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Review

# Hemoglobin polymorphisms in Atlantic cod – A review of 50 years of study

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ABSTRACT

Hemoglobin is one of the most studied proteins in nature, and evolutionary modifications of the interacting subunits seem to have refined the oxygen binding properties in the wide range of land- and/or water-living vertebrates. The adaptation of fish to varying environments seems to involve multiple hemoglobins, and polymorphic variants may further increase the diversity of functional properties. The pioneering study of Knud Sick on the hemoglobin polymorphisms in Atlantic cod fifty years ago was accompanied by multiple population genetic, physiological and behavioral studies before the recent identification of the genetic basis of the protein variants. The Met-Lys and Val-Ala substitutions in the cod β1 globin subunit provided the link between genotype and physiological functions, and the geographical distribution of the variants in temperate and Arctic waters strongly indicate that hemoglobin is under adaptive evolution in Atlantic cod. The structural and regulatory polymorphisms of the cod β1 globin highlight the relationship between temperature and functional molecular variation in the hemoglobin system.



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## 1. Introduction

"*The cod-fish which occupy the banks lying between the latitudes of 41 and 45, are very different on the different banks, and are kept so distinct, and are so similar on the respective banks that a man acquainted with the fishing business will separate those caught on one bank from those caught on another with as much ease as we separate the apple from the pear*" (Hon. General Lincoln, 1791)

## 2. Hemoglobin mutations

Structural changes affecting the function of the oxygen transporting hemoglobin molecule have attracted researchers since (Pauling et al., 1949) described the sickle cell anemia mutation by demonstrating alteration in electrophoretic mobility (Pauling et al., 1949). In 1956 Vernon Ingram demonstrated that the charge difference is due to substitution of valine for glutamic acid in position six of the β-globin peptide chain (Ingram, 1956). This observation was the first to prove that mutations can alter the amino acid sequence of a protein, and the study of human hemoglobin mutations opened the era of molecular genetics. While the majority of these mutations have been found to be deleterious (Alanazi et al., 2011), the positively charged hemoglobin variants commonly found in Mediterranean breeds of domestic animals might represent an adaptation to the arid climate (Pieragostini et al., 2010). Contrasting with Antarctic notothenioid fishes, species inhabiting tropical, temperate and Arctic waters typically

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have multiple hemoglobins (isohemoglobins) that show diverse functional properties (di Prisco and Tamburrini, 1992; Pérez et al., 1995). Genetic polymorphism within populations of a species may further increase the repertoire of hemoglobins. Hemoglobin polymorphism has been reported in diverse teleost species, including Atlantic cod (*Gadus morhua*) whiting (*Merlangius merlangus*), toadfish (*Opsanus tau*, *Thalassophryne maculosa*), eelpout (*Zoarces viviparous*), Arctic charr (*Salvelinus alpinus*) and turbot (*Scophthalmus maximus*) (Sick, 1961; Fyhn and Sullivan, 1974; Hjorth, 1975; Pérez, 1986; Giles, 1991; Imsland et al., 1997). However, the gene mutation(s) underlying the polymorphic subunit(s) has been identified only in Atlantic cod (Andersen et al., 2009; Borza et al., 2009). This paper reviews five decades of population genetic, physiological, behavioral and molecular studies of the hemoglobin polymorphisms in one of the world's most important commercial fish species.

## 3. Commercial and ecological importance of Atlantic cod

The *skrei* (Old Norse *skríða* means migrating) of the Northeast Arctic population has been the foundation of a huge fishing industry since the end of the 17th century. The mature skrei migrate yearly from feeding areas in the Barents Sea and near Svalbard to the main spawning grounds off the Lofoton Islands in North Norway, and dried salt cod has been exported worldwide for centuries as the main ingredient in the famous bacalao dish. Cod fishing was a principal occupation and source of food for the early American colonists. The first cod fisheries on the western side of the Atlantic Ocean occurred in the local waters off Maine and Massachusetts in the 1600 s, and cod catches from Georges Bank have been a major component of the USA groundfish fishery since the late 1800 s (Serchuk and Wigley, 1992). The more recent decline, or even collapse, of cod populations such as the overexploited Newfoundland and Labrador populations has promoted the commercial interest in intensive cod farming and selective breeding for improved production performance.

Atlantic cod comprises multiple more or less reproductively isolated populations inhabiting the continental shelves and banks in most areas of the North Atlantic. The cod populations display extreme plasticity, and the hemoglobins operate under greatly varying physicochemical conditions, which affect oxygen availability, oxygen demand and oxygen binding properties. Cod survive water temperatures ranging from near freezing (−1.5 °C) to almost 20 °C, although the temperature range is much narrowed during spawning season (Righton et al., 2010). The fish tolerates $O_2$ levels down to 16.5% air saturation at 5 °C, and exposure to chronic hypoxic conditions involves numerous cardiorespiratory adjustments that can improve oxygen transport and utilization, including increased ventilatory effort and elevated hemoglobin levels, although not sufficient to offset the negative effects of hypoxia on cardiac pumping capacity (Schurmann and Steffensen, 1997; Petersen and Gamperl, 2011). Atlantic cod is classified as a marine fish, but tolerates reduced salinities as low as 3‰ and spawn successfully in saline lakes and brackish waters like the Baltic Sea and Bras d'Or Lakes (Nelson et al., 1994, 1996; Tomkiewicz et al., 1998; Hardie et al., 2008). This predatory species performs frequent vertical migrations from water surface down to depths of 400 m (van der Kooij et al., 2007; Steingrund and Ofstad, 2009), and neutral buoyancy is dependent on hemoglobins with high Root effect to fill the closed swimbladder with oxygen via *rete mirable* (Scholander et al., 1956). The failure of fish blood to become fully saturated when exposed to elevated $pCO_2$ was demonstrated by August Krogh and Isabella Leitch already in 1919, but has later been known as the Root effect (Krogh and Leitch, 1919; Root, 1931; Scholander and Van Dam, 1954). Krogh and Leitch (1919) did not comment on their own results documenting the dramatic Root effect in Atlantic cod, but has later been confirmed by measuring maximal Root effect of 83%, which is the highest among teleosts (Berenbrink et al., 2005, 2011). The extraordinary high pH sensitive $O_2$ binding of cod hemoglobin was explained by the very low histidine content and thus low buffer value (Berenbrink et al., 2005).

## 4. Cod hemoglobin genotypes

In 1961 the Danish researcher Knud Sick reported three different hemoglobin phenotypes in the two gadoid species Atlantic cod and whiting by demonstrating two allelic variants named HbI-1 and HbI-2 differing in electrophoretic mobility (Sick, 1961) (Fig. 1). Cod in the Baltic Sea were found to be almost exclusively homozygous HbI-2/2, while HbI-1/1 cod were more prevalent in Danish waters. The distribution of the HbI variants in cod populations on the eastern side of the North Atlantic Ocean showed a clinal reduction in the HbI-1 allele frequency with increasing latitude from 69.0% to 7.3%, while a much less clear north–south gradient was found on the western side (Frydenberg et al., 1965; Sick, 1965). The gradient has been highly conserved along the Norwegian coast, and HbI-1 allele frequencies ranging from 62.5% in the southern population at Helgoland to 7.5% in the Barents Sea were recently reported (Andersen et al., 2009), whereas a similar gradient on the western side was not supported by recent data (Wetten et al., 2012). Sick (1965) concluded that the heterogeneous distribution of the cod Hb alleles was undoubtedly the combined results of the distributional history of the species and the different selective forces acting in different areas under recent and current environment conditions. The cod population in the North Sea experience large seasonal temperature fluctuations between 4 °C and 18 °C, whereas cod from the Barents Sea live in stable colder waters at temperatures between 2 °C and 4 °C. Thus, water temperature is intuitively a potentially strong selective factor in the distribution of the hemoglobin variants as evidenced by the preference of HbI-2/2 cod for colder water (8.2 °C) compared to the HbI-1/1 cod (15.4 °C) at normoxic conditions (Petersen and Steffensen, 2003). The advantage of the genotypes at different temperatures was supported by *in vitro* studies demonstrating different temperature optimum with regard to oxygen affinity. The hemoglobin oxygen dissociation curve of whole blood indicated that homozygous HbI-2/2 cod was the most efficient oxygen carrier at low temperatures (<10 °C), while HbI-1/1 showed highest oxygen affinity at elevated temperatures (>14 °C) with heterozygous HbI-1/2 cod intermediate in performance (Karpov and Novikov, 1980) (Fig. 2). These results were more or less reaffirmed by analyzing the effects of temperature and pH on the oxygen binding of stripped hemoglobin using a modified tonometric method (Brix et al., 1998). At 4 °C the oxygen affinities of HbI-2/2 cod were significantly higher than in HbI-1/1 cod, which showed affinities completely insensitive to temperature changes at pH 7.5.

## 5. Cod hemoglobin genes and polymorphisms

The tetrameric composition of the adult cod hemoglobins was found by purifying the α and β globin subunits comprising the three tetramers Hb 1 (α1, α1, β1, β1), Hb 2 (α2, α2, β2, β2) and Hb 3 (α1, α1, β2, β2) (Verde et al., 2006). Additional globin genes were recently identified in the Atlantic cod genome, and the total of four α and five β globin genes are organized in two clusters β5-α1-β1-α4 and β3-β4-α2-α3-β2 mapped to linkage group 2 and 18, respectively (Borza et al., 2009, 2010; Wetten et al., 2010). The cod globin genes were shown to be differentially expressed in the developing fish, and the α1, α2, β1 and β2 transcripts predominated in the adult fish, in agreement with the corresponding protein subunits (Verde et al., 2006; Borza et al., 2009; Wetten et al., 2010). The structural basis for the Root effect of fish hemoglobins is far from understood, but putative key residues, including Asp95α, Asp99β and Asp101β (Yokoyama et al., 2004; Mazzarella et al., 2006), are conserved in the cod globins, except for β1 and α3. Thus, whereas the cod HB3 tetramer exhibited a marked Root effect (Verde et al., 2006), the β1-containing Hb 1 tetramer has probably no Root effect

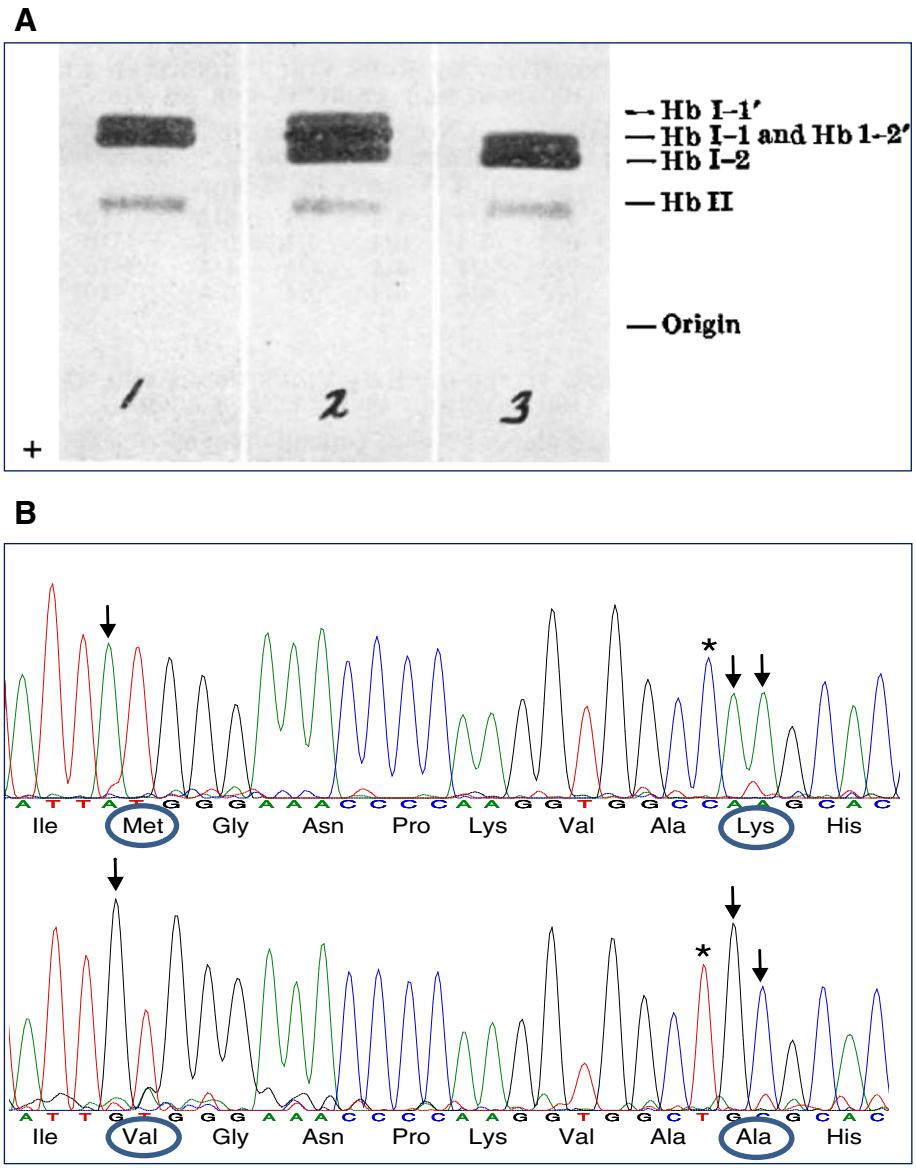


**Fig. 1.** A. Hemoglobin polymorphism in Atlantic cod shown by three distinct electrophoretic patterns. From Sick (1961). B. Sequence chromatogram of the mutated region of cod Hb β1encoding the two globin variants Met55-Lys62 (upper) and Val55-Ala62 (lower) variants. From Andersen et al. (2009).

and might function as an emergency oxygen supplier when fish exercise vigorously (Andersen et al., 2009).

The molecular background of the hemoglobin polymorphism was finally revealed by the identification of non-synonymous mutations in the β1 gene causing three amino acid substitutions, Met55Val, Lys62Ala and Leu123Met (Andersen et al., 2009; Borza et al., 2009). Whereas the latter replacement showed no relationship with the HbI-1/HbI-2 protein variants, the Met-Lys and Val-Ala haplotypes were found to be unambiguously associated with HbI-1 and HbI-2, respectively. Consistently, the higher isoelectric point estimated for the Met-Lys variant compared to the Val-Ala type was in agreement with the cathodic band of the HbI-1 type visualized by IEF analysis (Sick, 1961; Fyhn et al., 1994). Furthermore, the Met55Val replacement explains the appearance of the HbI-1 specific cathodic fragment in the fingerprinting analysis of chymotrypsin-digested cod hemoglobin (Rattazzi and Pik, 1965). These authors identified a His-containing peptide, which matches perfectly the acidic octapeptide from position 56 to 62 after cleavage at Met55, while the alternative Val residue at position 55 would leave an uncleaved neutral peptide. The polymorphic β1 globin gene was abundantly expressed in the juvenile and adult cod, while very low levels were quantified in the hatched larvae (Wetten et al., 2010). Consistently, the electrophoretic different components of the HbI genotypes could not be identified reliably until the cod were about 80 mm (Fyhn et al., 1995).

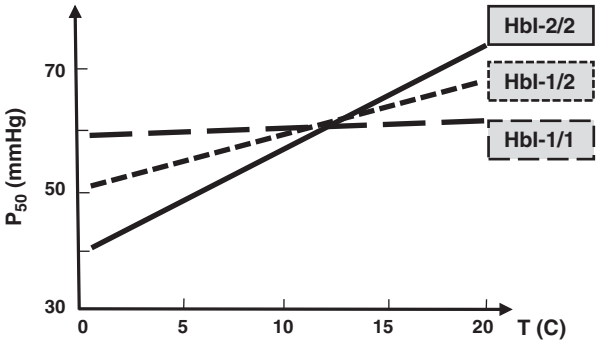


**Fig. 2.** Temperature effect on $O_2$ affinity ($P_{50}$) of the polymorphic variants of Atlantic cod hemoglobin at pH of 7.5. Rewritten from Karpov and Novikov (1980).

Rare subtypes of cod hemoglobin have been reported on both sides of North Atlantic (Sick, 1965; Fyhn et al., 1994; Husebø et al., 2004), and probably represent recombinations of the three amino acid substitutions identified in the β1 globin subunit. Accordingly, high resolution melting analysis of trans-Atlantic populations revealed low allelic frequencies of Val-Lys and Met-Ala recombinants (Wetten et al., 2012), while the Leu123Met substitution was identified in the Val-Ala allele as either Val-Ala-Leu or Val-Ala-Met (Borza et al., 2009). The distinct distribution of different subtypes in the Danish Belt, Lofoten region and Barents Sea was proposed to represent adaptation to specific conditions (Fyhn et al., 1994; Husebø et al., 2004), The HbI-2/2b subtype was shown to be an efficient oxygen carrier (Brix et al., 1998) and, together with the HbI-1/2b subtype, showed higher growth rates than the three main genotypes at 10 °C and 13 °C (Imsland et al., 2007).

## 6. Structural and regulatory polymorphisms of cod β1 globin

The molecular mechanisms underlying the functional properties of the cod hemoglobin variants were elucidated by studying structure-function relationship in the 3D modeled quaternary structure of the β1-containing tetramer Hb 1 (Andersen et al., 2009). The Met55Val substitution is located on the α1-β1 interface, and the gap is increased by replacing Val with the larger Met residue (Fig. 3). The subunit contact is crucial to the stability of the dimers, and increased oxygen affinity was induced in human hemoglobin by site-directed mutagenesis of Met55β→Ser, which excluded van der Waals contact between the subunits (Jessen et al., 1991). Intriguingly, this key position is mutated in the hemoglobin of the hypoxia-tolerant Andean goose (Leu55β→Ser) when compared with the low-land greylag goose (Hiebl et al., 1987). The exploitation of the same mutated position for tolerating hypoxia in such diverse species as the water-breathing cod and air-breathing goose supports the hypothesis of Perutz (1983) that adaptive changes in hemoglobins have evolved by only a few amino acid substitutions in key positions. The Lys62Ala replacement of the cod β1 globin did not seem to introduce any significant steric effects on the adjacent His63 (Andersen et al., 2009), which plays a key role in controlling the access of ligands to the heme pocket (Olson et al., 1988). However, the strong water interaction of the polar Lys62 compared to the aliphatic Val residue was proposed to reduce the oxygen affinity of the Met-Lys (HbI-1/1) variant (Andersen et al., 2009). Furthermore, the two exothermic reactions of internal water interaction and oxygen binding to heme might explain the much reduced temperature-sensitivity of the HbI-1/1 type (Brix et al., 2004).

The structural polymorphism of the cod β1 globin was recently found to be accompanied with a polymorphic promoter regulating the gene expression (Star et al., 2011). The cod β1 gene is positioned head-to-head to the adjacent α1 gene with an intergenic promoter region of about 1.7 kb (Wetten et al., 2010). The promoter harbors several mutations including a 73-bp indel, and the resulting long and short promoter variants are strongly associated with the Val-Ala (HbI-2) and Met-Lys (HbI-1) haplotypes, respectively (Fig. 4). Different transcriptional activities of the two promoter variants were demonstrated by transfecting salmon kidney cells with a firefly luciferase gene inserted downstream of either of the two promoters (Star et al., 2011). Whereas the two variants expressed the luciferase gene at similar levels at 4 °C, the long promoter displayed a two-fold higher transcriptional activity compared to the short promoter at both 15 °C and 20 °C. The promoter polymorphism might therefore provide a molecular mechanism that helps maintaining the total oxygen carrying capacity in Val-Ala (HbI-2/2) cod at increased temperatures despite reduced oxygen affinity. This compensatory mechanism is probably related to the increased levels of the most cathodic component in HbI-2/2 cod acclimated at 15 °C compared to 4 °C (Brix et al., 2004). The increase was paralleled by a reduction in the anodic component that otherwise might have resulted in critical high hemoglobin levels causing polymerization and red cell sickling, a serious phenomenon in gadoid fishes (Hàrosi et al., 1998; Koldkjær and Berenbrink, 2007; Riccio et al., 2011). The temperature sensitive regulation of cod β1 globin resembles the expression of cod myoglobin, but in contrast to β1 globin, the myoglobin mRNA and protein levels were significantly higher at low (4 °C) than at high (10 °C) temperatures (Lurman et al., 2007). Both the Northeast Arctic and North Sea cod populations displayed temperature sensitive myoglobin expression, but the increments were largest in the Arctic population, which might be due to polymorphism in the myoglobin promoter as well. In gilthead seabream (*Sparus aurata*), hypoxia and low salinity induced a change in the ratio between the genetic distinct hemoglobin components, which, however, showed identical functional properties (Campo et al., 2008). No responsive elements

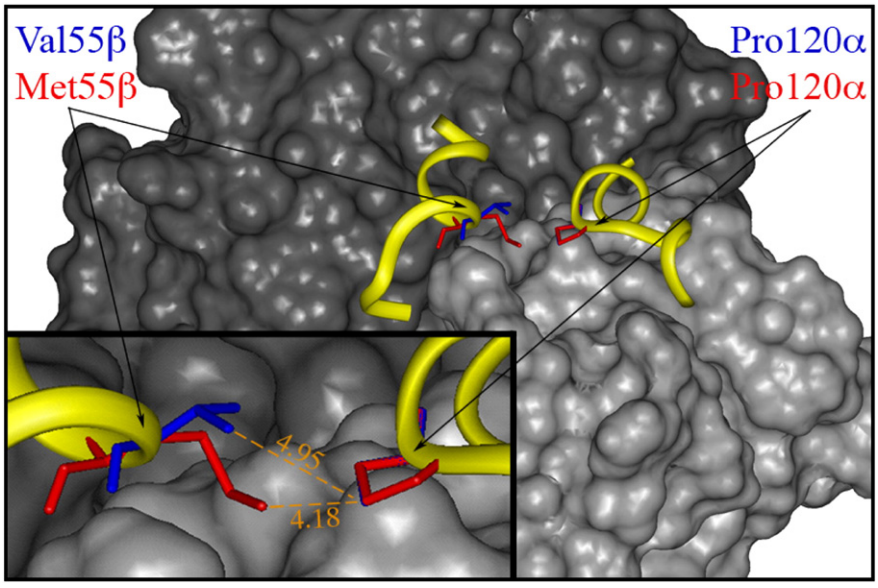


**Fig. 3.** The Met55Val substitution modifies the gap between the α1-β1 subunits in Atlantic cod. The 3 D model shows the superimposition of Met55 (red) and Val55 (blue) at the contact between the α1 (black) and β1 (grey) subunits. The frame depicts the distance of the CG atom of Pro120α to the CE atom of Met55β and to the CG2 atom of Val55β. From Andersen et al. (2009).

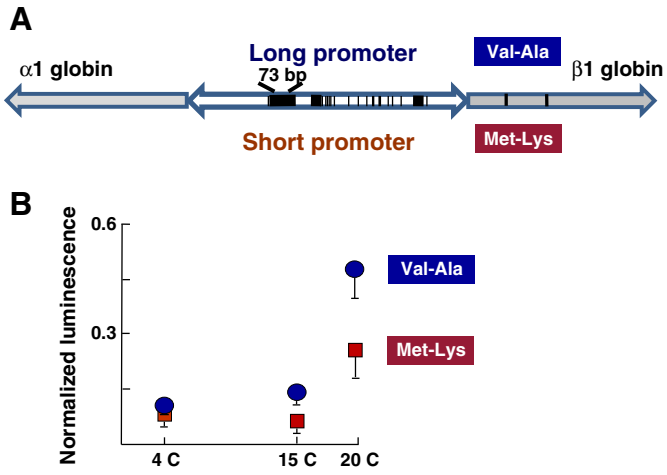


**Fig. 4.** A. Polymorphisms of the intergenic promoter of the head-to-head organized Hb-α1 and Hb-β1 genes in Atlantic cod. The 73-bp indel gives two promoter variants of different lengths. B. The long promoter construct displayed significantly higher luciferase activity than the short variant in transfected salmon kidney cells at 15 °C and 20 °C. Ratios were normalized using a cotransfected SV40/Renilla factor. Rewritten from Star et al. (2011).

for relevant transcription factors were identified in the 73-bp insert of the long cod β1 promoter, while hypoxia-responsive elements were identified in medaka Hb-α and Hb-β, which were both upregulated under hypoxic stress (Warowski et al., 2011).

## 7. Cod hemoglobin genotypes and adaptation

The opposing roles of hemoglobin during oxygen loading at the respiratory surfaces and unloading in peripheral tissues imply that both decreased and increased oxygen binding affinities can be interpreted as beneficial. However, safeguarding post-branchial oxygen saturation seems to be given the first priority in a low-oxygen medium, and the importance of oxygen loading by high-affinity hemoglobins in fish is supported by comparative studies of multiple water-breathing, bimodal and air-breathing fish species (Graham, 2006). HbI-2/2 cod were hypothesized to be more tolerant to short-term hypoxic episodes than HbI-1/1 fish, which displayed significantly higher plasma cortisol and lactate levels after hypoxia exposure at 5 °C, 10 °C and 15 °C (Methling et al., 2010). The HbI-2/2 (Val-Ala) type is almost exclusive in the seasonal cold Baltic Sea (Sick, 1961; Brix et al., 1998; Andersen et al., 2009), where the brackish water restricts cod to deeper water with higher salinity but low oxygen levels (Tomkiewicz et al., 1998). Thus, the efficient oxygen loading of this variant during the cold season together with increased synthesis during summer regulated by the temperature sensitive promoter seem to be of significant advantage for the Baltic cod population. This compensatory mechanism for maintaining the oxygen carrying capacity could also be beneficial for the *skrei* of the Northeast Arctic population during spawning migration to warmer regions.

The high affinity HbI-2/2 variant found in cod inhabiting the ice-cold Barents Sea might represent an adaptation to the higher maintenance costs in polar areas. Acclimation of fishes to cold waters is associated with rising mitochondrial densities and/or increased capacities of mitochondrial enzymes, which are crucial traits in thermal adaptation (Guderley, 2004: Pörtner et al., 2006). Permanent cold adaptation of muscle aerobic metabolism together with permanently elevated oxygen consumption rates were reported in cod populations at higher latitudes and reflected higher maintenance costs in cold-adapted versus cold-acclimated cod (Lannig et al., 2003; Lucassen et al., 2006). In contrast, no evidence of metabolic cold adaptation was found by comparing the oxygen consumption in Arctic and temperate teleost species, including Atlantic cod caught off Greenland (Steffensen et al., 1994). The authors suggested that the passive leak of ions across membranes in polar fishes might be reduced by lowering the diffusional loss, and not by increasing the capacity of active ion transport. Consistently, HbI-2/2 cod were reported to possess fewer, but thicker muscle fibres, compared to HbI-1/1 fish to reduce the ion leakage across the membrane, thus reducing the energy costs of maintaining ionic homeostasis (Johnston et al., 2006). The adaptive value of the Val55-Ala62 genotype in the permanent cold Barents sea is supported by physiological and molecular studies of the hemoglobins in the related ice cod (*Arctogadus glacialis*) and polar cod (*Boreogadus saida*) (Verde et al., 2006). Both species are strictly cold-water species, but the more sluggish ice cod inhabits permanently ice-covered waters even farther north than the highly migratory polar cod. Consistently, the tetrameric Hb 3 molecule displayed lower oxygen affinity in the ice cod compared with both polar cod and Atlantic cod, and may be preferentially related to lifestyle (Verde et al., 2006). Intriguingly, the ice cod and polar cod differ by possessing Met and Val, respectively, at position 55 in the β1 globin (Fig. 5). It was therefore hypothesized that the low-affinity Met55 variant transports sufficient amounts of oxygen in the sluggish ice cod, whereas the polar cod displaying Val55 is probably better fitted to higher oxygen demands like Atlantic cod inhabiting these waters (Andersen et al., 2009).

Both oxygen binding affinity and capacity decreased when blood from 7–8 °C acclimated cod was exposed to high temperatures of 20 °C and 24 °C using fish from Newfoundland, which probably comprised more than 90% HbI-2/2 (Gollock et al., 2006). Thus, the less temperature sensitive HbI-1/1 variant might be a significant advantage in warmer waters to ensure sufficient oxygen transport. However, the importance of physiological *versus* genetic adaptation to temperature were addressed by measuring the functional properties of oxygen affinity ($P_{50}$) and cooperative index ($n_{50}$) in HbI-1/1 and HbI-2/2 cod acclimated at 4 °C and 12 °C (Colosimo et al., 2003). When the parameters were measured at 12 °C, significant differences were found between the two genotypes albeit of much lesser magnitude than the effect of acclimation. No significant effect of either acclimation or genotype could be detected when the functional features were measured at 4 °C, and physiological adaptation was proposed to be more relevant than the genetically one. Accordingly, juvenile cod of the different Hb genotypes kept at temperatures from 10 °C and 22 °C showed no differences in basal and maximum metabolic rates as well as thermal tolerance when exposed to acute temperature increase (Gamperl et al., 2009). The authors concluded that there is no selective advantage in having a particular hemoglobin genotype with regards to the capacity to withstand ecologically relevant environmental challenges. Thus, *in vitro* studies demonstrating functional differences between the Hb genotypes have probably ignored *in vivo* compensatory mechanisms that enhance oxygen binding capacity or ensure adequate oxygen delivery to the tissues (Gamperl et al., 2009). Intriguingly, the promoter polymorphism of cod β1 globin might represent such a compensatory mechanism by maintaining the oxygen carrying capacity in HbI-2/2 cod at increased temperatures (Star et al., 2011).

A better utilization of oxygen would lower the energy spent in metabolism, and more energy could be allocated to growth. Hemoglobin genotype-dependent differences in growth performance of juvenile cod have been documented, but the magnitude of the growth differences and the genotype(s) displaying the highest growth rate vary depending on experimental conditions. Juveniles carrying the HbI-2/2b subtype, being the most efficient in terms of oxygen loading at high temperatures (10-20 °C) (Brix et al., 1998), showed higher growth rates than the main genotypes at 10, 13 and 16 °C (Imsland et al., 2007). However, Imsland et al. (2004) reported that HbI-2/2 displayed the overall highest growth rate at 13–16 °C, while the HbI-1/1 cod grew fastest at the lowest temperature of 7 °C (Imsland et al., 2004), thus contrasting with the different oxygen binding properties at high and low temperatures. HbI-2/2 was also found to possess enhanced competitiveness, feed efficiency and energy retention compared to HbI-1/1 (Salvanes and Hart, 2000;


**Fig. 5.** Alignment of the polymorphic region of Atlantic cod Hb-β1 with sequences from other gadoid species, including haddock (*Melanogrammus aeglefinus*) and burbot (Lota lota).The residues at position 55 and 62 of the β1 globin are indicated by one letter code, and consensus codons are indicated by nucleotides in black.

Jordan et al., 2006). Gamperl et al. (2009) found no differences in growth between the three genotypes after the juvenile stage of PIT tagging, while HbI-1/1 cod displayed highest growth rates after hatching but showed significantly lower survival than HbI-1/2 and HbI-2/2 fish at the early stages.

## 8. Evolution of hemoglobin polymorphism in gadoids

The pioneering studying of Knud Sick documented polymorphic hemoglobins in both Atlantic cod and whiting (Sick, 1961), and screening a whiting transcriptome (www.codgenome.no) revealed two β1 globin genes encoding proteins differing at four positions, including Lys62Arg (Fig. 5). The alignment of β1 globin sequences from diverse gadoid species suggests that the ancient gadoid β1 globin contained Val55 and Lys62 based on the consensus coding sequence. A small residue at the α-β subunit interface and a polar residue in the heme binding pocket were proposed to increase the oxygen affinity and lower the temperature sensitivity, respectively (Andersen et al., 2009), which seem to be beneficial features for ectothermic organisms living in habitats with high or fluctuating temperatures. Surprisingly, a β globin harboring Val55Lys62 was identified in only one non-gadoid species, the European conger eel (*Conger conger*), among 249 vertebrate species (Colafranceschi et al., 2010). This marine species lives in the northeastern Atlantic, and the cathodic hemoglobin (HbC) lacks significant pH effects (Pellegrini et al., 2003), but the low overall sequence identity with gadoid globins restricts further comparison. Structural and functional modifications of the putative Val55Lys62-containing ancestral codfish globin probably paralleled the climatic changes, which seem to have occurred during the speciation of the gadoids. *Merlangius* and *Melanogrammus* separated from *Gadus*/*Boreogadus* about 8.5 MYA, while the two latter genera diverged about 5.6 MYA (Bakke and Johansen, 2005). The climate at the Earth during Pliocene, which lasted from 5.4 to 2.5 MYA, was for the most part much warmer than today (Dowsett et al., 1996). Every summer the sea ice cap of the Arctic Ocean thawed out completely, and conifer forests grew on the northern coast of Greenland. Following the very warm interval of the Mid-Pliocene, ice began to accumulate during a series of short, successive cold periods, in the northern regions of America and Europe, and icebergs appeared in the North Atlantic. Today, the whiting is found in the Northeast Atlantic from Portugal to Iceland and the south-western Barents Sea, but information about the geographical distribution of the two hemoglobin variants is lacking. The more northern-living populations of Atlantic cod seem to have become evolutionary adapted to Arctic and temperate waters predominated by the Val-Ala and Met-Lys variants, respectively, while the putative ancestral Val-Lys haplotype is found in only about 10% of this successful species (Wetten et al., 2012).

Altogether, the recent molecular studies of the Atlantic cod hemoglobins have provided novel evidence for the coevolution of structural and regulatory adaptation within this species as well as a common mechanism for tolerating hypoxia in water-breathing and air-breathing species.

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