate (BP and 1 Mb each side)	CNV	Genomic coordinates (GRCh37/hg19) chr22:
DECIPHER 249397	**Primary amenorrhea**, aganglionic megacolon, hyperactivity, short stature, psychosis, short attention span	Unkown	46,XX		~900 kb del LCR22-C/-D inh	21032298–21939922
DECIPHER 262934	**Secondary amenorrhea**, congenital heart defect, abnormal dental morphology, coarse facial features, intellectual deficiency	Unkown	46,XX		~3 Mb del LCR22-A/-D dn	18919742–21440655
1	**Low ovarian reserve** at 38, dysmenorrhea, primary infertility, hypotrophic ovaries, uterine adenomyosis, endometriosis, short stature, body mass index = 34 AMH = 0.4 ng/mL, AFC = 0, FSH = 6 UI/L, E2 = 24 ng/L	Breast cancer in one maternal aunt	46,XX		~1.5 Mb del LCR22-A/-B ND	min: 18894835–20311763 (1.4 Mb); max: 18651673–20719112 (2 Mb)
2	**Low ovarian reserve** at 37, regular menstruation with dysmenorrhea, two miscarriages, short stature, body mass index = 28 AMH = 1.3 ng/mL, AFC <3, FSH = 17 UI/L, E2 = 51 ng/L.	Premature ovarian insufficiency in one sister; Breast cancer in a paternal aunt; Ovarian cancer in paternal grand-mother	46,XX		~1.5 Mb dup LCR22-A/-B ND	min: 18894835–20311763 (1.4 Mb); max: 18651673–20719112 (2 Mb)
3	**Low ovarian reserve** at 16, irregular menstruation, varus foot and torticollis at birth, mild developmental delay AMH = 0.6 ng/mL, FSH = 7 UI/L, E2 = 111 ng/L	Mother: irregular menstruation, one miscarriage, oligoamnios during her third pregnancy (one healthy younger brother)	46,XX		~3 Mb dup LCR22-A/-D mat	min: 18894835–21464119 (2.6 Mb); max: 18651673–21798705 (3.1 Mb)
4	**Premature ovarian insufficiency** at 30, irregular menstruation, one miscarriage, one intrauterine fetal death, two children with oocyte donation, body mass index = 25 AMH <0.4 ng/mL, AFC = 1, FSH = 39 UI/L	Infertility in one brother (no molecular data)	46,X,t(X;8)	Chromosome X IGSF1 (OMIM 300137) FIRRE (OMIM 300999) STK26 (OMIM 300547) FRMD7 (OMIM 300628) **RAP2C-AS1 - BP** MBNL3 (OMIM 300413) HS6ST2 (OMIM 300545) USP26 (OMIM 300309) TFDP3 (OMIM 300772) Chromosome 8 STMN2 (OMIM 600621) HEY1 (OMIM 602953) **MRPS28 (OMIM 611990) - BP** TPD52 (OMIM 604068) PAG1 (OMIM 605767)	~3 Mb dup LCR22-A/-D ND	min: 18937937–21464119 (2.5 Mb); max: 18920001–21798705 (2.9 Mb)

Table 1 (continued)

Patient	Clinical features	Familial features	Karyotype	Genes candidate (BP and 1 Mb each side)	CNV	Genomic coordinates (GRCh37/hg19) chr22:
DECIPHER 268195	**Delayed puberty**, short stature	Unkown	46,XX		~3 Mb dup LCR22-A/-D dn	18844632–21462353
Vaz et al. [4]	**Ovarian cyst**, short stature, developmental delay, hearing impairment, upper respiratory infections, restrictive ventricular septal defect, and membranous subaortic stenosis.	Seven normal siblings; mild intellectual disability and learning difficulties in the father	46,XX		~3 Mb trp LCR22-A/-D pat	18661724–21917251

Bold entries refer main ovarian features, *BP* breakpoint, *del* deletion, *dup* duplication, *trp* triplication, *mat* maternal, *pat* paternal, *inh* inherited, *dn* de novo, *ND* not determined, *min* minimal (first and last abnormal probes), *max* maximal (last and first normal probes surrounding the imbalance)

and is associated with a highly variable, milder and less characteristic phenotype than the 22q11.2 deletions, with some overlap existing [4]. Many of the affected patients have inherited their 22q11.2 duplication from a phenotypically normal parent [5]. The most frequently reported features are learning difficulties and psychiatric disorders. Facial dysmorphism, congenital heart malformations, and GU abnormalities are also reported. Among the genes involved in the 22q11.2 imbalances, *TBX1* haploinsufficiency seems to be largely responsible for the clinical findings, particularly the malformations; however, it cannot explain all the features observed and several other genes have been implicated in the pathogenesis [1]. *Crkl* for example, has been recently proposed to contribute to GU defects and to female subfertility in a mouse model [6].

Although 22q11.2 rearrangements have been associated with GU abnormalities, ovarian deficiency does not appear to be a commonly described feature. Ovarian reserve (OR) represents the number of available follicles/oocytes within ovaries and it can be assessed by follicle stimulating hormone (FSH) levels, anti-Müllerian hormone (AMH) levels, and/or antral follicle count (AFC) determined by ultrasounds [7]. A low OR (LOR), leading to poor fertility outcomes, can be defined by AMH <1.1 ng/mL or AFC <5–7 [8]. This condition is mostly the consequence of age but it can be considered premature if it occurs before the age of 40. Premature ovarian insufficiency (POI) is characterized by the occurrence of irregular or absent menstruation before the age of 40 with high FSH and low estradiol levels. This loss of ovarian function represents one of the main causes of female infertility. POI has often a genetic basis with many genes causing POI in a monogenic manner, but it is likely that the reproductive phenotype can also result from interactions of many genes in a polygenic manner. These genes can affect various processes such as gonadal development, meiosis, DNA repair, folliculogenesis, steroidogenesis, apoptosis, and mitochondrial function. Although the knowledge concerning the genetic basis of POI has grown, particularly due the implementation of whole-exome sequencing, the majority of patients remains without a genetic diagnosis [9].

Here we report 22q11.2 copy number variations (CNVs) in four of 80 women with LOR or POI assessed by array-CGH. These results indicate that ovarian deficiency (LOR and/or POI) may be a more frequent and underreported finding associated with 22q11.2 CNVs.
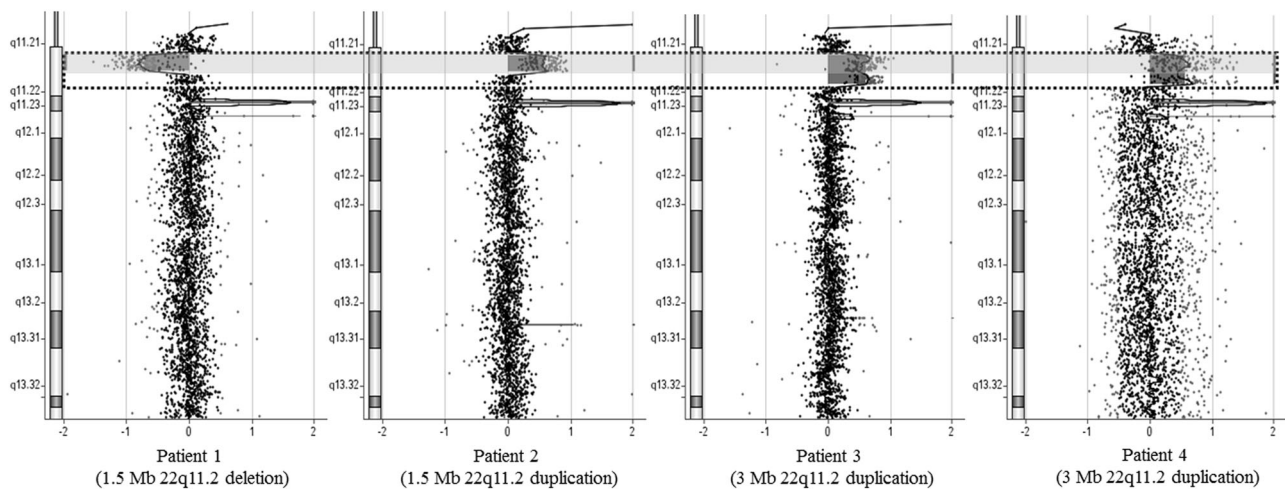
Fig. 1 Chromosome 22 array-CGH profiles in the four patients with ovarian defects. Vertical axis: chromosome 22q bands and sub-bands; horizontal axis: $\log_2$ ratio; dotted box = ~3 Mb CNVs; gray box = ~1.5 Mb CNVs

Patients and methods

Patients

The four patients of the study belong to a cohort of 80 infertile women under the age of 40, presenting with disorders of menstruation, FSH >20 UI/L and/or AMH <1.1 ng/mL and/or AFC <5, enrolled in an oocyte donation program. Sixty patients were previously described [10]. Additional features and familial history of the four patients are detailed in Table 1. The genetic assessment of their condition included *FMR1* screening, karyotype, and array-CGH. Material of the patients' parents was not available for patients 1, 2, and 4. Women were recruited from Rennes Hospital and all individual participants gave an informed consent for the genetic analysis of their condition. The study was approved by the local ethic committee of Rennes University Hospital, France.

Methods

Karyotype

Conventional R-banded karyotypes were performed on metaphase cells prepared from PHA-stimulated cultured peripheral blood cells according to standard protocols [11].

Microarray

Oligonucleotide array-CGH was performed using the Agilent Human Genome CGH microarray 105K or 180K (Agilent Technologies, Santa Clara, CA, USA) according to the protocol provided by the manufacturer. Microarrays

were scanned using the Agilent scanner G2565BA. Images were extracted using Agilent Feature Extraction software. A graphical overview and analysis of the data were obtained using the Agilent CytoGenomics software (statistical algorithm: ADM-2, sensitivity threshold: 6). Identification of the probes with a significant gain or loss was based on the $\log_2$ ratio plot deviation from 0 with cut-off values over 0.5 and under −0.5, respectively. The Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home) was used as a control population to identify benign CNVs. This DGV lists common CNVs detected in healthy individuals with more than 500,000 merged-CNVs. Nevertheless, information from this database has to be interpreted with caution since it represents healthy individuals without information concerning fertility and that variants annotated in DGV can be disease causing in some patients according to the phenotype. The CNVs are described using the version GRCh37 of the human genome (hg19).

Fluorescence in situ hybridization (FISH)

FISH was performed using the Vysis DiGeorge Region Probe TUPLE1 (22q11.21) / ARSA (22q13.33) (Abbott Molecular, Des Plaines, IL, USA) according to the protocol provided by the manufacturer. For patient 4, further analyses were performed using BAC and Fosmide clones as previously described (GRCh37/hg19) [10].

Results

FMR1 screening was normal for all the patients. Karyotype was 46,XX in patients 1, 2, and 3. In patient 4, a reciprocal translocation involving chromosomes X and 8: t(X;8)
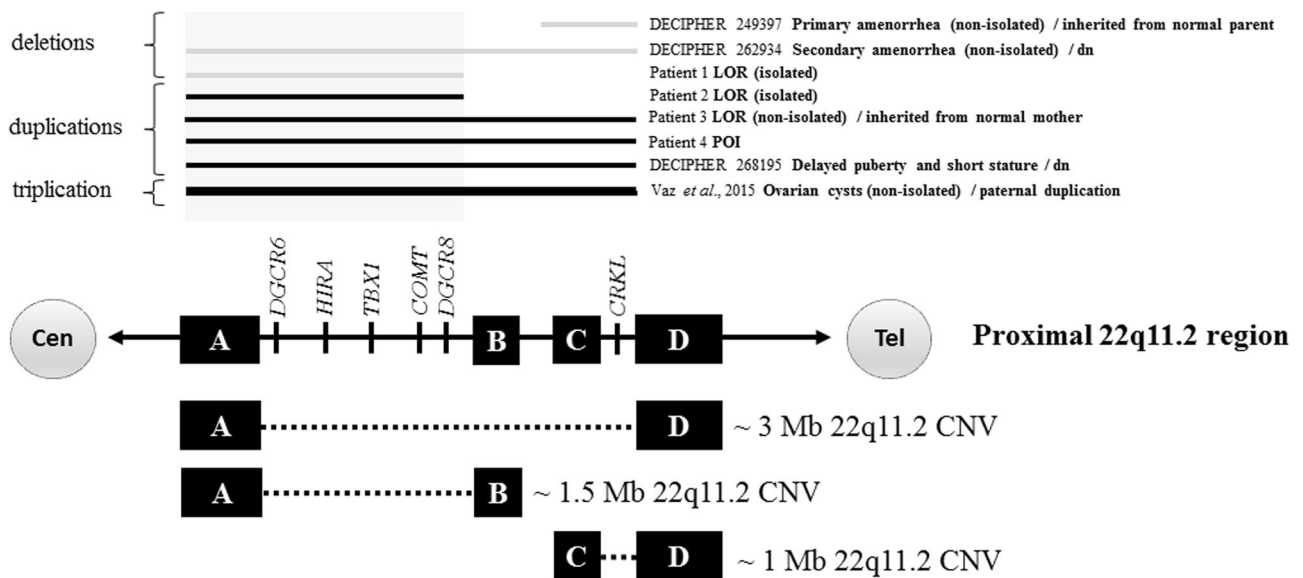
Fig. 2 CNVs in the proximal 22q region (hg19) observed in patients with ovarian defects (patients 1 to 4 of the study, DECIPHER, and published literature patients). Gray lines: deletions; black lines: gain (duplications, triplication); gray box: most common region of overlap. Cen centromeric side, Tel telomeric side, dn de novo

(q26.2;q21.1) was observed. The Xq breakpoint (BP) was located by FISH at Xq26.22 (POF1 locus), between WI2–1836L24 (chrX: 131,337,240–131,378,438) and WI2–2760E14 (chrX: 131,388,632–131,427,483), with one gene within the interval between the probes (intron 2): *RAP2C-AS1* (member of RAS oncogene family RAP2C antisense RNA1). The 8q BP was located at 8q21.13, between RP11–89I14 (chr8: 80,585,939–80,756,484) and RP11–92K15 (chr8: 80,915,956–81,107,121), with one gene in the interval between the probes: *MRPS28* (mitochondrial ribosomal protein S28, OMIM 611990). Although the reproductive phenotype was thought to be linked to this X chromosomal rearrangement, an array-CGH was performed as it has been demonstrated that additional CNVs can be observed in 40% of apparently balanced translocations associated with an abnormal phenotype [12].

A genomic variant in the 22q11.2 region was observed in the four patients (5% of the cohort) (Fig. 1): a ~1.5 Mb deletion (LCR22-A/LCR22-B) for patient 1 (LOR, dysmenorrhea, primary infertility, hypotrophic ovaries, uterine adenomyosis, short stature), a ~1.5 Mb duplication (LCR22-A/LCR22-B) for patient 2 (LOR, regular menstruation with dysmenorrhea, two miscarriages, short stature), a ~3 Mb duplication (LCR22-A/LCR22-D) for patients 3 (LOR, irregular menstruation, varus foot and torticollis at birth, mild developmental delay), and 4 (POI, irregular menstruation, one miscarriage, two pregnancies with oocyte donation), maternally inherited for patient 3 (with no obvious reproductive phenotype in the mother apart from irregular menstruation). Patients 1, 2, and 4 did not carry the typical 22q11.2 clinical features but isolated

ovarian deficiency while clinical spectrum of 22q11.2 deletion syndrome was observed in patient 3 (mild developmental delay, musculoskeletal system problems). A search for patients with a 22q11.2 deletion or duplication associated with ovarian features including POI, menstruation troubles/amenorrhea, early menopause, ovarian defects (e.g., cysts), or delayed puberty was performed in the DECIPHER database and in the published literature to find further corresponding cases. The smallest region of overlap (duplicated in 5/8 patients and deleted in 2/8 patients) was ~1.5 Mb in size, between LCR22-A and LCR22-B, thus excluding the *CRKL* gene associated with GU malformations and subfertility in mice [6]. Among the 40 genes present in the minimal overlapping region, particular attention was paid to those that showed a link with ovary in the literature (*COMT, HIRA*, and *DGCR6*). Out of the eight patients with ovarian features, only one (DECIPHER 249397 with primary amenorrhea) had a more telomeric CNV located between LCR22-C/LCR22-D and including the previously described *CRKL* candidate (Table 1 and Fig. 2).

Discussion

Human sex development is sensitive to dosage gene effects and the 22q11.2 region in particular may have dosage sensitive gene(s) that play a role in the development of the GU system. GU malformations are indeed a known clinical feature observed in the 22q11.2 deletions, with approximately 31% of patients exhibiting such defects, including

renal features and genital features in males like cryptorchidism/hypospadias/micropenis or females like absent or abnormal uterus/Müllerian duct aplasia [6, 13]. In 22q11.2 duplications, quite similar GU features have been reported [6] along with cases of ambiguous genitalia and 46,XX SRY-negative testicular disorder of sex development (DSD) [14]. For the congenital anomalies of the kidney and urinary tract (CAKUT), the smallest region covered by the 22q11.2 CNVs have been localized between LCR22-C and LCR22-D, including *CRKL*, which has been recently proposed to contribute to the GU defects [6]. Among GU defects and DSD, the proper development of the ovaries is necessary to avoid ovarian deficiency and to ensure the protection, support, and development of the oocytes they contain. We describe in our study four patients with ovarian deficiency and a 22q11.2 CNV, either duplication or deletion, suggesting a gene dosage effect, which could explain, at least in part, their phenotype.

Our discovery of 5% of our LOR/POI cohort carrying genomic rearrangements of the 22q11.2 region suggests its likelihood in ovarian deficiency pathogenesis. It remains possible that the discovery of these rearrangements was coincidental, given that previous pangenomic studies to identify CNVs on more than 500 POI women did not detect any CNV in the 22q11.2 region [15, 16]. Importantly, however, analysis of 10,118 published controls and 6,813 samples of unselected newborns failed to identify any deletion in this region, and detected only 5 (0.05%) and 6 (0.09%) individuals with duplication, respectively [5, 17]. This is vastly different from the incidence in our cohort. Furthermore, the individuals with duplication of this region may have had unreported/undetected infertility, as it is a relatively minor phenotype that may not present until adulthood. On the other hand, pathogenicity has been shown in patients with neurodevelopmental features with detection of a 22q11.2 deletion syndrome in 0.6% of the patients and a 22q11.2 duplication syndrome in 0.2–0.3% of them [17, 18].

Phenotypic difference with previously reported patients can be linked to the variability in the severity and extent of expression between individuals, even within the same family. Furthermore, there is no correlation between the presence of one particular feature and another [2]. The reason for this phenotypic variability has not yet been elucidated. Nevertheless, BP localization does not have a major role in it. It could be linked to the presence of additional CNVs involving miRNA combined to the abnormal gene dosage of *DGCR8* [19]. It also remains possible that other additional factors, such as moderating genes or the environment, may contribute to the phenotype. For patient 4, in particular, a reciprocal translocation t(X;8) was observed on the karyotype, which is likely to be linked to the reproductive phenotype. Women with balanced X-autosome translocations are a rare and clinically heterogeneous group of patients whose most common phenotype is POI, with consequences depending on the location of the BPs and on the X-chromosome inactivation pattern [20]. The maintenance of ovarian function and normal reproductive lifespan is linked with Xq critical regions, POF1 in Xq26-Xq28 and POF2 in Xq13-Xq21. Apart from disruption of a gene, position effect may alter the expression of genes flanking the BP, or meiotic arrest due to improper chromosomal alignment can be responsible for POI. Potential candidate genes located at, or near, the BPs for patient 4 include *HS6ST2* (Heparane sulfate 6-O-sulfotranferase 2, OMIM 300545), located in POF1, previously linked to POI and with its paralog, *egl*, in *Drosophila* expressed in all stages of oogenesis and involved in establishing oocyte polarity [21]. Another candidate gene is *MRPS28* (8q21.13), which encodes a mitochondrial protein, as many of the genes known to underlie cases of POI are involved in metabolism or mitochondrial function [9]. Chromosomal alterations involving the X chromosome have also been identified in women with normal ovarian function [22], suggesting for patient 4 the potential involvement of both rearrangements in the phenotype.

In order to further establish a link between 22q11.2 CNVs with ovarian features, we focused on genes in the smallest region of overlap that could be involved in the reproductive phenotype, due to gene dosage effect. One interesting finding concerns *COMT* (Catechol-O-methyltransferase, OMIM 116790), which is one of the major mammalian enzymes involved in the metabolic degradation of catecholamines. High expression has been observed in mammal granulosa cells [23] and in human ovary (GTex database). It allows, in particular, the conversion of 2-hydroxyestrogen to 2-methoxyestradiol (2-ME2), and is synthesized in the ovarian follicles in human, where it plays a potential physiologic role in follicle homeostasis [24]. Any alteration of COMT activity (gain or loss of function) would then lead to a perturbation of folliculogenesis. It has been shown, in human and porcine granulosa cell lines, that an increased expression of COMT, leading to increased levels of 2-ME2 in the follicular microenvironment, is associated with inhibited steroidogenesis and granulosa cell arrest and thus with anovulation, a condition observed in polycystic ovary syndrome [24] and often associated with subsequent follicular depletion. Conversely, a reduced COMT activity induces the accumulation of catecholestrogens, followed by an estrogen oxidative DNA damage effect, especially high in steroid-synthesizing tissues, which

leads to cellular apoptosis, corpus luteum regression, and follicular atresia [23]. The variant rs4680, resulting in a less active COMT enzyme form, has been linked to POI in two association studies and could be a predictive factor for POI [23, 25].

Other genes can affect the various processes mandatory for normal development and function of the reproductive system and thus represent candidate genes for the ovarian phenotype. *HIRA* (Histone cell cycle regulation defective, S. cerevisiae, Homolog of, A, OMIM 600237) encodes a histone chaperone implicated in histone exchange and is expressed in human ovary (GTex database). In vitro studies of mouse primordial germ cells (PGCs) have shown that when PGCs are residing in the gonads, major changes occur in nuclear architecture with an extensive erasure of several histone modification marks along with exchange of histone variants. At the same time, Hira shows accumulation in PGCs nuclei undergoing reprogramming suggesting its involvement in the erasure of epigenetic memory in PGCs [26]. Thus, depletion of Hira in primordial oocytes causes chromatin abnormalities and extensive oocyte loss during mouse oogenesis [27]. *DGCR6* (DiGeorge syndrome critical region gene, OMIM 601279) encodes a protein with homology to the *Drosophila* gonadal (gdl) protein, which participates in gonadal and germ-line cell development [28]. In *Drosophila*, *gdl* is expressed exclusively during gametogenesis and in the adult ovaries and testes, which suggests a germ cell-specific function. Consistent with this, in human, *DGCR6* is widely expressed in both fetal and adult tissues, in particular in the adult ovary [29]. Thus, a reduced dosage of *HIRA* or *DGCR6* in 22q11.2 deletions could contribute to the ovarian defects, in conjunction with other factors still to elucidate.

Conclusion

This study highlights the possibility of expanding the phenotype of the 22q11.2 CNVs to include ovarian deficiency, LOR, or POI. Ovarian deficiency may be a rare manifestation or may be underreported. Advances in genetic diagnosis and care may lead to the growing recognition of adults with 22q11.2 CNVs and associated ovarian defects. This study demonstrates that assessment of the ovarian function in young patients with a 22q11.2 imbalance may be warranted to detect ovarian deficiency in anticipatory care and on the contrary, the analysis of the 22q11.2 region may be considered in the patients experiencing ovarian deficiency, particularly when related phenotypic features are concurrently observed.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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