

DATA NOTE

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Whole-genome sequence of *Macaca fascicularis*: liver tissue

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Abstract

Objectives Thrombocytopenia is a condition that causes a low amount of blood platelets. Platelets are blood cells that play an essential role in blood coagulation. Therefore, thrombocytopenia can put the patient at risk for mild to severe bleeding. Thrombocytopenia is caused by a decrease in platelet production in the bone marrow or by a drug or immune system problem when production is normal. In particular, in some ASO-induced thrombocytopenia, the mechanism is not clear. Therefore, whole genome sequencing (WGS) was performed to discover genetic differences that affect thrombocytopenia and individual susceptibility to drugs between normal and reduced platelet monkeys despite administering the same ASO.

Data description Three antisense oligonucleotide (ASO) substances were injected into the subcutaneous tissue of monkeys for 12 weeks in two experiments. The monkeys were classified into three groups: monkeys with thrombocytopenia, monkeys without thrombocytopenia, and control monkeys not treated with ASO substances. Whole genome sequencing data was generated using liver tissues of monkeys. These data will be useful for identifying genetic differences that affect thrombocytopenia and drug sensitivity.

Keywords *Macaca fascicularis*, *Cynomolgus* monkeys, Whole genome sequencing

Objective

Platelets, also known as thrombocytes, are one type of blood cell involved in blood clotting and hemostasis. They are formed in the bone marrow, a spongelike tissue in the bone. Usually, platelet count is 150,000–400,000 per microliter of blood. Thrombocytopenia is diagnosed when platelet count is reduced to less than 150,000 per microliter [1]. The low amount of platelets in the blood

increases the risk of bleeding, leading to life-threatening bleeding such as cerebral hemorrhage. Thrombocytopenia is caused by several mechanisms, including decreased platelet production, increased splenic sequestration, and increased platelet destruction [2]. Platelet production is reduced in the bone marrow as a representative cause among them. When production is normal, thrombocytopenia is caused by drug or immune system problems.

Antisense oligonucleotides (ASO) are single-stranded DNA-based disease-modifying drugs that bind specifically to complementary mRNA and regulate protein expression through several mechanisms such as mRNA degradation, splicing modulation, and steric block of the ribosome [3–5]. It is already used to treat various diseases [5–12]. However, recent toxicity studies have shown that ASO substances cause thrombocytopenia in proportion to drug concentration, and some drugs also cause severe platelet reduction at low concentrations. However, the mechanism for this problem is not yet clear.

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This data investigates the genetic differences in individual sensitivity to drugs and platelet reduction in the monkey liver. Specifically, these data compare genomic differences among monkeys with varying degrees of reduction in platelet counts despite the same ASO administration.

The data was initially produced to elucidate the genetic differences for drug toxicity, but they were not published due to the small number of samples and the limitations of observing significant genetic differences responsible for differential drug toxicity. However, since it contains a lot of information on the animal model used in this experiment, it will be helpful to researchers using the animal model.

Data description

We used Cynomolgus monkeys (*Macaca fascicularis*) with a high level of genetic homology to humans for our experiments. We used three ASO substances to confirm the effect of ASO on thrombocytopenia. Cynomolgus monkeys were purchased from Nafovanny (Tam Phuoc Hamlet, Bien Hoa City, Dong Nai Province, Vietnam) and animal study carried out at Korea Institute of Toxicology (KIT, Daejeon, Korea), an accredited animal facility, complying with the AAALAC International Animal Care Policies. The Animal Care and Use Committee of the KIT reviewed and approved all the study protocols. Imported monkeys were SRV, SIV, STLV and B-virus free and quarantined at the nonhuman primate facility at KIT for at least 30 days. Before the ASO treatment, animals were permitted an acclimation period of 1 day to the laboratory environment. The ASO substance was administered to the subcutaneous tissue of monkeys for 12 weeks. The dose volume for each animal was calculated using the most recently measured body weight (i.e. weekly body weight measurements) at a volume of 0.4 mL/kg. It was administered once every two days in the first week and then once a week from the following weeks. We prepared five Cynomolgus monkeys and two ASO substances in the first experiment. One monkey was a control that was not treated with the drug, and the other four monkeys were treated with ASO #1 and #2 at

a dosage of 25 mg/kg and 30 mg/kg, respectively. Blood samples were collected from animals via cephalic vein for evaluation of hematology including platelets counts. Then, the number of platelets was measured and classified into Normal and LowPLT (TCP) groups. (Table 1, Additional file 1) [13]. We prepared 12 Cynomolgus monkeys in the second experiment and treated them with ASO #3 dose-dependently. Doses ranged from 0 to 40, and the number of platelets was measured and classified into normal and LowPLT(TCP) groups (Table 1, Additional file 2) [14]. The animals were euthanized by exsanguination while under deep anesthesia prior to necropsy. Liver tissues were collected for NGS analysis and the samples were flash frozen in liquid nitrogen at necropsy.

Total genomic DNA was extracted from monkey liver tissues using the DNeasy Blood and Tissue Kit (Qiagen). The sequencing library was prepared using the Illumina TruSeq Nano DNA library prep kit (Illumina). Sequencing was performed based on the Hiseq X Ten platform (Illumina) to generate 150 bp paired-end reads. The sequenced reads were mapped to the rheMac3 reference genome using the BWA-MEM algorithm (v0.7.12-r1039) [19]. The resulting SAM files were transformed into BAM files using samtools. Duplicate reads were eliminated using Picard MarkDuplicates [20].

Whole genome sequencing (WGS) data includes mapping rate, genome coverage, mapping quality, and duplicate reads, as shown in Additional file 3 [15]. In the first experiment (Table 1, data set_1st analysis) [16, 18], Each sample produced approximately 6.2 million to a maximum of 7.7 million reads, an average mapping rate of more than 98%, and a mapping quality score of more than 31. Duplicate reads were about 13%, and the coverage was about 31 on average. In the second experiment, each sample produced approximately 7.2 million to a maximum of 8.4 million reads, an average mapping rate of more than 98%, and a mapping quality score of more than 31 (Table 1, data set_2nd analysis) [17, 18]. Duplicate reads were about 14%, and the coverage was about 35 on average. The sequencing coverage required

Table 1 Overview of Additional files/data set

Label	Name of Additional file/data set	File Types (file extension)	Data repository and identifier (DOI or accession number)
Additional file 1	1st analysis PLT counts	Dataset (.xlsx)	FigShare (https://doi.org/10.6084/m9.figshare.19736242) [13]
Additional file 2	2nd analysis PLT counts	Dataset (.xlsx)	FigShare (https://doi.org/10.6084/m9.figshare.19738171) [14]
Additional file 3	Quality and quantity of the sequencing data	Dataset (.xlsx)	Figshare (https://doi.org/10.6084/m9.figshare.19738336) [15]
Data set	1st analysis and 2nd analysis	Fastq file (fastq.gz)	Korea Nucleotide Archive (Accession no.: KRA2200718) [16] Korea Nucleotide Archive (Accession no.: KRA2200719) [17] Sequence Read Archive (https://identifiers.org/ncbi/insdc.sra:SRP409432) [18]

to identify genomic variants may vary depending on the purpose. A coverage above 30X is sufficient to detect most variation, copy number variation (CNV), and structural variation in the genome [21].

Limitations

Since the number of samples for each condition is small, performing a genome-wide association study to identify genomic regions associated with drug toxicity is challenging.

Abbreviations

ASO	Anti-Sense Oligonucleotide
WGS	Whole Genome Sequencing
GATK	Genome Analysis Tool Kit

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12863-023-01114-9>.

Additional file 1. 1st analysis PLT counts.

Additional file 2. 2nd analysis PLT counts.

Additional file 3. Quality and quantity of the sequencing data.

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Authors' contributions

EHS, JHK, DHK and JHO generated and summarized the sequencing and toxicity data. EHS, JHO, and SYK wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The Additional files 1, 2 and 3 described in this Data Note can be freely and openly accessed on FigShare (<https://figshare.com/>) [13–15]. Data set was deposited in the Korea Nucleotide Archive (KoNA, <https://kobic.re.kr/kona>) with open accession ID KRA2200718 and KRA2200719 [16, 17] and the NCBI Sequence Read Archive (SRA, <https://www.ncbi.nlm.nih.gov/sra>) with open accession ID SRP409432 [18].

Declarations

Ethics approval and consent to participate

All the protocols of animal study were approved by the Association for Assessment and Accreditation of Laboratory Animal Care international and Institutional Animal Care and Use Committee, Korea Institute of Toxicology (Daejeon, Korea, IACUC No. 1407–0235).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Gauer RL, Braun MM. Thrombocytopenia. *Am Fam Physician*. 2012;85(6):612–22.
- Smock KJ, Perkins SL. Thrombocytopenia: an update. *Int J Lab Hematol*. 2014;36(3):269–78.
- Watts JK, Corey DR. Silencing disease genes in the laboratory and the clinic. *J Pathol*. 2012;226(2):365–79.
- Roberts TC, Langer R, Wood MJA. Advances in oligonucleotide drug delivery. *Nat Rev Drug Discov*. 2020;19(10):673–94.
- Scoles DR, Minikel EV, Pulst SM. Antisense oligonucleotides: a primer. *Neurol Genet*. 2019;5(2):e323.
- Lim KR, Maruyama R, Yokota T. Eteplirsen in the treatment of Duchenne muscular dystrophy. *Drug Des Devel Ther*. 2017;11:533–45.
- Benson MD, Waddington-Cruz M, Berk JL, Polydefkis M, Dyck PJ, Wang AK, Plante-Bordeneuve V, Barroso FA, Merlini G, Obici L, et al. Inotersen treatment for patients with hereditary transthyretin amyloidosis. *N Engl J Med*. 2018;379(1):22–31.
- Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, Chiriboga CA, Saito K, Servais L, Tizzano E, et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N Engl J Med*. 2017;377(18):1723–32.
- Miller T, Cudkowicz M, Shaw PJ, Andersen PM, Atassi N, Bucelli RC, Genge A, Glass J, Ladha S, Ludolph AL, et al. Phase 1–2 trial of antisense oligonucleotide Tofersen for SOD1 ALS. *N Engl J Med*. 2020;383(2):109–19.
- Clemens PR, Rao VK, Connolly AM, Harper AD, Mah JK, Smith EC, McDonald CM, Zaidman CM, Morgenroth LP, Osaki H, et al. Safety, tolerability, and efficacy of viltolarsen in boys with duchenne muscular dystrophy amenable to exon 53 skipping: a phase 2 randomized clinical trial. *JAMA Neurol*. 2020;77(8):982–91.
- De Velasco MA, Kura Y, Sakai K, Hatanaka Y, Davies BR, Campbell H, et al. Targeting castration-resistant prostate cancer with androgen receptor antisense oligonucleotide therapy. *JCI Insight*. 2019;4(17):e122688.
- Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, Wild EJ, Saft C, Barker RA, Blair NF, Craufurd D, Priller J, Rickards H, et al. Targeting huntingtin expression in patients with huntington's disease. *N Engl J Med*. 2019;380(24):2307–16.
- Seo, EH. 1st analysis PLT counts. figshare. Dataset. 2022. <https://doi.org/10.6084/m9.figshare.19736242>.
- Seo, EH. 2nd analysis of PLT counts. figshare. Dataset. 2022. <https://doi.org/10.6084/m9.figshare.19738171>.
- Seo, EH. Quality and quantity of the sequencing data. figshare. Dataset. 2022. <https://doi.org/10.6084/m9.figshare.19738336>.
- 1st analysis: Korea Nucleotide Archive. 2022. <https://kobic.re.kr/kona>.
- 2nd analysis: Korea Nucleotide Archive. 2022. <https://kobic.re.kr/kona>.
- NCBI Sequence Read Archive. Whole genome seq of Macaca fascicularis: liver tissue. 2022. <https://identifiers.org/ncbi/insdc.sra:SRP409432>.
- Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Preprint at arXiv. 2013. <https://arxiv.org/abs/1303.3997>.
- Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thibault J, et al. From FastQ data to high confidence variant calls: the Genome analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics*. 2013;43:11 10 11-11 10 33.
- Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008;456(7218):53–9.

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