Screening for Familial Hypercholesterolemia in Small Towns: Experience from 11 Brazilian Cities at the HipercolBrasil Program

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Abstract

Background: Familial hypercholesterolemia is an autosomal dominant disease clinically characterized by elevated serum levels of low density lipoprotein-cholesterol (LDL-C) and associated with the occurrence of early atherosclerotic cardiovascular disease. In Brazil, HipercolBrasil, currently the largest screening program underway, exists since 2012 and has already identified more than 2000 individuals with causal genetic variants for FH. The standard approach of HipercolBrasil is based on cascade-screening of refereed index cases, hypercholesterolemic individuals with a clinical suspicion of FH.

Methodology: to assess a new methodology for identifying new individuals with genetic alterations for FH we performed a comprehensive city-wide screening in 11 small Brazilian cities (up to 60 thousand inhabitants). The selection of cities occurred in 3 ways: 1) cities with suspicious founder effect (4 cities); 2) Cities in a region with high rates of dyslipidemic individuals as described by the National Health System database (DATASUS) (2 cities); and 3) Cities geographically close to other cities with a high incidence of individuals with FH (5 cities).

Results: One-hundred and five (105) index cases and 409 first-degree relatives were enrolled. The yield of such approach was significantly better than the general HipercolBrasil positivity rate in molecular screening. We identified 36 IC with a pathogenic or likely-pathogenic variants for FH and 240 affected first-degree relatives.

Conclusion: our data suggest that once detected, specific geographical regions warrant a target approach for the identification of clusters of FH individuals.

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disease clinically characterized by elevated blood levels of low density lipoprotein-cholesterol (LDL-C) and associated with the occurrence of early atherosclerotic cardiovascular disease (ASCVD).[1 2]

The prevalence of FH in the world is estimated to be approximately 1:250 in the heterozygous form and 1: 600.000 in homozygosity[3].A study conducted by the ELSA-Brasil cohort estimated that the prevalence of individuals with clinical criteria for FH in Brazil is 1: 263. Considering these estimates, there are approximately 760,000 people with FH in Brazil[4].

However, although relatively frequent, FH is still an underdiagnosed disease[5]. To assist in the identification of individuals with the disease, genetic cascade screening has been used in several countries, such as the Netherlands [6], United Kingdon[7] and Spain[8]. This method has already been recognized as cost effective for the identification as well as for the prevention of early ASCVD in individuals with FH [9].

In Brazil, HipercolBrasil, currently the largest screening program underway, exists since 2012[10] and has already identified more than 2000 individuals with causal genetic variants for FH. The program currently performs genetic testing on any index-cases (ICs) individual with LDL-cholesterol (LDL-C) ≥ 230 mg/dL[11] and in first-degree relatives of those with pathogenic or likely-pathogenic variants. In these cases, after receiving the genetic report, the patients are referred to an outpatient clinic for FH treatment.

Between July 2017 and July 2019, we tested another methodology for identifying new individuals with genetic alterations for FH. In this new model, the HipercolBrasil program performed comprehensive city-wide screening in 11 small Brazilian cities (up to 60 thousand inhabitants) that showed signs of a higher prevalence of people with FH. Here we describe the methodology and first results of a new FH screening strategy based on small cities with some evidence of increased FH prevalence.

2. Methods

1.1 Aim

Between July 2017 and July 2019, we tested another methodology for identifying new individuals with genetic alterations for FH. In this new model, the HipercolBrasil program performed comprehensive city-wide screening in 11 small Brazilian cities (up to 60 thousand inhabitants) that showed signs of a higher prevalence of people with FH. Here we describe the methodology and first results of a new FH screening strategy based on small cities with some evidence of increased FH prevalence.

1.2 Study Design

This study is an open prospective study evaluating a cohort of patients who were ascertained by the FH genetic screening cascade program (HipercolBrasil). The study was conducted at the Laboratory Genetics and Molecular Cardiology of the Heart Institute (InCor), University of São Paulo Medical School Hospital, São Paulo, Brazil. The protocol was approved by the Institutional Ethics Committee (CAPPesq protocol I00594212.0.1001.0068).

1.3 Study population

Figure 1 shows inclusion criteria and study design. We enrolled individuals from 11 selected cities with up to 60,000 inhabitants throughout the Brazilian territory. The selection of cities (Fig. 1) occurred in 3 ways: 1) cities with a suspicious founder effect, i.e. occurrence of homozygous individuals, but with non-related parents and from distinct geographic origins - Major Vieira, Papanduva, Lagoa do Mato and Passagem Franca; 2) Cities in a region with high rates of myocardial infarction as described by the National Health System database (DATASUS) – Bom Despacho and Moema[12]; and 3) Cities geographically close to other cities with a high incidence of individuals with FH - Bambuí, Pimenta, Luz, Colinas and Buriti Bravo.

1.4 Enrolment of index cases and relatives

In all cities, initial contact was made with the local secretary of health to explain the project and agreement on the partnership. Contact was made via telephone before visiting each city and an agreement was set by both parties via e-mail. Once in the city, the team was assisted by a health agent appointed by the health secretary. In the cities where there were evidence of a founder effect and in the ones where there were reports of high myocardial infarction incidence, the sample collection started from family members of these ICs. In these cities, there was also an active search for new ICs from medical records and cholesterol tests carried out in the clinical analysis laboratories of the local healthcare units. Individuals were considered as ICs when total cholesterol (TC) > 300 mg/dL and/or LDL-c ≥ 210 mg/dL with triglycerides < 300 mg/dL. In these cases, a blood sample was collected to perform a second measurement of cholesterol fractions in our laboratory, since many cities only performed TC exams. Those with LDL-c ≥ 210 mg/dl were selected for genetic sequencing. Individuals who did not reach this value received a report with the values of total cholesterol and fractions and were excluded from the study.

Figure 1: Methodology for selecting cities, capturing ICs and relatives and training health care professionals to continue the genetic cascade.

1.5 Genetic sequencing and cascade screening

Blood samples were collected (10 ml of peripheral blood in EDTA tubes) and sent to the Laboratory of Genetics and Molecular Cardiology at InCor/HCFMUSP for genetic analysis. Genomic DNA was extracted using QIAmp DNA MiniKit (QIAGEN), following the manufacturer's instructions. IC were sequenced by Next Generation Sequencing in a gene panel comprising the following dyslipidemia-related genes: *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *STAP1*, *LIPA*, *APOE*, *ABCG5* and *ABCG8*. Bioinformatics analyses were performed in Varstation and CLC genomic workbench 9.0 (QIAGEN). MLPA (Multiplex Ligation-dependent Probe Amplification) in *LDLR* was used to screen for copy-number variants (CNVs) in ICs without any missense, nonsense or frameshift variants identified in NGS. The screening of relatives was performed with Sanger sequencing (for point mutations or small Indels) or MLPA (for CNVs). Variants were classified following the recommendations of the American College of Medical Genetics and Genomics (ACMG)[13].

1.6 Data Analysis

For continuous variables, the mean and standard deviation were calculated. Categorical variables are shown as frequencies. The differences between frequencies were compared using the chi-square test. The differences between means were compared with Student's T-test or ANOVA, if necessary. Statistical significance was considered at a p-value < 0.05.

3. Results

Table 1 shows characteristics of the 11 visited cities, state of location at the Brazilian federation, number of inhabitants and date of each visit. The city with the lowest number of total inhabitants was Moema with 7,028 and the largest was Bom Despacho with 45,624 inhabitants, both in the state of Minas Gerais. The first cities to be visited were Major Vieira and Papanduva (Sep/2017) and the last were Buriti Bravo and Colinas (Feb/2019).

Table 2 shows the number of sequenced ICs and relatives by region and their genotype regarding the presence of pathogenic or likely pathogenic variants (Positive), no pathogenic variants (Negative) or presence of a variant of uncertain significance (VUS), as well as the number of new cases by IC. The cities with the highest number of enrolled individuals were Passagem Franca (n = 108), Papanduva (n = 103), Major Vieira (n = 96), Bom Despacho (n = 96), Moema (n = 73) and Lagoa do Mato (n = 60). The cities of Bambuí (n = 2), Buriti Bravo (n = 4), Colinas (n = 12) and Luz (n = 28) were the ones with the lowest number of enrolled individuals. Regarding the enrollment of family relatives per IC, the highest numbers were in Papanduva (n = 13.7), Moema (n = 13.6) Lagoa do Mato (n = 11) and Passagem Franca (n = 12.5). In the Hipercol Brasil program, the average number of family members per IC is 1.6.

Table 1
City locations, number of inhabitants, dates of visit, number of expected FH cases and number of found cases.

City	Brazilian Federation State	Total Inhabitants (IBGE* Census)	Visit date	N of expected cases (1:263)[4]	N of positive cases found
Bambuí	Minas Gerais	22.709	Dec/2018	86	2
Bom Despacho	Minas Gerais	45.624	Aug/2018	173	45
Buriti Bravo	Maranhão	23.827	Feb /2019	91	0
Colinas	Maranhão	42.196	Feb/2019	160	4
Lagoa do Mato	Maranhão	10.955	Apr/2018	42	32
Luz	Minas Gerais	17.492	Dec/2018	67	6
Major Vieira	Santa Catarina	8.103	Sep/2017	31	47
Moema	Minas Gerais	7.028	Aug/2018	27	36
Papanduva	Santa Catarina	18.013	Sep/2017	68	48
Passagem Franca	Maranhão	17.296	Apr/2018	66	50
Pimenta	Minas Gerais	8.236	Dec/2018	31	6
* Brazilian index of geography and statistics					

Table 2
ICs and relatives collected by region and their genotypes for the presence of pathogenic or likely pathogenic (Positive), no pathogenic variant (Negative) or VUS.

Origin	Index Cases			Relatives			Number of relatives per total of ICs	Total of genotyped individuals per city
	Negative	Positive	VUS	Negative	Positive	VUS		
Bambuí	0	1	0	0	1	0	1	2
Bom Despacho	15	11	2	34	31	3	2.3	96
Buriti Bravo	4	0	0	0	0	0	0	4
Colinas	6	1	1	1	3	0	0.5	12
Lagoa do Mato	3	2	0	25	30	0	11	60
Luz	21	4	1	0	2	0	0.08	28
Major Vieira	1	3	0	48	44	0	23	96
Moema	1	4	0	36	32	0	13.6	73
Papanduva	4	2	1	50	46	0	13.7	103
Passagem Franca	3	5	0	55	45	0	12.5	108
Pimenta	6	2	1	0	4	0	0.4	13
Total	64	35	6	249	238	3		595

Table 3 shows the three ICs groups (negative, positive or VUS) and their clinical and biochemical data. In total, 105 ICs were sequenced, and pathogenic or likely-pathogenic variants were found in 36 (37.8%) and VUS in 5 (5.25%) individuals. Most of ICs were females (67.6%) and when the clinical and biochemical characteristics were evaluated among the three groups, there was, as expected, a statistically significant difference regarding baseline (untreated) TC and LDL-c, with the positive group presenting the highest values of TC and LDL-c respectively 382 ± 150 mg/dL and 287 ± 148 mg/dL.

Figure 2 shows the geographic distribution of the 11 cities distributed in the 3 Brazilian federation states, the number of registered cases, as well as the number of individuals genotyped and with a pathogenic variant.

Table 4 shows the clinical and biochemical characteristics of the relatives. In total, 490 relatives were enrolled, of which 240 (49%) had the same pathogenic or likely pathogenic variant as their respective IC. In the group of relatives, 55% were females and, regarding clinical and biochemical data, there was a significant difference between the positive and negative groups in all evaluated variables, showing that in the group of individuals carrying pathogenic or likely pathogenic variants, there was a greater use of lipid-lowering medications, presence of early coronary artery disease, xanthomas, xanthelasmas and corneal arcus. Baseline TC (318 ± 97 mg/dL) and LDL-c (243 ± 82 mg/dL) values were also higher in relatives with a positive genetic result.

Figure 2: Geographical distribution of cases and the rate of genotyped individuals and individuals with an identified pathogenic variant (positive).

Legend for Fig. 2: From top to bottom Brazilian federation states of Maranhão, Minas Gerais and Santa Catarina

Table 3
Clinical and biochemical characteristics of negative, positive and VUS-altered ICs.

	Negative IC	(64)	Positive IC	(36)	CI VUS	(5)	p-value
Females %	45 (70.3)	64	21 (58.3)	36	5 (100)	5	0.134
Males %	19 (29.7)	64	15 (41.7)	36	-	5	
Age (years)	54 ± 15	64	44 ± 19	36	56 ± 16	5	0.015
Use of lipid lowering drugs	32 (50.0)	64	24 (66.7)	36	3 (60.0)	5	0.261
Early CAD	2 (3.1)	64	4 (11.1)	36	-	5	0.297
Xanthomas	3 (4.7)	64	3 (8.3)	36	1 (20.0)	5	0.365
Xanthelasmas	4 (6.3)	64	1 (2.8)	36	-	5	0.696
Corneal arcus	2 (3.1)	64	3 (8.3)	36	-	5	0.345
Current TC	279 ± 65	62	316 ± 107	36	302 ± 28	5	0.102
Current LDL-c	195 ± 56	64	234 ± 104	36	207 ± 35	5	0.051
Baseline TC	322 ± 33	60	382 ± 150	32	305 ± 43	5	0.008
Baseline LDL-c	233 ± 24	59	287 ± 148	34	229 ± 20	4	0.022

Legend for Table 3: CAD- coronary artery disease ; early CAD defined as ASCVD event < 55 and 60 years of age respectively in males and females; lipids in mg/dL; baseline lipids = untreated ;

Table 4
Clinical and biochemical characteristics of negative and positive relatives.

	Negative relatives	N (249)	Positive relatives	N (240)	p value
Females %	136 (54.6)	249	135 (56.3)	240	0.504
Males %	113 (45.4)	249	105 (43.8)	240	
Age (years)	40 ± 21	249	38 ± 21	240	0.710
In use of lipid lowering drugs	31 (12.4)	249	93 (38.8)	240	0.001
Early CAD	2 (0.8)	249	9 (3.8)	240	0.034
Xanthomas	6 (2.4)	249	17 (7.1)	240	0.013
Xanthelasmas	11 (4.4)	249	34 (14.2)	240	0.001
Corneal arcus	1 (0.4)	249	9 (3.8)	240	0.009
Current TC	198 ± 51	114	309 ± 86	127	0.001
Current LDL-c	124 ± 42	192	233 ± 75	198	0.001
Baseline TC	220 ± 191	97	318 ± 97	130	0.001
Baseline LDL-c	126 ± 41	169	243 ± 82	178	0.001

Legend for Table 4: CAD- coronary artery disease ; early CAD defined as ASCVD event < 55 and 60 years of age respectively in males and females; lipids in mg/dL; baseline lipids = untreated

Table 5 shows all the encountered variants and the location where they were identified. In total, 21 different variants were identified with 3 variants appearing more frequently: *LDLR* duplication from promoter to exon 6 in Passagem Franca (49) and Lagoa do Mato (29); *LDLR* duplication from exon 4 to exon 8 in Major Vieira (45) and Papanduva (41); and the variant p.Asp224Asn in Bom Despacho (39) and Moema (34). These frequencies suggest that these variants have founder effects in these localities. Six homozygous patients and one compound heterozygous in trans were found.

Table 5
FH pathogenic variants, likely pathogenic variants and VUS found per city.

Gene	Variant	Variant Classification	Bambuí	Bom Despacho	Luz	Pimenta	Moema	Buriti Bravo	Colinas	Lagoa do Mato	Passagem Franca	Major Vieira	Pa
LDLR	Duplication from exon 4 to 8 (b)	Pathogenic	0	0	0	0	0	0	0	0	0	45[b]	41
LDLR	Duplication from promoter to exon 6	Pathogenic	0	0	0	0	0	1	4	29	49[a]	0	0
LDLR	p.Asp224Asn	Pathogenic	0	39	4	0	34	0	0	0	0	0	0
LDLR	p.Cys222*	Pathogenic	0	0	0	0	0	0	0	0	0	0	5
LDLR	c.1359-1G > C	Pathogenic	0	0	0	5	0	0	0	0	0	0	0
LDLR	p.Gly592Glu	Pathogenic	0	0	0	0	0	0	0	0	0	2	0
LDLR	p.Ala771Val	Pathogenic	0	0	1	0	0	0	0	0	0	0	0
LDLR	p.Pro699Leu	Pathogenic	0	0	1	0	0	0	0	0	0	0	0
LDLR	p.Asp601His	Likely Pathogenic	2	0	0	0	2	0	0	0	0	0	0
LDLR	p.Cys34Arg	Likely Pathogenic	0	1	0	0	0	0	0	0	0	0	0
LDLR	p.Arg257Trp	Likely Pathogenic	0	0	0	0	0	0	0	0	0	0	1
LDLR	p.Ser854Gly	Likely Pathogenic	0	2	0	0	0	0	0	0	0	0	0
LDLR	c.-228G > C	VUS	0	0	0	0	0	0	1	0	0	0	0
LDLR	p.Ala30Gly	VUS	0	0	0	1	0	0	0	0	0	0	0
APOB	p.Ala2790Thr	VUS	0	0	0	0	0	0	0	0	0	0	1
APOB	p.Met499Val	VUS	0	1	0	0	0	0	0	0	0	0	0
PCSK9	p.Arg237Trp	VUS	0	4	0	0	0	0	0	0	0	0	0
PCSK9	p.Arg357Cys	VUS	0	0	1	0	0	0	0	0	0	0	0
STAP1	p.Pro176Ser	VUS	0	0	0	1	0	0	0	0	0	0	0
LDLR	p.Cys222*	Pathogenic	0	0	0	0	0	0	0	0	0	0	1[c]
LDLR	Duplication from exon 4 to 8	Pathogenic											
PCSK9	p.Arg215Cys	Likely Pathogenic	0	0	0	0	0	0	0	1	0	0	0
APOB	p.Asp2213Asn	VUS											
APOB	p.Val3290Ile	VUS											
PCSK9	p.Arg215Cys	Likely Pathogenic	0	0	0	0	0	0	0	1	0	0	0
APOB	p.Val3293Ile	VUS											
PCSK9	p.Arg215Cys	Likely Pathogenic	0	0	0	0	0	0	0	1	0	0	0
APOB	p.Asp2213Asn	VUS											

(a) 2 homozygotes (b) 4 homozygotes (c) compound heterozygous in trans.

4. Discussion

This study describes the result of implementing a genetic cascade screening system for FH in 11 small Brazilian cities. FH is a high frequency disease worldwide and in Brazil[4]. A Spanish study pointed that 55% of men and 24% of women with FH in their 50 s present manifestations of coronary heart disease, such as myocardial infarction and angina pectoris, which makes FH a public health problem and the proper identification and treatment are of primary importance [1415]. Genetic cascade screening is considered a cost-effective method for identifying undiagnosed cases of FH [9].

Despite the known cost benefits of cascade screening in FH, its implementation worldwide has been suboptimal. Different local barriers and implementation hurdles have to be identified and overcome. How to implement cascade screening in small localities, for example, has been mainly overlooked. This challenge is greater in a continental country like Brazil, where in addition to the enormous geographic distances there is inequality in access to health services. Here we describe the HipercolBrasil experience in conducting comprehensive cascade screening in small towns in Brazil. In this new model, genetic cascade screening was carried out in cities that showed evidence of a higher prevalence of FH due to previous finding of individuals with the HoFH phenotype from the same city, or because those regions had elevated reported frequency of myocardial infarction or if the cities were geographically close to either.

Cities that had evidence of a founder effect were the ones that presented a higher identification of individuals affected per each IC analyzed (respectively Major Vieira, Papanduva, Lagoa do Mato and Passagem Franca). In these cities, we started from homozygous individuals whose parents were non-related and were born in different geographic regions.

Of importance, the rate of family members per IC was higher in cities with suspected founder effects: Major Vieira, Papanduva, Moema, Passagem Franca and Lagoa do Mato. This probably occurred because these cities had a small number of inhabitants and since most relatives had some degree of familial rapport there was a low detection of new ICs. This did not occur in Bom Despacho, a city considerably larger than the others (45,624 inhabitants) and, although the number of family members collected was similar to that of other cities, there was a higher number of ICs collected (67) decreasing the rate of relatives/IC to 2.3.

Cities visited geographically close to cities with suspected founder effects, (Bambuí, Buriti Bravo, Colinas, Pimenta and Luz) had a low uptake of ICs and consequently a low number of identified relatives. Bambuí was the city with the lowest number of IC uptake and no pathogenic or likely-pathogenic variants were identified. Particularly for Bambuí there was a low adherence from the local health care professionals and most medical reports lacked the biochemical profiles of patients. In the other cities, although we could detect ICs with FH associated variants, the genetic cascade screening was not continued by trained health agents. However, it is noteworthy that these were the last cities visited by the program and that it remains open for the genotyping of new cases. Initially, some cities had high IC uptake from exams with TC > 300 mg/dL. However, a second and more specific determination of LDL-c in our laboratory discarded the genotyping of many individuals that were below the LDL-c cutoff (≥ 210 mg/dL), decreasing the actual number of cases.

5. Conclusion

Cascade screening in small cities (less than 60 thousand inhabitants) proved to be effective in those with a suspected founder effect. However, some points might be of great importance for the cascade screening to be effective, and those might be executed before the decision of what city to track: establishment of a formal partnership and explicit interest of the local health department in receiving the program and performing the cascade screening; availability of clinical analysis laboratories datasets to carry out a retrospective survey of cholesterol tests; dissemination via radio stations and social media about the disease and the program for greater adherence by the inhabitants.

This study is limited by the relative number of cities evaluated considering the continental size of Brazil. However, it suggests that the designed approach may be useful for detecting individuals with FH. In conclusion, our data suggest that once detected, specific geographical regions warrant a targeted approach for the identification of clusters of FH individuals.

Abbreviations

FH- Familial Hypercholesterolemia

LDL-C - low density lipoprotein-cholesterol

ASCVD - early atherosclerotic cardiovascular disease

IC - Index case

IBGE - Brazilian index of geography and statistics

Declarations

Authors contributions: All the authors listed in the article contributed significantly to this work, and all of them meet the full criteria and requirements for authorship. All the authors contributed to conception and design of the study, analysis and interpretation of data, revising the article and all of them approve the article submitted. RDS is recipient of a scholarship from the Conselho Nacional de Pesquisa e Desenvolvimento Tecnologico (CNPq) process # 303734/2018-3, Brazil.

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Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

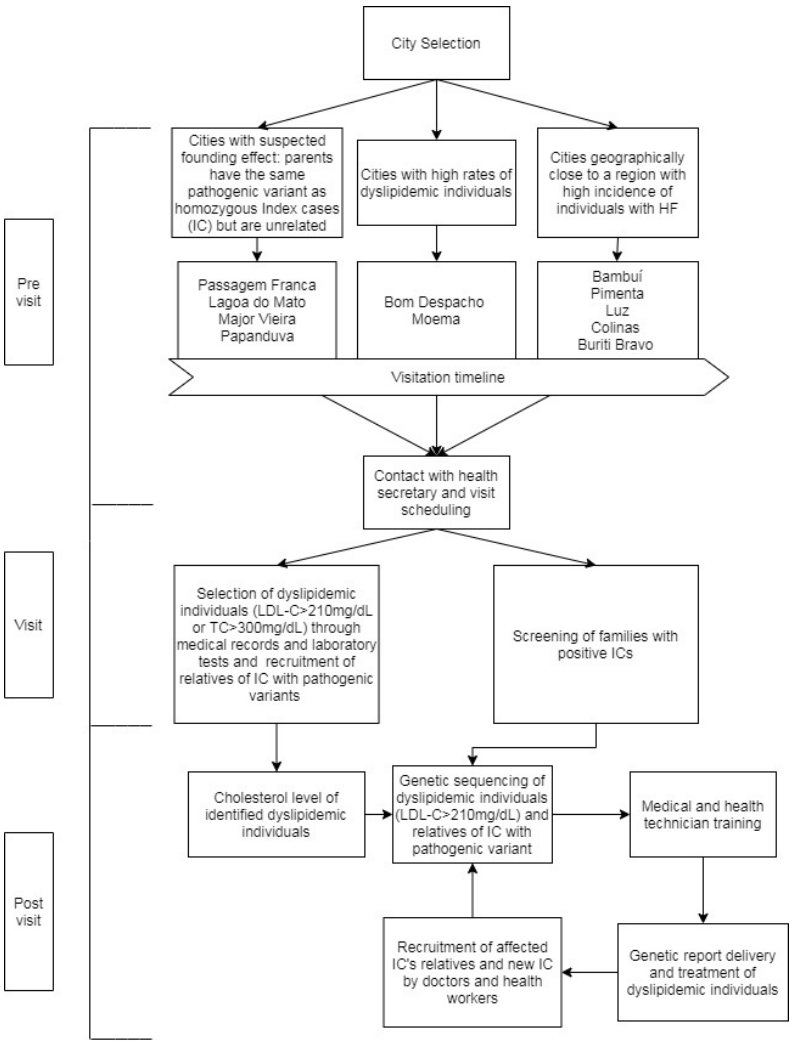
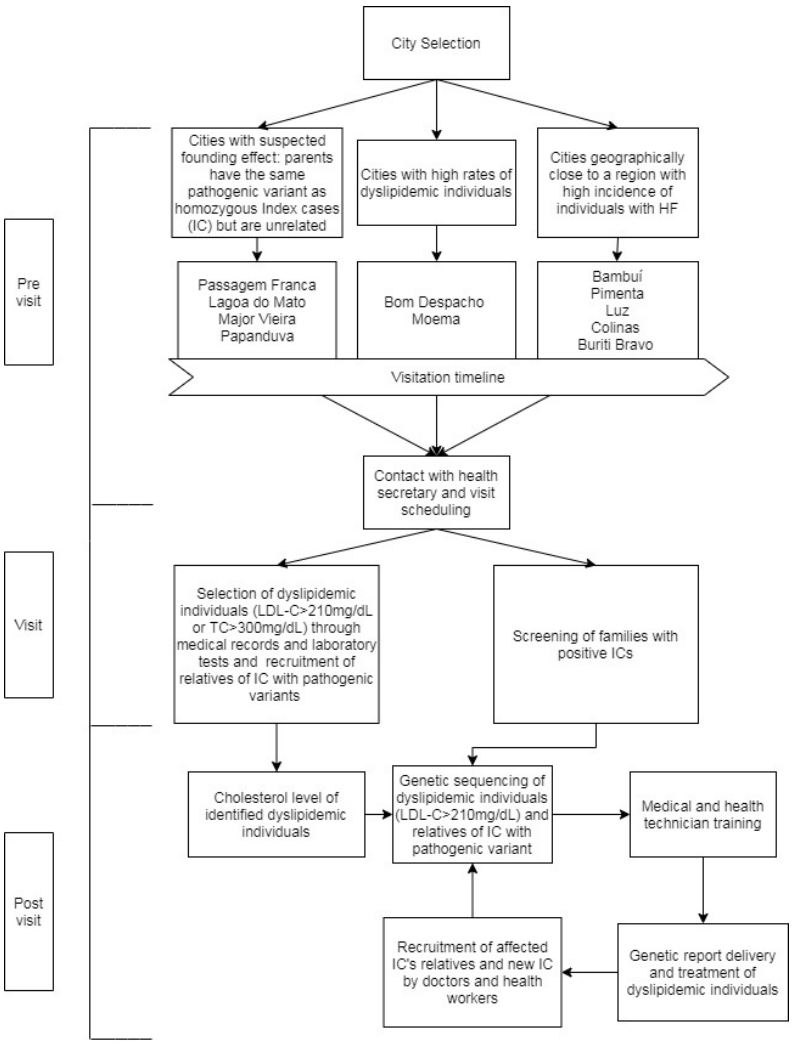


Figure 1

Methodology for selecting cities, capturing ICs and relatives and training health care professionals to continue the genetic cascade.



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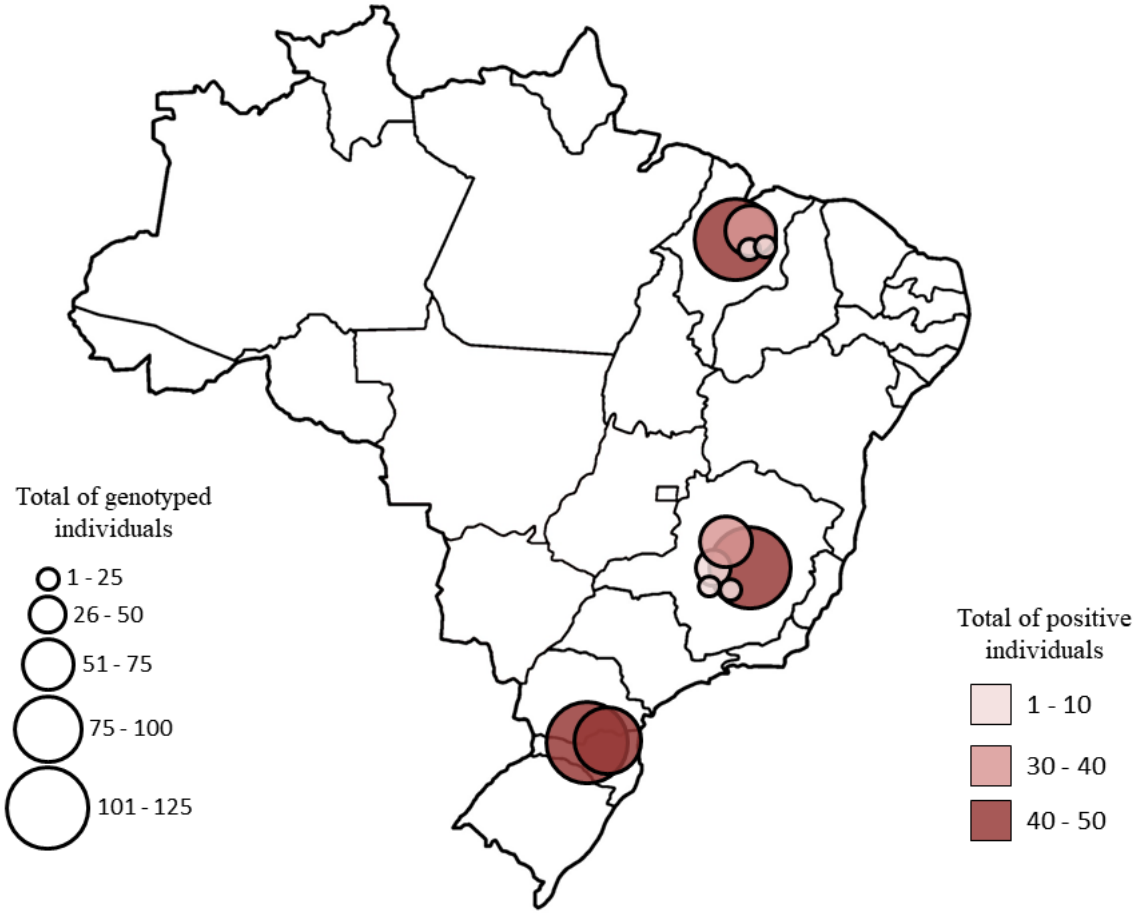
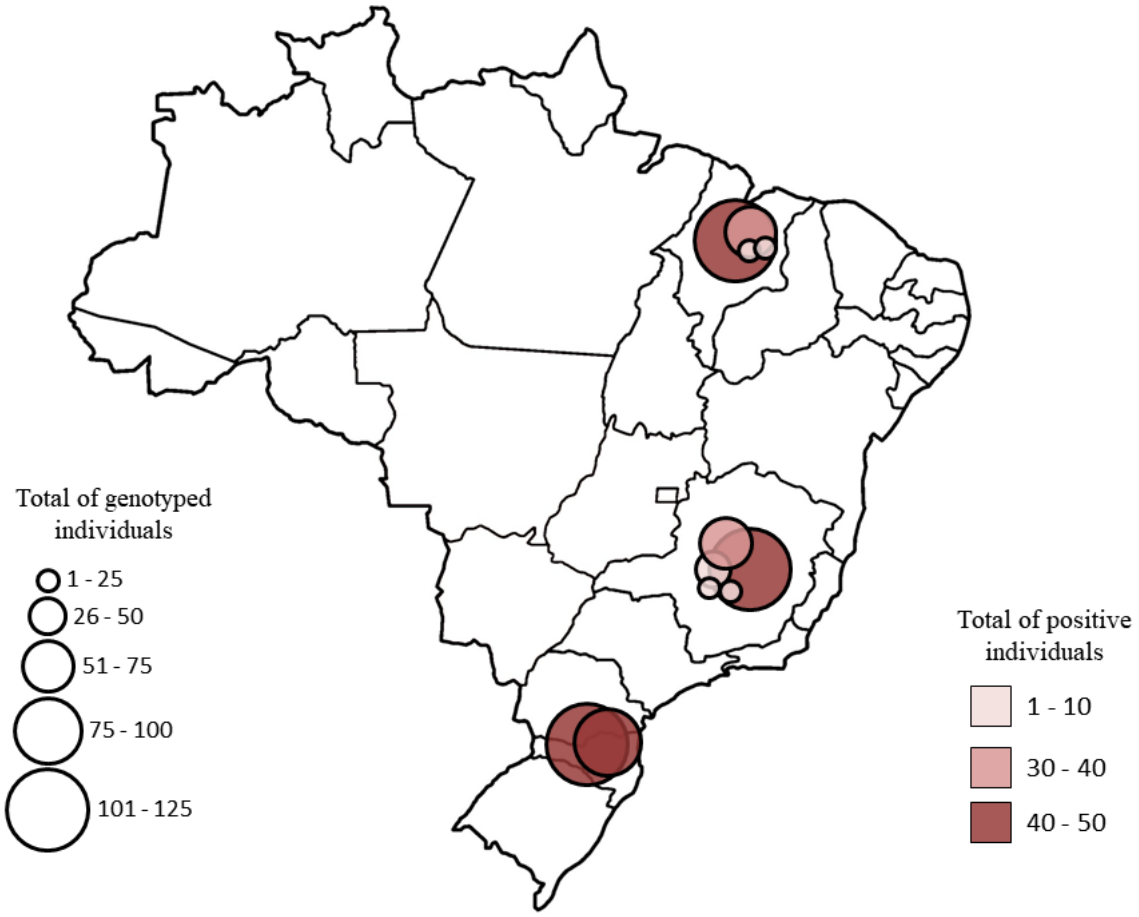


Figure 2

shows the geographic distribution of the 11 cities distributed in the 3 Brazilian federation states, the number of registered cases, as well as the number of individuals genotyped and with a pathogenic variant. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



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