A Single-Nucleotide Polymorphism (rs1131243) of the Transforming Growth Factor Beta Signaling Pathway Contributes to Risk of Acute Rejection in Chinese Renal Transplant Recipients

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Background: Acute rejection (AR) is a common complication of kidney transplantation. The transforming growth factor beta (TGF-β) signaling pathway has been observed to be involved in several cellular functions. Our study aimed to investigate the correlations between single-nucleotide polymorphisms (SNPs) in TGF-β-related genes and the risk of AR in renal transplant recipients.

Material/Methods: This retrospective, single-center study included 200 Chinese renal transplant recipients. All exons, exon/intron boundaries, and flanking regions of the TGF-β signaling pathway were detected by targeting sequencing (TS) based on next-generation sequencing technology. Tagger SNPs and haplotypes were identified after adjustment. A general linear model (GLM) was used to explore the confounding effect of clinical variables. Five adjusted inheritance models were utilized to investigate the influence of SNPs on AR, and Banff score was applied to evaluate the effect of related SNPs on pathological changes.

Results: A total of 188 SNPs on TGF-β genes were detected. Analysis of adjustment led to identification of 31 tagger SNPs and 10 haplotype blocks. After the analysis of a general linear model and 5 sirolimus-adjusted multiple inheritance models, 1 of the SNPs – rs1131243 on the TGF-βR3 gene – was observed to be significantly associated with the occurrence of AR. Based on Banff score, no significant association was observed between SNPs and pathological changes.

Conclusions: In this study, we observed that the SNP rs1131243 on the TGF-βR3 gene was significantly associated with the occurrence of AR in Chinese renal transplant recipients.

MeSH Keywords: Kidney Transplantation • Polymorphism, Single Nucleotide • Transforming Growth Factor beta

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/918142
Renal transplant is considered as the most effective therapeutic treatment for end-stage renal disease [1,2]. Compared with dialysis treatments, renal transplant allows patients to have a better quality of life and longer survival [3]. However, various complications, such as acute rejection (AR), chronic allograft dysfunction, and immunosuppressive agent-related nephrotoxicity, still strictly limit its wide application [4]. Graft loss, increased risk of chronic allograft dysfunction, and poor long-term outcomes are some of the clinical concerns related to AR [5,6]. Therefore, understanding the pathogenesis of AR is imperative for improving long-term and short-term prognosis in patients.

The TGF-β family of polypeptides includes 3 TGF-β isoforms, activins, nodal, and bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs) [7]. In contrast to the large number of TGF-β ligands, SMAD proteins, as fewer receptors and downstream intracellular effectors, mediate the transduction of intracellular signaling. In mammals, 7 type I receptors and 5 type II receptors were identified and were shown to form a heteromeric complex of type I and type II transmembrane receptors [8–10].

The TGF-β signaling pathway is widely involved in regulation of cellular responses, including cell growth and differentiation, apoptosis, homeostasis, and many other cellular functions [11,12]. TGF-β has various regulatory functions which range from specifying tissue pattern formation as morphogens during embryonic development to maintaining physiological homeostasis as cytokines in adult organisms. It is now widely accepted that TGF-β is a bifunctional regulator. TGF-β is a suppressor of early-stage tumors and has also been observed to promote tumor growth and progression by inducing epithelial-to-mesenchymal transition (EMT) [13–15]. TGF-β has immune-suppressive functions in several diseases [16,17]. Moreover, TGF-β, as an immune-regulatory cytokine, plays a crucial role in the development, homeostasis, and tolerance of T cells [18]. The immune response mediated by T cells is the main cause of AR, but the association of the TGF-β signaling pathway with AR is yet to be fully determined.

A genome-wide association study (GWAS) identified genetic variants and their association with human diseases, which enables analysis of millions of single-nucleotide polymorphisms (SNPs) in the genome. GWAS may be applied to identify novel molecules and pathways involved in acute rejection and to predict transplant outcomes [19]. The aim of this retrospective, single-center study was to investigate the correlation between SNPs in TGF-β signaling pathway-related genes and the susceptibility to AR by use of target sequencing (TS) based on next-generation sequencing (NGS) at our center.

Material and Methods

Study design and population

This work is a retrospective, single-center, cohort study, which was carried out to explore the influence of SNPs in TGFb signaling pathway-related genes (TGF-β1, TGF-β2, TGF-β3, TGF-βR1, TGF-βR2, TGF-βR3, SMAD2, SMAD3, SMAD4) on the risk of AR in renal transplant recipients. The Ethics Committee of the First Affiliated Hospital of Nanjing Medical University approved the protocols followed in this study (2016-SR-029). Written informed consents were obtained from all transplant recipients. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Recipients in this study were strictly limited to living-related transplantation of donors to lineal or collateral relatives not beyond the third degree of kinship or transplantation of kidney donors after cardiac death, from 2011 to 2015.

This study included 200 renal transplant recipients who received renal transplant between 1 February 2011 and 1 December 2015 at the kidney transplant center of the Nanjing Medical University First Affiliated Hospital, as detailed in our previous study [20]. Briefly, we enrolled adult patients who underwent single-kidney transplantation, with or without AR period confirmed by biopsy. Medical records of enrolled patients were meticulously extracted and reviewed by 2 clinicians (ZJ Wang and RY Tan).

Clinical data on age, sex, height, AR incidence, delayed graft function (DGF), and immunosuppressive protocols were also extracted independently by 2 authors (Ming Zheng and Jiajun Zhou). AR after kidney transplantation was diagnosed by 2 independent pathologists through application of histological examination of hematoxylin-eosin staining and immunohistological staining based on the Banff 15 criteria [21]. AR scores were classified by the degree of interstitial infiltration and intimal arteritis according to the type/grade of AR based on the Banff 15 criteria.

Ethics approval and consent to participate

The Ethics Committee of the First Affiliated Hospital of Nanjing Medical University approved the protocols followed in this study (2016-SR-029). Written informed consent was obtained from all transplant recipients. Peripheral blood samples (2 mL) from each recipient were collected. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration.
and its later amendments or comparable ethical standards. Recipients in this study were strictly limited to living-related transplantation of donors to lineal or collateral relatives not beyond the third degree of kinship or transplantation of kidney donors after cardiac death, from 2011 to 2015.

Immunosuppressive protocols

All recipients in our center underwent routine immunosuppressive protocols that included 3 or 4 drugs. Briefly, the basic protocol consisted of tacrolimus taken at an initial dosage of 0.2 mg/kg/day (q12h), with mycophenolate mofetil (MMF) at an initial dosage of 0.75–1.0 g/day (q12h) 24–48 h after transplantation, and prednisone, combined with or without sirolimus at an initial dosage of 1 mg/day (qd). The combined usage of sirolimus or not depended on drug concentrations, immunoreaction, and clinical symptoms of recipients. These dosages were later calibrated according to the serum creatinine levels and drug concentrations. As determined by the tolerance and response of recipients, tacrolimus could later be changed to cyclosporin A during follow-up. For patients who had AR episodes, methylprednisolone was administered intravenously at a dosage of 200 mg/day for 3–5 days. Detailed information on the immunosuppressive agents used in our center can be found in our previous study [22].

Sample collection, preparation, and TS

Peripheral blood samples (2 mL) from each recipient were collected. After DNA extraction, the concentration and purity of genomic DNA (gDNA) was quantitatively analyzed and gene integrity was accessed through application of agarose gel electrophoresis. A pool containing upstream and downstream oligonucleotides was selected as gDNA hybrids specific to target regions of interest. Then, the gDNA was fragmented and the adapter-ligated DNA was amplified through selective, limited-cycle polymerase chain reaction. The captured libraries were denatured and loaded into an Illumina cBot instrument as per the manufacturer’s instructions. Then, sequencing data based on the human reference sequence UCSC hg19 assembly (NCBI build 37.2) was analyzed using the Genome Analysis Tool Kit, Picard software, and dbSNP 132. During this procedure, putative somatic variant cells with 2 separate programs – MuTect 1.1.5 and VarScan 2.3.6 – were also observed.

Statistical analysis

Data are presented as mean±standard deviation (SD) except when stated otherwise. We explored minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) by using R packages genetics (genetics: Population Genetics, R package version 1.3.8.1.). Linkage disequilibrium (LD) blocks were analyzed by using Haploview version 4.2 (Broad Institute, Cambridge, MA, USA). The general linear model (GLM) was applied to examine the influence of clinical variables on AR. We used R package SNPassoc (SNPassoc: SNPs-based whole-genome association studies, R package version 1.9–2.) to perform 5 sirolimus-adjusted multiple inheritance models, including codominant model 1 (major allele homozygotes vs. heterozygotes), codominant model 2 (major allele homozygotes vs. minor allele homozygotes), dominant model (major allele homozygotes vs. minor allele homozygotes plus heterozygotes), recessive model (major allele homozygotes plus heterozygotes vs. minor allele homozygotes), over-dominant model (heterozygotes vs. major allele homozygotes plus minor allele homozygotes), and log-additive model (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes). The Bonferroni correction method (the α value for each comparison equal to the fixed α value divided by the total number of comparisons) was performed to avoid the inflation of p-values from multiple comparisons [23]. Chi-square analysis and exact chi-square analysis of variance were used to compare Banff score values when considering 2 or 3 genotypes. All data were analyzed by SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered statistically significant.

Results

Baseline characteristics of study participants

Table 1 presents the baseline clinical characteristics of the renal transplant recipients: age, sex, weight, and incidence of DGF. A total of 200 recipients (124 men and 76 women) who underwent first ABO-matched single-kidney transplantation were included in this study. Of these, 69 recipients (42 men and 27 women) had experienced at least 1 AR episode. None of...
the 200 recipients had detected panel reactive antibody (PRA) before transplantation. Comprehensive and detailed information on recipients and other clinical information can be found in our previous study [20].

Association analysis between tagger SNPs and AR

A total of 188 SNPs on TGF-β signaling pathway genes were detected by target sequencing. Detailed information on chromosome, position, function, and details are presented in Supplementary Table 1. Among these, 47 novel SNPs were reported for the first time. SNPs with a MAF >0.05 were identified as normal frequency, whereas MAF <0.05 were considered as rare frequency SNPs. The analysis of HWE highlighted 38 SNPs with MAF >0.05 and HWE >0.05 (Supplementary Table 2). During the study, Haploview version 4.2 was used to further analyze haplotypes by evaluating LD block and haplotype among 38 SNPs. After the adjustment of LD analysis, 31 tagger SNPs with 10 blocks (Block1: rs11466512-rs2228048, Block2: rs2241716-rs2241717-rs1800470, Block3: rs1065080-rs2289261-rs2289259-rs7179893, Block4: rs2289790-rs2289791, Block5: rs3917187-rs3917201, Block6: rs284878-rs2038931, Block7: rs1805113-rs1750641, Block8: rs10783002-rs2306888-rs11165376-rs12124904, Block9: rs1805110-rs1805109, Block10: rs11568753-rs11568778-rs334354) were included for further research (Figure 1, Supplementary Figure 1, Supplementary Table 3). However, no significant correlation was observed between the haplotypes and AR.

GLM analysis was undertaken to investigate the influence of the distribution of various clinical variables on the occurrence of AR. The use of sirolimus or not was found to be significantly related with the distribution of AR with a P value of 0.015 after the analysis of Pillai’s Trace, Wilks’ Lambda, Hotelling’s Trace, and Roy’s Largest Root (Supplementary Table 4). Other clinical variables, including age, sex, weight, and DGF showed no significant relation with AR (Supplementary Table 4). Thereafter, 5 models adjusted by the use of sirolimus were used to analyze the effect of tagger SNPs on AR by a corrected statistically significant P value according to Bonferroni correction method (corrected-P=0.01). The tagger SNP rs1131243 on TGF-β Receptor 3 (TGFBR3) gene exhibited significant correlation with

### Table 2. Results of logistic analysis of rs1131243 with the occurrence of acute rejection in 5 models adjusted by the usage of sirolimus.

| SNP    | Model      | Genotype | AR n (%) | non-AR n (%) | OR     | Lower | Upper | P value   |
|--------|------------|----------|----------|--------------|--------|-------|-------|-----------|
| rs1131243 | Codominant | CC       | 38 (55.07) | 104 (79.39) | 3.71   | 1.82  | 7.58  | 0.00037   |
|         |            | CT       | 27 (39.13) | 24 (18.32)  | 4.18   | 2.14  | 8.21  | 0.00006   |
|         |            | TT       | 4 (5.80)   | 3 (2.29)    | 1.41   | 0.37  | 5.16  | 0.63      |
|         | Dominant   | CC vs. TT+CT | 38 (55.07) | 27 (20.68)  | 3.9    | 1.97  | 7.72  | 7.79E-05  |
|         | Recessive  | CC+CT vs. TT | 65 (94.20) | 3 (2.29)    | 3.57   | 0.77  | 16.54 | 0.11      |
|         | Overdominant| CT vs. CC+TT | 27 (39.13) | 107 (81.68) | 3.31   | 1.65  | 6.65  | 0.00073   |
|         | Log-additive | CC vs. CT vs. TT | 131 (65.50) | 69 (34.50)  | 3.04   | 1.7   | 5.43  | 0.00011   |

Number of comparison: 5; Alpha: 0.05; Corrected Alpha: 0.01.
Table 3. Association analysis of rs1131243 genotypes with Banff score (A) and combined Banff score (B).

### A

| Genotype | CC   | %    | CT   | %    | TT  | %    |
|----------|------|------|------|------|-----|------|
| Borderline | 27   | 56.25| 10   | 55.56| 1   | 33.33|
| IA       | 18   | 37.50| 4    | 22.22| 2   | 66.66|
| IB       | 1    | 2.08 | 0    | 0    | 0   | 0    |
| IIA      | 2    | 4.16 | 3    | 16.67| 0   | 0    |
| IIB      | 0    | 0    | 1    | 5.56 | 0   | 0    |
| Sum      | 48   | 100  | 18   | 100  | 3   | 100  |

df=8, χ²=8.386, P=0.40.

### B

| Genotype | CC   | %    | CT   | %    | TT  | %    |
|----------|------|------|------|------|-----|------|
| Borderline+IA+IB | 46   | 95.83| 14   | 77.78| 3   | 100  |
| IIA+IB   | 2    | 4.16 | 4    | 22.22| 0   | 0    |
| Sum      | 48   | 100  | 18   | 100  | 3   | 100  |

df=2, exact χ²=4.86, P=0.055.

the occurrence of AR in 4 of the 5 models as P=0.00037 in co-dominant model (OR1=3.71, 95% CI1: 1.82–7.58, OR2=5.41, 95% CI2: 1.14–25.75), P=7.79E-05 in dominant model (OR=3.9, 95% CI: 1.97–7.72), P=0.11 in recessive model (OR=3.57, 95% CI: 0.77–16.54), P=0.00073 in over-dominant model (OR=3.31, 95% CI: 1.65–6.65), and P=0.00011 in log-additive model (OR=3.04, 95% CI: 1.7–5.43) (Table 2). No corrected statistical significance was observed in the other 30 SNPs (Supplementary Table 5).

### Effect of SNPs on histological examination outcome

Of the 200 recipients included in this study, 69 were diagnosed as having AR by allograft biopsy. The degree of AR was determined based on histological examination and according to Banff 15 criteria. Of these 69 recipients, 38 were classified as borderline, 24 were classified as Banff IA, 2 were classified as Banff IB and Banff IIB (1 recipient each), and 5 were classified as Banff IIa. No significant correlation was observed between the type of rs1131243 and the degree of AR (df=8, χ²=8.386, P=0.3967, Table 3A). As the presence of endarteritis confirmed in biopsy is the criterion that distinguishes between I and II degree of AR by Banff 15, the level of AR was divided into 2 groups. The degree of borderline, IA, and IB were regarded as the other group. We found that recipients who carried the rs1131243 T variant were more likely to have endarteritis and a higher level of AR. However, no significant difference was identified between rs1131243 and the 2 groups with a P value of 0.055 (df=2, exact χ²=4.86, Table 3B).

### Discussion

In this study, TS assay was performed based on NGS technology to identify the associations of SNPs of the TGF-β signaling pathway with AR following kidney transplantation. Mutations on rs1131243 of TGF-β3 gene were observed for the first time and found to be significantly correlated with increasing risk of AR in renal transplant recipients.

Changes in TGF-βR1 and TGF-βR2 gene can lead to growth inhibition in cells by TGF-β signaling pathway mediation [24]. Kim et al. reported that a synonymous SNP – rs2228048 of the TGF-βR2 gene – is associated with acute rejection in Korean renal transplant recipients [25]. In our study, we also detected SNP rs2228048 in Chinese recipients. However, the SNP rs2228048 showed a P value of 0.8146 based on the HWE analysis in our cohort, which indicated that equilibrium had been achieved. Variations of SNPs among human populations may be the reason for differences in these results.

TGF-βR3, also known as betaglycan, is the most abundant of the TGF-β receptors [26]. It has a high affinity for both homodimeric and heterodimer TGF-β1 and TGF-β2 [27]. Recent genetic studies of TGF-βR3 have reported its role in several diseases. According to Kao et al., SNP rs6696224 of TGF-βR3 gene was significantly associated with heart failure and preserved ejection fraction in the Cardiovascular Health Study (CHS) [28]. The rs1192415 of TGF-βR3 gene has been observed to be associated with primary open angle glaucoma among various
human populations [29,30]. In the white population, a SNP rs1805110 on the TGF-βR3 gene was found to be associated with Behçet’s disease and idiopathic intermediate uveitis [31].

The present results show that rs1131243, an SNP on the TGF-βR3 gene located in 3’-untranslated region sequences, is significantly correlated with the occurrence of post-transplantation AR episodes in first-time renal transplant patients. Recipients carrying the rs1131243 T variant appear to have a higher risk of AR after kidney transplantation. Kumar et al. stated that the TGF-βR3 gene in acute rejection recipients was significantly upregulated among non-rejection recipients after intestinal transplantation in children based on quantitative real-time PCR [32]. TGF-βR3, which has no known signaling domain, is reported to regulate the TGF-β signaling pathway by enhancing the binding of TGF-β ligands to TGF-β type II receptors by binding TGF-β and presenting it to TGF-βR2 [33,34]. Variants of TGF-βR3 can lead to the activation of diverse downstream substrates and regulatory proteins, influencing the transcription of various target genes that function in differentiation, proliferation, and activation of many types of immune cells [12]. Our research indicates that the rs1131243 variant of 3’-UTR on the TGF-βR3 gene alters the function of TGF-βR3, thereby affecting the occurrence of AR.

This study did not observe any statistically significant difference between the genotype of rs1131243 and the level of AR in the 69 patients confirmed by histological examination. The results of the present study indicate that rs1131243 T variant causes a higher risk of AR but does not influence the severity. Since the presence of endarteritis confirmed by biopsy is the dividing criteria between Banff I and II degree of AR based on Banff 15, the AR patients were categorized into 2 groups. We observed that recipients who carried the rs1131243 T variant were more likely to have endarteritis and a higher level of AR. However, after analysis using the exact chi-square test, no statistically significant difference was observed with a P value of 0.055. The relatively low number of AR recipients may have contributed to the border line P value. More recipients confirmed by biopsy should be included in further research to verify the result.

This study has certain limitations. This was a single-center study of 200 patients from eastern China who received renal transplantation and it may not have comprehensively covered the influence of SNPs in AR. Some SNPs which occur in a specific cohort may have been inadvertently ignored in this study. Also, SNPs with a MAF <0.05 in the cohort of our center were not sufficiently included in this study and thus may have led us to miss certain crucial findings. Negative results of other TGF-β and SMAD genes in this study might not be adequate to rule out the function of related genes and downstream proteins in the occurrence of AR.

**Conclusions**

We found that an SNP – rs1131243 on the TGF-βR3 gene – is significantly related to the risk of AR in renal transplant recipients but does not influence the severity of AR.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Genetic expression files are posted on the Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra; SRP133091).

**Conflict of interests**

None.

**Abbreviations**

AR – acute rejection; TGF-β – transforming growth factor beta; TGF-βR – transforming growth factor beta receptor; SNPs – single-nucleotide polymorphisms; TS – targeting sequencing; GLM – general linear model; MAF – minor allele frequency; HWE – Hardy-Weinberg equilibrium; LD – linkage disequilibrium.

**Supplementary Data**

**Supplementary Figure 1.** Linkage disequilibrium analysis of tagger SNPs.
**Supplementary Table 1.** Detailed information of 188 SNPs on TGF-β signaling pathway genes.

| Chromosome | Position | Reference allele | Alternation allele | Gene name | Function | avsnp144 | Gene detail |
|------------|----------|------------------|--------------------|-----------|----------|----------|-------------|
| chr18      | 45368162 | C                | T                  | SMAD2     | UTR3     | .        | NM_001003652: c.36G>A; NM_001135937: c.36G>A; NM_005901: c.36G>A |
| chr18      | 45368395 | C                | T                  | SMAD2     | Intrinsic| .        |             |
| chr18      | 45368457 | C                | T                  | SMAD2     | Intrinsic| rs79502327|             |
| chr18      | 45371509 | C                | T                  | SMAD2     | Intrinsic| rs1787186|             |
| chr18      | 45371546 | T                | G                  | SMAD2     | Intrinsic| .        |             |
| chr18      | 45371623 | T                | C                  | SMAD2     | Intrinsic| rs781465847|             |
| chr18      | 45371791 | C                | T                  | SMAD2     | Exonic   | rs1804712| SMAD2: NM_001135937: exon9: c.G1110A: p.Q370Q; SMAD2: NM_001003652: exon10: c.G1200A: p.Q400Q; SMAD2: NM_005901: exon10: c.G1200A: p.Q400Q |
| chr18      | 45375054 | T                | C                  | SMAD2     | Exonic   | rs2286256| SMAD2: NM_001135937: exon7: c.697G>A; p.L233S; SMAD2: NM_001003652: exon8: c.789G>A: p.L263L; SMAD2: NM_005901: exon8: c.789G>A: p.L263L |
| chr18      | 45375138 | C                | T                  | SMAD2     | Intrinsic| rs368276908|             |
| chr18      | 45377682 | T                | C                  | SMAD2     | Exonic   | rs146872557| SMAD2: NM_001135937: exon6: c.521G>A: p.L174R; SMAD2: NM_001003652: exon7: c.611G>A: p.L204R; SMAD2: NM_005901: exon7: c.611G>A: p.L204R |
| chr18      | 45377752 | G                | A                  | SMAD2     | Intrinsic| rs187015964|             |
| chr15      | 67391336 | C                | T                  | SMAD3     | Intrinsic| rs18653184|             |
| chr15      | 67394653 | G                | A                  | SMAD3     | Intrinsic| rs72661145|             |
| chr15      | 67394919 | T                | C                  | SMAD3     | Intrinsic| rs1787177|             |
| chr15      | 67422862 | T                | C                  | SMAD3     | Intrinsic| .        |             |
| chr15      | 67423208 | A                | G                  | SMAD3     | Intrinsic| .        |             |
| chr15      | 67358478 | G                | A                  | SMAD3     | UTR5     | rs10861427| NM_005902: c.15G>A |
| chr15      | 67358558 | G                | A                  | SMAD3     | Exonic   | rs187952791| SMAD3: NM_005902: exon1: c.G66A: p.E22E |
| chr15      | 67391336 | C                | T                  | SMAD3     | Intrinsic| rs184408275|             |
| chr15      | 67391497 | C                | A                  | SMAD3     | Intrinsic| rs1866319|             |
| chr15      | 67430466 | T                | C                  | SMAD3     | Intrinsic| .        |             |
| chr15      | 67430492 | A                | G                  | SMAD3     | Intrinsic| .        |             |
| chr15      | 67457335 | A                | G                  | SMAD3     | Exonic   | rs10865080| SMAD3: NM_001145103: exon2: c.A177G; p.Q59G; SMAD3: NM_005902: exon2: c.A309G; p.L103G |
| Chromosome | Position | Reference allele | Alternation allele | Gene name | Function | avsnp144 | Gene detail |
|------------|----------|-----------------|-------------------|-----------|----------|---------|------------|
| chr15      | 67457485 | G               | C                 | SMAD3     | Intronic | rs2289261|            |
|            | 67457647 | C               | T                 | SMAD3     | Exonic   | rs145380987|            |
|            | 67457807 | C               | T                 | SMAD3     | Intronic | rs2289260|            |
|            | 67457815 | A               | G                 | SMAD3     | Intronic | rs56336520|            |
|            | 67457850 | G               | A                 | SMAD3     | Intronic | rs2289259|            |
|            | 67458930 | C               | T                 | SMAD3     | Intronic | rs719840 |            |
|            | 67459013 | C               | T                 | SMAD3     | Intronic | rs719893 |            |
|            | 67459307 | A               | T                 | SMAD3     | Intronic |          |            |
|            | 67462729 | G               | A                 | SMAD3     | Intronic | rs3743341|            |
|            | 67473847 | G               | C                 | SMAD3     | Intronic | rs549919159|            |
|            | 67476952 | T               | C                 | SMAD3     | Intronic | rs2289791|            |
|            | 67476970 | T               | C                 | SMAD3     | Intronic | rs2289790|            |
|            | 67476986 | C               | T                 | SMAD3     | Intronic | rs54596731|            |
|            | 67479198 | C               | T                 | SMAD3     | Intronic | rs19127838|            |
|            | 67479524 | G               | A                 | SMAD3     | Intronic |          |            |
|            | 67479591 | G               | A                 | SMAD3     | Intronic |          |            |
|            | 67482696 | A               | G                 | SMAD3     | Intronic | rs8410524|            |
|            | 48573689 | A               | G                 | SMAD4     | Intronic | rs77389132|            |
|            | 48573718 | T               | A                 | SMAD4     | Intronic |          |            |
|            | 48575389 | T               | C                 | SMAD4     | Intronic | rs2276163|            |
|            | 48575544 | T               | C                 | SMAD4     | Intronic |          |            |
|            | 48577782 | G               | C                 | SMAD4     | Intronic | rs7229678|            |
|            | 48577894 | G               | A                 | SMAD4     | Intronic | rs118185031|            |
|            | 48584663 | T               | G                 | SMAD4     | Intronic | rs556951898|          |
|            | 48586175 | A               | G                 | SMAD4     | Intronic |          |            |
|            | 48586184 | A               | G                 | SMAD4     | Intronic | rs948589 |            |
|            | 48586328 | T               | A                 | SMAD4     | Intronic | rs758408030|          |
|            | 48586344 | C               | T                 | SMAD4     | Intronic | rs948588 |            |
|            | 48593617 | T               | C                 | SMAD4     | Intronic | rs19746173|            |
|            | 41836947 | G               | C                 | T/GFB1    | UTR3     | NM_000660: c.10C>G |            |
|            | 41837123 | C               | T                 | T/GFB1    | Splicing | NM_000660: exon8: c.1015-8G>A |          |
|            | 14847933 | A               | T                 | T/GFB1    | Exonic   | rs569594975| T/GFB1: NM_000660: exon5: c.715A|          |
|            | 41847956 | G               | A                 | T/GFB1    | Intronic | rs563345701|            |

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| Chromosome | Position | Reference allele | Alteration allele | Gene name | Function | avsnp144 | Gene detail |
|------------|----------|-----------------|------------------|-----------|----------|----------|------------|
| chr19      | 41848038 | C               | T                | T/GFB1    | Intronic | rs200134934 | .          |
| chr19      | 41848075 | C               | T                | T/GFB1    | Exonic   | rs376729112 | .          |
| chr19      | 41850710 | C               | T                | T/GFB1    | Exonic   | rs766221068 | .          |
| chr19      | 41850742 | G               | A                | T/GFB1    | Exonic   | rs753852676 | .          |
| chr19      | 41854052 | G               | A                | T/GFB1    | Exonic   | rs2241717   | .          |
| chr19      | 41854086 | C               | T                | T/GFB1    | Intronic | rs2241717   | .          |
| chr19      | 41854400 | C               | T                | T/GFB1    | Exonic   | rs376729112 | .          |
| chr19      | 41854452 | C               | T                | T/GFB1    | Intronic | rs376729112 | .          |
| chr19      | 41854534 | T               | A                | T/GFB1    | Intronic | rs8108632   | .          |
| chr19      | 41854883 | T               | A                | T/GFB1    | Exonic   | rs1800470   | .          |
| chr19      | 41858867 | G               | A                | T/GFB1    | Exonic   | rs1800470   | .          |
| chr19      | 41858892 | G               | A                | T/GFB1    | Exonic   | rs1800470   | .          |
| chr19      | 41859047 | A               | C                | T/GFB1    | UTR3     | rs376729112 | .          |
| chr19      | 218607317| G                | A                | T/GFB2    | Intronic | rs376729112 | .          |
| chr19      | 218607796| C               | T                | T/GFB2    | Splicing | rs376729112 | .          |
| chr19      | 218607992| A               | G                | T/GFB2    | Intronic | rs376729112 | .          |
| chr19      | 218610691| G               | T                | T/GFB2    | Exonic   | rs376729112 | .          |
| chr14      | 76425464 | G               | T                | T/GFB3    | UTR5     | rs531039494 | .          |
| chr14      | 76427103 | A               | G                | T/GFB3    | Intronic | rs115411167 | .          |
| chr14      | 76427473 | C               | G                | T/GFB3    | Intronic | rs531039494 | .          |
| chr14      | 76429555 | C               | T                | T/GFB3    | Intronic | rs3917201   | .          |
| chr14      | 76429868 | A               | G                | T/GFB3    | Intronic | rs3917200   | .          |
| chr14      | 76431817 | A               | G                | T/GFB3    | Intronic | rs3917200   | .          |
| chr14      | 76432136 | T               | C                | T/GFB3    | Intronic | rs3917187   | .          |
| chr14      | 76434199 | A               | G                | T/GFB3    | Intronic | rs3917187   | .          |
| chr14      | 76436149 | G               | A                | T/GFB3    | Intronic | rs3917187   | .          |
| chr14      | 76437614 | A               | G                | T/GFB3    | Intronic | rs3917187   | .          |
| chr14      | 76437638 | C               | T                | T/GFB3    | Intronic | rs201310311 | .          |
| chr14      | 76437817 | C               | T                | T/GFB3    | Intronic | rs554067491 | .          |
| chr14      | 76438137 | C               | G                | T/GFB3    | Intronic | rs537980685 | .          |
| chr14      | 76446750 | C               | A                | T/GFB3    | Intronic | rs537980685 | .          |

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| Chromosome | Position | Reference allele | Alternation allele | Gene name | Function | avsnp144 | Gene detail |
|------------|----------|-----------------|-------------------|-----------|----------|----------|-------------|
| chr14      | 76446782 | G               | C                 | T/GFB3    | Intronic | -        |             |
| chr14      | 76446943 | C               | T                 | T/GFB3    | Exonic   | rs778214495 | T/GFB3: NM_0033239: exon1: c.C294A: p.S98S |
| chr14      | 76447049 | G               | T                 | T/GFB3    | Exonic   | rs757664433 | T/GFB3: NM_0033239: exon1: c.C1188A: p.T363N |
| chr9       | 101890227 | C               | T                 | T/GFB1    | Intronic | rs11568753 |             |
| chr9       | 101890294 | A               | G                 | T/GFB1    | Intronic | rs189740990 |             |
| chr9       | 101891294 | A               | G                 | T/GFB1    | Exonic   | rs70413113 |             |
| chr9       | 101891447 | G               | C                 | T/GFB1    | Intronic | rs56402414 |             |
| chr9       | 101900392 | C               | A                 | T/GFB1    | Intronic | rs26521429 |             |
| chr9       | 101900410 | A               | G                 | T/GFB1    | Intronic | rs11568778 |             |
| chr10       | 99767222 | G               | T                 | T/GFB1    | Intronic | rs26521429 |             |
| chr9       | 101908915 | G               | A                 | T/GFB1    | Intronic | rs334354 |             |
| chr3       | 30648248 | C               | G                 | T/GFB2    | Exonic   | rs32086586 | NM_001024847: c.-128C>G;NM_003242: c.-128C>G |
| chr3       | 30648636 | T               | G                 | T/GFB2    | Intronic | rs200111443 |             |
| chr3       | 3064732  | A               | C                 | T/GFB2    | Intronic | rs200111443 |             |
| chr3       | 30686264 | C               | T                 | T/GFB2    | Exonic   | rs769570752 | T/GFB2: NM_0033242: exon2: c.C120T: p.V40T;T/GFB2: NM_001024847: exon3: c.C195T: p.D65T |
| chr3       | 30686414 | A               | G                 | T/GFB2    | Splicing | rs1155705 | NM_001024847: exon3: c.338+7A>G;NM_003242: exon2: c.263+7A>G |
| chr3       | 30691692 | C               | A                 | T/GFB2    | Intronic | rs17998227 |             |
| chr3       | 30713246 | A               | C                 | T/GFB2    | Intronic | rs117998227 |             |
| chr3       | 30713292 | C               | T                 | T/GFB2    | Intronic | rs150022335 |             |
| chr3       | 30713314 | C               | T                 | T/GFB2    | Exonic   | rs200332401 | T/GFB2: NM_0033242: exon2: c.C692T: p.T231M |
| chr3       | 30713619 | C               | T                 | T/GFB2    | Exonic   | rs34838818 | T/GFB2: NM_0033242: exon1: c.C364T: p.T122I;T/GFB2: NM_001024847: exon5: c.C786G: p.T262C |

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| Chromosome | Position | Reference allele | Alternation allele | Gene name | Function | avsnp144 | Gene detail |
|------------|----------|-----------------|-------------------|-----------|----------|----------|-------------|
| chr3       | 30713842 | C               | T                 | T/GFBR2   | Exonic   | rs2228048 | T/GFBR2: NM_003242: exon4: c.C1167T: p.N389N,T/GFBR2: NM_001024847: exon5: c.C1242T: p.N414N |
| chr3       | 30730096 | G               | A                 | T/GFBR2   | Intrinsic|          |             |
| chr3       | 30732821 | C               | A                 | T/GFBR2   | Intrinsic| rs2276767 |             |
| chr3       | 30731100 | G               | A                 | T/GFBR2   | UTR3     |          | NM_003242: exon5: c.*60:+1G>A, NM_001024847: c.*60:+1G>A |
| chr3       | 30733102 | G               | A                 | T/GFBR2   | UTR3     |          | NM_001024847: c.*11G>A, NM_003242: c.*11G>A |
| chr1       | 92149139 | C               | T                 | T/GFBR3   | UTR3     | rs1805115 | NM_001195683: c.*157G>A, NM_001915684: c.*157G>A, NM_003243: c.*157G>A |
| chr1       | 92149277 | C               | T                 | T/GFBR3   | UTR3     | rs1131243 | NM_001195683: c.*19G>A, NM_001915684: c.*19G>A, NM_003243: c.*19G>A |
| chr1       | 92161307 | G               | A                 | T/GFBR3   | Intrinsic| rs141883791 | T/GFBR3: NM_001195683: exon16: c.G2356A: p.V786M, T/GFBR3: NM_001195684: exon17: c.G2356A: p.V786M |
| chr1       | 92161515 | T               | A                 | T/GFBR3   | Intrinsic| rs2253316 |             |
| chr1       | 92163682 | C               | G                 | T/GFBR3   | Exonic   | rs17882828 | T/GFBR3: NM_001195683: exon16: c.G2299C: p.G764R, T/GFBR3: NM_003243: exon15: c.G2299C: p.G764R, T/GFBR3: NM_001195684: exon16: c.G2290C: p.G764R |
| chr1       | 92163786 | G               | T                 | T/GFBR3   | Intrinsic| rs2296621 |             |
| chr1       | 92174260 | A               | G                 | T/GFBR3   | Exonic   | rs284878 | T/GFBR3: NM_001195683: exon14: c.T2244C: p.T748T, T/GFBR3: NM_003243: exon14: c.T2244C: p.T748T, T/GFBR3: NM_001195684: exon15: c.T2244C: p.T748T |
| chr1       | 92174383 | A               | G                 | T/GFBR3   | Intrinsic| rs78893665 |             |
| chr1       | 92177740 | C               | T                 | T/GFBR3   | Intrinsic| rs53738501 |             |
| chr1       | 92177938 | A               | G                 | T/GFBR3   | Exonic   | rs1805113 | T/GFBR3: NM_001195683: exon13: c.T2025C: p.F675F, T/GFBR3: NM_003243: exon13: c.T2025C: p.F675F, T/GFBR3: NM_001195684: exon14: c.T2025C: p.F675F |
| chr1       | 92184673 | G               | A                 | T/GFBR3   | Intrinsic| rs6664260 |             |

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| Chromosome | Position | Reference allele | Alternation allele | Gene name | Function | avsnp144 | Gene detail |
|------------|----------|------------------|-------------------|-----------|----------|----------|-------------|
| chr1       | 92184744 | C                | T                 | T/GFBR3   | Intronic | rs4658261 |             |
| chr1       | 92185059 | A                | T                 | T/GFBR3   | Intronic | rs61748118 |             |
| chr1       | 92185134 | A                | C                 | T/GFBR3   | Intronic | rs186586693 |             |
| chr1       | 92185136 | C                | T                 | T/GFBR3   | Intronic | rs57773521 |             |
| chr1       | 92185185 | T                | C                 | T/GFBR3   | Intronic | rs2279455 |             |
| chr1       | 92185657 | C                | T                 | T/GFBR3   | Intronic | rs1805112 |             |
| chr1       | 92185715 | A                | G                 | T/GFBR3   | Exonic   | rs752061   |             |
| chr1       | 92185881 | G                | A                 | T/GFBR3   | Intronic | rs10783002 |             |
| chr1       | 92195221 | G                | A                 | T/GFBR3   | Exonic   | rs752061   |             |
| chr1       | 92195299 | C                | T                 | T/GFBR3   | Intronic |            |             |
| chr1       | 92195555 | T                | G                 | T/GFBR3   | Intronic |            |             |
| chr1       | 92195601 | G                | A                 | T/GFBR3   | Intronic | rs10783002 |             |
| chr1       | 92200376 | G                | A                 | T/GFBR3   | Exonic   | rs376528004 |             |
| chr1       | 92200382 | T                | C                 | T/GFBR3   | Exonic   | rs2306888 |             |
| chr1       | 92200389 | A                | G                 | T/GFBR3   | Exonic   | rs186259544 |             |
| chr1       | 92200513 | A                | C                 | T/GFBR3   | Exonic   | rs759218481 |             |
| chr1       | 92200520 | G                | C                 | T/GFBR3   | Splicing | rs138007142 |             |
| chr1       | 92200593 | A                | G                 | T/GFBR3   | Intronic | rs11165376 |             |
| chr1       | 92200597 | G                | A                 | T/GFBR3   | Intronic | rs12124904 |             |
| chr1       | 92200601 | T                | G                 | T/GFBR3   | Intronic |            |             |
| chr1       | 92200634 | C                | T                 | T/GFBR3   | Intronic | rs11165377 |             |
| chr1       | 92224067 | C                | T                 | T/GFBR3   | Intronic | rs3738441 |             |
| chr1       | 92225516 | G                | A                 | T/GFBR3   | Intronic | rs1316541 |             |
## Supplementary Table 2. HWE and MAF analysis of 188 SNPs.

| SNP          | Position  | HWE       | MAF  | REF: ALT |
|--------------|-----------|-----------|------|----------|
| rs10874913   | 92200627  | 7.24E-58  | 0.45 | C: T     |
| rs1866319    | 67391497  | 2.86E-31  | 0.11 | C: A     |
| rs7179840    | 67458930  | 4.08E-22  | 0.15 | C: T     |
| rs2275391    | 92185185  | 6.00E-04  | 0.092| T: C     |
| rs2276163    | 48575389  | 0.0053    | 0.043| T: C     |
| rs8108632    | 41854534  | 0.013     | 0.14 | T: A     |
| rs7041311    | 101890980 | 0.015     | 0.007| A: G     |
| rs2029354    | 92181670  | 0.10      | 0.018| C: A     |
| rs2029355    | 92181678  | 0.10      | 0.018| T: G     |
| rs4658261    | 92184744  | 0.10      | 0.018| C: T     |
| rs11165377   | 92200634  | 0.11      | 0.07 | C: T     |
| rs2810094    | 92262874  | 0.14      | 0.20 | G: A     |
| rs2038931    | 92174415  | 0.29      | 0.4  | G: A     |
| rs45515293   | 30713945  | 0.22      | 0.025| T: C     |
| rs1805115    | 92149139  | 0.26      | 0.028| C: T     |
| rs2289261    | 67457485  | 0.29      | 0.39 | G: C     |
| rs1131243    | 92149277  | 0.43      | 0.16 | C: T     |
| rs1805110    | 92227045  | 0.31      | 0.39 | G: A     |
| rs1805109    | 92227126  | 0.31      | 0.39 | C: T     |
| rs12124904   | 92200597  | 0.31      | 0.48 | G: A     |
| rs1613463    | 92178260  | 0.33      | 0.25 | G: A     |
| rs1824716    | 41854086  | 0.36      | 0.28 | C: T     |
| rs11165376   | 92200593  | 0.38      | 0.49 | G: A     |
| rs10783002   | 92195601  | 0.43      | 0.45 | G: A     |
| SNP          | Position   | HWE  | MAF  | REF: ALT |
|-------------|------------|------|------|----------|
| rs2289791   | 67476952   | 0.47 | 0.48 | G: T     |
| rs1116541   | 92224347   | 0.49 | 0.22 | G: A     |
| rs11466512  | 30713126   | 0.50 | 0.38 | T: A     |
| rs2276767   | 30732821   | 0.50 | 0.11 | C: A     |
| rs2289793   | 67496603   | 0.56 | 0.33 | T: C     |
| rs2289790   | 67476970   | 0.57 | 0.47 | T: C     |
| rs2296621   | 92163786   | 0.60 | 0.043| T: A     |
| rs1155705   | 30686414   | 0.63 | 0.33 | G: A     |
| rs1061427   | 67358478   | 0.67 | 0.20 | G: A     |
| rs3917201   | 76429555   | 0.68 | 0.48 | T: C     |
| rs11568753  | 101890227  | 0.73 | 0.41 | T: C     |
| rs1805113   | 92177938   | 0.73 | 0.10 | A: G     |
| rs3917187   | 76432136   | 0.77 | 0.47 | T: C     |
| rs334354    | 101908915  | 0.77 | 0.44 | G: A     |
| rs1750641   | 92178259   | 0.79 | 0.37 | T: C     |
| rs729678    | 48577782   | 0.81 | 0.46 | G: C     |
| rs2228048   | 30713842   | 0.81 | 0.25 | T: C     |
| rs2289259   | 67457950   | 0.88 | 0.25 | G: A     |
| rs3738441   | 92224067   | 0.90 | 0.3  | T: C     |
| rs2306856   | 30648248   | 1    | 0.007| C: G     |
| Chr3: 3064736 | 3064736   | 1    | 0.003| C: G     |
| Chr3: 30648636 | 30648636 | 1    | 0.003| T: G     |
| rs200111443 | 30664732   | 1    | 0.005| A: C     |
| rs11798227  | 30654864   | 1    | 0.007| T: C     |
| Chr3: 30686107 | 30686107 | 1    | 0.003| A: C     |
| rs76950752  | 30686264   | 1    | 0.003| C: T     |
| Chr3: 30687092 | 30687092 | 1    | 0.003| T: C     |
| rs56105708  | 30713246   | 1    | 0.022| G: A     |
| rs150022335 | 30713292   | 1    | 0.003| T: C     |
| rs135022401 | 30713314   | 1    | 0.003| T: C     |
| rs34833812  | 30713619   | 1    | 0.022| C: T     |
| Chr3: 30730096 | 30730096 | 1    | 0.003| G: A     |
| Chr3: 30730100 | 30730100 | 1    | 0.003| G: A     |
| Chr3: 30730102 | 30730102 | 1    | 0.003| G: A     |
| Chr19: 41836947 | 41836947 | 1    | 0.003| G: C     |
| rs199982059 | 41837123   | 1    | 0.013| C: T     |
| rs570977266 | 41837997   | 1    | 0.003| C: T     |
| rs190566789 | 41838174   | 1    | 0.013| C: T     |
| rs8179181   | 41838206   | 1    | 0.003| G: A     |
| rs13306709  | 41838287   | 1    | 0.003| C: T     |
| rs542695848 | 41847736   | 1    | 0.003| T: C     |
| rs569594975 | 41847933   | 1    | 0.003| A: T     |

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CLINICAL RESEARCH

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| SNP          | Position   | HWE | MAF    | REF: ALT |
|-------------|------------|-----|--------|----------|
| rs763345073 | 41847956   | 1   | 0.003  | G: A     |
| rs200134934 | 41848038   | 1   | 0.005  | C: T     |
| Chr19: 41848075 | 41848075 | 1   | 0.003  | C: T     |
| rs766221068 | 41850710   | 1   | 0.003  | C: T     |
| rs531039494 | 41850921   | 1   | 0.003  | C: T     |
| rs376729112 | 41854400   | 1   | 0.003  | C: T     |
| Chr19: 41854452 | 41854452 | 1   | 0.003  | C: T     |
| Chr19: 41854477 | 41854477 | 1   | 0.003  | A: C     |
| Chr19: 41858838 | 41858838 | 1   | 0.003  | T: A     |
| Chr19: 41858867 | 41858867 | 1   | 0.003  | G: A     |
| rs1800470   | 41858921   | 1   | 0.46   | G: A     |
| Chr19: 41859047 | 41859047 | 1   | 0.003  | A: C     |
| Chr18: 45368162 | 45368162 | 1   | 0.003  | C: T     |
| Chr18: 45368395 | 45368395 | 1   | 0.003  | C: T     |
| rs79502327  | 45368457   | 1   | 0.005  | C: T     |
| Chr18: 45371546 | 45371546 | 1   | 0.003  | T: G     |
| rs781465847 | 45371623   | 1   | 0.003  | T: C     |
| rs1804712   | 45371791   | 1   | 0.003  | C: T     |
| rs150503321 | 45374824   | 1   | 0.003  | C: T     |
| rs2282656   | 45375054   | 1   | 0.003  | T: C     |
| rs1287177   | 45379138   | 1   | 0.003  | C: T     |
| rs146872557 | 45377682   | 1   | 0.003  | T: C     |
| rs187015964 | 45377752   | 1   | 0.003  | G: A     |
| rs187348971 | 45391368   | 1   | 0.003  | T: C     |
| rs72661146  | 45394653   | 1   | 0.02   | G: A     |
| rs72661145  | 45394662   | 1   | 0.02   | C: A     |
| rs17866177  | 45399419   | 1   | 0.015  | T: C     |
| Chr18: 45399548 | 45399548 | 1   | 0.003  | T: C     |
| Chr18: 45422862 | 45422862 | 1   | 0.003  | T: C     |
| Chr18: 45423208 | 45423208 | 1   | 0.003  | A: G     |
| rs77389132  | 48573689   | 1   | 0.02   | A: G     |
| Chr18: 48573718 | 48573718 | 1   | 0.003  | T: A     |
| Chr18: 48575544 | 48575544 | 1   | 0.003  | T: C     |
| rs118185031 | 48577894   | 1   | 0.015  | G: A     |
| rs556951898 | 48584863   | 1   | 0.003  | T: G     |
| Chr18: 48586175 | 48586175 | 1   | 0.003  | A: G     |
| rs948589    | 48586184   | 1   | 0.003  | A: G     |
| rs758408003 | 48586328   | 1   | 0.003  | T: A     |
| rs948588    | 48586344   | 1   | 0.043  | C: T     |
| rs139741673 | 48593617   | 1   | 0.03   | T: C     |
| rs36221703  | 67358470   | 1   | 0.025  | C: T     |
| rs187952791 | 67358558   | 1   | 0.02   | G: A     |
| SNP       | Position | HWE | MAF   | REF: ALT |
|-----------|----------|-----|-------|----------|
| rs760598093 | 67391319 | 1   | 0.003 | C: T     |
| rs184408275 | 67391336 | 1   | 0.003 | T: C     |
| Chr15: 67430466 | 67430466 | 1   | 0.003 | A: G     |
| rs1291440 | 67430492 | 1   | 0.003 | G: A     |
| rs1065080 | 67457335 | 1   | 0.003 | C: T     |
| rs145380987 | 67457647 | 1   | 0.003 | C: T     |
| rs2289200 | 67457886 | 1   | 0.003 | C: T     |
| Chr15: 67457815 | 67457815 | 1   | 0.003 | C: T     |
| rs56336520 | 67457840 | 1   | 0.005 | G: A     |
| Chr15: 67459307 | 67459307 | 1   | 0.003 | G: C     |
| rs3743341 | 67457840 | 1   | 0.003 | G: A     |
| rs549919159 | 67467384 | 1   | 0.007 | C: T     |
| rs54596731 | 67467986 | 1   | 0.013 | C: T     |
| rs191278238 | 67479198 | 1   | 0.005 | C: T     |
| Chr15: 67479524 | 67479524 | 1   | 0.003 | C: T     |
| Chr15: 67479591 | 67479591 | 1   | 0.003 | C: T     |
| rs28410524 | 67482696 | 1   | 0.01  | A: G     |
| rs188123116 | 76425446 | 1   | 0.005 | G: T     |
| rs115411167 | 76427103 | 1   | 0.005 | G: C     |
| Chr14: 76427473 | 76427473 | 1   | 0.003 | G: C     |
| Chr14: 76427473 | 76427473 | 1   | 0.003 | G: C     |
| chr14: 76437409 | 76437409 | 1   | 0.003 | G: C     |
| chr14: 76437409 | 76437409 | 1   | 0.003 | G: C     |
| rs55467491 | 76437638 | 1   | 0.018 | A: G     |
| rs20111860 | 76437640 | 1   | 0.005 | G: C     |
| rs200181092 | 76437641 | 1   | 0.003 | G: C     |
| rs778214495 | 76446943 | 1   | 0.003 | G: C     |
| rs757664433 | 76447049 | 1   | 0.003 | G: C     |
| Chr1: 92149503 | 92149503 | 1   | 0.003 | G: C     |
| rs141883791 | 92161307 | 1   | 0.005 | A: T     |
| rs17882828 | 92163682 | 1   | 0.037 | C: G     |
| rs573785401 | 92178172 | 1   | 0.003 | G: A     |
| rs4658260 | 92184673 | 1   | 0.003 | G: A     |
| rs7524066 | 92184814 | 1   | 0.092 | G: T     |
| SNP         | Position  | HWE | MAF  | REF– ALT |
|-------------|-----------|-----|------|----------|
| rs61748118  | 92185059  | 1   | 0.03 | A: T     |
| Chr1: 9218513 | 9218513  | 1   | 0.003| C: T     |
| rs57773521  | 92185136  | 1   | 0.003| C: T     |
| rs1805112   | 92185657  | 1   | 0.468| T: C     |
| Chr1: 92185715 | 92185715 | 1   | 0.003| A: T     |
| Chr1: 92185881 | 92185881 | 1   | 0.003| G: A     |
| Chr1: 92195221 | 92195221 | 1   | 0.003| C: T     |
| Chr1: 92195229 | 92195229 | 1   | 0.003| C: T     |
| Chr1: 92195555 | 92195555 | 1   | 0.003| T: G     |
| rs11466584  | 92195652  | 1   | 0.003| T: C     |
| rs37628004  | 92200376  | 1   | 0.003| G: A     |
| rs2306888   | 92200382  | 1   | 0.11 | T: C     |
| rs18625944  | 92200389  | 1   | 0.003| A: G     |
| rs759218481 | 92200513  | 1   | 0.003| A: C     |
| rs138007142 | 92200520  | 1   | 0.003| G: C     |
| rs17881268  | 92263079  | 1   | 0.003| A: G     |
| rs72716444  | 92266656  | 1   | 0.035| A: G     |
| rs12123363  | 92268363  | 1   | 0.043| A: T     |
| rs189740990 | 101890294 | 1   | 0.003| A: G     |
| Chr9: 101891294 | 101891294 | 1   | 0.003| A: G     |
| Chr9: 101891478 | 101891478 | 1   | 0.003| C: T     |
| Chr9: 101900392 | 101900392 | 1   | 0.003| C: G     |
| rs11568778  | 101900410 | 1   | 0.438| A: G     |
| rs192662552 | 101907072 | 1   | 0.003| T: C     |
| rs56251429  | 101907222 | 1   | 0.007| C: T     |
| Chr1: 218607317 | 218607317 | 1   | 0.003| G: A     |
| Chr1: 218607796 | 218607796 | 1   | 0.003| C: T     |
| Chr1: 218607922 | 218607922 | 1   | 0.003| A: G     |
| Chr1: 218610691 | 218610691 | 1   | 0.003| G: T     |

REF—referential allele; ALT—alternative allele.
### Supplementary Table 3. Detailed information of 31 tagger SNPs.

| SNP             | POS         | HWE | MAF  | REF: ALT |
|-----------------|-------------|-----|------|----------|
| rs11165377      | 92200634    | 0.11| 0.07 | C: T     |
| rs7524066       | 92184814    | 1   | 0.092| G: T     |
| rs1805113       | 92177938    | 0.73| 0.10 | A: G     |
| rs2276767       | 30732821    | 0.49| 0.11 | C: A     |
| rs2306888       | 92200382    | 1   | 0.11 | T: C     |
| rs1131243       | 92149277    | 0.30| 0.12 | C: T     |
| rs1065080       | 67457335    | 1   | 0.19 | G: A     |
| rs2810904       | 92262874    | 0.14| 0.20 | C: T     |
| rs1061427       | 67358478    | 0.67| 0.20 | G: A     |
| rs11165441      | 92224347    | 0.48| 0.22 | G: A     |
| rs284878        | 92174260    | 1   | 0.23 | G: A     |
| rs2228048       | 30713842    | 0.81| 0.25 | C: T     |
| rs2289259       | 67457850    | 0.88| 0.25 | G: A     |
| rs2241716       | 41854086    | 0.35| 0.28 | C: T     |
| rs3738441       | 92224067    | 0.90| 0.3  | T: C     |
| rs1155705       | 30686414    | 0.62| 0.33 | G: A     |
| rs7179893       | 67459013    | 0.56| 0.33 | C: T     |
| rs2241717       | 41854052    | 0.60| 0.36 | C: A     |
| rs1750641       | 92178259    | 0.79| 0.37 | T: C     |
| rs11466512      | 30713126    | 0.49| 0.38 | T: A     |
| rs2289261       | 67457485    | 0.28| 0.39 | G: C     |
| rs1805109       | 92327126    | 0.31| 0.39 | C: T     |
| rs2038931       | 92174415    | 0.28| 0.4  | G: A     |
| rs11568753      | 101890227   | 0.73| 0.41 | C: T     |
| rs10783002      | 92195601    | 0.43| 0.45 | G: A     |
| rs1800470       | 41858921    | 1   | 0.46 | G: A     |
| rs7229678       | 48577782    | 0.81| 0.46 | G: C     |
| rs1805112       | 92185657    | 1   | 0.46 | T: C     |
| rs3917187       | 76432136    | 0.77| 0.47 | C: T     |
| rs2289791       | 67476952    | 0.47| 0.48 | G: T     |
| rs11165376      | 92200593    | 0.37| 0.49 | G: A     |

REF – referential allele; ALT – alterative allele.
## Supplementary Table 4. General linear model for clinical variables on the occurrence of acute rejection.

| Effect   | Method       | Value   | F    | P value |
|----------|--------------|---------|------|---------|
| Intercept| Pillai’s Trace| 0.72    | 58.52| 0       |
|          | Wilks’ Lambda | 0.28    | 58.52| 0       |
|          | Hotelling’s Trace | 2.53   | 58.52| 0       |
|          | Roy’s Largest Root | 2.53   | 58.52| 0       |
| Gender   | Pillai’s Trace | 0.015  | 0.36 | 0.94    |
|          | Wilks’ Lambda | 0.99   | 0.36 | 0.94    |
|          | Hotelling’s Trace | 0.015  | 0.36 | 0.94    |
|          | Roy’s Largest Root | 0.015  | 0.36 | 0.94    |
| Age      | Pillai’s Trace | 0.027  | 0.65 | 0.74    |
|          | Wilks’ Lambda | 0.97   | 0.65 | 0.74    |
|          | Hotelling’s Trace | 0.028  | 0.65 | 0.74    |
|          | Roy’s Largest Root | 0.028  | 0.65 | 0.74    |
| Weight   | Pillai’s Trace | 0.042  | 1.02 | 0.42    |
|          | Wilks’ Lambda | 0.96   | 1.02 | 0.42    |
|          | Hotelling’s Trace | 0.061  | 1.02 | 0.42    |
|          | Roy’s Largest Root | 0.061  | 1.02 | 0.42    |
| ISD      | Pillai’s Trace | 0.057  | 1.40 | 0.20    |
|          | Wilks’ Lambda | 0.97   | 1.40 | 0.20    |
|          | Hotelling’s Trace | 0.034  | 1.40 | 0.20    |
|          | Roy’s Largest Root | 0.034  | 1.40 | 0.20    |
| Duration | Pillai’s Trace | 0.033  | 0.78 | 0.62    |
|          | Wilks’ Lambda | 0.97   | 0.78 | 0.62    |
|          | Hotelling’s Trace | 0.034  | 0.78 | 0.62    |
|          | Roy’s Largest Root | 0.034  | 0.78 | 0.622   |
| Sir      | Pillai’s Trace | 0.096  | 2.44 | 0.015   |
|          | Wilks’ Lambda | 0.91   | 2.44 | 0.015   |
|          | Hotelling’s Trace | 0.11   | 2.44 | 0.015   |
|          | Roy’s Largest Root | 0.11   | 2.44 | 0.015   |
| DGF      | Pillai’s Trace | 0.071  | 1.77 | 0.085   |
|          | Wilks’ Lambda | 0.93   | 1.77 | 0.085   |
|          | Hotelling’s Trace | 0.077  | 1.77 | 0.085   |
|          | Roy’s Largest Root | 0.077  | 1.77 | 0.085   |

ISD – immunosuppressive drug; Sir – sirolimus; DGF – delayed graft function.
Supplementary Table 5. Results of logistic analysis of 31 tagger SNPs in 5 model adjusted by the usage of sirolimus.

| SNP       | Codominant | Dominant    | Recessive | Overdominant | log-additive |
|-----------|------------|-------------|-----------|--------------|--------------|
| rs1131243 | 0.00036    | 7.79E-05    | 0.10      | 0.00073      | 0.00011      |
| rs1805113 | 0.049      | 0.021       | 0.16      | 0.055        | 0.01         |
| rs1155705 | 0.062      | 0.026       | 0.13      | 0.22         | 0.01         |
| rs7524066 | 0.11       | 0.040       | 0.51      | 0.055        | 0.04         |
| rs2810904 | 0.24       | 0.15        | 0.18      | 0.43         | 0.09         |
| rs3738441 | 0.28       | 0.12        | 0.43      | 0.26         | 0.12         |
| rs1061427 | 0.39       | 0.17        | 0.66      | 0.21         | 0.18         |
| rs2276767 | 0.016      | 0.054       | 0.09      | 0.014        | 0.19         |
| rs1805112 | 0.085      | 0.79        | 0.04      | 0.065        | 0.33         |
| rs3917187 | 0.23       | 0.11        | 0.92      | 0.14         | 0.3          |
| rs7179893 | 0.60       | 0.52        | 0.35      | 0.95         | 0.35         |
| rs2241717 | 0.083      | 0.85        | 0.04      | 0.10         | 0.36         |
| rs2038931 | 0.61       | 0.54        | 0.34      | 0.89         | 0.36         |
| rs1800470 | 0.35       | 0.98        | 0.17      | 0.26         | 0.41         |
| rs11165376| 0.44       | 0.90        | 0.22      | 0.34         | 0.42         |
| rs2289259 | 0.70       | 0.40        | 0.75      | 0.48         | 0.43         |
| rs10783002| 0.14       | 0.68        | 0.09      | 0.08         | 0.49         |
| rs11165377| 0.80       | 0.51        | 0.85      | 0.53         | 0.54         |
| rs11466512| 0.16       | 0.71        | 0.09      | 0.15         | 0.58         |
| rs284878  | 0.89       | 0.77        | 0.65      | 0.93         | 0.68         |
| rs2289791 | 0.30       | 0.25        | 0.57      | 0.12         | 0.70         |
| rs11568753| 0.23       | 0.60        | 0.18      | 0.12         | 0.70         |
| rs2289261 | 0.92       | 0.69        | 0.88      | 0.78         | 0.72         |
| rs1750641 | 0.93       | 0.79        | 0.74      | 0.98         | 0.72         |
| rs2306888 | 0.88       | 0.83        | 0.62      | 0.94         | 0.75         |
| rs11165441| 0.24       | 0.79        | 0.11      | 0.39         | 0.77         |
| rs2228048 | 0.80       | 0.97        | 0.53      | 0.71         | 0.81         |
| rs1065080 | 0.50       | 0.56        | 0.40      | 0.34         | 0.83         |
| rs2241716 | 0.096      | 0.29        | 0.17      | 0.04         | 0.86         |
| rs7229678 | 0.89       | 0.91        | 0.68      | 0.66         | 0.86         |
| rs1805109 | 0.95       | 0.88        | 0.83      | 0.76         | 0.98         |
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