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Research article

Application of the Short Tandem Repeat Marker (D21S11) in the Molecular Diagnosis of Down Syndrome in Libya

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Abstract

Background: Down syndrome (DS) is the most common genetic anomaly linked to varying degrees of intellectual disability, distinct physical traits and health issues. PCR-based DNA diagnostic method is sensitive, robust and the most efficient for trisomy 21 diagnosis. **Aim:** This study aimed to demonstrate the sensitivity of DNA diagnosis of DS using the short tandem repeat (STR) marker D21S11. **Methods:** This study was conducted in Tripoli, Libya. Blood samples were collected from 55 individuals, of which 50 were successfully amplified and included in the analysis. In addition, 102 healthy controls were included to assess the sensitivity of the D21S11 marker for detecting chromosome 21 (Ch21) nondisjunction (NDJ). The GlobalFiler™ IQC PCR Amplification kit and a 3500 Genetic Analyzer were used to detect trisomy 21 by the D21S11 locus on Ch21. **Results:** Diagnosis of Ch21 trisomy was achieved in all 50 successfully amplified samples (100%), including one mosaic case. Of the 50 cases, 26 (52%) showed a diallelic pattern, 21 cases (42%) showed a triallelic pattern and 3 cases (6%) showed a monoallelic pattern. **Conclusion:** This study successfully diagnosed, for the first time, Ch21 NDJ in individuals with DS cases, including one mosaic case, using the STR marker (D21S11) and genetic analyzer in the Libyan population. This method may be applied to prenatal and postnatal diagnosis as well as preimplantation genetic diagnosis, as it is rapid, simple, accurate, and requires only a small amount of biological material compared with conventional cytogenetic analyses.

Keywords: Down syndrome, diagnosis, PCR, short tandem repeats, D21S11 marker

Introduction

Down syndrome (DS) is the most frequent aneuploidy condition in humans that survives to delivery. John Langdon Down, a physician from Cornwall, England, discovered that DS is associated with intellectual disability and described the clinical features of the syndrome for the first time in 1866 (Down, 1866). However, Dr. Jerome Lejeune in Paris first discovered the link between the DS phenotype and Ch21 NDJ in 1959 (Lejeune *et al.*, 1959). The first mouse models for DS research were developed in 1990 (Davisson *et al.*, 1990). A decade later, a global team of scientists published the entire nucleotide sequence of *Homo sapiens* autosome 21 (HSA21) (Hattori *et al.*, 2000). In subsequent years, significant progress has been made in the molecular pathophysiology of the various phenotypes of DS (Antonarakis *et al.*, 2020). More recently, a team of Japanese scientists succeeded, for the first time, in removing Ch21 NDJ from human trisomy 21 induced pluripotent stem cells and fibroblasts, proving its feasibility and efficacy as a therapeutic intervention for DS (Hashizume *et al.*, 2025).

There is a range of physical traits exhibited in DS individuals, including almond-shaped eyes, folded ears, a small mouth, a protruding tongue, a small chin, a single crease of the palms, short and broad hands and short feet (Sureshbabu *et al.*, 2011; Antonarakis *et al.*, 2020; Bull *et al.*, 2022). Moreover, the DS phenotype includes symptoms that affect several physiological systems, especially the nervous, cardiovascular, and musculoskeletal systems. Additional health issues that people with DS are more likely to develop include autoimmune disorders, infections, vision and hearing problems, hematological disorders, anxiety disorders, epilepsy and Alzheimer's disease (Antonarakis *et al.*, 2020).

The prevalence of DS varies across countries due to economic and sociocultural factors (Weijerman & Winter, 2010). Between 2013 and 2017, one in 787

babies in the United States was born with DS (Heinke *et al*., 2021). Ireland has the highest rate of babies with DS at 1/364, while Iceland has the lowest prevalence of DS (DoctorSolve, 2024). In the Middle East, the prevalence of DS is relatively high because of regional traditions. The highest rate was in the United Arab Emirates, with a rate of 1/374 live births, followed by Qatar at 1/513, Libya at 1/516, Saudi Arabia at 1/554 and Egypt at 1/555, and the lowest in Tunisia at ~1/1000 (Hafez *et al*., 1984; Verma *et al*., 1990;Niazi *et al*., 1995; Wahab *et al*., 2006; Chelli *et al*., 2008; Aburawi *et al*., 2015).

Conventional cytogenetic analysis (karyotyping) is the standard diagnostic method for prenatal and postnatal diagnosis of DS. However, it is time-consuming and expensive, often leading to delays in diagnosis (Mačkić-đurović *et al*., 2014). Therefore, quicker prenatal results regarding common chromosome aneuploidies, to lower the birth defect rate, raise awareness among DS families and reduce maternal concern, have been increasingly in demand (El Mouatassim *et al*., 2004). Rapid Aneuploidy Screen (RAS) tests, such as fluorescence in situ hybridization (FISH) and quantitative fluorescent PCR (QF-PCR), have been used to quickly detect trisomy 21 and common aneuploidies in high-risk pregnancies, enabling prenatal diagnosis of chromosomal abnormalities to be completed within 24-48 hours (Spathas *et al*., 1994; Sun *et al*., 2006). Although FISH is highly sensitive and specific for the rapid detection of aneuploidies when utilizing commercially available probes, the process is labor-intensive and FISH probes or kits are relatively expensive. As a result, QF-PCR, which employs polymorphic microsatellites unique to each chromosome, is easier, more sensitive and less expensive than FISH analysis (Mann *et al*., 2001).

Polymerase chain reaction-based molecular-genetic analyses have been launched as alternatives to traditional methods, with Short Tandem Repeats (STRs) being promoted as potential tools for the diagnosis of common aneuploidies such as DS (Chishti *et al*., 2014). These STRs, a type of microsatellite, are highly polymorphic nucleotide sequences that account for approximately 3% of the human genome (Lander *et al*., 2001). They are widely used in applications such as paternity testing, kinship analysis and population genetic studies (Zhan *et al*., 2018).

Autosomal STRs are diploid loci that typically have two similar (homozygous) or different (heterozygous) alleles. Thus, one or a maximum of two peaks per locus are anticipated in an electropherogram when autosomal STRs are genotyped using capillary electrophoresis (CE). Furthermore, third alleles may exist as in a chromosomal abnormality known as DS (Chishti *et al*., 2014). Pertl and colleagues in the United Kingdom, in 1994, evaluated a rapid technique for prenatal DS diagnosis using the D21S11 marker. DNA from blood, amniotic fluid and tissues was amplified and DS samples had three peaks with a 1:1:1 ratio, two STR peaks with a 2:1 or homologous peaks (Pertl *et al*., 1994). Additionally, an Iranian study evaluated the diagnostic utility of STR marker assays for DS and reported that highly polymorphic STR markers had 99% sensitivity and 100% specificity (Aleyasin *et al*., 2004). Another study using the D21S11 marker found a 100% success rate in detecting trisomy 21 cases (Sun *et al*., 2006). In India, Jain and colleagues used two tetranucleotide STR markers, D21S2055 and D21S11, to identify DS cases in 86.7% of cases (Jain *et al*., 2010).

The D21S11 polymorphism with a complex sequence structure is situated at chromosomal location 21q11.2 that was initially discovered in 1992 (Sharma & Litt, 1992). The use of the D21S11 marker helps physicians and technicians diagnose prenatal DS cases (Liou *et al*., 2004). Importantly, it is needed by *in vitro* fertilization (IVF) specialists to avoid suspected cases by preimplantation genetic diagnosis (PGD) and to help researchers in genetic mapping (Edwards *et al*., 1991; Blake *et al*., 1999). Therefore, this study aimed to demonstrate the sensitivity of DNA-based diagnosis of DS at the molecular level using the D21S11 marker in selected DS cases in Libya.

Materials and methods

Sample collection

This study was conducted at the National Authority for DNA Research and Analysis (NADNARA), Tripoli, Libya. Individuals with DS were selected, and the blood sampling procedures were performed following the approved protocol of NADNARA. A written consent form was obtained and signed by the parents of the children with DS and the control participants prior to blood sample collection. A total of 55 blood samples were collected from children with clinically diagnosed DS, of which only four cases were confirmed by karyotyping. In three DS cases, there was free trisomy, and one case was a mosaic type. The samples were collected from the following centers in Tripoli: Libyan Society for DS, Children's Teaching Hospital and Tareq Almatar, Tripoli, Libya. Two milliliters (ml) of blood were drawn from each participant using evacuated tubes containing ethylenediaminetetraacetic acid (EDTA). The blood samples were coded to ensure the confidentiality of the participants in accordance with the NADNARA protocol.

DNA extraction and quantification

DNA was extracted from whole blood using the PrepFiler™ Forensic DNA Extraction kit (Applied Biosystems, Foster City, USA) and an Automated Express DNA Expression System (Applied Biosystems, Foster City, USA) according to the manufacturer's guidelines. Briefly, 25µl of blood sample was transferred into the column/tube assembly then the 500µl of freshly prepared lysis solution was added. The lysate was transferred to a sample tube after being thermally shaken and centrifuged. The extraction process was then performed automatically.

Genomic DNA concentration was quantified using the Quantifiler Trio DNA Quantification kit (Applied Biosystems, Foster City, USA) with a Quant Studio 5 RT-PCR (Applied Biosystems, Foster City, USA).

DNA amplification and fragment analysis

DNA amplification was performed using the GlobalFiler™ IQC PCR Amplification kit (Applied Biosystems, Foster City, USA) with a Veriti 96-well Thermal Cycler (Applied Biosystems, Foster City, USA). The appropriate volume of nuclease-free water solution for each sample was added to each Eppendorf

tube. Then, 1µl of DNA sample was added to each tube to achieve a total of 1ng of DNA in a final reaction volume of 15µl. The reaction mixture was prepared using 7.5µl of master mix and 2.5µl primer set, and distributed into each MicroAmp™ tube following the manufacturer's guidelines.

For fragment analysis, 0.4µl of GeneScan™ 600 LIZ™ Size Standard v2.0 was added to 9.6µl Hi Di™ Formamide. Then, the 10µl of the formamide\size standard mixture and 1µl of PCR product or Allelic Ladder were added to every well of a MicroAmp™ Optical 96-well reaction plate. The plate was then heated for 3 minutes at 95°C. The plate was then placed on ice for 3 minutes and electrophoresis was performed using a 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA). The fragment data were analyzed using GeneMapper ID X software version v1.6 (Applied Biosystems, Foster City, USA), which included genotyping assignment, peak detection, and allele calling. To guarantee accuracy and reliability across samples, each genotyping result was manually examined and verified.

Data analysis

Genotypes for each participating sample were obtained using GeneMapper™ ID-X software version v1.6 (Applied Biosystems, Foster City, USA) for the D21S11 marker. For children with DS, this marker was used to detect trisomy 21 by either a "diallelic" pattern of two peaks with a 2:1 ratio, a "triallelic" pattern of three STR peaks of similar intensity with a 1:1:1 ratio or a monoallelic pattern of homologous peaks.

Ethical approval

This study was approved by the Ethics Committee at the National Center for Disease Control, Libya, under the reference number; NBC: 002.H-23.19.

Results

The D21S11 marker, utilizing the GlobalFiler™ IQC PCR Amplification kit and the 3500 Genetic Analyzer, was employed to evaluate diagnostic sensitivity in a cohort of 55 children with suspected DS. While all 55 samples underwent successful PCR amplification, definitive DNA profiles were obtained for 50 cases, which were then used for the final genetic analysis.

Molecular diagnosis of DS by D21S11 marker

Genetic analysis of the D21S11 marker using the GlobalFiler™ IQC PCR Amplification kit and size measurements provided conclusive proof of DS in all 50 (100%) children. It was not possible to obtain the DNA profile of 5 samples due to technical procedure problems that led to the incomplete genotyping using the 3500 Genetic Analyzer. Normal samples had two amplification peaks of similar magnitude with a 1:1 ratio or homologous peaks, whereas DS samples exhibited two peaks with a 1:2 or 2:1 ratio (diallelic) in 26 (52%) cases, three distinct peaks (triallelic) in 21 (42%) cases, or homologous peak (monoallelic) in 3 (6%) cases. Examples of STR profiles of the D21S11 marker analysis of a child with DS and normal sample are shown in table 1 and figure 1.

Table1. Examples of STR profiles in Down syndrome cases and normal samples.

Samples	Genotype	The ratio of alleles
DS case 1	29, 30, 31.2	1:1:1
DS case 2	31.2, 31.2, 32.2	2:1
DS case 3	29, 30, 30	1:2
DS case 4	29, 29, 29	Homologous
NS 1	29, 31	1:1
NS 2	29, 29	Homologous

STR: short tandem repeats, DS: Down syndrome, NS: normal sample

Discussion

The current study investigated the molecular detection of trisomy 21 using the D21S11 marker in Libyan children with Down syndrome. While all 55 samples were suitable for initial PCR amplification, high-quality DNA profiles were obtained for 50 of the DS cases. The remaining five samples yielded incomplete genotyping results on the 3500 Genetic Analyzer, likely due to technical limitations or sample-specific factors, and were thus excluded from the final analysis. Each DNA sample profile was then obtained from the data collection program and analyzed using GeneMapper ID X software.

Down syndrome is the most prevalent fetal genetic condition identified by prenatal and postnatal diagnostics (El Mouatassim *et al*., 2004). Therefore, the demand for faster prenatal results of common chromosome aneuploidies has increased to reduce maternal anxiety, lessen the birth defect rate and promote awareness among DS families. Moreover, parents and their families experience severe emotional and psychological strain over long waiting periods during diagnosis (Evers-Kiebooms *et al*., 1988). However, a rapid and accurate diagnosis is essential for initiating the necessary procedures. Multiple studies have applied the PCR and STR-based approaches for diagnosing DS, using the D21S11 or other markers (Chishti *et al*., 2014; Mačkić-đurović *et al*., 2014). This study utilized the GlobalFiler™ IQC PCR Amplification kit as a tool for detecting DS via the D21S11 locus on Ch21, in addition to its forensic use. In the current study, the success rate for detection of DS cases was 100%. Similar findings were reported in Pakistan, where 44 cases of DS were detected out of 74 blood samples with different chromosomal aneuploidies (Chishti *et al*., 2014). They used the Identifiler™ kit to check its capability of detecting aneuploidies.

Although this study used only one marker, DS was definitively proven by the genetic analysis of D21S11 marker products and measurement of their fragment sizes. Among the DS samples, 52% exhibited a diallelic pattern with a 2:1 ratio, 42% showed a triallelic pattern with a 1:1:1 ratio, and 6% displayed a monoallelic pattern. Additionally, similarities in diallelic and triallelic patterns were observed between the present study and those reported by Sun *et al*., (2006). However, there were no cases with a "monoallelic" pattern in their

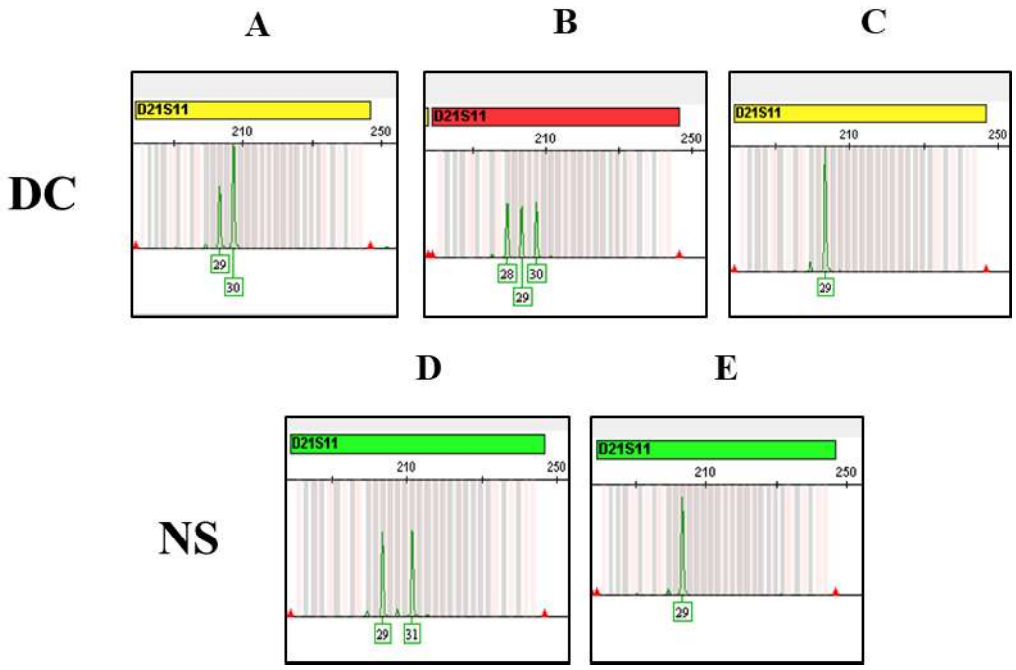

Figure 1. Electropherogram profiles of DS cases and normal samples with the D21S11 marker. A. DS child has two peaks with a 2:1 ratio in the D21S11 marker. **B.** DS child has three distinct peaks with a 1:1:1 ratio. **C.** DS child has three alleles in homologous peaks in allele 29. **D.** Normal sample has two peaks with a 1:1 ratio. **E.** Normal sample has two alleles in homologous peaks in allele 29. DS: Down syndrome, NS: Normal sample.

study (Sun *et al*., 2006). On the other hand, the findings of the present study agree with those of previous study in the United Kingdom, in which a small number of cases exhibited a monoallelic pattern (Pertl *et al*., 1994).

The present results are also consistent with a previous study reporting the appearance of three allelic patterns in DS children (Aleyasin *et al*., 2004). Furthermore, a study conducted in India identified DS cases using two STR markers (D21S2055 and D21S11) (Jain *et al*., 2010). In that study, 13 of 15 DS children (86.7%) were identified using PCR and analysis on 15% polyacrylamide gel. The remaining two cases could not be confirmed because densitometric measurements were required, as visual interpretation alone was not sufficient for diagnosis.

Although some previous studies have reported difficulty in confirming mosaic Down syndrome using STR markers, the present investigation successfully identified Ch21 NDJ in all 50 Libyan cases (100%) using the D21S11 marker and capillary electrophoresis. This high detection rate included the successful molecular confirmation of the mosaic case, which exhibited a peak height ratio consistent with the non-mosaic trisomy samples. This finding was consistent with an Iranian study that used STRs to confirm three DS children with the mosaic type (known karyotype) (Aleyasin *et al*., 2004). In contrast, the mosaic DS type was not confirmed in 2 out of 8 children with mosaic DS in Bosnia and Herzegovina (Mačkić-đurović *et al*., 2014). The authors suggested that this result may be due to the small proportion of cells in mitotic division, with one child having 22% and the other 8% of cells carrying trisomy 21. In addition, the success of this study in diagnosing mosaic-type DS may be due to the use of a highly sensitive and more advanced technique. However, further confirmation in a larger number of mosaic DS cases in future studies is needed to identify the sensitivity of this technique.

Conclusion

The present study successfully detected, for the first time, Ch21 NDJ in 50 (100%) DS cases in Libya using the D21S11 marker and genetic analyzer, including one case of DS mosaicism. Although the results are promising, further confirmation with a greater number of mosaic cases is required for a more accurate sensitivity assessment of the technique. The current study also emphasizes the importance of early DS diagnosis in improving healthcare outcomes for affected families in Libya.

Author contributions

Mansur E. Shmela: Study design, experimental supervision, data analysis, result interpretation, and manuscript drafting and revision. Amnnah E. Buthaynah: Sample collection, investigation (experimental performance), data analysis, result interpretation, manuscript drafting, and ethical approval acquisition. Fahima A. Alnagar: Study design, experimental assistance, manuscript drafting and revision, and ethical approval acquisition. Fauzia M. Mohamed: Manuscript revision and intellectual content review. Abdulmaula M. Abogrein: Manuscript revision and intellectual content review. Safa A. Fandi: Experimental assistance and manuscript revision. Hanin A. Abukliesh: Data analysis and validation. Nuri M. Alkhder: Experimental assistance and data analysis.

Conflict of interest

The authors declare no conflict of interest related to the publication of this work.

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