Human Immunology xxx (2024) 110791

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Research article

Donor genetic determinant of thymopoiesis, rs2204985, and stem cell transplantation outcome in a multipopulation cohort

Julia Nihtilä [a], Urpu Salmenniemi [b,c], Maija Itälä-Remes [d], Rachel E Crossland [e], David Gallardo [f], Katarzyna Bogunia-Kubik [g], Piotr Łacina [g], Maria Bieniaszewska [h], Sebastian Giebel [i], Kati Hyvärinen [a], Eliisa Kekäläinen [j], Jarmo Ritari [a], Jukka Partanen [a,*]

[a] Research and Development, Finnish Red Cross Blood Service, Helsinki, Finland
[b] Comprehensive Cancer Center, Stem Cell Transplantation Unit, Helsinki University Hospital, Finland
[c] University of Helsinki, Finland
[d] Turku University Hospital, Finland
[e] Translational and Clinical Research Institute, Faculty of Medical Science, Newcastle University, UK
[f] Department of Hematology, ICO Girona, Spain
[g] Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
[h] Department of Hematology and Transplantology, Medical University of Gdansk, Gdansk, Poland
[i] Department of Bone Marrow Transplantation and Hematology-Oncology, Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Gliwice Branch, Gliwice, Poland
[j] Department of Clinical Microbiology, Helsinki University Hospital, and Translational Immunology Research Program, University of Helsinki, Helsinki, Finland

ARTICLE INFO

Keywords:
Stem Cell Transplantation
Thymus
Genetic association
Allogeneic
Multipopulation
Thymopoiesis

ABSTRACT

Background: A genetic polymorphism, rs2204985, has been reported to be associated with the diversity of T-cell antigen receptor repertoire and TREC levels, reflecting the function of the thymus. As the thymus function can be assumed to be an important factor regulating the outcome of stem cell transplantation (SCT), it was of great interest that rs2204985 showed a genetic association to disease-free and overall survival in a German SCT donor cohort. Tools to predict the outcome of SCT more accurately would help in risk assessment and patient safety.

Objective: To evaluate the general validity of the original genetic association found in the German cohort, we determined genetic associations between rs2204985 and the outcome of SCT in 1,473 SCT donors from four different populations.

Study design: Genetic associations between rs2204985 genotype AA versus AG/GG and overall survival (OS) and disease-free survival (DFS) in 1,473 adult, allogeneic SCT from Finland, the United Kingdom, Spain, and Poland were performed using the Kaplan-Meier analysis and log-rank tests. We adjusted the survival models with covariates using Cox regression.

Results: In unrelated SCT donors (N = 425), the OS of genotype AA versus AG/GG had a trend for a similar association (p = 0.049, log-rank test) as previously reported in the German cohort. The trend did not remain significant in the Cox regression analysis with covariates. No other associations were found.

Conclusion: Weak support for the genetic association between rs2204985, previously also associated with thymus function, and the outcome of SCT could be found in a cohort from four populations.

1. Introduction

Clave and colleagues[1] reported, based on a genome-wide association study, that a genetic polymorphism, assigned as rs2204985 and located in the T-cell antigen gene segment, was associated with the diversity of T-cell antigen receptor repertoire and TREC levels, reflecting the function of the thymus. They also reported that the transplantation of human genotype rs2204985 AA stem cells into a mouse model resulted in lower thymocyte numbers and T-cell antigen receptor repertoire than the AG and GG genotypes. Hence, potentially a very useful genetic biomarker for thymus function and thymopoiesis was identified. The function of the thymus, which is the major tissue for T lymphocyte education, can be regarded as one of the important factors for a successful outcome following allogeneic stem cell transplan-

* Corresponding author.
E-mail address: jukka.partanen@veripalvelu.fi (J. Partanen).

https://doi.org/10.1016/j.humimm.2024.110791
Received 14 December 2023; Revised 21 February 2024; Accepted 21 March 2024
Available online xxxx

tation (SCT)[2], whereby its declining function increases, for example, the risk of relapse after SCT. It, therefore, was of great interest that Tsamadou and colleagues[3] reported that the donor AA genotype of rs2204985 was associated with inferior overall survival (OS, p = 0.003) and disease-free survival (DFS, p = 0.001), particularly in 9/10 HLA-matched unrelated SCT. This effect was reported to be driven by a higher risk for relapse (p = 0.026) and non-relapse mortality (p = 0.042). Their study cohort comprised of 2,016 adult unrelated-donor SCTs from Germany, transplanted between 2000 and 2013. As biomarkers reliably predicting the outcome of SCT would be of interest for better risk assessment and consequent safer treatment, we aimed to see whether the associations can be confirmed in other populations. Hence, we studied the genetic association of rs2204985 with the outcome of SCT in an independent cohort of 1,473 adult allogeneic SCT donors.

2. Materials and methods

2.1. SCT cohort

The SCT cohort consisted of 1,473 allogeneic SCT donors, of whom 765 were from Finland (Helsinki and Turku University Hospitals), 334 from the UK (The Freeman Hospital, Newcastle Hospitals NHS Foundation Trust), 272 from Spain (IDIBGI Biobank), and 102 from Poland (Hematological Departments of the Medical University of Gdansk and Maria Skłodowska-Curie Memorial Cancer Centre, Institute of Oncology in Gliwice). The study participants gave informed consent, and the Ethical Review Boards of each collaborating hospital granted ethical permits for use of the data. The permit numbers are V/74832/2017 (Finland), HUS/2152/2020 (Finland), ETMK 78/2012 (Finland), Biobank IDIBGI B.0000872 (Spain), 14/NE/1136 (UK), and KB-561/2019 (Poland). The clinical characteristics are summarized in Table 1.

2.2. Genotyping and imputation

Genomic DNA samples from the SCT donors underwent genome-wide genotyping at the Finnish Institute of Molecular Medicine, Helsinki, Finland, with the following arrays: Illumina Immunochip v1, Immunoarray v2, Illumina Global Screening Array v2 or v3. A subset of the Finnish samples was genotyped using an exome sequencing pipeline[4] at the McGill Genome Centre, Montreal, Canada. Following the genotyping, the data were harmonized with a lift-over[5] to the same human reference genome build, GRCh38/hg38, followed by genome-wide SNP imputation[6]. In lift-over and imputation, the reference panels used were the THL Biobank's SISu v3 reference panel (obtained from THL Biobank, study number: BB2019_12) for the Finnish samples and a reference panel of the European samples of the 1000Genomes project for the other samples.

Table 1
Characteristics of the SCT cohort

		All donors	Unrelated donors
Number of SCT donors, n		1473	427
Country of origin, n (%)	Finland	765 (52)	219 (51)
	UK	334 (23)	171 (40)
	Spain	272 (18)	0 (0)
	Poland	102 (7)	37 (9)
HLA match level, n (%)	10/10	1194 (81)	294 (69)
	9/10	57 (4)	44 (10)
	Other**	222 (15)	89 (21)
Donor type, n (%)	Unrelated	427 (29)	427 (100)
	Related	1046 (71)	0 (0)
SCT time, years		1984–2022	1993–2022
	Missing, n (%)	25 (2)	0 (0)
Recipient age in years, median (range)*		49 (18–73)	50 (18–72)
	Missing, n (%)	4 (0)	0 (0)
Donor age in years, median (range)*		41 (4–78)	34 (16–71)
	Missing, n (%)	447 (30)	45 (11)
Donor-recipient gender, n (%)	Male-male	519 (35)	205 (48)
	Male-female	366 (25)	111 (26)
	Female-male	292 (20)	41 (10)
	Female-female	277 (19)	69 (16)
	Missing	19 (1)	1 (0)
Stem cell source, n (%)	Peripheral blood	1051 (71)	335 (78)
	Bone marrow	416 (28)	89 (21)
	Both	3 (0)	2 (0)
	Missing	3 (0)	1 (0)
Conditioning regimen, n (%)	Myeloablative	860 (58)	217 (51)
	Reduced intensity	606 (41)	210 (49)
	Missing	7 (0)	0 (0)
aGvHD, n (%)	grade II-IV	380 (26)	112 (26)
	Missing	13 (1)	9 (2)
cGvHD, n (%)	Limited or extensive	656 (45)	201 (47)
	Missing	214 (15)	59 (14)
Relapse, n (%)	Yes	451 (31)	142 (33)
	No	1012 (69)	279 (65)
	Missing	10 (1)	6 (1)
Overall survival, n (%)	Found	1443 (98)	425 (100)
	Missing	30 (2)	2 (0)
Disease-free survival, n (%)	Found	1443 (98)	425 (100)
	Missing	30 (2)	2 (0)

GvHD, graft-versus-host disease; aGvHD, acute GvHD; cGvHD, chronic GvHD
*Missing ages were imputed; ** HLA matches worse than 9/10.

After imputation, the variants were filtered with an INFO score threshold > 0.5, leaving rs2204985 in the data. INFO scores for rs2204985 were 0.98 in the donors from Finland (imputed in parts, an average of 0.94, 0.97, 1, 1), 1 in the UK, 0.85 in Spain, and 0.55 in Poland. This was followed by quality control using Plink1.9[7] with the following filters: missing genotype rate of 0.05 for variants, missing genotype rate of 0.1 for samples, Hardy-Weinberg equilibrium test threshold 1e-6, and minor allele frequency 0.05.

We imputed[8] the alleles of all classical HLA genes using the genome data and used this information for calculating HLA matches between donors and recipients.

2.3. Statistical analyses

For studying OS and DFS for the genotypes AA vs. AG/GG, we used Kaplan-Meier analysis and log-rank tests (ggsurvfit R package version 0.3.1). Further, we adjusted the survival models with covariates using Cox regression (survival R package, version 3.5–7). We also performed genotypic association testing for the endpoints acute GvHD grade II-IV, chronic GvHD, and relapse with Plink2[9]. The covariates used in multivariate survival analyses and association testing were recipient and donor ages, donor type, graft, recipient – donor sex combination, preconditioning, country, diagnosis (AML or MDS vs. others), HLA matching level, and transplantation year. The subgroups in which the analyses were run were all donors, all 9/10 HLA-matched donors, all 10/10 HLA-matched donors, unrelated donors, 9/10 HLA-matched unrelated donors, and 10/10 HLA-matched unrelated donors.

We also performed meta-analyses combining our multivariate survival results with those of Tsamadou and colleagues[3] using meta the R package[10]. Random effects models were used to account for the results coming from different populations and between-study heterogeneity indicated by tauˆ2 and Iˆ2 statistics. We combined the results separately in 9/10 HLA-matched unrelated donors, 10/10 HLA-matched unrelated donors, and all results (all 9/10 and 10/10 HLA-matched unrelated donors; 9/10 and 10/10 HLA-matched unrelated donors from Tsamadou and colleagues and all our unrelated donors).

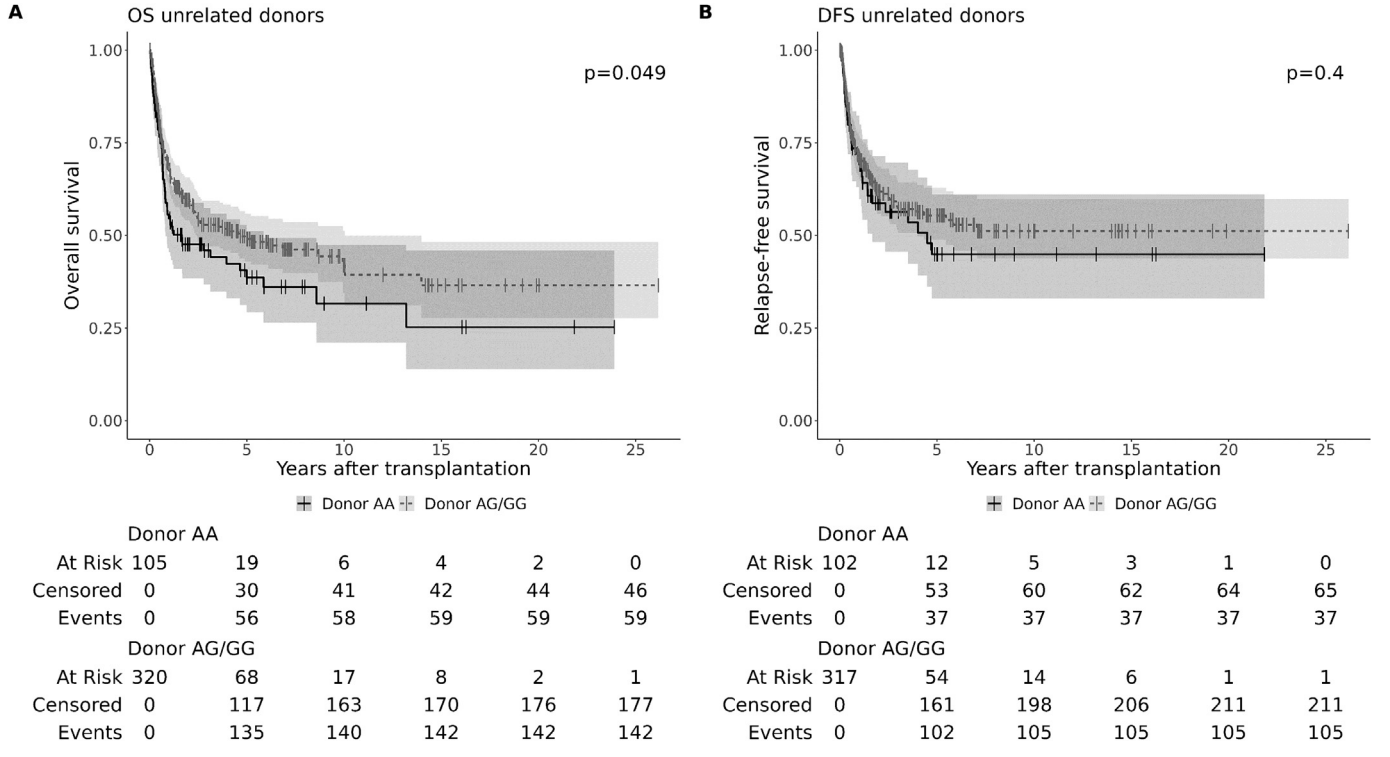
3. Results

3.1. Survival analysis

The results of the Kaplan-Meier analysis for donor rs2204985 genotypes AA vs. AG/GG were: (i) OS p = 0.4 (Supplementary Fig. 1) for all donors (N = 1442), (ii) DFS p = 0.8 (Supplementary Figure 6) for all donors (N = 1393), (iii) OS p = 0.049 (Fig. 1) for unrelated donors (N = 425), and (iv) DFS p = 0.4 (Fig. 1) for unrelated donor SCT (N = 419). When adjusted with covariates in the Cox regression, the p-values for the AA genotype were 0.4 for OS in all donors, >0.9 for DFS in all donors, 0.081 for OS in unrelated donors, and 0.6 for DFS in all donors. More detailed results for these and other subgroups can be found in the Supplementary material, including 9/10 HLA-matched unrelated SCT (overall survival: Supplementary Fig 5 and disease-free survival Supplementary Fig 10). The results of the meta-analyses combining the present results with those of Tsamadou and colleagues, are shown in Supplementary Figs 17–20.

3.2. Association analysis

For the endpoints in the association testing, none were statistically significant in any of the subgroups tested. The results are shown in the Supplementary material. As the Finnish SCT donors (N = 765) accounted for more than half of the donors, we also analysed associations in each population separately but found no evidence for associations (Supplementary material).



Donor AA						
At Risk	105	19	6	4	2	0
Censored	0	30	41	42	44	46
Events	0	56	58	59	59	59
Donor AG/GG						
At Risk	320	68	17	8	2	1
Censored	0	117	163	170	176	177
Events	0	135	140	142	142	142

Donor AA						
At Risk	102	12	5	3	1	0
Censored	0	53	60	62	64	65
Events	0	37	37	37	37	37
Donor AG/GG						
At Risk	317	54	14	6	1	1
Censored	0	161	198	206	211	211
Events	0	102	105	105	105	105

Fig. 1. Effect of donor rs2204985 genotype (AA versus AG/GG) on overall survival (OS, Panel A) and disease-free survival (DFS, Panel B) in unrelated SCTs. Risk tables below the survival curves indicate the number of donors still in the study at a timepoint and the number of donors lost due to censoring and the event occurring.

4. Discussion

The outcome of SCT is a complex multifactorial trait, whose genetic components are not readily dissected. It is therefore not surprising that we found, at best, only modest support for the original findings; the AA genotype showed a borderline association with inferior overall survival in unrelated donor SCT in our cohort. A key factor affecting thymus function is age; there were no differences between the cohort of Tsamadou and colleagues[3] and that of ours, the median ages of the patients were just above 50 years in both cohorts. Moreover, no clear difference in sex distribution was evident and the cohort sizes were close to each other. The present cohort, however, was more heterogeneous and included both sibling and unrelated donors, and SCTs were from many hospitals and four different countries. The year of transplantation was one of the covariates in the present analyses, but the fact that some SCTs dated back to the 1980s certainly increased heterogeneity. We subdivided our cohort into relevant groups and included covariates in the analyses to account for heterogeneity, but obviously, these lead to lower numbers of cases per subgroup. We furthermore were not able to test all endpoints reported by Tsamadou and colleagues[3], limiting our approach. As the hazard ratios were relatively small in the study of Tsamadou and colleagues, the heterogeneity and limitations of our cohort may hide a possible effect. We performed a meta-analysis combining the results of the present and the original study. It was clear that for 9/10 HLA-matched SCT, our cohort provided little additional data; there were only 57 cases. As the strongest evidence in the original report[3] was in 9/10 HLA-matched SCT, the sparse number of these SCTs in the present study is a major limitation. Nevertheless, the trend and direction of the associations remained. It is not clear why the thymopoiesis effect should be detectable primarily in the 9/10 HLA-matched SCTs. One explanation, albeit missing any real evidence, could be the higher alloantigenic load caused by the mismatched HLA that, together with impaired thymus function, could affect the outcome of SCT. It is, however, of note that the level of HLA matching showed no associations in the multivariate analyses of the present cohort.

To conclude, our present study provided modest evidence and support for a genetic association between rs2204985 and the outcome of SCT. We consequently must agree with Tsamadou[3] and colleagues stating that “confirmatory studies in larger independent cohorts are warranted before conclusions are drawn”.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The SISu v3 Imputation reference panel used for the research was obtained from THL Biobank (study number: BB2019_12). We thank all study participants for their generous participation in biobank research.

CRediT authorship contribution statement

Julia Nihtilä: Conceptualization, Methodology, Software, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Urpu Salmenniemi:** Investigation, Resources. **Maija Itälä-Remes:** Investigation, Resources. **Rachel E Crossland:** Investigation, Resources. **David Gallardo:** Investigation, Resources. **Katarzyna Bogunia-Kubik:** Investigation, Resources. **Piotr Łacina:** Investigation, Resources. **Maria Bieniaszewska:** Investigation, Resources. **Sebastian Giebel:** Investigation, Resources. **Kati Hyvärinen:** Methodology, Software. **Eliisa Kekäläinen:** Conceptualization. **Jarmo Ritari:** Conceptualization, Methodology, Software, Data Curation, Writing - Original Draft, Supervision, Project administration, Funding acquisition. **Jukka Partanen:** Conceptualization, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Funding

The present study was supported by research grants from the TEKES (currently Business Finland) for the SalWe project (for JP), the Academy of Finland (for JP), Cancer Foundation Finland (for JP and JR), VTR funding from the Government of Finland to the Blood Service, COST Eurograft ActionCA17138 (for K B-K, PL, JN, JP, RC), the National Science Centre (Poland) (grant No. 2018/31/B/NZ2/03065) (for K B-K, PL, MB and SG), National Institute of Health Carlos III (ISCIII) (Spain), co-funded by the European Union (grants number PI17/00815 and PI20/01353) (for DG), and Newcastle Hospitals Charity (REC).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2024.110791.

References

[1] E. Clave, I.L. Araujo, C. Alanio, et al, Human thymopoiesis is influenced by a common genetic variant within the TCRA-TCRD locus, Sci. Transl. Med. 10 (457) (2018) eaao2966, https://doi.org/10.1126/scitranslmed.aao2966.

[2] W. Krenger, B.R. Blazar, G.A. Holländer, Thymic T-cell development in allogeneic stem cell transplantation, Blood 117 (2011) 6768–6776, https://doi.org/10.1182/blood-2011-02.

[3] C. Tsamadou, S. Gowdavally, U. Platzbecker, et al, Donor genetic determinant of thymopoiesis rs2204985 impacts clinical outcome after single HLA mismatched hematopoietic stem cell transplantation, Bone Marrow Transplant. 57 (10) (2022) 1539–1547, https://doi.org/10.1038/s41409-022-01751-1.

[4] A. Morin, T. Kwan, B. Ge, et al, Immunoseq: the identification of functionally relevant variants through targeted capture and sequencing of active regulatory regions in human immune cells, BMC Med. Genomics 9 (1) (2016) 59, https://doi.org/10.1186/s12920-016-0220-7.

[5] K. Pärn, J. Nunez Fontarnau, M.A. Isokallio, et al. Genotyping chip data lift-over to reference genome build GRCh38/hg38 v2. *FIMMHumGen Sequencing Informatics*. Published online 2019. doi: 10.17504/protocols.io.xbhfij6.

[6] Pärn K, Isokallio MA, Nunez Fontarnau J, Palotie A, Ripatti S, Palta P. Genotype imputation workflow v3.0 V.2. FIMMHumGen Sequencing Informatics. Published online 2019. doi:dx.doi.org/10.17504/protocols.io.xbgfijw.

[7] S. Purcell, B. Neale, K. Todd-Brown, et al, PLINK: a tool set for whole-genome association and population-based linkage analyses, Am. J. Hum. Genet. 81 (3) (2007) 559–575, https://doi.org/10.1086/519795.

[8] J. Ritari, K. Hyvärinen, J. Clancy, P.J. FinnGen, S. Koskela, Increasing accuracy of HLA imputation by a population-specific reference panel in a FinnGen biobank cohort, NAR Genom Bioinform. 2 (2) (2020), https://doi.org/10.1093/nargab/lqaa030.

[9] C.C. Chang, C.C. Chow, L.C.A.M. Tellier, S. Vattikuti, S.M. Purcell, J.J. Lee, Second-generation PLINK: rising to the challenge of larger and richer datasets, GigaScience 4 (1) (2015) 7, https://doi.org/10.1186/S13742-015-0047-8/2707533.

[10] S. Balduzzi, G. Rücker, G. Schwarzer, How to perform a meta-analysis with R: a practical tutorial, Evid. Based Ment. Health 22 (4) (2019) 153–160, https://doi.org/10.1136/EBMENTAL-2019-300117.