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Donor HLA Class 1 Evolutionary Divergence Is a Major Predictor of Liver Allograft Rejection

A Retrospective Cohort Study

Cyrille Féray, MD, PhD; Jean-Luc Taupin, PharmD, PhD; Mylène Sebagh, MD, PhD; Vincent Allain, MD, PhD; Zeynep Demir, MD; Marc-Antoine Allard, MD, PhD; Christophe Desterke, PhD; Audrey Coilly, MD, PhD; Faouzi Saliba, MD; Eric Vibert, MD, PhD; Daniel Azoulay, MD, PhD; Catherine Guettier, MD, PhD; Arthur Chatton, MSc; Dominique Debray, MD, PhD; Sophie Caillat-Zucman, MD, PhD*; and Didier Samuel, MD, PhD*

Background: The HLA evolutionary divergence (HED), a continuous metric quantifying the peptidic differences between 2 homologous HLA alleles, reflects the breadth of the immunopeptidome presented to T lymphocytes.

Objective: To assess the potential effect of donor or recipient HED on liver transplant rejection.

Design: Retrospective cohort study.

Setting: Liver transplant units.

Patients: 1154 adults and 113 children who had a liver transplant between 2004 and 2018.

Measurements: Liver biopsies were done 1, 2, 5, and 10 years after the transplant and in case of liver dysfunction. Donor-specific anti-HLA antibodies (DSAs) were measured in children at the time of biopsy. The HED was calculated using the physicochemical Grantham distance for class I (*HLA-A* or *HLA-B*) and class II (*HLA-DRB1* or *HLA-DQB1*) alleles. The influence of HED on the incidence of liver lesions was analyzed through the inverse probability weighting approach based on covariate balancing, generalized propensity scores.

Results: In adults, class I HED of the donor was associated with acute rejection (hazard ratio [HR], 1.09 [95% CI, 1.03 to 1.16]), chronic rejection (HR, 1.20 [CI, 1.10 to 1.31]), and ductopenia of 50% or more (HR, 1.33 [CI, 1.09 to 1.62]) but not with other histologic lesions. In children, class I HED of the donor was also associated with acute rejection (HR, 1.16 [CI, 1.03 to 1.30]) independent of the presence of DSAs. There was no effect of either donor class II HED or recipient class I or class II HED on the incidence of liver lesions in adults and children.

Limitation: The DSAs were measured only in children.

Conclusion: Class I HED of the donor predicts acute or chronic rejection of liver transplant. This novel and accessible prognostic marker could orientate donor selection and guide immunosuppression.

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* Drs. Caillat-Zucman and Samuel contributed equally to this work.

Liver transplant remains the only life-saving treatment for many patients with end-stage liver disease. However, in the absence of immunosuppression, graft rejection is almost constant because of the recipient's T- and B-cell responses to the donor's HLA antigens (1, 2). A person's HLA genotype consists of a pair of alleles at each class I and class II locus where polymorphism is concentrated in the exons that encode the peptide-binding groove of the HLA molecule. The divergent allele advantage hypothesis predicts that HLA genotypes with more divergent heterozygous alleles (that is, a larger number of amino acid differences in peptide-binding domains) enable the presentation of a more diverse repertoire of peptides, called the immunopeptidome, which in turn increases the probability of triggering a specific immune response (3). The advantage of HLA allelic differences has been initially estimated by comparing homozygous and heterozygous persons. A strong advantage for heterozygosity has been seen in the control of viral diseases and the response to cancer immunotherapies (4–8).

More recently, HLA allele divergence was measured as a continuous metric using the Grantham distance (9), which takes into account the differences in the composition, polarity, and volume of each amino acid within the peptide-binding groove of 2 homologous HLA alleles and has been found to better capture the functional properties of HLA than other common distance metrics (3). Experimental evidence confirmed that the HLA evolutionary divergence (HED) between 2 homologous alleles was directly correlated with the number of pathogen- or tumor-derived peptides bound by these 2 alleles (3, 10). The HED emerged as a strong determinant of survival in patients with cancer who received immune checkpoint inhibitors and in patients with leukemia who had allogeneic hematopoietic stem cell transplant (10, 11).

We hypothesized that HED may have an effect on the occurrence of liver transplant rejection, a situation where the donor and recipient are poorly HLA-matched given the marginal effect of HLA mismatching on liver rejection (12). In such a context, a high HED of the donor may increase the diversity of the graft-derived immunopeptidome targeted by the recipient's T cells, whereas a

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high HED of the recipient may allow the recipient's antigen-presenting cells to present a broader immunopeptidome to effector T cells (**Supplement Figure 1**, available at Annals.org). Therefore, we investigated whether the HED of the donor or the recipient was associated with the histologic lesions of acute or chronic rejection.

METHODS

Study Design and Participants

We conducted a retrospective study in Paul Brousse Hospital (adult cohort) and Necker Hospital (pediatric cohort). Information was collected and merged from the national database of liver transplanted patients named Cristal (www.agence-biomedecine.fr/), the local histologic database in which liver histology has been classified prospectively since 1990, the HLA typing database from the Histocompatibility Laboratory at Saint-Louis Hospital, and the local databases prospectively collecting posttransplant events. Characteristics of the patients and donors are shown in **Table 1** and the **Appendix Figure** (available at Annals.org).

Among the 1628 adult patients who received a first liver transplant between January 2004 and January 2018, donor HLA typing and posttransplant liver biopsy data were available for 1154. Among them, recipient HLA typing data were available in 909 cases (79%). Measurement of donor-specific anti-HLA antibodies (DSAs) was not done. Liver biopsies were done in the event of abnormal liver test results and no biliary obstruction. Routine biopsies were systematically done at 1, 2, 5, and 10 years after liver transplant. Immunosuppression consisted of tacrolimus, withdrawn corticosteroids, and mycophenolate. In the event of combined kidney or heart transplant, antithymoglobulins, higher doses of immunosuppressive drugs, and the maintenance of corticosteroids were used. Patients with hepatitis B virus received hyperimmune anti–hepatitis B surface antigen polyclonal immunoglobulins (13) with nucleos(t)ide anti–hepatitis B virus analogue. All patients with HIV were receiving antiretroviral therapy. Before 2014, whenever possible, patients with hepatitis C virus received pegylated interferon-α and ribavirin. From 2014, they received direct antiviral agents and were cured before or after the transplant.

Of the 172 children who received a first, noncombined liver transplant between January 2010 and January 2018, a total of 113 children for whom histologic follow-up was available constituted the pediatric cohort. Routine biopsies were done at 1, 5, and 10 years. Donor and recipient HLA typing data were available for all patients. The DSAs were measured on the day of biopsy. Immune suppression was similar to that in adults, but the patients were weaned from corticosteroids during the first month after transplant.

The study was done in accordance with the Declaration of Helsinki. Given that this was a retrospective cohort study without therapeutic intervention, no informed consent was required by French law.

Procedures

Liver biopsy specimens were routinely paraffin embedded and stained with hematein-eosin-safran and

Table 1. Recipient and Donor Characteristics in the Adult and Pediatric Cohorts

Characteristic	Value
Adult cohort, *n*	1154
Recipient	
Median age (IQR), *y*	53 (42–60)
Female, *n* (%)	381 (34)
Blood group	
O	501 (43.41)
A	468 (40.55)
B	141 (12.21)
AB	44 (3.81)
Median MELD score (IQR)	18 (11–29)
Principal indication, *n* (%)	
Alcoholic cirrhosis	293 (25.39)
Hepatocellular carcinoma	241 (20.88)
Genetic disease	165 (14.30)
Fulminant hepatitis	113 (9.79)
HCV cirrhosis	97 (8.41)
Secondary biliary cirrhosis	49 (4.25)
HBV cirrhosis	39 (3.38)
Primary sclerosing cholangitis	29 (2.51)
Autoimmune cirrhosis	22 (1.91)
Other liver tumor	17 (1.47)
Primary biliary cholangitis	17 (1.47)
Other indications	72 (6.24)
HCV, *n* (%)	213 (18.46)
HBV, *n* (%)	84 (7.28)
HIV, *n* (%)	73 (6.33)
Combined graft, *n* (%)	99 (8.58)
Kidney	95 (8.23)
Heart	4 (0.35)
Donor/graft	
Median age (IQR), *y*	56 (42–69)
Female, *n* (%)	510 (44)
Cause of death, *n* (%)	
Vascular stroke	639 (55.37)
Trauma	261 (22.61)
Anoxia	149 (12.90)
Other	48 (4.15)
Unknown	49 (4.24)
Living donor, *n* (%)	8 (0.69)
Median cold ischemic time (IQR), *min*	473 (404–565)
Number of HLA identities, *n* (%)	
0–2	727 (80.0)
3–8	182 (20.0)
Recipient HLA genotyping not available	245
Pediatric cohort, *n*	113
Recipient	
Median age (IQR), *y*	1.75 (0.91–4.41)
Female, *n* (%)	46 (40.7)
Blood group, *n* (%)	
O	54 (47.7)
A	45 (39.8)
B	11 (9.7)
AB	3 (2.6)
Median MELD score (IQR)	7 (6–17)
Principal indications, *n* (%)	
Biliary atresia	62 (54.8)
Genetic liver disease	13 (11.5)
Metabolic disorder	11 (9.7)
Fulminant hepatitis	11 (9.7)
Tumor	5 (4.4)
Autoimmune	3 (2.6)
Other indications	8 (7.0)
HCV, *n* (%)	0 (0.0)
HBV, *n* (%)	0 (0.0)
HIV, *n* (%)	0 (0.0)
Combined graft, *n* (%)	0 (0.0)

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Table 1–Continued

Characteristic	Value
Donor/graft	
Median age (IQR), *y*	21 (17–27)
Female, *n* (%)	54 (47.7)
Living donor, *n* (%)	8 (7.0)
Cause of death, *n* (%)	
Trauma	61 (53.0)
Stroke	15 (13.2)
Anoxia	14 (12.3)
Other	12 (10.6)
Unknown	11 (9.7)
Full liver, *n* (%)	20 (17.6)
Left lobe, *n* (%)	93 (82.4)
Median cold ischemic time (IQR), *min*	303 (120–619)
Number of HLA identities, *n* (%)	
0–2	79 (70.0)
3–8	34 (30.0)

HBV = hepatitis B virus; HCV = hepatitis C virus; IQR = interquartile range; MELD = Model for End-Stage Liver Disease.

picrosirius. Throughout the study, all liver biopsies done in adult recipients were analyzed by 2 experienced pathologists (M.S. and C.G.) and reviewed with hepatologists during weekly staff meetings. For this study, we took the following into account for the original diagnosis made at the time of biopsy: acute rejection, chronic rejection, ductopenia, steatofibrosis, pattern of biliary obstruction, chronic hepatitis, cirrhosis, nodular regenerative hyperplasia, veno-occlusive disease, and de novo immune hepatitis (14). The diagnosis of acute or chronic rejection was made according to the Banff Classification of Allograft Pathology (15, 16). Acute cellular rejection was classified using the Banff Rejection Activity Index, combining portal or perivenular inflammation, bile duct inflammation damage, and venous endothelial inflammation. Chronic rejection was based on bile duct dystrophy, ductopenia, and hepatic venules fibrosis. Late chronic rejection was based on a rate of ductopenia of 50% or more. The histologic diagnoses were prospectively coded, and the corresponding histologic slides were not reexamined for the study.

Biomarkers

The HLA typing results were available at a first field (2-digit) resolution for the *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* loci (but not the *HLA-C* and *HLA-DPB1* loci). For recipients and living donors, HLA typing was done using Luminex reverse transcriptase polymerase chain reaction sequence-specific oligonucleotides (One Lambda) with an increasing level of resolution over time. For deceased donors, HLA typing was done using polymerase chain reaction sequence-specific primers (Olerup until 2016, then Linkage Biosciences). Typing of a donor using complementary, high-resolution, sequence-specific primers was done if DSAs were detected after transplant in any recipient having received an organ from this donor. Retyping was not done during this study. Second field (4-digit) resolution typing was imputed from the most probable allele listed among ambiguities of the typing results. When more than 1 probable allele was proposed, we retained the most frequent allele based on both the description of the most frequent haplotypes encountered in the Paris region (17) and the results from the HaploStats online tool (www.haplostats.org), which was run on the White and Black populations that correspond to our recruitment. In our laboratory, such an imputation was found to be very consistent with high-resolution HLA genotyping using next-generation sequencing in more than 1500 persons. (Taupin J, Allain V, Caillat-Zucman S. Unpublished data from the routine Histocompatibility Laboratory at Saint-Louis Hospital.) The HLA matching was calculated for each recipient as the number of *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* identities with the donor. Sequence divergence (at the amino acid level) between HLA alleles was computed for all possible combinations of allele pairs among alleles encountered in both cohorts for *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* loci. The respective protein sequences of the peptide-binding groove (exons 2 and 3 for HLA class I and exon 2 for HLA class II) were extracted from the international ImMunoGeneTics/HLA database (18). The calculation of HED between aligned allele pairs of a given locus was based on the Grantham distance metric (3), a quantitative pairwise distance accounting for the physicochemical differences between 2 amino acids. For each recipient and each donor, the mean class I HED and mean class II HED were calculated as the mean of the 2 pairwise divergences at the *HLA-A* and *HLA-B* and *HLA-DRB1* and *HLA-DQB1* loci, respectively, assuming that each locus contributes equally to the presentation of peptides (10). By definition, there was a null divergence in case of homozygosity. Importantly, the HEDs of donors and recipients were calculated in 2020, and, therefore, did not influence any diagnosis or therapeutic decision between the time of organ proposal and the end of patient follow-up.

Statistical Analysis

To estimate the marginal effect of HED on the different outcomes, we used the inverse probability weighting approach (19) based on covariate balancing, generalized propensity scores (CBGPS) (20).

First, we defined a set of 21 demographic and clinical variables on the basis of their clinical relevance (**Supplement Table 1**, available at Annals.org) (21, 22). In the adult population, each of these variables was associated with at least 1 histologic outcome in repeated multivariable Cox analysis (data available on demand). We followed the recommendations of Fong and colleagues (20) by adding the squares of the continuous variables in the CBGPS and by using a Box–Cox transformation of continuous exposure (23). For handling missing values, we applied the missing indicator method, which consisted of adding a missing data category for categorical covariates and setting the missing data to 0 and adding supplementary binary covariates in the propensity score, indicating whether the value is missing or not for each continuous covariate (24). When we had fewer than 10 missing values for a covariate, we excluded the persons to avoid convergence issues. Once fitted, the individual stabilized weights were obtained from the CBGPS (19). The balance of covariates was verified graphically with the reduction of the weighted absolute Pearson correlation between the exposure and each covariate (**Supplement Figure 2**, available at Annals.org) (20). The positivity assumption was

Table 2. Posttransplant Histologic Patterns in 1154 Adults*

Histologic Pattern	Adults, *n* (%)	Median Time After Liver Transplant (IQR), *d*	Remarks
Subnormal	339 (29.37)	450 (367–867)†	
Steatofibrosis	294 (25.48)	727 (378–1166)	
Chronic hepatitis	260 (22.53)	422 (362–777)	119 HCV, 55 steatohepatitis, 40 de novo immune hepatitis, and 11 HBV
Acute rejection	249 (21.58)	33 (11–398)	
Banff score ≥3	214 (18.54)	22 (10–383)	
Biliary obstruction	179 (15.51)	37 (11–331)	
Ductopenia ≥20%	145 (11.69)	691 (362–1395)	
Ductopenia ≥30%	111 (9.61)	705 (353–1466)	
Regenerative hyperplasia	105 (9.09)	576 (364–1072)	
Chronic rejection	92 (7.90)	746 (376–1561)	
De novo immune hepatitis	54 (4.68)	451 (262–858)	
Veno-occlusive disease	54 (4.68)	321 (57–394)	
Cirrhosis	46 (3.98)	1056 (723–1887)	24 HCV, 10 de novo immune hepatitis, 6 biliary, 4 chronic rejection, and 2 HBV
Ductopenia ≥50%	26 (2.25)	739 (354–1693)	

HBV = hepatitis B virus; HCV = hepatitis C virus; IQR = interquartile range.
* For each lesion, the delay of detection was calculated from the time of liver transplant to the first detection.
† Last available liver graft histology.

verified through the distribution of the individual stabilized weights (Supplement Figure 3, available at Annals.org) (25).

Second, we fitted a weighted, cause-specific, proportional hazard Cox model with the exposure, that is HED, as the only explanatory variable (26). The variance was obtained with a robust sandwich-type estimator to account for the weighting (19). The corresponding hazard proportionality was tested using the Grambsch–Therneau test (27). If this assumption did not hold, 2 different periods were considered to model the level of the relationship. The log-linearity assumption of the relationship between the HED and the outcome was checked if the Bayesian information criterion was not reduced using natural spline transformation compared with the inclusion of the covariate in its natural scale. In case of violation, a transformation of variables was used. We computed the E-values to assess the exchangeability assumption–that is, no unmeasured confounding (28).

Furthermore, we estimated adjusted survival curves from the weights obtained with the CBGPS and the weighted Kaplan–Meier estimator (26). We reported the marginal cumulative incidences at 3, 5, and 10 years after transplant. We also did an explanatory analysis using the quartiles of the exposure to illustrate the cumulative incidences of the 2 main outcomes according to different levels of HED.

All analyses were done with R, version 3.6.0 (R Foundation) using the "survival," "CBPS," "ipw," "splines," "EValue," and "forestplot" packages.

Role of the Funding Source

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RESULTS

Characteristics of the Adult Cohort

Among the 1154 adult recipients, 248 died without a retransplant, 65 received a retransplant, and 15 died after a retransplant. The median follow-up was 1464 days (interquartile range, 707 to 2785 days). One- and 5-year patient survival rates reached 93% and 80%, respectively, and 1- and 5-year graft survival rates were 92% and 76%, respectively. The median time elapsing between transplant and the last available biopsy was 715 days (interquartile range, 355 to 1826 days). During follow-up, the mean number of biopsies per patient was 2.9. In patients with normal liver function, the adherence to 1-, 2-, and 5-year routine biopsies was 92%, 56%, and 85%, respectively. The last available biopsy was normal in 339 patients (29%). The main histologic patterns are shown in Table 2.

Distribution of HED

We did a hierarchical clustering of HED for all pairwise allele combinations at the *HLA-A*, *HLA-B*, *HLA-DRB1*, and *HLA-DQB1* loci in donors and recipients in the adult and pediatric cohorts. Distinct clusters of high and low divergence between alleles were seen (Supplement Figure 4, available at Annals.org). Class I and class II HEDs of the donors and recipients were not normally distributed (Supplement Figure 5, available at Annals.org). Pairwise divergences for *HLA-B* were higher than for *HLA-A* ($P < 0.001$), as previously reported (10). There was no association between the HEDs of donors and those of recipients (Supplement Table 2, available at Annals.org) or between donor or recipient HEDs and covariates (Supplement Table 3, available at Annals.org).

Marginal Effect of Donor and Recipient HEDs on Liver Graft Histologic Lesions

The adjusted cumulative incidences at 3, 5, and 10 years of the 12 histologic outcomes are presented in Figure 1. The rates of acute rejection at 3 and 10 years after transplant were 0.261 (95% CI, 0.229 to 0.291) and 0.338 (CI, 0.289 to 0.383), respectively. The rates of chronic rejection at 3 and 10 years after transplant were 0.085 (CI, 0.063 to 0.106) and 0.238 (CI, 0.17 to 0.297), respectively.

Figure 1. Adjusted cumulative incidences of histologic lesions at 3, 5, and 10 years after liver transplant in adults.

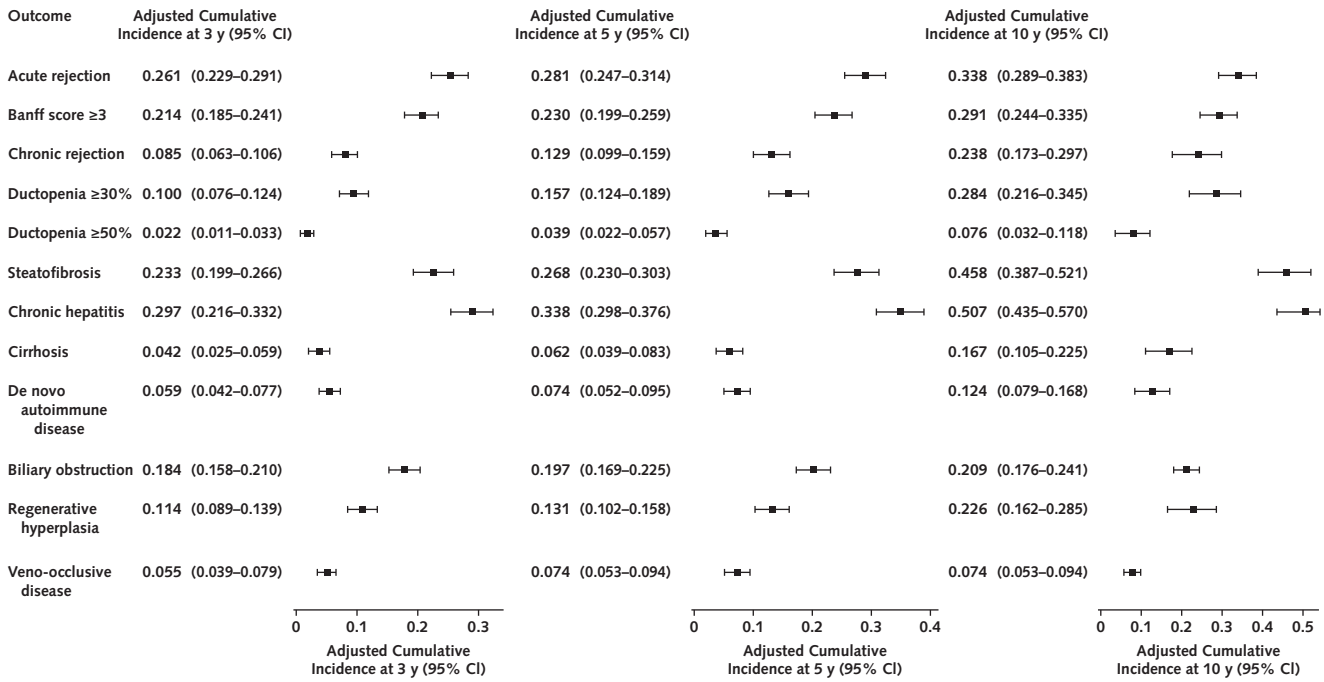


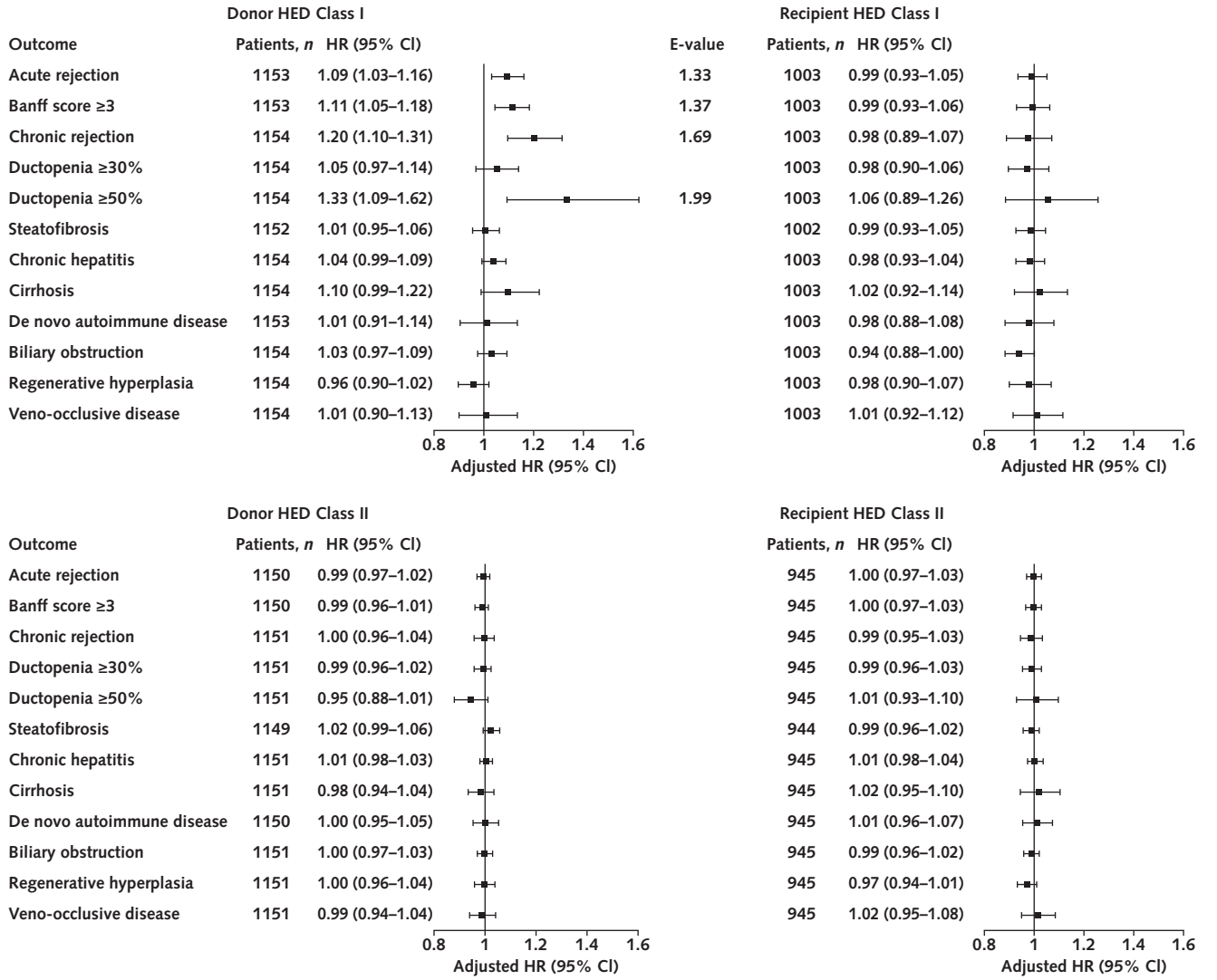
Figure 2 summarizes the adjusted hazard ratios (HRs) of class I and class II HEDs of donors and recipients for the 12 histologic outcomes. We saw a statistically significant association of the donor class I HED exposure with acute rejection (HR, 1.09 [CI, 1.03 to 1.16]; E-value, 1.33), acute rejection with Banff score of 3 or greater (HR, 1.11 [CI, 1.05 to 1.18]; E-value, 1.37), chronic rejection (HR, 1.20 [CI, 1.10 to 1.31]; E-value, 1.69), and ductopenia of 50% or more (HR, 1.33 [CI, 1.09 to 1.62]; E-value, 1.99). In other words, the higher the donor class I HED, the greater the risk for rejection. When considering donor *HLA-B*–only HED instead of the mean class I HED (*HLA-A* and *HLA-B*), association with acute and chronic rejection remained statistically significant (HR, 1.06 [CI, 1.02 to 1.11]; E-value, 1.26 and HR, 1.15 [CI, 1.07 to 1.22]; E-value, 1.55, respectively) (Supplement Figure 6, available at Annals.org). When considering *HLA-A*–only HED, an association was found only with chronic rejection (HR, 1.07 [CI, 1.0 to 1.14]; E-value, 1.34) (Supplement Figure 7, available at Annals.org). Of note, the same analysis applied to donor class II HED, and to recipient class I or class II HED, did not find any association with any histologic pattern (Figure 2; Supplement Table 4, available at Annals.org).

The cumulative incidences of acute and chronic rejection by quartiles of donor class I HED are shown in Figure 3. After 10 years of follow-up, the restricted mean times for acute rejection were 7.02 years (CI, 5.55 to 8.49), 8.12 years (CI, 6.95 to 9.28), 7.59 years (CI, 6.39 to 8.79), and 5.92 years (CI, 4.66 to 7.19) for the lowest to the highest quartile, respectively. The restricted mean times for chronic rejection were 9.19 years (CI, 8.25 to 10.00), 9.36 years (CI, 8.56 to 10.00), 8.17 years (CI, 6.83 to 9.50), and 7.99 years (CI, 6.84 to 9.13) for the lowest to the highest quartile, respectively. Because the median value of donor class I HED, which was 7.52, seemed to be the threshold for chronic rejection, we checked whether it was associated with the rate of ductopenia, which reflects the severity of chronic rejection. Among the 145 patients with ductopenia of 20% or more, we saw a strong, positive relationship between the rate of ductopenia and donor class I HED above the median value ($P < 0.001$) (Figure 3, *C*). Resolution of rejection, assessed by the disappearance of histologic lesions on subsequent liver biopsy, was seen in 58% and 27% of acute and chronic rejection cases, respectively, and was not associated with the HEDs of donors or recipients (data not shown).

Marginal Effect of Donor and Recipient HEDs in the Pediatric Cohort

Finally, we studied the role of HED in a distinct cohort of 113 children with liver transplant. The median follow-up was 1668 days (interquartile range, 858 to 2521 days). Acute rejection and liver fibrosis were diagnosed in 63 (56%) and 61 (54%) children, respectively. The other histologic lesions seen in adults were scarce: cirrhosis ($n = 2$), steatosis ($n = 3$), chronic rejection ($n = 4$), and de novo immune hepatitis ($n = 3$). The covariates considered for the propensity scores were the recipient's age, ABO blood group and donor or recipient HLA identities (as in adults), and the presence of class I and class II DSAs. All of these variables were related to acute

Figure 2. Effect of class I and class II HEDs of donors and recipients on the 12 histologic lesions identified in adult patients.



Results show adjusted HRs with 95% CIs and E-values. Donor class I HED was associated with acute and chronic rejection but not with other lesions. All other HEDs were not associated with any histologic lesion. HED = HLA evolutionary divergence; HR = hazard ratio.

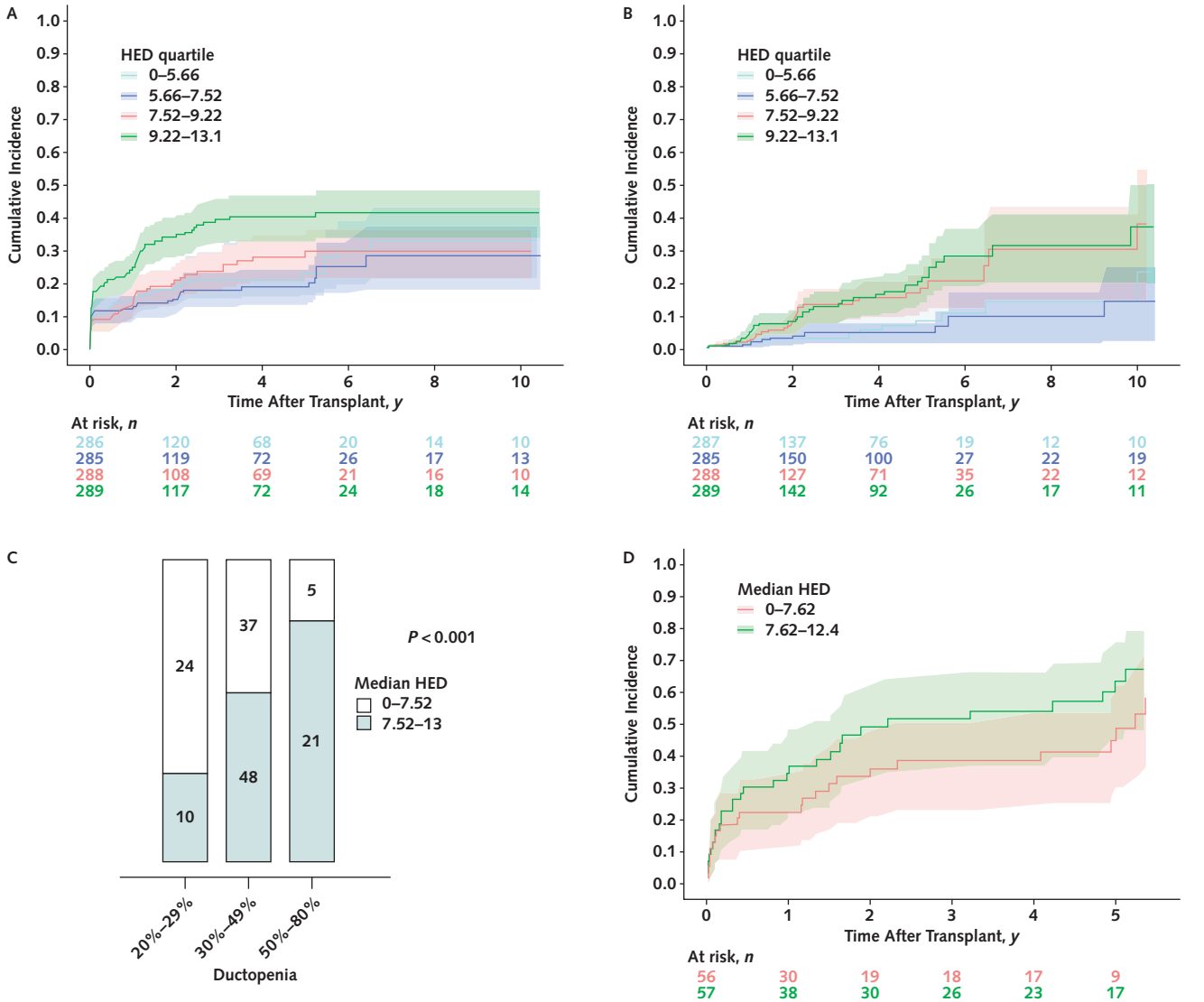
rejection or fibrosis in multivariable Cox analysis (data available on request). Figure 4 shows the effect of class I and class II HEDs of donors and recipients on acute cellular rejection and fibrosis. Like in adults, we saw a statistically significant association of donor class I HED with acute rejection (HR, 1.09 [CI, 1.03 to 1.16]; E-value, 1.45) but not with fibrosis. The cumulative incidence of acute cellular rejection according to the median value of donor class I HED (median, 7.62) is shown in panel D of Figure 3.

Discussion

The divergent allele advantage assumes that HLA genotypes with more divergent heterozygous alleles may allow for the presentation of a larger set of peptides for T-cell recognition than less divergent alleles. Recent results in patients receiving anticancer immunotherapy (7, 10) highlighted the important role of class I HED in shaping the immunopeptidome and thus enhancing the antitumor T-cell response. Through this latter finding, not only has the HLA divergence hypothesis been reinforced, but a direct clinical application is offered by this metric of immunogenicity. We show that a high class I (*HLA-A* and *HLA-B*) HED of the donor is associated with the occurrence of acute and chronic rejection in a large, single-center cohort of adult recipients of liver transplant with long-term follow-up and systematic and diagnostic liver biopsies. The effect of class I HED of the donor on acute rejection was replicated in a smaller, independent pediatric cohort.

The HED is an intrinsic metric of diversity at the HLA-peptide complex for each person Therefore, our data

Figure 3. Adjusted cumulative incidences of acute and chronic rejection.

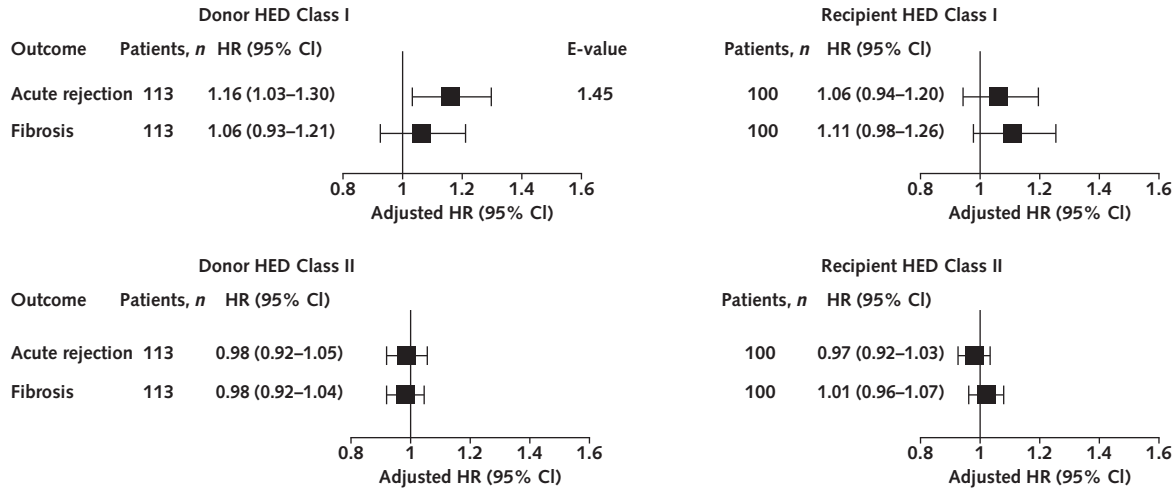


HED = HLA evolutionary divergence. **A and B.** The cumulative incidence of acute rejection and chronic rejection, respectively, by quartiles of donor class I HED in the adult cohort. **C.** The relationship between the rate of ductopenia and donor class I HED ($\chi^2 = 16$, $P < 0.001$) in adults. **D.** The cumulative incidence of acute rejection according to the median value of donor class I HED in the pediatric cohort.

suggest that the more divergent the HLA class I molecules of the donor are, the more diverse the graft-derived peptides they present to the recipient's cytotoxic T cells are, and the higher the risk for rejection. Donor class II HED was not associated with rejection. However, because it was calculated for *HLA-DRB1* and *HLA-DQB1* alleles only, we cannot exclude that class II HED did not capture the entire divergence of DR and DQ molecules (which are composed of 2 polymorphic chains) and thus partially reflected their contribution to peptide presentation. Importantly, HLA allele divergence of the recipient was not predictive of any histologic outcome. Thus, what seems important is the diversity of the repertoire of (allogeneic) peptides bound to the donor HLA molecules expressed on the graft and not the ability of the HLA molecules expressed on the recipient's antigen presenting cells to present a more diverse (self or allogeneic) peptide repertoire. This observation is consistent with the marginal effect of donor–recipient HLA matching on liver allograft survival, which has been described by others (12) and was seen in the current study both for adults and children. However, the association of donor HED class I with rejection was independent of the number of HLA identities between the donor and recipient.

Donor-specific HLA antibodies are produced through the indirect presentation of allogeneic epitopes by recipient HLA class II molecules, providing CD4 T-cell help for the generation of antibody-producing B cells. Because the incidence of antibody-mediated rejection is low and this issue still debated in the liver transplant setting, DSA

Figure 4. Adjusted HRs of class I and class II HEDs of donors and recipients on acute rejection and fibrosis in children.



Results show adjusted HRs with 95% CIs and E-values. Donor class I HED was associated with acute rejection only. All other HEDs were not associated with any histologic lesion. HED = HLA evolutionary divergence; HR = hazard ratio.

determination has only recently been done on a routine basis (16). Therefore, we could not determine if HED was associated with the development of a humoral response to the graft in the adult cohort. In children, the donor HED class I was associated with rejection independent of the presence of class I or class II DSA. It will be interesting to further evaluate the relationship between HED and the occurrence of DSAs in prospective studies in liver transplant settings but also in other solid organ transplantation settings (kidney, lung, and heart) where the humoral response is crucial.

As well as HED, other known variables were involved in the risk for rejection. Recipient age is determinant in the case of acute rejection (29), explained by adherence problems in young recipients or so-called immune senescence (30) in older recipients. Rejection is less frequent in patients having a combined transplant in whom immunosuppression is stronger than with liver transplant alone. Less evidence of acute rejection in patients with hepatitis B virus has been seen previously (31). The donor's age is also associated with chronic rejection (32).

This study has limitations because of its retrospective design. To minimize observer bias, we did not review the histologic slides and took into account only the prospectively coded histologic diagnosis. Concerning misclassification bias, beside the main diagnosis of acute or chronic rejection, we also analyzed nested subgroups according to the Banff score or the level of ductopenia. Another limitation is the absence of measured DSAs in adults. We showed only in children that donor class I HED was related to rejection independent of the DSA.

From a practical standpoint, HED can be determined rapidly at no additional cost as soon as the HLA genotype of the donor is available. The HED can be calculated for all combinations of HLA class I alleles and made available. In adults, liver transplants with normal histology are infrequent (33, 34), and long-term prognosis is a major issue in young recipients (35). The availability of donor HED could allow for better allocation of liver grafts when possible; for example, by avoiding donors with high HED values for recipients at high risk for rejection. Alternatively, HED could be considered in personalized strategies to optimize immunosuppression as a function of the risk for rejection (36). Finally, the role of HED in other transplant types where HLA matching (and occurrence of DSA) is crucial, such as kidney or lung transplant, should be investigated.

From Centre Hépato-Biliaire, Hôpital Paul-Brousse, Assistance Publique-Hôpitaux de Paris, Université Paris-Saclay, unité Institut National de la Santé et de la Recherche Médicale 1193, Villejuif, France (C.F., M.A., C.D., A.C., F.S., E.V., D.A., D.S.); Laboratoire d'Immunologie et Histocompatibilité, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris, Université de Paris, and unité Institut National de la Santé et de la Recherche Médicale 976, Université de Paris, Paris, France (J.T., S.C.); Laboratoire d'Anatomopathologie, Assistance Publique-Hôpitaux de Paris, Hôpital Paul-Brousse, Université Paris-Saclay, unité Institut National de la Santé et de la Recherche Médicale, Physiopathogénèse et traitement des maladies du Foie, and FHU Hepatinov, Villejuif, France (M.S., C.G.); Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris, Université de Paris, Laboratoire d'Immunologie et Histocompatibilité, and Institut National de la Santé et de la Recherche Médicale, Paris, France (V.A.); Hôpital Necker Enfants Malades, Assistance Publique-Hôpitaux de Paris, Université de Paris, and Unité d'Hépatologie pédiatrique, Paris, France (Z.D., D.D.); and Institut National de la Santé et de la Recherche Médicale UMR 1246–SPHERE, Nantes University, Tours University, Nantes, and IDBC, Pacé, France (A.C.).

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Corresponding Author: Cyrille Féray, MD, PhD, Centre Hepato Biliaire, Hôpital Paul Brousse, 14 avenue Paul Vaillant couturier, Villejuif, France 94800; e-mail, cyrille.feray@aphp.fr.

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Current author addresses and author contributions are available at Annals.org.

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Current Author Addresses: Drs. Féray, Allard, Desterke, Coilly, Saliba, Vibert, Azoulay, and Samuel: Hôpital Paul Brousse, 14 avenue Paul Vaillant couturier, Villejuif, France 94800.
Drs. Taupin, Allain, and Caillat Zucman: Hôpital Saint-Louis, 1 avenue Claude Vellefaux Paris, France 75010.
Drs. Sebagh and Guettier: Hôpital de Bicetre, 78 avenue du Général Leclerc, Kremlin-Bicetre, France 94270.
Drs. Demir and Debray: Hôpital Necker Enfants Malades, 149 rue de Sevre, Paris, France 75015.
Dr. Chatton: A2COM, 1 Boulevard du Trieux, Pacé, France, 35740.

Author Contributions: Conception and design: C. Féray, S. Caillat-Zucman.
Analysis and interpretation of the data: C. Féray, J.-L. Taupin, M. Sebagh, V. Allain, M. Allard, F. Saliba, D. Azoulay, A. Chatton, D. Debray, S. Caillat-Zucman, D. Samuel.
Drafting of the article: C. Féray, J.-L. Taupin, D. Azoulay, S. Caillat-Zucman, D. Samuel.
Critical revision of the article for important intellectual content: C. Féray, J.-L. Taupin, V. Allain, A. Coilly, F. Saliba, E. Vibert, D. Azoulay, A. Chatton, D. Debray, S. Caillat-Zucman, D. Samuel.
Final approval of the article: C. Féray, J.-L. Taupin, M. Sebagh, V. Allain, Z. Demir, M. Allard, C. Desterke, A. Coilly, F. Saliba, E. Vibert, D. Azoulay, C. Guettier, A. Chatton, D. Debray, S. Caillat-Zucman, D. Samuel.
Statistical expertise: V. Allain, C. Desterke, A. Chatton.
Collection and assembly of data: C. Féray, J.-L. Taupin, M. Sebagh, Z. Demir, M. Allard, C. Desterke, S. Caillat-Zucman.

Appendix Figure. Flow diagrams for the adult and pediatric groups.