Single Nucleotide Polymorphism Discovery in the Avian *Tapasin* Gene[1]

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ABSTRACT *Tapasin* is a transmembrane glycoprotein located in the endoplasmic reticulum. Its function is to assist the assembly of major histocompatibility complex class I molecules. The chicken *Tapasin* gene includes 8 exons and is localized inside the major histocompatibility complex between the 2 class IIβ genes. The aim of the current study was the estimation of single nucleotide polymorphism frequency within the avian *Tapasin* gene. The *Tapasin* gene sequence from exon 5 to exon 6 was amplified for the chicken, turkey, and pheasant, and sequences of different lengths were obtained. The sequence analysis based on PolyBayes identified 25 putative single nucleotide polymorphism sites when the 3 species were compared. The coding sequences were further translated and analyzed to identify amino acid substitutions. The results indicated that polymorphisms within this region of the gene was mainly observed in the heterozygous state. The level of conservation of the *Tapasin* gene sequence among species is likely to be related to the functional importance of the gene.

Key words: major histocompatibility complex, *Tapasin*, single nucleotide polymorphism, sequence homology, amino acid comparison

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INTRODUCTION

Cell-mediated immunity is important as a component of the action of surveillance against viral infection and tumor avoidance (Grandea and Van Kaer, 2001). In the cell, cytosolic antigens are subjected to degradation by a large proteolytic complex, the proteasome. To be exposed on the cell surface bound to MHC class I molecules, the derived peptides are transported inside the endoplasmic reticulum (**ER**) via the transporter associated with antigen processing (**TAP**) and loaded onto MHC class I molecules. This process results from the action of several molecular chaperones (reviewed in Pamer and Cresswell, 1998; Bouvier, 2003), and among these, *Tapasin* is a specific accessory molecule (Antoniou et al., 2003).

Mammalian *Tapasin* is a 48-kDa transmembrane protein member of the Ig superfamily (Sadasivan et al., 1996; Ortmann et al., 1997) localized inside the ER. The only known function of *Tapasin* is to assist the assembly of class I molecules (Grandea and Van Kaer, 2001), acting as a bridge between class I molecules and TAP (Sadasivan et al., 1996). Because the protein creates a bridge between TAP and class I molecules, it is also credited as contributing to the loading of the peptide on MHC class I molecules (Brocke et al., 2002). Moreover, *Tapasin* might enhance transport of peptides, as it increases TAP levels (Lehner et al., 1998) and stabilizes both the TAP1-2 heterodimer (Raghuraman et al., 2002; Garbi et al., 2003) and the peptide-binding site of TAP (Li et al., 2000), contributing to retention and stabilization of empty MHC class I molecules in the ER (Ortmann et al., 1997; Brocke et al., 2002). Instead, it might retain MHC class I molecules in the ER until optimal binding with a peptide is established (Barnden et al., 2000; Grandea and Van Kaer, 2001). Tapasin might also participate in the selection of peptides that will be carried by the class I MHC molecule, optimizing their transport, either by facilitating without discriminating the binding of different peptides (Zarling et al., 2003) or by acting as a peptide editor involved in the selection of high-affinity peptides (Purcell et al., 2001; Brocke et al., 2002).

In humans, the mammal *Tapasin* gene referred to as TAPBP, is organized in 8 exons, and the coding sequence of 12,357 bp (Herberg et al., 1998) is localized within the extended class II region (The MHC Sequencing Consortium, 1999; Beck and Trowsdale, 2000). The chicken *Tapasin* gene includes 8 exons (as in the human), but 2 introns are much smaller than the corresponding human ones, leading to a shorter gene sequence. The homology between chicken and human amino acidic sequences is relatively low (36%). Nevertheless, the predicted 3-D

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structure of the 2 proteins seems to be very similar (Frangoulis et al., 1999). The chicken *Tapasin* gene is localized between the 2 MHC class IIβ genes (Jacob et al., 2000) and is reported to be very polymorphic (Kaufman, 1999). Characteristic of the chicken B complex is the simplicity and compactness of the B-F/B-L region (Kaufman et al., 1995). This results in a very low level of recombination within the complex, and as Kaufman et al. (1999) suggest, "genes within the chicken MHC can co-evolve, giving rise to distinct allelic combinations (or haplotypes) that are relatively stable in evolution." The present study aimed at estimating the frequency of single nucleotide polymorphisms (**SNP**) within the avian *Tapasin* gene.

MATERIALS AND METHODS

Sample Collection and Preparation

Blood samples were collected in 3.2% CTAD Vacuette tubes (Greiner Bio-One GmbH, Kremsmuenster, Austria) from unrelated animals of different chicken (*Gallus gallus*) local breeds (*Pepoi*, *Ermellinato di Rovigo*, *Robusto Maculato*, and *Robusto Lionato*) and commercial lines (18 layers, 18 broilers, and 9 light breeders). Further blood samples were collected from 5 wild pheasants (*Phasianus colchicus*), a turkey (*Meleagris gallopavo*) commercial line (19 birds), and from 2 turkey local breeds (4 *Ermellinato di Rovigo* and *Comune Bronzato* birds). Genomic DNA was extracted using commercial kits (Puregene, DNA isolation kit, blood kit, Gentra Systems, Minneapolis, MN).

Primer Design

Tapasin-specific sequences available in Genbank (Accession no. AL023516, AJ004999) were aligned. Primers (forward: 5′-gggACACAgTgATggACAgC-3′; reverse: 5′-gTAgAgCCAACggATgAggC-3′) were designed to amplify the gene region spanning from exon 5 to exon 6. Amplification conditions were optimized to produce single amplicons in each species.

Amplification and Sequencing

Following optimization, PCR reactions were carried out in a total volume of 15 μL with 40 ng of genomic DNA, 0.4 pmol of each of forward and reverse primer/μL, 1.5 μL of 10× buffer, 1.5 m*M* of $MgCl_2$, 0.2 m*M* of deoxyribonucleotides triphosphate, and 1.5 U of AmpliTaq Gold (Applied Biosystems, manufacturered by Roche, Branchburg, NJ) on an Eppendorf Mastercycler gradient (Eppendorf Italia, Milano, Italy). With regard to chicken samples, the PCR cycles were 95°C for 10 min; 28 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s; and a final extension step at 72°C for 5 min. For turkey and pheasant samples, the PCR cycles were 95°C for 10 min, 30 cycles of 94°C for 30 s, annealing at 59°C for 30 s, and 72°C for 30 s followed by a final extension step at 72°C for 5 min. The resulting PCR products were visualized after electrophoresis in 2% ethidium bromide-stained 1× Tris-acetate-EDTA agarose gel and purified with QIAquick PCR purification kit (cat. no. 28104, Qiagen S.p.A., Milano, Italy). Sequencing reactions were performed with BigDye (Applied Biosystems, Monza, Milano, Italy) terminator chemistry and resolved on an ABI PRISM 3100 DNA analyzer (Applied Biosystems) according to the protocols from the manufacturer. To minimize sequencing errors, all fragments were sequenced in both directions, and only high-quality sequences were considered.

Sequence Analysis

Raw sequence quality was ascertained with Phred (Ewing and Green, 1998; Ewing et al., 1998) with the aim to retain only sequences showing an average quality value >20. High-quality sequences were edited with Chromas 1.45 (http://www.technelysium.com.au/chromas.html) and were converted to FASTA format. Forward and reverse sequences were aligned, and consensus sequences were created. Homology with sequences in public databases (GenBank) was ascertained by BLASTn (Altschul et al., 1990, 1997). To recover only significant homologies, the e-value threshold of $1e^{-10}$ was set. BLASTn was also used to compare the present sequences with the expressed sequence tag (**EST**) data available at http://www.chick.umist.ac.uk/ (Boardman et al., 2002). The same database was queried to assess for the presence of already identified SNP in *Tapasin* sequences. Contig assembly and sequence alignments were performed with CAP3 (Huang and Madan, 1999) on all of the current sequences plus those retrieved from chickEST.

Putative SNP Identification

A semiautomatic pipeline was set up to analyze sequences from electropherograms to putative SNP detection. The PhredPhrap script was used for automatic electropherogram reading (Phred) and sequence assembly (Phrap). The Phrap stringency parameters were set to obtain 3 contigs containing all of the sequences and reflecting the species of origin (-forcelevel 6,-repeat_stringency 0.8). The SNP detection was carried out with PolyBayes (Marth et al., 1999) and was edited with Consed (Gordon, 2004). The expected a priori polymorphism rate was set to 0.005, and the PolyBayes SNP probability score of 0.99 was used as threshold for putative SNP identification.

Amino Acid Comparison

The amino acid sequences inferred from DNA sequences were obtained using BioEdit software (Hall, 1999). Putative proteins were aligned using CAA06328 (Frangoulis et al., 1999) as a reference sequence. Species-specific protein consensus sequences were created and blasted toward the GenBank nr DB using the BLASTp program (Altschul et al., 1997); BLAST was restricted to *Gallus gallus* (organism) and $1e^{-10}$ (e-value).

RESULTS

Tapasin-specific primers were designed based on genomic DNA sequences available in GenBank (Accession no. AL023516; AJ004999), and all PCR reactions were carried out using genomic DNA as template. Amplicons of different sizes were obtained from each species: chicken, 448 bp; turkey, 554 bp; and pheasant; 564 bp. The correspondent 188 electropherograms were initially analyzed with Phred to assess their quality. Average Phred quality values were >20 for 45% of the sequences and were >30 for an additional 41%. All sequences were accepted. For each animal, both forward and reverse sequences were produced, aligned, and edited to generate reliable consensus sequences. All sequences obtained with *Tapasin*-specific primers were analyzed with BLASTn vs. the GenBank nr database. The current sequences showed homologies ranging between 92 and 99% with public *Tapasin* sequences. Thus, one sequence representative of each species was blasted against chicken EST sequences and chicken genome sequences available at http://www.chick.umist.ac.uk/ and http://genome.wustl.edu/projects/chicken/index.php, respectively. Two homologous EST contigs (032628.1, 032628.2) and 1 homologous genomic sequence mapping on chromosome 16 (NW_060541.1) were identified.

All sequences were aligned and analyzed with CAP3. Three species-specific contigs were obtained. Chicken sequences were further clustered according to breed. The same was done with turkey sequences, even though the limited number of animals for each line allowed us to split sequences only in 2 major groups, namely, the commercial line and local breeds.

Nucleotide substitutions were identified in all groups: broilers (26.78 substitutions/kb), layers (15.62 substitutions/kb), breeders (26.78 substitutions/kb), chicken local breeds (*Ermellinato di Rovigo* = 8.93 sustitutions/kb; *Pepoi* = 6.69 substitutions/kb; *Robusto Lionato* = 11.16 substitutions/kb, and *Robusto Maculato* = 4.46 substitutions/kb), turkey commercial line (1.81 substitutions/kb), turkey local breeds (14.36 substitutions/kb), and pheasants (17.73 substitutions/kb).

Tapasin SNP Discovery

The sequences produced were compared with the EST sequences present in the chickEST database. Sequences in this database are currently clustered into 85,486 contigs. All of the species-specific consensus sequences obtained from sequences in the current study were run against the EST submitted to chickEST and the homology assessed through BLASTn. Thus, sequences belonging to the homologous contigs were retrieved. None of the homologous EST sequences participated to the 11,000 high confidence SNP submitted to chickEST.

PolyBayes Analysis

Three contigs of homologous sequences (reflecting the species of origin of the samples) were obtained with the Phrap sequence assembly program and analyzed with PolyBayes for putative SNP identification. Eleven putative SNP sites have been identified in chickens, 7 in turkeys, and 7 in pheasants (Table 1). Most significant scores were attributed to those positions where alternative bases were present in homozygous condition, and the score was dramatically less significant when only one base was homozygous and the variant base was detected in the heterozygous state.

Table 1. In silico putative single nucleotide polymorphism (SNP) detection on *Tapasin* genomic sequences

Species and SNP position	Transition/transversion	P_{SNP}[1]
Chicken		
93	Transition (C/T)	0.999999996
94	Transition (A/G)	1
113	Transition (C/T)	1
125	Transition (C/T)	0.999999999
161	Transition (C/T)	1
167	Transition (C/T)	1
176	Transition (A/G)	1
196	Transition (A/G)	1
217	Transition (C/T)	0.999980768
243	Transition (A/G)	1
370	Transition (C/T)	1
Turkey		
79	Transition (A/G)	0.999999993
254	Transversion (G/T)	1
267	Indel (*/C)	1
268	Indel (*/G)	1
269	Indel (*/T)	1
476	Transversion (C/G)	0.999999997
506	Transition (A/G)	0.999996317
Pheasant		
93	Transition (C/T)	0.999999821
134	Transversion (C/G)	1
200	Transversion (A/T)	0.999950545
242	Transversion (A/C)	0.999999888
243	Transition (A/G)	1
275	Transition (A/G)	0.999999262
452	Transversion (C/G)	1

[1]P_{SNP} = SNP probability score.

Amino Acid Substitutions

Synonymous and Non-synonymous SNP. Sequences showing significant nucleotide substitutions were selected and aligned with the *Tapasin* cDNA sequence retrieved from GenBank (Accession no. AJ005072). This step allowed the location of the coding sequences present in the amplicons. The deduced coding sequences were then translated into the corresponding amino acid sequence. One amino acid sequence representative for each species was chosen and aligned to the sequence (CAA06328) derived from the previously mentioned GenBank nucleotide sequence. The analysis showed the existence of a substitution of isoleucine with valine in all of the current sequences, and of valine with isoleucine in all turkey and pheasant sequences (Figure 1). A small number of sequence-specific substitutions was also noted.

Inter- and Intraspecies Homologies

Homology Between Chicken Commercial Lines and Breeds. Primers were designed to amplify a 448 bp *Ta-*

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    5         15         25         35         45         55
CAA06328        DTVMDSWTSG HRQAADGTYS RTAAARLIPA RPQHHGDIYS CVVTHTALAK PMRVSVRLLL
B               .......... .......... .......... .......V.. .......... ..........
OVA             .......... .......... .......... .......V.. .......... ..........
T               .......... .......... .......... .......V.. .......... ..........
PHA             .......... .......... .......... .......V.. .......... ..........

                ....|....| ....|....| ....|....
                    65         75         85
CAA06328        AGTEGPHLED ITGLFLVAFV LCGLIRWLY
B               .......... .......... .........
OVA             .......... .......... .........
T               .......... .........I .........
PHA             .......... .........I .........
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Figure 1. Putative amino acid substitutions: B = broiler; OVA = layer; T = turkey; and PHA = pheasant. CAA06328 is the homologous protein sequence retrieved from GenBank. Numbers indicate the position of the amino acid residue.

pasin fragment corresponding to exon 5, intron 5, and exon 6. All amplicons showed the expected size. Moreover, all sequences had a homology e-value of 0.0 when blasted against sequences already available in public databases.

Sequence alignment showed that sequences of commercial lines and local breeds were almost equally conserved in the esonic and intronic regions, which showed a comparable level of polymorphism. Actually, for the 29,120 analyzed nucleotides, most of the observed differences were present in heterozygous condition, and all of the substitutions were transitions. Moreover, the SNP at position 196 (A/G) is typical of layers: 8 of the 11 SNP identified by PolyBayes were located in the exons, and 3 were in the intron sequence.

Homology Between Turkey Lines. With regard to the turkey, the same primers generated 554 bp amplicons with the exception of 2 local breed birds that showed a 557 bp PCR product. The 3 extra nucleotides (CgT) were located next to each other and coded for one extra amino acid (R). It is worthwhile to mention that in turkeys, all sequences seem to represent one single open reading frame, i.e., no stop codons were observed in the sequence even if, based on sequence similarity with the other species, an intronic region between exons 5 and 6 was expected to exist. Based on the sequence data produced in the present study, the location of the putative turkey intron 5 and its size can be predicted only by comparing turkey and chicken *Tapasin* sequences. Running the SplicePredictor (http://bioinformatics.iastate.edu/cgi-bin/sp.cgi) splicing site detection program, the same couple of donor-acceptor sites has been detected for turkey and chicken sequences. This supports the conclusion that the extra amino acid described previously is actually encoded within intron 5, thus suggesting that no additional polymorphism is present within the coding sequence. Also in turkeys, similar to chickens, most of the nucleotide substitutions were present in heterozygous state. BLASTn comparison of turkey sequences vs. the GenBank nr database showed homology e-values ranging between e^{-108} and e^{-109} with the public *Tapasin* sequences.

The observed variations were represented by a single transition typical of the commercial line and by 3 transversions, 2 transitions, and a 3 bp indel in the local breeds. Three of the 7 SNP observed with PolyBayes were located in the exons.

Homology Among Pheasant Sequences. The same primer pair amplified pheasant genomic DNA. The PCR reaction produced 564 bp amplicons with an estimated substitution rate of 17.73 substitutions/kb. The level of homology estimated blasting the pheasant sequences against the sequences available in public databases ranges between $4e^{-78}$ and e^{-103}. The observed variations were represented by 3 transitions and 4 transversions. Five of the 7 putative SNP identified by PolyBayes were located at intron level, only 2 were present in the exon 5 sequence.

Homology Among Different Species. The pheasant and turkey sequences showed a significant difference in amplicon length when compared with the chicken sequences. This difference seems to be mainly due to extra nucleotides organized in 2 major clusters of 93 and 21 bases located after chicken nucleotides 264 and 317, respectively (Figure 2).

DISCUSSION

Several studies support the existence of associations between polymorphism within the MHC region and the immune response in vertebrates. Chicken MHC is far simpler and smaller than the mammalian homologous region, and it is characterized by low recombination frequency (Kaufman et al., 1999). It was also suggested that within MHC, coevolution of genes leads, throughout evolution, to the establishment of stable haplotypes (Kaufman, 1999; Kaufman et al., 1999). The amplified region here investigated stretches from exon 5 to exon 6 and

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                    .    :    .    :    .    :    .    :    .    :    .    :     60

B                   GGGACACAGTGATGGACAGCTGGACTTCAGGTCACCGCCAGGCAGCCGATGGAACCTACA     60
T                   GGGACACAGTGATGGACAGCTGGACATCGGGTCACCGCCAGGCTGCTGATGGAACCTACA     60
PHA                 GGGACACAGTGATGGACAGCTGGACATCGGGTCACCGCCAGGCTGCTGATGGAACCTACA     60

                    .    :    .    :    .    :    .    :    .    :    .    :    120
B                   GCCGGACGGCGGCAGCACGGCTGATCCCCGCACACCCCCAACACCACGGGGATGTCTACA    120
T                   GCCGGACGGCGGCAGCACGGCTGATCCCTGCACGTCCCCAACACCACGGGGACGTCTACA    120
PHA                 GCCGGACGGCGGCAGCACGGCTGATCCCTGCACGTCCCCAGCACCACGGGGACGTCTACA    120

                    .    :    .    :    .    :    .    :    .    :    .    :    180
B                   GCTGCGTTGTCACCCACACTGCACTGGCCAAACCAATGCGTGTCTCCGTCCGACTGCTCC    180
T                   GCTGCGTTGTCACCCACACTGCACTGGCCAAACCAATGCGCGTCTCCGTCCGACTGCTCC    180
PHA                 GCTGTGTTGTCACCCACACTGCACTGGCCAAACCAATGCGCGTCTCCGTCCGACTGCTCC    180

                    .    :    .    :    .    :    .    :    .    :    .    :    240
B                   TGGCTGGTGAGGGGGGATGTGGGGATATTGGAAACACGTGGAGGTATTGGGATGCTGGGA    240
T                   TGGCTGGTGAGGGGGGATGTGGGGGTGTTGGAGACACGTGGAGGTATTGGGATGGTGGGA    240
PHA                 TGGCTGGTGAGGGGGGATGGGGGGATACTGGAGACATGTGGAGGTATTGGGATGGTGGGA    240

                    .    :    .    :    .    :    .    :    .    :    .    :    300
B                   CCGTGGTTAGGAGGGTCTGAGGGA-----------------------------------     264
T                   CCGTGGTTAGG--------AGGGATATTGA--TGATGGCTGGGGACACCAAGGGACACCG    290
PHA                 GAGTGGTTAGGTGGGTCTAAGGGACATTGAGATCATGGCTGGGGACACCAAGGGACACTG    300

                    .    :    .    :    .    :    .    :    .    :    .    :    360
B                   ------------------------------------------------------------CA     266
T                   GAACTGAGGTTGGGGACATCAAGAGACATTGGGACCGTGGTTGGGAGTGTGGCAGGGACA    350
PHA                 GAACTGAGGTTGGGGACATCAAGAGACATTGGGACAATGGTTAGGAGTGTCACAGGGACA    360

                    .    :    .    :    .    :    .    :    .    :    .    :    420
B                   TCAGGACCATGGCCTGGGACAATGGGAGAT-CATGGATTTGGGTTGGGGACC--------    317
T                   TCAGGACCGTGGCTTGGGATAATGAGAGATGCATGGATTTGGGTTGGGGACCGTAGCCCC    410
PHA                 TCAGGACCATGGCTTGGGACAAGAAGAGATGCATGGATTTGGGTTGGGGACCACAGCCCC    420

                    .    :    .    :    .    :    .    :    .    :    .    :    480
B                   -------------CCACCCAGGATGGTGACACTGTGCTTAGGGCTGTCGTTGTCCCCACA    364
T                   AGGGTTGGAGACCCCACCCAGGGTGGTGACACCATGCTTAAGGCTGATGTTGTCCCCAAA    470
PHA                 AGGGCTGGAGACCCCACCCAGGGTGGTGACAGCATGCTTCAGGCTGTTGTTGTCCCCAAA    480

                    .    :    .    :    .    :    .    :    .    :    .    :    540
B                   GGCACCGAGGGACCGCACCTGGAGGACATCACGGGGCTCTTCTTGGTGGCCTTTGTCCTC    424
T                   GGCACTGAGGGGCCGCACCTGGAGGACATCACGGGGCTCTTCTTGGTGGCTTTTATCCTC    530
PHA                 GGCACAGAGGGGCCACACCTGGAGGACATCACGGGGCTCTTCTTGGTGGCTTTTATCCTT    540

                    .    :    .    :    .    :    .    :    .    :    .    :    600
B                   TGTGGCCTCATCCGTTGGCTCTAC                                        448
T                   TGTGGCCTCATCCGTTGGCTCTAC                                        554
PHA                 TGTGGCCTCATCCGTTGGCTCTAC                                        564
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Figure 2. Alignment of broiler (B), turkey (T), and pheasant (PHA) genomic consensus sequences with the MAP software (Huang, 1994).

448 bp (chicken), 554 bp (turkey), and 564 bp (pheasant) amplicons can be amplified from different species with the same primer pair. The difference in length between the chicken and the other 2 avian species was mainly due to the insertion of 2 nucleotide clusters. These 93 and the 21 nucleotide clusters in the turkey were inserted after the chicken bases 264 and 317, respectively. Within the first cluster, pheasants present 2 further extra nucleotides for a total of 95 bases. Eight more bases, absent in turkeys, were present in both the pheasant and chicken and were

located between the chicken nucleotides 251 and 260. Sequences reported in this paper are the first turkey and pheasant *Tapasin* sequences described so far.

The translation of turkey genomic sequences results in a continuous open reading frame that covered the whole amplicon. However, splicing sites analysis identified putative splicing sites corresponding to those present in the chicken and pheasant sequences, where open reading frames were discontinuous in the intronic region. Assuming that the *Tapasin* gene undergoes analogous intron splicing in the 3 species, the deduced amino acid sequences showed identical length, as the 2 clusters identified in turkey and pheasant were localized within intron 5. A substitution of isoleucine with valine in all sequences was found, together with a substitution of valine with isoleucine present in all turkey and pheasant sequences. The amino acid sequences produced in the present study were homologous to the amino acid sequence CAA06328 from GenBank (Frangoulis et al., 1999) and to the amino acids characteristic of the Ig-like domains Y-S-C-V-V-T-H. A further search was carried out with the genomic consensus sequences for each species in the Conserved Domain Database (Marchler-Bauer and Bryant, 2004), and homology between the region encoded by exons 5 and 6 in chicken, turkey, and pheasant and the Ig superfamily molecule was confirmed with e-values down to $9e^{-6}$.

Mayer and Klein (2001) have recently underlined the homology existing between human *Tapasin* and MHC class I, MHC class II genes, and immunoglobulin. The BLASTx analysis against the GenBank nr database was performed to identify homologies among the avian *Tapasin* sequences produced in the present study and other immune response genes. Significant sequence similarities were identified only among avian *Tapasins*. It is noteworthy though, that the tBLASTn analysis showed homology (e-value = e^{-4}) also with mammalian classical class I genes.

Finally, polymorphisms found in the *Tapasin* sequences and described here were mainly found in heterozygous state. The existence of between- and within-species conservation is likely to be related to the functional importance of this gene. *Tapasin* functions as a bridge between MHC class I and TAP molecules (Sadasivan et al., 1996), and in its absence, the class I and TAP assembling is disrupted (Sadasivan et al., 1996). It is noteworthy that the same level of conservation was also observed in other genes located within the MHC region and sequenced by the authors (data not shown).

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REFERENCES

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389–3402.

Antoniou, A. N., S. J. Powis, and T. Elliott. 2003. Assembly and export of MHC class I peptide ligands. Curr. Opin. Immunol. 15:75–81.

Barnden, M. J., A. W. Purcell, J. J. Gorman, and J. McCluskey. 2000. Tapasin-mediated retention and optimization of peptide ligands during the assembly of class I molecules. J. Immunol. 165:322–330.

Beck, S., and J. Trowsdale. 2000. The human major histocompatibility complex: Lessons from the DNA sequence. Annu. Rev. Genomics Hum. Genet. 1:117–137.

Boardman, P. E., J. Sanz-Ezquerro, I. M. Overton, D. W. Burt, E. Bosch, W. T. Fong, C. Tickle, W. R. Brown, S. A. Wilson, and S. J. Hubbard. 2002. A comprehensive collection of chicken cDNAs. Curr. Biol. 12:1965–1969.

Bouvier, M. 2003. Accessory proteins and the assembly of human class I MHC molecules: A molecular and structural perspective. Mol. Immunol. 39:697–706.

Brocke, P., N. Garbi, F. Momburg, and G. J. Hammerling. 2002. HLA-DM, HLA-DO and tapasin: Functional similarities and differences. Curr. Opin. Immunol. 14:22–29.

Ewing, B., and P. Green. 1998. Basecalling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8:186–194.

Ewing, B., L. Hillier, M. Wendl, and P. Green. 1998. Basecalling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8:175–185.

Frangoulis, B., I. Park, F. Guillemot, V. Severac, C. Auffray, and R. Zoorob. 1999. Identification of the Tapasin gene in the chicken major histocompatibility complex. Immunogenetics 49:328–337.

Garbi, N., N. Tiwari, F. Momburg, and G. J. Hammerling. 2003. A major role for tapasin as a stabilizer of the TAP peptide transporter and consequences for MHC class I expression. Eur. J. Immunol. 33:264–273.

Gordon, D. 2004. Viewing and editing assembled sequences using consed. In Current Protocols in Bioinformatics. A. D. Baxevanis and D. B. Davison, ed. John Wiley & Co., New York, NY.

Grandea, A. G., III, and L. Van Kaer. 2001. Tapasin: An ER chaperone that controls MHC class I assembly with peptide. Trends Immunol. 22:194–199.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids. Symp. Ser. 41:95–98.

Herberg, J. A., J. Sgouros, T. Jones, J. Copeman, S. J. Humphray, D. Sheer, P. Cresswell, S. Beck, and J. Trowsdale. 1998. Genomic analysis of the Tapasin gene, located close to the TAP loci in the MHC. Eur. J. Immunol. 28:459–467.

Huang, X. 1994. On global sequence alignment. Comput. Appl. Biosci. 10:227–235.

Huang, X., and A. Madan. 1999. CAP3: A DNA sequence assembly program. Genome Res. 9:868–877.

Jacob, J. P., S. Milne, S. Beck, and J. Kaufman. 2000. The major and the minor class II β-chain (B-LB) gene flank the *Tapasin* gene in the B-F/B-L region of the chicken major histocompatibility complex. Immunogenetics 51:138–147.

Kaufman, J. 1999. Co-evolving genes in MHC haplotypes: The "rule" for nonmammalian vertebrates? Immunogenetics 50:228–236.

Kaufman, J., S. Milne, T. W. Gobel, B. A. Walker, J. P. Jacob, C. Auffray, R. Zoorob, and S. Beck. 1999. The chicken B locus

is a minimal essential major histocompatibility complex. Nature 401:923–925.
Kaufman, J., H. Volk, and H. J. Wallny. 1995. A "Minimal Essential MHC" and an "Unrecognized MHC:" Two extremes in selection for polymorphism. Immunol. Rev. 143:63–68.
Lehner, P. J., M. J. Surman, and P. Cresswell. 1998. Soluble tapasin restores MHC class I expression and function in the tapasin-negative cell line .220. Immunity 8:221–231.
Li, S., K. M. Paulsson, S. Chen, H. O. Sjogren, and P. Wang. 2000. Tapasin is required for efficient peptide binding to transporter associated with antigen processing. J. Biol. Chem. 275:1581–1586.
Marchler-Bauer, A., and S. H. Bryant. 2004. CD-Search: Protein domain annotations on the fly. Nucleic Acids Res. 32:327–331.
Marth, G. T., I. Korf, M. D. Yandell, R. T. Yeh, Z. Gu, H. Zakeri, N. O. Stitziel, L. D. Hillier, P. Y. Kwok, and W. R. Gish. 1999. A general approach to single-nucleotide polymorphism discovery. Nat. Genet. 23:452–456.
Mayer, W. E., and J. Klein. 2001. Is tapasin a modified MHC class I molecule? Immunogenetics 53:719–723.
Ortmann, B., J. Copeman, P. J. Lehner, B. Sadasivan, J. A. Herberg, A. G. Grandea, S. R. Riddell, R. Tampè, T. Spies, J. Trowsdale, and P. Cresswell. 1997. A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. Science 277:1306–1309.
Pamer, E., and P. Cresswell. 1998. Mechanism of MHC class I-restricted antigen processing. Annu. Rev. Immunol. 16:323–358.
Purcell, W. A., J. J. Gorman, M. Garcia-Peydrò, A. Paradela, S. R. Burrows, G. H. Talbo, N. Laham, C. A. Peh, E. C. Reynolds, J. A. Lopez de Castro, and J. McCluskey. 2001. Quantitative and qualitative influences of tapasin on the class I peptide repertoire. J. Immunol. 166:1016–1027.
Raghuraman, G., P. E. Lapinski, and M. Raghavan. 2002. Tapasin interacts with the membrane-spanning domains of both TAP subunits and enhances the structural stability of TAP1-TAP2 complexes. J. Biol. Chem. 277:41786–41794.
Sadasivan, B., P. J. Lehner, B. Ortmann, T. Spies, and P. Cresswell. 1996. Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP. Immunity 5:103–114.
The MHC sequencing consortium. 1999. Complete sequence and gene map of a human major histocompatibilty complex. Nature 401:921–923.
Zarling, A. L., C. J. Luckey, J. A. Marto, F. M. White, C. J. Brame, A. M. Evans, P. J. Lehner, P. Cresswell, J. Shabanowitz, D. F. Hunt, and V. H. Engelhard. 2003. Tapasin is a facilitator, not an editor, of class I MHC peptide binding. J. Immunol. 171:5287–5295.