

High-throughput sequencing reveals similar molecular signatures for class switch recombination junctions for the γ and α isotypes

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1 High throughput sequencing reveals similar molecular signatures for class switch
2 recombination junctions for the γ and α isotypes.

3

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19

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23 After encountering antigen, B-cells undergo class switch recombination (CSR) that substitutes
24 the C_μ gene with C_γ , C_ϵ or C_α , thereby generating IgG, IgE and IgA antibodies with same
25 antigenic specificity but new effector functions (1). The DNA-editing enzyme activation-
26 induced deaminase (AID) is essential for CSR by targeting switch (S) regions preceding C_μ
27 (namely the S_μ donor region) and C_γ , C_ϵ and C_α genes (namely the $S_{\gamma,\epsilon,\alpha}$ acceptor regions) (1).
28 *Cis*- and *trans*-controlled DNA double strand breaks are generated during this process (2-6).
29 The recruitment of DNA repair factors that facilitate the end-joining process is a crucial step
30 of class switch recombination. Two pathways are implicated in this end joining. The classical
31 non-homogenous end joining (c-NHEJ) pathway ligates DNA ends with no or little
32 homology. In contrast, the alternative end joining (A-EJ) pathways is used to ligate DNA ends
33 with microhomology (3-6). Previous reports have suggested that IgG and IgA CSR might be
34 differently regulated with a preferential use of c-NHEJ for γ CSR and A-EJ for α CSR (7, 8).
35 We recently reported a computational tool (CSReport) for automatic analysis of CSR
36 junctions sequenced by high-throughput sequencing (9) and used it to analyze the rare S_μ - S_δ
37 junctions form during IgD CSR (10, 11). We thus used CSReport and high-throughput
38 sequencing to analyze the molecular signature of S_μ - $S_{\gamma3}$, S_μ - $S_{\gamma1}$ and S_μ - S_α junctions in *wt* mice
39 more in depth.

40 Our research has been approved by our local ethics committee review board (Comité Régional
41 d'Ethique sur l'Expérimentation Animale du Limousin, Limoges, France) and carried
42 according the European guidelines for animal experimentation. Single-cell suspensions of
43 spleen cells from wild-type (*wt*) 129 mice were cultured 4 days at 1×10^6 cells/ml in RPMI
44 1640 with 10% fetal calf serum (FCS) and 5 μ g/ml LPS, with or without addition of 20 ng/ml
45 IL-4 or 2 ng/ml TGF β (PeproTech, Rocky Hill, NJ). Splenocyte DNA was then extracted for
46 investigation of S_μ - $S_{\gamma3}$, S_μ - $S_{\gamma1}$ and S_μ - S_α junctions. As previously described in detail (9),
47 junctions were PCR amplified. Libraries of 200bp were prepared from the 1-2kb PCR

48 products of S_{μ} - $S_{\gamma 1}$, S_{μ} - $S_{\gamma 3}$ and S_{μ} - S_{α} amplification for Ion Proton sequencing (“GénoLim
49 platform” of the Limoges University, France). Sequenced reads were then mapped to S_{μ} and
50 acceptor $S_{\gamma 1}$, $S_{\gamma 3}$ and S_{α} regions using BLAST algorithm. The computational tool developed
51 for experiments performs junction assembly, identifies breakpoints in S_{μ} , $S_{\gamma 1}$, $S_{\gamma 3}$ S_{α} , identifies
52 junction structure (blunt, micro-homology or junction with insertions) and outputs a statistical
53 summarization of identified junctions.

54 LPS, LPS+IL4 and LPS+TGF β stimulated B-cell CSR to IgG3, IgG1 and IgA, respectively
55 (2, 5, 12). We detected 4140, 3798 and 1955 S_{μ} - $S_{\gamma 1}$, S_{μ} - $S_{\gamma 3}$ and S_{μ} - S_{α} junctions, respectively.
56 The structural profiles of all these junctions (blunt, micro-homology or junction with
57 insertions) are reported in Fig. 1A. The positions of IgG₁, IgG₃ and IgA junctions in terms of
58 distance from the forward PCR primer in S_{μ} are reported in Fig. 1B. Localizations of S_{μ}
59 breakpoints within AID hotspots (AGCT, WRCY, RGYW) and other motifs are shown in Fig.
60 1C (both displayed along S_{μ} region and expressed in % of junctions). Analysis of 5000
61 synthetic junctions simulated from the random association of 100-bp S_{μ} segments with 100-bp
62 segments of $S_{\gamma 1}$, $S_{\gamma 3}$ or S_{α} revealed a similar pattern of blunt and micro-homology junctions
63 (junction with insertions are not produced with this numerical approach) (Fig. 1D) compared
64 with true junctions (Fig. 1A). As shown in Fig1E, the frequency of S_{μ} - $S_{\gamma 1}$, S_{μ} - $S_{\gamma 3}$ and S_{μ} - S_{α}
65 junctions with large (>5 bp) micro-homology is found higher in S_{μ} - S_{α} compared to S_{μ} - $S_{\gamma 1}$ and
66 S_{μ} - $S_{\gamma 3}$, both for sequenced junctions and for randomly simulated junctions. Fig. 1F shows
67 dotplots for S_{μ} vs $S_{\gamma 1}$, S_{μ} vs $S_{\gamma 3}$ and S_{μ} vs S_{α} sequence comparisons.

68 Confirming the validity of our technical approach, the structural profile of our S_{μ} - $S_{\gamma 1}$
69 junctions was similar to that reported few weeks ago using a high-throughput translocation
70 sequencing method (5). We also confirm the previously reported slight increase of small
71 insertions for S_{μ} - S_{γ} compared to S_{μ} - S_{α} (8). Finally we demonstrated that the slight increase of

72 S_{μ} - S_{α} junctions with large micro-homology can be numerically reproduced using randomly
73 generated synthetic junctions. As this simulation mimics a pure NHEJ process (linking two
74 free DNA ends without any resection), it evidences that it is not necessary to invoke another
75 molecular mechanism (such A-EJ) to explain the observed structure alteration of S_{μ} - S_{α}
76 junctions. Those micro-homologies arise solely by chance and are favored by the repetitive
77 structure of the S_{α} region and its high degree of similarity to S_{μ} region. In conclusion the
78 structural profiles of S_{μ} - $S_{\gamma 1}$, S_{μ} - $S_{\gamma 3}$ and S_{μ} - S_{α} junctions are similar indicating same CSR
79 process and partners whatever cytokine stimulations, length of the S acceptor region and its
80 distance with the S_{μ} donor region. Analysis of the molecular signature of CSR junctions does
81 not argue in favour of a preferential use of c-NHEJ and A-EJ for γ CSR and α CSR,
82 respectively.

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121 **Legend to Figure 1.**

122 **CSR in *wt* mice.**

123 A: Structure profiles of S_{μ} - $S_{\gamma1}$, S_{μ} - $S_{\gamma3}$ and S_{μ} - S_{α} junctions. Junctions are classified in terms of
124 junction types (junction with insertions, blunt junction or junction with micro-homology). B:
125 Breakpoint localizations in S_{μ} for S_{μ} - $S_{\gamma1}$, S_{μ} - $S_{\gamma3}$ and S_{μ} - S_{α} junctions. C: (top) Location of
126 breakpoints in respect of AID hotspots AGCT, WRCY, RGYW and other motifs along the
127 first 1kb in S_{μ} . Identified breaks are shown as a black line, colocation with a sequence motif is
128 indicated with a colored asterisk. (bottom) Frequency of hotspot/break colocation events. D:
129 Structure profiles of synthetic S_{μ} - $S_{\gamma1}$, S_{μ} - $S_{\gamma3}$ and S_{μ} - S_{α} junctions. Three sets of 5000 synthetic
130 junctions were simulated from the random association of 100-bp S_{μ} segments with 100-bp
131 segments of $S_{\gamma1}$, $S_{\gamma3}$ or S_{α} and analyzed with the same computational tool (CSReport) as
132 sequencing reads. E: Frequency of junctions with long micro-homology (>5bp) for S_{μ} - $S_{\gamma1}$, S_{μ} -
133 $S_{\gamma3}$ and S_{μ} - S_{α} junctions. Simulated datasets are compared to the junctions identified from
134 high-throughput sequencing. F: Sequence similarity dotplots of S_{μ} vs $S_{\gamma1}$, S_{μ} vs $S_{\gamma3}$ and S_{μ} vs
135 S_{α} . Similarity was evaluated with a 20-bp window and reported when greater than 60%.

