# Appendix

## Altered hematopoietic system and self-tolerance in Bardet-Biedl Syndrome

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**Appendix Table S1**

Counts of BBS patients from the CRIBBS registry presenting with more than one autoimmune condition.

| | Count of all cases | Rheumatoid arthritis | Celiac disease | Ulcerative Colitis | Type I Diabetes Mellitus | Hashimoto's thyroiditis |
|---|---|---|---|---|---|---|
| **Rheumatoid arthritis** | 17 | x | 0 | 1 | 0 | 1 |
| **Celiac disease** | 2 | | x | 0 | 0 | 0 |
| **Ulcerative Colitis** | 2 | | | x | 0 | 1 |
| **Type I Diabetes Mellitus** | 6 | | | | x | 1 |
| **Hashimoto's thyroiditis** | 6 | | | | | x |

**Appendix Table S2**

Counts of BBS patients from the Great Ormond Street Hospital & Guy's Hospital cohort presenting with more than one autoimmune condition.

| | Count of all cases | Inflammatory Bowel Diseases | Type I Diabetes Mellitus | Celiac disease | Psoriasis | Sarcoidosis | Henoch-Schönlein purpura | Immune thrombocytopenic purpura | Vitiligo | Hypothyroidism |
|---|---|---|---|---|---|---|---|---|---|---|
| **Inflammatory Bowel Diseases** | 3 | x | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Type I Diabetes Mellitus** | 3 | | x | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| **Celiac disease** | 4 | | | x | 0 | 0 | 0 | 0 | 0 | 1 |
| **Psoriasis** | 2 | | | | x | 0 | 0 | 0 | 0 | 0 |
| **Sarcoidosis** | 1 | | | | | x | 0 | 0 | 0 | 0 |
| **Henoch-Schönlein purpura** | 1 | | | | | | x | 0 | 0 | 0 |
| **Immune thrombocytopenic purpura** | 1 | | | | | | | x | 0 | 1 |
| **Vitiligo** | 1 | | | | | | | | x | 1 |
| **Hypothyroidism** | 22 | | | | | | | | | x |

**Appendix Table S3**

Counts of BBS patients from the Great Ormond Street Hospital & Guy's Hospital cohort with/without an autoimmune disease and grouped by BBS genes with causative mutation.

| **Gene with causative mutation** | **Autoimmune disease** | | |
|---|---|---|---|
| | **NO** | **YES** | **%** |
| BBS1 | 67 | 13 | 16,25% |
| BBS2 | 18 | 1 | 5,26% |
| BBS3 | 1 | 0 | 0,00% |
| BBS4 | 2 | 2 | 50,00% |
| BBS6 | 2 | 1 | 33,33% |
| BBS7 | 4 | 1 | 20,00% |
| BBS9 | 6 | 2 | 25,00% |
| BBS10 | 26 | 8 | 23,53% |
| BBS12 | 12 | 0 | 0,00% |
| BBS14 | 1 | 0 | 0,00% |
| Unknown | 26 | 5 | 16,13% |
| **Total** | **165** | **33** | 16,67% |

p = 0.28
(two-sided
Fisher's
exact test)

**Appendix Table S4**

Counts of BBS patients from the Great Ormond Street Hospital & Guy's Hospital cohort with/without an autoimmune disease and grouped by functional groups of BBS genes with causative mutation.

| Causative mutation (group of genes) | Autoimmune disease | | |
|---|---|---|---|
| | NO | YES | % |
| BBS1, 2, 4, 5, 7, 9 (the BBSome) | 100 | 19 | 15,97% |
| BBS6, 10, 12 (Chaperonins for the BBSome) | 41 | 10 | 19,61% |
| Other/Unknown | 24 | 4 | 14,29% |

p = 0.83
(two-sided
Fisher's
exact test)

**Appendix Table S5**

5'-3' DNA sequences of the primers used in the study.

| Primer name | 5'-3' DNA sequence | Purpose |
|---|---|---|
| F-GAPDH | TGCACCACCAACTGCTTAGC | qPCR |
| R-GAPDH | GGCATGGACTGTGGTCATGAG | |
| F-Tubb2A | AACCAGATCGGCGCTAAGT | |
| R-Tubb2A | TGCCAGCAGCTTCATTGTA | |
| F-eEF1a1 | ACACGTAGATTCCGGCAAGT | |
| R-eEF1a1 | AGGAGCCCTTTCCCATCTC | |
| F-BBS1 | ATCGGATTCTGACAGCGGG | |
| R-BBS1 | CCACCAGCTTGTACTCCCCA | |
| F-BBS2 | TGCCCCGATTCACCATGTAT | |
| R-BBS2 | CACGTGACCATCCTCTGTGTG | |
| F-BBS4 | AGCTTGGGATGAAAACTCAGGT | |
| R-BBS4 | GCTGTTCTTTGATCACAGCCTT | |
| F-BBS5 | GCGACCAGGGGAATTTAGGA | |
| R-BBS5 | ATGACAAGCGCCAAACCAAA | |
| F-BBS7 | AGGGCTACACAAAAGGTGGT | |
| R-BBS7 | TTCTCCTGAGGCGTGTTGAC | |
| F-BBS8 | CTTATGATCAGGCGGCTTGGA | |
| R-BBS8 | GTGGGACCTGAGCAATAGCA | |
| F-BBS9 | ACTCCAGACCGACAGGTATT | |
| R-BBS9 | GGCTGACCAGGTAGGCAAAT | |
| F-BBS18 | AGCCCCTGATCGCTTACCTA | |
| R-BBS18 | GACAATGTCTCACTCGTCAGC | |
| F-CXCL12 | AAAGCTTTAAACAAGGGGCG | |
| R-CXCL12 | GCAGGAAGCGGGGAACTA | |
| F-IL7 | AAAGCCAGAGCGCCTGGGTG | |
| R-IL-7 | CTGGGCAGGGCAGTTCAGGC | |
| F-BBS4 Exon5/6 | GTGGCCAGATCTTTGTTTCTTCT | |
| F-BBS4 Exon 5 | GACATGTGCTGTTCTCAGCC | End-point PCR |
| R-BBS4 Exon 6 | GGCAGCTTTGTGTTTTCCCA | |
| F-BBS4 Exon 6 | GGGAAAACACAAAGCTGCCA | |
| R-BBS4 Exon 7 | GTGTAGCACACTCCCAGGTT | |
| F-BBS4 Exon 7 | CCATAACCTGGGAGTGTGCT | |

| | | |
|---|---|---|
| R-BBS4 Exon 8 | CCAGGTCTCCCTGCAGTAAA | |
| F-BBS4 Exon 8 | GGGAGACCTGGATAAAGCCATC | |
| R-BBS4 Exon 10 | AGGTCAGTGCATTCCCAAGAT | |
| F-BBS4 Exon 10 | TGGGAATGCACTGACCTACG | |
| R-BBS4 Exon 11 | GTCCCCATGAGTCTGCATCA | |
| F-EcoRI-BBS4 | TCGGAATTCATGGCTGAAGTGAAGCTTGGGAT | BBS4-FLAG cloning |
| R-BBS4-Flag | GTCTTTGTAGTCTTTTTCTTTCTTTTGTTCTGATGCTTC | |
| R-ClaI-Flag | TTTATCGATCTACTTGTCGTCATCGTCTTTGTAGTCTTTTTC | |
| F-BBS18 | CGGTTACCTCCGTTTCTCTG | BBS18 mice genotyping |
| R-BBS18 | TGAAGGTGTTTGGTGACTGC | |

## Appendix Table S6

Genetic modifications in ST2 cell clones obtained by CRISPR/Cas9 targeting of BBS4. Table comprises the modifications in *Bbs4* and the derived protein sequences. #1 – sgRNA #1, #2 – sgRNA #2, bold - amino acid sequence different from WT, * - stop codon.

| ST2 clones | BBS4 ORF nucleotide modification 5' to 3' | protein sequence from the START to STOP codon |
|---|---|---|
| B4 | #1: deletion of T(131) | MAEVKLGMKTQVPASVESQKPRSKKAPDFPIVEKQNWLIHLHY**TGRIMKLARL*** |
| B7 | #2: deletion of C(235) | MAEVKLGMKTQVPASVESQKPRSKKAPDFPIVEKQNWLIHLHYIRKDYEACKAVIKEQLQETQGLCEYAIYVQALIFR**WKEISKNP*** |
| D7-2 | #1: deletion of AT(130-131) | MAEVKLGMKTQVPASVESQKPRSKKAPDFPIVEKQNWLIHLHY**PEGL*** |
| F7 | #1: deletion of TA(129-130) | MAEVKLGMKTQVPASVESQKPRSKKAPDFPIVEKQNWLIHLHY**PEGL*** |
| F8 | none | WT sequence |