Concordance of Genotypes in Pre– and Post–Lung Transplantation DNA Samples

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Genetic epidemiology studies of end-stage lung disease are potentially hindered by low numbers of participants due to early death of patients from the underlying disease, or due to exclusion from studies after patients have had lung transplants, because of concern about bias of genotype data due to chimerism. The number of participants enrolled in genetic studies of end-stage lung disease could be increased by including those individuals who have undergone lung transplant. We hypothesized that individuals who have had lung transplants can be included in genetic epidemiology studies that use single nucleotide polymorphism and short tandem repeat marker data, without confounding due to chimerism. Ten probands with severe, early-onset chronic obstructive pulmonary disease were included in this analysis. Pre– and post–lung transplant DNA samples were used in the investigation of concordance of genotype results for 12 short tandem repeat markers and 23 single nucleotide polymorphisms. Concordance was observed for all genotypes before and after lung transplant. We conclude that the risk of biasing genetic epidemiology studies due to donor lung–related DNA microchimerism is low, and that the inclusion of post–lung transplantation participants will allow for larger genetic epidemiology studies of individuals with end-stage lung disease.

Keywords: genetic epidemiology; lung; chimerism; transplantation

Genetic investigation of end-stage lung diseases, such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), and pulmonary hypertension, proceeds with the goal of identifying genetic susceptibility for disease that may influence diagnosis, treatment, and prevention strategies. These end-stage lung diseases are often treated with lung transplantation; although individuals with the most severe and end-stage forms of disease could be valuable participants in genetic studies, DNA chimerism has been reported throughout the body after lung transplantation (4).

Genetic investigation of complex disease has often used the strategy of studying the most extremely affected individuals, who may provide a better opportunity to dissect genetic influences. With regard to COPD, this means investigating those individuals with severe lung disease at a young age (1). However, obtaining probands with early-onset severe disease for genetic epidemiology studies can be limited by death of affected individuals at an early age. Lung transplantation has become a treatment option for people with end-stage lung disease due to COPD, and the number of individuals enrolled in genetic studies of COPD could be increased by including individuals who have undergone lung transplantation.

In the setting of lung transplantation, the possibility for genetic chimerism has led to the exclusion of post-transplant individuals in genetic studies of end-stage lung disease such as the Boston Early-Onset COPD Study (1). In genetic epidemiologic investigation, the presence of donor DNA due to chimerism could bias genotyping results, and contribute to false-positive or false-negative observations in genetic linkage or association studies. We hypothesized that individuals who have undergone lung transplantation can be included in genetic epidemiologic investigations without a risk of inaccurate genotyping results due to DNA chimerism. To pursue this hypothesis, we analyzed pre– and post–lung transplant DNA samples of 10 probands in the Boston Early-Onset COPD Study, investigating the presence of genotype discordance for 12 short tandem repeat (STR) markers and 23 single nucleotide polymorphisms (SNPs).

MATERIALS AND METHODS

Participants

Recruitment criteria and methods for the Boston Early-Onset COPD study have been previously reported (1). In brief, probands with severe, early-onset COPD were enrolled primarily from pulmonary clinics at Brigham and Women's Hospital and Massachusetts General Hospital. Probands were defined as having a physician's diagnosis of COPD, $FEV_1 < 40\%$ predicted, age younger than 53 yr, and no evidence of severe α_1-antitrypsin deficiency. For the 10 probands included in this study, peripheral blood samples were collected for DNA extraction from pre– and post–lung transplantation time periods. Lung allograft donor sex was known for 9 of 10 probands. All participants provided written informed consent. The protocol was approved by the Partners/Brigham and Women's Hospital Institutional Review Board.

DNA Extraction

Pre-transplant blood samples were collected as part of the Boston Early-Onset COPD Study protocol at the time of study enrollment. Post-transplant samples were collected at variable times from the date of lung transplantation. Puregene kits (Gentra Systems, Minneapolis, MN) were used to extract DNA per standard protocol.

STR Markers

STR markers, also known as microsatellite markers, were selected from previously optimized markers in our laboratory. A total of twelve STR markers were evaluated for evidence of discordance between pre- and post-transplant specimens. PCR was performed in a standard fashion using AmpliTaq Gold DNA polymerase. All genotyping reactions were performed using fluorescent-labeled and unlabeled primers on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Genotyping calls were performed manually and checked by a second reviewer blinded to the preoperative genotypes until the end of the study. Questionable calls or discordant results were assessed by two other reviewers. The maximum number of times that a sample was repeated was three.

SNPs

SNPs were selected at random from a series of polymorphisms under investigation in the Boston Early-Onset COPD Study. Genotyping for

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TABLE 1. CHARACTERISTICS OF PARTICIPANTS AND TIME ELAPSED BETWEEN LUNG TRANSPLANTATION AND COLLECTION OF THE POST-TRANSPLANT DNA SAMPLE

Patient No.	Sex-Lung Recipient	Sex-Lung Donor	Type of Transplant	Time elapsed*
1	Female	Female	Single	3 yr, 4 mo
2	Female	Female	Single	2 yr, 9 mo
3	Female	Female	Single	7 yr, 2 mo
4	Female	Female	Single	8 mo
5	Female	Female	Single	2 yr, 1 mo
6	Female	Female	Double	3 mo
7	Male	Unknown	Double	3 yr, 9 mo
8	Female	Female	Single	1 yr, 6 mo
9	Female	Male	Single	2 yr, 6 mo
10	Male	Male	Single	2 yr, 4 mo

* Time elapsed represents the time between the lung transplant and the post-transplant blood sample, rounded to the nearest month.

all SNPs was performed using the SEQUENOM platform (Sequenom, San Diego, CA), using 2.5 ng of genomic DNA per reaction. Minor allele frequencies in the founders (parents of the probands included in this study) are listed in Table 3. Two SNPs (rs1921663 and rs2450738) had poor genotyping completion rates and were excluded from the analysis.

Sex-Related Chimerism Detection

For the investigation of potential chimerism in the instance of sex-mismatch between lung transplant donor and recipient, sex-determining region Y (SRY, Yp11.31) gene expression was investigated. Samples from 100 male and 100 female control subjects were evaluated for SRY expression to provide a range of SRY expression in normal males and females (most likely due to nonspecific amplification). For post-transplantation samples, two protocols of real-time PCR were evaluated for quantification of SRY expression (the first round with 50 cycles of PCR, the second round with 40 cycles). Research assistants performing reactions were blinded to the sex of all participants.

RESULTS

The characteristics of the 10 participants are listed in Table 1. All individuals received a lung transplant for the underlying diagnosis of COPD; all transplanted lungs were obtained from cadaveric donors. Only one individual was known to be a female recipient–male donor mismatch. The donor sex for one male recipient was not known. The mean time elapsed since the lung transplant and the collection of the post-transplant blood sample was 29 mo (with a range of 3–56 mo). Information regarding parity of the female lung recipients, number of blood transfusions and dates of most recent transfusions, and timing of rejection episodes was not available.

TABLE 2. SHORT TANDEM REPEAT MARKERS GENOTYPED IN THE PRE- AND POST-TRANSPLANT BLOOD SAMPLES

STR	Chromosomal Location	No. of Alleles Observed
D10S191	10p31	9
D10S197	10p12.1	5
D10S212	10q26.3	6
D10S583	10q23.33	7
D11S901	4q12	6
D12S1052	12q21.1	4
D12S379	12q21.31	3
D19S420	19q13.31	8
D9S161	9p21.2	6
D9S175	9q21.13	5
D9S279	9q32	7
D9S287	9q22.32	3

Definition of abbreviation: STR, short tandem repeat.

TABLE 3. SINGLE NUCLEOTIDE POLYMORPHISMS GENOTYPED IN PRE– AND POST–LUNG TRANSPLANT BLOOD SAMPLES

Gene	SNP	Chromosomal Location	Minor Allele Frequency
ADAM23	rs1448905	2	0.37
	rs759844	2	0.42
	rs759845	2	0.35
IL8RA	rs1008563	2	0.48
	rs1567868	2	0.05
IL8RB	rs1126580	2	0.49
	rs1126579	2	0.37
MMP12	rs505770	11	0.06
	rs652438	11	0.07
	rs737693	11	0.08
MGST1	rs1913263	12	0.26
	rs11875	12	0.08
	rs3759207	12	0.32
	rs1913262	12	0.24
RAI3	rs2241228	12	0.42
	rs1061047	12	0.20
MGP	rs1800802	12	0.21
	rs1049897	12	0.38
ICAM1	rs5490	19	0.01
ICAM3	rs2230399	19	0.05
TGFB1	rs1800469	19	0.30
LTBP4	rs1051303	19	0.39
	rs1131620	19	0.39

Definition of abbreviations: ADAM23, a disintegrin and metalloproteinase domain 23; ICAM1, intercellular adhesion molecule 1; ICAM3, intercellular adhesion molecule 3; IL8RA, interleukin-8 receptor alpha; IL8RB, interleukin-8 receptor beta; LTBP4, latent transforming growth factor beta–binding protein 4; MGP, matrix Gla Protein; MGST1, microsomal glutathione S-transferase 1; MMP12, matrix metalloproteinase 12; RAI3, retinoic acid induced 3; SNP, single nucleotide polymorphism; TGFB1, transforming growth factor-β1.

STR Markers

The 12 STR markers and number of alleles observed in these 10 individuals are listed in Table 2. Of 120 possible genotype pairs, (10 participants, 12 STRs, each participant with one pre- and one post-transplant sample, 240 total genotypes) there were no instances of discordance between pre- and post-transplant DNA samples. One hundred ninety-one of the 240 reactions had genotypes that could be determined on the first cycle of evaluation. Forty samples required duplication for verification, and 9 were done in triplicate. All samples requiring triplicate, and 11 requiring duplicate genotyping were for D9S279, which had characteristic evidence of interference by polyadenylation signals. Of the others requiring duplication, nine were for missing data due to PCR failure, with the remainder due to difficulty in adequately designating a genotype because of low amplitude signals or due to interference by polyadenylation signals.

SNPs

The SNPs and minor allele frequencies in the founders are listed in Table 3. Of the 230 SNP genotype pairs on pre– and post–lung transplant DNA samples, there was possible discordance in only one individual for rs1913263, after initial genotyping. In the pre-transplant sample the genotype was a questionable homozygous GG, and in the post-transplant sample it was a questionable GA. This SNP was genotyped again for all participants pre- and post-transplant; upon duplication there was no discordance for this individual and the final genotype was GA.

SRY Gene Expression

For the control population, all male control subjects expressed SRY in the expected range (25–38 cycles of RT-PCR). Three female control subjects had low-level expression of SRY (between 36 and 44 cycles of RT-PCR). From these control subjects we selected 50 and 40 cycle lengths of PCR for investigation in our pre– and post–lung transplant samples. One female proband was a recipient of a lung from a male donor, and Y chromosome–related chimerism was also evaluated in this individual. This individual was 2 yr and 6 mo post-transplant. Although evidence of circulating Y chromosome was detected using 50 PCR cycles in this female proband and in two other female probands that received allografts from female donors, there was no detectable SRY with 40 PCR cycles, suggesting that in the three women with Y chromosome signals after 50 PCR cycles the higher PCR cycle length contributed to nonspecific amplification.

DISCUSSION

Lung transplantation is often offered as a therapeutic intervention for end-stage lung disease in young individuals. As such, in genetic epidemiology studies of such end-stage lung diseases, confounding of genotype results by lung donor DNA is a theoretical concern that could bias the results of genetic linkage and association studies if DNA samples are collected after lung transplantation. Our assessment of STR markers and SNPs in 10 individuals with severe, early-onset COPD before and after lung transplantation suggests that donor DNA chimerism does not interfere with routine STR and SNP genotyping.

Although the degree of peripheral chimerism associated with solid organ transplantation is not as high as chimerism associated with bone marrow transplant, low levels of donor cells have been detected in the skin, lymph nodes, and blood of liver transplant recipients even several years after transplantation (2). In bronchoalveolar lavage samples from 10 patients who underwent lung transplant, donor lymphocytes were not detectable in any of 10 patients after 3 mo; however, low level of donor macrophages remained detectable 2 yr post-operatively. Wiebe and colleagues concluded that chimerism in the lung at the macrophage level may persist (3). Using Y chromosome–specific DNA probes in female recipients of lungs from male donors, Kubit and coworkers quantified circulating donor cells in the blood, and observed that although donor cells could be detected in a variety of organs (such as heart, skin, kidney, lymph nodes, and spleen, as well as native lung) as a result of migration from the lung, the number of cells was relatively small (4). Through investigation of donor-specific HLA-DRB1, Knoop and colleagues reported the detection of donor cells in single lung and heart-lung transplant recipients (5). This group also noted that although blood chimerism as measured with HLA DRB1 was somewhat elevated in all patients in the immediate postoperative setting and during the first three post-transplant months, this decreased to a low level at 1 yr (6). Because all of our study participants were at least 3 mo post-transplant, we cannot exclude chimerism in the very early postoperative period. Reinsmoen and colleagues observed blood microchimerism in 77% of lung and 91% of heart allograft recipients at a range of 12–18 mo after transplantation (7). In another report they detected blood microchimerism in 47% of lung allograft recipients more than 11 mo post-transplant (8). This same group has observed a positive correlation between blood microchimerism levels and FEV_1 after lung transplantation (9).

The assessment of chimerism may be most efficient through the use of techniques to assess donor versus recipient HLA loci. In the case of female recipients who receive lungs from male donors, this assessment may be undertaken by quantitative polymerase chain reaction amplification of SRY antigens (10). STR markers have also been used to quantify mixed chimerism (11–13). Concordance between pre- and post transplant results for STR genotypes of a panel of highly polymorphic STRs in our study suggests an absence of chimerism of a level that would influence large-scale genetic epidemiology studies. Kleeberger and coworkers demonstrated that for detection of microchimerism in liver transplant recipients, STR PCR assays may detect a chimeric state down to 5% (14). One important advantage of the use of STRs is that they are independent of sex mismatching (which is necessary for use with X and Y chromosome specific antigen probes). This has relevance in genetic epidemiology studies, as the donor sex (or HLA information) is not readily collected as part of routine protocols in epidemiology studies that may include post-transplant individuals. Although microchimerism may exist, it is unlikely to influence the validity of genotyping results for genetic epidemiology studies of STR markers and SNPs assessed with more routine genotyping protocols.

Weaknesses of our approach to this hypothesis include lack of HLA donor and recipient information and lack of knowledge of the donor genotypes at each SNP locus. If we had been able to obtain donor genotype information, genotyping protocols with an increased PCR cycle number could have been used to determine if donor genotype could be detected. However, the probability of matching between a lung donor and recipient at each locus for all microsatellites and SNPs tested in this study is highly unlikely. Based on the estimated genotype frequencies for each SNP calculated from the allele frequencies observed in the parents (Table 3), the probability of an identical random donor match for all 23 SNPs was $< 1 \times 10^{-6}$. This exceedingly small probability suggests that all of the genotypes that we observed were likely those of transplant recipients uninfluenced by chimerism. As suggested above, one advantage of STR markers to detect chimerism is that they are likely independent of sex mismatching between lung donors and recipients. However, in our retrospective assessment, we had only one sex-mismatched recipient–donor pair, which limits our ability to directly compare STR and SRY approaches to detecting chimerism. Benefits of using STRs and SNPs to assess for chimerism include that the methods are readily automated at a low cost. Demonstrating the fidelity of these methods increases the possible number of participants in genetic epidemiology studies of end-stage lung diseases.

In conclusion, genetic epidemiology studies of end-stage lung disease can include post–lung transplant individuals, as the risk of bias due to contribution of donor DNA chimerism in investigations of STR markers and SNPs appears to be quite low. Expanding the pool of potential participants in genetic epidemiology studies by not excluding post-transplant individuals will provide the opportunity for larger scale studies with higher power to detect genetic associations relevant for advancing the understanding of a variety of severe lung diseases.

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