Prenatal diagnosis of a 16p11.2p11.1 mosaic small supernumerary marker chromosome (sSMC)

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Abstract

**Background.** Small supernumerary marker chromosome (sSMC) is a challenge in prenatal diagnosis. Its’ presence is associated with advanced maternal age and distinct ultrasound findings, prediction of postnatal clinical consequences and prenatal counselling is difficult. Exact characterization of an sSMC is augmented by the application of novel molecular methods such as fluorescent *in situ* hybridization (FISH) and array comparative genome hybridization (aCGH).

**Case presentation.** Chorion villous sampling of a fetus of a 42-year old secundipara was carried out due to advanced maternal age and the conventional banding cytogenetic examination revealed a mosaic sSMC. Detailed prenatal ultrasound scan showed no fetal malformations. The karyotype from confirmatory amniocentesis was 47,XY,+mar[45]/46,XY. The level of mosaicism was 70% from chorionic villus and amnion cells as well. By using FISH and SNP based aCGH the exact origin of the marker was described and the final prenatal karyotype was determined as 47,XY,+mar[70].arr[GRCh38]16p11.2p11.1(31699804_35989873)x3dn/46,XY[30]. The increase in DNA dosage was 4,29 Mb affecting 20 genes with two OMIM ones (*ZNF267* and *TP53TG3*), the SNP analysis excluded the possibility of uniparental disomy (UPD) of the chromosome. The application of the molecular cytogenetic methods allowed us the differentiate the mosaic marker chromosome from the known 16p11.2 duplication syndrome in close neighboring that is connected to developmental delay and autism spectrum disorder. The present case was identified as a harmless *de novo* euchromatic variant (EV) of the short arm of chromosome 16. Postnatal karyotyping from neonatal peripheral blood confirmed the presence of the marker. FISH analysis with probe D16Z2 on cultured lymphocyte and buccal smear samples revealed a 64% and 45% mosaic state of the sSMC, respectively. The precise karyotype was finally defined as 47,XY,+min(16)(:16p11.2->p11.1:)dn[64]/46,XY.

**Conclusions.** Novel microarray methods combined with molecular cytogenetic analysis is particularly effective in the rapid and accurate diagnosis of sSMCs, especially in a prenatal situation when the exact characterization of a genomic imbalance is of utmost importance. Precise diagnosis facilitates proper genetic counseling, allows informed decision making and helps avoiding unnecessary
pregnancy termination.

Background
Small supernumerary marker chromosome (sSMC) is a structurally abnormal chromosome that cannot be clearly characterized by conventional banding cytogenetics and is equal or smaller in size than a chromosome 20 of the same metaphase spread [1]. Supernumerary marker chromosomes occur at a rate of 0.44 per 1000 newborns and at 0.72-0.75 per 1000 fetuses in prenatal settings possibly caused by higher rate of spontaneous loss and termination of pregnancy (TOP) [2,3]. The incidence of sSMCs has been estimated higher in prenatal cases with ultrasound anomalies [2], but the most frequent reason leading to the prenatal identification of an sSMC is advanced maternal age (AMA) [4]. Clinical phenotypes associated with a marker chromosome are highly variable from normal to severely affected patients. It is generally accepted that about 70% of de novo sSMC carriers are phenotypically normal and less than one third of the patients show clinical symptoms [2]. The risk of phenotypic abnormalities associated with an sSMC depends on many factors, including the chromosomal imbalance, the mode of inheritance, the chromosomal origin, the morphology, content and structure of the marker and the imprinting effects of uniparental disomy (UPD) [5]. The vast majority of marker chromosomes originate from acrocentric chromosomes, almost 30% from chromosome 15 [1]. Approximately two thirds of sSMCs are de novo and a significant proportion of them are mosaics [6]. Proper genetic counselling of a de novo sSMC remains a challenge for clinicians and it is especially true in a prenatal situation when the clinical outcome is uncertain and the time that is necessary for the genetic diagnosis is of utmost importance. Except for certain sSMCs when the outcome is well established (such as isochromosome 12p- Pallister-Killian syndrome, tetrasomy 18p, i(5p), i(9p)) [7], accurate characterization of the marker chromosome is mandatory. The presence of a sSMC is usually identified by G-banding, however in most cases application of molecular cytogenetic techniques is necessary for proper diagnosis. Fluorescence in situ hybridization (FISH) based methods have been considered as the gold standard for decades in the evaluation of the origin of the marker [8,9]. To
overcome the limitations of FISH (such as accuracy or resolution) array comparative genome hybridization (aCGH) has been applied in the diagnostic of sSMCs that can also determine the origin of the marker chromosome, moreover can also detect the exact size, the breakpoints, the genomic copy number changes and the genes involved [10,11].

Here we report a case of a prenatally diagnosed case of mosaic sSMC derived from chromosome 16 and characterized by molecular cytogenetic methods.

Case Presentation

The 42-year-old gravida (G:2, P:1) was referred to our prenatal center at 13 weeks 1 day of the gestation because of advanced maternal age (AMA). First trimester ultrasound scan showed normal fetal anatomy, nuchal translucency fell into normal range (NT:1.65 mm), nasal bone was visible, crown-rump length (CRL) was 70 mm, biparietal diameter (BPD) 22 mm. No abnormalities were seen at screening according to the Fetal Medicine Foundation (FMF) protocol (GE Voluson® E8 - GE Medical System Kretztechnik GmbH & Co OHG). Following proper genetic counselling the couple opted for chorionic villus sampling (CVS) that was done at 14w+1d. Second trimester ultrasound scan was carried out at 17w2d according to the protocol of the Hungarian Society of Ultrasound in Obstetrics and Gynecology showing normal fetal anatomy. Due to the result of the CVS examination parents accepted genetical amniocentesis (AC) that was performed at the day of the midtrimester scan. Detailed fetal echocardiology examination did not detect any structural fetal heart abnormality and further ultrasound screening did not confirm any fetal minor or major malformations between the pregnancy week 20-22. Based on the genetic results and the normal serial fetal ultrasound and echocardiography scans parents decided to continue the pregnancy. At the 38th weeks of gestation a normal healthy male neonate was born in the course of vaginal delivery with a body weight of 3170 grams. Confirmatory postpartum genetic tests were done from neonatal blood and buccal smear. The boy has shown normal somatomental, motor and neurological development at two months of age.

Methods

Cytogenetic analyses were performed on direct and cultured chorionic villus samples, amniotic fluid cells, postpartum venous blood (leukocytes) and buccal smear. GTL-banding was done according to
standard methods. Fluorescence in situ hybridization was carried out with probes specific to chromosome regions DXZ1, DYZ3, D18Z1, 13q14.2, 21q22.13 (CytoCell FAST FISH Prenatal Enumeration probe kit) to exclude the most frequent aneuploidies. To determine the origin of the marker chromosome FISH was applied with the following further probes: D17Z1 for chromosome 17 centromere, SNRPN for the Prader-Willi/Angelman region, D14,22Z1 for the centromere of chromosome 14 and 22, Chromoprobe Multiprobe OctoChrome™ for all chromosomes, and after the microarray analysis D16Z2 for chromosome 16 centromere (CytoCell). All FISH reactions were done according to the manufacturer’s instructions. Karyotyping from short and long-term preparations of chorion tissue was performed on 68 cells altogether. Confirmatory cytogenetic analysis was done on 64 amniocytes from in situ cultures. Parental karyotypes from peripheral lymphocyte cultures were performed in line with the prenatal examinations. Karyotype results were evaluated according to the International System for Human Cytogenetic Nomenclature (ISCN).

SNP microarray analysis was performed using the Affymetrix Cytoscan Optima platform which uses 315 608 for copy number and single nucleotid polymorphism (SNP) probes. Cytoscan optima array has whole-genom coverage and increased probe coverage targeting 396 regions relevant for prenatal and perinatal research applications. 250 ng of total genomic DNA extracted from cell suspension was digested with NsPl and then ligated to NSPI adaptors respectively and amplified using Titanium Taq with the Sensoquest PCR system. PCR products were purified using AMPure beads and quantified using Nanodrop ND1000. Purified DNA was fragmented, biotin labeled and hybridized to the Affymetrix Cytoscan Optima GeneChip. Data was analyzed using the Chromosome Analysis Suite. The analysis is based on human genome version GRCh38/hg19.

Results

Cytogenetic analysis of direct and cultured chorionic villus samples revealed the presence of a mosaic sSMC in trophoblast cells and in mesenchymal placental cells. CVS karyotype was:

47,XY,+mar[46]/46,XY[22]. The karyotype from confirmatory AC was 47,XY,+mar[45]/46,XY[19]. The level of mosaicism was 70% from chorionic villus and amnion cells as well. No probes except for Chromoprobe system and D16Z2 hybridized to the marker. After intensive workup with Chromoprobe
on metaphases from amniocytes we have found hybridization pattern with WCP16 on the marker chromosome, parallel with the result of the microarray analysis. Parental karyotypes showed no alterations, so the fetal chromosome aberration was specified as de novo.

SNP microarray analysis of amniocytes finally described the exact origin of the marker as arr[GRCh38]16p11.2p11.1(31699804_35989873)x3. The increase in DNA dosage was 4,29 Mb affecting 20 genes with two OMIM ones. SNP analysis excluded the possibility of uniparental disomy (UPD) of chromosome 16. The prenatal karyotype was determined as 47,XY,+mar[70].arr[GRCh38]16p11.2p11.1(31699804_35989873)x3dn/46,XY[30].

Postnatal karyotyping from neonatal peripheral blood confirmed the presence of the marker. FISH analysis with probe D16Z2 on cultured lymphocyte and buccal smear samples revealed a 64% and 45% mosaic state of the sSMC, respectively. The precise karyotype was finally defined as 47,XY,+min(16)(:16p11.2->p11.1:)dn[64]/46,XY[36]. In the evaluation of the final karyotype we took into consideration the notions of Liehr regarding the nomenclature problems of ISCN in the definition of sSMCs [12].

**Figure 1.** A. GTL-banded karyotype from an amniocyte displaying the min sSMC. B. FISH with Chromoprobe Multiprobe OctoChrome WCP16 showing hybridization pattern on the sSMC from metaphase spread of an amniocyte. C. FISH with D16Z2 probe on metaphase chromosomes from the newborn’s peripheral lymphocyte culture. D. Interphase amniotic fluid cell displaying three signals, including the one on the marker, hybridized with Chromoprobe Multiprobe OctoChrome WCP16. E. Buccal epithelial cell showing three FISH signals hybridized with D16Z2 probe. F. CMA on cultured amniotic fluid cells defining extra chromosomal material as gain in 16p11.2-11.1.

**Discussion And Conclusion**
Prenatal identification and characterization of an sSMC and proper genetic counselling is a challenging task for clinicians. Although it is regarded that about 30% of all de novo sSMC carriers have associated abnormalities, prenatal prediction of the phenotypical consequences is problematic. The first study evaluating the overall risk for an abnormal phenotype in case of a de novo sSMC was based on a large number of amniocentesis samples and found it to be 13% prenatally [13]. By
utilizing molecular genetic methods such as FISH, Crolla et al. confirmed that, excluding chromosome 15 derived markers, the risk increased to 28% [14]. A study of 108 prenatally detected cases of marker chromosomes collected from 12 laboratories found that the risk for phenotypical abnormality was 26 % and it was reduced to 18% when the prenatal ultrasound was normal [15]. They reported the highest chance of abnormalities in case of a ring chromosome probably due to the largest amount of genetic material necessary to form the ring appearance. The risk was significantly reduced when the marker appeared in a monocentric and non-satelitited form, while it was higher with bisatellited dicentrics, acentrics or isodicentrics.

It seems that somatic mosaicism is often associated with sSMCs. Chromosomal mosaicism is one of the main difficulties in prenatal diagnosis. It represents the phenomenon of the presence of two or more chromosomally different cell lines in an individual arising from a single zygote. The main mechanism of mosaicism forming an sSMC involves the maternal meiosis I. or II. chromosomal non-disjunction error followed by incomplete trisomy rescue in the dividing pre-implantation embryo [16]. It is predicted that meiosis II. segregation errors occur more frequently then meiosis I. errors and it is strictly connected to AMA. It has been established that ovarian aging is the most important factor not only in aneuploidies but in the formation of de novo sSMCs as well [17]. Chromosomal mosaicism in CVS and in amniocytes is well-known and occurs in 1-2% of CVS and 0.1-0.3% of amniocentesis samples [18]. The differentiation of the cells and the tissues begins at the early post-fertilization stage. The distribution of normal and abnormal cell lines in the fetus and the placenta depends on the stage and the mechanism of the differentiation. When trisomy rescue occurs soon after fertilization, the mosaic formation regards both placental and fetal tissues, when it occurs at a later stage (following the separation of the fetal and the placental compartments), the aneuploid cell line can be confined to the placenta, to the fetus or both. The prenatal study by Graf et al. reported a total of 61% rate of mosaicism in sSMC and found no difference between the groups with or without phenotypic abnormality [15]. According to a large review that studied 3124 sSMC cases previously reported in the literature, the authors found 52% overall rate of somatic mosaicism. Non-acrocentric derived sSMCs were more involved in mosaicism. The authors emphasized that in the vast majority of
the cases there was no correlation between the grade of somatic mosaicism detected in the peripheral blood or in amnion cells and the severity of the clinical status [19]. In a recent survey of 143,000 consecutive prenatal diagnosis the frequency of overall mosaic sSMC was 69% and the risk of confirmation in amniotic fluid following mosaic CVS result was 33.3%, suggesting a high rate of confined placental mosaicism (CPM) [3]. The main indication for the invasive procedure was AMA and ultrasound anomaly. It seems that sSMCs derived from chromosome 16 are relatively rare and it was found that 91% were mosaics [19]. sSMCs can be associated with uniparental disomy, either in complete or segmental forms, as a result of trisomic zygote rescue [4,5]. Mosaic trisomy with UPD occur at a significantly high frequency from chromosome 16. However, chromosome 16 does not seem to be involved in imprinting mechanisms with clinical consequences.

According to GRCh38/hg19 chromosome 16 has a size of 90.4 Mb. The proximal short arm of the chromosome contains a several copy number variation (CNV) hotspots that predispose to deletions and duplications. The chromosome 16p11.2 duplication syndrome (OMIM 614671) represent a continuous gene duplication syndrome with genomic coordinates (GRCh38:28,500,000-35,300,000). The typical region is an approx. 600 kb genomic duplicate/ deletion from 29,5-30,1 Mb associated with developmental delay and obesity [20]. More distant starting from the centromere is a large microscopically visible region of 8-9 Mb in 16p11.2-16p12.1 that was reported with developmental delay and autism spectrum disorder (ASD) [21,22]. Although most affected patients show different dysmorphic features, mental retardation and behavioral problems, the wide range of phenotypical spectrum refers to incomplete penetrance and variable expressivity of these genomic abnormalities [23]. Moreover, while patients with a deletion of that region has severe obesity besides developmental delay, affected individuals with a duplication are characterized by reduced postnatal weight and low BMI [24]. Thus, it seems that the phenotypes of the duplication carriers mirror those of the deletion carriers [25]. From centromere to telomere, the proximal part of the short arm close to the heterochromatic region is prone to CNV formation. The whole region can undergo duplication together with the heterochromatic blocks forming visible, unusual G-banding pattern [26]. The centric euchromatic region of chromosome 16 is in close proximity to the large block of heterochromatin and
this centromere-near region colocalizes with an euchromatic variant (EV) [27]. The EV that is mapped to 32.0-34.4 Mb needs to be distinguished from the potentially pathological duplications found in 16p11.2-12.1 region [27,28]. Euchromatic variants of proximal 16p11.2 are not associated with phenotypical consequences and can be mistaken for the more distantly positioned 16p11.2 duplication syndrome (Figure 2.). However, the overlapping genetic position of the two genomic regions can make the differentiation difficult. The differentiation between them is not feasible during the conventional cytogenetic analysis but can be distinguished at molecular level using FISH or chromosome microarray analysis (CMA, aCGH). Recently published studies indicated that the novel microarray methods such as CMA combined with molecular cytogenetic analysis is particularly effective in the rapid and accurate diagnosis of sSMCs or copy number variations. These combined protocols are especially useful in identifying rare structural chromosomal aberrations prenatally and in assessing the prognosis of fetuses carrying such abnormalities [29]. It is worth of noting that although aCGH identifies the whole gene component of sSMCs and the other underlying chromosomal anomalies too (except for balanced translocations) in one test, it may miss to diagnose sSMCs formed of heterochromatin and the cases with low level of mosaicism [30].

By reviewing the literature and the genome browsers we found altogether 3 cases with copy number variations that were reported in the same chromosomal region that our case. In the Decipher v9.30 database one patient (356289) with a dedicated 1.75 Mb heterozygous de novo microduplication was indicated to have a pathogenic abnormality, another patient (356289) with a 1.54 Mb heterozygous de novo microduplication a likely pathogenic and a third patient (402189) with a 3.1 Mb de novo mosaic microduplication was indicated also likely pathogenic. We did not find the corresponding references in the literature and the morbid genes in the abovementioned chromosomal regions.

According to the sSMC database by Liehr [31] the centromere-near region of chromosome 16 contains altogether 96 cases, of them 38 cases (39.5%) are without any clinical symptoms, 16 cases (16.7%) are associated with clinical findings and 37 cases (38.5%) remain without clear clinical correlation. It is interesting to note that in the cases without any clinical phenotype the rate of prenatal diagnosis was about 47%, in cases with clinical findings it was about 19% (3/16) and in cases of uncertain
findings it was approx. 81%. It seems that most patient with a sSMC and with clinical findings were diagnosed postnatally and presumably the symptoms of the newborn made the genetic investigations necessary. At the same time in the cases with unclear clinical correlation most diagnosis was performed prenatally underlining the difficulties of the prenatal assessment diagnosing an sSMC. In terms of breakpoints and the corresponding chromosomal regions none of the cases in the database were similar to our case. However, one fetus (case 16-U-39) was diagnosed by amniocentesis with a mosaic marker forming a ring of the proximal short arm (r(16)(::p12.2→p11.2::)), that resembles the most in terms of the chromosomal region. The data of the clinical symptoms are not available. Albeit, the distal breakpoint of that sSMC was involved in 16p12.2 and the shape of the marker was a ring chromosome. It is also important to emphasize that supposedly the sSMC cases without clinical consequences are less likely reported in the literature.

In our case we identified a marker chromosome of 4.29 Mb derived from proximal 16p11.2-11.1. The exact position of the marker’s endpoint is uncertain as we did not have appropriate FISH probes to the heterochromatin of the q arm. Furthermore, array CGH cannot detect heterochromatic targets.

The 16p11.1 and the proximal 16p11.2 bands are gene-poor regions of the chromosome. According to the Decipher database GRCh38/hg19 the chr16p:31,699,804-35,989,873 region contains only 20 genes, presumably pseudogenes or transcript variants, and only two of them are OMIM genes, namely **ZNF267** and **TP53TG3** (Figure 2.). The Zinc finger protein 267 (**ZNF267**) gene that localizes in the middle of 16p11.2 (GRCh38/chr16:31,873,806-31,917,356) is a transcriptional factor. The gene modulates the gene expression and functions as a negative transcriptional regulator of matrix metalloproteinase-10 (MMP-10). The **ZNF267** gene by inhibiting MMP-10 might promote liver fibrosis through diminished matrix degradation [32]. It is demonstrated that **ZNF267** mRNA is up-regulated in liver cirrhosis and may be a risk factor for hepatocellular carcinoma [33]. Tp53 target gene 3 (**TP53TG3**) was mapped to the proximal short arm of Chr16 and is located at GRCh38/chr16:32,673,518-32,676,128 [34]. It is one of the numerous TP53 genes, those transcription factors that are involved in cell cycle arrest, apoptosis, DNA repair, chromosomal stability, and inhibition of angiogenesis. The **TP53TG3** gene has no proven phenotypic gain of function effects
described so far in the databases. The function of the other 18 genes in that region remains unknown.

Exact identification of an sSMC is especially important in a prenatal situation and the time necessary for the diagnosis is of great importance. Utilization of a clear algorithm and diagnostic protocol is a valuable tool in the management of a prenatally detected sSMC and can prevent the unnecessary delays during the diagnostic process [35]. Regarding our case, the characterization of the marker chromosome before the molecular genetic era would not have been carried out properly and in many instances that pregnancies would have been terminated. FISH with whole chromosome paints was very useful in the analytical processes to give information about the origin of sSCMs and might predict the euchromatic content of the markers. [36]. However, precise genotype-phenotype correlation can only be determined via chromosomal microarray technology. By applying SNP microarray analysis, we could exclude UPD, determine and specify the gene content and the region to be a harmless EV block. Our statement was also strengthened by the notion that the detailed fetal and fetal cardiac ultrasound examination did not confirm any malformation [16]. The child now is two months old and has not shown any sign of somatomental retardation or dysmorphic feature. However, we emphasize that those clinical symptoms such as developmental delay or ASD can manifest later in life. It is important to note that another prenatal case of a 16p copy number variation was diagnosed by our team recently. Genetic analysis of a fetus showing mild bilateral ventriculomegaly and partial dysgenesis of the corpus callosum (by ultrasound and MRI) at pregnancy week 20 revealed normal male karyotype and a microdeletion of 1.385 Mb of the short arm of Chr16 by aCGH (Chr16:32,542,904-33,928,095). The genomic position of that microdeletion is inside the region of the child with the sSMC in the present case. The association between the CNV and the corpus callosum dysgenesis is uncertain, the pregnancy is ongoing now (unpublished own data, Figure 2.).

In summary, we present prenatal diagnosis and molecular cytogenetic characterization of an sSMC. Exact identification of the marker chromosome as an EV enabled us to provide proper genetic counseling, to allow informed decision making and to avoid the unnecessary pregnancy termination.

**Figure 2.** Schematic representation of the pericentromeric region of Chr16 showing the centromere
and the neighboring genomic positions. Figure indicates the known region of 16p11.2-p12.2 microduplication syndrome, the position of the euchromatic variants (EV), the present prenatal case of an sSMC (chr16p:31,699,804-35,989,873) with the 2 OMIM genes (ZNF267 and TP53TG3) and another prenatal case of a 16p copy number variation with fetal findings diagnosed by our team (Chr16:32,542,904-33,928,095, unpublished own data).

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Abbreviations
AC- amniocentesis, AMA- advanced maternal age, ASD- autism spectrum disorder, aCGH- microarray comparative genome hybridization, BPD- biparietal diameter, BMI- body mass index, CMA- chromosomal microarray analysis, CNV- copy number variants, CPM- confined placental mosaicism, CRL- crown- rump length, CVS- chorionic villos sampling, EV- euchromatic variant, FISH- fluorescence in situ hybridization, FMF- Fetal Medicine Foundation, ISCN- International System for Human Cytogenetic Nomenclature, MMP- matrix-metalloproteinase, NT- nuchal translucency, PCR- polymerase chain reaction, SNP- single nucleotid polymorphism, sSMC- small supernumerary marker chromosome, TOP- termination of pregnancy, UPD- uniparental disomy.

Declarations

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Authors’ contributions

ZT performing ultrasound, genetical counselling, invasive procedures and writing the manuscript. EPT genetical counselling, cytogenetic and data analysis, writing the manuscript. IB and ES literature research and data analysis. HP carrying aCGH and writing the manuscript. AB, JS, JK, VG and JD revision of the manuscript. All authors reviewed and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was performed with the approval of Medical Ethics Committee of the Institution.

Consent for publication

The patient in this report provided their consent for publication.

Competing interests

The authors have no conflict of interest to declare.

Figures
A. GTL-banded karyotype from an amniocyte displaying the min sSMC. B. FISH with Chromoprobe Multiprobe OctoChrome WCP16 showing hybridization pattern on the sSMC from metaphase spread of an amniocyte. C. FISH with D16Z2 probe on metaphase chromosomes from the newborn’s peripheral lymphocyte culture. D. Interphase amniotic fluid cell displaying three signals, including the one on the marker, hybridized with Chromoprobe Multiprobe OctoChrome WCP16. E. Buccal epithelial cell showing three FISH signals hybridized with D16Z2 probe. F. CMA on cultured amniotic fluid cells defining extra chromosomal material as gain in 16p11.2-11.1.
Figure 2

Schematic representation of the pericentromeric region of Chr16 showing the centromere and the neighboring genomic positions. Figure indicates the known region of 16p11.2-p12.2 microduplication syndrome, the position of the euchromatic variants (EV), the present prenatal case of an sSMC (chr16p:31,699,804-35,989,873) with the 2 OMIM genes (ZNF267 and TP53TG3) and another prenatal case of a 16p copy number variation with fetal findings diagnosed by our team (Chr16:32,542,904-33,928,095, unpublished own data).