11-2022

The Role of Dynamic DNA Methylation in Liver Transplant Rejection in Children.

Mylarappa Ningappa

Xiaojian Shao

Chethan Ashokkumar

Qingyong Xu

Adriana Zeevi

See next page for additional authors

Recommended Citation

Ningappa M, Shao X, Ashokkumar C, et al. The Role of Dynamic DNA Methylation in Liver Transplant Rejection in Children. Transplant Direct. 2022;8(11):e1394. Published 2022 Oct 14. doi:10.1097/TXD.0000000000001394

OPEN

The Role of Dynamic DNA Methylation in Liver Transplant Rejection in Children

Mylarappa Ningappa, PhD,[1] Xiaojian Shao, PhD,[2] Chethan Ashokkumar, PhD,[1] Qingyong Xu, PhD,[3] Adriana Zeevi, PhD,[4] Elin Grundberg, PhD,[5] Tomi Pastinen, MD, PhD,[5] and Rakesh Sindhi, MD[1]

Background. Transcriptional regulation of liver transplant (LT) rejection may reveal novel predictive and therapeutic targets. The purpose of this article is to test the role of differential DNA methylation in children with biopsy-proven acute cellular rejection after LT. **Methods.** Paired peripheral blood DNA samples were obtained before and after LT from 17 children, including 4 rejectors (Rs) and 13 nonrejectors (NRs), and assayed with MethylC capture sequencing approach covering 5 million CpGs in immune-cell–specific regulatory elements. Differentially methylated CpGs (DMCs) were identified using generalized linear regression models adjusting for sex and age and merged into differentially methylated regions (DMRs) comprising 3 or more DMCs. **Results.** Contrasting Rs versus NRs, we identified 2238 DMCs in post-LT and 2620 DMCs in pre-LT samples, which clustered in 216 and 282 DMRs, respectively. DMCs associated with R were enriched in enhancers and depleted in promoters. Among DMRs, the proportion of hypomethylated DMRs increased from 61/282 (22%) in pre-LT to 103/216 (48%, $P < 0.0001$) in post-LT samples. The highest-ranked biological processes enriched in post-LT DMCs were antigen processing and presentation via major histocompatibility complex (MHC) class I, MHC class I complex, and peptide binding ($P < 7.92 \times 10^{-17}$), respectively. Top-ranked DMRs mapped to genes that mediate B-cell receptor signaling (*ADAP1*) or regulate several immune cells *(ARRB2)* ($P < 3.75 \times 10^{-08}$). DMRs in MHC class I genes were enriched for single nucleotide polymorphisms (SNPs), which bind transcription factors, affect gene expression and splicing, or alter peptide-binding amino acid sequences. **Conclusions.** Dynamic methylation in distal regulatory regions reveals known transplant-relevant MHC-dependent rejection pathways and identifies novel loci for future mechanistic evaluations in pediatric transplant subcohorts.

(*Transplantation Direct* 2022;8: e1394; doi: 10.1097/TXD.0000000000001394).

INTRODUCTION

Epigenetic changes may be better suited to aid management of liver transplant (LT) rejection in children, who are at risk for cumulative toxicity of lifelong immunosuppression.[1] These changes regulate gene transcription by affecting the binding of transcription factors (TFs) and should therefore precede transcription and its clinical consequences. In turn, patterns of TF binding or the loci, which bind to these factors can reveal the particular mechanism of rejection in a given recipient, and the immunosuppressant best able to target that mechanism. This task is not fulfilled by currently available diagnostic tests. Cell

Received 21 July 2022.

Accepted 14 August 2022.

[1] Department of Transplant Surgery, University of Pittsburgh Medical Center (UPMC) Children's Hospital of Pittsburgh and the University of Pittsburgh, Pittsburgh, PA.

[2] Digital Technologies Research Centre, National Research Council Canada, Ottawa, ON, Canada.

[3] Department of Pathology, Histocompatibility Laboratory, University of Pittsburgh Medical Center (UPMC) and the University of Pittsburgh, Pittsburgh, PA.

[4] Department of Pathology, The Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA.

[5] Genomic Medicine Center, Children's Mercy Kansas City, Children's Mercy Research Institute, Kansas City, MO.

M.N. and X.S. contributed equally.

This study was supported by Hillman Foundation of Pittsburgh. E.G. holds the Roberta D. Harding and William F. Bradley, Jr. Endowed Chair in Genomic Research, and T.P. holds the Dee Lyons/Missouri Endowed Chair in Pediatric Genomic Medicine.

The authors declare no funding or conflicts of interest.

M.N. participated sample collection and sequencing studies with C.A. X.S. participated data analysis and writing of the article. Q.X., A.Z., E.G., and T.P. participated in data interpretation and writing of the article. R.S. participated in research design, obtained funding, and wrote the article.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

Correspondence: Rakesh Sindhi, MD, FACS, Pediatric Transplant Surgery, Pediatric Transplant Research, University of Pittsburgh, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA 15201. (rakesh.sindhi@chp.edu); or Tomi Pastinen, MD, PhD, Genomic Medicine Center, Children's Mercy Research Institute, Children's Mercy Kansas City, Kansas City, MO. (tpastinen@cmh.edu).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001394

function assays predict rejection-risk and not the appropriate immunosuppressant.[2] Molecular diagnostics detect rejection and its progression using predominantly upregulated genes, which may not be mechanistic.[3,4]

As a clinically usable epigenetic mechanism, DNA methylation is attractive for several reasons.

Epigenetic regulation turns genes on to allow transcription or turns off genes to silence them. DNA methylation is a common epigenetic change that causes genes to compact around histones, thereby silencing gene expression. Hypomethylation unwinds gene that promote transcription. Epigenetic regulation can also be inferred from chromatin states but requires complex sample preparation procedures.[5]

As a test substrate, DNA can be easily acquired from several sources: blood, biopsy or body fluids. Commercially available arrays use small quantities of DNA to screen methylation status of nearly a million methylation (CpG) sites genome-wide.[6] Early DNA methylation studies have identified differentially methylated regions (DMRs) near genes encoding cytokines as well as other genes within interferon and mammalian target of rapamycin signaling pathways, as being relevant in renal transplant rejection.[7,8] No such work has been performed in pediatric LT recipients.

We and others have shown that trait-associated and dynamic epigenetic variants are enriched in distal regulatory enhancer regions and we further showed that these features are also seen downstream of the transcription start sites—both genomic regions being underrepresented on commercially available arrays.[5-11] To overcome this limitation, we implemented the methylC-capture sequencing (MCC-Seq) approach for customized DNA methylation profiling of millions of CpGs located in regulatory elements known to be active specifically in immune cells.[11,12] Specifically, this immune cell MCC-Seq panel covers (1) the majority of human gene promoters, blood-cell-lineage–specific enhancer regions and methylation footprint regions observed in peripheral blood,[12] (2) CpGs from Illumina Human Methylation 450 Bead Chips, and (3) published autoimmune-related single nucleotide polymorphisms (SNPs) and SNPs in their linkage disequilibrium regions with $r^2 > 0.8$. Here, we test whether MCC-Seq applied to pre- and post-LT blood samples obtained in the first 90 d after LT from 17 children with LT can reveal potential mechanisms of LT rejection for further investigation. Four children experienced early biopsy-proven acute cellular rejection (rejectors [Rs]) and 13 did not (nonrejector [NRs]).

MATERIALS AND METHODS

Human Subjects

Archived DNA extracted from blood samples from 17 children with LT was tested under University of Pittsburgh Institutional Review Board approved study #19030279. Mean ± SD age of subjects was 6.3 ± 8.2 y and male:female gender distribution was 6:11. Indications for LT included primary diagnoses such as biliary atresia in 6, maple syrup urine disease in 4, urea cycle in 5, Propionic acedimia in 1, and cystic fibrosis in 1. Four of the 17 children experienced acute cellular rejection within 90 d of transplant and were termed rejectors (Rs). Thirteen of the 17 children had no rejection. DNA samples were collected before and within the first 90 d after LT. For the 4 rejectors, post-LT samples were collected at 9.75 ± 10.8 d before the rejection event. All 4 patients experienced steroid-responsive rejection. None needed antilymphocyte treatment for steroid-resistant rejection. The number of HLA mismatches in Rs and NRs was not significant (**Table S1, SDC,** http://links.lww.com/TXD/A466).

MCC-Seq

One μg of DNA was used for whole genome bisulfite sequencing library preparation (KAPA Biosystems, Wilmington, MA), bisulphite conversion (Epitect Fast DNA Bisulfite Kit [Qiagen]), and enrichment (12-plex) using the custom probes[10,13] according to the Roche NimbleGen SeqCapEpi Enrichment System protocol (Wilmington, MA). We compared DNA methylation differences between Rs and NRs using a general linear regression model adjusted for age and sex. Correction of immune cell proportion was performed with constrained linear projection via the projectMix function of the RefFreeEWAS package (version 2.2), using a custom panel of 30 455 cell-type–specific hypomethylated and hypermethylated CpGs.[14] The blood reference epigenome profiles include megakaryocyte, neutrophil, monocyte, B cell, and T cell. Differentially methylated CpGs (DMCs) were further filtered if the DNA methylation profile is correlated with any of the estimated blood proportion at nominal $P < 0.05$. To assess potential regional clustering of significant DMCs, candidate regions surrounding the blood corrected DMCs were expanded for up to 200-bp distance both upstream and downstream. Within these candidate regions, all consecutive CpGs with methylation changes in the same direction and with nominal $P < 0.01$ were merged. Regions with at least 3 CpGs fulfilling these criteria were considered DMRs. Gene ontology function enrichment analysis of rejection-associated DMCs was performed using Genomic Regions Enrichment of Annotations Tool.[15]

RESULTS

CpGs that Characterize Rejectors are Enriched in Distal Regions and Hypomethylated After LT

Of the ~2.5 million autosomal CpGs tested in the association analysis, we identified 3357 DMCs including 1894 hypomethylated and 1463 hypermethylated CpGs in post-LT samples from R compared with NR. The genome-wide distribution of significant CpGs is shown in Figure 1A. Among the 3357 DMCs, 1117 were significantly correlated with blood cell proportions and were excluded. Among the remaining 2238 DMCs, 1108 were hypermethylated and 1130 were hypomethylated (Tables 1 and **Table S2, SDC,** http://links.lww.com/TXD/A466). To further explore how the DMCs mapped to regions, we identified DMRs containing clusters of 3 or more DMCs with the same directional change in methylation. Of 216 DMRs that clearly distinguished R and NR groups, 113 (52%) were hypermethylated and 103 (48%) were hypomethylated (Figure 1B, **Table S3, SDC,** http://links.lww.com/TXD/A466). Examples include intronic regions in *ADAP1* (50 DMCs, chr7:948678-949115, Figure 1C) and *LHX6* (34 DMCs) and the intergenic region near *ARRB2* (24 DMCs) (**Table S3, SDC**, http://links.lww.com/TXD/A466).[16-18] Enrichment analysis of genomic regions revealed that rejection-associated DMCs and DMRs were enriched in the distal regulatory regions and depleted in the promoter regions (**Table S3, SDC,** http://links.lww.com/TXD/A466). Next, we identified significant genome-wide DMCs in pre-LT samples from Rs compared with NRs. After filtering DMCs (n = 665), which correlated significantly with blood proportions, 2620 DMCs remained, of which 1413 were hypermethylated

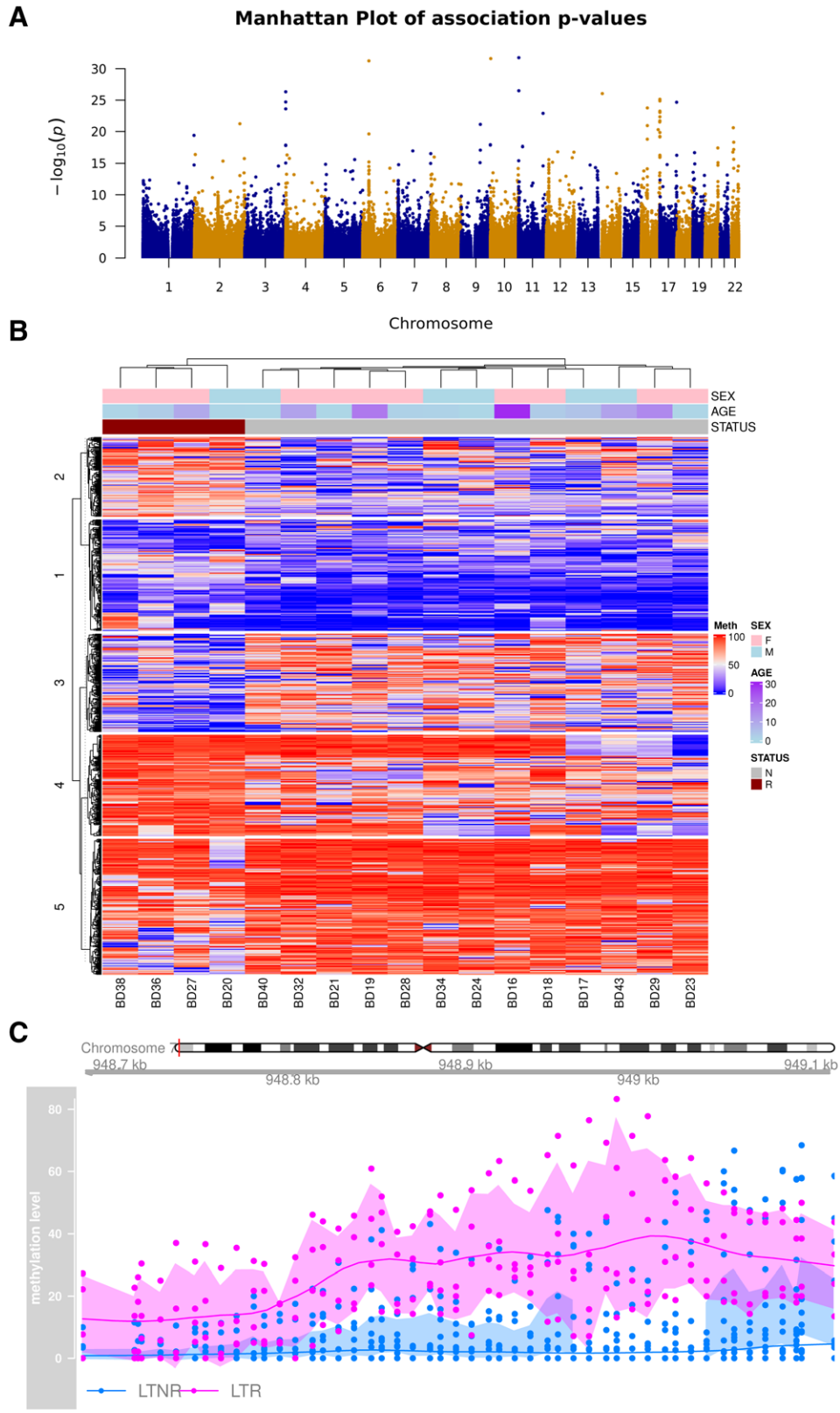

FIGURE 1. Identification of differentially methylated CpGs (DMCs) for LT rejection. (A) Manhattan plot of *P* values from association analysis to identify differentially methylated CpGs. (B) Heatmap of DMCs in methylation profiles for all individuals. Phenotype features including sex, age onset, and LT rejection status are illustrated in the bars on top of the heatmap. (C). DNA methylation pattern at the differentially methylated region (DMR) for *ADAP1* (chr7:948678-949115). This region shows consistent hypermethylation among R (pink) compared with NR (blue) over 50 DMCs. CpGs, methylations; LT, liver transplant; LTNR, liver transplant nonrejector; LTR, liver transplant rejection; NR, nonrejector.

TABLE 1.

Summary of differentially methylated CpGs and regions

	Post-LT	Pre-LT
No. CpGs	2 544 280	2 545 085
No. DMCs	2238	2620
No. Hypermethylated DMCs	1108	1413
No. Hypomethylated DMCs	1130	1207
No. DMRs	216	282
No. Hypermethylated DMRs	113	221
No. Hypomethylated DMRs	103	61

DMC, differentially methylated CpG; DMR, differentially methylated region; LT, liver transplant.
Counts of DMCs (**Tables S2** and **S4, SDC**, http://links.lww.com/TXD/A466) and DMRs (**Tables S3** and **S5, SDC**, http://links.lww.com/TXD/A466) in samples obtained from 4 rejectors compared with those from 13 nonrejectors within 90 d post LT) and pre LT.

and 1207 were hypomethylated (**Table S4, SDC,** http://links.lww.com/TXD/A466). Among these DMCs, we identified 282 DMRs consisting of 221 (78%) hypermethylated and 61 (22%) hypomethylated DMRs (**Table S5, SDC,** http://links.lww.com/TXD/A466). Thus, as proportion of all DMRs, hypomethylated DMRs increased from 61/282 (22%) in pre-LT to 103/216 (48%, $P < 0.0001$) in post-LT samples.

Functional Enrichment Analysis Reveals Mechanisms for Additional Investigation

The genes associated with DMCs located within the DMRs regions in posttransplant samples were enriched for the biological processes of antigen processing and presentation of exogenous peptide antigen via major histocompatibility complex (MHC) class I, ($P = 4.4\times10^{-21}$) and cellular component of "MHC protein complex" ($P = 1.7\times10^{-23}$) and molecular function of peptide antigen binding ($P = 7.9\times10^{-17}$) (Figure 2, **Table S6, SDC,** http://links.lww.com/TXD/A466). DMCs in pretransplant samples were enriched for the top-ranked biological processes of "regulation of thyroid-stimulating hormone secretion" ($P < 3.1\times10^{-58}$) and several developmental and morphogenesis processes. "Antigen processing and presentation of exogenous peptide antigen via MHC class" ranked 17th among

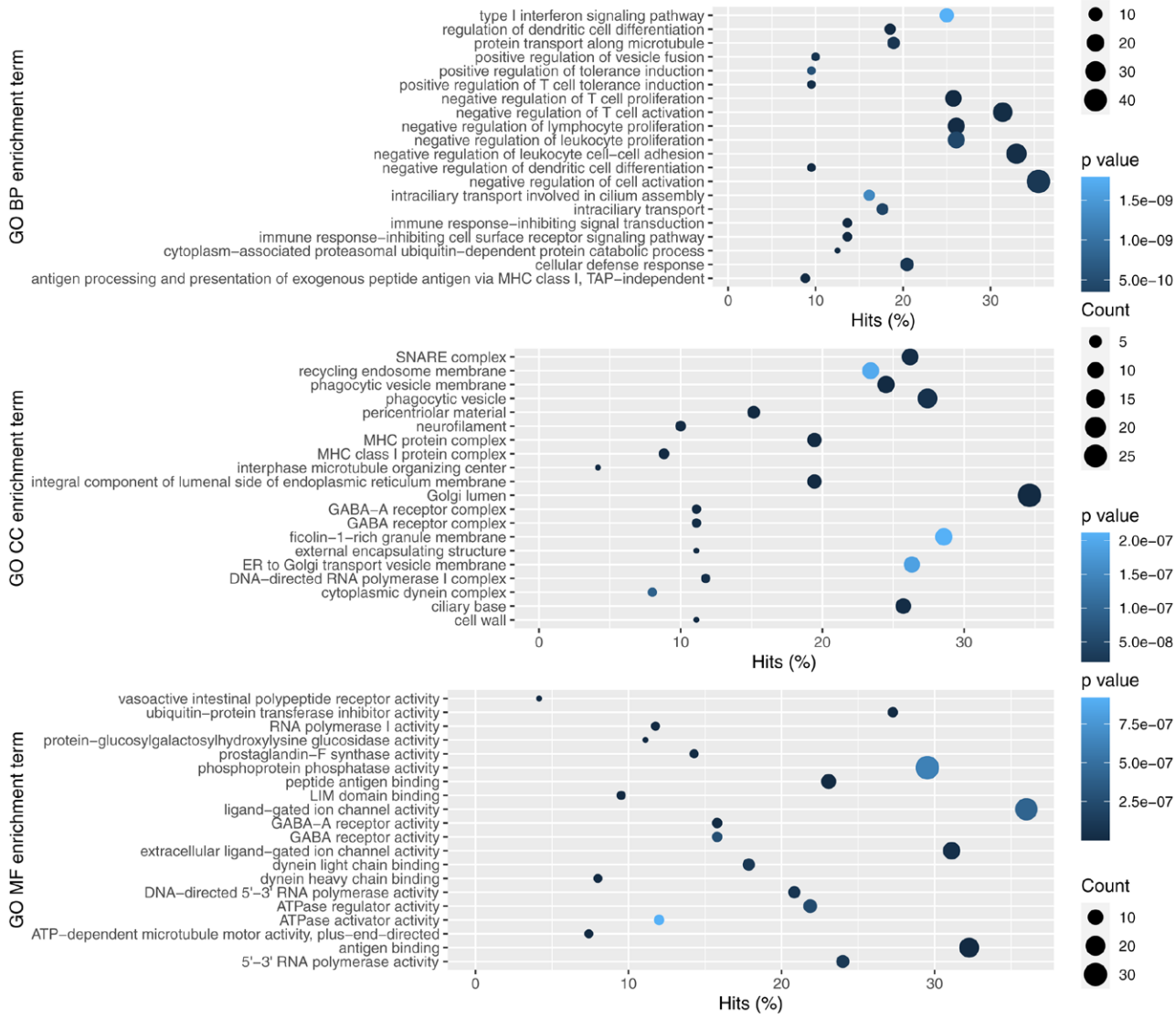

FIGURE 2. Enrichment in posttransplant samples. Top-ranked 20 gene ontology (GO) biological processes (BP), cellular component (CC), and molecular function (MF) enriched in R compared with NR in DMCs located within regions of DMRs in posttransplant samples. DMC, differentially methylated CpG; DMR, differentially methylated region; NR, nonrejector; R, rejector.

these processes ($P < 9.5 \times 10^{-34}$) (Figure 3, **Table S7, SDC,** http://links.lww.com/TXD/A466). Enriched top-ranked cellular components included "MHC class I protein complex" ($P < 9.1 \times 10^{-36}$), and top-ranked molecular functions included "thyroid-stimulating hormone receptor activity" ($P < 2.6 \times 10^{-55}$)" and "peptide antigen binding" ($P < 1.8 \times 10^{-30}$) (Figure 3, **Table S7, SDC,** http://links.lww.com/TXD/A466). The top-ranked enriched pathways among pretransplant DMC were consistent with the developmental nature of liver disease affecting children who present for transplantation. Liver development is regulated by thyroid hormones, manifested in the top-ranked pathways of regulation of thyroid-stimulating hormone secretion P = 4.06E-54 (**Table S7, SDC,** http://links.lww.com/TXD/A466).[19,20] Liver components like hepatic stellate cells and sinusoidal epithelium originate from the mesoderm, as does the metanephric collecting duct system, represented by the pathway "regulation of metanephric nephron tubule epithelial cell differentiation, $P = 3.7 \times 10^{-36}$" (**Table S7, SDC,** http://links.lww.com/TXD/A466).[21] Enrichment of DMRs in MHC class I molecules among rejectors was demonstrable in coding sequences of *HLA-C* and intronic reqions of *HLA-F* in pretransplant samples and the *HLA-B* promoter sequence in posttransplant samples.

Public databases reveal that all 3 HLA DMRs (*HLA-C, HLA-F,* and *HLA-B*) contain known single nucleotide polymorphisms, which are known to bind to TFs, alter expression of the corresponding gene, or affect peptide antigen binding (SNPs) (**Table S8A–C, SDC,** http://links.lww.com/TXD/A466, **Figure S1, SDC,** http://links.lww.com/TXD/A465). SNPs associated with altered gene expression or expressed quantitative trait loci are shown in **Figure S1, SDC,** http://links.lww.com/TXD/A465. However, the differential methylation between R versus NR was not associated with the degree of mismatches between the donor and recipient in traditional pretransplant HLA typing of HLA-B (mean ± SD 1.5 ± 0.58 versus 1.69 ± 0.48, P = 0.577, (not significant) and HLA-C alleles (mean ± SD 1 ± 0.82 versus 1.38 ± 0.51, P = 0.42, not significant).

DISCUSSION

Our study shows that rejection after LT in children is associated with differential DNA methylation affecting distal

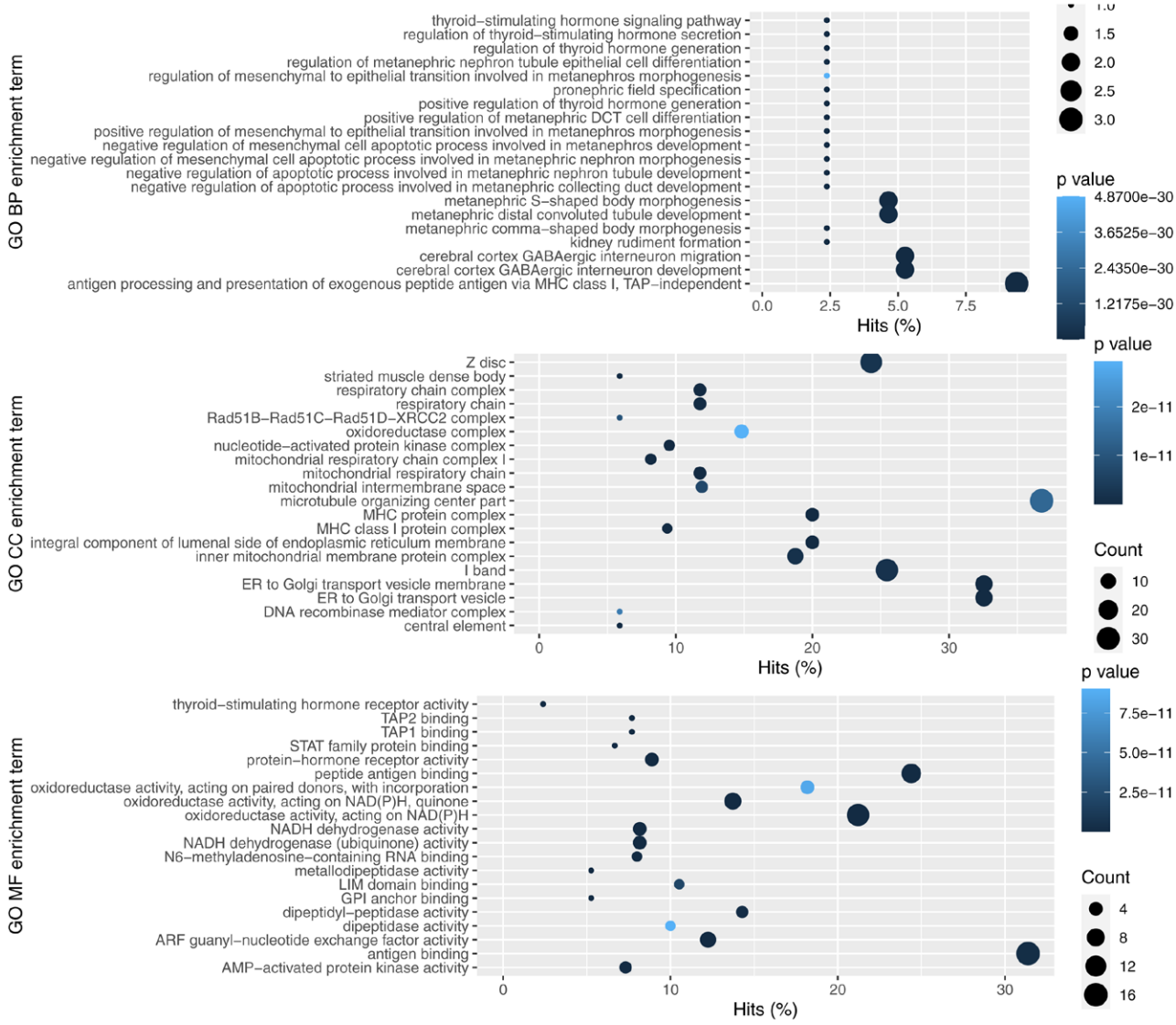

FIGURE 3. Enrichment in pretransplant samples. Top-ranked 20 gene ontology (GO) bological processes (BP), cellular component (CC), and molecular function (MF) enriched in R compared with NR in DMCs located within regions of DMRs in pretransplant samples. DMC, differentially methylated CpG; DMR, differentially methylated region; NR, nonrejector; R, rejector.

regions of genes, and with 3-fold increase in hypomethylated: hypermethylated regions (103:113) compared with pretransplant status (61:221; P = 0.0001) (Figure 1B and Table 1). This is to be expected because dynamic changes in methylation that are induced by disease predominantly affect distal regions such as enhancers, for which the MCC-seq platform is especially suited. Differentially methylated loci also reveal several new directions for future study. First, DMRs containing the highest number of DMCs are present in several loci that could regulate the rejection alloresponse (**Table S3, SDC**, http://links.lww.com/TXD/A466). These loci include *ADAP1* (Figure 1C), which participates in B cell receptor signaling[17]; *ARRB2*, which negatively regulates inflammatory responses of many immune cells including T cell and B cell[18]; and *LHX6* which regulates the development of many cells including lymphoid cells.[22] A larger association study and functional experiments are needed to confirm this possibility.

Second, enrichment analyses suggest that posttransplant rejection may recruit MHC I molecules, which classically present endogenous antigen, to present exogenous (transplant) antigens.[23] Exogenous antigens are usually presented by MHC class II molecules. This possibility is based on the emergence of transporter associated with antigen processing (TAP)–independent antigen processing and presentation of peptide antigen via MHC class I, as the top-ranked biological process, and MHC class I protein complex and peptide antigen binding as the top-ranked cellular component and molecular function, respectively, in posttransplant samples from rejectors compared with NRs (Figure 2, **Table S6, SDC**, http://links.lww.com/TXD/A466). The TAP proteins bind to the complex between MHC class I molecules and endogenous peptide antigens, aiding transport of this complex to the cell surface.[16] Presentation of exogenous antigen via MHC class I does not require TAP. Consistent with a shift to TAP-independent antigen presentation after transplantation, pretransplant DMCs are enriched for molecular functions of TAP1- and TAP2-binding and peptide antigen binding, and the cellular component, MHC class I protein complex. Pre-LT samples were also enriched for several biological processes related to morphogenesis, for example, pronephric field specification and nephron morphogenesis, reflecting the contribution of underlying developmental diseases that require liver transplantation, to the pretransplant blood methylome (Figure 3, **Table S7, SDC**, http://links.lww.com/TXD/A466).

Third, these enrichment results suggests that dynamic methylation could add to the clinical utility of the MHC locus by predicting transplant rejection. Currently, donor–recipient mismatches at polymorphic HLA alleles are used to assess histocomatibility. These mismatches lead to donor-specific anti-HLA antibodies (DSA), which are increasingly implicated in late graft loss, and DSA specificity.[23-28] Highly polymorphic HLA genes, for which corresponding proteins show high expression on the cell surface induce DSA more frequently (*HLA-DR*, *HLA-DQ*, and *HLA-B*).[29-31] Polymorphisms in *HLA-B*, which is enriched for DMRs in posttransplant samples from rejectors are also associated with TAP-independent MHC class I antigen processing and presentation of exogenous antigens and have been used to explain CD8-mediated cytotoxicity in a variety of autoimmune diseases.[32-34] TAP-independent class I antigen presentation after LT may also explain why alloresponsive CD8-memory cells are also being used clinically to predict acute LT rejection in children.[2] Others HLA genes are less polymorphic and have low surface expression because of predominantly intracellular location, which is influenced in part by binding to TAP. These genes are either associated with a low incidence of DSA (*HLA-C*), or have no known function *(HLA-F)*. *HLA-C* is largely intracellular where it remains bound to TAP.[29] *HLA-F* expression is restricted to the B cell lineage in the resting state, also partly influenced by binding to TAP.[30] DMRs in *HLA-C* and *HLA-F* are enriched in pretransplant samples from rejectors in our study.

Finally, public databases reveal how sequence variants in DMR of the HLA genes may affect the rejection alloresponse via dynamic methylation, highlighting a complexity that has yet to be understood fully. The expression of MHC molecules is regulated by binding of TFs to regulatory elements.[35-37] This binding is influenced by polymorphisms at TF binding sites. An example is the CCCTC binding TF, CTCF, which regulates the expression of several MHC genes.[38-39] We have previously shown the effect of altered methylation on CTCF-mediated expression of *HLA-DOA*, a B cell–specific MHC gene, and the relationship of SNPs in these regulatory loci to B cell presentation of donor antigen during LT rejection in children.[40,41] Consistent with these reports, each DMR in the *HLA-B, -C,* and *-F* genes contains known SNPs, which either bind TFs, bring about amino acid changes in the peptide-biding regions of corresponding MHC molecules, or may explain cell–cell interactions such as those between HLA-F and natural killer cells, which underlie immunity and tolerance (**Table S8, SDC**, http://links.lww.com/TXD/A466). For example, the SNPs rs2076177 and rs1736924 in the DMR of *HLA-F* in rejectors are, respectively, associated with enhanced and decreased expression and splicing in public databases (**Figure S1, SDC**, http://links.lww.com/TXD/A465). Thus, the study of dynamic DNA methylation can reveal potential causal links between genetic variation and transcriptional regulation, and facilitate preventive intervention by predicting rejection.

In conclusion, and consistent with several previous studies in nontransplant settings, our study localizes dynamic DNA methylation associated with LT rejection in children, to mostly nonpromoter distal regulatory regions. The emergence of the well-known transplant-relevant MHC-dependent antigen presentation as an enriched pathway, and of associated MHC class I-dependent mechanisms among rejectors in this exploratory cohort adds to the validity of DNA methylation as an investigative tool. Given that this enrichment is associated with novel but plausible differentially methylated regulatory loci, DNA methylation analysis can identify novel mechanisms and hypotheses for further investigation in the rare pediatric LT subcohort of transplant recipients. Limitations to be addressed in follow-up studies include a larger sample size, and evaluation of methylation status of DNA derived from intragraft immune cells in addition to DNA derived from unfractionated peripheral blood leukocytes.

REFERENCES

1. Soltys KA, Mazariegos GV, Squires RH, et al; SPLIT Research Group. Late graft loss or death in pediatric liver transplantation: an analysis of the SPLIT database. *Am J Transplant*. 2007;7:2165–2171.
2. Ashokkumar C, Soltys K, Mazariegos G, et al. Predicting cellular rejection with a cell-based assay: preclinical evaluation in children. *Transplantation*. 2017;101:131–140.
3. Deng MC, Eisen HJ, Mehra MR, et al; CARGO Investigators. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. *Am J Transplant*. 2006;6:150–160.

4. Ningappa M, Rahman SA, Higgs BW, et al. A network-based approach to identify expression modules underlying rejection in pediatric liver transplantation. *Cell Rep Med*. 2022;3:100605.
5. Corradin O, Scacheri PC. Enhancer variants: evaluating functions in common disease. *Genome Med*. 2014;6:85.
6. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489:57–74.
7. Soyoz M, Pehlivan M, Tatar E, et al. Consideration of IL-2, IFN-γ and IL-4 expression and methylation levels in CD4+ T cells as a predictor of rejection in kidney transplant. *Transpl Immunol*. 2021;68:101414.
8. Zhu C, Xiang W, Li B, et al. DNA methylation modulates allograft survival and acute rejection after renal transplantation by regulating the mTOR pathway. *Am J Transplant*. 2021;21:567–581.
9. Allum F, Hedman ÅK, Shao X, et al. Dissecting features of epigenetic variants underlying cardiometabolic risk using full-resolution epigenome profiling in regulatory elements. *Nat Commun*. 2019;10:1209.
10. Cheung WA, Shao X, Morin A, et al. Correction to: functional variation in allelic methylomes underscores a strong genetic contribution and reveals novel epigenetic alterations in the human epigenome. *Genome Biol*. 2019;20:89.
11. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biol*. 2016;17:208.
12. Burger L, Gaidatzis D, Schübeler D, et al. Identification of active regulatory regions from DNA methylation data. *Nucleic Acids Res*. 2013;41:e155.
13. Shao X, Hudson M, Colmegna I, et al. Rheumatoid arthritis-relevant DNA methylation changes identified in ACPA-positive asymptomatic individuals using methylome capture sequencing. *Clin Epigenetics*. 2019;11:110.
14. Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 2012;13:86.
15. McLean CY, Bristor D, Hiller M, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nat Biotechnol*. 2010;28:495–501.
16. Pamer E, Cresswell P. Mechanisms of MHC class I-restricted antigen processing. *Annu Rev Immunol*. 1998;16:323–358.
17. Montesinos-Rongen M, Purschke FG, Brunn A, et al. Primary central nervous system (CNS) lymphoma B cell receptors recognize CNS proteins. *J Immunol*. 2015;195:1312–1319.
18. Zhou WJ, Wang DD, Tao J, et al. Deficiency of β-arrestin2 exacerbates inflammatory arthritis by facilitating plasma cell formation. *Acta Pharmacol Sin*. 2021;42:755–766.
19. Slotkin TA, Seidler FJ, Kavlock RJ, et al. Thyroid hormone differentially regulates cellular development in neonatal rat heart and kidney. *Teratology*. 1992;45:303–312.
20. Chi HC, Chen CY, Tsai MM, et al. Molecular functions of thyroid hormones and their clinical significance in liver-related diseases. *Biomed Res Int*. 2013;2013:601361.
21. Yang L, Li LC, Lamaoqiezhong, et al. The contributions of mesoderm-derived cells in liver development. *Semin Cell Dev Biol*. 2019;92:63–76.
22. Liu WB, Jiang X, Han F, et al. LHX6 acts as a novel potential tumour suppressor with epigenetic inactivation in lung cancer. *Cell Death Dis*. 2013;4:e882.
23. Miyagawa-Hayashino A, Yoshizawa A, Uchida Y, et al. Progressive graft fibrosis and donor-specific human leukocyte antigen antibodies in pediatric late liver allografts. *Liver Transpl*. 2012;18:1333–1342.
24. Feng S, Bucuvalas JC, Demetris AJ, et al. Evidence of chronic allograft injury in liver biopsies from long-term pediatric recipients of liver transplants. *Gastroenterology*. 2018;155:1838–1851.e7.
25. Wozniak LJ, Hickey MJ, Venick RS, et al. Donor-specific HLA antibodies are associated with late allograft dysfunction after pediatric liver transplantation. *Transplantation*. 2015;99:1416–1422.
26. Grabhorn E, Binder TM, Obrecht D, et al. Long-term clinical relevance of de novo donor-specific antibodies after pediatric liver transplantation. *Transplantation*. 2015;99:1876–1881.
27. Dao M, Habès D, Taupin JL, et al. Morphological characterization of chronic antibody-mediated rejection in ABO-identical or ABO-compatible pediatric liver graft recipients. *Liver Transpl*. 2018;24:897–907.
28. Kaneku H, O'Leary JG, Banuelos N, et al. De novo donor-specific HLA antibodies decrease patient and graft survival in liver transplant recipients. *Am J Transplant*. 2013;13:1541–1548.
29. Neisig A, Melief CJ, Neefjes J. Reduced cell surface expression of HLA-C molecules correlates with restricted peptide binding and stable TAP interaction. *J Immunol*. 1998;160:171–179.
30. Wainwright SD, Biro PA, Holmes CH. HLA-F is a predominantly empty, intracellular, TAP-associated MHC class Ib protein with a restricted expression pattern. *J Immunol*. 2000;164:319–328.
31. Zernich D, Purcell AW, Macdonald WA, et al. Natural HLA class I polymorphism controls the pathway of antigen presentation and susceptibility to viral evasion. *J Exp Med*. 2004;200:13–24.
32. Casp CB, She JX, McCormack WT. Genes of the LMP/TAP cluster are associated with the human autoimmune disease vitiligo. *Genes Immun*. 2003;4:492–499.
33. Kamei H, Masuda S, Nakamura T, et al. Association of transporter associated with antigen processing (TAP) gene polymorphisms in donors with acute cellular rejection in living donor liver transplantation. *J Gastrointestin Liver Dis*. 2013;22:167–171.
34. Kobayashi T, Yokoyama I, Hayashi S, et al. TAP1, TAP2, LMP2, DMA, and DMB genetic polymorphisms in renal transplantation. *Transplant Proc*. 1998;30:29–30.
35. Denzin LK, Sant'Angelo DB, Hammond C, et al. Negative regulation by HLA-DO of MHC class II-restricted antigen processing. *Science*. 1997;278:106–109.
36. Majumder P, Boss JM. CTCF controls expression and chromatin architecture of the human major histocompatibility complex class II locus. *Mol Cell Biol*. 2010;30:4211–4223.
37. Majumder P, Boss JM. DNA methylation dysregulates and silences the HLA-DQ locus by altering chromatin architecture. *Genes Immun*. 2011;12:291–299.
38. Majumder P, Gomez JA, Chadwick BP, et al. The insulator factor CTCF controls MHC class II gene expression and is required for the formation of long-distance chromatin interactions. *J Exp Med*. 2008;205:785–798.
39. Majumder P, Scharer CD, Choi NM, et al. B cell differentiation is associated with reprogramming the CCCTC binding factor-dependent chromatin architecture of the murine MHC class II locus. *J Immunol*. 2014;192:3925–3935.
40. Sindhi R, Higgs BW, Weeks DE, et al. Genetic variants in major histocompatibility complex-linked genes associate with pediatric liver transplant rejection. *Gastroenterology*. 2008;135:830–9, 839.e1.
41. Ningappa M, Ashokkumar C, Higgs BW, et al. Enhanced B cell alloantigen presentation and its epigenetic dysregulation in liver transplant rejection. *Am J Transplant*. 2016;16:497–508.