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Croatia and Slovenia have a maximum peak in multiple sclerosis prevalence at 7.54°C

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ABSTRACT

Introduction: The high rate of multiple sclerosis (MS) in the Gorski Kotar Region of Croatia is currently unexplained. This is the only region in Croatia with an average yearly temperature below 9°C. Colder temperatures corresponds to the immune system regulator TRPM8.

Aim: To determine if average yearly temperature is associated with MS prevalence rates in Croatia and what average yearly temperature is the most significantly associated when variation is considered.

Materials and methods: Weather data from the Croatian Meteorological and Hydrological Service was compared to MS prevalence rates from 28 locations in Croatia. Multivariate analysis was used to determine the significance of a correlation between average yearly temperature and MS prevalence. When this was determined the same method was repeated with variation towards a particular temperature.

Results and discussion: The results were significant for all areas combined ($P < 0.013$) and were highly significant in non-coastal areas ($P < 0.002$) but not significant in coastal areas ($P < 0.44$). 10.87°C was found to be the most significantly associated temperature ($P < 1.60 \times 10^{-4}$) and variation was found to be significantly associated ($P < 0.05$) between 7.9°C and 8.29°C. Swimming can have the same effects on the body as colder weather and this could explain the lack of association in coastal areas. MS prevalence rates also shows corresponding metrics with specific cancers which are known to be linked to the TRPM8 receptor.

Conclusions: MS prevalence in Croatia is significantly associated with average yearly temperature. MS also has the highest prevalence rates in areas which correspond to peak activity of the TRPM8 receptor.

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1. INTRODUCTION

Croatia has one of the widest variations in multiple sclerosis (MS) prevalence rates for a geographic area of it's size in the world.[1] Variations range from a low of 22 cases per 100 000 population in Rab County to 194/100 000 in Cabar County (Figure 1) – with these locations less than 100 km from each other.[1] The high of 194/100 000 – part of an overall average of 124/100 000 in the Gorski Kotar region[1] – has been a topic of considerable scientific interest and investigation. Environmental factors have been accepted as being a contributing factor to the development of MS and the one defining environmental factor in the Gorski Kotar region is the fact that the region is the only one in Croatia with an average temperature below 9°C (Figure 1). The TRPM8 receptor has been shown to peak in activity between 8°C–10°C[2] and is a mediator of IL-6 levels in the body.[3] The average yearly temperature of the Gorski Kotar region is roughly 7.4°C in Cabar County and 8.4°C in Delnice.[4]

The TRPM8 receptor is mediated by voltage and temperature: each can independently trigger the activity of the TRPM8 receptor. As temperature falls from 8°C to 20°C the half maximum voltage – half the voltage necessary to trigger a response in the receptor – and the number of gating charges – the number of charges needed to activate the receptor – both fall by about half.[5] This change reflects a temperature mediated change in the Gibbs free energy (the minimum activation energy) of the TRPM8 receptor: as the temperature falls the energy required to activate the receptor falls as well (Figure 2). Mutations have been documented in this channel that have been shown to alter the functioning of the temperature dependent response and the voltage dependent response.[6] In order to determine if average yearly temperature is responsible for the wide range in MS rates within Croatia average yearly temperature was compared to MS prevalence rates as compiled in Materljan and Sepcic (2002)[1] as well as previous research done in the Rijeka area[7] and additional research done in the Sibenik-Knin and Zadarska county regions.[8] This was compared to the documented temperature dependent voltage activity[5] to determine if MS prevalence rates in Croatia match documented normal TRPM8 receptor activity.

2. AIM

To determine if average yearly temperature is associated with MS prevalence rates in Croatia, what average yearly temperature is the most strong associated when variation is considered, what variation towards a particular temperature has the greatest impact on MS prevalence rates, and to examine more specific effects within those trends if relevant.

3. MATERIAL AND METHODS

Areas which had weather data available from the Croatian Meteorological and Hydrological Service[4] were compiled and compared against the available MS prevalence rates for Croatia. Prevalence that did not have available corresponding weather locations were compared with a reasonable close weather station (Table 1).[9] Lika county's average yearly temperature was averaged between Senj, Otacac and Gospic,[4] which between these three locations represent the majority of the population of that country. Sibenik-Knin county's average yearly temperature was averaged between Sibenik and Knin,[4] which constitute the majority of the population of that county. Ivanec did not have a weather station within a very close distance (20 km) and thus it's prevalence rate was not included in analysis. Vrbovsko had a more recent prevalence estimated of 66.3/100 000[7] and thus this prevalence was used instead of the earlier study by Materljan. Average yearly temperature was compared to the prevalence rate for that location and regression analysis was

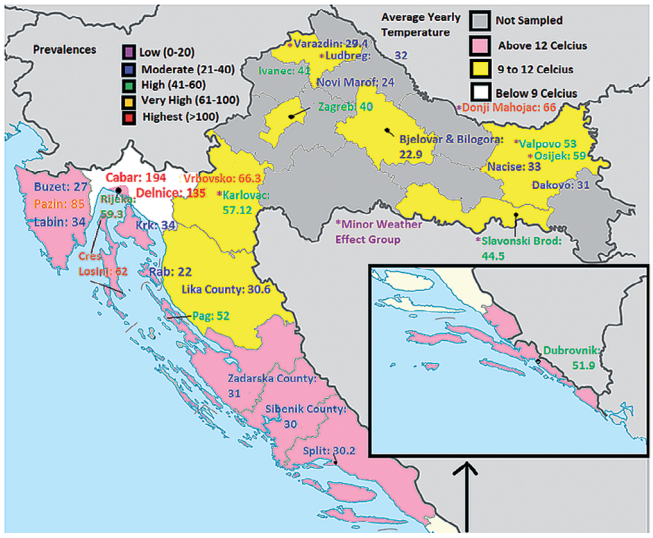

Figure 1. Map of Croatia, with average yearly temperature of each county colored, MS prevalence for each tested location, and the locations of a minor weather effect examined in the analysis. Note that Ivanec has an MS prevalence rate but that no corresponding weather station was available, thus it was not included in the analysis.

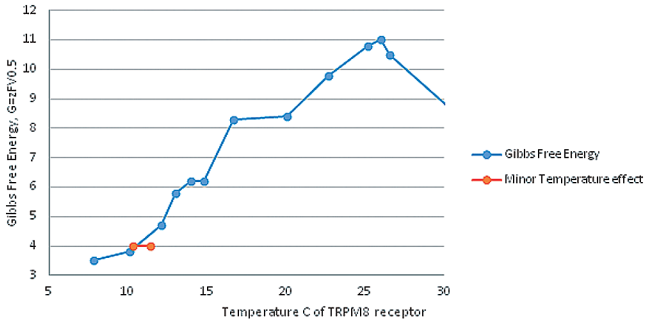

Figure 2. Gibbs free energy for TRPM8 receptor as a function of temperature. Gibbs free energy required to activate the TRPM8 receptor, as defined by Raddatz et al. (2014). As the temperature falls from 25.0°C to 7.8°C the energy required for this function declines. In the study it was found that a minor effect of temperature crossed this threshold.

Table 1. Locations of study, their MS prevalence, average yearly temperature as defined by the Croatian Meteorological and Hydrological Service (or denoted alternative), and alternative Weather Stations as necessary. All temperatures are sourced from the Croatian Meteorological and Hydrological Service. Vrbovsko is sourced from a location other than its location of prevalence rate. Years of meteorological data are listed in the far right column.

Location of study	MS prevalence per 100000	Average temperature, °C	Alternative weather station, distance from prevalence site[9]	Data range
Bjelovar and Bilogora	22.9[1]	10.76	–	1949–2015
Zadarska County	31[8]	15.13	Zadar, 0 km	1961–2015
Sibenik County	30[8]	14.32	Sibenik and Knin (averaged), 0 km	1949–2015 (Sibenik) 1949–2015 (Knin)
Donji Miholjac	66[1]	11.16	–	1955–2015
Valpovo	53[1]	10.92	–	1963–2015
Osijek	59[1]	11.04	–	1899–2015
Nasice	33[1]	11.29	–	1982–2015
Dakovo	31[1]	11.00	–	1981–1988, 1990, 1995–2015
Lika County	30.6[1]	11.11	Gospic, Senj and Otacac (averaged), 0 km	1872–2015 (Gospic) 1949–2015 (Senj) 1995–2015 (Otocac)
Dubrovnik	52[1]	16.61	–	1961–2015
Rijeka (Rijeku)	59.6[1]	14.11	–	1948–2015
Split Marjan	30.2[1]	16.29	–	1948–2015
Zagreb Gric	40[1]	11.58	–	1861–2015
Karlovac	57.12[7]	11.03		1949–2015
Slavonski Brod	44.5[1]	11.02		1963–2015
Cres Losini	62[1]	15.46	Mali Losinj, 0 km	1961–2015
Rab	34[1]	15.49		1978–2015
Krk	22[1]	14.08		1981–1984, 1995–2011, 2013–2015
Pag	52[1]	15.82	–	1978–2015
Buzet	27[1]	12.45	–	1981–2015
Pazin	85[1]	11.43	–	1961–2015
Labin	34[1]	13.38		1994–2001
Vrbovsko	66.3[7]	7.07	Stara Susica, 5 km	1960–1985
Delnice	135[1]	8.33	–	1981–1984, 1992–1993, 2008–2015
Cabar	194[1]	7.43	Parg, 1 km	1950–2015
Ludbreg	32[1]	10.54	–	1982–2015
Varazdin	29.4[1]	10.35	–	1949–2015
Novi Marof	24[1]	11.00		1981–2015

conducted on the resulting trendline in data analysis of Excel 2013 (Table 1). The results were considered significant if the *P* value was less than 0.050. In order to determine an average yearly temperature with the highest MS prevalence rate the average yearly temperatures was subtracted from temperatures between 7°C–17°C and the absolute value of the result was calculated in data analysis of Excel 2013 (Table 1) as multivariate analysis with MS prevalence rate and average temperature variation set as independent variables and the absolute difference calculated as the dependent variable. Variation was determined in this test by determining standard deviation of temperature throughout the year. The way a temperature with the highest significance was determined was to use the intercept of the absolute value of all locations to determine the point at which the significance level was the highest. The result in this test was determined to be significant if *P*value was less than 0.05.

An additional test was also done for variation. The way variation was calculated was that the variation from that temperature in a given month was subtracted from the temperature of highest MS prevalence that was determined first and an absolute value was taken for that difference. Each of these values was collected monthly and then added together from the entire year. Multivariate analysis was then conducted with the added difference as the dependent variable and the set difference from the determined temperature of high significance for each location and MS prevalence rates as independent variables. This was done under regression in

'Data analysis' of Excel 2013. Significance was determined if *P* was less than 0.05 and if that significance was

higher than that of the test for average yearly temperature with variation accounted for. Finally, a separation was made between coastal and non-coastal areas to determine if there was a difference in average yearly temperature association significance between those regions.

4. RESULTS

The results were significant ($P < 0.013$) for temperature and were also significant when variation was taken into account ($P < 0.009$). Of additional note was the division in association with average yearly temperature between non-coastal areas and coastal areas: Areas which were near a coastline ($n = 10$) followed the trend line less distinctively than those which were not ($n = 18$) (Figure 3). When these coastal areas were analyzed separately by regression analysis it was found that the results were not significant ($P < 0.44$). Regression analysis of the non-coastal areas, however, demonstrated a very significant correlation ($P < 0.002$) (Figure 3). The average yearly temperature with the highest significance of association with MS prevalence when variation was accounted for was determined to be 10.87°C, with a peak significance of 1.60×10^{-7} (Figure 4). Results for variation towards a particular temperature showed that 8.29°C was the point at which variation would reach significance ($P < 0.05$) (Figure 4). This was by a gradient curve, however, and was not more significant that average yearly temperature with variation accounted for.

5. DISCUSSION

The distinct line documented in Figure 3 crosses the threshold of TRPM8 activity as defined by Gibbs Free Energy.[5] The increases in MS prevalence, 29.4–66 cases per 100 000, are indicative of these small temperature increases from 10.3°C to 11.16°C crossing the 10.87°C temperature that was the most significant for MS prevalence when average yearly temperature and variation are accounted for. This could cause individuals with TRPM8 receptors that possess polymorphisms that alter temperature dependent functions[6] to be at risk of MS development – which is the same reason that areas with an average temperature around 8°C could be expected to have very high MS prevalence rates. At those temperatures (7°C–9°C) any deficiency or reduced temperature responsiveness could cause a heightened risk of MS prevalence. The minor effect noted for the distinct line is also due to minute changes in average variation that cross the threshold of the highest MS prevalence association at 10.87°C: When the seven data points that make up this line are considered the results are significant regarding variation from the given average yearly temperature ($P < 0.031$) further defining temperature variation as a reason for this feature in Figure 3.

Additionally, the significance of association above 10.87°C is one of exponential decrease (Figure 4) whereas that above 9.60°C is one of near exponential increase. The increase in significance above that demonstrated for 10.87°C occurs at approximately 7.90°C, suggesting that this is the lower limit of where variation towards a particular temperature would increase above that of average yearly temperature with variation accounted for regarding significance. The upper limit is the 8.29°C defined in the variation towards a particular temperature test, although this did not exceed the significance of the first test for average yearly temperature with variation accounted for. The difficulty in achieving this result for that analysis is due to the extraordinarily high level of significance for the first test, which largely makes the second analysis somewhat superfluous. This result, it should be recognized, is due to the very detailed analysis of the population of Croatia that was examined: some counties that were tested for MS prevalence had populations of less than 100 000 people, making the results very detailed and amplifying the significance of the first analysis.

With this minor effects noted the fact remains that the overall trend is still one of increasing MS prevalence with increasing demands on the TRPM8 receptor (that is, decreasing temperature up to 8°C). If the body was physiologically suited for the TRPM8 receptor to be active and it was reduced in function or not functioning at all, this could account for

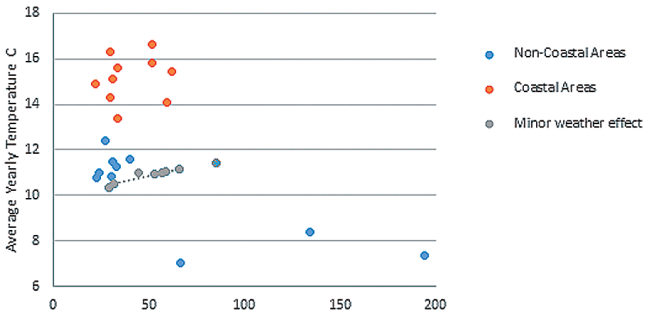

Figure 3. MS prevalence rates vs. average yearly temperature (°C). The minor weather effect from variation across the peak MS prevalence temperature and the Gibbs free energy boundary is marked with a trendline. All data points on this line of the minor weather effect are non-coastal areas. Coastal areas are non-signficant ($P < 0.44$) whereas non-coastal areas are significant ($P < 0.002$). The overall trend was significant ($P < 0.013$).

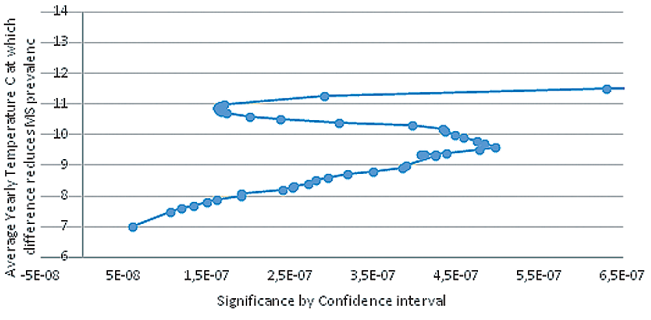

Figure 4. Significance of the intercept for the collective difference from an average yearly temperature.

all these effects. The TRPM8 receptor also ceases to be reactive below 8°C, and this explains the sudden drop in MS prevalence (66.3/100 000) at Vrbovsko, which has an average yearly temperature of 7.07°C but of which it's variation in temperature from month to month would cross that threshold. The apparent lack of correlation between MS prevalence and temperature in coastal areas may be due to an amorphous confound distinctive to areas on a coastline: water immersion from swimming may cause the same effects on MS development by producing the same physiological stresses as atmospheric cooling, and thus may cause notable increases in MS prevalence which are hard to define by weather patterns because not everyone will engage in this activity and be exposed to the physiological risks it would present.[10]

In addition to this possibility, some correlations between cancer and MS in Croatia have been made.[11,12] TRPM8 has been shown to be abnormally expressed in a number of different tumors.[12,13] It has been directly associated with pancreatic cancer, which was the strongest association noted with MS prevalence and incidence in Croatia (non-coastal areas: $P < 0.0003$, coastal areas: $P < 0.009$).[13] In addition TRPM8 has been shown to be strongly expressed in lung epithelial cells[6] and associated with lung cancer in at least one study[14] which could explain the otherwise difficult to explain association between lung cancer and MS prevalence (non-coastal: NS, coastal: $P< 0.03$). By comparison stomach cancer does not correlate with MS prevalence[12] even though colorectal cancer was associated with MS prevalence in both coastal and non-coastal areas (non-coastal areas $P < 0.0007$, coastal $P < 0.04$). Colon cancer was also associated with MS prevalence ($P < 0.045$) in non-coastal areas. This makes the connection between abnormal TRPM8 expression and these particular cancers strong and shows that the same receptor may be responsible for cancers that have been correlated with MS.

6. CONCLUSIONS

Average yearly temperature is associated with MS prevalence in Croatia. It is significantly associated with MS prevalence in non-coastal areas and not in coastal areas. The average yearly temperature with the highest association with MS prevalence when variation is accounted for is 10.87°C, and the variation towards which MS prevalence demonstrates the greatest difference is between 7.90°C and 8.29°C, which is close to where the TRPM8 has been demonstrated to cease being reactive.[5] An increase in MS prevalence in some locations with increasing temperature from 10.32°C to 11.16°C is due to crossing the threshold of TRPM8 receptor activity and crossing the temperature of 10.87°C that demonstrates peak MS prevalence when variation is considered.

Conflict of interest

Author declare to have no conflict of interest.

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