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Assessment of sodium channel mutations in Makah tribal members of the U.S. Pacific Northwest as a potential mechanism of resistance to paralytic shellfish poisoning

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ABSTRACT

The Makah Tribe of Neah Bay, Washington, has historically relied on the subsistence harvest of coastal seafood, including shellfish, which remains an important cultural and ceremonial resource. Tribal legend describes visitors from other tribes that died from eating shellfish collected on Makah lands. These deaths were believed to be caused by paralytic shellfish poisoning, a human illness caused by ingestion of shellfish contaminated with saxitoxins, which are produced by toxin-producing marine dinoflagellates on which the shellfish feed. These paralytic shellfish toxins include saxitoxin, a potent Na^+ channel antagonist that binds to the pore region of voltage gated Na^+ channels. Amino acid mutations in the Na^+ channel pore have been demonstrated to confer resistance to saxitoxin in softshell clam populations exposed to paralytic shellfish toxins present in their environment. Because of the notion of resistance to paralytic shellfish toxins, the study aimed to determine if a resistance strategy was possible in humans with historical exposure to toxins in shellfish. We collected, extracted and purified DNA from buccal swabs of 83 volunteer Makah tribal members and sequenced the skeletal muscle Na^+ channel (Nav1.4) at nine loci to characterize potential mutations in the relevant saxitoxin binding regions. No mutations of these specific regions were identified after comparison to a reference sequence. This study suggests that any resistance of Makah tribal members to saxitoxin, if present, is not a function of Nav1.4 modification, but may be due to mutations in neuronal or cardiac sodium channels, or some other mechanism unrelated to sodium channel function.

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1. Introduction

Human consumption of shellfish that feed on toxic algae can cause paralytic shellfish poisoning (PSP), a public health hazard that can cause severe economic losses globally due to bans on harvesting of contaminated shellfish. Paralytic shellfish toxin (PST)-producing dinoflagellates (e.g. *Alexandrium* spp.) cause toxic blooms ('red tides') in North America and worldwide. Records of PSP in the Pacific Northwest date back as early as June 15, 1793 (Vancouver, 1798), when a member of Captain George Vancouver's exploration team died from eating contaminated mussels in the uncharted coastline of what is now known as British Columbia. Additionally, 100 Russian hunters died from consuming toxic mussels in 1799 near Sitka, Alaska (Halstead, 1965).

The death of three people and illness of two others after their consumption of mussels and butter clams from the beach in Sekiu, WA in 1942 was the first evidence of high levels of PSTs in Washington State. Three members of the Ucluelet Tribe died after eating mussels containing PSTs on the west coast of Vancouver Island, British Columbia, Canada three days prior to the mortalities

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in Sekiu, suggesting that this event was probably widespread in the Pacific Northwest (Trainer et al., 2003). From 1942 to 1957, Washington State monitoring was sporadic, and temporarily stopped in 1946 due to blanket closures that were in effect at this time (Lilja, 1978), however these PSP outbreaks resulted in a mandatory monitoring program for PSTs that was implemented in Washington State in 1957. Annual shellfish harvest closures (except for razor clams) on the outer coast of Washington State continue in the present day and are enforced from Port Angeles on the Strait of Juan de Fuca, south to the mouth of the Columbia River (Fig. 1) for the period from April 1 to October 31 (Horner et al., 2011).

Paralytic shellfish toxins block conduction of the nerve impulse by interfering with the voltage-dependent increase in Na^+ ion conductance that generates the action potential (Hille, 1968; Narahashi and Moore, 1968), leading to neuromuscular paralysis and the human illness known as paralytic shellfish poisoning. Saxitoxin (STX) and tetrodotoxin (TTX) bind to a single site in the outer pore of the Na^+ channel, formed by the amino acid residues in the outer pore loops located between the S5 and S6 segments of each of the four homologous domains (I–IV) of the α-subunit (Fozzard and Hanck, 1996; Catterall, 2000; Zhang et al., 2013). The classic symptoms of PSP include vomiting, tingling of the lips, numbness of extremities, and breathing difficulties. Symptoms can occur within 30 min and death by respiratory failure can occur within 12 h (Halstead, 1978).

The level of PST bioaccumulation in shellfish varies widely among species (Bricelj and Shumway, 1998), which has been associated with differential sensitivity of nerves to STX (Twarog et al., 1972; Twarog, 1974). Twarog (1974) also theorized that recurrently exposed shellfish populations may undergo a genetic adaptation to PSTs with natural selection against individuals

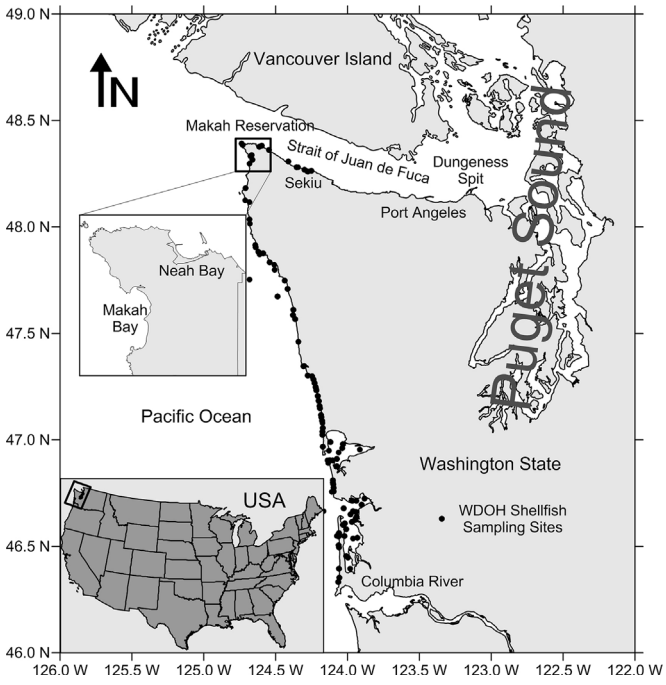

Fig. 1. Location of Makah Nation on the northwestern most portion of Washington State. The black dots indicate WDOH shellfish sampling sites from which PST data were obtained for use in this study (no data from locations east of Sekiu or Puget Sound are shown). The dots inside the box denoting the Makah Reservation refer to the "Makah Only" sites (data shown in Figs. 2, 3). PST test records spanned from October 1957 through October 2013. Species tested include: razor clam, California mussel, rock scallop, blue mussel, pacific oyster, butter clam, horse clam, Dungeness crab, littleneck clam, varnish clam, pink scallop, barnacle, cockle, manila clam, eastern softshell clam, hairy triton, weathervane scallop, and Olympia oyster.

sensitive to the toxins. Research on the soft-shelled clam (*Mya arenaria*) has shown an adaptation in clam populations from geographical regions with prior history of exposure to PSTs where the substitution of a single amino acid can confer resistance to STX (Bricelj et al., 2005, 2010; Connell et al., 2007; MacQuarrie and Bricelj, 2008). The specific substitution elucidated in *M. arenaria* was a simple change from adenine (A) to cytosine (C) in the domain II pore region of the alpha-subunit of the voltage-gated Na^+ channel (Bricelj et al., 2005). This modification caused a 1000 fold decrease in affinity of STX in Na^+ channels of resistant clams compared to sensitive clams with no prior history of PST exposure. This resistance mechanism is thought to allow clams to accumulate higher concentrations of PSTs (MacQuarrie and Bricelj, 2008).

Makah tribal folklore tells of potlatches (tribal parties) where visitors came over the mountains to eat seafood with locals. These stories suggested that the visitors became ill, while the Makah, who rely on shellfish in their diet, did not. One anthropological study reported that members of the Makah Tribe, located at the northwestern corner of Washington State (Fig. 1), believed that they were immune to shellfish poisoning due to their long history of exposure (Sepez, 2001). Shellfish are a major source of protein to the Makah Tribe and are the most widely consumed subsistence resource harvested in Neah Bay, where many Makah tribal members reside, and where almost 70% of households participate in harvest activities (Sepez, 2001). The Makah are aware of the risks of eating contaminated shellfish, but will often harvest from areas that they deem as safe, so incidents of PSP are rare.

The study investigated whether natural selection for resistant skeletal muscle Na^+ channels occurred in this community that has been chronically exposed to PSTs. It was hypothesized that natural selection for a Na^+ channel mutation could have occurred in ancestral Makah and passed down through generations to present day tribal members. Therefore, the expectations are to see prevalence of Na^+ channel mutations in the progeny of ancestral Makah. To this end, skeletal muscle Na^+ channel DNA was sequenced and questionnaires were distributed addressing ancestry and shellfish consumption in Makah tribal members.

2. Methods

2.1. PSTs in shellfish

Paralytic shellfish toxin test results were provided by the Washington State Department of Health (WDOH) to determine PSP risk in areas where members of the Makah tribal members typically harvest shellfish. Paralytic shellfish toxin data were acquired for the entire period of record (1957–2013) during which WDOH has been testing shellfish using the mouse bioassay. These data were divided into two groups to assess risk specific to the Makah Tribe. The first group included all of the WDOH sampling sites that were within the boundaries of the Makah reservation (Makah Only) and the second group included all of the sites on the WA State Pacific coast as well as sites in the Strait of Juan de Fuca (from Cape Flattery to Sekiu) where survey participants indicated they had harvested shellfish (WA coast excluding Makah, Fig. 1). The number of tests for PSTs, the number of positive tests (i.e. detectable levels of PSTs), percent positive tests, and the maximum PST concentration was determined for each group on a monthly, yearly, and species-specific basis to determine the PST exposure risk. To reflect a worst-case scenario when calculating average PST concentrations, a value of 30 μg STX eq./100 g shellfish meat was assigned to mouse bioassay tests (Association of Official Analytical Chemists, 1990) for which a "trace" concentration (i.e. toxic effects were observed but the concentration was not quantifiable) was