Int J Mol Epidemiol Genet 2021;12(5):102-111
www.ijmeg.org /ISSN:1948-1756/IJMEG0134118

Original Article

Land use as an effective factor on the occurrence of chromosomal diseases in Brazil

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Received April 10, 2021; Accepted July 9, 2021; Epub October 15, 2021; Published October 30, 2021

Abstract: Background: The occurrence of chromosomal diseases is a worldwide health problem. The use of agrochemicals, urbanization processes, and solar radiation can be predictive factors of the elevated risk of congenital malformations. In this sense, predicting the geographical potential of the distribution of chromosomal diseases has high relevance for public health. Objectives: This study aimed to describe chromosomal prevalence in Brazil regions, from 2005 to 2015, to model a potential distribution of chromosomal disease occurrence probability associated with land use. Methods: We used chromosomal prevalence to model a potential distribution of chromosomal diseases using machine learning algorithms. As the predictors of the models, we used the variables *global forest canopy height*, *distance from the built-up area*, and *solar radiation*. We characterized the predictive areas as potential occurrence of chromosomal diseases by land use and occupation. Results: Georeferenced data of 43,672 karyotypes detected 7,237 cases of chromosomal diseases and used 5,362 to build the models. The models generated were accurate (TSS>0.5). Discussion: The areas with greater occurrence of chromosomal diseases present a significant association with pasture areas, crops and agroforestry systems, and urbanized areas. This research is the first Brazilian study with this approach that seems promising in predicting the potential distribution of chromosomal diseases. Therefore, it can be an excellent management tool in public health.

Keywords: Chromosomopathies distribution, ENM, landscape, SDM

Introduction

A chromosomal disorder is any syndrome caused by an odd chromosome number or constitution, characterized by malformations or dysfunctions in any body system. Several factors can promote chromosome disorders, including hereditary, advanced maternal age (especially numerical chromosome disorders), exposure to teratogens such as alcohol, drugs, medications, viral infections, and even environmental ones, as the exposure to physical and chemical agents, industrial residues, and pesticides [1, 2].

The chromosomal alterations may be associated with uncontrolled exposure of non-target organisms to xenobiotic compounds, mainly agrochemicals [3] and products related to industrialization and urbanization processes [4]. With the search for high agricultural productivity and transgenic products in 2009, Brazil was the world's largest agrochemical consumer, using around one million tons of these substances. It is estimated that each inhabitant consumed the equivalent of 5.2 kg per year [5], corresponding to a broad risk for developing such anomalies.

Through the superficial percolation of agricultural areas, agrochemicals discharge organic or inorganic substances, natural or synthetic, into water systems and soil. These substances can pose risks to human health and the environment due to their genotoxic effects, interactions with the nitrogenous bases present in the nucleotides, promoting phosphorylation and alkylation, and increase reactive oxygen species to a toxic level for cells, inducing chromosomal, DNA, and epigenetic alterations [6].

Excessive radiation exposure associated with xenobiotics can damage the DNA, and such facts have already been verified amongst farmers [7].

It is worth emphasizing the importance of knowing the mechanisms involved in chromosomal diseases, especially in countries with growing agrochemical consumption, such as Brazil. There is also an epidemiological interest in identifying the geographic regions with the most significant potential for chromosomal changes. As a step to solve this fundamental and growing health issue, it is necessary to carry out an investigation at the spatial level to direct the efforts of public health policies and scientific research. Thus, the present study proposed to create a potential risk map for chromosomal disturbances in Brazil through machine learning algorithms associated with landscape and solar radiation predictors.

Methodology

Data collect

Our research involved a retrospective documentary study of the results of samples from patients with indications of diagnostic exams for chromosomal changes in Álvaro Laboratory (DASA), which is a national reference in the segment of laboratory tests in Brazil.

In the karyotype analysis, peripheral blood samples from patients were collected and sent to the "operational technical nucleus" to be processed, obtaining the images of the metaphases [8]. The images of the metaphases were scanned and analyzed by the Case Data Manager v.7.1 (App. Biosystems) and Ikaros Karyotyping System v. 2015 (Metasystem) programs. The diagnostic data of the karyotypes were collected in the database of this referred laboratory, establishing as inclusion criterion the period corresponding to 2005-2015. The variables related to the genetic-clinical evaluation procedure were inventoried, including location and date of biological material collection, karyotype (normal or altered), and the critical analysis of chromosomal diseases found.

Characterization of chromosomal diseases

We evaluated chromosomal diseases for their absolute and relative frequencies, classified by Brazilian region, with the primary purpose of evaluating the prevalence in the studied period. The characterization was performed by types of alteration from these frequencies, classifying them as autosomal, sexual, numerical, or structural.

Spatial modeling

We base the models on machine learning concepts used to model the niche and distribution of species (ENM/SDM, [9]). After searching for records of chromosomal diseases, we found 7,237 georeferenced cases. Only one case was considered for each cell (pixel) of the grid (raster) that contained environmental information, excluding duplicated coordinates. After this process, 5,362 records regarding chromosomal diseases remained.

We used three variables to infer the large-scale effect on the geographic delimitation of chromosomal diseases: *Distance from Built-up Area*, *Global Forest Canopy Height,* and *Solar Radiation*. These data are grids (raster) in GeoTiff format, with a geographic coordinate system ("lat/long"), Datum WGS-84, and spatial resolution of 5 arcminutes (~86 km^2). The *Distance from the Built-up area* dataset represented the distance between a chromosome disease case and the nearest building area (unit: km). The *Global Forest Canopy Height* dataset represents global tree heights (unit: m) based on a fusion of spaceborne-lidar data (2005) from the Geoscience Laser Altimeter System (GLAS) and ancillary geospatial data [10]. Solar radiation is radiant energy emitted by the sun from a nuclear fusion reaction that creates electromagnetic energy (unit: $kJ.m^{-2}.day^{-1}$).

The models were built based on machine learning algorithms belonging to the three classes of methods: 1) presence only method-Bioclim-climatic envelope [11]; 2) presence/background method-Maximum Entropy-MaxEnt [12] and Support Vector Machines (SVM) [13]; and 3) presence and absence method-RandomForest [14]. The combined use of these algorithms can improve results by considering different tolerances in the potential distribution [15].

To evaluate the constructed models, the case records were randomized in two subsets for training (70%) and testing (30%) the models.

Table 1. Characterization of chromosomal alterations (n=7244)

Variables	Categories	FR (n)
Numeric and Structural	Numeric	77% (5,558)
	Structural	16% (1,163)
	Mixed	7% (516)
Autosome (79.2%; n=5,736)	Numeric autosome	78% (4,482)
	Structural autosome	17% (989)
	Both	5% (265)
Sexual (20.3%; n=1,466)	Sexual Numeric	73% (1,071)
	Sexual Structural	11% (159)
	Both	17% (236)
autosomes and Sexual		0.5% (35)
Mosaicism	Mosaicism	9% (663)
	Single lineage	91% (6,574)

The partition of the records was randomized ten times using the bootstrap technique (random partition) to decrease the data's correlation [9], totalizing forty different models (10 replicates × 4 algorithms).

We used the ensemble technique with "maximum specificity and sensitivity" as a threshold [15, 16]. The threshold is an adequacy value that defines the presence or absence of a phenomenon. Thus, the value of each cell in the final map ranged from 0 to 40, demonstrating the frequency of presence prediction among models. We divided these values by the total number of models built to obtain the relative frequency of presence per pixel, varying between 0 and 1.

Statistical analysis

After the descriptive statistics about the characterization of chromosomal diseases, we evaluated association at types of chromosomal alteration and Brazilian regions. These analyses were performed through Pearson Chi-square, using the Monte Carlo Permutational method when was necessary, and the post-hoc Adjusted Residuals test. We used the XLStat program version 2016 (<https://www.xlstat.com/en/>) for the analysis with a significance level of 0.05.

For the evaluation of the models, we used two metrics: AUC and TSS. The area under the curve (AUC) measures performance for classification problems in various threshold configurations. This metric tells how well a model can distinguish between hits and misses in prediction. The higher the AUC, the better the model will predict 0 s as 0 s and 1 s as 1 s. By analogy, the higher the AUC, the better the model distinguishes the areas suitable for chromosomes diseases from those not suitable [9].

True Skill Statistic (TSS) were estimated to assess each of the models. TSS values range from -1 to 1 so that negative values or close to 0 indicate that the models are not statistically different from randomly generated models, and values close to 1 indicate excellent models. However, it is assumed that models with values above 0.5 are considered adequate to infer the distribution of species [17].

After defining the sites with the highest potential for chromosomal diseases, we made landscape characterization (%) using Mapbiomas (2015) database (<https://mapbiomas.org/), evidencing Forest, Non-Forest Natural Formation, Agriculture and Urban areas in localities with the occurrence and no occurrence of chromosomal diseases. The R <https://www.R-project.org/> and QGis 3.4 <http://qgis.org> software created these spatial layers.

Results

43,672 karyotypes were evaluated, 83% of them were normal (n=36,435), being 52% of females (n=18,946) and 48% of males (n=17,489). Chromosomal abnormalities were found in 17% (n=7,237) of the karyotypes, being 52% of females (n=3,763) and 48% of males (n=3,474), following the same proportions of normal karyotypes.

Numerical alterations corresponded to 77% (n=5,558), structural 16% (n=1,163) and both 7% (n=516). Autosomal abnormalities were found in 79.2% (n=5.736), sex chromosomes, 20.3% (n=1,466) and 0.5% (n=35) with both autosomal and sexual disorders. The presence of mosaicism was detected in 9% (n=663) among the alterations. The autosomal and sex chromosomal abnormalities were subdivided into numerical and/or structural (**Table 1**).

Characterization by Brazilian region

As for chromosomal diseases in the Brazilian regions, it was possible to observe that the highest frequencies occurred in the North and

Table 2. Absolute frequencies of types of alteration and comparison among Brazilian regions

Variables	Categories	Southeast	South	Northeast	Central-Western	North	*p*-value
Alteration	Normal	12,278[a]	1,0366[a,b]	7,817[b]	3,252[a,b]	2,722[b]	<0.0001
	Changed	2,018[b]	2,020[a,b]	1,819[a]	616[a,b]	769[a]	
Autosomic	Numeric	1,246	1,226	1,155	358	497	
	Structural	309[a]	277[a,b]	228[a,b]	90[a,b]	85[b]	0.003
	Both	68[a,b]	57[b]	72[a,b]	26[a,b]	42[a]	
Sexual	Numeric	297	329	246	93	101	
	Structural	37	53	40	17	12	0.262
	Both	48	70	68	26	24	
Autosomic and Sexual		11	6	6	5	7	0.147**
Mosaicism	Mosaic	158[b]	201[a]	175[a]	68[a]	66[a,b]	0.059
	Unic	1,860[a]	1,819[b]	1,644[b]	548[b]	703[a,b]	

P-value of Pearson Chi-Squared test, with Monte Carlo method, **K proportions. Different letters (a, b) indicate statistically significant differences between variable categories.

Table 3. Threshold, AUC and True Skill Statistic (TSS) for all algorithms

Algorithm	Threshold	AUC	TSS
Bioclim	0.140±0.007	0.843±0.006	0.582±0.017
Maxent	0.502±0.030	0.932±0.005	0.793±0.008
Randomforest	0.473±0.038	0.929±0.004	0.790±0.010
SVM	0.760±0.0120	0.927±0.005	0.778±0.010

Northeast regions (P<0.0001; **Table 2**). However, as for the number of chromosome diseases by the number of inhabitants, we observe that the highest proportions came from the Southern, with 6 cases/100,000 inhabitants. The Central-West and North regions presented a prevalence of 4 cases/100,000 inhabitants, the Northeast region, 3 cases/100,000 inhabitants, and the Southeast region, 2 cases/100,000 inhabitants (P<0.0001).

Regarding the autosomal chromosomal diseases, we verified that the autosomal numerical alterations did not present statistical differences between the regions. The autosomal structures were more frequent in the Southeast region, and both changes occurred more frequently in the North region (P=0.003; **Table 2**). We do not observe statistical differences among the regions for the sexual chromosomal diseases and the joint autosomal and sexual alterations (P=0.262 and P=0.147, respectively; **Table 2**). For mosaicism, it was observed more frequently in the South, Northeast, and Central West regions, while the single lineage was significantly more frequent in the Southeast region (P=0.059; **Table 2**).

Spatial modeling

The models provided reliable predictions in all used algorithms (**Table 3**). It is observed that the different algorithms predict different thresholds for the prediction of localities with greater suitability for the occurrence of chromosomal diseases, but always with fair values of adjustments of the models (AUC>0.8 and TSS>0.5). Therefore, using the specific thresholds for each model, the ensemble technique was used to generate a consensual map of chromosomal pathology distribution (**Figure 1**), with models predicting the geographical range of chromosomal diseases in Brazil based on predictor variables.

According to the information on land use and occupation obtained from the MapBiomas database, we found a greater area of forest and natural non-forested formation in places where there is less prediction of chromosomal diseases (**Table 4**). It can be raised the doubt that places with these characteristics naturally have a lower demographic density, however even in places where there is a larger population group (as part of eastern Brazil, **Figure 1**), there is less prediction of the occurrence of chromosomal diseases. These results corroborate our hypothesis that forest and natural formations promote a protective effect against these dis-

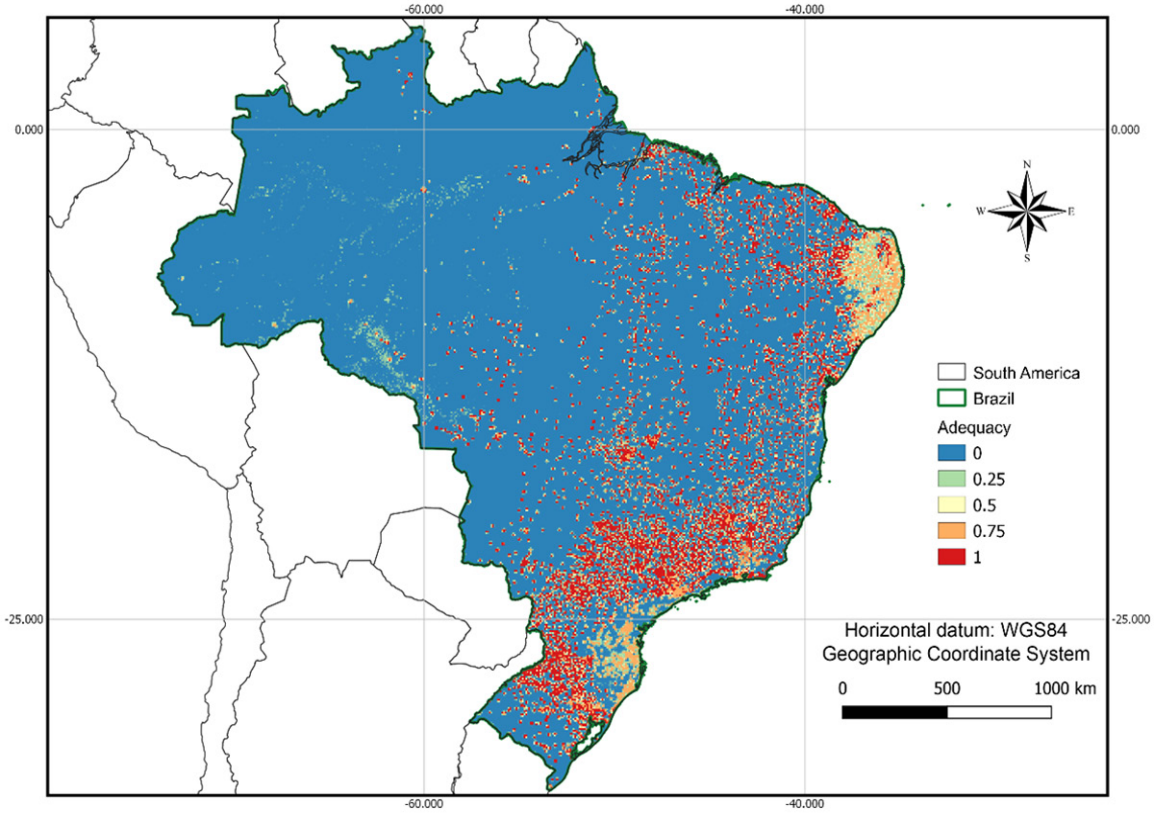

Figure 1. Environmental suitability map of the occurrence of chromosome diseases throughout the Brazilian territory.

Table 4. Landscape characterization (%), by Mapbiomas (2015), in localities with the occurrence and no occurrence of chromosomal diseases

Landscape characterization	Localities	
	Occurrence of chromosomal diseases	No occurrence of chromosomal diseases
Forest	35.7	68.0
Non-Forest Natural Formation	4.6	7.9
Agriculture	55.7	21.9
Urban area	1.8	0.1

turbances. Differences in the predictor variables were evident when compared between pixels with environmental suitability for the occurrence, or not, of chromosomal diseases.

The variable *Distance from Built-up areas* was used in the present study to reflect the distance of each case of chromosomal disease and urbanized, industrialized, and highly waterproofed soils. In places where disease occurrences were not predicted, the distance values were 6.28 times higher than the places where they are suitable for the occurrence of chromosomal diseases ($\bar{\chi}$ ± 95% *ci*; no occurrence: 105.2±0.9 km *vs.* occurrence: 16.8±0.6 km) (**Figure 2A**).

We used the *Global Forest Canopy Height* variable to reflect the degree of environmental preservation of the locations under study. In places where chromosomal diseases were not predicted, the canopy heights were 2.23 times higher than in places where chromosomal diseases were predicted (no occurrence: 19.6±0.10 km *vs.* occurrence: 8.8±0.10 m) (**Figure 2B**).

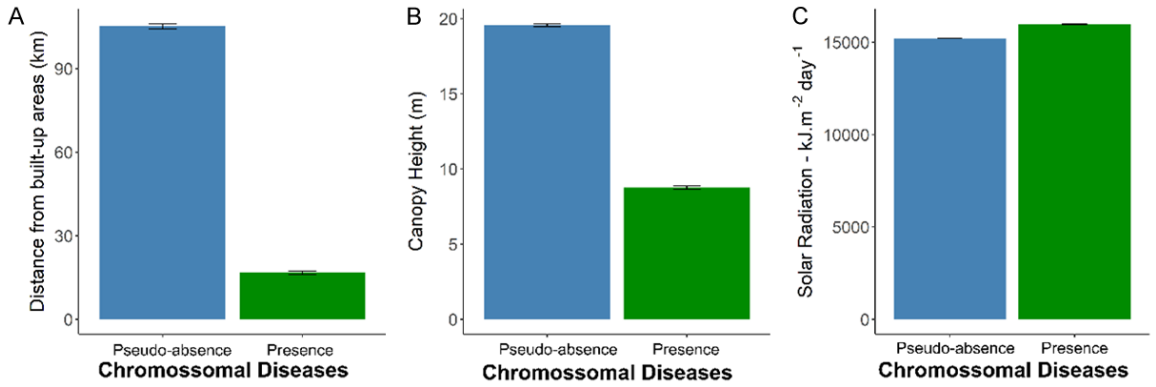

Figure 2. Predictor variables for the occurrence of chromosomal diseases. A. Distance from built-up areas; B. Canopy height; C. Solar radiation.

We used the variable solar radiation because it is considered a significant risk factor for mutational processes, considered a natural filter for these pathologies. In the places where the cases of chromosomal diseases were predicted, the radiation values were 1.05 times higher than the places where the cases were not predicted (no occurrence: 15216±9 km *vs.* occurrence: 15986±18 km) (**Figure 2C**).

Discussion

General characterization

Our study has comprehensive temporal coverage, from 2005 to 2015, comprising all geographic regions of Brazil (North, Northeast, Center-West, Southeast, and South), with many post-natal karyotype exams (n=43,672). Chromosomal alterations were detected in 17% of the total cytogenetic analyses; the frequencies of autosomal alterations were 79.2% and sex chromosomopathies 20.3%, results similar to those found by Ghazaey et al. (2013) [18] and Kim et al. (1999) [19]. Our results showed that 77% of the obtained chromosomal changes were numerical, and 16% were structural. Moreira et al. (2011) [20] analyzed 813 exams and showed a high frequency of numerical alterations (61.9%) and a similar frequency of structural alterations (19.4%).

Frequencies of chromosomal diseases in Brazilian regions

We verified that the urbanized regions presented the highest probability of chromosomal diseases in all the Brazilian states. Samples are collected in laboratories with fixed addresses, creating a bias in the study as soon as patients go to these addresses for the exams. Going to the lab can mean moving away from where the person lives.

*The Southern region: T*he southern region had the highest prevalence of cases (6 cases/100,000 inhabitants) and is characterized by 85% of the urbanized population. The State of Paraná has 53% of its territory occupied by permanent crops, with an average consumption of agrochemicals of 5.5 kg.ha^{-1} [21]. The excessive use of pesticides poses risks to the onset of congenital diseases due to the genotoxic potential of their ingredients, altering the functioning of organs, cells and damaging proteins and nucleic acids (DNA or RNA) [22].

Dutra and Ferreira (2017) [23] analyzed records of live births in 20 years (1994 to 2014), showing an association between the increase in the consumption of pesticides and the increase in the rate of congenital malformations. Another interesting fact is the results of a survey on the use of pesticides and adverse events in pregnancy in the three states of the Southern Region of Brazil, which suggest an association between the greater frequency of births before 22 weeks of gestation and with a bad Apgar score, and the highest per capita consumption of pesticides [24].

The Southeast region: The Southeast region presented a prevalence of 2 cases/100,000 inhabitants. Being predominantly urban (91.98%), the region is the one that most consumes pesticides in Brazil, with an average of approximately 9 kg/ha. The state of São Paulo is the most significant purchaser of pesti-

cides in Brazil, with an average of over 10 kg/ha, and 30% of its land is occupied by permanent crops [21].

Regarding the urbanization aspects, in regions with higher industrialization, it was observed that workers presented higher frequencies of chromosomal alterations [25, 26].

The Center-Western region: The Center-Western region presented the prevalence of 4 cases/100,000 inhabitants. This region has large agricultural establishments (23.17% and 16.38%, respectively), denoting the high consumption of pesticides. The states of Goiás, Mato Grosso, and Mato Grosso do Sul stood out for the increase in the commercialization of agrochemicals between 2000 and 2012 [21, 27].

Studies on congenital malformations were carried in Center-Western regions, associating their occurrence with exposure to pesticides [2]-Moreira et al. (2012) [28] associated malformation in anuran with water contaminated with pesticide residues. Palma et al. (2014) [29] showed that a type of pesticide had contaminated 100% of the human milk samples, and 85% of the samples contained more than one substance, which poses a risk to mothers and children.

The Northeast region: The Northeast region presented a prevalence of 3 cases/100,000 inhabitants. Despite the precariousness of soil, two states in this region are among the ten largest consumers of agrochemicals in Brazil: Bahia (9th) and Maranhão (10th) [27]. In some areas, tomato producers increase the number of applications of agrochemicals preventively and, with no rigorous use of personal protective equipment, grows the number of intoxications [21, 27]. Parental exposure to pesticides increases the likelihood of children presenting with congenital disabilities, linking this fact to the low socioeconomic level of the parents [30]. Soil and water contamination are worrying factors, and in the state of Ceará, deep well water analyzes were carried out and revealed contamination by three to ten active pesticide ingredients in all samples [31].

The Northern region: The Northern region presented a prevalence of 4 cases/100,000 inhabitants. Amorim et al. (2000) [32] demonstrated that methyl mercury contamination is related to chromosomal damage in an Amazonian population living on the banks of the Tapajós River in the state of Pará. More significant contamination of the water and soil by pesticides may occur in other regions where there is greater use of soil for crops, pastures, and agroforestry systems. A study carried out with a group of vegetable producers from Boa Vista, Roraima, showed that 98% said they use some pesticide, and only 41% of them consult technicians about buying the product [33], increasing the probability of cases of chromosomal diseases.

Spatial modeling

We used the variable *Distance from Built-up areas* to reflect the distance of each chromosomal disease from nearby urbanized locations. We found the highest prediction of the occurrence of chromosomal diseases in urbanized areas, corroborating with the findings of Munshi-South, Zolnik, and Harris (2016) [34]. The authors mention that genome-wide variation is inversely related to urbanization as measured by percent impervious surface cover and, to a lesser extent, human population density. They also report that urbanization results in enhanced genome-wide differentiation between populations in cities, indicating that urban populations suffering isolation by environment models deviate much more strongly from global allele frequencies than suburban or rural populations.

We used the *canopy height* variable to reflect the degree of environmental preservation of the locations under study and the potential of human exposure to monoterpenes, which are substances released by aromatic plants. Monoterpenes have a wide range of biological applications as antioxidants, anticancer, anticonvulsants, antiulcer, antihypertensive, and anti-nociceptive compounds [35]. Bach et al. (2020) [36] demonstrated that monoterpenes are potential determinants of the beneficial effects on human health induced by exposure to the forest. Our result corroborates this fact since places with greater canopy height, and therefore have a higher emission of monoterpenes, predicted minor occurrence of chromosomal diseases.

We used the variable *solar radiation* because it is considered a significant risk factor for mutational processes, considered a natural predic-

tor for chromosomal pathologies. Sunlight is a continuous spectrum of electromagnetic radiation, mainly containing the ultraviolet wavelength (UVR), whose overexposure is the severe risk factor for photoaging and skin cancer. The damaging effects of UVR on the skin are thought to be caused by direct cellular damage and alterations in immunologic function [37]. Our results corroborate these facts when we observe that the places with the highest prediction of chromosomal diseases are regions with an increased incidence of solar radiation, with a smaller forest area and closer to an urbanized area.

In conclusion, it was possible to associate *soil and occupation use of the land* with human chromosomal diseases from Brazilian regions, comprising ten years of laboratory analysis records (from 2005 to 2015). The Southern region presented the highest proportions of chromosomal diseases and probability of alterations occurrence, characterized as the primary consumers of agrochemicals in Brazil. The Southeast is the most urbanized region, presented the highest frequencies of autosomal structural alterations. The Central-Western is a region characterized by a large area of monocultures and high use of agrochemicals. The highest frequencies of chromosomal changes, in absolute numbers, occurred in the North and Northeast regions.

In spatial modeling, we demonstrated strong associations of chromosomal diseases with extensive areas of urbanization or agriculture, characterizing these environments as being at higher risk for chromosomal diseases, mainly due to high exposure to pesticides. Nevertheless, it is noteworthy that the samples in this study were collected in laboratories with fixed addresses and not in the patient's address of origin, which certainly maximizes the associations of chromosomes with the regions of the laboratories.

Acknowledgements

We want to thank Laboratorio Álvaro/DASA for granting the data and the Postgraduate Program in Biosciences and Health at the Universidade estadual do Oeste do Paraná to grant a master's degree Marcos Cochak. We are grateful for the collaboration of the authors (Marcos Cochak-interpretation and writing of the results; Marília M. Favalesso-making of the models and suggestions in writing; Rose Brancalhão-suggestions in writing; Ana Guimarães-making of the models, interpretation, and writing; Lucineia Ribeiro-suggestions in writing).

Disclosure of conflict of interest

None.

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