Insufficiency of Genomic Structure in Immunoglobulin Locus for Inducing High-Frequency Gene Conversion in Variable Regions

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Abstract

Mammals, including humans and mice, employ V(D)J recombination and somatic hypermutation (SHM) to diversify their immunoglobulin (Ig) variable region repertoires, while domestic poultry, such as chickens and ducks, rely heavily on gene conversion (GCV) to achieve this diversification. Given the differences in the arrangement of Ig gene loci between chickens/ducks and humans/mice, we posited that the structural arrangement of the Ig locus might play a pivotal role in driving the pronounced frequency of GCV in poultry. To test this hypothesis, we engineered a novel mouse model, Igλ $^{-/-}$Igκ $^{-/-}$DIgL, characterized by the incorporation of the entire duck IgL gene locus and the simultaneous absence of the mouse endogenous IgL locus. These transgenic mice produced chimeric antibodies comprising mouse heavy chains and duck light chains, thereby offering partial support for B cell development. However, when subjected to repeated immunization with a variety of antigens, the humoral immune response in Igλ $^{-/-}$Igκ $^{-/-}$DIgL mice was notably diminished compared to that in wild-type mice. High-throughput sequencing of IgL repertoires unveiled distinct differences between Igλ $^{-/-}$Igκ $^{-/-}$DIgL mice and ducks in terms of V gene utilization frequencies, characteristics of the CDR3 region (CDRL3), and nucleotide additions at junctions (NP nucleotides). Despite the increased expression of duck IgL due to the absence of endogenous mouse IgL, the level of GCV observed in duck IgL of Igλ $^{-/-}$Igκ $^{-/-}$DIgL mice (2.01%) remained significantly lower than that in ducks (35.3%). These findings collectively indicate that the structural configuration of the Ig locus may not be a critical determinant of GCV and provide valuable insights into the mechanisms underpinning the high frequency of GCV observed in poultry species.

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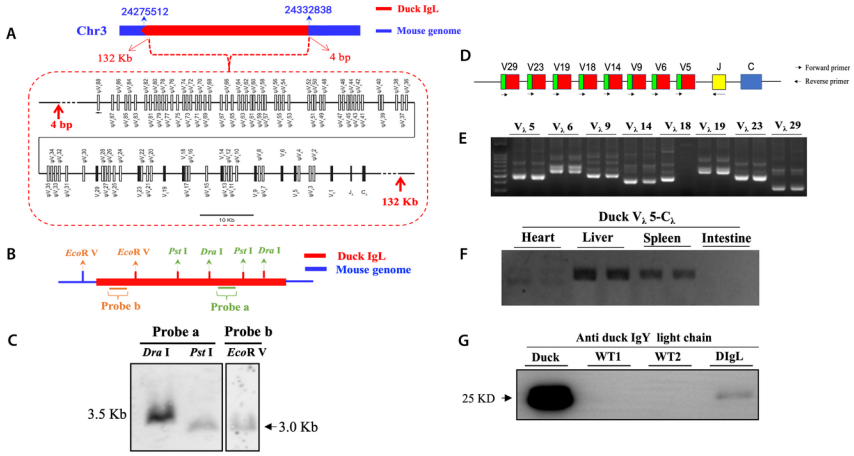
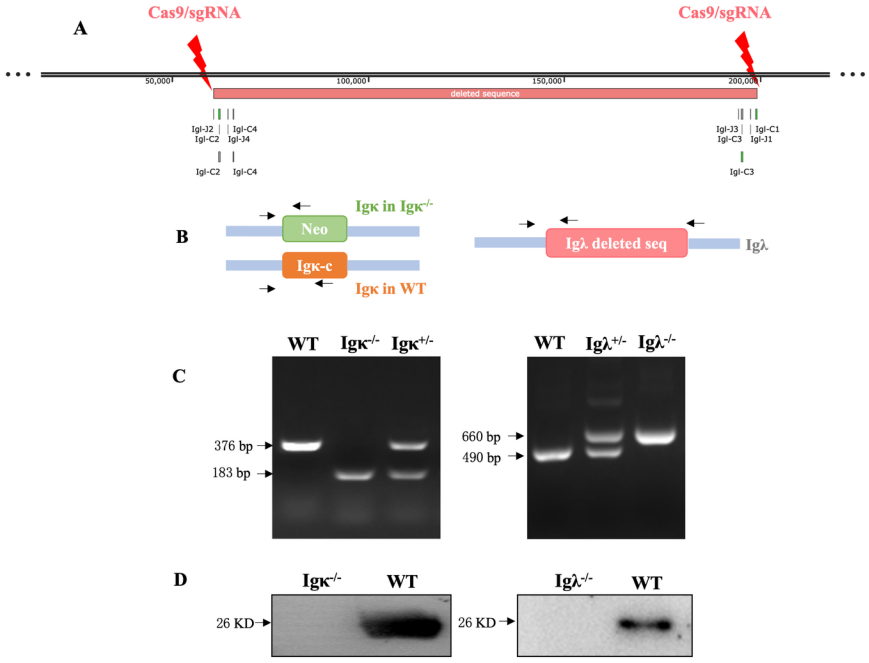
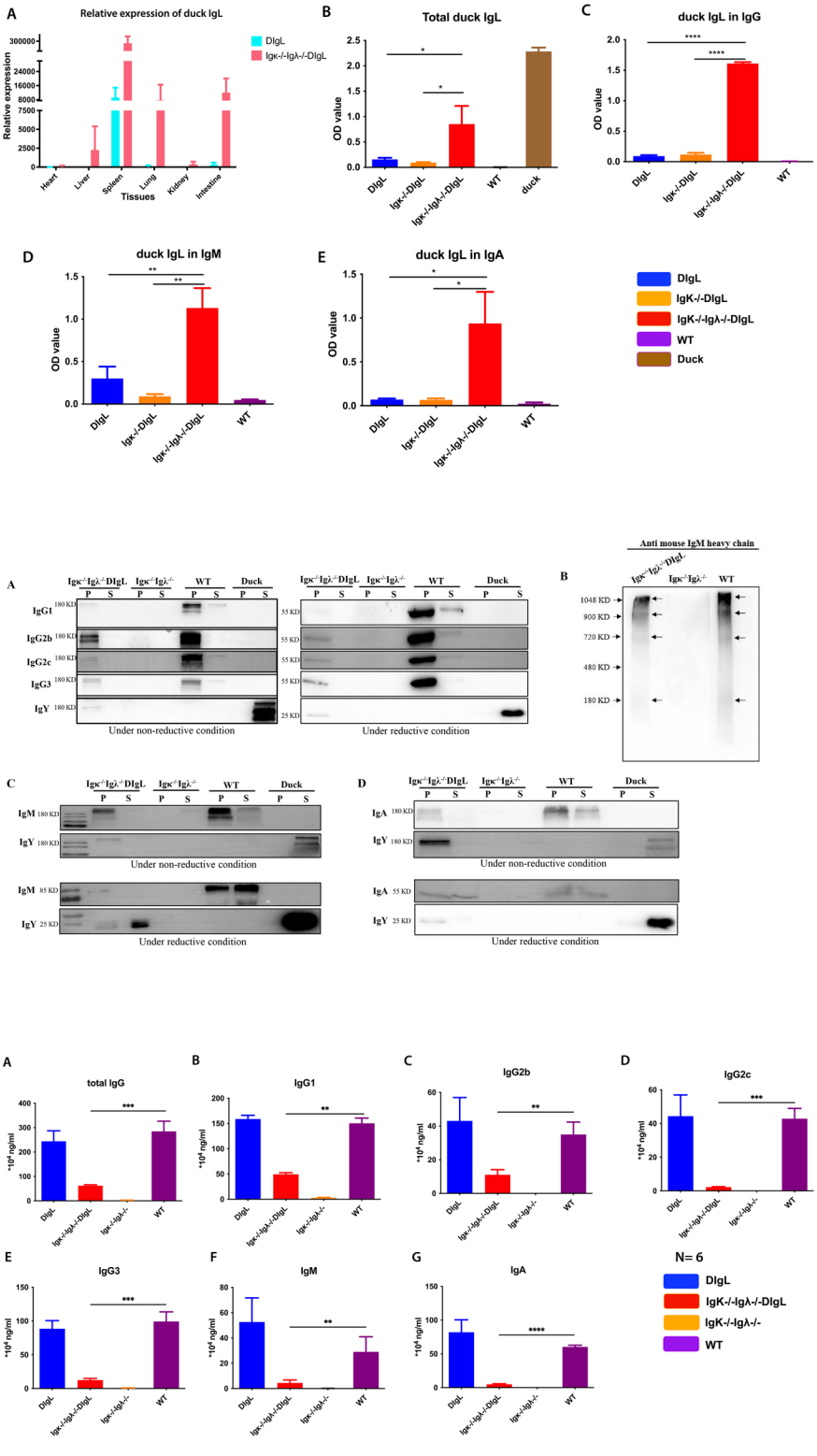
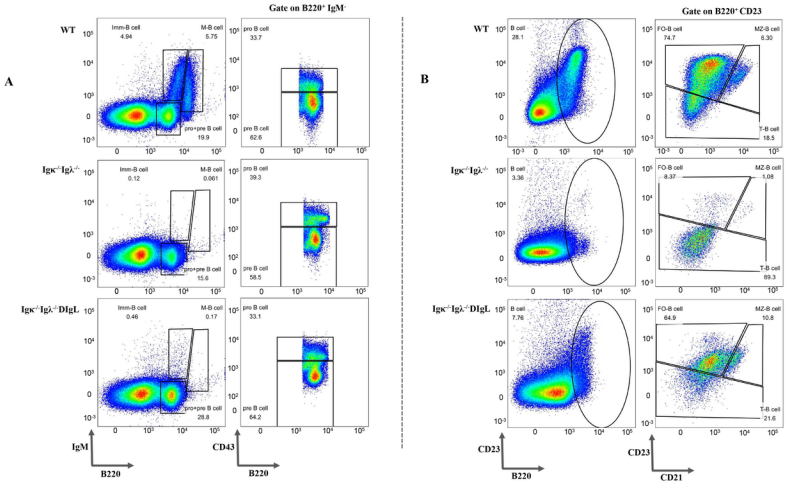
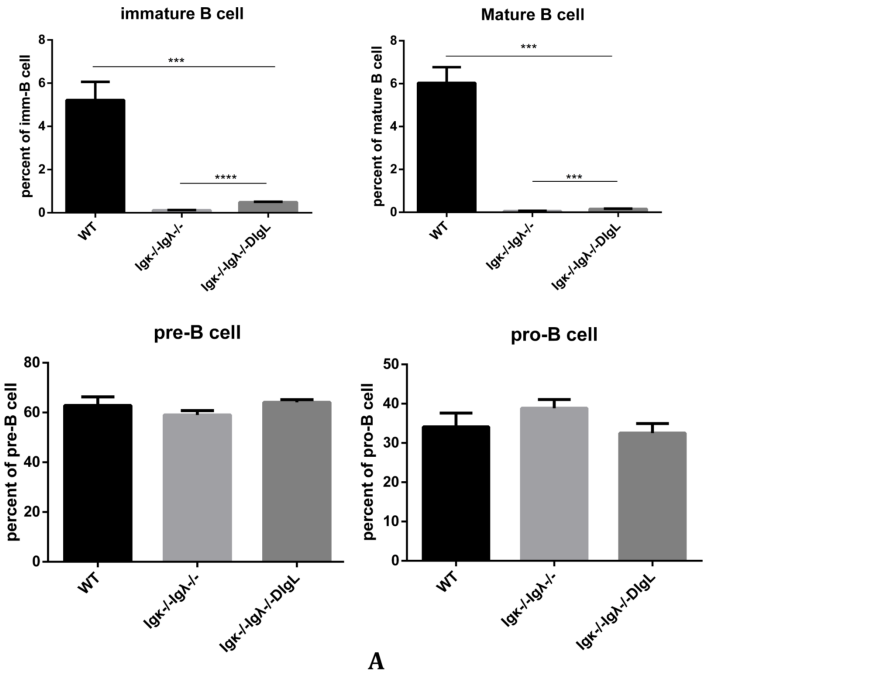


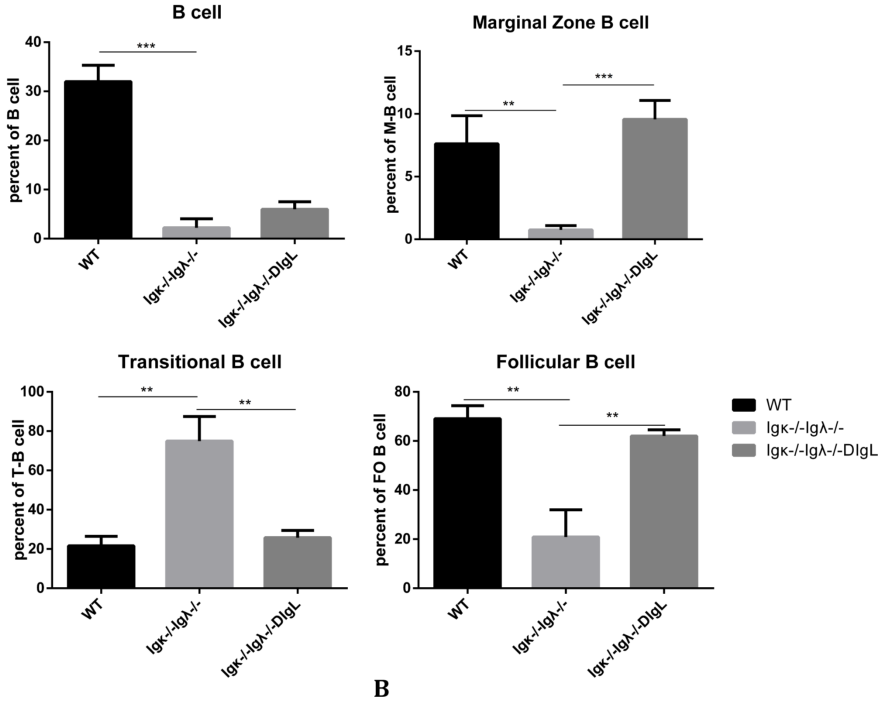








A



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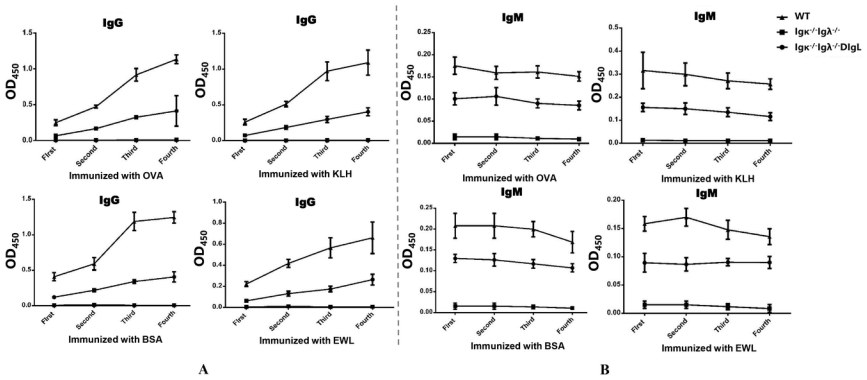

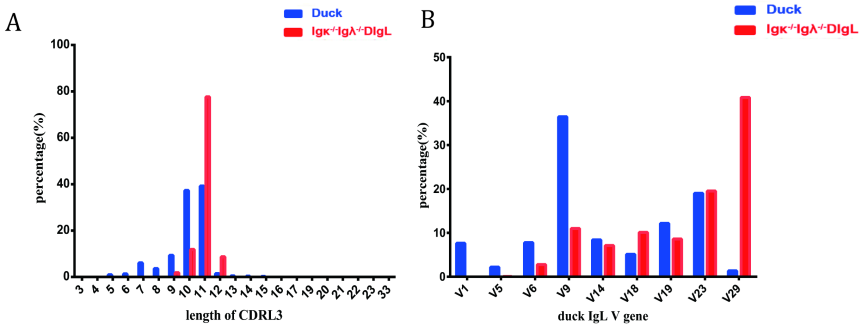

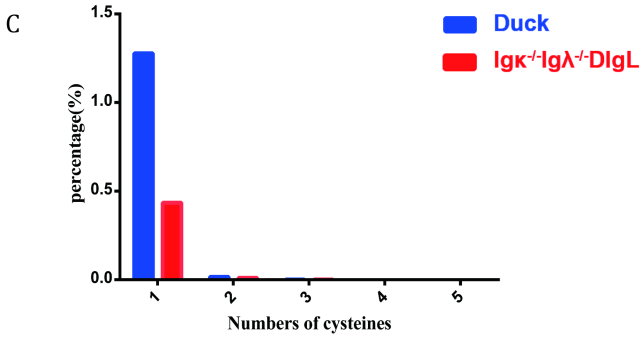

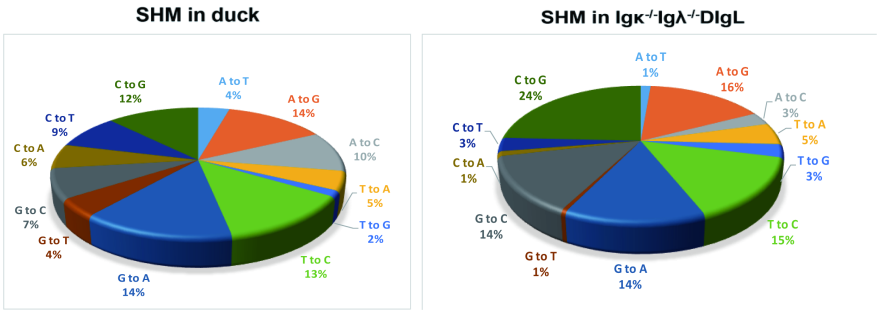

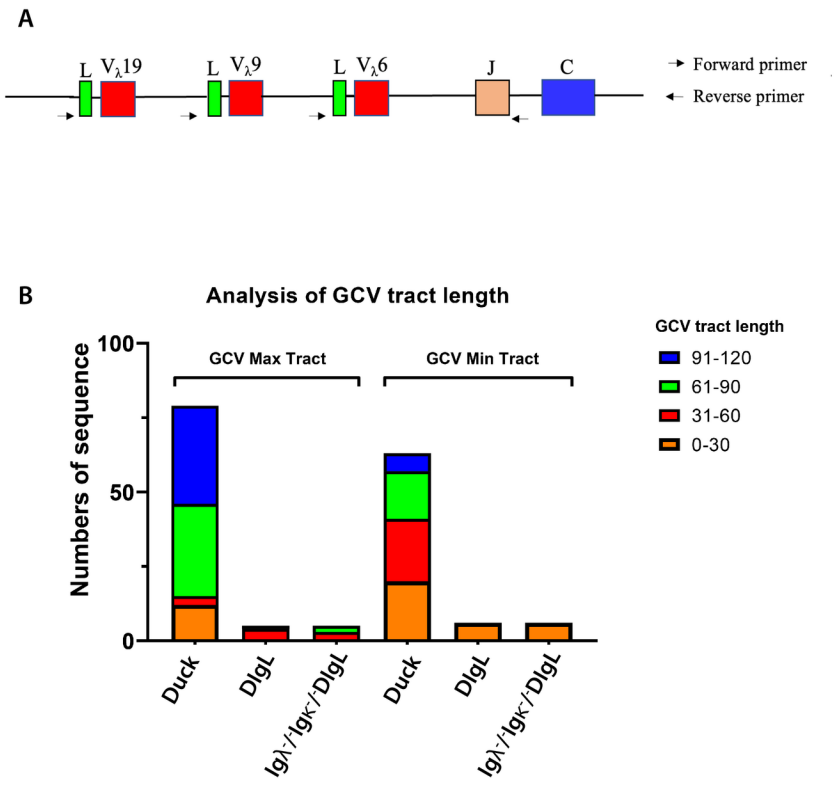

Sample	Unique clones	% with GCV	Max GCV score	Avg Tract (nt)
Igκ$^{-/-}$Igλ$^{-/-}$DIgL-Vλ6	125	1.6	9	37
Igκ$^{-/-}$Igλ$^{-/-}$DIgL-Vλ9	191	2.09	21	50±26
Igκ$^{-/-}$Igλ$^{-/-}$DIgL-Vλ19	218	2.29	23	57
Igκ$^{-/-}$Igλ$^{-/-}$DIgL-total	**534**	**2.01**	**23**	**49±23**
DIgL-Vλ6	111	1.8	12	42±29
DIgL-Vλ9	172	2.33	18	64±38
DIgL-Vλ19	132	2.27	19	56
DIgL-total	**415**	**2.25**	**19**	**52±32**
duck-Vλ6	118	52.7	18	84±28
duck-Vλ9	102	4.9	6	72±58
duck-Vλ19	167	29.8	22	96±48
duck-total	**387**	**35.3**	**22**	**88±51**

Table. 2a

Sample	Unique CDR3	Average length	CDR3 reads
Duck	13711	10.015±1.343	908195
Igκ$^{-/-}$Igλ$^{-/-}$DIgL	5401	10.932±0.525	1966937

Table. 2b

Sample	Total reads	SHM reads	SHM Frequency
Duck	45998645	33947	0.000738
Igκ$^{-/-}$Igλ$^{-/-}$DIgL	49733258	22933	0.000461