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Case Report

Importance and application of WES in fetal genetic diagnostics: Identification of novel *ASPM* mutation in a fetus with microcephaly

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ABSTRACT

Background: Prenatal whole exome sequencing (WES) approaches can provide genetic diagnosis with rapid turnaround time and high diagnostic rate when conventional tests are negative. Here we report a family with multiple pregnancy loss and with repeated occurrence of fetal microcephaly.

Methods and results: Because of positive family history and recurrent structural abnormality during the pregnancies that may lead postnatal neurodevelopmental consequences, WES analysis was indicated. Umbilical cord blood sampling was carried out and WES was performed using Twist Human Core Exome Kit and Illumina sequencing technology. The presence of pathogenic variants was confirmed by Sanger sequencing. WES analysis revealed a known pathogenic c.8506_8507delCA (p.Gln2836Glufs*35, rs587783280) and a novel pathogenic c.3134_3135delTC (p.Leu1045Glnfs*17) *ASPM* mutations in the fetus in compound heterozygous state. The c.3134_3135delTC has never been reported in the literature.

Conclusions: Our findings serve additional evidence that WES can be an efficient and relevant tool to diagnose certain genetic disorders with appropriate indication and to assess the recurrence risk of a disease. With the application of WES in combination with pre-implantation genetic tests, we can avoid the transmission of pathogenic mutations and we can achieve a decreased abortion rate in obstetric care.

1. Introduction

Congenital abnormalities are a substantial cause of neonatal and childhood morbidity and mortality. Fetal anomalies are identified in 2–5% of pregnancies and are responsible for 20% of perinatal deaths [1,2]. A major challenge in fetal diagnostics is that many Mendelian diseases may not have a known prenatal phenotype, moreover a prenatal feature may be atypical compared to the postnatally described phenotype. In addition, well-defined fetal phenotypes are often not available because of the limited health history and the examination is only indirectly accessible via prenatal sonography in obstetric care [2,3]. Actually, prenatal cytogenetic tests include G-banded karyotyping, fluorescence in situ hybridization (FISH), and chromosomal microarray analysis (CMA) are limited by their resolution. Using the combination of these techniques, still leaving the majority of cases undiagnosed (60%) [4]. Whole exome sequencing (WES) provide a greater resolution, and focuses the DNA regions containing protein-coding exons and splice sites, including more than 85% of all disease-causing mutations [4]. In cases of fetal anomalies with undetermined underlying cause, prenatal exome sequencing can define the responsible pathogenic variants in an additional 20–80% when conventional genetic testing is negative [5,6]. The incremental diagnostic yield of WES can add clinically relevant prognostic information that could contribute management of a pregnancy [7]. The detection rate is dependent on the indication (increased for fetuses with multiple anomalies vs single organ system affected) and applied methods (increased in trio WES vs proband-only WES) [3,8,9].

Microcephaly (MIC) is one of those fetal structural anomalies, which is detectable by ultrasonography at 18–20 weeks of gestation. Diagnosis of microcephaly in utero relies on the sonographic measurement of an abnormally small fetal head circumference 3SD below the mean for gestational age [10]. Human brain size is determined via complex processes, including neural stem cell proliferation, expansion, migration,

Abbreviations: WES, Whole Exome Sequencing; CMA, Chromosomal microarray analysis; FISH, Fluorescence in situ hybridization; MIC, Microcephaly; MCPH, Autosomal recessive primary microcephaly; CGH, Comparative genomic hybridization; ACMG, American College of Medical Genetics and Genomics; OMIM, Online Mendelian Inheritance in Man; VOUS, Variant of unknown significance.

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organization, synaptogenesis, and apoptosis [11]. Several neurodevelopmental consequences such as intellectual disability, autism spectrum disorders and epilepsy are associated with abnormal brain growth causing morbidity and mortality in infancy or early childhood [12]. Genetic and environmental factors can also contribute to the development of abnormally small brain size. Previously identified mutations in MIC-associated genes have been well described to lead a wide variety of centrosomal and cell cycle defects via abnormal structure and function of centrosome and microtubule and aberrant spindle-kinetochore assembly [13]. One of the most important gene is *ASPM* (Abnormal Spindle Microtubule Assembly, 1q31.3, GenBank accession number AF509326) causing autosomal recessive primary MIC (MCPH). *ASPM* gene mutations are estimated to account for 10–40% of autosomal recessive congenital MIC [14]. Previous studies that performed comprehensive mutation screen of the *ASPM* gene reported that mutations occurred throughout the gene and were all predicted to be protein truncating nonsense or frameshift mutations. Identified mutations were homozygous or compound heterozygous in the patients [15,16]. Autosomal recessive primary microcephaly-5 (MCPH5, OMIM#608716) is characterized by decreased occipitofrontal circumference (< -3SD) and associated with mental retardation and speech delay. Other features may include a simplified cerebral cortical gyral pattern, short stature, and mild seizures in some cases [15,17,18].

In this report, we would like to highlight the feasibility and importance of WES in fetal diagnostic via a presentation of a fetus with in utero identified autosomal recessive primary microcephaly.

2. Materials and methods

2.1. Consents and samples

After genetic counselling, written informed consent was obtained from participating members prior to their inclusion in the study. During the collection and analysis of DNA samples and processing of the accompanying clinical and personal data the guidelines and regulations of the Helsinki Declaration in 1975 and the currently operative national regulations (Hungarian law; XXI/2008) were followed. Genomic DNA was extracted from umbilical cord blood of the fetus and from peripheral leukocytes of the parents, using E.Z.N.A. Blood DNA Maxi extraction kit (OMEGA®, Bio-tek, Inc., GA, USA). The concentration and purity of extracted DNAs were measured with the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

2.2. Patients

A non-consanguineous couple was referred to our genetic counselling unit. In the family history of the examined couple, an artificial abortion (II/1.) was reported for the paternal aunt of the male patient because of fetal microcephaly presumably due to teratogenic harm (Fig. 1.). Regarding the female member of the couple (II/4.) in the obstetric history an artificial abortion (III/2.) and a delivery of a healthy daughter (III/3.) were noted. At 18 week of third pregnancy (III/4.) of the 28-year-old gravida a bilateral choroid plexus cysts was detected by ultrasound examination. During further observations, the size of the fetal skull progressively lags behind the values appropriate for the gestational age. At 23 weeks of gestation, for the request of the couple, amniocentesis and pregnancy termination was carried out due to microcephaly and the complete absence of *gyral pattern*. In the macroscopic pathology finding, a deceased male fetus (III/4.) was reported with microcephaly without an associated developmental disorder. The results of karyotyping test, array CGH (comparative genomic hybridization) examination and sequencing of *LIS1* gene were negative. Six years later, during the fifth pregnancy (III/6.) the size of the fetal skull showed already two weeks delay at the 19 weeks of gestation compared to a normal development. Fetal brain MRI confirmed fetal microcephaly with delayed gyrification at the 20 week of gestation. For the request of the couple, pregnancy termination was performed at the 21 weeks of gestation; meanwhile umbilical cord blood sampling was carried out. Pathological examination confirmed a female fetus characterized by microcephaly without an associated developmental disorder. Based on the anamnesis and clinical data, primary recessive microcephaly was suspected, therefore an exome sequencing analysis was considered as the next step in establishing an accurate genetic diagnosis. In the following pregnancy (III/7.) having knowledge of the mutations causing microcephaly in previous pregnancies, targeted genetic test was performed.

2.3. Generation of sequence data

Exome sequencing was performed using DNA samples obtained from the fetal umbilical cord blood. Exomic libraries were prepared using the Twist Human Core Exome Kit Library Prep Kit, and sequencing was performed on Illumina NovaSeq 6000 instrument according to the manufacturer's protocol, using paired-end 100 bp reads. The mean sequencing depth of on target regions was 77.3×. Reads were aligned to the human reference genome (GRCh37:hg19) using Burrows-Wheeler Aligner.

For classification and interpretation of genomic data the guideline of American College of Medical Genetics and Genomics (ACMG) were followed [19]. Moreover, databases, such as ClinVar, The Genome Aggregation Database (gnomAD) Genomes/Exomes coverage, in silico prediction tools, such as Mutation taster, PhastCons and PhyloP were used. All of the potential variants were then manually searched with literature using PubMed and Online Mendelian Inheritance in Man (OMIM) leading to the identification of the presented variants.

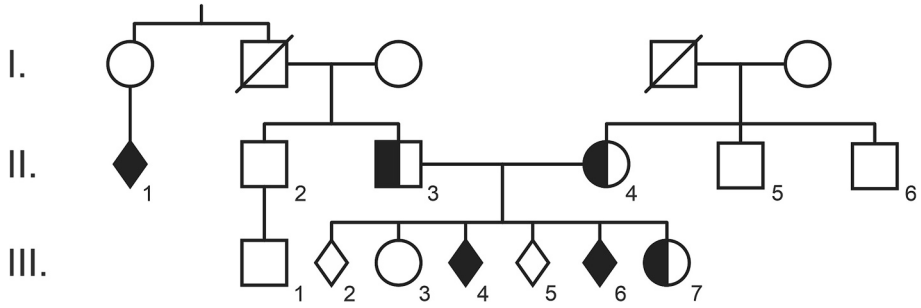

Fig. 1. Pedigree of the family with *ASPM* mutations.
Circles represent females and squares males. A slash through the symbol indicates that the family member is deceased. Half-shaded symbol indicates that the family member is heterozygous carrier of *ASPM* mutation. Filled symbol indicates fetal microcephaly. Diamond shaped symbol indicates artificial abortion. III/6 was the proband fetus. DNA samples were obtained from individuals III/6 (underwent exome sequencing), II/3, II/4 and III/7 (underwent targeted Sanger sequencing).

3. Results

The family members were evaluated and ascertained at the University of Pecs, Department of Medical Genetics. Regarding the examined fetus (III/6.), exome sequence analysis identified a previously described c.8506_8507delCA (p.Gln2836Glufs*35, rs587783280) pathogenic and a novel c.3134_3135delTC (p.Leu1045Glnfs*17) pathogenic variants in *ASPM* (NM_018136.5) gene in compound heterozygous state.

The *ASPM* c.8506_8507delCA frameshift variant is classified by ClinVar database as pathogenic (based on multiple consistent submissions and two citing articles) and associated with autosomal recessive primary microcephaly 5 [14,20]. The ACMG (American College of Medical Genetics and Genomics) classification of the novel c.3134_3135delTC mutation in *ASPM* gene is pathogenic, since this mutation is predicted to cause a recessive disease, detected in trans with a pathogenic variant and this identified variant is not found either in gnomAD genomes or gnomAD exomes (PVS1, PM2, PM3) (https://varsome.com/, https://www.ncbi.nlm.nih.gov/clinvar/).

No other variants were identified that are compatible with the phenotype. The presence of detected variants were verified via Sanger sequencing using genomic DNA obtained from the fetus. These variants were present in his parents, as well.

4. Discussion

In current study, we purposed to emphasize the importance and summarize the advantages of the application of WES in fetal mutation screening, beside the limitations of this method. We presented a family with recurrent fetal microcephaly and multiple pregnancy loss. In those pregnancies when more miscarriages reported - hence, multiple fetus affected probably with the same disorder - and the clinician presume a monogenic disorder, WES can be a useful tool to make diagnosis with high diagnostic rate. Additionally, pre-implantation genetic testing - with embryo selection - giving the couple the chance of starting pregnancy with the confidence that the expected baby will be unaffected [21]. With these therapeutic interventions, we could avoid the transmission of these genetic defects [22,23]. Consequently, we can elevate pregnancy rates and decrease abortion rates.

WES also indicated to the assessment of the genetic diagnosis, when the characteristic features are detectable by ultrasonography in utero with the involvement of particular anatomical systems, namely lymphatic, skeletal, central nervous, cardiovascular and renal system. In several diseases, such as intellectual disability, epilepsy, autism spectrum disorder, attention deficit hyperactivity disorder there is no prenatally detectable structural malformation.

Prenatal knowledge of a disease may allow accurate prediction the postnatal condition, complications and the immediate implementation the appropriate and efficient clinical treatment and comprehensive follow-up. In point of prenatal data, the diagnostic yield of WES is variable; the highest reported rate is 57.1% in published articles, in carefully chosen cases [8]. Further potential benefit of exome sequencing can be the cost- and time efficacy. The prenatal diagnosis obtained by WES technique, the medical expense and the length of the hospital stay may be reduced, as we can avoid the implementation of unnecessary postnatal tests [24,25]. The rapid turnaround time is a very important factor in prenatal diagnostic, if we consider the ultrasound examination performed at 18–22 weeks of gestation and the fact, that the primary testing (microarray, panel tests) is usually accomplished prior to WES. Rapid production of results is critical for the logistics of delivery or termination of pregnancy. Trio WES can reduce the time of interpretation in case of de novo mutations, furthermore with trio analysis we can improve the diagnostic rates and facilitate the use of sequencing data in a subsequent pregnancy for the same couple [26].

Based on positive family history, ultrasonography and pathological findings suggesting primary microcephaly, WES analysis was indicated. After WES and Sanger sequencing, it was found, that the examined fetus was compound heterozygous, the members of the examined couple were heterozygous carrier for the detected *ASPM* frameshift mutations. The presence of pathogenic *ASPM* gene mutations were responsible for the frequent incidence of abortions and recessive microcephaly during sequent pregnancies in the examined family.

The *ASPM* gene contains 28 exons. All previously reported mutations are scattered all along the coding sequence with no hot spots [20]. Out of the reported variants from this study, the formerly reported *ASPM* (NM_018136.5) mutation (c.8506_8507delCA) is located in the largest exon (in exon 18) of the gene, the novel mutation (c.3134_3135delTC) is located in exon 12, evolving the IQ/calmodulin binding domain and calponin homology (CH) domain of the ASPM protein, respectively. ASPM protein is relevant for symmetric, proliferative divisions of neuroepithelial cells during brain development so it plays an important role in the determination of human cortex size [27]. Defects in *ASPM* gene have been considered as the most common cause of autosomal recessive primary microcephaly (MCPH).

Among patients with MCPH5 (OMIM #608716) the phenotypic spectrum is broad. Individuals with this disorder are essentially characterized by severe microcephaly (3–11 standard deviations below the mean). Further characteristic symptoms affect the central nervous system. Patients can also display behavioral manifestations, including hyperactivity and attention deficit. Intellectual disability and epilepsy are the majorly reported clinical features in patients with *ASPM* gene mutations [15,28].

Exome sequencing is clinically indicated when the patient is characterized by multiple congenital anomalies or neurodevelopmental delay. The application of WES is recommended either when the presumed disease is genetically heterogeneous or targeted genetic test is not available [19]. In obstetric care, prenatal WES approaches often provide genetic diagnoses, after negative findings in karyotype testing and chromosomal microarray. Despite, there are technical, interpretation and ethical challenges, evidences demonstrate, that the limitations of WES is minor compared to its diagnostic value. However, the challenges of interpretation of WES are less compared to WGS data [29]. Although, there are several benefits (low amounts of fetal DNA required, rapid turnaround, and great sequencing depth) of applying WES prenatally, this technique on fetal material has not yet become routine practise partly because of financial constraints in some countries [30].

Regarding limitations of exome sequencing, those factors are mentionable, that may cause difficulty during either the application of the technique or the interpretation of results. In the laboratory diagnostic, we can detect single nucleotide variants and small indels (insertions, deletions) by WES, but these methods are inapplicable in the detection of copy number variants, aneuploidy, repeat expansions, mosaicism or structural rearrangements [26]. The limited documented fetal phenotypic data, the different time of disease onset and other phenomena such as incomplete penetrance and variable expressivity can also contribute to the complexity and difficulty of the clinical interpretation. Thorough pre- and post-test counselling managed by experts is very important in regards critical information and results, such as the interpretation of variants of unknown significance (VOUS), communication of negative result, and revelation of false paternity or consanguinity [3,4].

A multidisciplinary team approach is necessary with clinical geneticist, genetic counsellor, molecular geneticist, and bioinformaticians. This collaboration is essential to the evaluation and review the findings to provide accurate results and appropriate explanation.

An additional difficulty is that currently there are no expert consensus guidelines, including criteria, technical and interpretational standard for the reporting of incidental or secondary findings (unrelated to the indication) when prenatal sequencing is carried out. Moreover, a shared common database would be useful, which is available for laboratories and clinics to additional understanding and classification of variants and prenatal genotype-phenotype correlation.

Our study has demonstrated the application of WES as a useful

technique in fetal genetic diagnosis. Prenatal WES would have been the best method to detect pathogenic mutations even earlier and avoid multiple abortions. This is particularly important in cases such as the family described above, as parents carrying pathogenic mutations of a recessive disorder are at higher risk of disease recurrence than in sporadic cases. In our work, the greatest practical significance of the use of WES in fetal umbilical cord blood samples was that, knowing the carrier status of the parents and the risk of disease recurrence, we were able to perform targeted mutation testing, which provided accurate prognostic information for the next pregnancy.

5. Conclusion

In conclusion, WES could prove to be a valuable method in diagnostics, in case of carefully chosen group of patients with appropriate indication. Thus, it would be important to make it more widely available and applicable in clinical practice. Allow for pitfalls and limitations of next generation sequencing, an improved detection rate can be obtained by exome sequencing, which can provide clinically relevant information to manage a pregnancy, in those cases, when ultrasound findings indicate the application of WES or previously performed conventional tests were negative. The correct diagnosis offers an opportunity for early intervention and effective treatment in prenatal or in postpartum period.

CRediT authorship contribution statement

Renata Szalai: Data curation, Writing – original draft. **Agnes Till:** Data curation. **Attila Gyenesei:** Investigation. **Judit Bene:** Conceptualization. **Kinga Hadzsiev:** Conceptualization, Supervision.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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References

[1] T.J. Matthews, MacDorman MF, M.E. Thoma, Infant mortality statistics from the 2013 period linked birth/infant death data set, Natl. Vital Stat. Rep. 649 (2015) 1–30.
[2] P.A. Boyd, A.M. Tonks, J. Rankin, C. Rounding, et al., Monitoring the prenatal detection of structural fetal congenital anomalies in England and Wales: register-based study, J. Med. Screen. 181 (2011) 2–7, https://doi.org/10.1258/jms.2011.010139.
[3] N. Jelin Acvora, Whole exome sequencing: applications in prenatal genetics, Obstet. Gynecol. Clin. N. Am. 451 (2018) 69–81, https://doi.org/10.1016/j.ogc.2017.10.003.
[4] S. Best, K. Wou, N. Vora, I.B. Van der Veyver, et al., Promises, pitfalls and practicalities of prenatal whole exome sequencing, Prenat. Diagn. 381 (2018) 10–19, https://doi.org/10.1002/pd.5102.
[5] S. Drury, H. Williams, N. Trump, C. Boustred, et al., Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities, Prenat. Diagn. 3510 (2015) 1010–1017, https://doi.org/10.1002/pd.4675.
[6] S.M. Yadava, E. Ashkinadze, Whole exome sequencing (WES) in prenatal diagnosis for carefully selected cases, Am. J. Obstet. Gynecol. 2161 (2017) S87–S88, https://doi.org/10.1016/j.ajog.2016.11.029.
[7] S. Petrovski, V. Aggarwal, J.L. Giordano, M. Stosic, et al., Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study, Lancet 39310173 (2019) 758–767, https://doi.org/10.1016/S0140-6736(18)32042-7.
[8] C.L. Alamillo, Z. Powis, K. Farwell, L. Shahmirzadi, et al., Exome sequencing positively identified relevant alterations in more than half of cases with an indication of prenatal ultrasound anomalies, Prenat. Diagn. 3511 (2015) 1073–1078, https://doi.org/10.1002/pd.4648.
[9] Y. Yang, D.M. Muzny, F. Xia, Z. Niu, et al., Molecular findings among patients referred for clinical whole-exome sequencing, JAMA 31218 (2014) 1870–1879, https://doi.org/10.1001/jama.2014.14601.
[10] Z. Leibovitz, T. Lerman-Sagie, Diagnostic approach to fetal microcephaly, Eur. J. Paediatr. Neurol. 226 (2018) 935–943, https://doi.org/10.1016/j.ejpn.2018.06.002.
[11] F. Pirozzi, B. Nelson, G. Mirzaa, From microcephaly to megalencephaly: determinants of brain size, Dialogues Clin. Neurosci. 204 (2018) 267–282, https://doi.org/10.31887/DCNS.2018.20.4/gmirzaa.
[12] S. Ashwal, D. Michelson, L. Plawner, W.B. Dobyns, et al., Practice parameter: evaluation of the child with microcephaly (an evidence-based review): report of the quality standards Subcommittee of the American Academy of neurology and the practice Committee of the Child Neurology Society, Neurology 7311 (2009) 887–897, https://doi.org/10.1212/WNL.0b013e3181b783f7.
[13] D. Alcantara, M. O'Driscoll, Congenital microcephaly, Am. J. Med. Genet. C: Semin. Med. Genet. 166C2 (2014) 124–139, https://doi.org/10.1002/ajmg.c.31397.
[14] A.K. Nicholas, E.A. Swanson, J.J. Cox, G. Karbani, et al., The molecular landscape of ASPM mutations in primary microcephaly, J. Med. Genet. 464 (2009) 249–253, https://doi.org/10.1136/jmg.2008.062380.
[15] S. Passemard, L. Titomanlio, M. Elmaleh, A. Afenjar, et al., Expanding the clinical and neuroradiologic phenotype of primary microcephaly due to ASPM mutations, Neurology 7312 (2009) 962–969, https://doi.org/10.1212/WNL.0b013e3181b8799a.
[16] J. Bond, S. Scott, D.J. Hampshire, K. Springell, et al., Protein-truncating mutations in ASPM cause variable reduction in brain size, Am. J. Hum. Genet. 735 (2003) 1170–1177, https://doi.org/10.1086/379085.
[17] C.G. Woods, J. Bond, W. Enard, Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings, Am. J. Hum. Genet. 765 (2005) 717–728, https://doi.org/10.1086/429930.
[18] A. Saadi, G. Borck, N. Boddaert, M.C. Chekkour, et al., Compound heterozygous ASPM mutations associated with microcephaly and simplified cortical gyration in a consanguineous Algerian family, Eur. J. Med. Genet. 524 (2009) 180–184, https://doi.org/10.1016/j.ejmg.2009.03.013.
[19] Directors ABo, Points to consider in the clinical application of genomic sequencing, Genet. Med. 148 (2012) 759–761, https://doi.org/10.1038/gim.2012.74.
[20] P. Letard, S. Drunat, Y. Vial, S. Duerinckx, et al., Autosomal recessive primary microcephaly due to ASPM mutations: an update, Hum. Mutat. 393 (2018) 319–332, https://doi.org/10.1002/humu.23381.
[21] S. Piyamongkol, K. Makonkawkeyoon, V. Shotelersuk, O. Sreshthaputra, et al., Pre-implantation genetic testing for Marfan syndrome using mini-sequencing, J. Obstet. Gynaecol. 427 (2022) 2846–2852, https://doi.org/10.1080/01443615.2022.2109957.
[22] W. Guo, Y. Lai, Z. Yan, Y. Wang, et al., Trio-whole-exome sequencing and preimplantation genetic diagnosis for unexplained recurrent fetal malformations, Hum. Mutat. 412 (2020) 432–448, https://doi.org/10.1002/humu.23935.
[23] J. Traeger-Synodinos, Pre-implantation genetic diagnosis, Best Pract. Res. Clin. Obstet. Gynaecol. 39 (2017) 74–88, https://doi.org/10.1016/j.bpobgyn.2016.10.010.
[24] C.A. Valencia, A. Husami, J. Holle, J.A. Johnson, et al., Clinical impact and cost-effectiveness of whole exome sequencing as a diagnostic tool: a pediatric center's experience, Front. Pediatr. 3 (2015) 67, https://doi.org/10.3389/fped.2015.00067.
[25] L.K. Willig, J.E. Petrikin, L.D. Smith, C.J. Saunders, et al., Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings, Lancet Respir. Med. 35 (2015) 377–387, https://doi.org/10.1016/S2213-2600(15)00139-3.
[26] A.N. Abou Tayoun, N.B. Spinner, H.L. Rehm, R.C. Green, et al., Prenatal DNA sequencing: clinical, counseling, and diagnostic laboratory considerations, Prenat. Diagn. 381 (2018) 26–32, https://doi.org/10.1002/pd.5038.
[27] J.L. Fish, Y. Kosodo, W. Enard, S. Paabo, et al., Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells, Proc. Natl. Acad. Sci. U. S. A. 10327 (2006) 10438–10443, https://doi.org/10.1073/pnas.0604066103.
[28] J. Shen, W. Eyaid, G.H. Mochida, F. Al-Moayyad, et al., ASPM mutations identified in patients with primary microcephaly and seizures, J. Med. Genet. 429 (2005) 725–729, https://doi.org/10.1136/jmg.2004.027706.
[29] S. Lacey, J.Y. Chung, H. Lin, A comparison of whole genome sequencing with exome sequencing for family-based association studies, BMC Proc. 8 (Suppl. 1) (2014), https://doi.org/10.1186/1753-6561-8-S1-S38. Genetic Analysis Workshop 18Vanessa Olmo: p. S38.
[30] J. Lord, D.J. McMullan, R.Y. Eberhardt, G. Rinck, et al., Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study, Lancet 39310173 (2019) 747–757, https://doi.org/10.1016/S0140-6736(18)31940-8.