Spinal Muscular Atrophy Diagnosis and Dried Saliva Spots

Subjects: Neurosciences

Contributor: yogik onky silvana wijaya

Spinal muscular atrophy (SMA) is a lower motor neuron disease, once considered incurable. The main symptoms are muscle weakness and muscular atrophy. More than 90% of cases of SMA are caused by homozygous deletion of survival motor neuron 1 (*SMN1*). Emerging treatments, such as splicing modulation of *SMN2* and *SMN* gene replacement therapy, have improved the prognoses and motor functions of patients. However, confirmed diagnosis by *SMN1* testing is often delayed, suggesting the presence of diagnosis-delayed or undiagnosed cases. To enable patients to access the right treatments, a screening system for SMA is essential. Even so, the current newborn screening system using dried blood spots is still invasive and cumbersome. Here, we developed a completely non-invasive screening system using dried saliva spots (DSS) as an alternative DNA source to detect *SMN1* deletion. In this study, 60 DSS (40 SMA patients and 20 controls) were tested. The combination of modified competitive oligonucleotide priming-polymerase chain reaction and melting peak analysis clearly distinguished DSS samples with and without *SMN1*. In conclusion, these results suggest that our system with DSS is applicable to SMA patient detection in the real world.

Keywords: dried saliva spot ; spinal muscular atrophy ; melting peak analysis ; nested PCR

1. Introduction

Spinal muscular atrophy (SMA) is one of the most devastating neuromuscular disorders, characterized by motor neuron degeneration[1]. The patients present with muscle weakness and muscular atrophy[1]. Clinically, SMA is divided into five subtypes: Type 0 (the most severe form, prenatal onset, death within weeks of birth); Type I (Werdnig–Hoffmann disease, severe form, onset < 6 months, non-sitter); Type II (Dubowitz disease, intermediate form, onset < 18 months, sitter); Type III (Kugelberg–Welander disease, mild form, onset > 18 months, walker); and Type IV (the mildest form, onset > 30 years) [2][3].

The majority of SMA cases (~95%) involve homozygous deletion of survival motor neuron 1 (*SMN1*), while some others (~5%) carry a deleterious *SMN1* mutation, thus *SMN1* is considered the disease-causing gene[1][2]. The homologous gene, *SMN2*, serves as an SMA-modifying gene because a high *SMN2* copy number is generally associated with a milder phenotype[4][5].

SMN1 and *SMN2* are identical except for five nucleotides located in intron 6, exon 7, intron 7, and exon 8[1]. Molecular diagnosis of SMA requires the detection of *SMN1*-specific nucleotides, especially the one located in exon 7. Several methods have been developed to achieve this, including single-stranded conformation polymorphism (SSCP) analysis[1], restriction enzyme digestion analysis[6], modified competitive oligonucleotide priming-polymerase chain reaction (mCOP-PCR)[7][8], multiplex ligation-dependent probe amplification (MLPA)[9], digital droplet PCR (ddPCR)[10] and next-generation sequencing (NGS)[11].

SMA has long been thought an incurable disease because of the absence of effective drugs. However, three new and effective drugs for SMA are now available: nusinersen[12], onasemnogene abeparvovec-xioi[13], and risdiplam[14]. These drugs give better outcomes when treatment is initiated at an early stage, before the onset of symptoms[12][13][14]. Therefore, there is a growing implementation of newborn screening programs for SMA (NBS-SMA) worldwide[7][15][16][17][18][19][20][21]. Technically, the current NBS-SMA program can detect all SMA patients with a homozygous deletion regardless of the clinical subtype.

Even though NBS-SMA has begun in many countries, there remain many older children or adults with SMA in the population who were not detected in early infancy. The majority of them may be diagnosed with SMA in a timely fashion, but the rest may be diagnosed late or may not be diagnosed, particularly those with a later onset of the disease[22][23][24][25]. Undiagnosed patients cannot access treatment with the new drugs. Therefore, to complement NBS-SMA, it is

desirable to establish a screening system that covers older children and adults. The samples for that purpose should be collected at schools, workplaces, or homes. However, one potential hurdle is the invasiveness of blood-based templates such as dried blood spots (DBS), which may require health care visitation of the individuals or blood collection by health care workers.

2. Saliva as a Good Source for Genetic Analysis

Saliva has been used for analysis of human DNA by PCR-based HLA typing[26], microarray SNP genotyping[27][28], TaqMan SNP genotyping assays[27][29], loop-mediated isothermal amplification-melting curve (LAMP-MC) analysis[29], PCR-Sanger sequencing[28], next generation sequencing[30], and whole genome or exome sequencing[31][32].

Saliva has now proved to be a good alternative source for genetic analysis. In the studies mentioned above, saliva was collected using a saliva collection kit with a special container, either in-house-made[26] or commercially available[27][28][29][30][31][32]. Later, DNA was extracted from the liquid saliva sample in the container[26][27][28][29][30][31][32].

Some previous studies used a different kind of saliva sample. Instead of a liquid saliva sample, some researchers prepared DSS samples and extracted DNA from them for various purposes[33][34][35]. Kisoi et al. identified insertion/deletion polymorphisms of the angiotensin-converting enzyme (*ACE*) gene by PCR amplification using DNA from DSS samples[33]. Our study also demonstrated genotyping analysis of *SMN* genes using DSS samples. These findings support the idea that saliva, even if dried, can be a good DNA source for PCR.

Kisoi et al. reported a simple DNA extraction method from the DSS samples: they boiled one DSS punch (4 mm in diameter) and used an aliquot of the supernatant for PCR amplification[33]. Hayashida et al. reported direct PCR from DSS to detect SNP in alcohol metabolism-related genes (*ADH1B* and *ALDH2)* using a TaqMan probe assay[36]. In agreement with their results, we provide further evidence that direct PCR amplification is feasible from DSS without boiling or DNA purification procedures.

3. Robustness and Accuracy of Our SMA Screening System

In this study, the basic technology to detect *SMN1*, mCOP-PCR, is a type of allele-specific PCR. Originally, COP-PCR was based on allele-specific amplification, in which two oligonucleotide primers compete for one target DNA sequence in one PCR tube[37]. The primers are short (10–11 bases) and highly identical except for one nucleotide change in the middle of the primers[8][37]. The modified version adopted in this study, mCOP-PCR, uses one gene-specific oligonucleotide primer (SMN1-COP) that preferentially binds the *SMN1* sequence rather than the competitor sequence, *SMN2*[8].

Here, we employed nested PCR to rescue low quantity and quality genetic material in the DSS. The first-round PCR amplified common regions of the target genes and provided enough template for the second-round PCR, even for quantitative assays[7][8][38]. In addition, the first-round PCR products contained no other sequences similar to the *SMN1*-specific mCOP-PCR primer, which avoids the amplification of unexpected primer binding sites in the second-round PCR and enhances the specificity of our system[7][8].

4. Limitation of PCR-Based Assays Using DSS

The use of saliva, either liquid or dried, has potential limitations for PCR-based assays. First, the quantity of amplifiable human DNA recovered from liquid saliva is lower than blood (37.3% in saliva versus 87.6% in the blood)[27]. Second, the DNA quality obtained from saliva does not always meet the quality standard for certain assays, even though the DNA obtained from saliva showed a good A260/A280 ratio[31]. DNA degradation during the storage period may be related to the quality. Third, PCR inhibitors in the saliva may confound the results[27][39].

We experienced one case of assay failure out of 61 DSS samples (1.6%) in this study. This failure might be due to the absence of DNA or the presence of PCR inhibitors. The possibility of DNA degradation can be ruled out because the storage period was less than two weeks. In our pilot study of NBS-SMA, there were no cases of assay failure out of 4157 DBS samples (0%)[7]. Compared with our previous study of DBS, this study with DSS showed a higher rate of assay failure.

5. Conclusions

We demonstrated the potential use of DSS sampling as a good alternative source for SMA detection using nested mCOP-PCR. The sample collection procedure is non-invasive, easy to handle, and requires no hospital visitation. DSS might be preferable for SMA screening for older children or adults. We hope that this will help in overcoming the delay in SMA diagnosis.

References

1. Suzie Lefebvre; Lydie Bürglen; Sophie Reboullet; Olivier Clermont; Philippe Burlet; Louis Viollet; Bernard Benichou; Co rinne Cruaud; Philippe Millasseau; Massimo Zeviani; et al. Identification and characterization of a spinal muscular atrop hy-determining gene. *Cell* **1995**, *80*, 155-165, 10.1016/0092-8674(95)90460-3.
2. W. David Arnold; Darine Kassar; John T. Kissel; Spinal muscular atrophy: Diagnosis and management in a new therape utic era. *Muscle & Nerve* **2014**, *51*, 157-167, 10.1002/mus.24497.
3. Amanda Carré; Candice Empey; Review of Spinal Muscular Atrophy (SMA) for Prenatal and Pediatric Genetic Counsel ors. *Journal of Genetic Counseling* **2015**, *25*, 32-43, 10.1007/s10897-015-9859-z.
4. Thomas W Prior; Narasimhan Nagan; Elaine A Sugarman; Sat Dev Batish; Corey Braastad; Technical standards and g uidelines for spinal muscular atrophy testing. *Genetics in Medicine* **2011**, *13*, 686-694, 10.1097/gim.0b013e318220d52 3.
5. Maite Calucho; Sara Bernal; Laura Alías; Francesca March; Adoración Venceslá; Francisco J. Rodríguez-Álvarez; Elen a Aller; Raquel M. Fernández; Salud Borrego; José M. Millán; et al. Correlation between SMA type and SMN2 copy nu mber revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscula r Disorders* **2018**, *28*, 208-215, 10.1016/j.nmd.2018.01.003.
6. G. van der Steege; P.M. Grootscholten; P. van der Vlies; T.G. Draaijers; J. Osinga; J.M. Cobben; H. Scheffer; C.H.C.M. Buys; PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy. *The Lancet* **1 995**, *345*, 985-986, 10.1016/s0140-6736(95)90732-7.
7. Masakazu Shinohara; Emma Tabe Eko Niba; Yogik Onky Silvana Wijaya; Izumi Takayama; Chisako Mitsuishi; Sakae K umasaka; Yoichi Kondo; Akihiro Takatera; Isamu Hokuto; Ichiro Morioka; et al. A Novel System for Spinal Muscular Atro phy Screening in Newborns: Japanese Pilot Study. *International Journal of Neonatal Screening* **2019**, *5*, 41, 10.3390/ijn s5040041.
8. Mawaddah Ar Rochmah; Nur Imma Fatimah Harahap; Emma Tabe Eko Niba; Kenta Nakanishi; Hiroyuki Awano; Ichiro Morioka; Kazumoto Iijima; Toshio Saito; Kayoko Saito; Poh San Lai; et al. Genetic screening of spinal muscular atrophy using a real-time modified COP-PCR technique with dried blood-spot DNA. *Brain and Development* **2017**, *39*, 774-782, 10.1016/j.braindev.2017.04.015.
9. Eva L. Arkblad; Niklas Darin; Kerstin Berg; Eva Kimber; Göran Brandberg; Christopher Lindberg; Eva Holmberg; Mar T ulinius; Margareta Nordling; Multiplex ligation-dependent probe amplification improves diagnostics in spinal muscular at rophy. *Neuromuscular Disorders* **2006**, *16*, 830-838, 10.1016/j.nmd.2006.08.011.
10. Noemi Vidal-Folch; Dimitar Gavrilov; Kimiyo Raymond; Piero Rinaldo; Silvia Tortorelli; Dietrich Matern; Devin Oglesbe e; Multiplex Droplet Digital PCR Method Applicable to Newborn Screening, Carrier Status, and Assessment of Spinal M uscular Atrophy. *Clinical Chemistry* **2018**, *64*, 1753-1761, 10.1373/clinchem.2018.293712.
11. Yanming Feng; Xiaoyan Ge; Linyan Meng; Jennifer Scull; Jianli Li; Xia Tian; Tao Zhang; Weihong Jin; Hanyin Cheng; X ia Wang; et al. The next generation of population-based spinal muscular atrophy carrier screening: comprehensive pan -ethnic SMN1 copy-number and sequence variant analysis by massively parallel sequencing. *Genetics in Medicine* **201 7**, *19*, 936-944, 10.1038/gim.2016.215.
12. Richard S. Finkel; Eugenio Mercuri; Basil Darras; Anne M. Connolly; Nancy L. Kuntz; Janbernd Kirschner; Claudia A. C hiriboga; Kayoko Saito; Laurent Servais; Eduardo Tizzano; et al. Nusinersen versus Sham Control in Infantile-Onset Sp inal Muscular Atrophy. *New England Journal of Medicine* **2017**, *377*, 1723-1732, 10.1056/nejmoa1702752.
13. Jerry R. Mendell; Samiah Al-Zaidy; Richard Shell; W. Dave Arnold; Louise R. Rodino-Klapac; Thomas W. Prior; Linda L owes; Lindsay Alfano; Katherine Berry; Kathleen Church; et al. Single-Dose Gene-Replacement Therapy for Spinal Mu scular Atrophy. *New England Journal of Medicine* **2017**, *377*, 1713-1722, 10.1056/nejmoa1706198.
14. Sohita Dhillon; Risdiplam: First Approval. *Drugs* **2020**, *80*, 1853-1858, 10.1007/s40265-020-01410-z.
15. Yin-Hsiu Chien; Shu-Chuan Chiang; Wen-Chin Weng; Ni-Chung Lee; Ching-Jie Lin; Wu-Shiun Hsieh; Wang-Tso Lee; Y uh-Jyh Jong; Tsang-Ming Ko; Wuh-Liang Hwu; et al. Presymptomatic Diagnosis of Spinal Muscular Atrophy Through N

ewborn Screening. *The Journal of Pediatrics* **2017**, *190*, 124-129.e1, 10.1016/j.jpeds.2017.06.042.

16. Jennifer N Kraszewski; Denise Kay; Colleen F Stevens; Carrie Koval; Bianca Haser; Veronica Ortiz; Anthony Albertorio; Lilian L Cohen; Ritu Jain; Sarah Andrew; et al. Pilot study of population-based newborn screening for spinal muscular a trophy in New York state. *Genetics in Medicine* **2017**, *20*, 608-613, 10.1038/gim.2017.152.

17. Katerina Kucera; Jennifer Taylor; Veronica Robles; Kristin Clinard; Brooke Migliore; Beth Boyea; Katherine Okoniewski; Martin Duparc; Catherine Rehder; Scott Shone; et al. A Voluntary Statewide Newborn Screening Pilot for Spinal Muscul ar Atrophy: Results from Early Check. *International Journal of Neonatal Screening* **2021**, *7*, 20, 10.3390/ijns7010020.

18. Didu S.T. Kariyawasam; Arlene M. D'Silva; Janine Vetsch; Claire E. Wakefield; Veronica Wiley; Michelle A. Farrar; “We needed this”: perspectives of parents and healthcare professionals involved in a pilot newborn screening program for s pinal muscular atrophy. *EClinicalMedicine* **2021**, *33*, 100742, 10.1016/j.eclinm.2021.100742.

19. Katharina Vill; Oliver Schwartz; Astrid Blaschek; Dieter Gläser; Uta Nennstiel; Brunhilde Wirth; Siegfried Burggraf; Wulf Röschinger; Marc Becker; Ludwig Czibere; et al. Newborn screening for spinal muscular atrophy in Germany: clinical re sults after 2 years. *Orphanet Journal of Rare Diseases* **2021**, *16*, 1-10, 10.1186/s13023-021-01783-8.

20. Tamara Dangouloff; Eva Vrščaj; Laurent Servais; Damjan Osredkar; Thierry Adoukonou; Omid Aryani; Nina Barisic; Fa had Bashiri; Laila Bastaki; Afaf Benitto; et al. Newborn screening programs for spinal muscular atrophy worldwide: Whe re we stand and where to go. *Neuromuscular Disorders* **2021**, *31*, 574-582, 10.1016/j.nmd.2021.03.007.

21. Hugh J. McMillan; Kristin D. Kernohan; Ed Yeh; Kim Amburgey; Jennifer Boyd; Craig Campbell; James J. Dowling; Her nan Gonorazky; Janet Marcadier; Mark A. Tarnopolsky; et al. Newborn Screening for Spinal Muscular Atrophy: Ontario Testing and Follow-up Recommendations. *Canadian Journal of Neurological Sciences / Journal Canadien des Science s Neurologiques* **2020**, *48*, 1-8, 10.1017/cjn.2020.229.

22. Chia-Wei Lin; Stephanie J. Kalb; Wei-Shi Yeh; Delay in Diagnosis of Spinal Muscular Atrophy: A Systematic Literature Review. *Pediatric Neurology* **2015**, *53*, 293-300, 10.1016/j.pediatrneurol.2015.06.002.

23. Tomokazu Kimizu; Shinobu Ida; Kentaro Okamoto; Hiroyuki Awano; Emma Tabe Eko Niba; Yogik Onky Silvana Wijaya; Shin Okazaki; Hideki Shimomura; Tomoko Lee; Koji Tominaga; et al. Spinal Muscular Atrophy: Diagnosis, Incidence, an d Newborn Screening in Japan. *International Journal of Neonatal Screening* **2021**, *7*, 45, 10.3390/ijns7030045.

24. Yanyan Cao; Miaomiao Cheng; Yujin Qu; Jinli Bai; Xiaoyin Peng; Xiushan Ge; Yuwei Jin; Hong Wang; Fang Song; Fact ors associated with delayed diagnosis of spinal muscular atrophy in China and changes in diagnostic delay. *Neuromus cular Disorders* **2021**, *31*, 519-527, 10.1016/j.nmd.2021.03.002.

25. Maria Carmela Pera; Giorgia Coratti; Beatrice Berti; Adele D’Amico; Maria Sframeli; Emilio Albamonte; Roberto De San ctis; Sonia Messina; Michela Catteruccia; Giorgia Brigati; et al. Diagnostic journey in Spinal Muscular Atrophy: Is it still an odyssey?. *PLoS ONE* **2020**, *15*, e0230677, 10.1371/journal.pone.0230677.

26. Prerana Madhusudhana Murthy; Anupama Cheleri Neduvat; Cheemalamarri Veenadhar; Sudarson Sundarrajan; Srira m Padmanabhan; Human Saliva and Dried Saliva Spots as Source of DNA for PCR based HLA Typing using a Combin ation of Taq DNA Polymerase and AccuPrimeTaq Polymerase. *Walailak Journal of Science and Technology (WJST)* **20 19**, *17*, 113-127, 10.48048/wjst.2020.4430.

27. Yueshan Hu; Erik A. Ehli; Kelly Nelson; Krista Bohlen; Christophina Lynch; Patty Huizenga; Julie Kittlelsrud; Timothy J. Soundy; Gareth E. Davies; Genotyping Performance between Saliva and Blood-Derived Genomic DNAs on the DMET Array: A Comparison. *PLoS ONE* **2012**, *7*, e33968, 10.1371/journal.pone.0033968.

28. Harini V. Gudiseva; Mark Hansen; Linda Gutierrez; David W. Collins; Jie He; Lana D. Verkuil; Ian D. Danford; Anna Sag aser; Anita S. Bowman; Rebecca Salowe; et al. Saliva DNA quality and genotyping efficiency in a predominantly elderly population. *BMC Medical Genomics* **2016**, *9*, 1-8, 10.1186/s12920-016-0172-y.

29. Catherine Drogou; Fabien Sauvet; Mégane Erblang; Liselot Detemmerman; Céline Derbois; Marie Claire Erkel; Anne B oland; Jean François Deleuze; Danielle Gomez-Merino; Mounir Chennaoui; et al. Genotyping on blood and buccal cells using loop-mediated isothermal amplification in healthy humans. *Biotechnology Reports* **2020**, *26*, e00468, 10.1016/j.bt re.2020.e00468.

30. Michael R. Goode; Soo Yeon Cheong; Ning Li; William C. Ray; Christopher W. Bartlett; Collection and Extraction of Sali va DNA for Next Generation Sequencing. *Journal of Visualized Experiments* **2014**, *90*, e51697-e51697, 10.3791/5169 7.

31. Roderick A. Yao; Oyediran Akinrinade; Marie Chaix; Seema Mital; Quality of whole genome sequencing from blood ver sus saliva derived DNA in cardiac patients. *BMC Medical Genomics* **2020**, *13*, 1-10, 10.1186/s12920-020-0664-7.

32. Omar Ibrahim; Heidi G. Sutherland; Larisa M. Haupt; Lyn R. Griffiths; Saliva as a comparable-quality source of DNA for Whole Exome Sequencing on Ion platforms. *Genomics* **2019**, *112*, 1437-1443, 10.1016/j.ygeno.2019.08.014.

33. Madoka Kisoi; Miwako Moritsugu; Miho Imai; Kae Fukumoto; Yui Sakaguchi; Shigenori Murata; Sayuri Kawai; Atsushi I chikawa; Kenji Kinoshita; Rapid and Cost-Effective Genotyping Protocol for Angiotensin-Converting Enzyme Insertion/ Deletion (Ins/Del) Polymorphism from Saliva.. *Biological and Pharmaceutical Bulletin* **2019**, *42*, 1345-1349, 10.1248/bp b.b19-00110.
34. Raffaele Brogna; Harriëtte Oldenhof; Harald Sieme; Willem F. Wolkers; Spectral fingerprinting to evaluate effects of stor age conditions on biomolecular structure of filter-dried saliva samples and recovered DNA. *Scientific Reports* **2020**, *10*, 1-12, 10.1038/s41598-020-78306-1.
35. Miho Imai; Madoka Kisoi; Yui Sakaguchi; Miwako Yamamura; Sayuri Kawai; Shigenori Murata; Atsushi Ichikawa; Kenji Kinoshita; Development of Novel Genotyping Protocol and Its Application for Genotyping of Alcohol Metabolism-related Genes. *YAKUGAKU ZASSHI* **2019**, *139*, 1111-1119, 10.1248/yakushi.19-00016.
36. Mariko Hayashida; Tomoko Ota; Minori Ishii; Kyoko Iwao-Koizumi; Shigenori Murata; Kenji Kinoshita; Direct Detection o f Single Nucleotide Polymorphism (SNP) by the TaqMan PCR Assay Using Dried Saliva on Water-soluble Paper and H air-roots, without DNA Extraction. *Analytical Sciences* **2014**, *30*, 427-429, 10.2116/analsci.30.427.
37. Richard A. Gibbs; Phi-Nga Nguyen; C. Thomas Caskey; Detection of single DNA base differences by competitive oligo nucleotide priming. *Nucleic Acids Research* **1989**, *17*, 2437-2448, 10.1093/nar/17.7.2437.
38. Yogik Onky Silvana Wijaya; Jamiyan Purevsuren; Nur Imma Fatimah Harahap; Emma Tabe Eko Niba; Yoshihiro Bouik e; Dian Kesumapramudya Nurputra; Mawaddah Ar Rochmah; Cempaka Thursina; Sunartini Hapsara; Seiji Yamaguchi; et al. Assessment of Spinal Muscular Atrophy Carrier Status by Determining SMN1 Copy Number Using Dried Blood S pots. *International Journal of Neonatal Screening* **2020**, *6*, 1-12, 10.3390/ijns6020043.
39. A S Ochert; A W Boulter; W Birnbaum; Newell Johnson; C G Teo; Inhibitory effect of salivary fluids on PCR: potency an d removal.. *Genome Research* **1994**, *3*, 365-368, 10.1101/gr.3.6.365.
