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Prevalence of chromosomal abnormalities identified by copy number variation sequencing in high-risk pregnancies, spontaneous abortions, and suspected genetic disorders

Rui Zhang[1,2], Xiangbin Chen[3], Dong Wang[2], Xuan Chen[4], Chao Wang[1], Yuhong Zhang[1], Mengnan Xu[3] and Jingcui Yu[2]

Abstract

Objective: High-throughput sequencing based on copy number variation (CNV-seq) is commonly used to detect chromosomal abnormalities including aneuploidy. This study provides evidence for the prevalence of chromosomal abnormalities in target populations.

Methods: A total of 160 samples, including 83 high-risk pregnancies, 37 spontaneous abortions, and 40 suspected genetic disorders, were analyzed by CNV-seq. Relationships between the incidence of these chromosomal abnormalities and risk factors (e.g. advanced maternal age, abnormal pregnancy history, and family history of congenital disease) were further analyzed by subgroup.

Results: A total of 37 (44.6%) high-risk pregnancies, 25 (67.6%) spontaneous abortions, and 22 (55%) suspected genetic disorders had chromosomal abnormalities including aneuploidy and CNVs. There was an increased risk association between the prevalence of aneuploidy and pathogenic-relevant CNV in the fetus or abortive tissue and advanced maternal age. Moreover, a family history of congenital disease was also positively correlated with fetal chromosomal abnormalities in high-risk pregnancies.

[1]Prenatal Diagnosis Center, The Second Affiliated Hospital of Harbin Medical University, Harbin, China
[2]Scientific Research Centre, The Second Affiliated Hospital of Harbin Medical University, Harbin, China
[3]Berry Genomics, Beijing, China
[4]Department of Obstetrics, The Second Affiliated Hospital of Harbin Medical University, Harbin, China

Corresponding author:
Jingcui Yu, Scientific Research Centre, the Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangang District, Harbin 150081, China.
Email: yujingcui@ems.hrbmu.edu.cn

Conclusion: A relatively high prevalence of chromosomal abnormalities was detected in high-risk pregnancies, spontaneous abortions, and suspected genetic disorders, indicating the importance of CNV detection in such populations.

Keywords
Chromosomal abnormalities, CNV-seq, high risk pregnancies, spontaneous abortions, suspected genetic disorders, aneuploidy

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Introduction

Aneuploidy and copy number variation (CNV), referring to segmental deletion or duplication ranging from thousands to millions of chromosomal bases, are the main types of chromosomal abnormalities.[1] As an important source of genetic variation, chromosomal abnormalities are considered to have biological importance in species evolution and genetic diversity. For instance, Zarrei et al.[2] identified CNV in around 10% of the human genome in a study involving multiple races. Together with aneuploidy, CNV is considered a major cause for several chromosomal disorders,[3,4] while aneuploidy is mostly responsible for congenital birth defects and infertility. Therefore, it is necessary to screen these birth defects and chromosomal disorders by detecting chromosomal CNV changes.

Recently, the development of high-throughput sequencing techniques has extended the detection of chromosomal CNV to karyotype analysis, fluorescence in situ hybridization (FISH), chromosomal microarray analysis (CMA) such as array comparative genomic hybridization (a-CGH) and single nucleotide polymorphism (SNP) array, as well as high-throughput-based CNV-sequencing (seq).[5–8] Although karyotype analysis is not effective at analyzing small CNV with clinical importance because of its complex procedures and low resolution of 5 Mb,[9] it remains the golden standard for identifying chromosomal abnormality. In contrast, FISH is simpler and more rapid, but the information that can be obtained is limited by probe coverage.[10] CMA shows higher resolution compared with karyotype analysis, but requires the preparation of microarray chips based on the chromosomal abnormality and clinical information from public databases. Moreover, its efficiency is defined by the screening of rare CNVs that are not included in the databases. Furthermore, its application is hampered in developing regions because of high technical demands and costs.[11] Recently, next-generation sequencing has offered high-throughput, higher accuracy and sensitivity, and lower costs, so is increasingly used in clinical research and detection, as well as in the screening of chromosomal CNVs.[8]

The detection of CNVs has been acknowledged in fetal screening, spontaneous abortions, and diseases with no confirmed clinical diagnosis. In this study, we aimed to investigate the efficiency of CNV-seq in the screening of these conditions by analyzing the effects of pregnancy age, family history of congenital disease, and abnormal pregnancy history on chromosomal CNV.

Materials and methods

Subjects

A total of 162 subjects who presented to the Second Affiliated Hospital of Harbin Medical University from September 2015 to October 2016 were enrolled in the study. Among them, 84 cases were high-risk pregnancies (aged 17–44 years) with a gestational age of 17–31 weeks. These subjects underwent amniocentesis to obtain amniotic fluid or umbilical vein puncture to obtain cord blood. Thirty-eight cases (aged 22–41 years) underwent spontaneous abortions with a gestational age of 7–37 weeks. The villus, umbilical cord, or fetal tissues were obtained under sterile conditions. The other 40 cases with suspected genetic disorders (aged 1–38 years) underwent venous blood collection. Each subject or their parents signed an informed consent form. The study protocols were approved by the Ethical Committee of the Second Affiliated Hospital of Harbin Medical University (No. 2015-research-185).

CNV-seq

Genomic DNA was extracted using the DNeasy Blood &Tissue Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions and measured using the Qubit quantitative kit (Thermo Fisher Scientific Inc., Rockford, IL, USA) with a Qubit fluorometer (Invitrogen Corp., Carlsbad, CA, USA) strictly adhering to the protocols. A DNA library was constructed as previously described.[12] Briefly, 50 ng genomic DNA was fragmented to an average size of 300 bp, and end-ligated with barcoded sequence adaptors. Tagged DNA fragments were amplified using primers with partial adaptor sequences to generate sequencing libraries. The CNV libraries were established after DNA purification, and sequenced on the HiSeq2000 platform (Illumina Inc., San Diego, CA, USA) to generate approximately 5 million 36-bp single-end reads. The raw data were analyzed to evaluate chromosomal copy number as previously described.[12]

Numerical analysis of chromosomal abnormalities

The number of subjects with each type of chromosomal abnormality in each subgroup was counted. Then the proportions of pathogenic-relevant chromosomal abnormalities were calculated and compared.

Results

Chromosomal abnormalities identified by CNV-seq

A total of 162 subjects were initially enrolled in the study (Figure 1). However, one subject with >90% maternal cell contamination was excluded from the group of 84 high-risk pregnancies, and one subject was excluded from 38 cases of spontaneous abortions because of high noise background in the sequencing analysis. Therefore, 160 samples (83 high-risk pregnancies, 37 spontaneous abortions, and 40 suspected genetic disorders) were eventually analyzed (Figure 1).

Findings of aneuploidy or CNV corresponding to each sample are listed in Supplemental Table 1, and the clinical importance of CNV was annotated according to Database of Genomic Variants, DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources, Online Mendelian Inheritance in Man, UCSC, and PubMed databases. Table 1 summarizes the prevalence of chromosomal abnormalities in subjects with high-risk pregnancies, spontaneous abortions, and suspected genetic disorders.

Of the 83 women with high-risk pregnancies who underwent prenatal testing, 37 (44.6%) showed aneuploidy or CNV; three cases had aneuploidy, 33 had CNV,

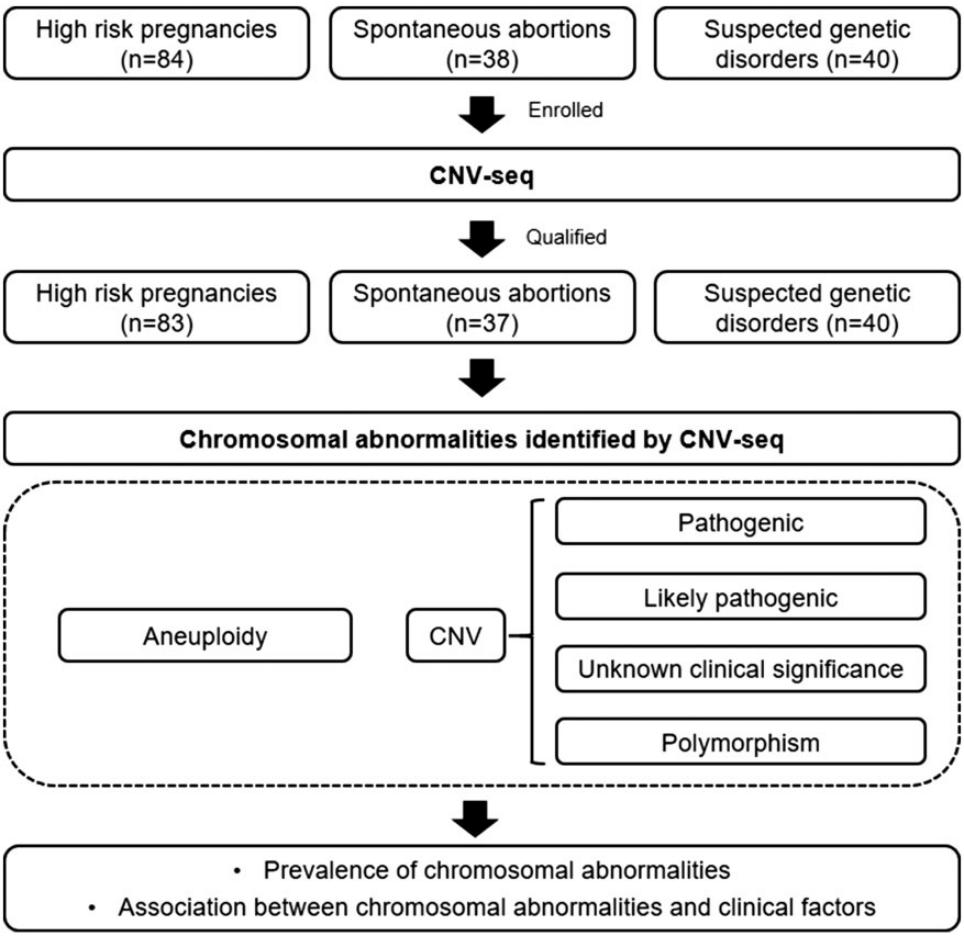

Figure 1. Study workflow, embodying study groups, methods, and aspects of data analyses.

Table 1. Number of cases carrying chromosomal abnormalities (CNV-seq) in high-risk populations.

CNV-seq	High risk pregnancies (n=83)	Spontaneous abortions (n=37)	Suspected genetic disorders (n=40)
Cases without aneuploidy or CNV	46	12	18
Cases with aneuploidy	3	15	0
Cases with CNV	33	8	22
Cases with both aneuploidy and CNV	1	2	0
Pathogenic CNV	4	3	6
Likely pathogenic CNV	4	0	2
Unknown clinical significance CNV	16	4	12
Polymorphic CNV	10	3	2

and one had both aneuploidy and CNV. These chromosomal abnormalities included four pathogenic CNVs, four likely pathogenic CNVs, 16 CNVs of unknown clinical significance, and 10 polymorphic CNVs.

For the 37 cases with spontaneous abortions, 25 (67.6%) showed aneuploidy or CNV; 15 cases had aneuploidy, eight had CNV, and two had both aneuploidy and CNV. These chromosomal abnormalities include three pathogenic CNVs, no likely pathogenic CNVs, four CNVs of unknown clinical significance, and three polymorphic CNVs.

For those 40 patients with suspected genetic disorders, 22 (55%) had CNVs, with no aneuploidy detected. Of these CNVs, six were pathogenic, two were likely pathogenic, 12 had unknown clinical significance, and two were polymorphic.

High-risk pregnancies

CNV-seq showed that the frequency of pathogenic-relevant chromosomal abnormalities (including aneuploidy and pathogenic and likely pathogenic CNVs) in women with high-risk pregnancies was 14.46% (12/83). To investigate the correlation between this frequency and clinical factors, we divided the 83 samples into subgroups according to the mother's age, history of congenital disease, history of aberrant pregnancy or delivery, and ultrasound examinations. On this basis, a comparative analysis was carried out to identify potential relationships.

Subjects were divided into the following groups: 20–24 years, 25–29 years, 30–34 years, 35–39 years, and 40–44 years. As shown in Figure 2a, the proportions of pathogenic-relevant chromosomal abnormalities were 9.09% (1/11), 10% (2/20), 14.81% (4/27), 16.67% (3/18), and 20% (1/5), respectively. The prevalence of pathogenic-relevant chromosomal abnormalities showed an age-dependent increase, especially in those aged $\geq$30 years who showed an increase of nearly 50% compared with their younger counterparts.

The 83 subjects were also divided into two groups depending whether they had a family history of certain congenital disorders. As shown in Figure 2b, the proportion of pathogenic-relevant chromosomal abnormalities was notably lower in those with no family history of congenital disorders than in those with a family history (11.54% (9/78) vs. 40% (2/5), respectively).

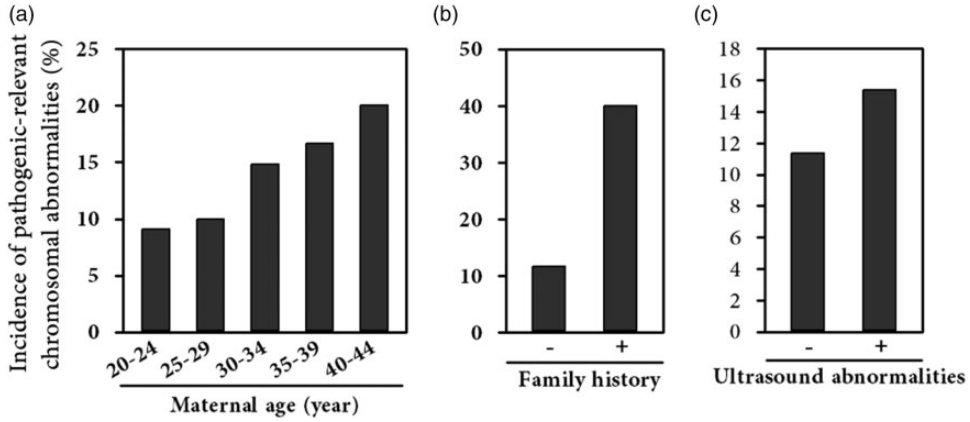

Figure 2. Incidence of pathogenic-relevant chromosomal abnormalities in high-risk pregnancy subgroups divided according to maternal age (a), family history (b), and ultrasound abnormalities (c).

Additionally, subgroup analysis was performed based on a history of aberrant pregnancy or delivery. Unexpectedly, the incidence of pathogenic-relevant chromosomal abnormalities was lower in those with an abnormal pregnancy history than in those without such a history (5.13% (2/39) vs. 20.45% (9/44), respectively).

Finally, the 83 subjects were divided into two groups according to ultrasonic findings. The incidence of pathogenic-relevant chromosomal abnormalities was lower in those with normal ultrasonic findings than in those with aberrant ultrasonic findings (11.36% (5/44) vs. 15.38% (6/39), respectively, Figure 2c).

Spontaneous abortions

To investigate the correlation between fetal chromosomal CNVs and abortive tissue CNVs, we divided the abortive tissue samples into subgroups according to the mother's age, history of an abnormal pregnancy, and ultrasonic findings. We also compared the incidence of aneuploidy and CNV based on CNV-seq findings.

Subjects were divided into three subgroups according to age: 20–29 years, 30–34 years, and 35–41 years. As shown in Figure 3a, the incidence of pathogenic-relevant chromosomal abnormalities was notably higher in subjects aged 30–34 years and 35–41 years than in those aged 20–29 years (57.14% (8/14) vs. 28.57% (4/14) and 66.67% (6/9) vs. 28.57% (4/14), respectively). This indicated that the incidence of pathogenic-relevant chromosomal abnormalities in the fetus was higher in women aged $\geq$30 years with a history of spontaneous abortion.

We also divided the 37 subjects into groups based on an abnormal pregnancy history and ultrasonic findings. The incidence of pathogenic-relevant chromosomal abnormalities in those with an abnormal pregnancy history was similar to that in those with no abnormal pregnancy history [40% (4/10) vs. 51.85% (14/27), respectively, Figure 3b]. It was also similar in those with aberrant ultrasonic findings and those with normal ultrasonic findings [42.86% (3/7) vs. 50% (15/30), respectively, Figure 3c].

Suspected genetic disorders

We divided subjects with suspected genetic disorders into two subgroups according to sex. As shown in Table 2, the incidence of CNVs in the male subgroup was similar to

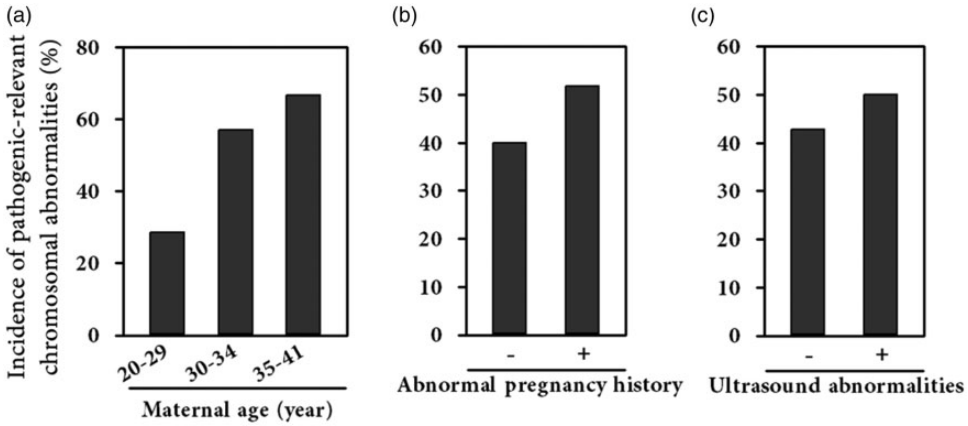

Figure 3. Incidence of pathogenic-relevant chromosomal abnormalities in spontaneous abortion subgroups divided according to maternal age (a), abnormal pregnancy history (b), and ultrasound abnormalities (c).

Table 2. Number of cases carrying CNV in suspected genetic disorders.

CNV-seq of suspected genetic disorders	Female (n=20)	Male (n=20)
Cases without CNV	10	8
Cases with pathogenic CNV	5	1
Cases with likely pathogenic CNV	1	1
Cases with unknown clinical significance CNV	3	9
Cases with polymorphic CNV	1	1

that of the female subgroup [60% (12/20) vs. 50% (10/20), respectively]. However, the incidence of pathogenic or likely pathogenic CNVs in female fetuses was slightly higher than in male fetuses [30% (6/20) vs. 10% (2/20), respectively].

Discussion

Chromosomal CNVs are an important source of genetic variation, but are a major cause of chromosomal disorders. CNV-seq is effective for the identification of chromosomal CNVs, and contributes to the screening of neonate deficits and investigating genetic or other potential causes of spontaneous abortions. In this study, CNV-seq was used to analyze CNVs in the prenatal screening of high-risk pregnancies, spontaneous abortions, and samples with suspected genetic disorders but no clinical diagnosis.

For women with high-risk pregnancies and aberrant findings in their serum biochemistry tests and/or ultrasonic examinations, CNV was confirmed in some cases after the collection of amniotic fluid or cord blood. Previously, pathogenic CNV has been used as a factor to terminate the pregnancy.[13] Moreover, age and congenital disease history of the mother were shown to be associated with an increased risk of chromosomal disease in the fetus.[14,15] Our findings were in agreement with this, with age and congenital disease history related to chromosomal CNV in the fetus (Figure 2). Additionally, we showed that the prevalence of pathogenic-relevant chromosomal abnormalities had an age-dependent increase, especially in those aged $\geq$30 years, while the risk of pathogenic CNV in the fetus also increased with maternal age. This is in line with a previous study.[16] For pregnant women with a congenital disorder or ultrasonic anomaly, the risk of pathogenic CNVs in their fetus was even higher, implying that family history or aberrant ultrasonic findings may be associated with an increased possibility of chromosomal CNV changes. Interestingly, an abnormal pregnancy history was not correlated with an increased risk of pathogenic-relevant chromosomal abnormalities in the fetus (data not shown), which differed from findings of a previous study in which a complete karyotype analysis was recommended for women with an abnormal pregnancy history before their second pregnancy.[17] In future, more studies are needed to further investigate the correlation between an abnormal pregnancy history and the increased risk of pathogenic-relevant chromosomal abnormalities in the fetus.

Fetal chromosomal CNVs, especially aneuploidy, have been reported as the major cause of spontaneous abortions.[18,19] Thus, it is reasonable to conclude that factors affecting fetal chromosomal CNVs are also associated with spontaneous abortions. In this study, almost half of abortive tissue samples were identified as aneuploid, resulting in a prevalence that was significantly higher than that of pathogenic-relevant CNV (45.9% (17/37) vs. 8.11% (3/37)). The age of the pregnant woman having spontaneous abortions was also shown to be correlated with pathogenic-relevant chromosomal abnormalities, with those aged $\geq$30 years showing an increased risk of

pathogenic-relevant chromosomal abnormalities (Figure 3a) and neonate deficiency (Figure 2a). Additionally, women with an abnormal pregnancy history showed an increased risk of pathogenic-relevant chromosomal abnormalities in the fetus (Figure 2b). This implied that aneuploidy is strongly associated with repeated abortions, which supports the findings of a study by Bianco et al.[20]

The detection of chromosomal abnormalities is important for the confirmation of patients with no clinical diagnosis. In this study, no aneuploidy was identified in 40 suspected genetic disorders. This has relevance for women who undergo abortions after the identification of severe manifestations induced by aneuploidy in clinical practice.[21,22] Several pathogenic or likely pathogenic CNVs were identified from these subjects. According to a previous study, CNV may have a sex bias in some diseases such as schizophrenia,[23] olfactory system disorder related retardation and aponoia,[24] and autism.[25] Our data supported this, with an observed higher incidence of pathogenic or likely pathogenic CNVs in women compared with men. In the future, we will investigate the survival tolerance of female fetuses with pathogenic CNV abnormalities.

When comparing clinical data with CNV data in previous publications, we found that pathogenic deletions or duplications could effectively explain patient clinical manifestations. For example, a deletion of 5.42 Mb (chr21: 42680001–48100000) was identified during the follow-up of a neonate with language barriers. This CNV was reported to be related to speech retardation, slight build, mental retardation, and unusual facial expressions.[26] Additionally, a female infant with wide-set eyes who was unable to walk or crawl showed mosaicism of a 34.82 Mb (chr12: 1–34820000) duplication and normal copy number (60.0%) on chromosome 12. Such duplication was reported to be associated with congenital deformities of the face, moderate to severe psychomotor development, and hypotonia,[27] and was characterized by mental retardation, blurred speech, poor intelligence, and supravalvular aortic stenosis. Moreover, a female fetus with Williams syndrome showed a 1.4 Mb deletion on chromosome 7 (chr7: 72720001–74120000). Williams syndrome was previously related to the deletion of such a DNA fragment, and was characterized by a congenital cardiac anomaly, dysmorphic facial features, mental retardation, a short attention span, mental retardation, and microsomia.[28,29]

Our study has some limitations. First, its sample size was small and all subjects were from a single center, which prevented us from drawing more concrete conclusions. In the future, a multicenter study using a bigger sample size is required. Second, although possible relationships between the incidence of chromosomal abnormalities and clinical factors were examined, more data are required to confirm these relationships.

Conclusions

This work provides insights into the prevalence of chromosomal abnormalities in high-risk populations, such as high-risk pregnancies, spontaneous abortions, and suspected genetic disorders. These three study groups could represent the main target population of CNV-seq technology, and indeed this study confirmed the efficacy of CNV-seq in identifying chromosomal abnormalities including aneuploidy and CNVs. The three groups all showed a relatively high prevalence of chromosomal abnormalities, specifically, 44.6% in high-risk pregnancies, 67.6% in spontaneous abortions, and 55% in suspected genetic disorders. Advanced maternal age is a major factor increasing the risk of aneuploidy and pathogenic-relevant CNV in high-risk

pregnancies and spontaneous abortions, while a family history of congenital disease may also play a role in the incidence of fetal chromosomal abnormalities in high-risk pregnancies. These observed high prevalences of chromosomal abnormalities shows the importance and need of CNV detection in such high-risk populations.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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ORCID iD

Jingcui Yu http://orcid.org/0000-0001-5182-987X

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