Unraveling of the Polymorphic Cλ2-Cλ3 Amplification and the Ke^+Oz^- Polymorphism in the Human Igλ Locus

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Two polymorphisms of the human Igλ (*IGL*) locus have been described. The first polymorphism concerns a single, 2- or 3-fold amplification of 5.4 kb of DNA in the Cλ2-Cλ3 region. The second polymorphism is the $Mcg^-Ke^+Oz^-$ isotype, which has only been defined via serological analyses in Bence-Jones proteins of multiple myeloma patients and was assumed to be encoded by a polymorphic Cλ2 segment because of its high homology with the $Mcg^-Ke^-Oz^-$ Cλ2 isotype. It has been speculated that the $Mcg^-Ke^+Oz^-$ isotype might be encoded by a Cλ gene segment of the amplified Cλ2-Cλ3 region. We now unraveled both *IGL* gene polymorphisms. The amplification polymorphism appeared to result from a duplication, triplication, or quadruplication of a functional J-Cλ2 region and is likely to have originated from unequal crossing over of the J-Cλ2 and J-Cλ3 region via a 2.2-kb homologous repeat. The amplification polymorphism was found to result in the presence of one to five extra functional J-Cλ2 per genome regions, leading to decreased Igκ:Igλ ratios on normal peripheral blood B cells. Via sequence analysis, we demonstrated that the $Mcg^-Ke^+Oz^-$ isotype is encoded by a polymorphic Cλ2 segment that differs from the normal Cλ2 gene segment at a single nucleotide position. This polymorphism was identified in only 1.5% (2 of 134) of individuals without J-Cλ2 amplification polymorphism and was not found in the J-Cλ2 amplification polymorphism of 44 individuals, indicating that the two *IGL* gene polymorphisms are not linked. ***The Journal of Immunology*****, 2002, 169: 271–276.**

The human Igλ (*IGL*)[2] locus is located on chromosome band 22q11.2 and spans a region of about one megabase (1). The *IGL* locus contains 73–74 Vλ gene segments, including 56–57 functional Vλ segments, which could be assigned to 11 subgroups based on nucleotide homology (1–6) (http://imgt.cnusc.fr:8104). Seven J-Cλ gene regions are located downstream of the Vλ segments. Each Cλ gene segment is preceded by a Jλ gene segment. Only four J-Cλ gene regions are functional: i.e., J-Cλ1, J-Cλ2, J-Cλ3, and J-Cλ7; the regions J-Cλ4, J-Cλ5, and J-Cλ6 are nonfunctional (pseudo) gene regions (7–9). This is due to a deletion of ~1150 bp in the J-Cλ4 region and the lack of some of the essential nucleotides in the recombination signal sequence (RSS) (3) of the Jλ4 gene segment (7, 10). In the 3′ end of the Cλ5 exon, 11 bp are deleted, and the RSS of the Jλ5 segment also lacks some of the essential nucleotides (7, 10). Finally, the Cλ6 exon contains a duplication of four nucleotides, which results in a premature stop codon (7, 9). Rearrangements of the J-Cλ6 segment can occur, but they encode a truncated Igλ protein (11).

The four different functional types of Igλ L chains have also been identified in human serum using the serological isotype markers Mcg, Kern (Ke), Oz, and Mcp (11–14). These serological isotype markers are based on amino acid differences found in the Cλ regions of various Bence-Jones proteins derived from multiple myeloma patients as well as from Igλ L chains isolated from intact Igs. The four functional J-Cλ gene regions, J-Cλ1, J-Cλ2, J-Cλ3, and J-Cλ7, encode the isotypes $Mcg^+Ke^+Oz^-$, $Mcg^-Ke^-Oz^-$, $Mcg^-Ke^-Oz^+$, and Mcp^+, respectively. A fifth isotype, termed $Mcg^-Ke^+Oz^-$, is highly homologous to $Mcg^-Ke^-Oz^-$ and is assumed to be encoded by a polymorphic Cλ2 gene segment, but this has not yet been confirmed by sequence analysis at the DNA level.

The human *IGL* locus also contains an ~5.4-kb amplification polymorphism in the Cλ2-Cλ3 region. As the amplified region can be present once, twice, or three times; this amplification might result in up to 10 J-Cλ gene regions per allele (3, 15–17). The polymorphic Cλ2-Cλ3 amplifications can be identified via Southern blot (SB) analysis as *Eco*RI fragments of 13.7, 19.1, or 24.5 kb, representing one, two, or three amplifications, respectively (15, 18). In the absence of an *IGL* amplification polymorphism, only an 8.3-kb *Eco*RI fragment is detected. In *Hin*dIII-digested DNA, the *IGL* polymorphism is visible as a 5.4-kb fragment, independent of the number of amplified regions (18). It has been reported that the frequency of the Cλ2-Cλ3 amplification polymorphism varies between populations in different geographical areas (3, 16, 17), but this polymorphism has not been fully characterized in detail.

Here we studied both the Cλ2-Cλ3 amplification polymorphism and the $Mcg^-Ke^+Oz^-$ polymorphism. Our aim was to investigate whether the $Mcg^-Ke^+Oz^-$ isotype is encoded by a polymorphic Cλ2 gene segment and whether the Cλ segment encoding this isotype is located on the amplified Cλ2-Cλ3 fragment, implying that the two *IGL* polymorphisms might be linked, as speculated previously (8, 14). Furthermore, we assessed the structure of the Cλ2-Cλ3 amplification polymorphism and determined whether the amplified region contains functional J-Cλ segments that might lead to higher frequencies of Igλ protein expression.

Materials and Methods

Cell samples

Peripheral blood (PB) samples were obtained from 96 healthy individuals. The expression of Igκ and Igλ on PB B cells was determined via Igκ/CD19

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[2] Abbreviations used in this paper: *IGL*, Igλ; *IGK*, Igκ; PB, peripheral blood; RSS, recombination signal sequence; SB, Southern blot.

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and Igλ/CD19 two-color immunofluorescence in 69 cases. DNA was isolated from the granulocytes of all 96 healthy individuals using phenol/chloroform extraction (18). In addition, DNA samples from 82 precursor-precursor B cell acute lymphoblastic leukemia samples and Igκ^+ B cell chronic lymphocytic leukemia with both *IGL* alleles in germline configuration were analyzed for the presence of the Mcg$^-$Ke$^+$Oz$^-$ polymorphism (19, 20).

Southern blot analysis

Fifteen micrograms of DNA from 80 healthy controls (60 of Caucasoid origin and 20 of Chinese origin) was digested with *Eco*RI (Life Technologies, Gaithersburg, MD), separated in 0.7% agarose gels, and transferred by vacuum blotting to Nytran-13N nylon membranes (Schleicher & Schuell, Dassel, Germany) (18). The filters were hybridized with the ^{32}P-labeled λ-IVS probe (15).

PCR analysis and fluorescent sequencing of the amplified Cλ2-Cλ3 region

Specific PCR primers for the Cλ2-Cλ3 amplification polymorphism (*IGL*amp-F and *IGL*amp-R) were designed using the OLIGO 6.2 software program (Dr. W. Rychlik, Molecular Biology Insights, Cascade, CO; Table I). PCR analysis was performed with the Expand Long Template PCR System (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. PCR products were analyzed on a 1% agarose gel and purified using a PCR purification kit (Qiagen, Hilden, Germany) before fluorescent sequencing. PCR products were sequenced on an ABI 377 fluorescent cycle sequencer (PE Applied Biosystems, Foster City, CA) with Big Dyes (PE Applied Biosystems) according to the manufacturer's instructions. The original PCR primers were used as sequence primers, and subsequently new sequence primers were designed in the newly sequenced regions. An extra PCR was performed using the primers Up-F and Down-R to sequence the gap between the primers *IGL*amp-R and *IGL*amp-F (see also Table I and Fig. 2*B*). The *IGL* gene sequence (accession no. X51755) was used as the reference sequence. Pairwise alignment algorithms were used from EMBL, European Bioinformatics Institute (Cambridge, U.K.; http://www.ebi.ac.uk/emboss/align/).

Analysis of the Cλ2 gene region for the presence of the Mcg$^-$Ke$^+$Oz$^-$ polymorphism

The Cλ2 region was amplified by PCR with the primers Cλ2-F and Cλ2-R (Table I) using the TaqGold amplification system (PE Applied Biosystems). The obtained PCR products were sequenced with the Cλ2-F primer and/or the Cλ2-Rseq primer (Table I) as described above.

Results

Southern blot analysis of the Cλ2-Cλ3 amplification polymorphism in Caucasoid and Chinese individuals

The *IGL* locus of 80 healthy controls (60 of Caucasoid origin and 20 of Chinese origin) was analyzed with respect to the number of amplifications using the λ-IVS probe in combination with *Eco*RI digests (Fig. 1*A*). Examples of Southern blot results for two individuals without a Cλ2-Cλ3 amplification polymorphism and four individuals with amplification polymorphisms are shown in Fig. 1*B*. Seventy-two percent of the Caucasians did not have the Cλ2-Cλ3 amplification polymorphism, in contrast to only 15% of the Chinese individuals (Table II). The *IGL* locus of individuals without a Cλ2-Cλ3 amplification polymorphism on both alleles is described as P_0/P_0. In the group of Caucasoid individuals the frequency of monoallelic amplifications was 7, 15, and 7% for one, two, or three amplifications, respectively (P_0/P_1, P_0/P_2, and P_0/P_3). These percentages were comparable to the percentages in the Chinese individuals. However, 55% of the Chinese individuals had biallelic amplifications (P_1/P_2, P_1/P_3, P_2/P_2, and P_2/P_3), which were not observed in the Caucasoid individuals.

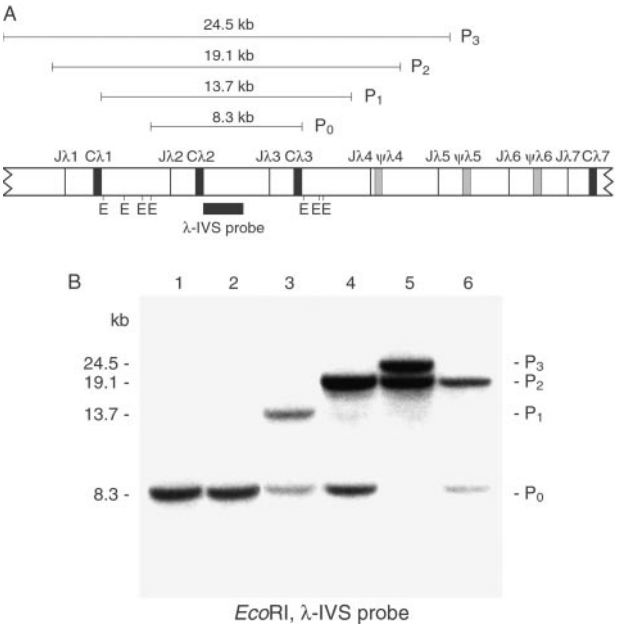

FIGURE 1. Detection of the *IGL* amplification polymorphism of the Cλ2-Cλ3 region. *A*, Schematic representation of the *IGL* locus with the amplification polymorphism of the Cλ2-Cλ3 region. The *Eco*RI restriction sites, the position of the λ-IVS probe, and the relevant restriction fragments are indicated. In cases without an *IGL* amplification polymorphism (P_0) the λ-IVS probe detects an 8.3-kb band. Dependent on the number of amplified regions (P_1, P_2, or P_3), a 13.7-, 19.1-, or 24.5-kb band is detected, respectively. *B*, Southern blot analysis of *Eco*RI-digested DNA of six healthy controls with the λ-IVS probe. *Lanes 1* and *2*, No *IGL* amplification polymorphism on both alleles (P_0/P_0); *lanes 3*, *4*, and *6*, one allele without *IGL* amplification polymorphism together with a polymorphic allele, P_0/P_1, P_0/P_2, and P_0/P_2, respectively; *lane 5*, two polymorphic alleles (P_2/P_3).

Sequencing strategy of the Cλ2-Cλ3 amplification polymorphism

To date, the polymorphic Cλ2-Cλ3 amplification has never been sequenced, probably because of the complexity of the *IGL* locus, especially because of the high homology between the J-Cλ2 region and the J-Cλ3 region. The primers (*IGL*amp-F and *IGL*amp-R) were designed tail-to-tail, just upstream of the *Hin*dIII restriction

Table I. *Sequences of used primers for PCR analysis and/or sequencing of the* IGL *locus*

Primer	Sequence (5′–3′)
*IGL*amp-F	TTGTGCTGCCACCAGGAT
*IGL*amp-R	TGCCCAGGGAACTGAGAAC
Up-F	GTGGGTTCTCAATTTGTGGT
Down-R	TGCCAGATGGATAAATGTGAC
Cλ2-F	CCTGCCCCTCATCCACC
Cλ2-R	GAGAGCTCACCAGAGTCACTGG
Cλ2-Rseq	TCAGGCGTCAGGCTCAGATAG

Table II. *Configuration of the* IGL *locus with respect to the number of* IGL *amplifications as determined by Southern blot analysis*

IGL Locus Configuration	Caucasians (n = 60)	Chinese (n = 20)	Total (n = 80)
P_0/P_0	43 (71.6%)	3 (15%)	46 (57.5%)
P_0/P_1	4 (6.7%)	1 (5%)	5 (6.3%)
P_0/P_2	9 (15%)	4 (20%)	13 (16.2%)
P_0/P_3	4 (6.7%)	1 (5%)	5 (6.3%)
P_1/P_1			
P_1/P_2		2 (10%)	2 (2.5%)
P_1/P_3		1 (5%)	1 (1.2%)
P_2/P_2		7 (35%)	7 (8.8%)
P_2/P_3		1 (5%)	1 (1.2%)
P_3/P_3			

site, such that in situations without polymorphic Cλ2-Cλ3 amplifications (P_0/P_0), no PCR product is formed (Fig. 2, *A* and *C*). In cases with a polymorphic amplification, a PCR product of ~5.4 kb was generated, independent of the number of amplifications (Fig. 2, *B* and *C*). The size of the PCR product was comparable to the size of the amplified region, as it was determined via Southern blot analysis using *Hin*dIII digests (18). The amount of PCR product was quantified using semiquantitative PCR with sampling after different cycles (7, 10, 13, 16, 19, and 22 cycles), followed by hybridization with the J-Cλ2-specific *IGL*C2D probe (19). The hybridization signals obtained correlated with the number of amplifications as determined via Southern blot analysis (data not shown). This result implies that in the case of multiple Cλ2-Cλ3 regions, all regions were represented in the sequence analysis. The 5.4-kb PCR product of four individuals with the genotypes P_1/P_2, P_2/P_2, P_2/P_2, and P_0/P_3 were completely sequenced. Sequence analysis was started with the *IGL*amp-F and *IGL*amp-R primers. Subsequently, sequence primers were designed in the newly generated sequence ("gene walking"). Finally, an extra primer set was designed to analyze the region between the two primers *IGL*amp-R and *IGL*amp-F by PCR and sequencing. The forward primer (Up-F) was designed in the newly generated sequence and the reverse primer (Down-R) downstream of the *IGL*amp-F primer (Fig. 2*B*). These primers also annealed in the homologous region just downstream of the J-Cλ2 region (Fig. 2*B*). The total sequenced region is indicated in Fig. 2*B*.

Structure of the Cλ2-Cλ3 amplification polymorphism

The region of the *IGL*amp-F *IGL*amp-R product together with the extended region that fills the gap between the ends of the two primers was further analyzed for the presence of restriction sites (Fig. 3*A*, white area). This sequence was subsequently aligned with the standard *IGL* gene sequence (P_0) to determine the structure of the amplification. The first 2.5 kb of the sequenced region appeared to be homologous to the region downstream of Cλ2 gene segment (Fig. 3*A*, dotted lines) with 99.3% homology. The second part of the sequenced region (2.9 kb) shared the highest homology with the J-Cλ2 region (Fig. 3*A*, dashed lines), which was also 99.3%. It should be noted that there is a highly homologous region of 2.2 kb between the J-Cλ2 and J-Cλ3 regions, with a similarity of 98.7%. These homologous regions are indicated as gray areas. Fig. 3*A* represents the experimental approach for characterization of the *IGL* amplification, but does not reflect the mechanism. In Fig. 3*B* the actual positioning of the amplification polymorphism is depicted in the total structure of a P_1 allele. The amplified region starts with a 2.2-kb homologous region and ends just before the 2.2-kb homologous region of J-Cλ3.

Due to the polymorphic amplification, a new *Hin*dIII is generated (Fig. 3*B*). SB analysis of *Hin*dIII-digested DNA from individuals with the amplification polymorphism will therefore show the 5.4-kb polymorphic *Hin*dIII fragment when using the λ-IVS probe. Consequently, two and three (identical) repeats of the amplified region (P_2 or P_3) also result in 5.4-kb *Hin*dIII restriction fragments.

Sequence analysis of the J-Cλ region within the IGL *amplification polymorphism*

The J-Cλ region of the amplified *IGL* region shares the highest homology with the J-Cλ2 region. The Jλ2amp gene segment is identical with the Jλ2 except for one amino acid (Fig. 4). The RSS of the Jλ2amp was compared with the RSS of the seven other RSS and appeared to be identical with that of Jλ2 and Jλ3; it contained

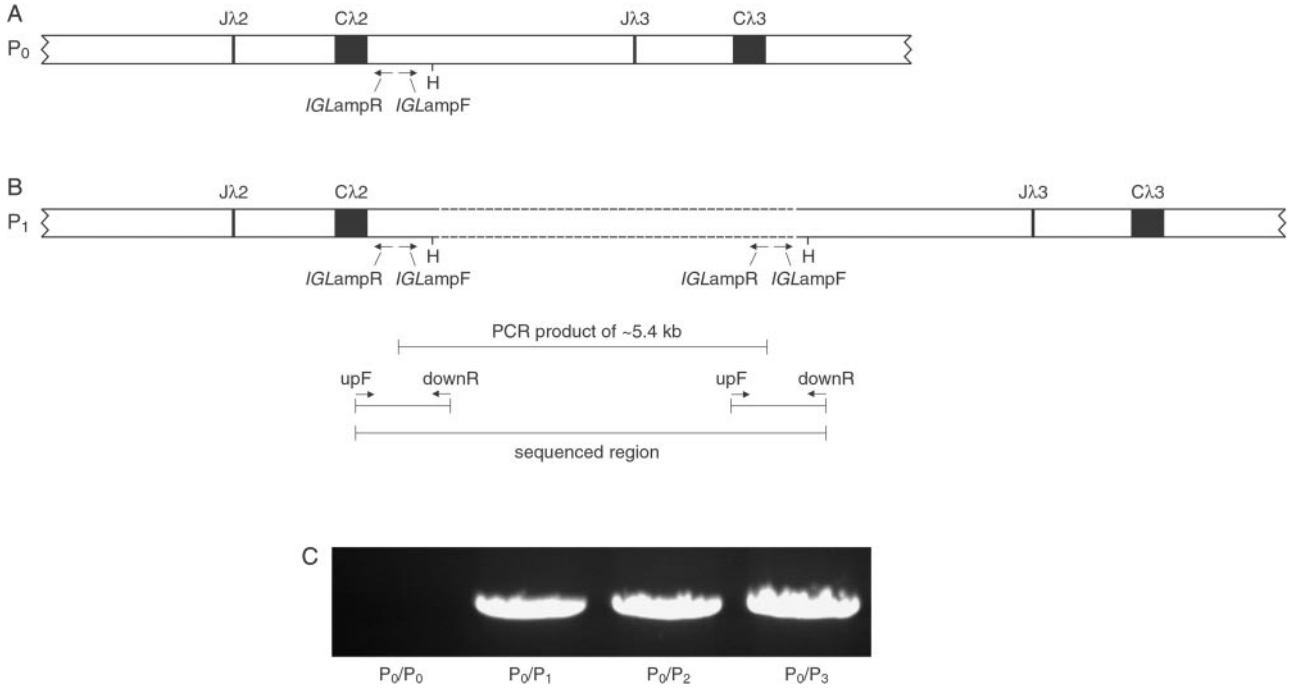

FIGURE 2. Strategy for PCR and sequencing analysis of the *IGL* amplification polymorphism. *A*, Cλ2-Cλ3 region without *IGL* amplification polymorphism (P_0) with the primers *IGL*amp-R and *IGL*amp-F positioned just upstream of the *Hin*dIII restriction site. *B*, Cλ2-Cλ3 region with one polymorphic *IGL* amplification (P_1) with the positions of the two *IGL*amp primers. PCR analysis with the primers *IGL*amp-F and *IGL*amp-R results in the generation of a fragment of ~5.4 kb. The PCR product generated with the *IGL*amp-F and *IGL*amp-R primers was totally sequenced starting with the PCR primers and subsequently with sequence primers designed in the newly generated sequence ("gene walking"). To analyze the region between the *IGL*amp-F and *IGL*amp-R primers, sequence analysis was performed using PCR products generated with primers Up-F and Down-R. The total sequenced region is indicated. *C*, PCR analysis with the primers *IGL*amp-F and *IGL*amp-R of one healthy individual without *IGL* amplification polymorphism (P_0/P_0) and three with an *IGL* amplification polymorphism (P_0/P_1, P_0/P_2, and P_0/P_3).

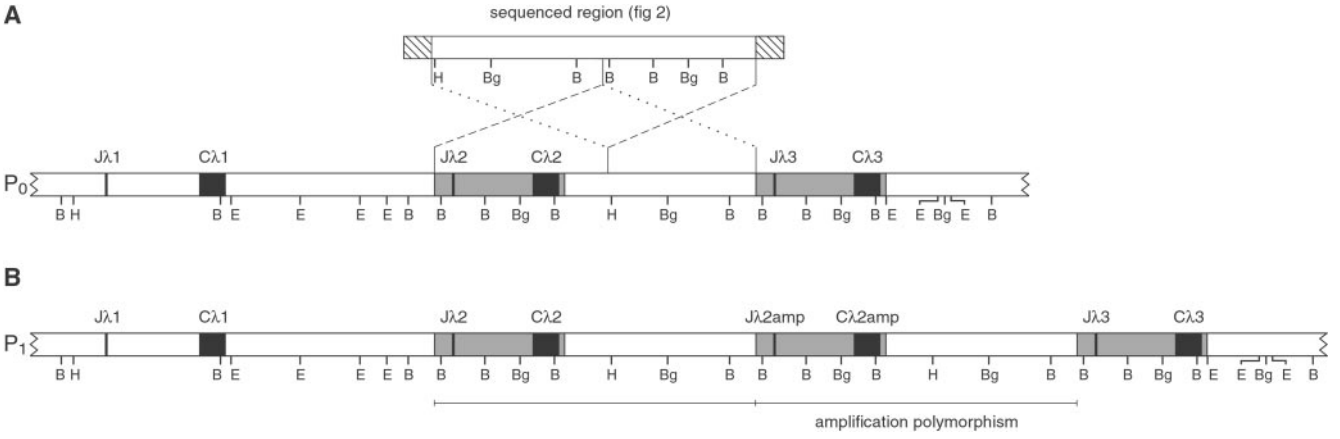

FIGURE 3. Experimental approach for characterization of the *IGL* amplification polymorphism resulting in the actual positioning within the *IGL* locus. *A*, Schematic representation of the total sequenced region, as defined in Fig. 2*B*. For further sequence analysis the region of the *IGL*amp-F *IGL*amp-R product together with the extended region to fill the gap between the ends of the two primers was used (white area); the remaining sequence was excluded (dashed area). *Bam*HI (B), *Bgl*II (Bg), *Eco*RI (E), and *Hin*dIII (H) restriction sites are indicated. The homologous repeats of the J-Cλ2 and J-Cλ3 regions with a similarity of 98.7% in the standard sequence (P_0) are marked as gray areas. The first part of the sequence could be aligned with the region downstream of the Cλ2 gene segment (indicated with dotted lines) with a homology of 99.3%. The second part of the sequence was aligned with the J-Cλ2 region (indicated with dashed lines) and had a homology of 99.3%. *B*, The Cλ1-Cλ2-Cλ3 region of a polymorphic allele (P_1) with the actual positioning of the polymorphic amplified region.

all essential nucleotides (Table III). The donor and acceptor splice sites of the J-Cλ2amp region were intact. The Cλ2amp gene segment was completely identical with the Cλ2 gene segment and also coded for the $Mcg^-Ke^-Oz^-$ (Table IV, comparison to all isotypes). Therefore, the *IGL* amplification polymorphism does not encode the $Mcg^-Ke^+Oz^-$ polymorphism and constitutes a different *IGL* polymorphism. Based on these data we conclude that the J-Cλ2amp region is functional. This conclusion was supported by homology search of the Jλ2amp segment using nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST), which resulted in a perfect match with >15 sequences of human mRNA for Igλ chains.

Effect of the number of IGL *amplifications on the Igκ:Igλ ratio on PB B cells*

As sequence analysis of the J-Cλ region on the *IGL* amplification showed that this region can undergo gene rearrangements and can be expressed, we studied the effect of the number of functional J-Cλ regions on the frequency of Igλ-positive B lymphocytes, by determining the Igκ:Igλ ratio on normal PB B cells. The combined Southern blot and Igκ:Igλ ratio data are summarized in Table V. The human *IGL* locus contains four functional J-Cλ regions, meaning that in cases without *IGL* amplification polymorphisms (P_0/P_0) a total of eight functional J-Cλ regions are present. In cases with the *IGL* amplification polymorphism, every amplified region represents one additional functional J-Cλ region. The Igκ:Igλ ratio of cases with an amplification (resulting in 9–13 functional J-Cλ gene regions) was significantly lower than the Igκ:Igλ ratio of cases without amplification (8 J-Cλ gene regions): 1.37 ± 0.18 vs 1.56 ± 0.26 (by *t* test, $p < 0.001$). One additional J-Cλ gene region appeared to be sufficient for a decrease in the Igκ:Igλ ratio (Table V).

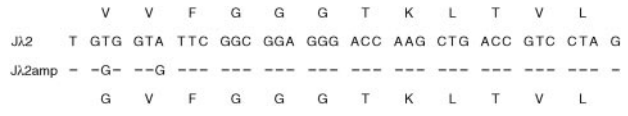

FIGURE 4. The Jλ gene segment of the *IGL* amplification polymorphism (Jλ2amp) compared with the normal Jλ2 region. The segments differ at two nucleotide positions, of which only one results in an amino acid substitution (V→G).

Frequency of $Mcg^-Ke^+Oz^-$ Cλ2 polymorphism

The sequence data demonstrated that the *IGL* amplification polymorphism does not coincide with the $Mcg^-Ke^+Oz^-$ polymorphism. Therefore, it is most likely that the $Mcg^-Ke^+Oz^-$ polymorphism is encoded by a polymorphic Cλ2 gene segment. This implies that at the DNA level the codon AGC for serine (S) at position 152 must be changed in GGC for glycine (G), which is the serological marker Ke^+. The Cλ2 region of 178 individuals was analyzed for the presence of the $Mcg^-Ke^+Oz^-$ polymorphism. The group consisted of 96 healthy controls (granulocyte DNA) as well as 82 precursor B cell acute lymphoblastic leukemia and $Ig\kappa^+$ B cell chronic lymphocytic leukemia samples, without *IGL* gene rearrangements, as determined by Southern blot analysis (19, 20). All samples were first screened with the *IGL*amp-F and *IGL*amp-R primers to define cases with the *IGL* amplification polymorphism. Twenty-five percent of cases (44 of 178) appeared to have the *IGL* amplification polymorphism. In cases without the *IGL* amplification polymorphism, the Cλ2 region was analyzed by PCR and sequencing. The Cλ2amp regions of individuals with the *IGL* amplification polymorphism were also sequenced. For this purpose the PCR products generated with the *IGL*amp-F and *IGL*amp-R

Table III. *RSS of all Jλ gene segments as compared to the consensus RSS*

	Nonamer[a]	Spacer	Heptamer[a]
Consensus RSS	GGTTTTTGT	12	CACTGTG
Jλ1	------G--	12	-------
Jλ2	---------	12	---A---
Jλ2amp	---------	12	---A---
Jλ3	---------	12	---A---
Jλ4	A--------	12	---C-CA
Jλ5	---------	12	---A-CA
Jλ6	-----G---	12	---A---
Jλ7	-----G---	12	-------

[a] Essential nucleotides are underlined.

Table IV. *The five Igλ isotypes described by the serological markers Mcg, Ke, Oz, and Mcp*

		Amino Acid Position						
		112	114	152	190	136	157	195
Cλ Segment	Serological Marker[a]	Mcg		Ke	Oz	Mcp		
Cλ1	$Mcg^+Ke^+Oz^-$	N	T	G	R	I	A	Q
Cλ2	$Mcg^-Ke^-Oz^-$	A	S	S	R	I	A	Q
Cλ2amp	$Mcg^-Ke^-Oz^-$	A	S	S	R	I	A	Q
Cλ2P[b]	$Mcg^-Ke^+Oz^-$	A	S	G	R	I	A	Q
Cλ3	$Mcg^-Ke^-Oz^+$	A	S	S	K	I	A	Q
Cλ7	$Mcp^+Ke^+Oz^-$	A	S	G	R	V	V	R

[a] The serological marker Mcg is determined based on amino acid N at position 112 and T at position 114, Ke is based on a G at position 152, Oz is based on K at position 190, and Mcp is based on V at positions 136 and 157 and R at position 195.
[b] Cλ2P, polymorphic Cλ2 region.

primers were sequenced with those generated with the Cλ2-F and Cλ2-Rseq primers.

In two of the 134 analyzed P_0/P_0 cases (1.5%) a heterozygous peak of nucleotides A and G was observed in the codon encoding the serological marker Ke. This implies that these individuals are heterozygous for the $Mcg^-Ke^+Oz^-$ polymorphism. In the Cλ2amp region of the 44 individuals with an *IGL* amplification polymorphism we did not detect the $Mcg^-Ke^+Oz^-$ polymorphism, indicating that the two polymorphisms are not linked.

Discussion

Two polymorphisms have been described in the human *IGL* locus. However, they have never been sequenced at the DNA level. The first polymorphism concerns an *IGL* amplification polymorphism of 5.4 kb of DNA in the Cλ2-Cλ3 region. The second polymorphism is the $Mcg^-Ke^+Oz^-$ isotype, which was only known from serological analyses of Bence-Jones proteins of multiple myeloma patients (11–14). Due to the high homology with the $Mcg^-Ke^-Oz^-$ isotype, which is encoded by the J-Cλ2 region, it was proposed that the $Mcg^-Ke^+Oz^-$ polymorphism is encoded by a polymorphic J-Cλ2 region that differs from the normal J-Cλ2 region at a single nucleotide position. It has been speculated that the two polymorphisms might be linked in such a way that the Cλ region on the polymorphic amplification encodes the isotype $Mcg^-Ke^+Oz^-$ (8, 14).

In this study we were able to sequence the 5.4-kb *IGL* amplification polymorphism. The *IGL* amplification polymorphism shares a homology of >99% with the J-Cλ2 region. The first 2.5 kb of the amplification, containing the Jλ and Cλ gene segments, coincides with a 2.2-kb region, which is also highly homologous to a 2.2-kb region in the J-Cλ3 cluster (98% homology; Fig. 3*B*). Taub et al. (15) proposed in 1983 a mechanism for the generation of this *IGL* polymorphism. Based on SB data and restriction fragment analysis, they proposed unequal crossing over between two 8-kb *Eco*RI allelic fragments through homologous recombination between two large homologous repeats. Our sequence information of the polymorphic *IGL* amplification together with the germline sequence of the complete *IGL* locus show that the homologous regions are 5.4 kb apart in the germline (P_0) situation. Therefore, we confirm that the amplification probably occurred during meiosis via unequal homologous recombination between the two 2.2-kb homologous J-Cλ2 and J-Cλ3 regions.

Table V. *Mean Igκ/gλ ratios for different numbers of functional J-Cλ gene regions*

No. of J-Cλ Gene Regions	Mean Igκ:Igλ Ratio	SD	No. of Analyzed Cases
8	1.56[a]	0.26	38
9–13	1.37[a]	0.18	31
9	1.30	0.35	4
10	1.36	0.17	13
11	1.39	0.14	6
12	1.40	0.15	7
13	1.42		1

[a] The Igκ/Igλ ratio in cases without amplification is significantly higher than that in cases with amplification (by *t* test, $p < 0.001$).

The Cλ2 and Cλ3 gene region might be susceptible to duplication, because of its high homology, but especially because of the two homologous J-Cλ regions of 2.2 kb. The maximum number of amplifications is three, which suggests that the region is indeed susceptible to duplication, but this phenomenon apparently has its limitations. The different amplification variants appeared to be exactly identical. No differences were observed in multiple amplifications on the same allele, which suggests that during evolution the various amplifications occurred simultaneously or shortly after each other.

The J-Cλ region of the polymorphic amplification appeared to have the highest homology with the J-Cλ2 region and contained all essential elements required for recombination and expression, i.e., a functional RSS, intact donor and acceptor splice-sites, as well as functional Jλ and Cλ gene regions. Since the amplified J-Cλ2 region does not encode the $Mcg^-Ke^+Oz^-$ isotype, we concluded that the human *IGL* locus has two separate polymorphisms.

As known from literature, the *IGL* amplification polymorphism is found up to three times per allele (15, 18). Our sequence analysis showed that this polymorphism concerns identical amplifications, implying that in cases with a P_2 amplification the first amplified region does not differ from the second. Screening of healthy controls showed that the frequency of the *IGL* amplification was 28% in Caucasoid individuals and involved only one allele. In Chinese individuals the frequency was much higher (85%) and involved both alleles in the majority of cases.

As the polymorphic amplified regions contain functional J-Cλ2 regions, we investigated the effect of the total number of J-Cλ regions on the frequency of $Ig\lambda^+$ B lymphocytes by studying the distribution between Igκ and Igλ L chain expression (Igκ:Igλ ratio). The presence of additional functional J-Cλ gene regions resulted in decreased Igκ:Igλ ratios of blood B lymphocytes compared with individuals without the J-Cλ2 amplification. One additional J-Cλ gene region was sufficient to decrease the Igκ:Igλ ratio, suggesting that this effect is dominant and that the addition of extra J-Cλ gene regions has little effect on the Igκ:Igλ ratio.

The Igκ:Igλ ratio can be decreased by extra copies of J-Cλ regions as well as by a decrease in Igκ (*IGK*) gene copies. In a

patient with a heterozygous de novo deletion of chromosome 2 region p11.2p13, including the *IGK* locus (2p12), the Igκ:Igλ ratio was strongly decreased to 0.7 (21). This decrease is stronger than that found in individuals with extra J-Cλ regions. This difference in effect on the Igκ:Igλ ratio can be explained by the fact that Ig L chain rearrangements take place in an ordered fashion, starting with rearrangements in the *IGK* locus, followed by *IGL* gene rearrangements, if no functional *IGK* rearrangement took place (20). If there is only one *IGK* allele, the chance of a functional rearrangement resulting in Igκ expression is ~50% reduced. Consequently, the rearrangement process will shift to the *IGL* locus in an earlier stage, which is in line with a reduction of the Igκ:Igλ ratio from 1.4 to 0.7. In contrast, there is a limited time period or a limited number of attempts for acquiring functional Ig gene rearrangements (22, 23). If the B cell is not able to generate an Ig molecule within that time frame or that number of attempts, the cell will die by apoptosis (22, 23). Finally, the presence of multiple functional J-Cλ regions also implies that consecutive *IGL* rearrangements with Vλ-Jλ replacements can occur on one allele.

The polymorphic $Mcg^-Ke^+Oz^-$ isotype is the second type of *IGL* polymorphism and was only known from serological analyses. Our sequencing data now show that this isotype is encoded by a polymorphic Cλ2 gene segment. The polymorphic Cλ2 region was detected in only two individuals, who were both heterozygous for this *IGL* polymorphism. The reported frequency of the $Mcg^-Ke^+Oz^-$ polymorphism in 70 multiple myeloma patients was 6% (13, 19, 24), which exceeds the frequency of 1.5% in the 134 analyzed individuals who did not carry the *IGL* amplification polymorphism. However, this difference is not statistically significant (by χ^2 test, $p > 0.05$). No $Mcg^-Ke^+Oz^-$ polymorphism was detected in the J-Cλ2 amplification polymorphism of 44 individuals, but this might be due to the low number of analyzed cases. Nevertheless, the data obtained indicate that the J-Cλ2 amplification polymorphism and the Cλ2 polymorphism are independent. The two polymorphisms might theoretically be present in one individual, but the frequency of such an event will be low (<0.5%).

In conclusion, two separate polymorphisms in the human *IGL* locus exist: the *IGL* amplification polymorphism and the Cλ2 polymorphism, resulting in the $Mcg^-Ke^+Oz^-$ isotype. Both polymorphisms involve the Cλ2 region, but they are not linked, although they might theoretically both be present in one individual. The presence of the *IGL* amplification polymorphism results in the presence of extra functional J-Cλ regions, which appeared to decrease the Igκ:Igλ ratio of normal blood B lymphocytes.

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