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REVIEW

REVIEWS IN Aquaculture

Infectious pancreatic necrosis virus in salmonids: Molecular epidemiology and host response to infection

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Abstract

Infectious pancreatic necrosis virus (IPNV) is one of the most pervasive pathogens in aquaculture worldwide. It causes a highly contagious disease in salmonids that has a significant economic impact in almost every country where salmonid fish are farmed. For decades IPNV studies have focussed on the molecular characterisation of isolates collected from around the world and the discovery of molecular markers associated with virulence and pathogenicity. The great success of selective breeding for resistance to IPNV has caused special emphasis to be put on the immune response of salmon genetically resistant/susceptible to IPNV. In this work, we review the classification of IPNV, summarise virulence markers and add recent findings about the molecular epidemiology and worldwide distribution of the virus. We also review genetic improvements for IPNV resistance, the kinetics of the immune response to IPNV and the transcriptomic response of salmonids, made possible through the use high-throughput technologies.

KEYWORDS
infectious pancreatic necrosis virus, molecular epidemiology, QTL, salmonids, virulence

1 | INTRODUCTION

One of most important viral diseases that affect salmonid aquaculture worldwide is Infectious Pancreatic Necrosis (IPN), a contagious disease that can cause high mortality rates in first-feeding fry, and in post-smolts shortly after transfer to seawater.[1,2] Its aetiological agent, IPN virus (IPNV), is an *Aquabirnavirus* (within the family *Birnaviridae*), and is considered the most pervasive pathogens of aquatic animals, with worldwide distribution and a wide range of hosts.[3,4] The term "IPN virus," however, has been used (and will be used in this review) to denote only aquabirnaviruses infecting or causing disease in salmonids.[4] Aquatic birnaviruses isolated from non-salmonid species will be referred to as marine aquabirnaviruses (MABV) or yellowtail ascites virus (YTAV) if they were isolated or caused disease in yellowtail fish (*Seriola quinqueradiata*).[5] The salmonid species typically affected by IPN are brook trout (*Salvelinus*

fontinalis), common trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), and several species of the genus *Oncorhynchus* spp.; including coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Oncorhynchus mykiss*).[6] The disease is transmitted both horizontally and vertically, and after an outbreak the surviving fish and those that become infected without developing clinical signs can persist as asymptomatic carriers of the virus,[7] thus presenting a risk for their progeny and other susceptible fish.[8]

Given the importance of the disease and the wide distribution of the virus throughout the world, a large body of literature on IPNV has been published. Several studies have reviewed the pathology of IPN, biophysical properties of the virus, its classification, pathogenesis, virulence, diagnostic methods, and control.[2-5,9,10] However, none have focussed on recent findings on the molecular classification of IPNV, the genetic progress of IPN resistance in salmonids or host gene expression in response to infection. Thus, the objective of this review is to present updated information on the molecular epidemiology of IPNV, first describing the history of the genetic characterisation of the virus and, secondly, the geographical distribution of its genogroups and new sequenced isolates. Aspects related to host response in salmonids will also be reviewed, emphasising recent findings on genetic improvement for resistance to the disease and the transcriptomic response in susceptible/resistant fish.

2 | AETIOLOGICAL AGENT

The detailed information regarding morphology, biophysical properties, and molecular biology of IPNV has been extensively described in previous reviews,[3,5,11,12] so only a brief summary will be included as an introduction. IPNV is non-enveloped with a single layer icosahedral capsid of about 60 nm in diameter, containing a bisegmented, double-stranded RNA (dsRNA) genome.[13,14] The two dsRNA segments have been designated A and B and are comprised of ~3100 and 2784 base pairs (bp), respectively.[3] Segment A contains two partially overlapping open reading frames (ORF), the larger ORF encodes a polyprotein (pVP2-VP4-VP3, 106 kDa) that is co-translationally cleaved by the virus encoded serine-lysine protease (VP4 or non-structural (NS), 30 kDa) to produce two polypeptides: pVP2 (63-kDa), the precursor of the major viral capsid protein (VP2, 54-kDa); and VP3 (31-kDa), an internal/minor capsid protein.[3,15,16] Segment A also has a smaller ORF, which codes for a 17-kDa arginine rich polypeptide, known as VP5 a NS protein.[17] Segment B, with one ORF, encodes the RNA-dependent RNA polymerase, VP1, of 94-kDa.[18,19] This protein is present in two forms, as a free polypeptide (VP1) and as a genome linked protein (VPg).[18]

3 | SEROLOGICAL CLASSIFICATION

Initially, *Aquabirnaviruses* were classified based on an antiserum neutralisation test against three recognised reference serotypes: VR299, Sp, and Ab.[20] However, as more viruses were isolated from different host species and areas around the world, it was clear that aquatic birnaviruses displayed a high degree of antigenic diversity. Consequently, Hill and Way[21] provided an extensive review about antigenic comparisons between IPNV isolates and other aquatic birnaviruses and proposed a standard serological classification scheme that was widely adopted; dividing the isolates into two serogroups (A and B). Serogroup A contains most of the IPNV isolates associated with disease in salmonids, further separated into nine serotypes (A_1–A_9). Serogroup B consists of only a single serotype with just a few isolates; only a few of which have been found to be pathogenic in salmonids.[22] More recent reviews have indicated that there are still issues concerning the serotyping of IPNV isolates due to complex serological relationships and a lack of analytical method uniformity.[4,9] These limitations have led scientists to develop new methods to study the relationship of aquatic birnaviruses at the genomic level; leading to the replacement of serological classification almost entirely using the molecular characterisation of IPNV and other *Aquabirnavirus*.

4 | MOLECULAR CHARACTERISATION

4.1 | Segment A

The first nucleotide sequences of IPNV was segment A of the Jasper (3097 bp) strain from Canada[23]and the N1 strain (3104 bp) from Norway.[24] Using the Sanger method on cDNA from segments cloned into vectors, IPNV sequences were compared with each other and a strain of infectious bursal disease virus (IBDV), another member of *Birnaviradae*.[24] Results showed that the precursor protein of VP2, pVP2, is highly conserved at the N and C termini, while an internal segment exhibited greater diversity between the strains, suggesting this hypervariable segment contained the serotype-specific epitopes of birnaviruses. Subsequently, Heppell et al.[25] amplified, cloned and sequenced a small 310 bp cDNA fragment located at the junction of the C-terminus of pVP2 and the NS coding regions of 17 IPNV strains that represented all serotypes from diverse geographical areas. The comparison between the deduced amino acid sequences and those previously reported showed that the serologically related viruses were highly homologous. The authors proposed that only three major genogroups exist among the many viral strains in Serogroup A; however, some serologically different strains had nearly identical amino acid sequences. Segment A of a Korean IPNV strain called DRT (3155 bp) was sequenced and also compared with the Jasper and N1 strains.[26] The comparison indicated that DRT was more closely related to the Canadian strain Jasper than to the Norwegian N1; suggesting that geographical distance was not correlated with nucleotide sequence homology. These early findings showed that more sequence information from a larger number of viral isolates would be necessary to better understand the genetic relationship and diversity between aquabirnaviruses.

One of the most important contributions to the molecular classification of IPNV was published by Blake et al.,[27] who conducted

the first phylogenetic analysis based on the large ORF of segment A from nine strains of serogroup A and the VP2 gene from 28 isolates from different geographical and host origins. Using Reverse Transcription Polymerase Chain Reaction (RT-PCR) the authors amplified a 2904 bp cDNA fragment that fully encompassed the larger ORF of segment A or a 1611 bp fragment of the entire VP2 coding region. The nucleotide and amino acid sequences were sequenced from purified PCR products, compared, and phylogenetic trees were constructed using maximum parsimony analysis. The results showed that the nine reference strains of serogroup A could be clustered into six genogroups, where Genogroup 1 was composed of several genotypes.[27] In contrast to earlier analyses based on shorter sequences of the pVP2/NSs coding region,[25] these genogroups showed a correlation with the serological classification and geographical origin of the strains. For example, Genogroup 5 corresponds to serogroup A2 and includes several isolates from Europe such as Sp, Fr10, and N1. Genogroup 1 correlates to serotype A1 and includes strains from the USA, such as West Buxton (WB), VR-299, and Buhl (Table 1). Genogroup 1 is composed of four genotypes that correspond to previously identified serological strains.[27]

Several other authors have presented phylogenetic analyses based on fragments of genome segment A of IPNV and other aquabirnaviruses isolated worldwide.[28-37] Many of them used the same reference strains sequenced by Blake et al.[27] and previous authors (Table 1). A seventh genogroup was reported based on the amplification and sequencing of a 310 bp fragment at the VP2/VP4 junction region from fourteen isolates from marine fish and molluscan shellfish.[29] Importantly, this genogroup was comprised of only Japanese aquabirnaviruses isolated from marine shellfish and fish (i.e. MABVs) including isolates belonging to the species YTAV.[29,38]

It is important to emphasise that there have been discrepancies in the numbering of the different IPNV genogroups, with several authors numbering the genogroups differently than that proposed by Blake et al..[27] Moreover, Blake et al.[27] swapped the numbers of Genogroups 2, 3, and 4 in the dendrogram of their publication and later an errata with the correct numbering was published. However, unaware of the error and subsequent correction, some authors have incorrectly numbered the IPNV genogroups. Others have made similar mistakes or even used Roman numerals to designate the phylogenetic groups of the virus. We recommend the classification and numbering proposed by Blake et al.,[27] with six genogroups numbered from 1 to 6, supplemented using a seventh genogroup as reported by Nishizawa et al..[29] From now on we will refer to this classification and numbering when discussing IPNV genogroups, unless otherwise stated.

4.2 | Segment B

Segment B of IPNV was first sequenced using the Sanger sequencing method on the Jasper (2784 bp) and Sp (2630 bp) strains from cloned cDNA, synthesised from RNA isolated from purified

TABLE 1 Aquabirnavirus reference strains used commonly in phylogenetic analysis of segment A of IPNV, and the genogroups they belong according to the classification proposed by Blake et al. (2001) and supplemented by Nishizawa et al. (2005)

Strain	Origin Country	Host	Serotype	Genogroup	GenBank	References
VR299	USA	*Salvelinus fontinalis*	A1	1	AF343572	27
DM	USA	*S. fontinalis*	A1	1	AF343571	27
Buhl	USA	*Oncorhynchus mykiss*	A1	1	AF343573	27
Reno	USA	*O. clarkii*	A1	1	AY026345	27
WB	USA	*O. mykiss*	A1	1	AF078668	43
Jasper (VR-1325)	Canada	*O. mykiss*	A9	1	AF342735	27
Jasper (JasperD)	Canada	*O. mykiss*	A1	1	M18049	23
DRT	Korea	*O. mykiss*	A1	1	D26526	26
Abild (Ab)	Denmark	*O. mykiss*	A3	2	AF342729	27
Tellina (Te)	Scotland	*Tellina tenuis*	A5	3	AF342731	27
ASV	Canada	*Salmo salar*	A6	3	AY026490	27
Canada 1 (C1)	Canada	*S. salar*	A6	3	AF342732	27
Canada 2 (C2)	Canada	*O. mykiss*	A7	4	AF342733	27
Canada 3 (C3)	Canada	*S. alpinus*	A8	4	AF342734	27
Spjarup (Sp)	Denmark	*O. mykiss*	A2	5	AF342728	27
N1	Norway	*S. salar*	A2	5	D00701	24
Bonnamy (Fr10)	France	*O. mykiss*	A2	5	AY026482	27
d'Honnincthun (Fr21)	France	*O. mykiss*	A2	5	AY026482	27
Hecht (He)	Germany	*Esox lucius*	A4	6	AF342730	27
YTAV	Japan	*S. quinqueradiata*	MABV	7	AB011440	38

virions.[19] The nucleotide sequences of Jasper and Sp, containing the large ORF encoding the VP1 protein, were 80.7% similar. However, the VP1 protein of IBDV, another member of the *Birnaviridae* family, was less similar to the IPNV isolates (41%).[19] The VP1 gene of the Korean strain DRT was later sequenced, using RT-PCR and cDNA cloning. The nucleotide sequence was more closely related to the Jasper strain than to the Sp strain.[39] The first phylogenetic analysis based on the cDNA sequences of segment B was done by Zhang and Suzuki,[40] who compared amino acid sequences of IPNV to other MABV, avibirnaviruses and entomobirnaviruses. The phylogenetic tree showed that the aquabirnavirus strains clustered into three Genogroups: I, II, and III; where Genogroup I consisted of IPNV strains of the A1 serotype and Genogroup II contained all MABV strains. Only IPNV Sp strain was clustered into Genogroup III.[40] Joh et al.[41] also compared the genetic relationship between amino acid sequences of the VP1 protein from aquatic birnaviruses and other genuses of the *Birnaviridae* family. The VP1 sequences clustered the aquabirnavirus strains into three groups, separated from the avibirnaviruses and entomobirnaviruses. Genogroup 1 included IPNV strains isolated from Korea (DRT), Japan (AM98), the USA (WB), and Canada (Jasper). Genogroup 2 included IPNV strains isolated primarily from Europe (Sp), and a MABV genogroup included marine aquatic birnaviruses such as YTAV isolates.[41] More recently, the VP1 gene from a Mexican IPNV isolate was characterised and compared with several IPNV isolates from Europe, North America, and Asia, as well as two MABV isolates.[42] The authors identified three genetic groups. Group 1 isolates were from Spain, Korea, Japan, Canada, the USA, and the Mexican isolate. Group 2 included isolates from Spain, Norway, Denmark, France, and Canada. Group 3 included only MABV isolates originating in Japan, in agreement with previous phylogenetic classifications. Barrera-Mejía et al.[42] suggest that since the VP1 gene is more conserved than VP2, phylogenetic analysis of VP1 is a more suitable method for identifying new genetic lineages of IPNV. Nevertheless, there have been no recent publications that rely only on segment B to classify IPNV; most focussing on segment A or the entire genome.

4.3 | Whole genome

Advances in molecular techniques and DNA sequencing has greatly increased the availability of whole genomes of IPNV and other aquabirnaviruses. In 1998, the first complete nucleotide sequences of IPNV segments A and B, including the precise 5′- and 3′-terminal sequences, were completed for strain WB.[43] To develop a reverse genetics system for IPNV, full-length cDNA clones of both viral segments were constructed and their complete nucleotide sequences were determined. Comparison of the nucleotide and amino acid sequences of strain WB segments A and B with those of the Jasper strain showed high identity at the nucleotide level (91.64% and 90.37%) and at the amino acid level (97.22% and 97.16%), indicating that these two North American strains were closely related.[43]

Romero-Brey et al.[33] characterised the complete genome of seven Canadian aquabirnavirus isolates using RT-PCR amplification and cDNA sequencing of segments A and B (including the 5′ and 3′ non-coding regions) through the dideoxy chain termination method. Phylogenetic analysis found that most of the isolates clustered in the same genogroups (1 and 2) when the sequences of either segment A or segment B were compared with IPNV reference strains and other aquatic birnaviruses. Analysis of segment B from viral isolates showed the same clustering as analysis of segment A. However, one isolate, 20G1D, clustered with North American type strains (Genogroup 1) using sequence from segment A, but clustered with European reference strain Ab (Genogroup 2) using segment B. The authors suggested the existence of natural genetic reassortment between two strains of aquabirnaviruses co-infecting a host, a process already demonstrated for other birnaviruses but not previously reported for aquabirnaviruses[33]; pointing out the importance of sequencing both genomic segments for classification of new isolates of aquabirnaviruses. Subsequent phylogenetic studies sequenced the whole genome of IPNV isolates looking for the possibility of natural reassortments.[36,37] In 2017, reassortment between IPNV strains was demonstrated *in vitro* through coinfections with different viral strains, and genome sequencing using the Sanger method and Next Generation Sequencing (NGS).[44] The use of NGS techniques has facilitated the sequencing of both genomic segment A and B for genotyping of new IPNV isolates,[45-48] and the identification of new inter-segment reassortments.[49,50]

5 | GEOGRAPHIC DISTRIBUTION OF GENOGROUPS

Infectious pancreatic necrosis virus was the first virus to be isolated from teleosts. Currently, IPNV is considered one of the most widely distributed aquaculture pathogens worldwide. The virus has been detected in almost every country where salmonids are farmed. It has been stated that "the geographical distribution of IPNV is mainly a reflection of aquaculture production"[4] as the transmission of IPNV has been primarily associated with the introduction of infected fish and/or eggs into other countries. Geographical distribution of sequenced IPNV isolates reported in peer-reviewed literature is shown in Table 2. Also, a phylogenetic tree based on the nucleotide sequence of a 1043 bp fragment of segment A (positions 248–1290) was constructed using most of the isolates in Table 2 and the reference strains presented in Table 1 (Figure 1 and Figure S1).

The first cases of IPN were reported in North America. The disease was initially described in Canada (originally named Acute Catarrhal Enteritis),[51] but the IPN virus was first isolated from brook trout (*Salvelinus fontinalis*) in the USA in 1957.[52] This isolate was known as reference strain VR-299 after being deposited in the American Type Culture Collection in 1963.[21] Later, it was classified within Genogroup 1 based on phylogenetic analysis of segment A.[27] After the isolation of strain VR-299 the frequency of IPN outbreaks increased markedly and the virus was found extensively throughout

TABLE 2 Geographical distribution, host species, and genogroups of sequenced IPNV isolates reported recently worldwide

World Region	Country	Host	Isolate ID	Genogroup	GenBank	Reference
North America	USA	*S. fontinalis*	Erwin	1	EU869269	36
		S. salar	Connecticut-1	4	JF440810	53
		S. fontinalis	PA1	1	MH010544	48
	Mexico (MEX)	*O. mykiss*	Mexican	1	nr	54
		O. mykiss	EdoMex07	1	nr	31
		O. mykiss	EdoMex11	1	JF714665	35
		O. mykiss	MEX2-CSM-07	1	MH708132	55
		O. mykiss	MEX3-CSM-05	1	AYQ58854	56
Europe	France (FRA)	*O. mykiss*	31-75	5	AJ622822	60
	Spain (ESP)	*S. salar*	2310	1	AJ489225	78
		O. mykiss	2284	2	AJ489223	78
		O. mykiss	1146	5	AJ489222	78
	Norway (NOR)	*S. salar*	Sp116	5	AY354520	66
		S. salar	NVI-013	5	AY379738	67
		S. salar	NVH-101	5	HQ457198	68
		S. salar	S-IPNV/FS2-01	5	HQ833318	69
		S. salar	B2-2_33	5	KX355240	70
		S. salar	H25Y10no1Sea20Y11	5	MH561993	71
	Scotland (GB-SCT)	*S. salar*	975/99	5	AJ829474	74
		S. salar	2003-0767-18	5	AJ880301	75
		S. trutta	2000-1288	3	AJ880284	30
		S. salar	2002-0757	5	AJ877116	30
		S. maximus	IPNV061	1	ERS2755598	50
		S. salar	IPNV063	2	ERS2755600	50
		S. salar	IPNV006	3	ERS2755566	50
		O. mykiss	IPNV007	5	ERS2755567	50
		S. salar	V1810–4	5	OK076707	47
	Ireland (IRL)	*S. salar*	F3202	5	JF430403	28
		O. mykiss	I-4	5	HQ457197	68
		S. salar	IRL-F1619	3	JX880107	34
		O. mykiss	F106-13	5	KJ801312	34
	Finland (FIN)	*O. mykiss*	ka1005/14	2	KR780997	79
		O. mykiss	ka640/12	5	KR781008	79
		O. mykiss	90/12	2	KY548513	46
		O. mykiss	639/12	5	KY548514	46
		S. salar	94/01	6	KY548509	46
	Croatia (HVR)	*O. mykiss*	CroIPNV/06	5	EU219618	57
		O. mykiss	Cro-IPNV-09/09	5	HM036118	58
		O. mykiss	Cro 111/10	5	JQ684179	59
	Bosnia and Herzegovina (BIH)	*O. mykiss*	BH2974/08	5	JQ684190	59
	Ukraine (UKR)	*O. mykiss*	Karpaty	5	KJ596654	61
	Italy (ITA)	*O. mykiss*	IPNV/O.mykiss/I/PN/208/Mar88	5	MG543567	49

(Continues)

TABLE 2 (Continued)

World Region	Country	Host	Isolate ID	Genogroup	GenBank	Reference
	Turkey (TUK)	*O. mykiss*	TR2/15	5	KY986935	62
		O. mykiss	Duzce/17	5	nr	63
		O. mykiss	Almus	5	MH614929	64
		O. mykiss	HAH-4	5	MH614932	64
	Poland (POL)	*O. mykiss*, *Salmo trutta m. fario*, *Salvelinus namaycush*	e.g., 47, 57, 100	5	nr	65
South America	Chile (CHL)	*O. kisutch*	V193/08	1	HQ738517	91
		S. salar	V70/06	5	HQ738515	91
		S. salar	C-1	5	HQ457171	68
		O. kisutch	VCh32523	1	JN642221	92
		S. salar	VCh3228	5	JN642216	92
		O. mykiss	IHJS1	1	KF954926	93
		S. salar	KJKB1	5	KF954917	93
		O. kisutch	VUV/84	1	KU609593	45
		O. kisutch	LKJH6	1	KU609589	45
		S. salar	EBPS1	5	KU609583	45
		O. mykiss	PITR2	1	KU605640	95
		S. salar	MPMA2	5	KU605642	95
		O. kisutch	1096-BC1	1	KX523824	94
		S. salar	1096-BC4	5	KX523803	94
Asia	Japan (JPN)	*O. rhodurus*	AM-98	1	AY283780	40
		O. mykiss	Nagano	1	AB179712	29
	Iran (IRN)	*O. mykiss*	IRFIPN2009	5	HQ383921	102
		O. mykiss	IRIPNV	5	KF279643	37
		O. mykiss	Ir-Ms-TR-IPN13	5	KF414729	104
		O. mykiss	S.AV-IR-IPNV	5	KX665156	105
	China (CHN)	*O. mykiss*	ChRtm213	1	KX234591	97
		O. mykiss	WZ2016	1	KX355401	98
		O. mykiss	Co/P-2018	1	MK213585	99
		S. trutta	HLJ2019-4	1	MW662103	100
		O. mykiss	GS2020-1	5	MW662091	100
Oceania	New Zealand (NZL)	*O. tshawytscha*	NZ6	5	EU869270	36
	Australia (AUS)	*S. salar*	TAB98	5	EU672429	36
		O. mykiss	TABV 13-03567	5	KP268663	107
		O. mykiss	VTAB 10-04677	7	KP268664	107
Africa	Kenya (KE)	*O. mykiss*	RTH1	5	MF280062	109

Note: Sequences shorter than 1043 bp, not included in the phylogenetic tree presented in Figure 1.

Only one isolate for each reported genogroup was selected in studies with several sequenced isolates. Genogroups determined for each isolate were assigned according to phylogenetic tree constructed here and/or in the study where it was originally reported, following the classification system proposed by Blake et al. (2001) supplemented by Nishizawa et al. (2005).

Abbreviation: nr, not reported.

the USA and Canada.[9] Other strains isolated in the USA, such as WB, Dry Mills (DM), Buhl, Reno, and Powder Mill, were also classified in Genogroup 1; however, they showed some genetic variation, and it was suggested that they form separate genotypes within the genogroup.[27] Canadian strains isolated from farmed and wild salmon and trout, belonged to Genogroup 1 (Jasper), Genogroup 3 (Canada 1 and ASV), and the Genogroup 4 (Canada 2 and Canada 3). Interestingly, isolates from the last two genogroups were more

closely related to European strains than to isolates from the USA.[27] More recently, an IPNV isolated from wild Atlantic salmon returning to the Connecticut River in Massachusetts was classified in Genogroup 4, along with the Canadian strains of IPNV.[53] The authors speculate that the infection occurred somewhere along the salmon migratory route or feeding grounds in the Northwest Atlantic.

The first IPN outbreak in Mexico was reported in 2000 in rainbow trout fry originally imported from the USA as ova. The virus isolated from this outbreak was clustered in Genogroup 1.[54] Subsequent phylogenetic analyses have continued reporting the presence of Genogroup 1 IPNV in Mexico.[31,35,42,55,56] Mexican isolates show a high genetic identity and form a phylogenetic subgroup closely related to the Buhl strain from USA.[35,54,55]

The majority of reports on isolates and phylogenetic characterisations of IPNV have been from Europe due to the large number countries that cultivate salmonids. The first known IPN outbreaks in Europe occurred in France in 1964 in rainbow trout fry.[21] The two isolates from these initial outbreaks, designated Bonnamy (Fr10) and d'Honnincthun (Fr21), were then classified in Genogroup 5 and clustered with other European strains.[27] The occurrence of IPN in France was followed by the detection of IPNV in Denmark, where reference strains Spjarup (Sp) and Abild (Ab) were isolated from rainbow trout.[21] Phylogenetic analysis showed that strain Ab belonged to Genogroup 2 and strain Sp to Genogroup 5.[27] Since then, most isolates identified in Europe belong to Genogroup 5. They are found in almost all salmon-producing countries in Europe and, according to molecular analysis, this genogroup is the only one reported in some countries including Croatia,[57-59] Bosnia and Herzegovina,[59] France,[27,60] Italy,[49] Ukraine,[61] Turkey,[62-64] Poland,[65] and Norway.[66-71]

In Norway, the largest Atlantic salmon worldwide producer, IPNV was isolated for the first time in 1975 from rainbow trout. In the 80s, the virus spread rapidly to most salmon farms and the reference strain N1 was isolated from Atlantic salmon during an IPN outbreak.[72,73] The N1 strain was later classified into Genogroup 5 using phylogenetic analysis, together with the Sp and French strains.[27] Subsequent molecular characterisations of Norwegian isolates from Atlantic salmon and rainbow trout, in both sea and freshwater, have continued to report viruses belonging to Genogroup 5; closely related to strains N1 and Sp[66,67]and to several Chilean and Irish isolates.[68] To understand the molecular basis of IPNV virulence in salmon many studies have compared the sequences of isolates related to strain Sp in Norway (a topic that will be further discussed in the next section). Importantly, some isolates displayed differences in their ability to cause mortality in field outbreaks and/or in experimental challenges.[66-69]

Infectious pancreatic necrosis virus isolates belonging to Genogroup 5 and 3 have been reported in Scotland[30,74,75] and Ireland.[28,34] Phylogenetic analysis showed that the majority of the Scottish and Irish isolates are classified in Genogroup 5, similar to the Sp and French strains.[30,34] However, a few isolates from wild and farmed salmonids have been classified into Genogroup 3; more closely related to strain Tellina (Te) isolated in Scotland decades earlier from the bivalve mollusc *Tellina tenuis*.[30,34,76] Recently, in Scotland, complete genome analysis of 57 isolates from 1982 to 2014 revealed that, in addition to Genogroup 3 and 5, Genogroup 1 viruses have also been found in the country.[50] Furthermore, the phylogenetic analysis of both viral genomic segments (A/B) revealed reassortment of Genogroup 3/2 viruses, indicating the presence of Genogroup 2 IPNV in the country as well. The authors suggest that reassortant virus may have been generated in wild fish and then infected farmed salmon and trout during the early phase of aquaculture in Scotland. More importantly, the isolation of a new variant of IPNV capable of infecting and causing disease in IPNV-resistant salmon has been reported in the country in recent years.[47] A whole genome analysis showed that the new isolates belong to genogroup 5; however, serotyping using neutralisation assay showed that they did not react with the classical European serotype Sp, suggesting that there are emerging variants resistant to Sp-specific antibodies.[47]

Genogroup 2 IPNV, associated with the Danish strain Ab, have been frequently isolated from salmonids only in Spain[77,78] and Finland.[46,79] In both of these countries, the isolation of Genogroup 5 viruses is also common. In Spain, a few isolates have been classified into Genogroup 1.[78] Finland is the only country where IPNV from Genogroup 6 have been isolated from farmed salmonids.[46] Genogroup 6 is composed of strain Hetch (He), an aquabirnavirus originally isolated from European pike (*Esox lucius*) in Germany.[22]

In some European countries, IPNV has been reported but molecular analyses have not yet been performed and viral isolates have only been serotyped. For instance, in Greece IPNV was found to be related to the Sp type strain (serotype A2),[80] and in Bulgaria Sp and Ab type strains (serotype A2 and A3, respectively) have been isolated.[81,82] In other countries, the virus has been occasionally or rarely detected and no serological or molecular classifications have been made. In Kosovo, for example, IPNV has only been detected using PCR in wild and farmed trout,[83,84] while in the Czech Republic the virus was isolated only once from rainbow trout.[85] In Iceland, long considered IPNV-free, the virus was recently detected in asymptomatic farmed salmon.[86]

In South America, IPNV has been detected extensively in Chile, and recently in Peru,[87,88] where no phylogenetic analysis has been performed. In Chile, the second largest salmon producer, IPNV was isolated and characterised for the first time in 1983 from rainbow trout[89,90]; but, it was not until the late nineties that the disease was confirmed in Atlantic salmon farms. The virus is now considered endemic in Chile and only two genogroups (Genogroup 1 and Genogroup 5) have been reported in the country. Genogroup 5 is more dominant and widely dispersed.[68,91-94] Therefore, the introduction of IPNV in Chile has been associated with trade in imported breeding materials (e.g., salmon eggs). For many years, the Chilean salmon industry imported salmon eyed eggs from North America and Europe, where these genogroups are prevalent. More recently, Torres et al.[95] suggested there is a host-specific relationship between the two reported genogroups and the salmonid species cultivated in the country: the isolates belonging to Genogroup 5 were mainly isolated from *S. salar*, while IPNV Genogroup 1 was mostly isolated from *O. mykiss* and *O. kisutch*.

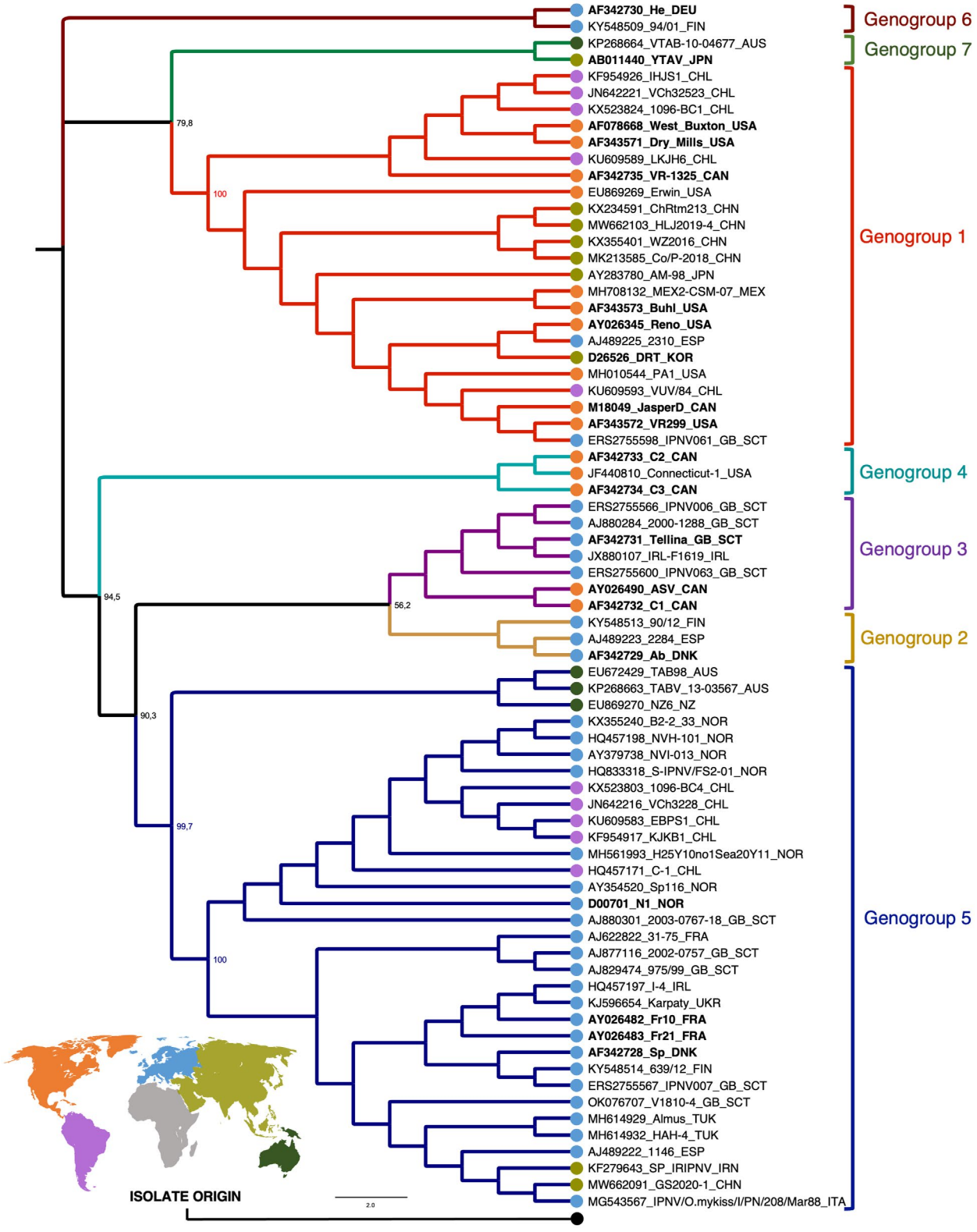

FIGURE 1 Phylogenetic tree based on the nucleotide sequence of a 1043 bp fragment in segment A (positions 248–1290) of IPNV isolates listed in Table 2 and reference strains (in bold letters) presented in Table 1. Their Genbank accession number, isolate ID, and country code are also shown. Multiple sequence alignments were performed using the ClustalW algorithm followed by the neighbour-joining method with p-distance and 1000 bootstrap replicates using Mega X version 10.2.6.[146] Branches are coloured according to the genogroups proposed by Blake et al.[27] and Nishizawa et al.,[29] and branch tips are coloured according to the world region where the virus was isolated

In Asia, IPNV has been reported in Korea, China, Japan, and more recently Iran. In Korea, the reference strain DRT isolated from rainbow trout was one the first IPNV to be sequenced.[96] It is classified as Genogroup 1 with the North American strains[29] (Figure 1). Genogroup 1 isolates have also been found in farmed salmonids affected by IPN in China[97-99] and Japan.[29] The Japanese and Chinese isolates from genogroup 1 showed great similarity and were clustered together with viruses from the USA and Mexico.[97] A recent study showing the phylogenetic evolution of IPNV in China revealed that while most Chinese isolates belong to Genogroup 1, Genogroup 5 viruses are also present in the country.[100] Isolates from both genogroups caused mortality in rainbow trout and other salmonids in China. On the other hand, several aquabirnaviruses isolated from marine shellfish and fish (no salmonids) in Japan were classified into the new Genogroup 7 by Nishizawa et al..[29] In Iran, one of the world's largest producers of rainbow trout, IPNV was confirmed for the first time in 2007. The virus was sequenced and showed close genetic identity to the Ab type strain (Genogroup 2).[101] However, since then, only isolates belonging to Genogroup 5 of the virus have been reported.[37,102-105]

In Oceania, one of the last regions that remained free of IPN, IPNV was detected in Australia and New Zealand and clustered in Genogroup 5.[36,106,107] An Australian isolate called VTAB constitutes part of an eighth genogroup; however, the phylogenetic analysis done for this review categorises VTAB in Genogroup 7, along with the Japanese aquabirnavirus isolates (Figure 1). In Africa, IPNV has been reported in South Africa[108] and Kenya. Phylogenetic analysis has only been done on the Kenya isolate, indicating that it belongs to Genogroup 5.[109]

Together, these reports show that IPNV strains from genogroups 1 and 5 (representing reference strains from North America and Europe, respectively), are the most prevalent, and most widely distributed worldwide (Figure 2). However, it is important to take into account that these strains occur in species that are widely cultivated and that represent areas where the number of analyses is greater, so there may be a bias.

6 | IPNV VIRULENCE

Infectious pancreatic necrosis virus infections cause a range of pathogenicity and virulence in salmonid species, with infections resulting in either sub-clinical or overt disease, and outbreak mortality ranging from insignificant to almost 100%. To define any major molecular determinants involved in pathogenicity and pinpoint the amino acid residues that most significantly affect virulence several authors have studied the molecular basis of virulence in IPNV by comparing the deduced amino acid sequences from isolates causing different mortality in field outbreaks and/or in experimental challenges. Table 3 summarises the work done by these authors. In Figure 3 we show a schematic representation with the molecular determinants in segment A involved in virulence found in IPNV. For more details, see the comprehensive review recently published by Dopazo[10] that thoroughly describes the processes followed to discover the viral determinants of virulence in IPNV.

Initially, Sano et al.[110] demonstrated that virulence of IPNV is associated with segment A and not with segment B through infection of rainbow trout with a reassortant virus between a virulent Buhl type isolate (Genogroup 1) and an avirulent aquabirnavirus isolated from eel (EVE). This was confirmed by Bruslind and Reno[111] who discovered a variation in the virulence between isolates of the same serotype (A1, Genogroup 1) in brook trout. They suggested VP2 is the major determinant of virulence, with residues 217 and 286 distinguishing the least and most virulent isolates, respectively. Subsequent studies have continued identifying amino acid residues within the VP2 coding region as possible molecular markers for virulence in IPNV; including residue 217 and others, e.g., 221, 247, and 500.[66,67,69,112] Using reverse genetics, Song et al.[113] described the Threonine and Alanine in positions 217 and 221 as the main determinants of virulence in IPNV. Therefore, isolates with the motif $T_{217}A_{221}$ are considered highly virulent, while isolates with $P_{217}T_{221}$ are considered avirulent. More recently, a phylogenetic analysis comparing sequences from Norwegian isolates from Atlantic salmon, identified specific genetic fingerprints in isolates associated with clinical and persistent infections. The analysis included amino acid positions in VP2 previously described, with the motif $V_{64}A_{137}P_{217}T_{221}A_{247}N_{252}S_{281}D_{282}E_{319}$ found in isolates associated with subclinical infections and $I_{64}T_{137}T_{217}A_{221}T_{247}V_{252}T_{281}N_{282}A_{319}$ in isolates from fish with clinical disease.[70]

It is important to emphasise that these virulence markers have been studied almost exclusively in strain Sp (Genogroup 5) infecting Atlantic salmon (Table 3). Nevertheless, virulence determinants of the Sp strain do not always correlate with field mortality rates seen in IPN outbreaks in salmon.[30,34,50,66,69,74,93,114] For example, Bain et al.[30] found that some Scottish isolates from Genogorup 5 with the low virulence motif ($P_{217}T_{221}$) where highly virulent, based on known field mortality. This means that other factors, in addition to virulence markers, can affect the outcome of an infection and the mortality seen in outbreaks of IPN. Interestingly, it has been shown that stress plays an important role in IPNV replication *in vivo* and can promote conditions, which will allow reversion from attenuated to virulent virus variants in Atlantic salmon.[115]

More recently, the virulence (e.g., mortality caused by infection) of different IPNV strains have been tested in experimental challenges with rainbow trout.[114,116] Results have shown that mortality in trout varied in relation to the genogroup of the virus infecting it, regardless of even the virulence motifs described above.[114] For instance, Finish isolates from Genogroups 5 and 2 caused moderate to high mortalities in rainbow trout, while a Genogroup 6 isolate caused only low mortalities.[116] However, a Norwegian Genogroup 5 isolate, characterised as highly virulent based on amino acid markers and associated with high mortality in Atlantic salmon (used as a positive control) caused the lowest mortality in the experiment.[116] Furthermore, the $P_{217}T_{221}$ motif in the Sp strain, associated with low or avirulence in Atlantic salmon, has been shown to be related to high mortality in rainbow trout.[105,117,118] Together, these results provide

further evidence that differences in IPNV virulence are host-related and dependent on virus strain.

7 | HOST SUSCEPTIBILITY AND RESISTANCE

For several decades significant effort has gone into selectively breeding fish resistant to IPNV. It has been demonstrated that there is genetic variation in the susceptibility to the disease in both Atlantic salmon and rainbow trout.[119,120] These efforts have resulted in the identification of a major quantitative trait locus (QTL) for IPNV resistance in Atlantic salmon in two separate populations, in Scotland[121] and Norway.[122] The QTL explains 80%–100% of genetic variation in fish susceptibility to IPNV at the first feeding fry (freshwater) and post-smolt (seawater) stages. There is marked differences in IPN mortality between homozygous resistant fish (QQ_{QTL}) and homozygous susceptible (qq_{QTL}). The implementation of this QTL in marker-assisted selection (MAS) of individuals carrying the resistance-related allele for the production of IPN-resistant eggs has led to the considerable reduction in outbreaks of IPNV in Norwegian salmon farms.[123] Fine mapping of the QTL region using NGS revealed an epithelial cadherin (*cdh1*) as the causative gene for IPN resistance in Atlantic salmon.[123]

Cdh1 is located in the cell membrane and functional analysis shows that it binds to IPNV virions *in vitro* and it co-locates with IPNV in liver cells of qq_{QTL} individuals, but not in the hepatocytes of QQ_{QTL} individuals. This suggests that Cdh1 is part of the cell machinery that IPNV uses for infection, acting as (co)-receptor for the virus; implying that in fish with the resistant genotype, IPNV is unable to attach and enter the host cell.[123] However, it has been confirmed that while susceptible fish show significant mortality, both resistant and susceptible Atlantic salmon fry can become infected with IPNV[124,125] indicating that resistance is not entirely due to the inability of IPNV to enter and replicate in the cells of the host. Instead, comparison of the gene expression profiles of resistant and susceptible fish challenged with IPNV indicates that better regulation of the immune response may contribute to the protection against the virus in resistant fish.[124,125]

It has recently been reported that there is significant genetic variation for resistance to IPNV in rainbow trout[120] and markers explain a low to moderate proportion of the genetic variance for the trait.[126] The lack of a QTL with a greater effect indicates that the application of MAS is not the most appropriate method to accelerate genetic progress of resistance to IPNV in rainbow trout. For this reason, it has been suggested that genomic selection could be the most appropriate approach, by incorporating high-resolution genomic information through the use of high density genetic markers.[127]

8 | GENE EXPRESSION ANALYSIS OF HOST RESPONSE

The host response to IPNV infection has been widely studied *in vitro*, in salmonid-derived cell lines, and *in vivo*, mostly in Atlantic salmon. The innate immune response has special relevance because

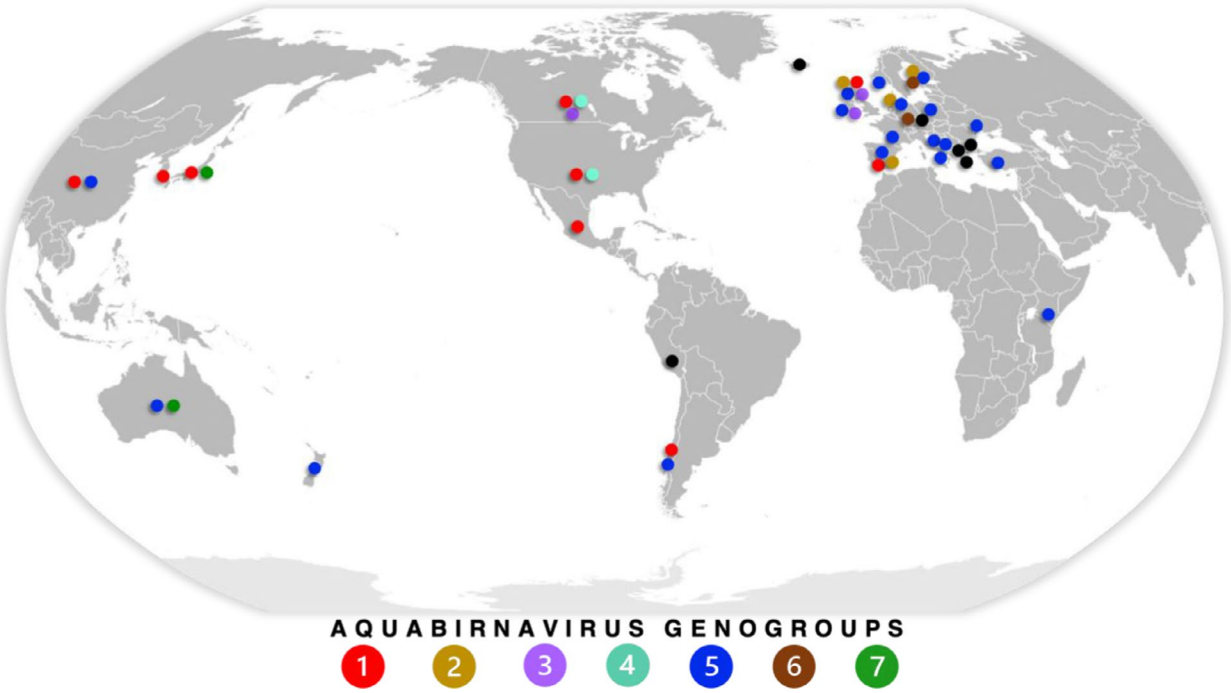

FIGURE 2 Global distribution of aquabirnavirus genogroups. Genogroups are determined based on segment A of IPNV according to the classification proposed by Blake et al.[27] and supplemented by Nishizawa et al.[29] Black dots represent countries where IPNV has been detected, but isolates have not been sequenced

TABLE 3 List of amino acids residues involved in virulence determined for different IPNV strains in salmonids

Fish species	IPNV Strain and genogroup	Segment and amino acids position	Reference
O. mykiss	Buhl (Genogorup 1)	segment A	110
S. fontinalis	Buhl (Genogorup 1)	segment A: 217, 286	111
S. salar	Sp (Genogroup 5)	segment A: 217, 221, 247, 500	67
S. salar	Sp (Genogroup 5)	segment A: 199, 217, 221, 247, 500	66
S. salar	Sp (Genogroup 5)	segment A: 217,221	113
S. salar	Sp (Genogroup 5)	segment A: 217, 221, 247	112
S. salar	Sp (Genogroup 5)	segment A: 217, 221, 247, 252, 314, 500	69
S. salar	Sp (Genogroup 5)	segment A: 64, 137, 217, 221, 247, 252, 281,282,319	70

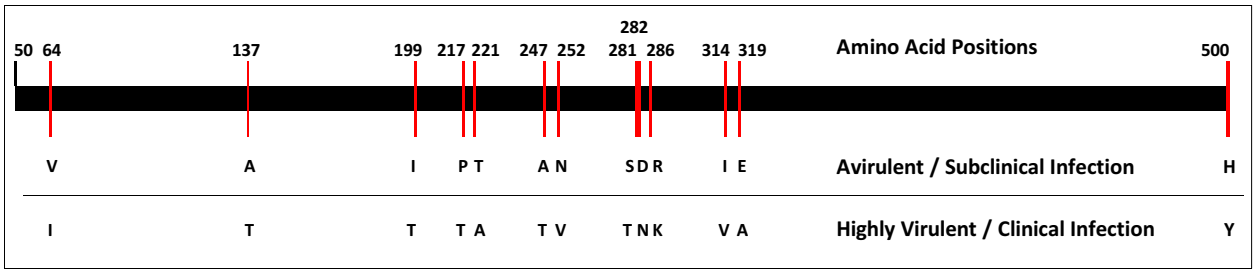

FIGURE 3 Schematic representation of main amino acid positions identified as determinants of virulence/clinical infection or avirulence/subclinical infection in segment A (VP2 region) of IPNV. V: Valine, I: Isoleucine, A: Alanine, T: Threonine, P: Proline, N: Asparagine, K: Lysine, S: Serine, D: Ac. Aspartic, R: Arginine, E: Ac. Glutamic, H: Histidine, Y: Tyrosine

it functions as first line of host defence and prevents the pathogen from spreading in the early hours after exposure. One of the key mediators of quick initiation of the innate immune response is the activation of cytokines, which are secreted proteins that function as communication intermediaries between different cell-types involved in the immune response. Information regarding the immune response in salmonids is still limited. Some reports have shown the modulation of cytokines in IPNV-infected fish. In brown trout (*Salmo trutta*), upregulation of the pro-inflammatory cytokines *il-1β* and *il-8* was found in head, kidney and spleen early in infection; 1–2 days post-intraperitoneal (ip) IPNV infection.[128] The same trend was observed for *il-1β* and *il-8* in non-salmonid fish species (Atlantic cod, *Gadus morhua*).[129] *il-1β* transcript was also observed in Atlantic salmon head kidneys.[130] The long-term effect of IPNV on the upregulation of *il-1β* (30–40 days post-infection [dpi]) and *il-8* (groups 17 dpi, and 30–40 dpi) showed both in trout head kidney. No differences compared with control were observed for *il-1β* and *il-8* in the 4–15 days post IPNV-infected groups. The inconsistent upregulation of pro-inflammatory cytokines after IPNV infection over time suggests the virus could induce some immune evasion mechanisms; interfering with an optimum host defence response.

Studies on the kinetics of IPNV infection using qRT-PCR revealed that interferon (IFN) type I and II and IFN-induced genes (e.g. *ifnα*, *ifnγ* and *mx*), are typically upregulated in salmonid fish infected with IPNV.[7,130-134] Type-I IFN (*ifnα*, *ifnβ*) is the first mediator in the activation of the antiviral response. Once IFN is recognised by its receptor, a signalling cascade is activated that acts upon transcription factors that bind to IFN-stimulated response elements located in the IFN-stimulated genes promoter (ISG)[135]; including several genes involved in the antiviral response such as *mx* and *pkr*.[136] The upregulation of type-I IFN (*ifnα*) in response to IPNV infection has been reported[112,132,133,137] and has been shown to be an effective suppressor of viral protein synthesis.[138] The high susceptibility of IPNV to Mx1 has been also determined.[139] However, in IPNV-ip infected trout, the activation of *ifnα* was only observed at 96 h post-infection in the spleen and head kidney but not in gills.[130] In addition, the protein kinase R (PKR), a Type I IFN stimulated gene, is suggested to be used by IPNV to promote virus replication during IPNV infection, given that PKR inhibition resulted in reduced virus titres and a decreased number of cells with compromised membranes in permissive cell lines.[140] These results suggest that IPNV could develop mechanisms to impede the protective effect mediated by the IFN response. It has been proposed that IPNV-encoded proteins may be involved in weakening IFN signalling.[138]

Regulation of inflammatory mechanisms is crucial for the regulation of the immune response to keep the tissue damage that is a consequence of the inflammatory process under control. While the expression of *il-10* is associated with an immune suppression effect on a variety of pro-inflammatory cytokines, the expression of *tfgβ1* blocks the activation of a repertoire of immune cell populations (lymphocytes, monocytes) and promotes local tissue repair after an inflammatory event. In salmonids, the expression of *il-10* was observed in rainbow trout head kidney, spleen and gills.[130] *il-10* mRNA levels were independent of the expression of both pro-inflammatory

(*il-1β*, *il-8*) and antiviral cytokines (*ifnα*). Importantly, the expression of *il-10* was also upregulated in head kidney in IPNV-naturally infected asymptomatic carrier fish[130]; suggesting an association between immune response suppression and viral infection.

When Atlantic salmon were infected with IPNV using immersion, the gene expression kinetics for *il-10* showed upregulation in all the time intervals evaluated in previous studies (4–15 dpi; 17 dpi; 30–40 dpi).[7] The expression of *tgfβ1* was identified only in Atlantic salmon head kidney with persistent IPNV infection.[7] A higher expression of *tgfβ1* at 5 dpi was also identified in IPNV-resistant Atlantic salmon families when compared with the susceptible phenotype.[124] Analyses of IPNV infected Atlantic salmon liver showed a correlation between tissue damage (lymphocytic infiltration, apoptotic hepatocytes) and the highest levels of IFN.[134] This data suggests that the expression of anti-inflammatory cytokines is important for control of the inflammatory outcome but also for the tissue damage provoked by such response. These results and others have established a relationship between the modulation of immune suppressive cytokines and the establishment of chronic viral infections[141-143]; suggesting that *tgfβ1* plays a role in IPNV-resistance and therefore, the development of IPNV persistence.

Studies have shown high variation in the expression levels of immune response-related genes at different time points of infection, based on the inoculation route (e.g., intraperiotenal injection or cohabitation),[132] tissues sampled,[134] the developmental stage of the fish (e.g., parr or smolt),[131] or if fish presented manifest disease or persistent subclinical infection.[7,130] Furthermore, several of these studies have also observed large individual variation among IPNV-challenged fish of the same age, receiving the same treatment, and sampled at the same time post-infection. This variation could be explained by differences in the individual levels of infection or genetic differences between fish; which would influence the expression of selected immune genes. Therefore, researchers have begun to look for new genomic approaches to better understand the salmonids response to IPNV infection.

9 | TRANSCRIPTOMIC RESPONSE TO IPNV

Over the past decade functional genomics studies have analysed the gene expression profiles of IPNV-challenged salmon using high-throughput technologies such as cDNA expression microarrays (chip) or mass transcriptome sequencing (RNAseq) (Table 4). Different factors related to the onset and development of disease (e.g. markers of virulence, resistance, genetic susceptibility) have been compared, with the aim of understanding the differences in the host response to IPNV infection at the transcriptomic level. For example, Skjesol et al.[112] analysed the response of Atlantic salmon to infection with IPNV isolates with high and low virulence using microarrays. They found that both mortality levels and expression of key genes of the immune response are positively associated with viral replication, which in turn is associated with the genetic characteristics of the virus. That is, fish infected with viruses containing the high virulence marker presented higher mortality and viral load, which was correlated to a greater magnitude in the expression of IFNs and ISGs.[112]

Studies analysing the differential gene expression response to IPNV infection between resistant and susceptible Atlantic salmon using cDNA microarrays have detected marked differences in different biological processes including the immune response.[124,125] Robledo et al.[125] analysed the transcriptomic response from resistant and susceptible salmon fry challenged with IPNV at day 1, 7, and 20 post-infection. Fish from susceptible families presented higher mortality and viral load and showed a considerably higher number of differentially expressed transcripts than resistant fish particularly at 7 and 21 dpi. Gene ontology analysis revealed that the susceptible salmon exhibited a characteristic transcriptomic pattern, with a marked overexpression of genes associated with the activation of cytokines, the inflammatory response and immune response at both times post-infection.[125] Additionally, in IPNV-susceptible fish, the expression of *ifnα* was upregulated at 7 and 20 dpi.[125] Accordingly, the same expression pattern was observed at 7 and 20 dpi for *ifnγ*. There was only a slight increase in *ifnγ* (but not *ifnα*) 24 hours post-infection.

Similarly, Reyes-López et al.[124] compared the transcriptomic response in the head kidney of Atlantic salmon fry resistant and susceptible to IPNV at days 1 and 5 post-infection by immersion. Functional annotation of differentially expressed genes revealed that IPNV-resistant families showed upregulation of genes related to endocrine function and ubiquitination, and pro-inflammatory genes, together with marked down-regulation of genes related to tissue differentiation, protein degradation, metabolism, and immune response. In fish from susceptible families an upregulation of inflammatory genes and IFNs (*il-10*, *il-12*, *ifnα*, *ifnγ*) was observed on the first day post-infection.[124] Unlike the results from Robledo et al.,[125] the highest *ifnα* expression peak was observed at 1 dpi for the susceptible families, which then dropped to basal levels.[124] In contrast, the expression for *ifnγ* in resistant families was upregulated at 1 and 5 dpi, although the expression at 1 dpi was lower than susceptible families.[124]

Taken together, these data suggest a moderated and controlled activation of the immune response in early infection is associated with resistance to IPNV, while a short, acute response, which is highly inflammatory and ineffective, is characteristic of susceptible fish.[124,125] In addition, it has been shown that miRNAs associated to regulation of the host anti-viral response are sensitive to viral load and disease progression, suggesting that these molecules may modulate immediate immune response and the subsequent inflammatory processes against IPNV in Atlantic salmon.[144]

More recently, Tarifeño-Saldivia et al.[145] evaluated the early response (24 h) to IPNV infection in head kidney of salmon post-smolt using RNAseq. The results showed a massive modulation of gene expression in relation to uninfected fish, with a marked upregulation of genes related to the antiviral immune response, biological pathways

TABLE 4 Transcriptomic studies describing the host response of Atlantic salmon infected with IPNV

Fish species and growth stage	Virus and challenge model	Tissue sample	Time post-challenge	Analysis method	Host response: main findings	References
S. salar Smolt	Genogroup 5, Immersion, 5×10^5 $TCID_{50}$/ml	Pancreas, Head kidney	6, 13, 29 dpc	Microarray	Immune response differ between infections with high and low virulence strains. Virulent strain induced the expression of IFNs and ISGs to a greater extent, which correlated with a higher viral load and greater mortalities.	112
S. salar Fry	Genogroup 5 and 6, Immersion, 1×10^5 $TCID_{50}$/ml	Head kidney	1, 5 dpc	Microarray	Resistant fish: moderate pro-inflammatory response accompanied by low expression levels of *ifnγ*. Susceptible Fish: Highly inflammatory but short early response.	124
S. salar Fry	Genogroup 5, Immersion, 5×10^5 $TCID_{50}$/ml	Whole fry	1, 7, 20 dpc	Microarray	Resistant fish: moderate immune response with macrophage-mediated modulation of inflammation. Susceptible Fish: Pronounced but ineffective immune response with marked overexpression of genes related to cytokine activity and inflammatory response	125
S. salar Parr	Chilean isolate Immersion, 10×10^5 PFU/ml	Head kidney, liver, RBC	24 hpc	RNAseq	IPNV infection induced a massive modulation of genes involved in iron metabolism, which was correlated with an increase in oxidative stress in the head-kidney as a result of iron overload.	145
S. salar Fry	Genogroup 5, Immersion, ~5×10^5 $TCID_{50}$/ml	Whole fry	1, 7, 20 dpc	RNAseq	Differences in the expression of miRNAs between resistant and susceptible fish were only observed at late infection (20 dpc). miRNAs are sensitive to viral load and disease progression and may regulate several major immune system pathways, acting as fine-tuners of the immediate immune response activation and the later inflammatory processes	144

of translation and transcription (which are probably hijacked by the virus), and with the metabolism of iron. IPNV infection increased oxidative stress in head kidney, suggesting that this is a consequence of the iron overload induced by the regulation of genes involved in the metabolism of this nutrient and pointing to a key role of nutritional immunity during infection with the virus in salmon.[145]

10 | CONCLUSIONS

Infectious pancreatic necrosis virus is by far one of the most persistent and recurrent viral pathogens in salmon and trout farms around the world. It is also one of the most studied viruses given its widespread distribution and the impact of its infection and the

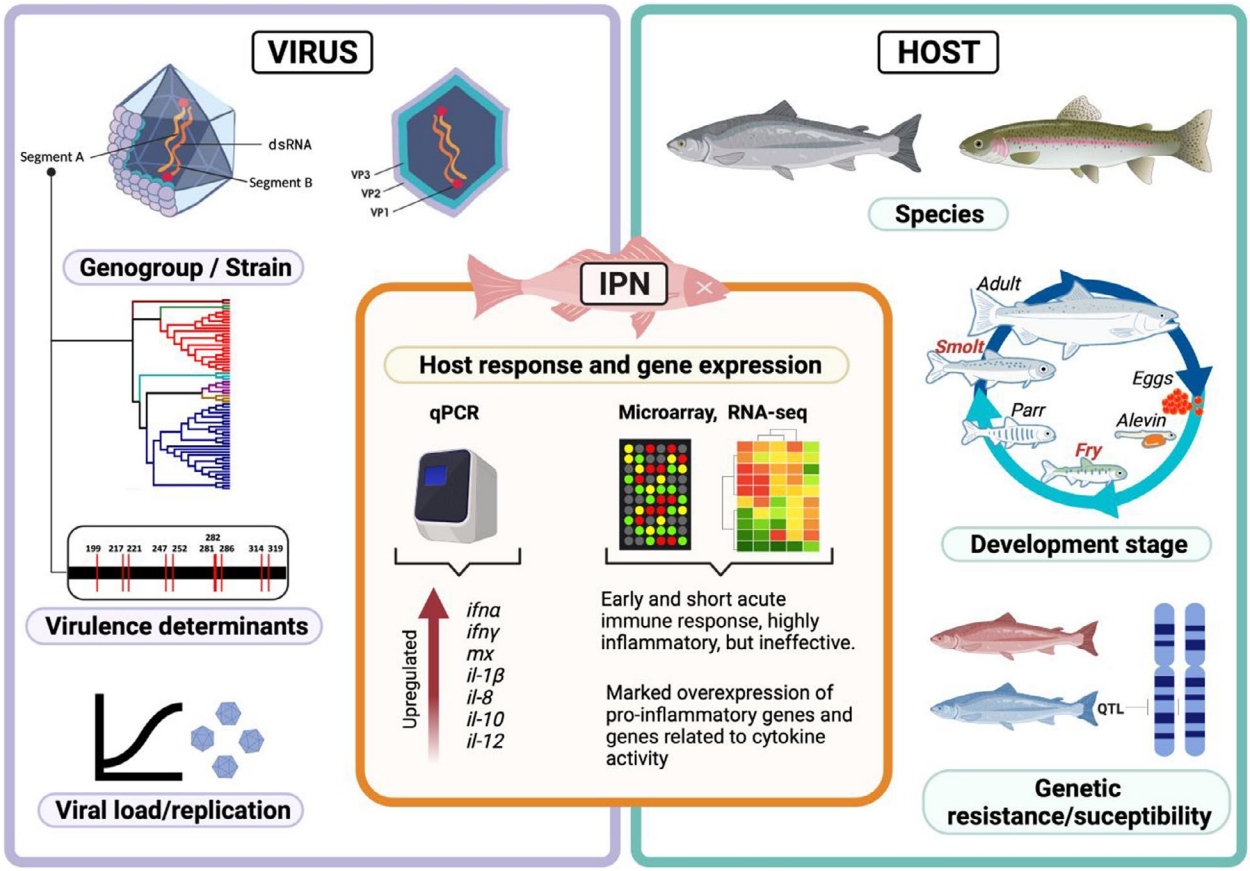

FIGURE 4 Main viral and host factors related to IPN disease outcome and host response during infection. The main viral factors affecting disease outcome are (i) the genogroup or strain; (ii) presence of virulence determinants; and (iii) replication and viral load, which in turn might depend on genogroup or strain, presence of virulence determinants and also characteristics related with the host. The main host factors affecting disease outcome are (i) the species affected; (ii) the developmental stage of fish; and (iii) the genetic susceptibility or resistance background of the infected fish. The host response to infection has been extensively studied by using gene expression approaches including qPCR, microarrays, and RNA-sequencing, demonstrating the activation of immune-related genes and pathways

disease it causes in farmed fish. Initial studies were focussed on the serological classification of IPNV. However, during the last few decades this strategy has been almost completely replaced by the molecular characterisation of IPNV isolates and other aquatic birnaviruses. The definition of genogroups based on the phylogenetic analysis of the VP2 protein of the virus has been widely adopted. Studies worldwide have shown that Genogroups 1 and 5, which represent North American and European reference strains, respectively, are the most prevalent in salmonid farms. Moreover, as long as salmon farming continues to expand, the distribution of these viruses will continue to expand as well, as demonstrated by the recent identification of IPNV in Iran and Peru. Thus, it is essential to maintain surveillance of the virus and to continue carrying out molecular epidemiological studies. The lower cost of sequencing technologies will allow for a greater number of sequenced isolates, making complete genomes more available, strengthening future phylogenetic analyses.

Although the host-pathogen interaction for IPNV infection is highly complex (Figure 4), it has been described that, at least in Atlantic salmon, there is a major locus controlling survival rates after infection in resistant and susceptible fish. Genetic variants present in the genomic region modulating IPNV resistance have been used to assist the rapid genetic improvement of farmed populations of Atlantic salmon, creating a great impact in controlling outbreaks and IPNV-related mortalities in the aquaculture industry, in the most important producer countries in the world. From a molecular epidemiology point of view, it will be very important to further monitor new viral variants and isolates to screen for more deadly strains. We expect that in the near future, research on IPNV and its interaction with different host species will provide novel insights into the immune response of fish to viral infections, and also on the epidemiological considerations for surveillance and different control measures for highly prevalent infectious diseases in aquaculture.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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