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Recipient *CCL5* −403G>A (rs2107538) Polymorphism and Acute Graft-Versus-Host Disease in Pediatric Hematopoietic Cell Transplantation

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Abstract

Acute graft-versus-host disease (aGvHD) is a clinically important immune complication after allogeneic hematopoietic cell transplantation (HCT). Chemokines play a pivotal role in regulating leukocyte migration and recruitment to inflamed tissues, thereby contributing to the immunopathogenesis of aGvHD. In particular, CCL5 mediates the recruitment of CCR1-, CCR3-, and CCR5-expressing immune cells, suggesting that regulatory polymorphisms in the *CCL5* gene may influence inflammatory cell trafficking and recipient susceptibility to aGvHD. In this retrospective single-center candidate-gene study, we evaluated recipient *CCL5* −403G>A (rs2107538), *CCR9* G926A (rs12721497), and *CXCL12* 3′A>G (rs1801157) polymorphisms in 62 pediatric HCT recipients. *CCR9* and *CXCL12* were analyzed as biologically selected comparator loci within chemokine receptor and hematopoietic trafficking pathways, whereas the primary association signal was observed for *CCL5*. Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and analyzed according to aGvHD status. Acute GvHD occurred in 25 patients (40.3%). Patients without aGvHD were classified as grade 0; grade 0–I and grade II–IV groups comprised 40 (64.5%) and 22 (35.5%) patients, respectively. The *CCL5* rs2107538 variant showed a significant association with aGvHD, with enrichment of the GA genotype and A allele among affected patients, whereas the GG genotype was more frequent in patients without aGvHD. No significant associations were detected for recipient *CCR9* rs12721497 or *CXCL12* rs1801157. This pattern suggests that a promoter-region *CCL5* variant, rather than the evaluated *CCR9* or *CXCL12* loci, may be the most relevant chemokine-related susceptibility signal in this pediatric cohort. The finding is exploratory and does not establish causality or clinical eligibility criteria for HCT, but it provides a focused rationale for donor–recipient paired studies and functional assessment of *CCL5* transcriptional regulation in aGvHD.

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Keywords: acute graft-versus-host disease; hematopoietic cell transplantation (HCT); *CCL5*; promoter polymorphism; recipient genotype; pediatric HCT; chemokine-mediated trafficking

1. Introduction

Allogeneic hematopoietic cell transplantation (HCT) can be curative for malignant and non-malignant hematologic disorders, but its benefit is constrained by relapse, infection, graft failure and graft-versus-host disease (GvHD). Acute graft-versus-host disease (aGvHD) is initiated when donor immune cells recognize host alloantigens and minor histocompatibility antigens in the context of tissue injury and inflammatory activation; the response is further amplified by recipient tissue-derived factors, including endothelial activation, innate immune signaling, complement activation, cytokines and chemokine gradients [1–6].

Chemokines are small secreted chemotactic cytokines that coordinate leukocyte migration, positioning and tissue localization during both homeostatic immune surveillance and inflammatory responses. Platelet factor 4 (PF4/CXCL4), a platelet-derived chemokine, was characterized in early biochemical studies [7]. In the setting of HCT, chemokine-mediated trafficking is particularly relevant to aGvHD because donor T cells, monocytes, dendritic cells and other effector populations must be recruited to recipient target tissues before inflammatory tissue damage becomes clinically apparent [8,9]. Genetic variation in this setting can be relevant in different ways [10]. Coding-region variants may alter protein sequence or receptor function, whereas non-coding promoter or regulatory variants may influence inducible gene expression, local chemokine abundance and the intensity of immune-cell recruitment without generating a new protein sequence [11,12].

The three polymorphisms examined in this study were selected a priori to represent complementary chemokine-related mechanisms relevant to HCT and aGvHD. Previous studies have reported an association between recipient *CCL5* promoter polymorphisms and chronic GvHD [13]; however, the relevance of these findings to recipient susceptibility to pediatric aGvHD remains uncertain. *CCL5* −403G>A (rs2107538) was selected because CCL5/RANTES (regulated on activation, normal T cell expressed and secreted) is an inducible inflammatory chemokine involved in recruitment of CCR1-, CCR3- and CCR5-expressing leukocytes, and rs2107538 maps to the promoter/regulatory region of *CCL5*. The *CCL5 (RANTES)* gene was mapped within a cluster of CC chemokine genes on chromosome 17q11.2 [14]. *CCR9* G926A (rs12721497) was included because the CCL25-CCR9 axis contributes to tissue-specific lymphocyte homing, and donor-derived *CCR9* variation has previously been linked to skin GvHD [15,16]. *CXCL12* 3′A>G (rs1801157) was included because the CXCL12-CXCR4 axis regulates hematopoietic cell trafficking, marrow niche biology and immune-cell localization [17]. Previous HCT studies have also examined *IL6* and *CCL2* polymorphisms [18], recipient *CXCL10* variation [19], and a *CCR5* deletion [20] in relation to transplantation outcomes.

A pediatric cohort may provide a relatively less confounded setting than adult HCT cohorts because children generally have fewer age-related comorbidities and cumulative environmental exposures. Nevertheless, pediatric HCT cohorts are often small and clinically heterogeneous; therefore, immunogenetic and transplant-related factors, including donor–recipient human leukocyte antigen (HLA) disparities within the major histocompatibility complex (MHC), minor histocompatibility antigen mismatches, donor category, graft source, conditioning intensity, and donor–recipient sex mismatch, should be considered when interpreting candidate-gene findings. Cytomegalovirus (CMV) serostatus and

post-transplant CMV infection or reactivation may also influence immune activation and inflammatory responses following HCT.

In the present study, we tested whether recipient *CCL5*, *CCR9* and *CXCL12* polymorphisms were associated with aGvHD in 62 pediatric HCT recipients. To address the limited evidence regarding recipient chemokine-related genetic variation in pediatric hematopoietic cell transplantation, this retrospective, single-center study was designed as an exploratory candidate-gene association analysis. The three recipient polymorphisms were selected a priori based on their biological relevance to leukocyte recruitment, tissue-specific lymphocyte homing, and hematopoietic cell trafficking. Genotype associations were evaluated according to the occurrence of aGvHD (grade 0 versus grades I–IV), whereas the grade 0–I versus grade II–IV classification was used exclusively for descriptive summaries of disease severity. The study aimed to identify potentially relevant genetic associations that could guide future validation in larger, independent pediatric cohorts and subsequent functional investigations. Regulatory annotation was performed solely to provide biological context for the observed associations, without establishing causality, individual clinical risk prediction, or transplant eligibility criteria.

2. Results

This section presents the findings according to the study design: a retrospective candidate-gene analysis of 62 pediatric HCT recipients, grouped by aGvHD occurrence and severity, followed by exploratory adjustment for available transplant-related variables. Recipient *CCL5* rs2107538, *CCR9* rs12721497 and *CXCL12* rs1801157 genotypes were evaluated, with *CCL5* defined as the main signal only if its association differed from the comparator chemokine loci.

2.1. Association of CCL5, CCR9 and CXCL12 Polymorphisms with Acute Graft-Versus-Host Disease

Among the three investigated polymorphisms, only *CCL5* −403G>A rs2107538 showed a significant difference according to aGvHD status. The GA genotype was enriched in patients who developed aGvHD compared with those without aGvHD (72.0% vs. 32.4%, $p = 0.004$), whereas the GG genotype was more frequent in the non-aGvHD group (62.2% vs. 24.0%, $p = 0.007$). At the allele level, the A allele was also more common among patients with aGvHD (40.0% vs. 21.6%, $p = 0.044$). By contrast, the *CCR9* rs12721497 AA genotype showed similar frequencies in patients with and without aGvHD (48.0% vs. 45.9%; $p = 0.873$), as did the *CXCL12* rs1801157 GG genotype (64.0% vs. 51.4%; $p = 0.434$). Furthermore, allele-level comparisons revealed no statistically significant differences for *CCR9* (A allele: 74.0% vs. 73.0%; $p = 0.899$) or *CXCL12* (A allele: 18.0% vs. 27.0%; $p = 0.284$) between the two groups (Table 1).

Table 1. Comparison of recipient *CCL5* rs2107538, *CCR9* rs12721497, and *CXCL12* rs1801157 genotype and allele frequencies between patients with and without acute graft-versus-host disease.

CCL5 (rs2107538)	**aGvHD (+)**	**aGvHD (−)**	**OR**	**95% CI**	***p***
Genotypes	**n = 25**	**n = 37**			
AA	1 (4.0%)	2 (5.4%)	0.729	0.062–8.506	0.800
GA	18 (72.0%)	12 (32.4%)	5.357	1.762–16.288	**0.004** *
GG	6 (24.0%)	23 (62.2%)	0.192	0.061–0.597	**0.007** *
Allele					
A	20 (40.0%)	16 (21.6%)	2.417	1.095–5.334	**0.044** *
G	30 (60.0%)	58 (78.4%)			

Table 1. *Cont.*

CCR9 (rs12721497)	aGvHD (+)	aGvHD (−)	OR	95% CI	*p*
Genotypes	**n = 25**	**n = 37**			
AA	12 (48.0%)	17 (45.9%)	1.086	0.392–3.002	0.873
AG	13 (52.0%)	20 (54.1%)			
GG	0 (0.0%)	0 (0.0%)			
Allele					
A	37 (74.0%)	54 (73.0%)	1.054	0.467–2.379	0.899
G	13 (26.0%)	20 (27.0%)			
CXCL12 (rs1801157)	**aGvHD (+)**	**aGvHD (−)**	**OR**	**95% CI**	***p***
Genotypes	**n = 25**	**n = 37**			
AA	0 (0.0%)	2 (5.4%)	0.278	0.012–6.055	0.510
AG	9 (36.0%)	16 (43.2%)	0.738	0.259–2.098	0.607
GG	16 (64.0%)	19 (51.4%)	1.684	0.594–4.768	0.434
Allele					
A	9 (18.0%)	20 (27.0%)	0.592	0.244–1.437	0.284
G	41 (82.0%)	54 (73.0%)			

OR, odds ratio; CI, confidence interval; *CCL5*, C-C motif chemokine ligand 5; *CCR9*, C-C motif chemokine receptor 9; *CXCL12*, C-X-C motif chemokine ligand 12; aGvHD, acute graft-versus-host disease; aGvHD (+), patients with acute graft-versus-host disease (grades I–IV; n = 25); aGvHD (−), patients without acute graft-versus-host disease (grade 0; n = 37). Genotype and allele frequencies are presented as n (%). * Nominally statistically significant at $p < 0.05$.

For descriptive reporting of aGvHD severity, patients without aGvHD were classified as grade 0, whereas patients who developed aGvHD were graded I–IV. In the full cohort, 40 patients (64.5%) were classified as grades 0–I, whereas 22 patients (35.5%) were classified as grades II–IV (Table 2).

Table 2. Baseline demographic and transplant characteristics of the study cohort.

Demographic Characteristics	All Patients
Number of patients, n (%)	62 (100%)
Sex (female/male), n (%)	21 (33.9%)/41 (66.1%)
Age (years), mean ± SD	11.31 ± 5.74
aGvHD grade 0 (no aGvHD), n (%)	37 (59.7%)
aGvHD grades 0–I, n (%)	40 (64.5%)
aGvHD grades II–IV, n (%)	22 (35.5%)
Age by sex (years), mean ± SD (female/male)	10.86 ± 6.01/11.75 ± 4.27
aGvHD, n (%)	25 (40.3%)
Skin involvement	15 (60.0%)
Gastrointestinal involvement	7 (28.0%)
Intraoral involvement	1 (4.0%)
Liver involvement	2 (8.0%)
Grade I	3 (12.0%)
Grade II	13 (52.0%)
Grade III	5 (20.0%)
Grade IV	4 (16.0%)

Table 2. *Cont.*

Demographic Characteristics	All Patients
Diagnosis, n (%)	
AML	8 (12.9%)
ALL	25 (40.3%)
FAA	7 (11.3%)
Thalassemia Major	6 (9.7%)
Aplastic Anemia	2 (3.2%)
Hodgkin Lymphoma	3 (4.8%)
Other diseases/rare diagnoses	11 (17.7%)
Conditioning, n (%)	
Busulfan/cyclophosphamide	7 (11.3%)
Fludarabine-based	17 (27.4%)
Other/unspecified conditioning	38 (61.3%)
GvHD prophylaxis, n (%)	
Cyclosporine A plus methotrexate	53 (85.5%)
Cyclosporine A alone	3 (4.8%)
CellCept/tacrolimus	6 (9.7%)
Graft source, n (%)	
Peripheral blood graft	24 (38.7%)
Bone marrow	34 (54.8%)
Cord blood	4 (6.5%)
Transplant type, n (%)	
Matched unrelated donor	40 (64.5%)
Matched sibling donor	15 (24.2%)
Haploidentical	7 (11.3%)
HLA and donor, n (%)	
HLA-identical sibling donor	15 (24.2%)
HLA-identical unrelated transplants	21 (33.9%)
Alternative donor	26 (41.9%)
Survival, n (%)	
Relapse	11 (17.7%)
Death	13 (21.0%)
Female donor-to-male recipient mismatch (available n = 56)	14/56 (25.0%); aGvHD: 5/21 (23.8%) vs. no aGvHD: 9/35 (25.7%); Fisher exact $p = 1.000$
Additional available donor and CMV variables	
Donor sex (available n = 56)	Male: 31/56 (55.4%); female: 25/56 (44.6%); missing: 6/62 (9.7%)
Recipient CMV IgG (available n = 15)	Positive: 14/15 (93.3%); negative: 1/15 (6.7%); missing: 47/62 (75.8%)
Recipient/donor CMV IgG serostatus combinations (available n = 13)	R+/D−: 9/13 (69.2%); R+/D+: 3/13 (23.1%); R−/D−: 1/13 (7.7%); R−/D+: 0/13 (0.0%)
Recipient CMV IgM (available n = 23)	Negative: 21/23 (91.3%); positive: 2/23 (8.7%); missing: 39/62 (62.9%)

Table 2. *Cont.*

Demographic Characteristics	All Patients
Recipient/donor CMV IgM serostatus combinations (available n = 21)	R−/D−: 19/21 (90.5%); R+/D−: 2/21 (9.5%); other combinations: 0/21 (0.0%)
Donor CMV IgG (available n = 42)	Negative: 31/42 (73.8%); positive: 11/42 (26.2%); missing: 20/62 (32.3%)
Donor CMV IgM (available n = 46)	Negative: 45/46 (97.8%); positive: 1/46 (2.2%); missing: 16/62 (25.8%)

Abbreviations: GvHD, graft-versus-host disease; aGvHD, acute graft-versus-host disease; SD, standard deviation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; FAA, Fanconi aplastic anemia; HLA, human leukocyte antigen; CMV, cytomegalovirus; R, recipient; D, donor; IgG, immunoglobulin G; IgM, immunoglobulin M.

2.2. Multivariable Analysis of Factors Associated with Acute Graft-Versus-Host Disease

A multivariable logistic regression model was used to explore whether the observed *CCL5* association persisted after considering available transplant-related variables (Table 3). The variables in the model were defined as follows: age at HCT; recipient sex; graft source (bone marrow, peripheral blood grafts or cord blood); transplant type/donor category (matched unrelated donor, matched sibling donor or haploidentical donor); diagnosis group; conditioning category (busulfan/cyclophosphamide, fludarabine-based or other/unspecified); HLA compatibility (fully matched versus mismatched/alternative donor categories in the available records); GvHD prophylaxis regimen; and recipient chemokine genotypes. In this exploratory model, *CCL5* GA genotype and transplant type/donor category showed nominal associations with aGvHD, whereas HLA compatibility did not show a discernible effect. The absence of a detectable HLA effect in this small retrospective cohort should not be interpreted as evidence that HLA disparity is biologically unimportant; rather, the model was limited by sample size, broad clinical categories and event number.

Table 3. Multivariable logistic regression analysis for factors associated with acute graft-versus-host disease.

Exploratory Variables in Multivariable Model		
Parameters	**OR (95% CI)**	***p***
Age	0.676 (0.428–1.990)	0.996
Recipient sex	0.818 (0.208–3.218)	0.074
Graft source	0.708 (0.303–1.655)	0.425
Transplant type (donor category)	2.329 (1.129–4.805)	**0.022** *
Diagnosis	0.789 (0.578–1.076)	0.134
Conditioning category	0.717 (0.392–1.312)	0.281
HLA compatibility	0.497 (0.172–1.432)	0.195
GvHD prophylaxis regimen	0.674 (0.437–2.095)	0.276
CCL5 rs2107538 GA genotype	3.000 (0.244–6.770)	**0.012** *
CCR9	0.921 (0.333–2.545)	0.874
CXCL12	0.486 (0.891–1.461)	0.754

OR, odds ratio; CI, confidence interval; HLA, human leukocyte antigen; *CCL5*, C-C motif chemokine ligand 5; *CCR9*, C-C motif chemokine receptor 9; *CXCL12*, C-X-C motif chemokine ligand 12. Transplant type denotes donor category (matched unrelated donor, matched sibling donor, or haploidentical donor). Graft source denotes bone marrow grafts, peripheral blood grafts, or cord blood grafts. Conditioning denotes the major documented categories available in the retrospective dataset. HLA compatibility denotes fully matched versus mismatched/alternative donor categories. * Nominally statistically significant: $p < 0.05$.

2.3. Exploratory Analysis of Recipient Sex and Donor–Recipient Sex Mismatch

Recipient sex and donor–recipient sex mismatch were examined because sex-related immune differences and female donor-to-male recipient mismatch may contribute to alloimmune risk through sex-associated minor histocompatibility antigens and pregnancy-related donor immune history. Among patients with aGvHD, the *CCL5* GA genotype was the most frequent genotype in both female and male recipients, whereas the GG genotype was observed in 2 of 12 female patients (16.7%) and 4 of 13 male patients (30.8%). Among patients without aGvHD, the GG genotype was observed in 5 of 10 female patients (50.0%) and 18 of 27 male patients (66.7%). Because of the small subgroup sizes, these findings should be interpreted cautiously. No descriptive sex-based difference was apparent for *CCR9* or *CXCL12* genotype distributions (Table 4).

Table 4. Exploratory distribution of *CCL5* genotypes according to sex and aGvHD status.

Patients with aGvHD	**Female (n = 12)**	**Male (n = 13)**
***CCL5*(403 G/A) (n = 25)**		
AA	1 (8.3%)	0 (0.0%)
GA	9 (75.0%)	9 (69.2%)
GG	2 (16.7%)	4 (30.8%)
Patients without aGvHD	**Female (n = 10)**	**Male (n = 27)**
***CCL5*(403 G/A) (n = 37)**		
AA	0 (0.0%)	2 (7.4%)
GA	5 (50.0%)	7 (25.9%)
GG	5 (50.0%)	18 (66.7%)

No apparent differences in chemokine genotype distributions were observed across organ involvement categories among patients with aGvHD.

Among cases with available donor sex data (n = 56), female-to-male donor–recipient sex mismatch was observed in 14 patients (25.0%). This mismatch was present in 5 of 21 patients with aGvHD (23.8%) and 9 of 35 patients without aGvHD (25.7%), without evidence of a significant difference in this exploratory comparison (Fisher exact $p = 1.000$). Recipient/donor CMV IgG serostatus combinations were available for 13 pairs: recipient-positive/donor-negative in 9 patients, recipient-positive/donor-positive in 3 patients, recipient-negative/donor-negative in 1 patient and recipient-negative/donor-positive in 0 patients. Because CMV serostatus was missing in a large proportion of the cohort, CMV variables were summarized descriptively and were not included in multivariable models.

3. Discussion

The principal finding of this study is that recipient *CCL5* −403G>A (rs2107538), but not the evaluated recipient *CCR9* or *CXCL12* polymorphisms, was associated with aGvHD in a pediatric HCT cohort. This more focused conclusion is important because the data do not support a general association between all three chemokine loci and aGvHD; rather, they identify *CCL5* as the only signal emerging from the selected candidate-gene panel.

The biological significance of this finding should be considered in relation to the type of variant detected. Donor T-cell recognition in aGvHD is directed against recipient tissues and host-derived alloantigens or minor histocompatibility antigens, while inflammatory amplification is shaped by endothelial activation, innate immune pathways, cytokines and chemokine gradients [2,8,9]. Because rs2107538 is located in the *CCL5* promoter/regulatory region [13], it is unlikely to generate a novel coding alloantigen. Its potential relevance, if validated, is more plausibly related to altered transcriptional responsiveness, local CCL5

abundance and recruitment of CCR1-, CCR3- and CCR5-expressing effector leukocytes to inflamed target tissues [8,9].

This framework also helps explain why *CCL5*, but not the evaluated *CXCL12* or *CCR9* variants, may have shown an association in the present dataset. CCL5 is an inducible inflammatory chemokine positioned directly within effector leukocyte recruitment pathways that are active during tissue injury [8,9]. By contrast, CXCL12 is primarily linked to CXCR4-dependent hematopoietic cell trafficking and marrow niche biology [17], and the analyzed *CXCL12* variant may not capture inflammatory target-organ recruitment in this setting. CCR9 remains biologically relevant to tissue homing, particularly through the CCL25-CCR9 axis, but prior evidence has emphasized donor-derived *CCR9* genotype and skin-specific GvHD [16]; the present study evaluated recipient CCR9 genotype and did not detect an association.

The pediatric design partly offsets, but does not eliminate, the limitations of cohort size. Pediatric HCT recipients generally have fewer age-related comorbidities, less cumulative environmental exposure and a shorter history of chronic inflammatory disease than adult recipients, which may reduce some sources of biological heterogeneity. However, other key variables, including HLA disparity, donor category, graft source, donor–recipient sex mismatch, conditioning intensity and CMV status, remain important potential confounders [1,2,10]. In the exploratory model, HLA compatibility did not show a discernible association with aGvHD, whereas *CCL5* genotype and donor category did; this should be interpreted cautiously and not as evidence that HLA disparity lacks clinical relevance.

Several limitations should be considered when interpreting these findings. The cohort size was limited, which reduced statistical power for subgroup and multivariable analyses. Median age/range, detailed diagnoses within the other-disease category and granular conditioning intensity categories could not be reliably reconstructed from the available anonymized retrospective dataset. Accordingly, these variables are reported at the level supported by the available records, which limits the granularity of subgroup analyses. Donor genotyping was not performed, and available donor sex and CMV serology data were incomplete; therefore, these variables were reported descriptively rather than incorporated into definitive inferential models. Finally, regulatory annotation provides biological plausibility but does not demonstrate that rs2107538 alters *CCL5* transcription or immune-cell recruitment.

Overall, the study extends existing knowledge by narrowing the chemokine-related signal in this pediatric HCT cohort to a recipient *CCL5* promoter polymorphism. The results do not support *CCR9* or *CXCL12* as associated recipient loci in this dataset and do not justify clinical patient selection based on *CCL5* rs2107538 alone. Instead, the data identify *CCL5* transcriptional regulation as a focused candidate mechanism for future validation using donor–recipient paired genotyping, allele-specific expression, promoter reporter assays and immune-cell trafficking studies.

4. Materials and Methods

4.1. Study Design and Patient Characteristics

This retrospective single-center study included 62 pediatric patients who underwent allogeneic hematopoietic cell transplantation at Medipol Hospital between 2014 and 2019. The study protocol was approved by the Clinical Research Ethics Committee of Istanbul Medipol University (26.07.2022, no.: 644, E-10840098-772.02-4237) and was conducted in accordance with the Declaration of Helsinki. Genotyping analyses were performed in 2022 at the Department of Medical Biology, Istanbul Faculty of Medicine. The mean follow-up period was 85 days (range, 5–395 days). Of the 62 patients, 41 (66.1%) were male and 21 (33.9%) were female. The mean age of the patients was 11.31 ± 5.74 years. The mean age

was 11.75 ± 4.27 years in male patients and 10.86 ± 6.01 years in female patients. Only aggregate age data were available in the retrospective dataset; therefore, median age and range could not be reliably recalculated without returning to the source clinical records. The underlying diagnoses are summarized in Table 2.

Regarding donor type, 40 patients (64.5%) received grafts from matched unrelated donors (MUD), 15 (24.2%) from matched sibling donors (MSD), and 7 (11.3%) from haploidentical donors. In the multivariable model, transplant type refers to these donor categories. With respect to graft source, 34 patients (54.8%) received bone marrow grafts, 24 (38.7%) peripheral blood grafts, and 4 (6.5%) cord blood grafts. The patients were followed at the Pediatric Bone Marrow Transplantation Unit of Medipol Hospital and were classified into two groups according to the development of aGvHD. Acute GvHD was assessed according to European Society for Blood and Marrow Transplantation (EBMT) guidance [21]. Patients without aGvHD were classified as grade 0, whereas patients with aGvHD were graded I–IV. This grading structure is consistent with published Consensus and Mount Sinai Acute GVHD International Consortium (MAGIC) descriptions, in which grade I generally indicates limited cutaneous involvement without hepatic or gastrointestinal manifestations [22,23]. For descriptive severity summaries, grades 0–I (no or mild aGvHD; n = 40) were grouped separately from grades II–IV (more extensive aGvHD; n = 22), reflecting a commonly reported clinical severity threshold [22,23]. This severity grouping was used only for descriptive reporting; Table 1 compares patients with any aGvHD (grades I–IV; n = 25) with patients without aGvHD (grade 0; n = 37) for the recipient-genotype association analyses. All patients with aGvHD had histopathological confirmation. The mean time to aGvHD onset was 54.20 ± 37.24 days (range, 13–133 days).

Conditioning regimens included busulfan/cyclophosphamide (n = 7, 11.3%) and fludarabine-based protocols (n = 17, 27.4%). The remaining 38 patients (61.3%) were recorded as other/unspecified conditioning in the available retrospective records. Individual-level classification as reduced-intensity conditioning (RIC), myeloablative conditioning (MAC), total body irradiation (TBI)-based conditioning, treosulfan-based conditioning or other chemotherapy-specific protocols could not be reliably extracted from the available anonymized retrospective dataset and therefore was not used for further stratified analyses. GvHD prophylaxis consisted mainly of cyclosporine A plus methotrexate (n = 53, 85.5%), while 3 patients (4.8%) received cyclosporine A alone and 6 patients (9.7%) received CellCept/tacrolimus.

The baseline demographic, clinical, and transplantation-related characteristics of the study cohort are summarized in Table 2. Acute GvHD developed in 25 of 62 patients (40.3%). Among the patients with aGvHD, skin involvement was observed in 15 cases, gastrointestinal involvement in 7 cases, intraoral involvement in 1 case, and liver involvement in 2 cases (Table 2).

Available donor sex and cytomegalovirus (CMV) serology variables were additionally extracted from the retrospective records and summarized descriptively. Female-to-male donor–recipient sex mismatch was specifically derived because of its known relevance to post-transplant alloimmune outcomes. Recipient/donor CMV IgG serostatus combinations were also summarized where both recipient and donor values were available. Because CMV variables had substantial missingness and were not complete at the patient level for all 62 cases, they were not used to infer causality or to build a clinical patient-selection model.

Data are presented as n (%) unless otherwise specified; ages are reported as mean ± standard deviation (SD). Percentages for aGvHD organ involvement and grades I–IV use patients with aGvHD (n = 25), whereas grades 0–I and II–IV use the full cohort (n = 62). Donor sex and CMV serology results use available records; missing percentages refer to the full cohort. Median age and range, individual diagnoses in the other-disease

category, and detailed conditioning subtypes were unavailable in the anonymized retrospective dataset. The grade 0–I versus II–IV categorization was used for descriptive reporting and not for the genotype comparisons presented in Table 1.

4.2. DNA Extraction and Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) Genotyping

Genomic DNA was extracted from peripheral blood samples using the Thermo Fisher PureLink Genomic DNA Mini Kit™ in accordance with the manufacturer's protocol (Thermo Fisher Scientific, Waltham, MA, USA). DNA concentration and purity were evaluated before the samples were stored at −20 °C until analysis. Polymerase chain reaction (PCR) amplification was performed for the target regions containing *CCL5* −403G>A (rs2107538), *CCR9* G926A (rs12721497), and *CXCL12* 3′A>G (rs1801157). Following restriction enzyme digestion, the PCR products were separated by gel electrophoresis, and genotypes were assigned according to the expected fragment patterns for each polymorphism.

4.3. Regulatory Annotation of Selected Variants

The selected variants were annotated using Ensembl Variant Effect Predictor (VEP) [24] to identify genomic context, transcript consequences and regulatory-region overlap. Regulatory potential was further reviewed using RegulomeDB [25] and HaploReg [26], and public expression quantitative trait loci resources, including the Genotype-Tissue Expression GTEx project [27], were consulted to determine whether the variants had reported expression associations in relevant tissues. These annotations were used only to contextualize the genetic results and were not treated as experimental evidence of causality.

4.4. Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA) and R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were presented as numbers and percentages and were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were assessed for normality and presented as mean ± standard deviation or median with interquartile range, according to their distribution.

Hardy–Weinberg equilibrium was assessed for each chemokine gene polymorphism using Haploview [28]. Genotype and allele frequencies were compared between patients with and without aGvHD. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of associations. A p value of <0.05 was considered nominally statistically significant. To account for multiple testing, false discovery rate (FDR)-adjusted q values were calculated using the Benjamini–Hochberg procedure [29]. aGvHD severity was summarized descriptively as grades 0–I versus II–IV across the full cohort; genetic association tests were based on aGvHD occurrence (grade 0 versus grades I–IV), not this severity categorization.

Multivariable logistic regression analysis was performed to identify factors associated with the development of aGvHD. Variables were selected a priori based on clinical relevance and included age, sex, donor type, graft source, HLA compatibility, GvHD prophylaxis, and chemokine polymorphisms. Given the limited number of aGvHD events, the number of variables included in the final model was restricted to reduce the risk of model overfitting, and the results were interpreted cautiously. Donor sex, female-to-male donor–recipient mismatch and CMV serology variables were summarized descriptively. Female-to-male mismatch was examined in an exploratory Fisher exact comparison among patients with available donor sex data, but CMV serostatus combinations were not included in the multivariable model because of incomplete availability across the cohort. Because of

the exploratory design and limited event number, regression estimates were not used to construct or validate a clinical prediction model.

5. Conclusions

In this pediatric HCT cohort, recipient *CCL5* −403G>A (rs2107538) was the only investigated chemokine polymorphism associated with aGvHD, whereas recipient *CCR9* rs12721497 and *CXCL12* rs1801157 did not demonstrate similar relationships. The findings support a focused hypothesis in which regulatory variation in *CCL5* may contribute to chemokine-mediated leukocyte recruitment during acute alloimmune injury. Across the full cohort, aGvHD severity was summarized as grade 0–I versus grade II-IV, and donor sex and CMV serostatus variables were reported descriptively where available. Several limitations should be acknowledged, including the retrospective, single-center study design, the relatively small pediatric cohort (n = 62), the absence of donor genotyping, and incomplete information regarding conditioning regimens and donor–recipient CMV serostatus. These limitations restricted the comprehensive assessment of potential confounding factors and the interpretation of exploratory multivariable analyses. Future prospective, multicenter studies involving larger pediatric cohorts, paired donor–recipient genotyping, systematically collected transplant-related clinical data, and functional investigations of *CCL5* transcriptional regulation and immune-cell trafficking are warranted to validate the observed genetic associations and clarify their underlying biological mechanisms. Until these findings are independently replicated and functionally validated, they should be considered hypothesis-generating rather than clinically actionable predictors for aGvHD risk assessment or transplant candidate selection.

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