

Abstract

Cândido Godói (CG) is a small municipality in South Brazil with approximately 10,000 inhabitants. It is known as the “Twins' Town” due to its high rate of twinning. It was claimed that such high frequency of twinning would be connected to a genetic experiment performed by the German Nazi doctor Joseph Mengele. It is known, however, that the town was founded by a small number of families and therefore a genetic founder effect may represent an alternatively explanation for the high twinning prevalence. In this study, we tested specific predictions of the “Nazi's experiment” and the founder effect hypotheses. We surveyed a total of 6,262 baptism records from 195 churches, and identified 91 twin pairs and one triplet. Contrary to the “Nazi's experiment hypothesis”, there is no spurt in twinning between the years (1964–1968) allegedly was in CG ($P=0.482$). Moreover, there is no temporal trend in twinning since the 1960s ($P=0.351$), and no difference in twinning prevalence considering two different periods: 1927–1958 and 1959–2008 ($P=0.123$). On the other hand, the “founder effect hypothesis” is supported by an isonymy analysis showing that women who gave birth to twins have a higher inbreeding coefficient compared to women who never had twins (0.0148, 0.0081, respectively, $P=0.0001$). Our results show no evidence for the “Nazi's experiment hypothesis” and support that the “founder effect hypothesis” is a much more likely alternative explanation for the high prevalence of twinning in CG. If this hypothesis is correct, then CG represents a valuable population where genetic factors linked to twinning are being identified.

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Introduction

The etiology of twin births in humans is still largely unclear and is the subject of many investigations [1]–[4]. Traditionally, twins are classified as monozygotic (MZ) or dizygotic (DZ). MZ twins are developed when an embryo splits soon after fertilization. DZ twins occur when two separate oocytes, released during the same ovulation cycle, are fertilized by separate sperm cells [1]. MZ twinning is rarer than DZ twinning and no maternal, genetic, or environmental factors have been associated with it. On the other hand, DZ twinning is a phenomenon of complex etiology, where genetic predisposition and environmental factors play a role [7]. Although the occurrence of DZ twinning has been known for a long time, few genes have been associated with it, including the receptor of FSH hormone (*FSHR*), growth differentiation factor 9 (*GDF9*), methylenetetrahydrofolate reductase (*MTHFR*), etc. [8]–[10]. Risk factors such as advanced maternal age, increased parity, lactation, diet, high body mass index and race are observed also as risk factors for DZ twinning.

Twinning rates show a wide geographical and temporal variation, being higher in Asian populations (5–6 in 1,000 maternities) and more frequent in European populations (23 in 1,000 maternities) [11]. Previous studies show that twinning rates in European countries started to decline around 1900, but have increased since the 1970s onwards [12], possibly as a result of both increased maternal age and the widespread use of assisted reproductive technology (ART) procedures. Even in a single continent such as Europe variations in twinning rates between countries are observed [15]. The variability in twinning rates has been attributed to variation in DZ twinning rates, as MZ twinning has a constant prevalence worldwide and time (4 in 1,000 maternities) [16].

Cândido Godói (CG) is a small town in South Brazil (27°57′07″S; 54°54′00″W) with approximately 6,000 inhabitants. It is known as the “Twins' Town” due to the high rate of twin births. According to the Brazilian Ministry of Health, between 1994 and 2004, 2% of the live births in CG were twins, compared to an average of 1% worldwide [17]. However, twinning may not be equally distributed throughout Brazil. In 1994, the twinning birth rate in Linha São Pedro (LSP), a small district in the state of Rio Grande do Sul, was as high as 10% [18]. CG and LSP were both founded at the beginning of the 20th century by a few families of German ancestry coming from other German-founded towns in Rio Grande do Sul. Presently, the population of LSP is less than 600 inhabitants, mostly catholic [19]. The reasons for the higher twinning rate in CG in particular, however, are still unclear.

Recently, a controversial theory was raised by an Argentinean journalist in a book alleging a possible link between the twinning phenomenon in CG and the experiments of the Nazi physician, Joseph Mengele. According to the book, Mengele could have lived and worked as a physician in CG in the beginning of the 20th century, before living in Buenos Aires. Even though Camarasa's suppositions were not supported by actual historical records available [21], his story has caught attention in the mass press, which created a worldwide “fuzz” around the “Brazilian Twinning Phenomenon” in countries such as the United Kingdom (<http://www.dailymail.co.uk/news/worldnews/article-1126504/The->

doctor-Mengele-Angel-Death-cause-twin-surge-South-American-to (http://revistaepoca.globo.com/Revista/Epoca/0,EMI24803-15228,0 NAZISTAJOSEFMENGELECRIUCIDADEDOSGEMEOSNORIOGRANDEDC USA (http://news.nationalgeographic.com/news/2009/11/091125-mengele.html).

In this study, we surveyed baptism records in CG and LSP to evaluate test the predictions of the “Nazi's experiment hypothesis”. More specifically, we asked the following questions: 1. Is there any temporal and geographical variation in twinning rate (and LSP)? 2. Is there any increase of the twinning rate around the time when Joseph Mengele was supposedly working as a physician there? We explored alternative explanations for the higher twinning rate in CG. Given that we suspected that a genetic founder effect may be involved in the higher twinning rate in CG (and LSP). To test this hypothesis, we asked, using isonymy analysis, if twin's mothers have a higher inbreeding coefficient as compared to women who gave birth to twins. Our results clearly show that contrary to the “Nazi's experiment hypothesis” there is no peak on the twinning rate around the 60 s. Our isonymy analysis supports the hypothesis that a founder effect is a plausible explanation for the higher prevalence of twinning in LSP and in CG.

Materials and Methods

Ethics Statement

This study used only secondary public data for analysis, which was not directly collected from participants. This research project was approved by the Hospital de Clinicas de Curitiba's Research Ethics Committee under the protocol number 09-359. Written informed consent was obtained from all participants before interviews were conducted.

Twinning patterns within CG

Live births were surveyed from baptism records available in CG. Catholic baptism records include the child first name, family names of both parents, sex, date and location (district) where the family lives. The earliest available baptism records in CG with complete and reliable data were available only from 1959 onwards. To test the suggestion that LSP may have an especially high twinning frequency, we used a chi-square test to assess whether LSP has a higher twinning rate as compared to the remaining CG districts (CG-LSP) considering 1959 to 2008. Because maternal age is a known factor affecting twinning [11] we compared MA distribution in LSP using data obtained from interviews to test if women living in LSP had a higher MA, thus increasing the chance of twin births. Mean maternal age between LSP and CG was compared using a Student *t*-test adjusted for unequal variances.

Temporal tendencies in LSP and CG

According to Camarasa [20], Mengele would have arrived in CG around 1961 and stayed in the city until 1968, but the exact timing for his stay is uncertain. To evaluate

hypothesis that Mengele's stay in CG would have increased the twinning rate. We used a chi-square test to compare the occurrence of twin births in 1964–1968 and the remaining years.

To have a more detailed view on the temporal variations of twinning rate, we used a chi-square test for trends to ask if there is any temporal trend over the frequency of twinning occurring after 1959, grouped by intervals of five years. We tested the frequency of twin births for CG as a whole, for LSP only, and for CG-LSP. The “Naz hypothesis” predicts a peak of twinning around the late 1960s followed by a constant or abrupt decline on the twinning rate towards the average twinning rate of the population, since the increase in twinning obtained by Mengele was only on the short-term.

Finally we used a chi-square test to evaluate if the relative frequency of twinning in LSP and CG-LSP was held constant when two periods are considered: 1959–1968 and 1969–2008. Because the earliest records (up to 1959) are only reliable for twinning – but not for overall births only the relative twinning frequency in different regions could be compared. All statistical tests, odds ratios, and their 95% confidence intervals were computed using BioEstat 5.0 [22].

MZ/DZ ratio

The proportion of the MZ twinning rate (MZr) over the DZ twinning rate (DZr) was estimated using Weinberg's differential method [23] in which DZr can be estimated by doubling the number of opposite sex twin pairs (OS) and dividing by the total number of maternities (N): $DZr=2OS/N$, while MZr can be estimated by subtracting the number of opposite sex twins from the number of same sex (SS) twins and dividing by N : $MZr=(SS-OS)/N$. We only have the total number of maternities for the period 1959–2008, therefore the period considered for MZr and DZr estimation. The corrected variance ($Var(MZr)$ and $Var(DZr)$, respectively) was estimated as in [24]. We compared the twinning rate between LSP and CG-LSP using a binomial test. Finally, we used the chi-square test to test if MZr and DZr are equally increased in LSP compared to CG-LSP. The total number of MZ and DZ twin pairs estimated by the numerator of Weinberg's method and the total number of maternities is not necessary for this estimation we used the twinning records from 1927–2008 to increase statistical power.

Pedigree and isonymy

To have a more detailed picture of the pedigree structure for families with twinning, the twinning rate is reported compared to families without familial history of twinning. We interviewed 42 women from independent households who gave birth to twins and 101 women from independent households who only had single births. All women were residents from CG. The aim of this questionnaire was to collect data that would provide a better description of these women and to access potential factors for twinning. More specifically, we asked familiar data, including the names of both parents and history of twinning in their family, and questions about twinning in each household, we used the software Progeny 7.0® to draw pedigrees. To analyze the surname data to calculate isonymy, a quantity that makes use of

surnames in a given population to measure deviations from panmixis. Isonymy is related to the inbreeding coefficient F [26], which was estimated in the present study. Analyses of population structure by isonymy methods have been conducted in several human populations [28]–[32]. The “founder effect hypothesis” predicts that the twinning rate in CG reflects, at least in part, a genetic founder effect when CG was settled by few families of German descent in the beginning of the 19th century. Thus, if such founder effect affects the twinning rate in CG, the inbreeding coefficient for cases (F_{cases}) and for controls ($F_{controls}$) should be different, and the statistical significance of this difference was assessed by permutation tests. The reported P -value thus represents the proportion of permutations where $F_{cases}/F_{controls}$ is equal or higher than the observed.

Results

From 1959 to 2008 there were a total of 6,262 baptisms in 14 CG districts, including 10 pairs of twins and one triplet. Table 1 presents the geographical distribution of twin births in CG according to the district where the parents lived. Using these records we estimated the frequency of twinning in these districts. The twinning rate of live born twins of 7.0% compared to 1.5% in CG as a whole. No other districts showed twinning frequencies above 3.5% (Table 1, Figure 1). It is noteworthy that while representing only 7.5% to all baptism records, it contributes almost 1/3 (33/92) to all twin births in CG, representing an odds ratio of 7.3 (CI 95% 4.75–11.38) for LSP compared to the remaining districts in CG ($P<0.0001$) (Table 2). There was no difference in maternal age between LSP and CG-LSP ($P=0.3036$; $MA_{LSP}=26.10\pm 4.71$ years; $MA_{CG-LSP}=26.10\pm 4.71$ years (mean \pm SD), suggesting that differences in maternal age is unlikely to explain the higher twinning rate in LSP.

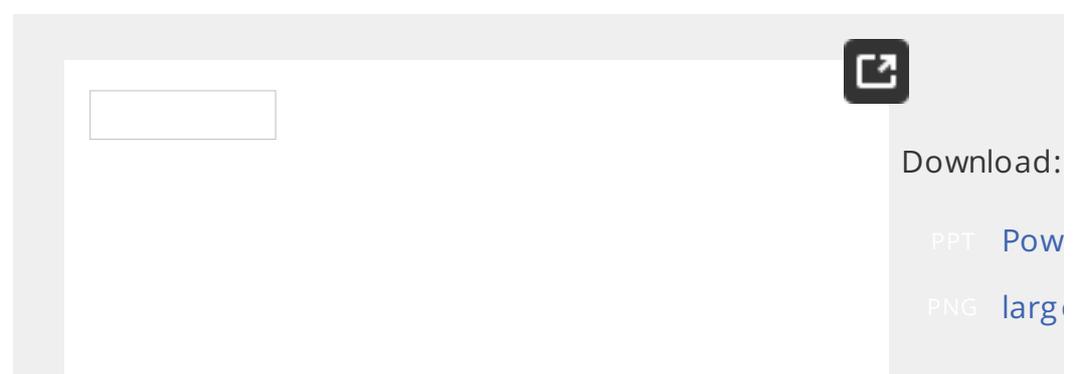
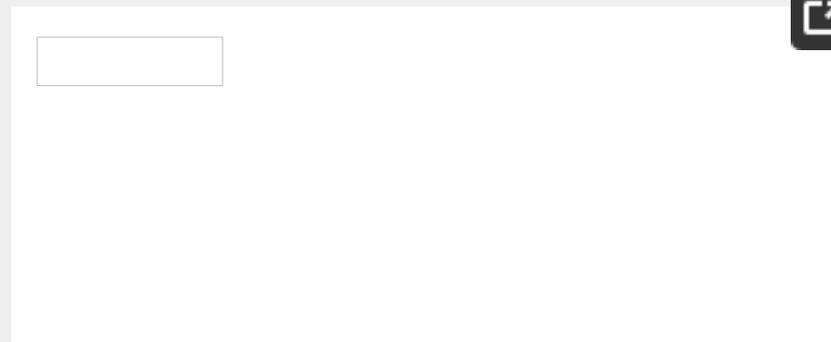


Table 1. Distribution of twin baptism records in Cândido Godói<https://doi.org/10.1371/journal.pone.0020328.t001>

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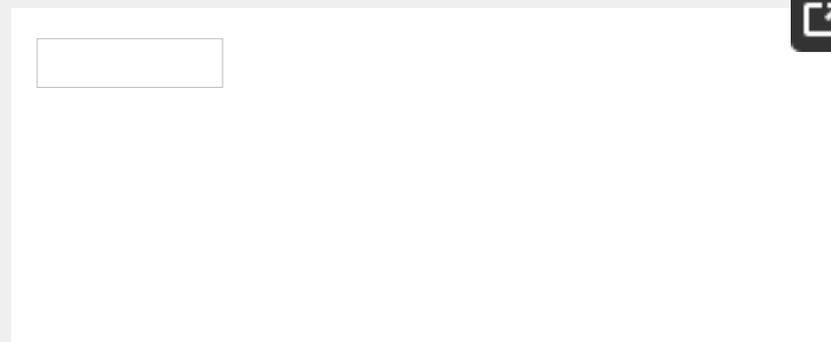
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Table 2. Twin and single baptism records in Linha São Pedro (LSP) and**Cândido Godói's (CG-LSP) districts, from 1959–2008.**<https://doi.org/10.1371/journal.pone.0020328.t002>

There is no increase on the twinning rate in CG between the period remaining years (Table 3, $P=0.482$). These results are consistent with those that are analyzed ($P=0.772$, $P=0.294$, respectively). There is also no temporal trend in the relative frequency of twinning between LSP and CG-LSP, considering the period from 1958 and 1959–2008 (Table 4, $P=0.638$), suggesting that LSP has been the major contributor for the overall higher twinning rate in CG. The test of the frequency of twin births reveals an interesting pattern (Table 5) for any temporal trend in either CG as a whole (Figure 2A; $P=0.351$) or LSP (Figure 2B; $P=0.486$). In contrast, for LSP alone there is a tendency for an increase in twinning across time (Figure 2C; $P=0.001$). Importantly, this trend goes in the opposite direction that expected according to the “Nazi's experiment hypothesis”, which was also rejected in all other tests we performed.



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Figure 2. Temporal twinning tendencies.

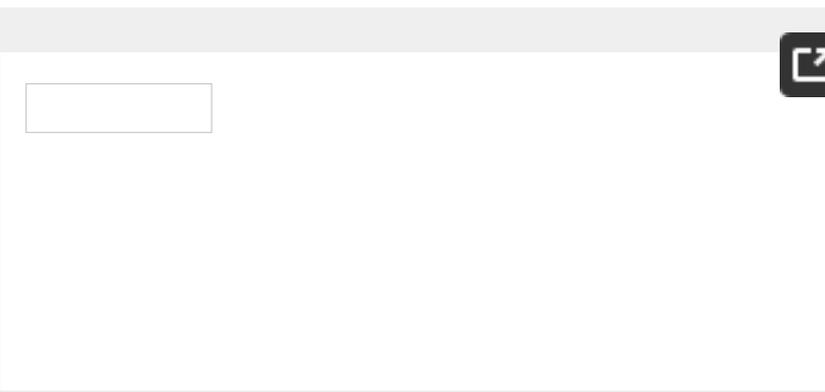
2a: Temporal twinning tendencies in Cândido Godói; 2b: Temporal twinning tendencies in Cândido Godói, excluded Linha Sao Pedro; 2c: Temporal twinning tendencies in Linha Sao Pedro only.

<https://doi.org/10.1371/journal.pone.0020328.g002>



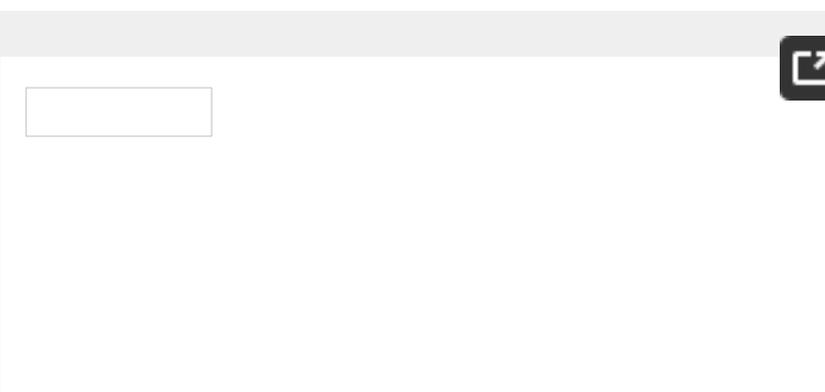
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Table 3. Twin baptism records between 1964–1968 and in the region of Linha Sao Pedro (LSP) and in others Cândido Godói's (CG-LSP) districts.
<https://doi.org/10.1371/journal.pone.0020328.t003>



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Table 4. Twin baptism records before and after 1958 in Linha Sao Pedro (LSP) and in others Cândido Godói's (CG-LSP) districts.
<https://doi.org/10.1371/journal.pone.0020328.t004>



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Table 5. Temporal distribution of twin baptism records from 1927–1958 in Linha Sao Pedro (LSP), in other districts of Cândido Godói (CG-LSP) and as a whole (CG).
<https://doi.org/10.1371/journal.pone.0020328.t005>

Table 6 shows for both LSP and CG-LSP the numbers of *SS* and *OS* for the period between 1927–1958, and *SS*, *OS* and total births for the period between 1959–2008. In the 1927–1958 period, *DZr* equals 3,84% for LSP but only 0,48% in CG-LSP, while *MZr* equals 0,64% in LSP and only 0,64% in CG-LSP. This difference is highly significant for both comparisons ($P < 0,0001$ for both comparisons). To test if *MZr* and *DZr* are increased in LSP we used the numerator of Weinberg formula to estimate the number of twin pairs and 11 MZ twin pairs were born in LSP between 1927–2008, w

period 42 DZ twin pairs and 46 MZ twin pairs were born in CG-LSP. This result is statistically significant ($P=0.004$). Thus, even though both DZr and MZr are increased, the increase seems to affect DZ twin births more strongly than MZ twin births. These factors have been only associated with DZ twinning, this result may suggest a suggestion that genetic factors are responsible for the increased twinning in CG (for which LSP is the major contributor). Such notion is reinforced by the twinning resulting from the reported familiar history, which allows us to observe twinning recurrent for several families living in CG (Figure 3). Twenty-eight out of 470 twins had female sisters, and in five out of these 28 kindreds (17.8%) twinning births was observed. The total number of sisters of index mothers versus the number of mothers having twins, which means an overall recurrence chance of having twins is 5.9%.

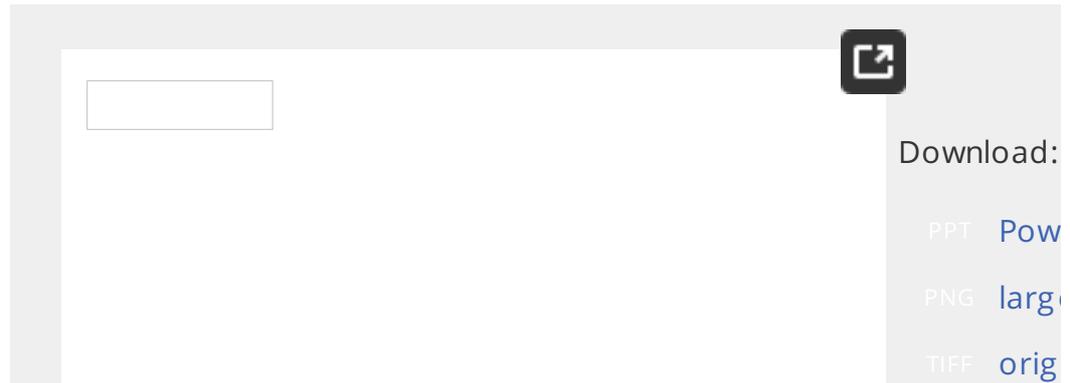


Figure 3. Illustrative pedigree of one family living in Linha São Paulo.
<https://doi.org/10.1371/journal.pone.0020328.g003>

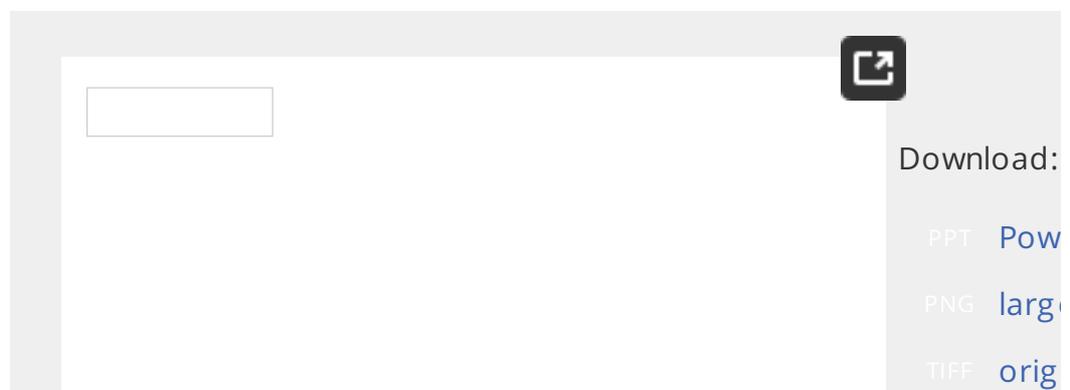


Table 6. Estimation of DZ twinning rate (DZr), MZ twinning rate (MZr), and absolute number of DZ and MZ twin pairs in LSP and CG-LSP.
<https://doi.org/10.1371/journal.pone.0020328.t006>

Finally, by analyzing the distribution of surnames between cases and controls, we found that the estimated F for cases is statistically higher than the estimated F for controls (0.019; Table 7), suggesting that, as predicted by the founder effect, women having twins in CG are more genetically related than women having twins in LSP.

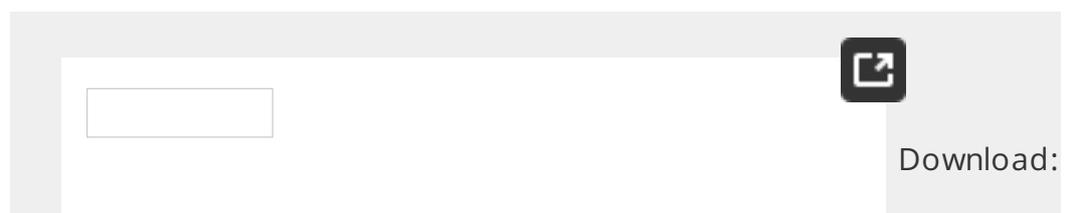


Table 7. Inbreeding coefficient by Isonomy (F) from cases and controls
<https://doi.org/10.1371/journal.pone.0020328.t007>

Discussion

Our results provide a robust refutation to the hypothesis that the high twinning rate in CG is due to Nazi's experiments in the 1960s, and strongly suggest that the effect occurred during the settlement of CG is a much better alternative hypothesis. On the other hand, we confirmed that the high prevalence of twinning is particularly concentrated in LSP and that this is a historical trend. The increase on the twinning rate at LSP is not due to fertility treatments, as such techniques have been extremely rare in Brazil due to its high costs, especially in areas like CG.

One of the limitations of this study is that it is based on baptism records, not on official birth certificates or hospital births records. However, baptism records provide reliable information for twinning only after 1990, and old hospital records are not available in CG. Moreover, in small communities such as CG was common in the first decades of the 20th century for women to have babies at home instead of in hospitals in the neighbor cities. Baptism records, on the other hand, are kept in the different churches located in the municipality, and these records include the name and sex of the children, date of birth, parent's name and locality where they are living. The fact that only catholic churches were included may also limit the representativeness of the data. The last demographic census in Brazil in 2007 showed that 76.6% of CG population are catholic. Baptism records may suffer from another bias, in that only live births are recorded. However, this would underestimate the actual twinning prevalence. The overall pattern of twinning is consistent between LSP and CG-LSP cohorts. Since the pattern of 1927–1958 and 1959–2008, it is unlikely that this would bring a significant change in the data.

Although the importance of environmental factors cannot be formally tested, factors which are important for twinning etiology such as the use of folic acid or acid folic supplementation [33]–[35] are unlikely to explain the high twinning rate already displayed by this population during the 1960s, when these supplements were not available in the Brazilian market. On the other hand, high levels of milk consumption are implicated in elevated twinning rates [4]. Since all CG districts are built in rural areas and most of the families consume dairy products, dietary factors may be considered a possible adjuvant for the high prevalence of twinning. However, because all districts have a similar lifestyle, milk consumption itself hardly explains the differences in twinning rates between LSP and CG-LSP. Similarly, differences in maternal age between LSP and CG-LSP are also unlikely to explain the observed pattern, since there is no significant difference in maternal age between LSP and CG-LSP.

When one analyses extreme twinning rates in small isolates one has to consider that the random fluctuations are also extreme. In Europe, a phenomenon known as Godoi has been registered in the Aland Islands, where higher twinning rates have historically been recorded compared to mainland areas of Finland and Sweden. The colonization history of CG is suggestive that a genetic founder effect played a key role in this process, leading to high shifts in allele frequency between the derived population. Because it is a random process, founder effects can lead to unpredictable phenotypic effects, which, in this specific case, may include twinning. Our results provide strong evidence that corroborates this hypothesis of the familiar aggregation of twin births and the high prevalence of twinning. These findings are both indicative that genetic factors are involved in twinning in the CG (Figure 3). Although causes for MZ twinning are still obscure, DZ twinning shows a clear familial aggregation, which is taken as evidence for genetic predisposition. Despite being a small district with few baptisms, LSP concentrates roughly 10% of all twins. The increased DZ twinning proportions in LSP thus indicate that twinning for overall twinning occurs because there are genetic factors enhanced in CG as a whole and in LSP in particular. Secondly, strong evidence for a genetic founder effect comes from the higher inbreeding coefficient observed in women who gave birth to twins compared to other women.

Currently, there are several reported examples of the importance of founder effects for some specific phenotypes which are more frequently observed in small populations. As a general rule, a relatively high frequency of an autosomal recessive disease in an isolated population suggests a founder effect [37]. For example, it was suggested that a subgroup of Native American Athabascan populations in Alaska and New Mexico have an unusually high incidence of severe combined immunodeficiency probably due to founder effect [38]. More recently, the importance of genetic founder effects has been made for explaining the prevalence of genes responsible for 'single-gene' disorders and disease predisposition in Ashkenazi Jews compared to Sephardi Jews and non-Jews [39]. Another example is the population of the Acadians in Canada. This population was founded by 8500 French Acadians who, for some genetic disease, a geographical distribution consistent with founder effects occurred during the migration of those settlers and their descendants [40]. It was also recently proposed that for the Sardinian population the high frequency of glycogen storage disease type Ib in this population may be due to a founder effect [41].

Finally, our results also suggest that CG in general and LSP in specific population isolates where specific genetic variants influencing twinning may be identified. For the citizens of CG, our results may also be relieving as they formally reject the possibility that CG twins are a result of Nazi experiments. In this sense, our study illustrates how knowledge of population history and its consequences may be of direct interest for the populations under study.

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Author Contributions

Performed the experiments: AT-R MZ-O AKS MO. Analyzed the data: NJRF LS-F. Contributed reagents/materials/analysis tools: MRR NJRF I paper: AT-R NJRF GS LS-F.

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