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Heritability of Blood Pressure in Families from Southwest Coastal Bangladesh

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Heritability of Blood Pressure in Families from Southwest Coastal Bangladesh

By

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B.S. Biology

University of Arizona

2015

Faculty Thesis Advisor: Matthew O. Gribble, Ph.D. D.A.B.T.

An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

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2020

Abstract

Heritability of Blood Pressure in Families from Southwest Coastal Bangladesh

By Ryan Threlkel

BACKGROUND

High blood pressure is a leading cause of morbidity and mortality, but it is a complex trait that can be attributed to high sodium intake, other environmental factors, and heritable genetics.

METHODS

Pedigrees were constructed with the RStudio package <Kinship2> and were analyzed alongside phenotype data collected in a cohort study in southwest coastal Bangladesh. The pedigree and phenotype data were run in the statistical software package SOLAR-Eclipse with the pedigree variance-component linkage method, generating heritability estimates for systolic blood pressure and diastolic blood pressure for this population.

RESULTS

This analysis included 1186 individuals with both relationship and phenotype data. This included 467 first degree relationship pairs (sharing about 50% genetics), 152 second degree relationship pairs (25% shared genetics), 152 third degree relationship pairs (12.5% shared genetics), 497 relationship pairs of fourth to seventh degree, and 90 pairs classified as “Other.” However, females in these families tended to be much less related due to patrilineal marriage practices. Heritability analyses demonstrated a significant heritability value of 0.176 (SE 0.089) for unstratified DBP. Other estimates were not statistically significant, but unstratified SBP was estimated at 0.113 (SE 0.087). DBP among females was estimated at 0.439 (SE 0.280) and SBP among females was estimated at 0.131 (SE 0.302). Male SBP and DBP heritability estimates were unable to be calculated.

DISCUSSION

The differences between males and females in relatedness were unsurprising since this is a patrilineal society. However, the lack of normality among males was surprising. This could indicate inaccurate relationships in the study’s pedigree data. The only heritability estimate that was significant was overall DBP, providing evidence that among this population, about 17.6% of the variation in DBP was explained by heritable genetics.

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BACKGROUND

Hypertension is a major cause of mortality and morbidity worldwide with complex etiology (1). Hypertensive individuals are thought to have a genetic predisposition along with exposure to certain environmental influences, and increased salt intake is a possible risk factor for increased blood pressure (2). A global burden of disease study in 2014 collected sodium intake data from surveys and conducted a comparative risk assessment to determine that 1.65 million deaths due to cardiovascular causes in 2010 were attributed to high sodium intake (3). However, blood pressure is a complex trait with numerous genetic and environmental factors (4). Previous studies have estimated the heritability of blood pressure to be about 40%, and the phenotypic variance due to genetics has been estimated between 30% and 70% (4, 5). For the purposes of this study, the focus was on blood pressure traits in families from the southwest coastal region of Bangladesh. The study region consists of three districts of Bangladesh: Bagerhat, Khulna, and Satkhira (6). These are rural districts with fewer than 750 people per square kilometer (6). In rural areas such as these, people primarily live in villages. Generally, households in these villages are extended with married sons occupying the same household as their father and remaining under his authority throughout his lifetime (7). When daughters marry, they will relocate to the household of their husbands (7). These households usually stay together until the head of the household dies, after which the sons within the household will divide into their own respective households (7). The aim of this study was to determine the heritability of systolic blood pressure (SBP) and diastolic blood pressure (DBP) among this population.

METHODS

Study Population

The blood pressure and relationship data that were the foundation for this study were collected in a cohort study in the southwest coastal population of Bangladesh.

Participants were surveyed to collect demographic data as well as family relationships, which were used to develop family trees for each family. An intervention was also conducted that reduced salt intake from drinking water in some participants. Households were visited five times during the study period to measure SBP and DBP between December 2016 and April 2017. This cohort included 1186 individuals from 297 families with both heritability and blood pressure data available (8).

Data Collection Protocols

For this study, the five longitudinal measures of SBP and DBP were used to estimate a person-level random intercept to be used as the outcome for this heritability analysis.

These person-level random intercept predictions were extracted from a multilevel model for longitudinal blood pressure, which included random effects for person, household, and community, adjusted for participant age and whether the blood pressure was measured during a community-level salt-intake-reduction intervention

The previously collected family trees were summarized as a pedigree file using RStudio, version 1.2.5033 (Boston, Massachusetts), with R, version 3.6.2 and the R package <Kinship2> (9, 10). This pedigree file was used alongside the blood pressure phenotype data for analysis using SOLAR-Eclipse: An Imaging Genetics Software version 8.5.1.

(11). SOLAR-Eclipse was initially used to produce counts of relationship types in the study. Overall relationships were shown as well as stratified by sex because there was a suspected difference in relationships between males and females.

Statistical Approach

Next, the heritability estimates were produced for SBP and DBP (overall and stratified by sex). This was performed with SOLAR-Eclipse as variance-components analysis, per the formula $\Omega = 2\Phi\sigma_g^2 + I\sigma_e^2$ (where Ω is the phenotypic covariance between relatives, Φ is the kinship matrix, σ_g^2 is the phenotypic variance due to additive genetics, I is the identity matrix, and σ_e^2 is phenotypic variance due to the environment) (11). This resulted in estimates for the phenotypic variance due to additive genetics (σ_g^2) and residual variance (σ_e^2), allowing the program to calculate h^2 (heritability) by dividing phenotypic variance due to additive genetics by total phenotypic variance ($h^2 = \frac{\sigma_g^2}{\sigma_p^2}$). Outcomes that were identified as non-normal by SOLAR-Eclipse were transformed via inverse normalization prior to variance-component heritability analysis (12).

RESULTS

Table 1 summarizes the relationship types and number of pairs for the cohort. There were 1186 individuals participating in the study, but additional individuals without blood pressure trait data were included in the pedigree file as placeholders to preserve reported family relationships between the study participants (e.g. grandparents may be included to show that the grandchildren are related, but the grandparents may not have been participants in the study or included in the final analysis since they lack phenotype data). There were 467 first-degree relationship pairs (relationship coefficient of 0.5), 152 second-degree relationship pairs (relationship coefficient of 0.25), 152 third-degree relationship pairs (relationship coefficient of 0.125), 177 fourth-degree relationship pairs (relationship coefficient of 0.0625), 148 fifth-degree relationship pairs (relationship coefficient of 0.0313), 112 sixth-degree relationship pairs (relationship coefficient of 0.0156), 60 seventh degree relationship pairs (relationship coefficient of 0.0078), and 90 relationship pairs designated as “Other.”

Tables 2 and 3 indicate differences in the structure of family relationships among the male (N=483) and female (N=703) participants in the cohort. Males in these households tended to be more related to each other while females tended to be unrelated (e.g., women have married into families). The male-male relationships in Table 2 seem similar to the overall relationships in Table 1, but the females in Table 3 tend to be unrelated. Despite the larger number of participants, the 703 females constitute only 58 relative pairs (Table 3), whereas the n=483 males in constitute 910 relative pairs (Table 2).

The distribution of family sizes in the cohort are shown in Table 4. Family sizes can range from 3 members (two parents and an offspring) to 69 members. However, these numbers include family members that do not have phenotype data. For example, the pedigree plot of the largest family (N=69) is illustrated in Figure 1, and the relationships counts are shown in Table 5. This family consists of 37 individuals that participated in the study and had phenotype data collected, while the other 32 individuals were included in the pedigree to show relatedness between the participating family members. Some of the three-person families (example in Figure 2) only included one study participant and two parents that did not participate.

Heritability estimates are shown in Table 6. Analyses were conducted for the overall cohort as well as stratified by sex. The unstratified analysis showed a DBP heritability estimate of 0.176 (SE of 0.089 and p-value of 0.022) and an SBP heritability estimate of 0.103 (SE of 0.087 and p-value of 0.332). Females had a DBP heritability estimate of 0.439 (SE of 0.280 and p-value of 0.057) and an SBP heritability estimate of 0.131 (SE of 0.302 and p-value of 0.332). SOLAR-Eclipse was not able to generate heritability estimates for males.

DISCUSSION

The difference in relationships between males and females are unsurprising, since Bangladesh is a patrilineal society. Brothers tend to stay together in the same household clusters, while sisters leave the village and join another household after getting married. This can be seen in Figure 1, where the descendants that are still part of the family are males, while the females are spouses of these individuals. This also explains why the females in households tend to be less related to each other as shown in Table 3. However, one surprising finding was the random effect estimates being non-normal. As shown in Table 6, many of these had to be inverse normalized during the analysis to account for a non-normal outcome variable. This could be due to inaccurate relationships in the pedigrees.

The heritability estimates in Table 6 seem to imply that DBP is more heritable than SBP, and both are more heritable among females. However, this is not conclusive because most of the heritability estimates had insignificant p-values. The only heritability estimate that was significant was overall DBP, providing evidence that among this population, about 17.6% of the variation in DBP was explained by heritable genetics. However, these findings are conditional on the reported pedigrees.

Strengths and Weaknesses

This study has a large sample size, which provides a lot of power to the analysis. Being a cohort study with multiple blood pressure measurements, the likelihood of biases and measurement errors are also reduced. However, the lack of a normalized heritability

outcome here is concerning, although it was addressed with inverse normalization. The heritability outcomes here are also much lower than previous studies, which usually report SBP and DBP heritabilities of ~ 40% (4). This could be due to information bias from a few sources of error; one of which being data entry error in the pedigrees. Non-paternity is also possible, which has been estimated by previous studies to occur at rates ranging from 0.8% to 30%, (median 3.7%) (13). Additionally, pedigree data used in this study did not identify identical twins, so treating any identical twins who may have participated in this study as siblings with relationship coefficients of 0.5 would underestimate relatedness of these pairs.

Future Directions

Given the concerns of pedigree accuracy, the next steps are to verify the pedigree accuracy for the study population through genotyping. Pedigrees can be validated by laboratory detection of genetic markers to verify relationships in the pedigree (14). If inconsistencies are detected, the pedigree could be corrected, and this heritability analysis could be conducted again.

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TABLES

Table 1. Overall relationships of study participants (N=1186)

Degree	Shared Genetics	Relationship	Number of Pairs	Totals
First	1/2	Siblings	150	467
		Parent-offspring	317	
Second	1/4	Avuncular	131	152
		Grandparent-grandchild	17	
		Half-siblings	9	
Third	1/8	First cousins	136	152
		Grand avuncular	13	
		Great grandparent or half avuncular	3	
Fourth	1/16	First cousins, once removed	163	177
		Half first cousins	11	
		Half grand avuncular	3	
Fifth	1/32	First cousins, twice removed	14	148
		Half first cousins, once removed	11	
		Double second cousins, once removed	6	
		Second cousins	117	
Sixth	1/64	Second cousins, once removed	100	112
		Half first cousins, twice removed	6	
		Double second cousins, twice removed	2	
		Half second cousins	4	
Seventh	1/128	Third cousins	32	60
		Half second cousins, once removed	2	
		Second cousins, twice removed	26	
N/A	N/A	Other	90	90
Total relative pairs				1358

Table 2. Relationships between male study participants (N=483)

Degree	Shared Genetics	Relationship	Number of Pairs	Totals
First	1/2	Siblings	116	212
		Parent-offspring	96	
Second	1/4	Avuncular	114	123
		Grandparent-grandchild	5	
		Half-siblings	4	
Third	1/8	First cousins	108	121
		Grand avuncular	11	
		Half-avuncular	2	
Fourth	1/16	First cousins, once removed	122	130
		Half first cousins or half grand avuncular	8	
Fifth	1/32	First cousins, twice removed	12	102
		Half first cousins, once removed	9	
		Double second cousins, once removed	3	
		Second cousins	78	
Sixth	1/64	Second cousins, once removed	84	94
		Half first cousins, twice removed	6	
		Double second cousins, twice removed or half second cousins	4	
Seventh	1/128	Third cousins	24	47
		Half second cousins, once removed	2	
		Second cousins, twice removed	21	
N/A	N/A	Other	81	81
Total relative pairs				910

Table 3. Relationships between female study participants (N=703)

Degree	Shared Genetics	Relationship	Number of Pairs	Totals
First	1/2	Siblings	12	41
		Parent-offspring	29	
Second	1/4	Avuncular or grandparent-grandchild	4	4
Third	1/8	First cousins	7	7
Fourth	1/16	First cousins, once removed	1	1
Fifth	1/32	Second cousins	4	4
Eighth	1/256	Third cousins, once removed	1	1
Total relative pairs				58

Table 4. Distribution of pedigree sizes

Number of family members	Counts of families
3	36
4	112
5	18
6-10	72
11-15	22
16-20	11
21-25	8
26-30	3
31-35	5
36-40	5
41-45	2
46-50	1
51-55	0
56-60	0
61-65	1
66-69	1
Total number of families	297

Table 5. Relationships of study participants in largest family (N=37)

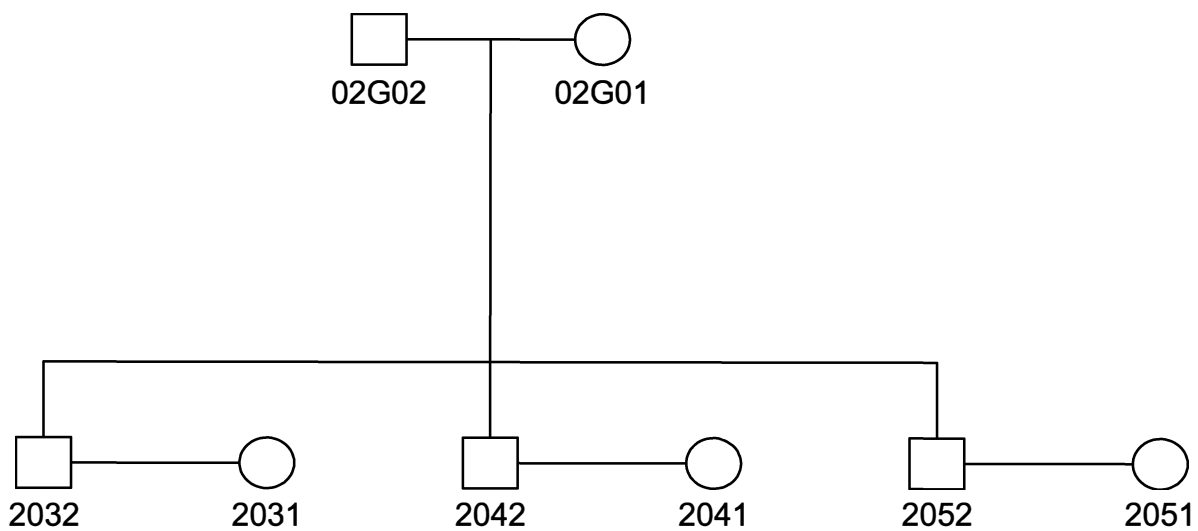
Degree	Shared Genetics	Relationship	Number of Pairs	Totals
First	1/2	Siblings	6	18
		Parent-offspring	12	
Second	1/4	Avuncular	14	14
Third	1/8	First cousins	7	7
Fourth	1/16	First cousins, once removed	6	6
Fifth	1/32	Second cousins	11	11
Sixth	1/64	Second cousins, once removed	18	30
		Half first cousins, twice removed	12	
Seventh	1/128	Third cousins	10	10
N/A	N/A	Other	48	48
Total relative pairs				144

Table 6. Heritability analysis of adjusted random intercepts, stratified by sex

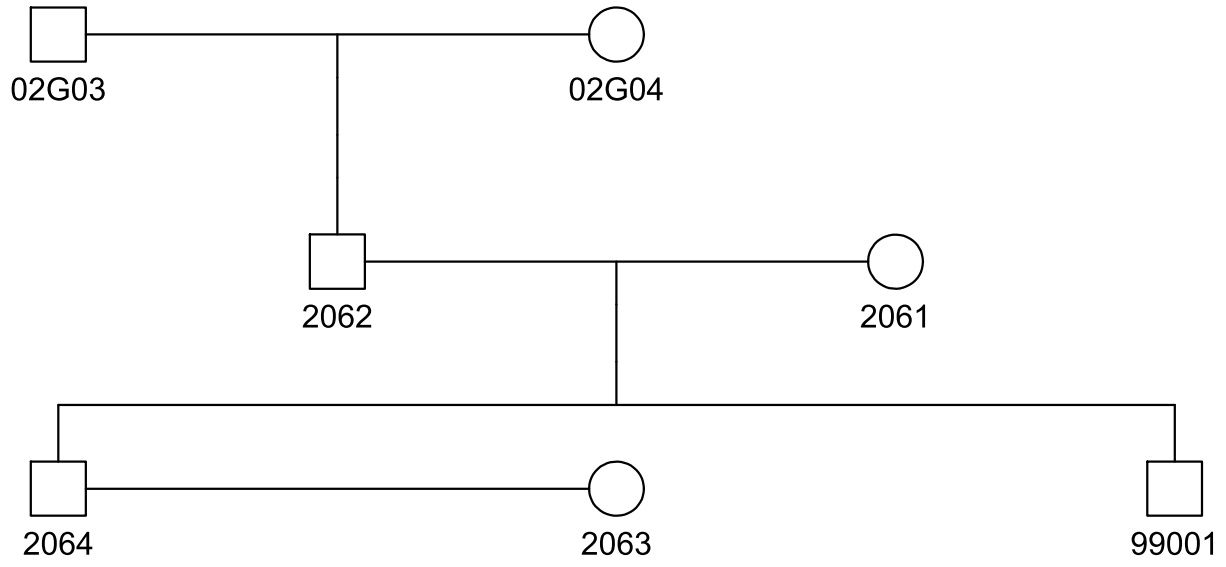
Model	N	Heritability	P-value	Standard error	Inverse normalized
Overall DBP	1186	0.176	0.022	0.089	No
Female DBP	703	0.439	0.057	0.280	Yes
Male DBP	483	0.000	0.500	--	No
Overall SBP	1186	0.103	0.113	0.087	Yes
Female SBP	703	0.131	0.332	0.302	Yes
Male SBP	483	0.000	0.500	--	Yes

APPENDIX: Pedigree plots of all families

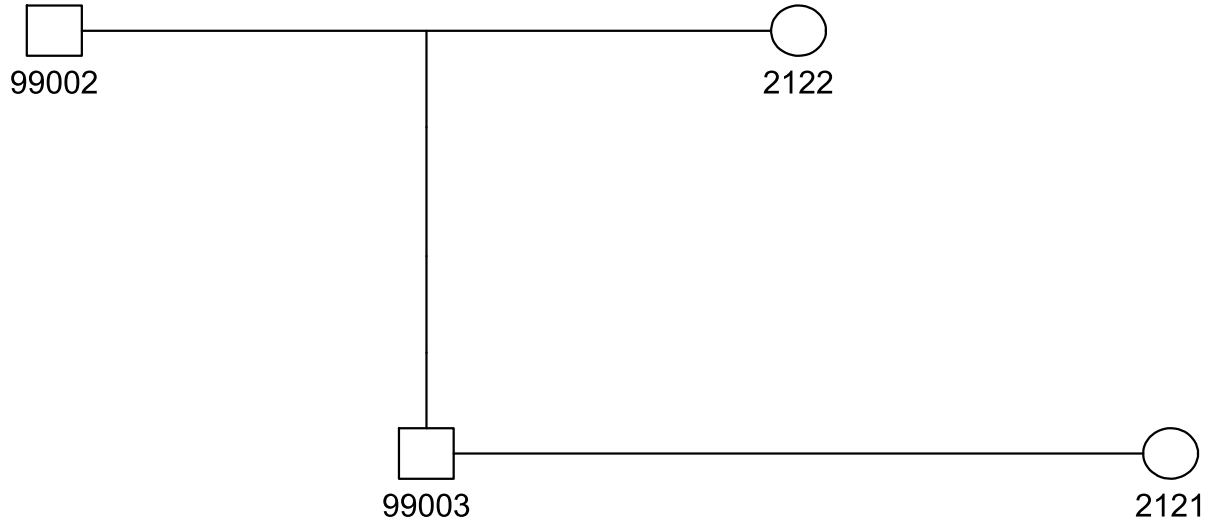
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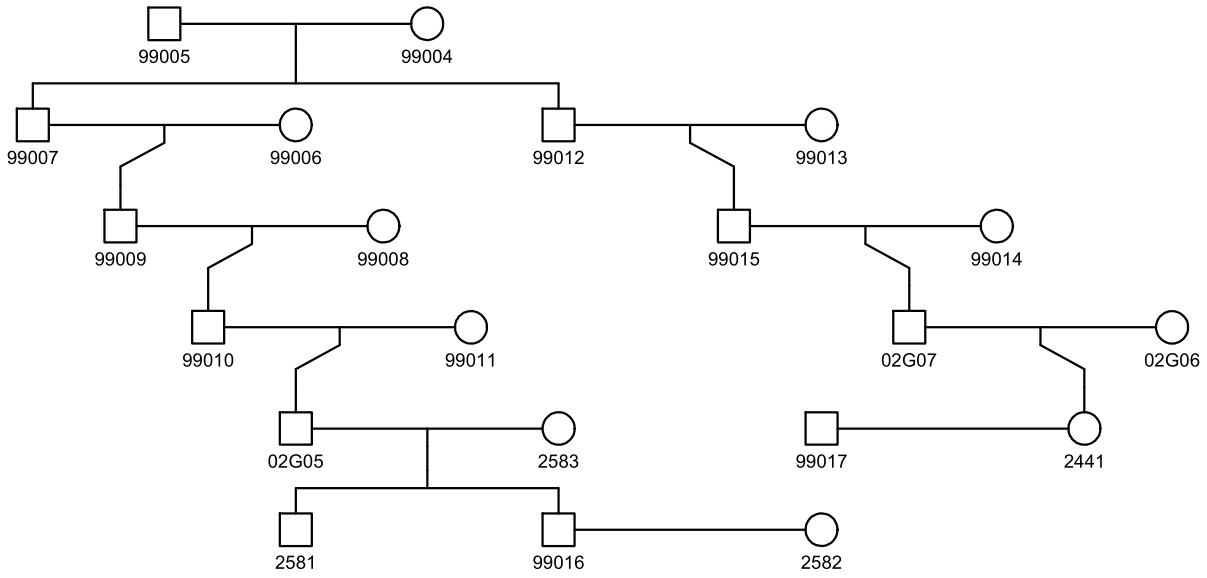
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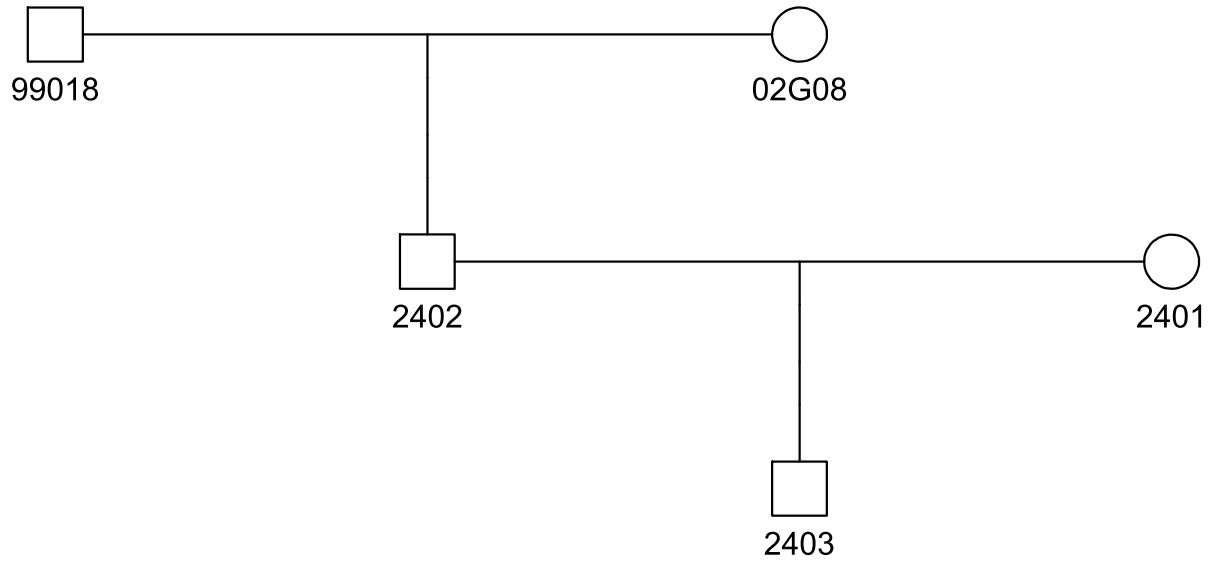
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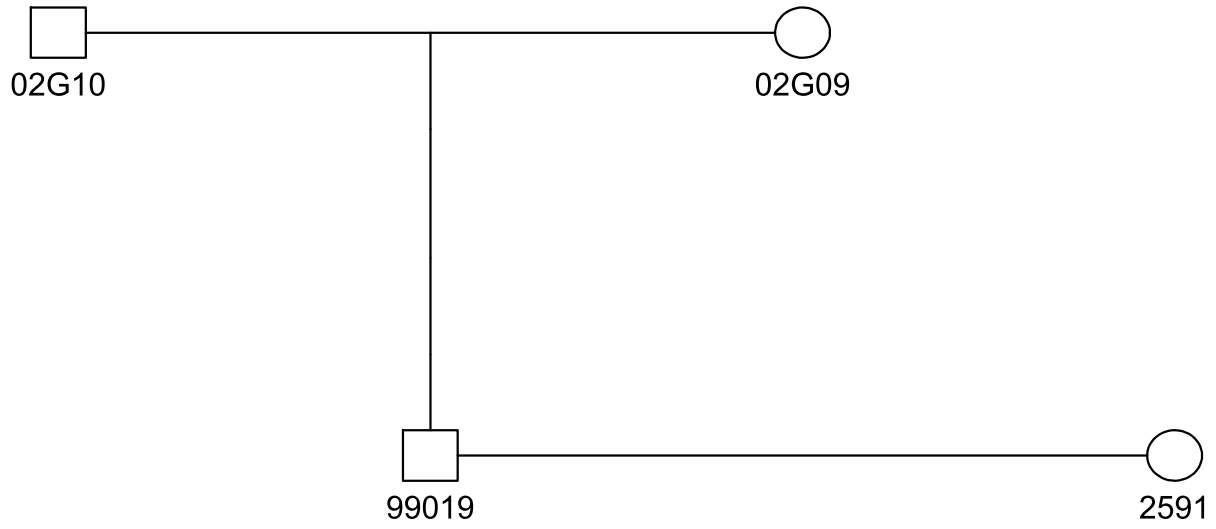
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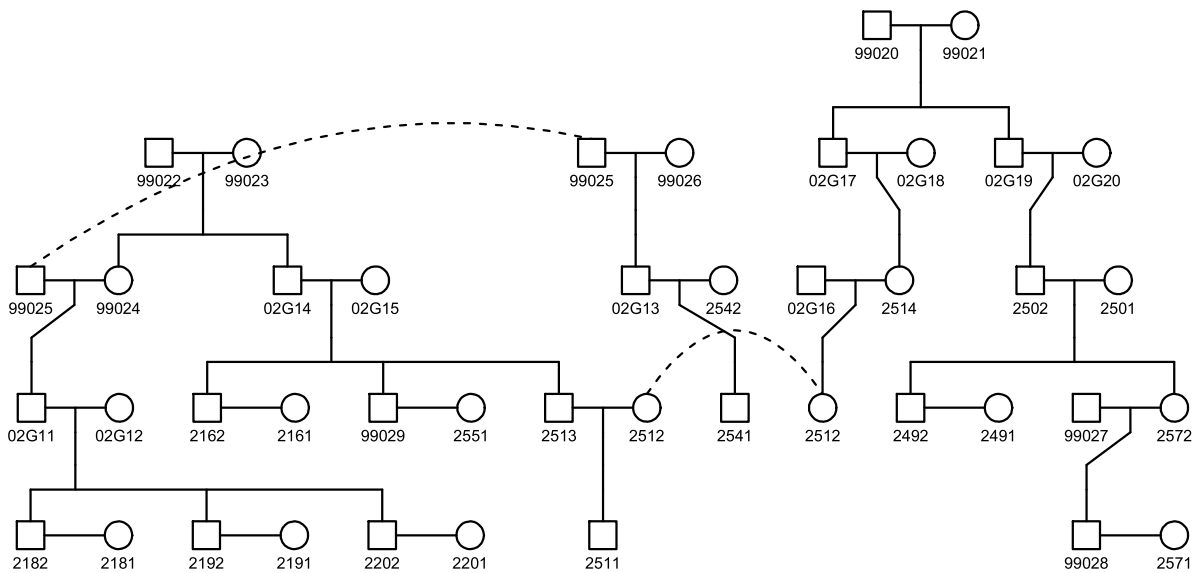
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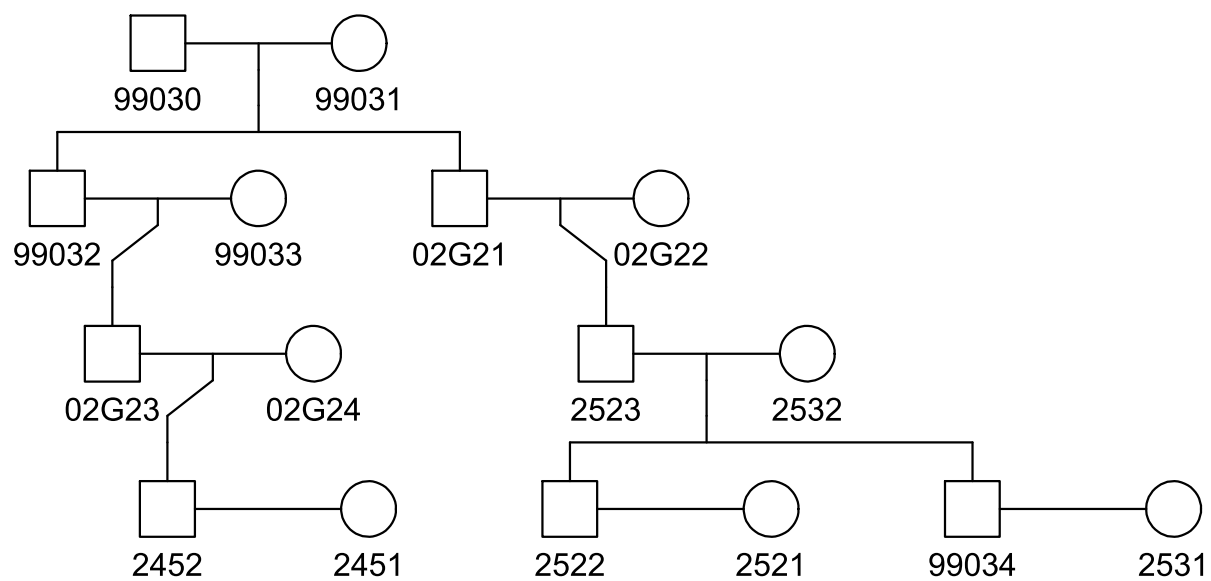
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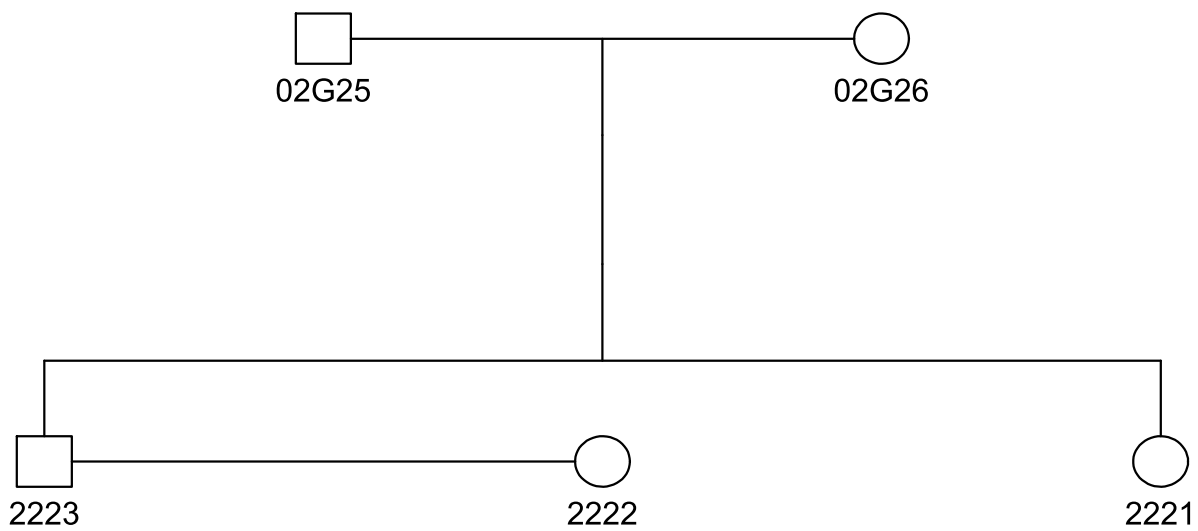
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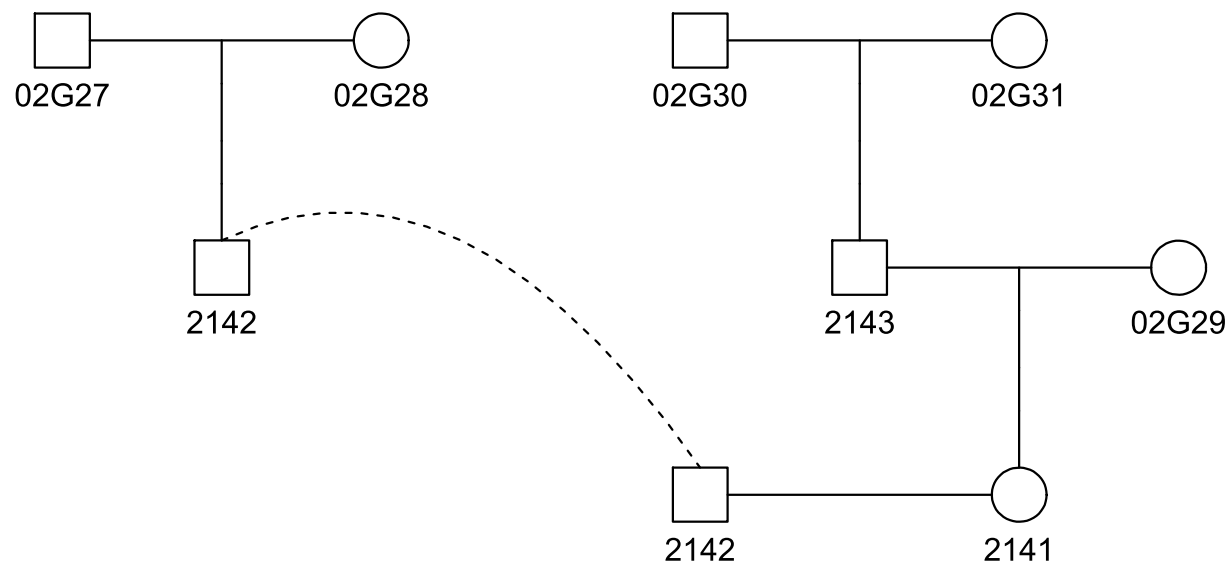
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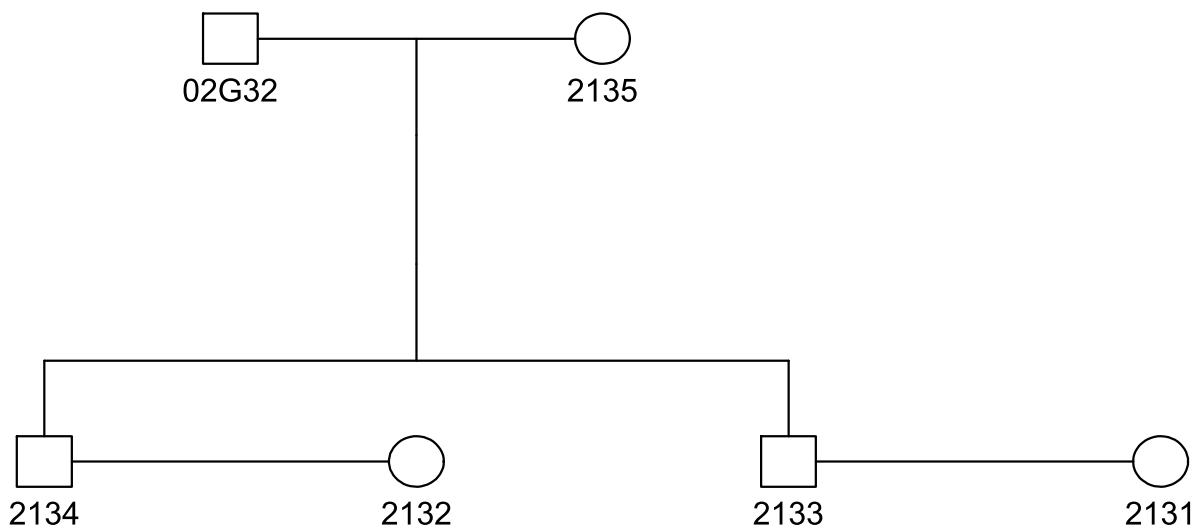
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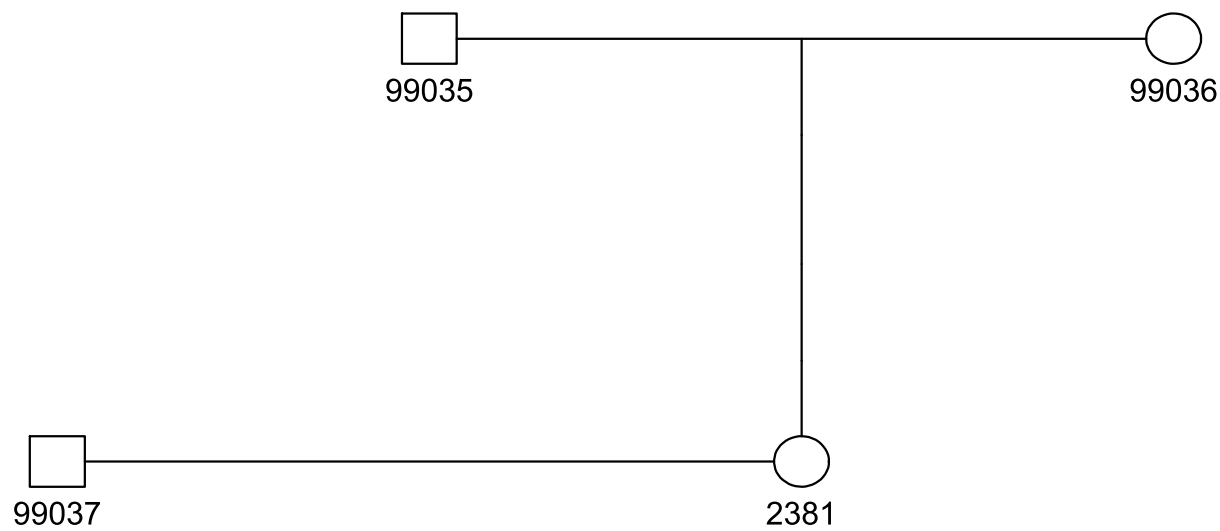
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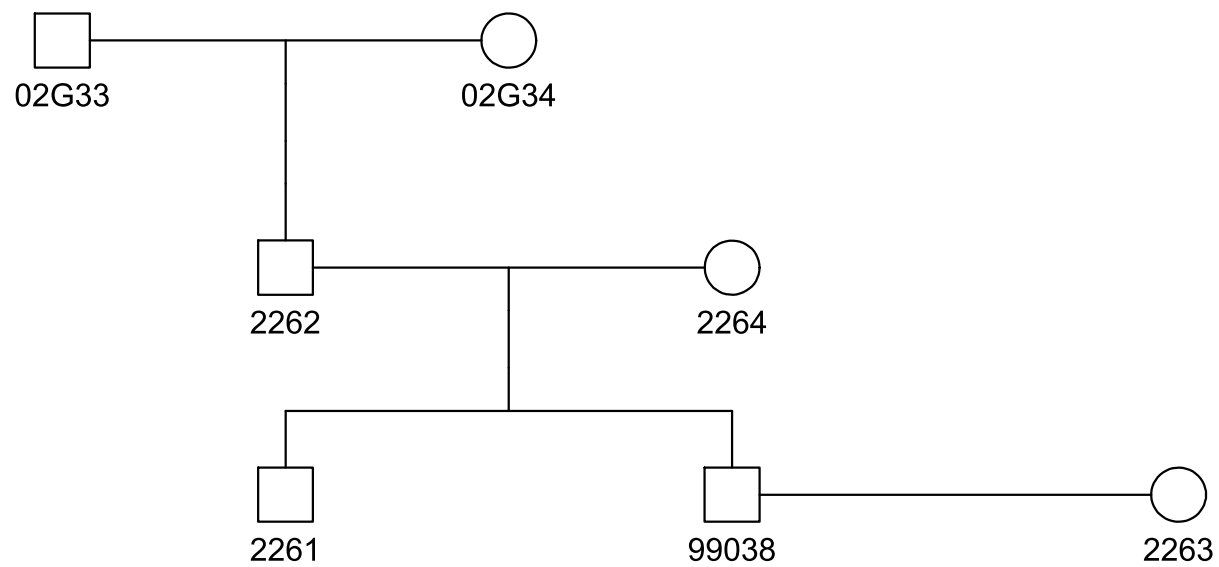
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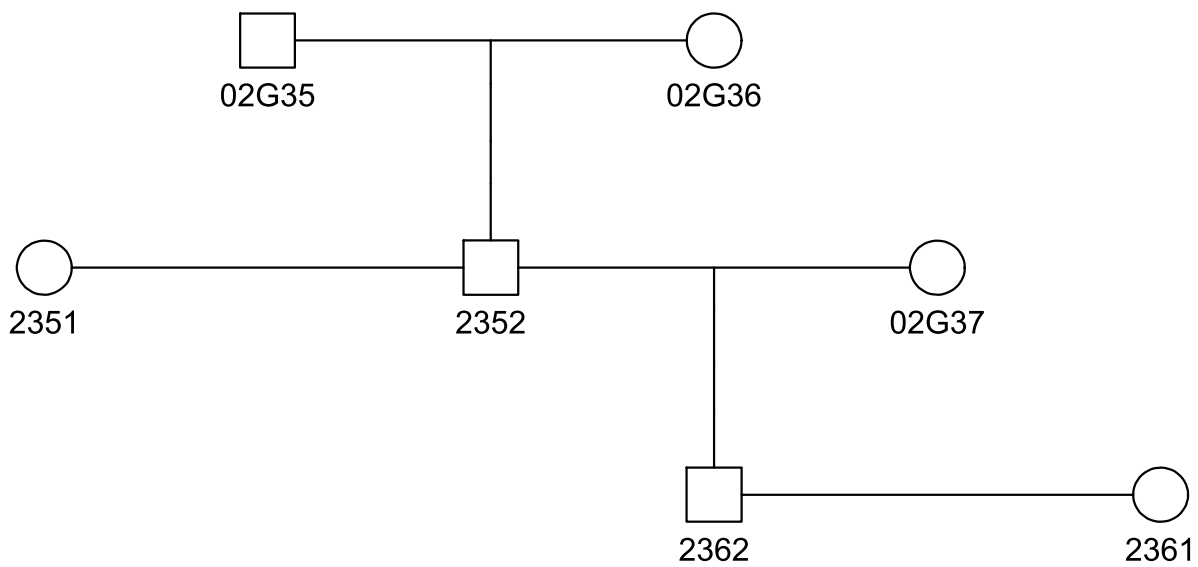
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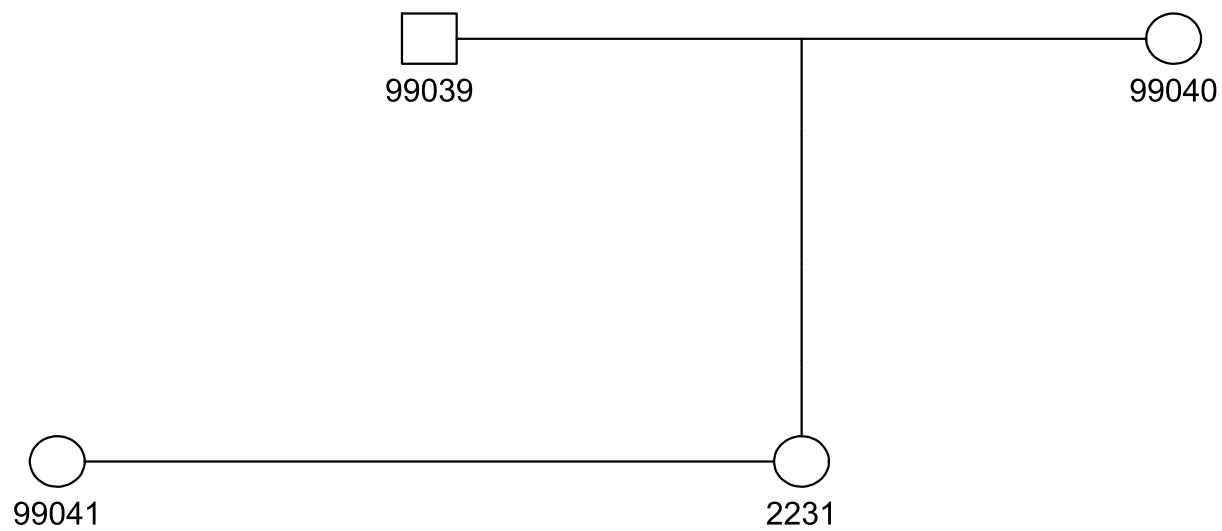
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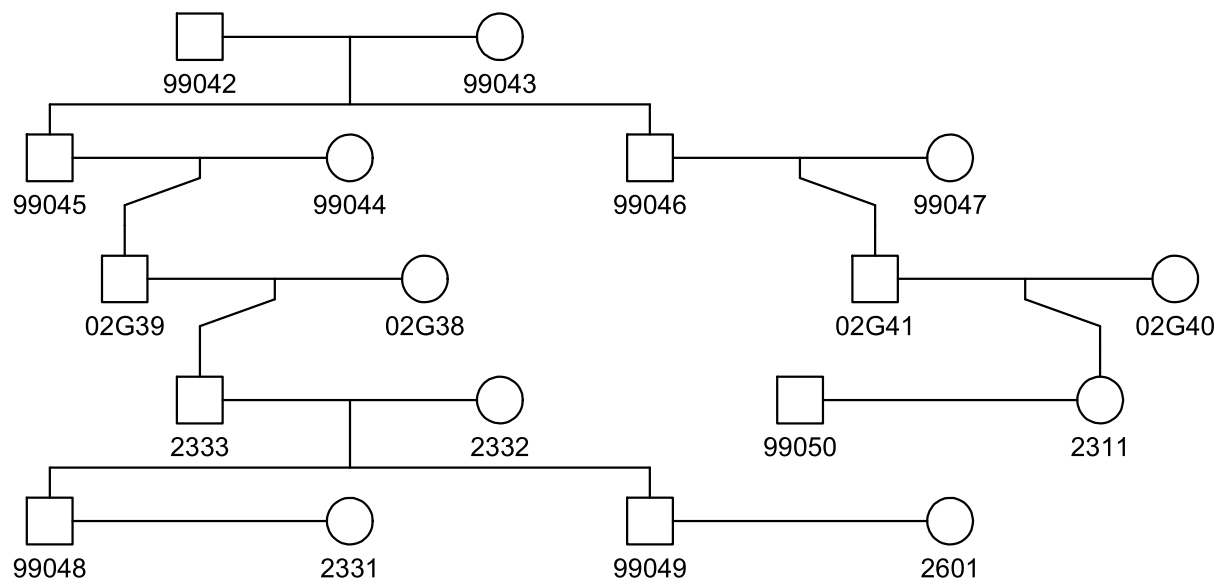
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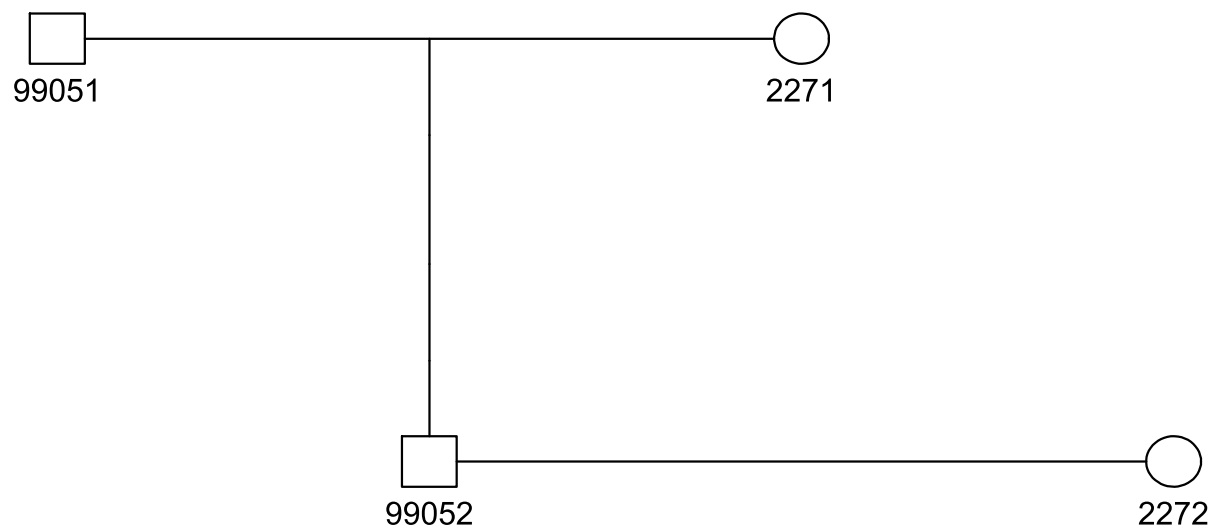
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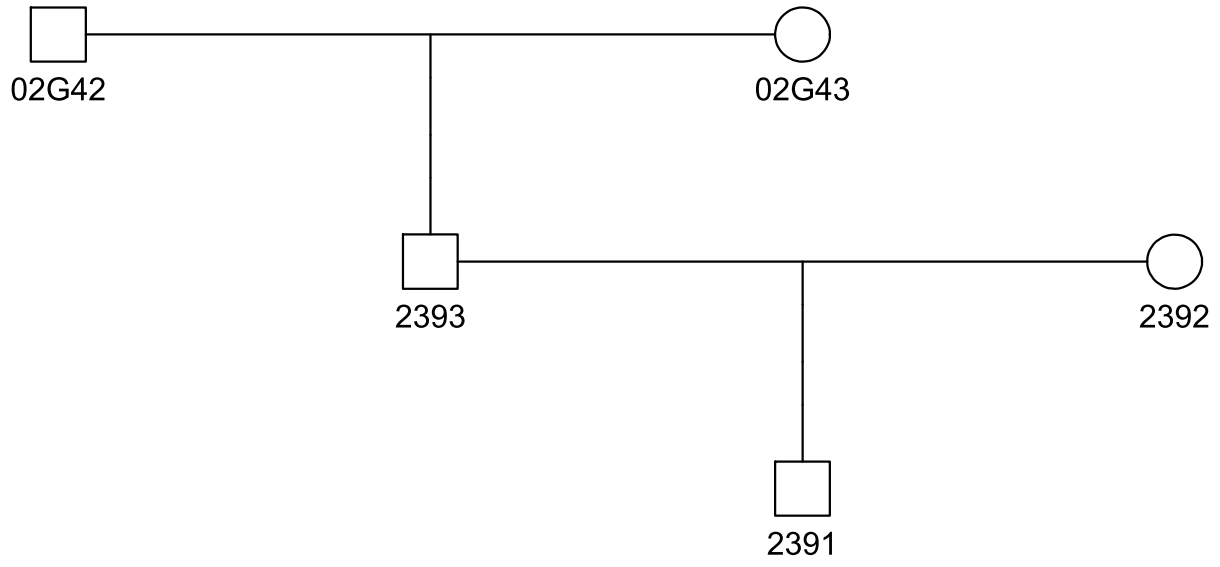
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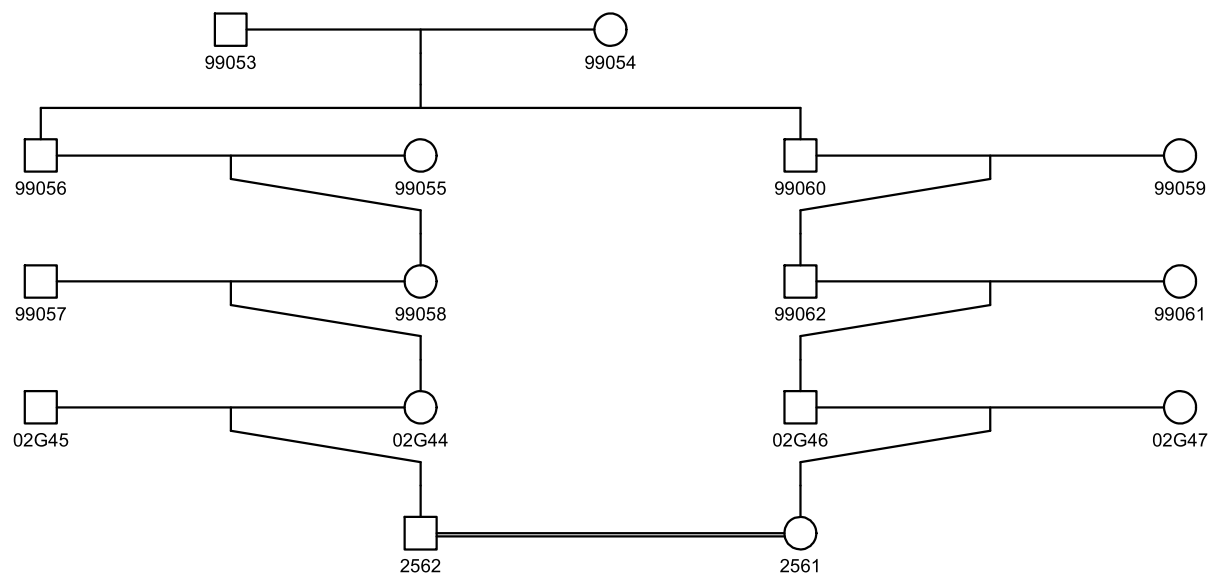
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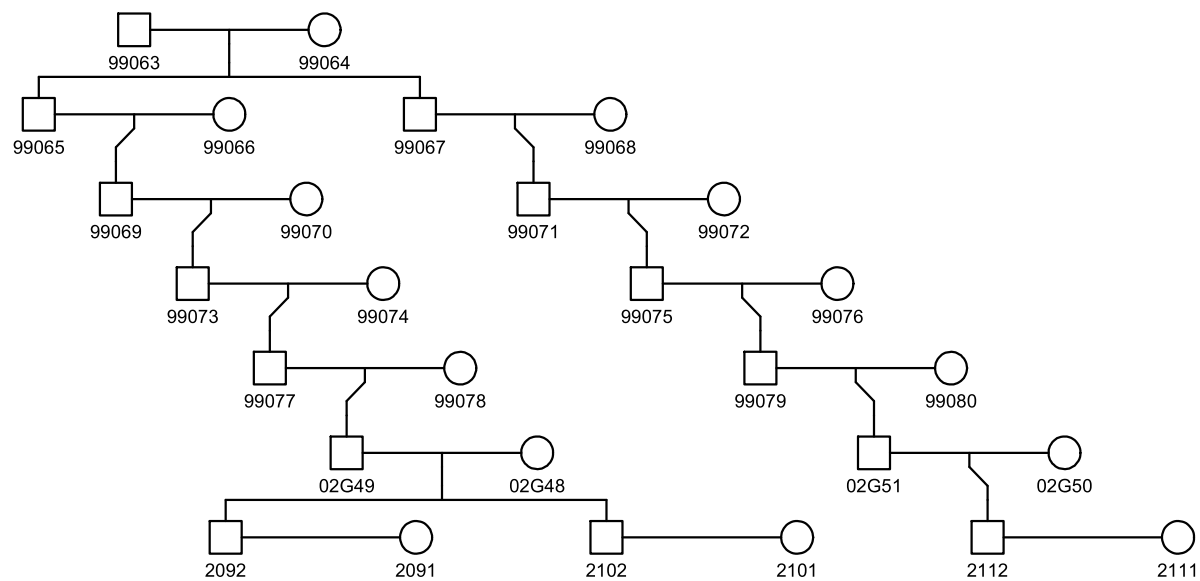
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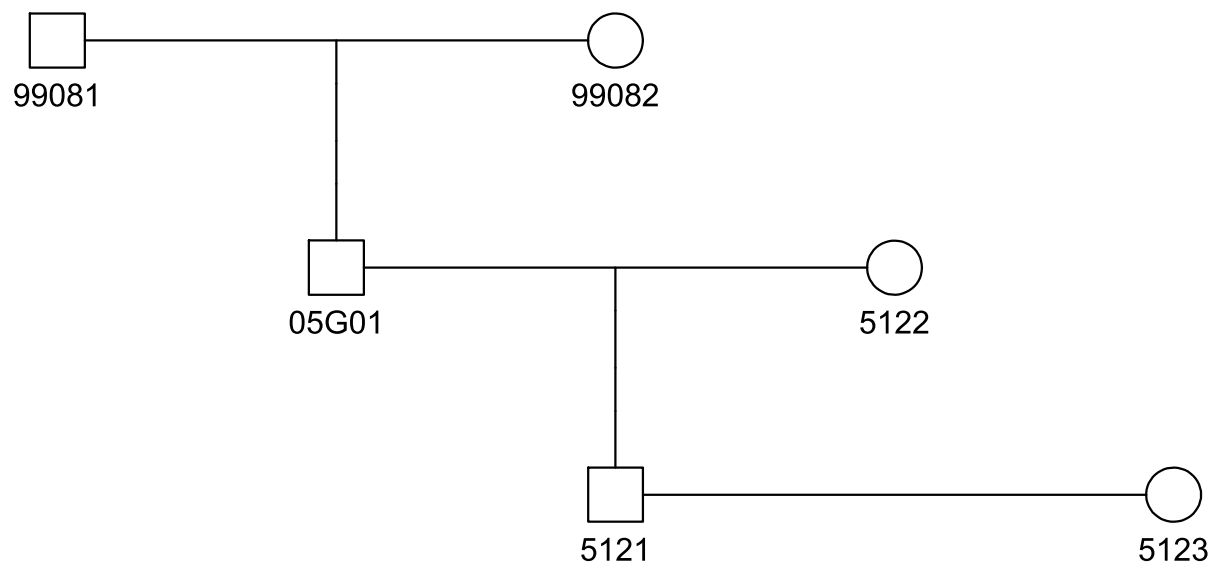
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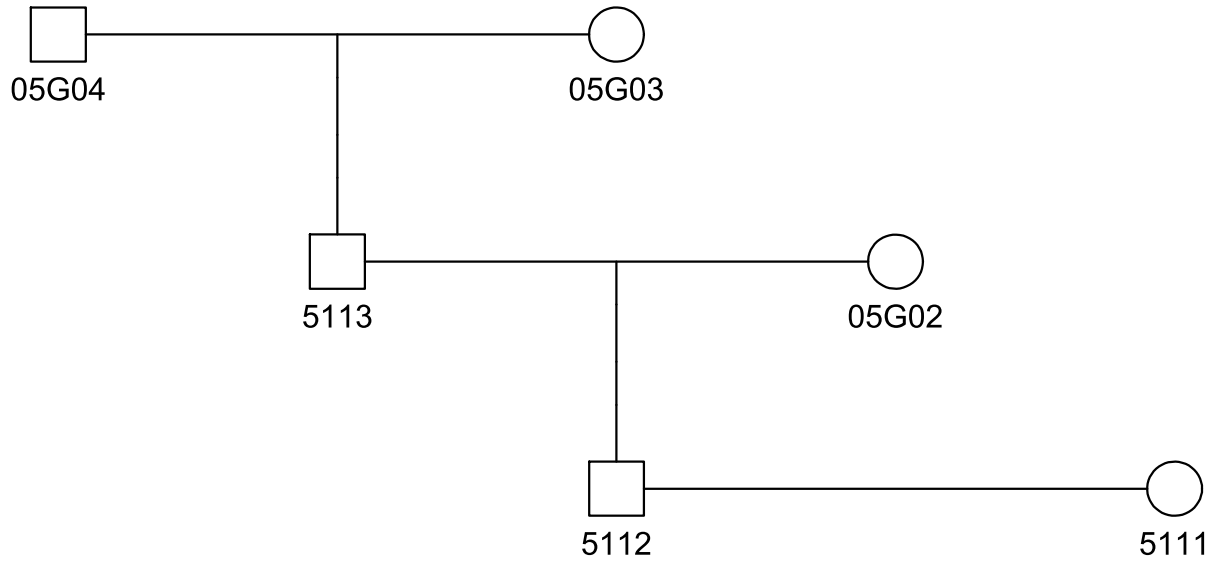
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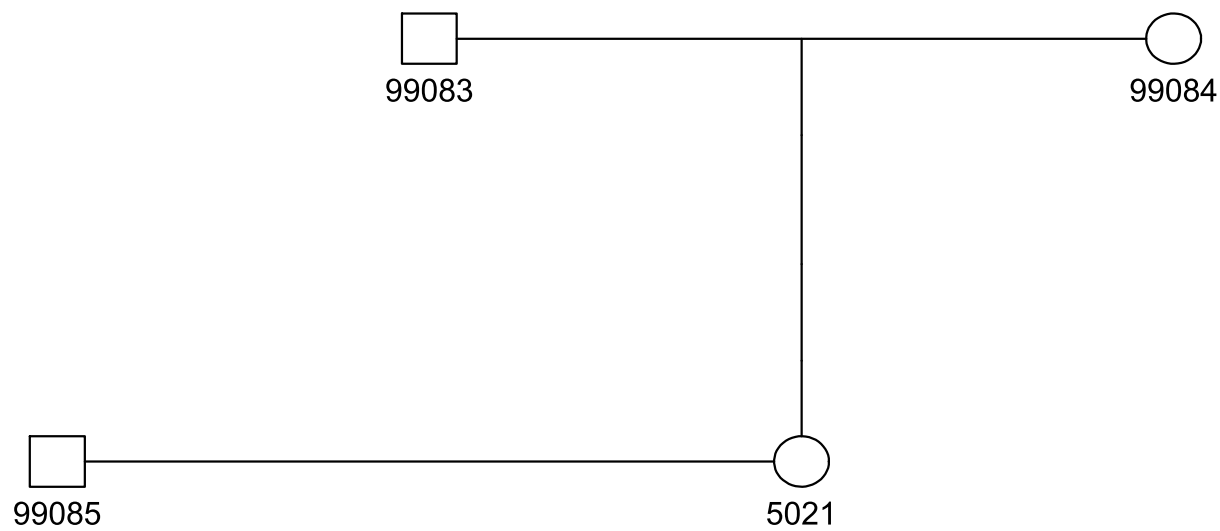
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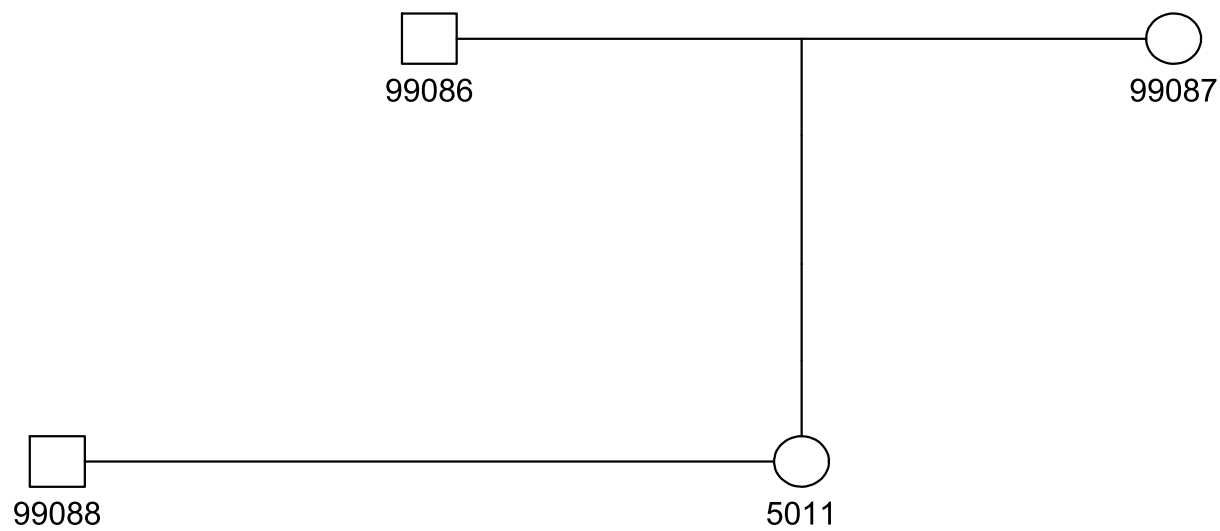
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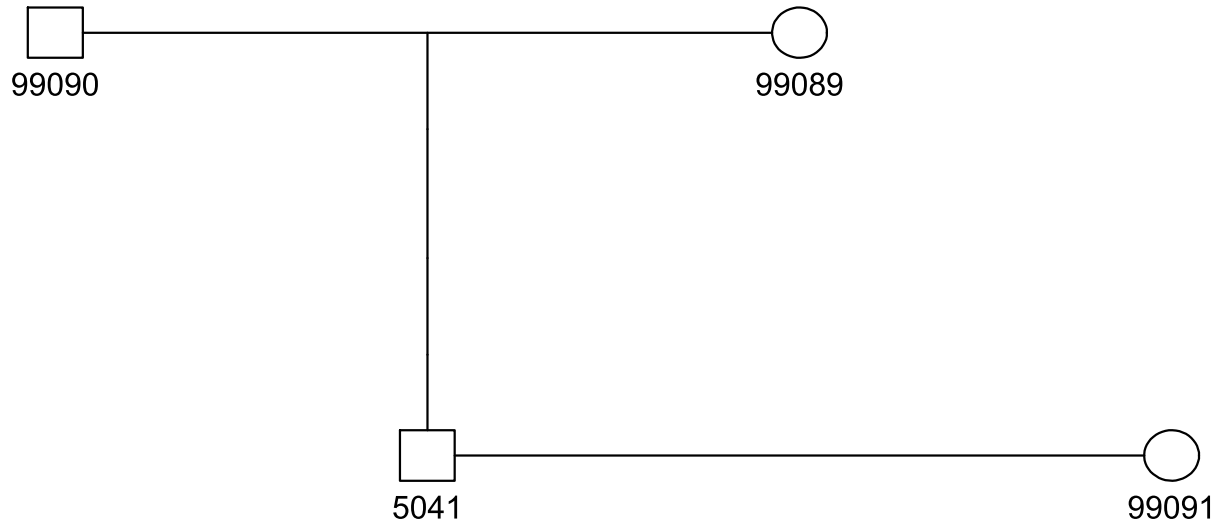
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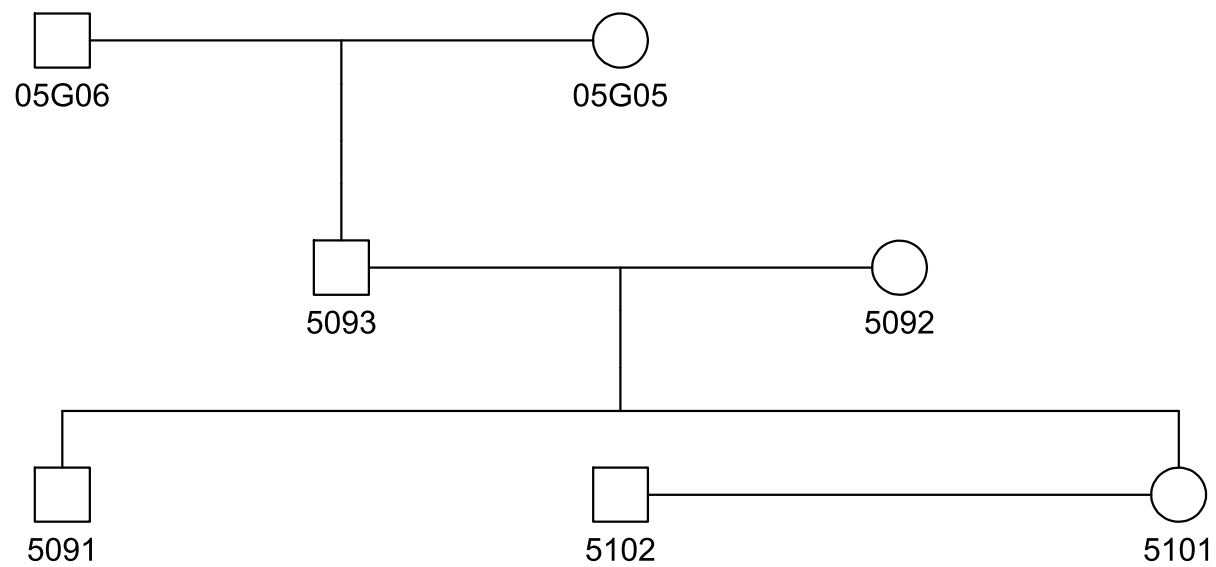
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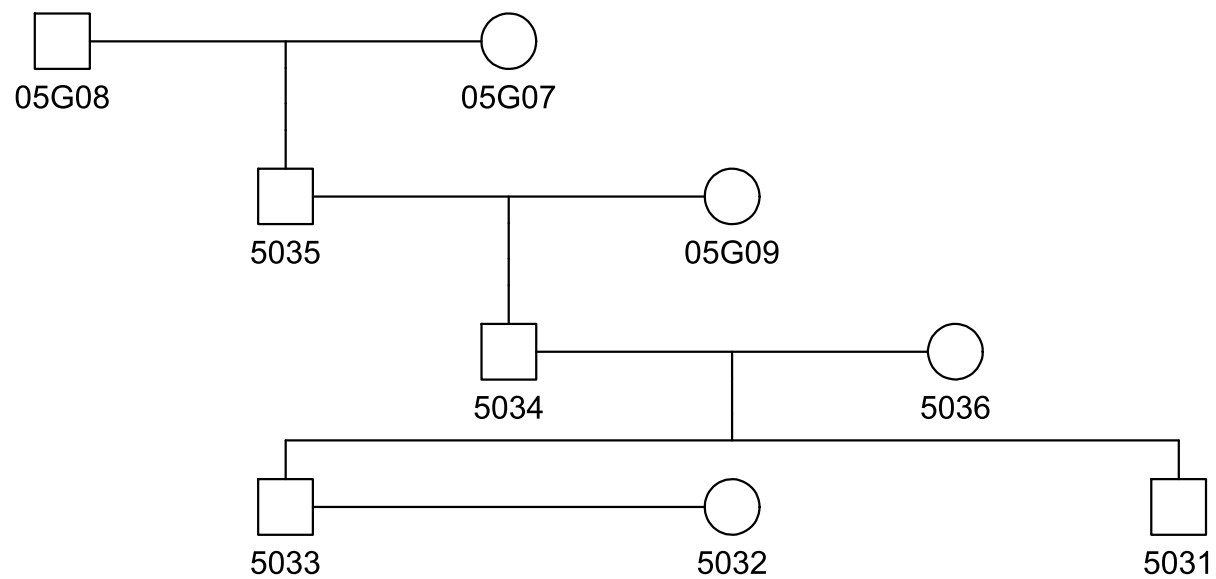
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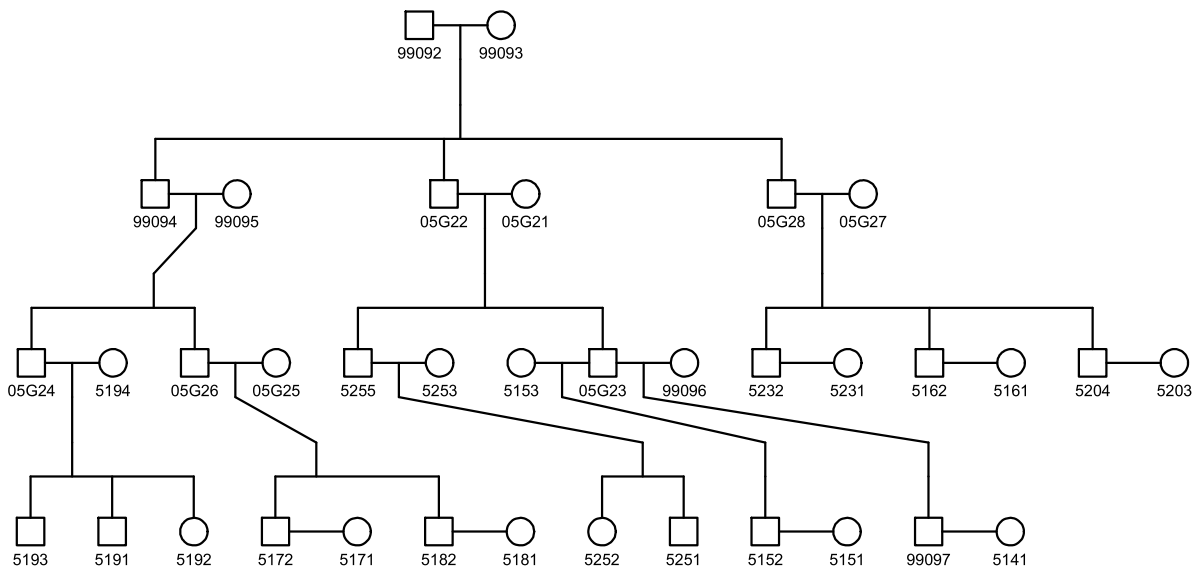
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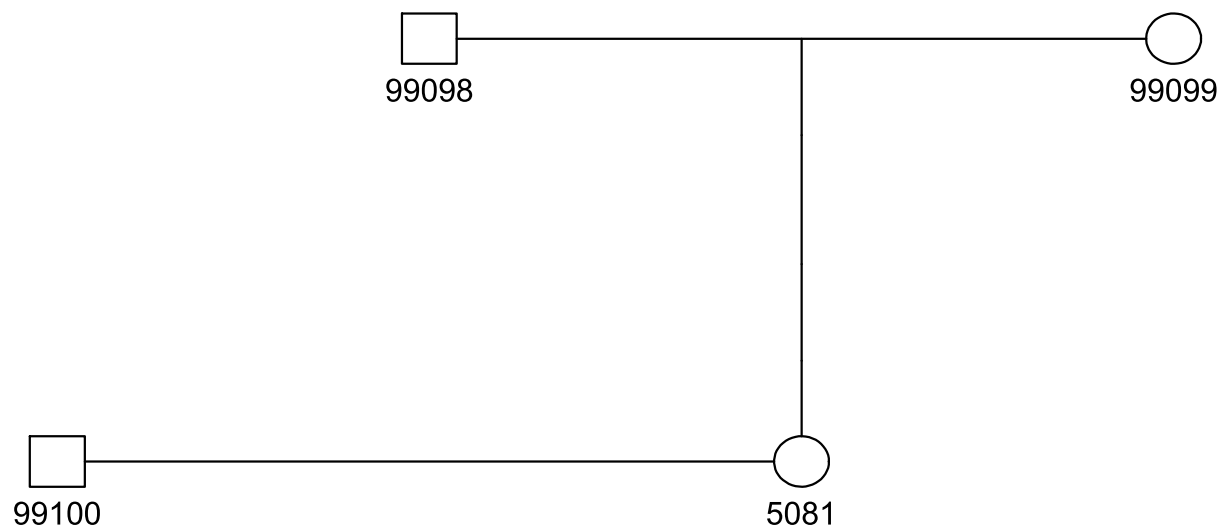


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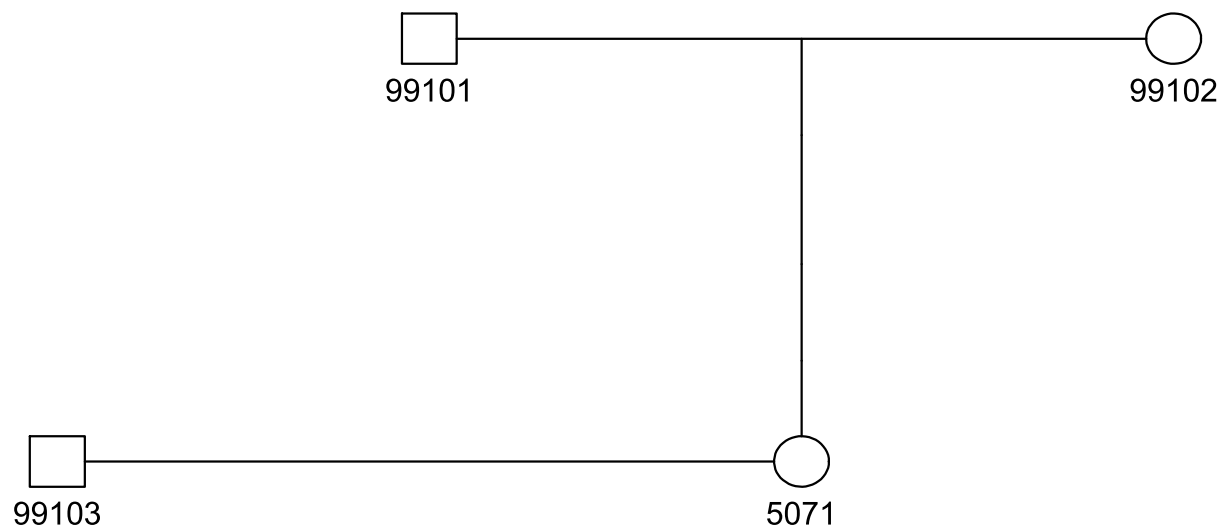


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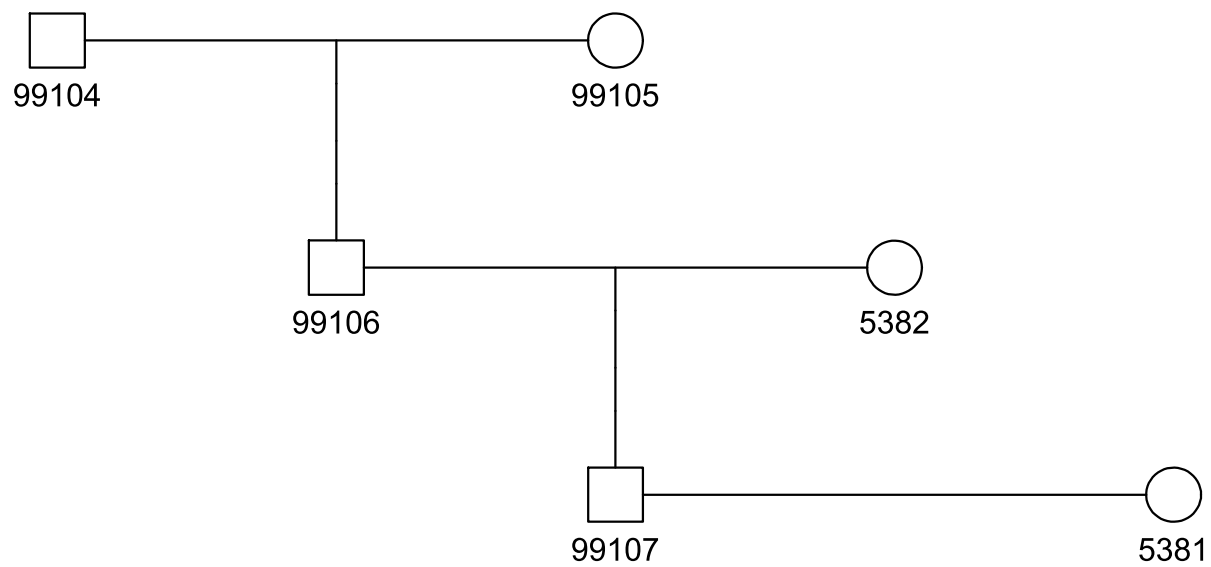
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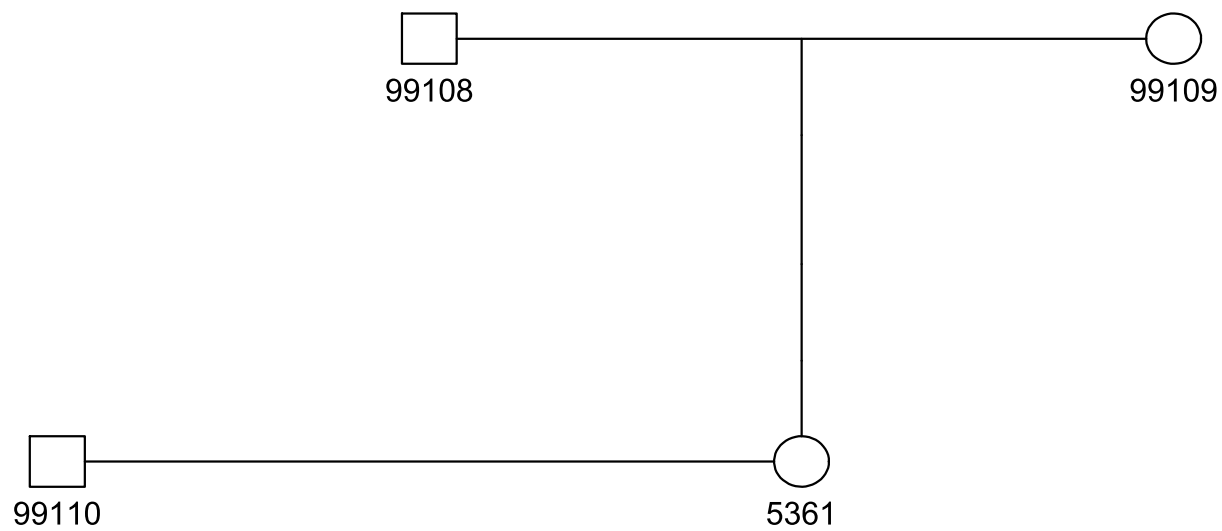
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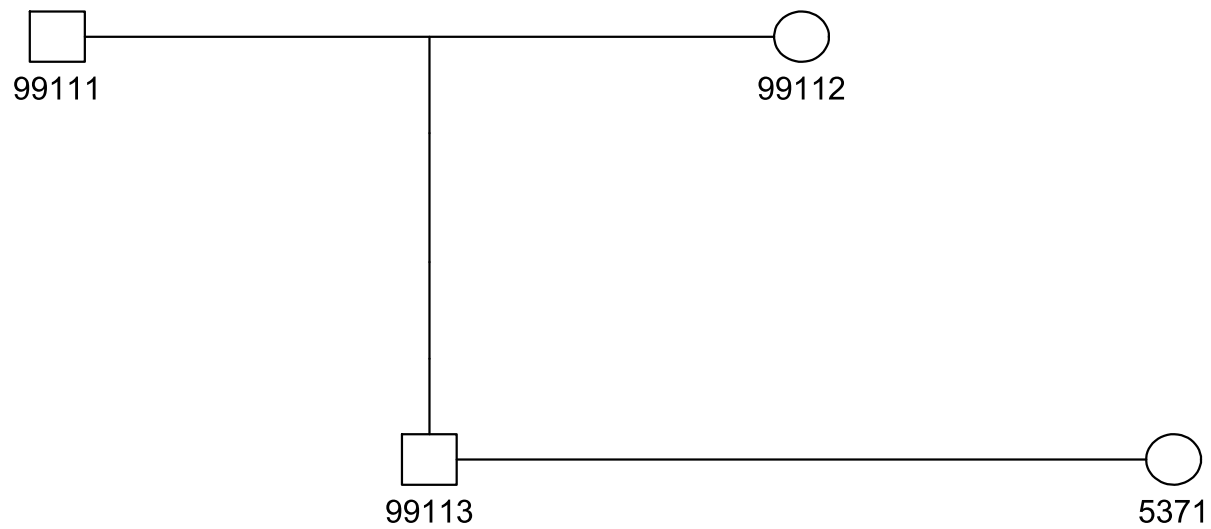
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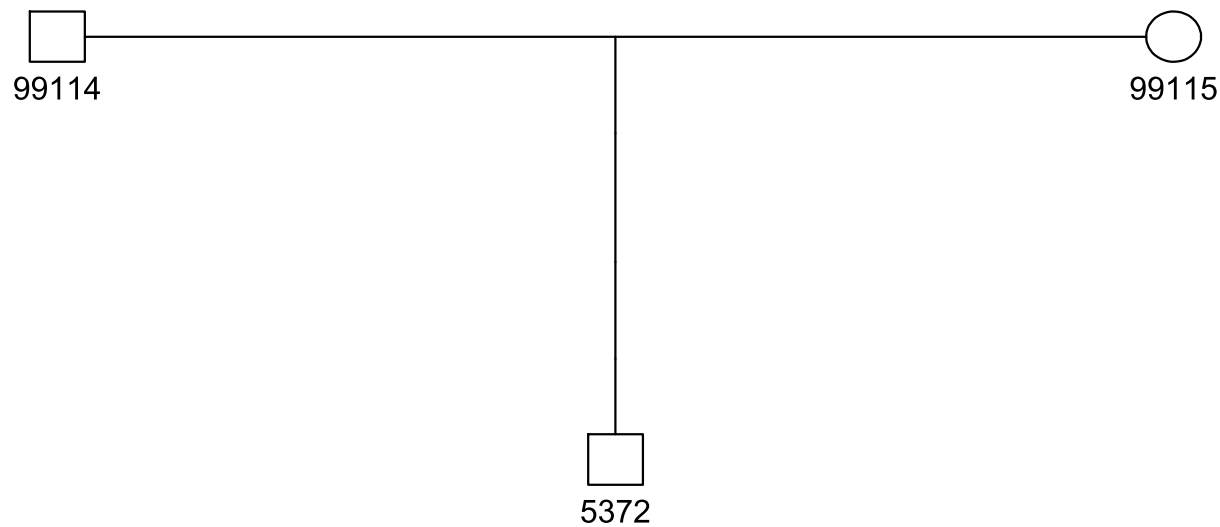
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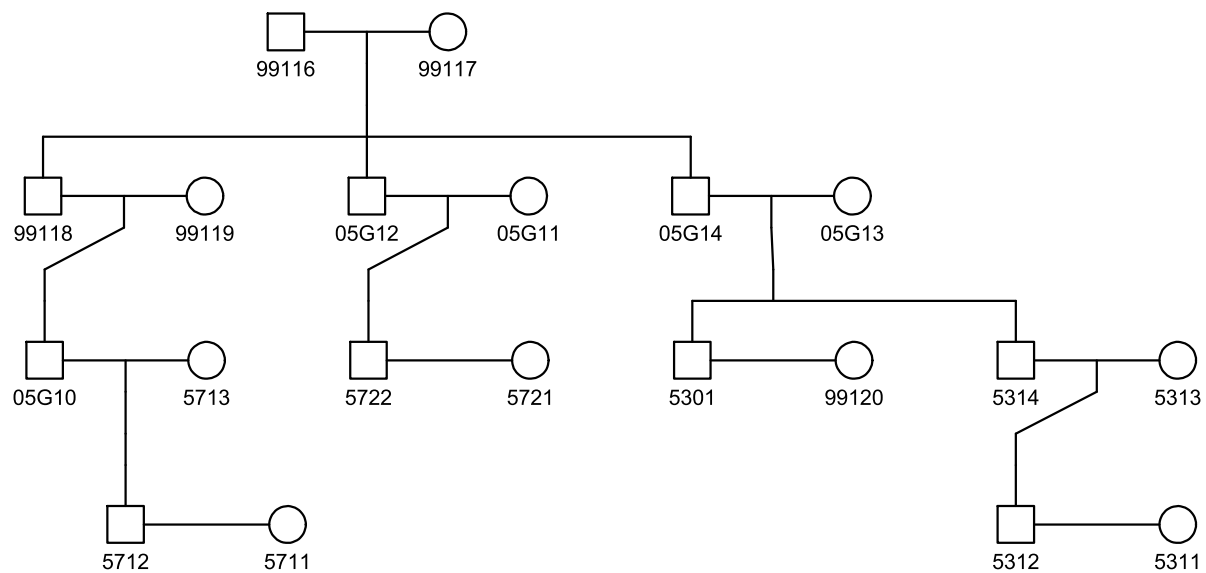
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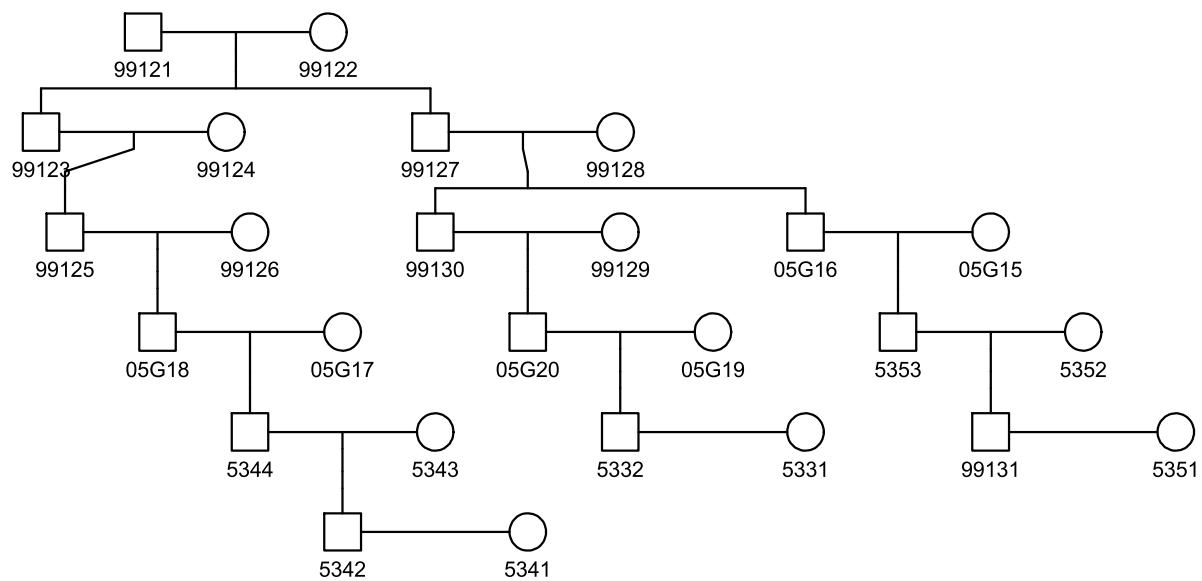
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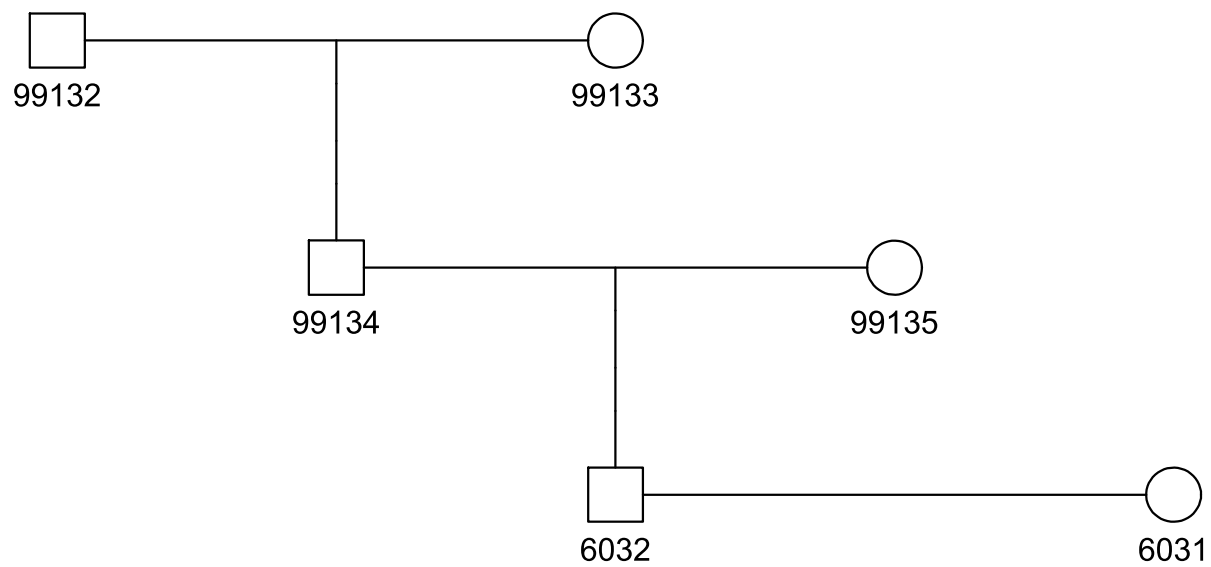
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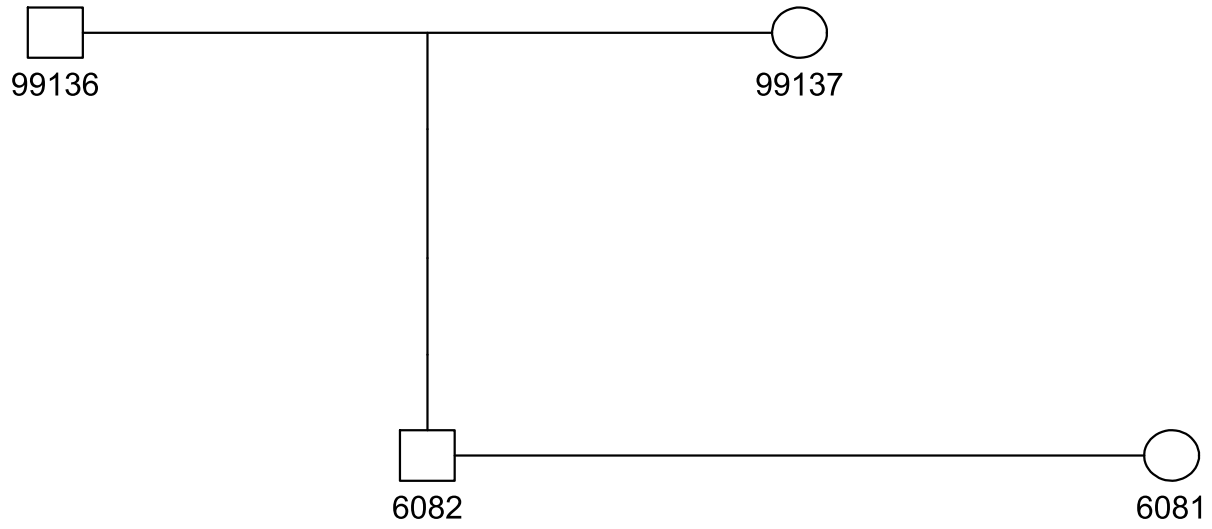
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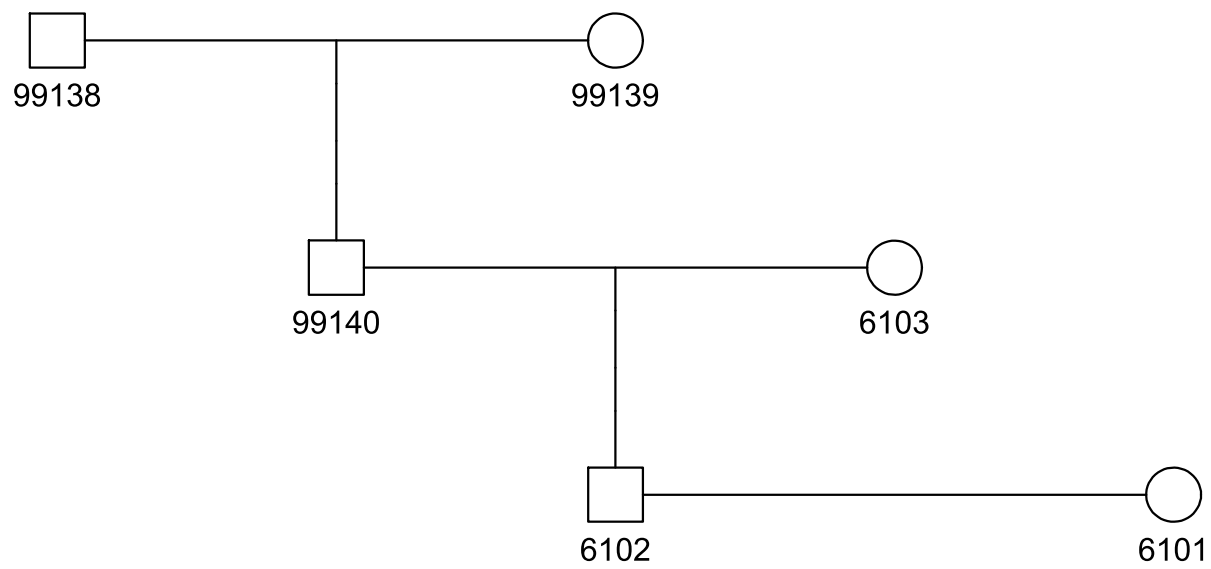
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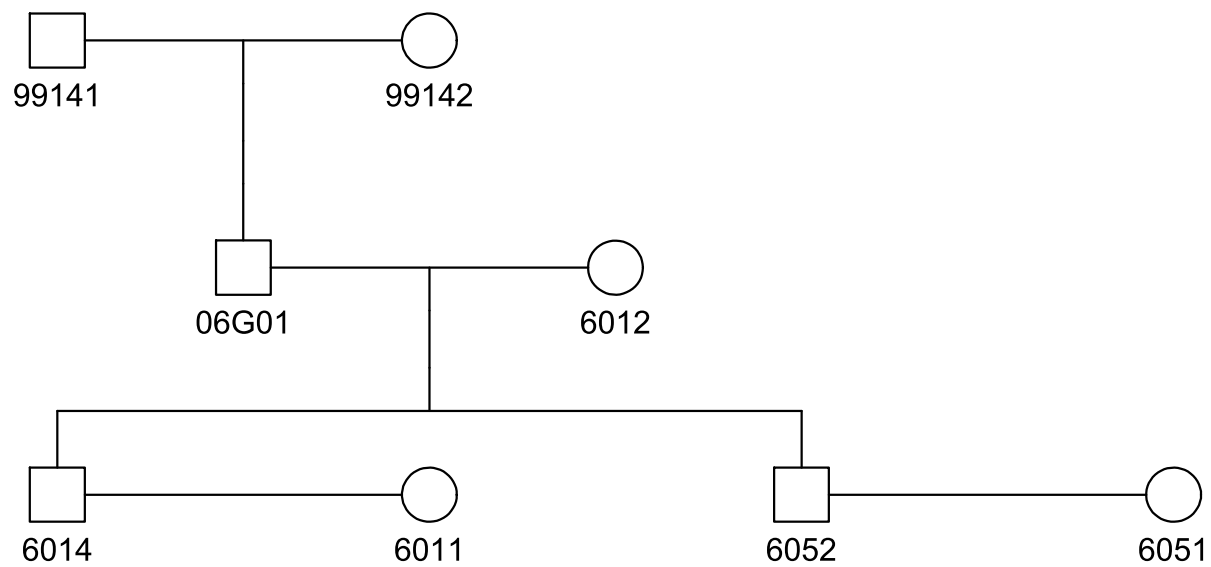
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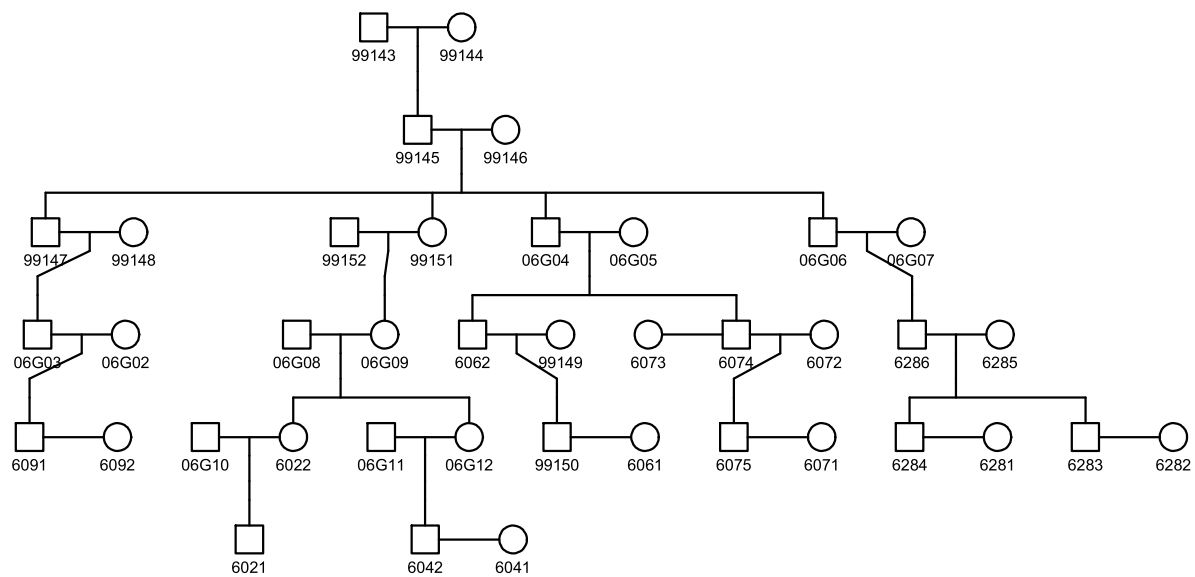
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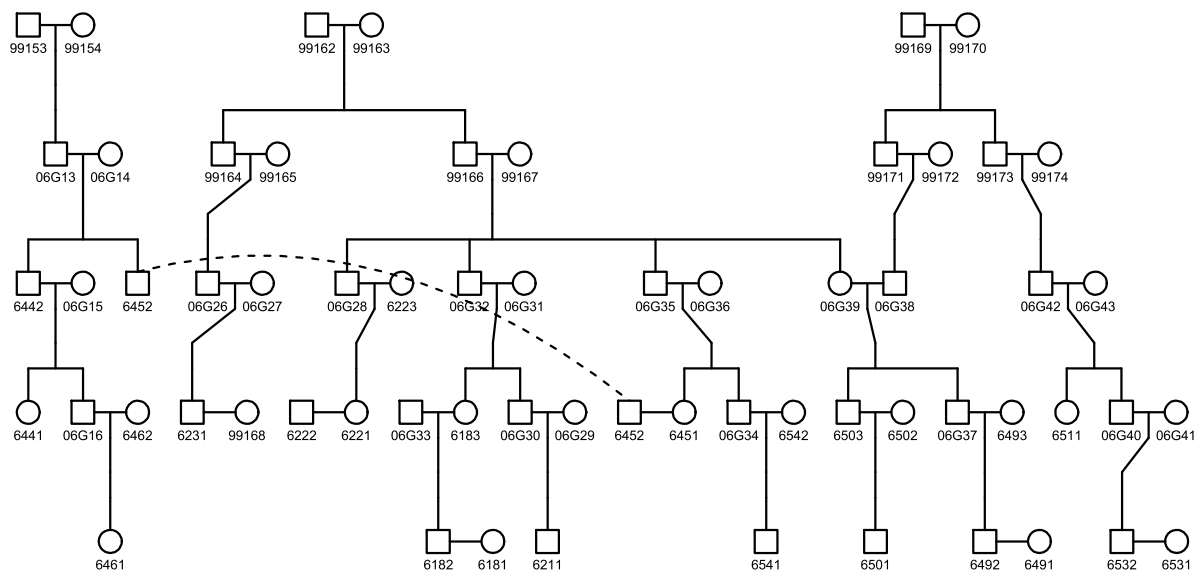
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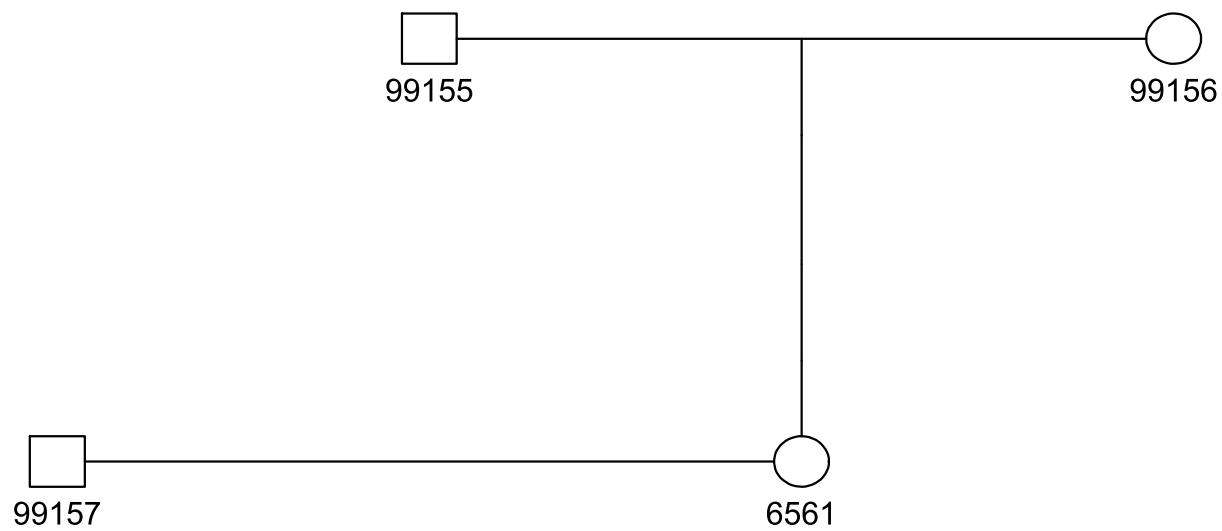
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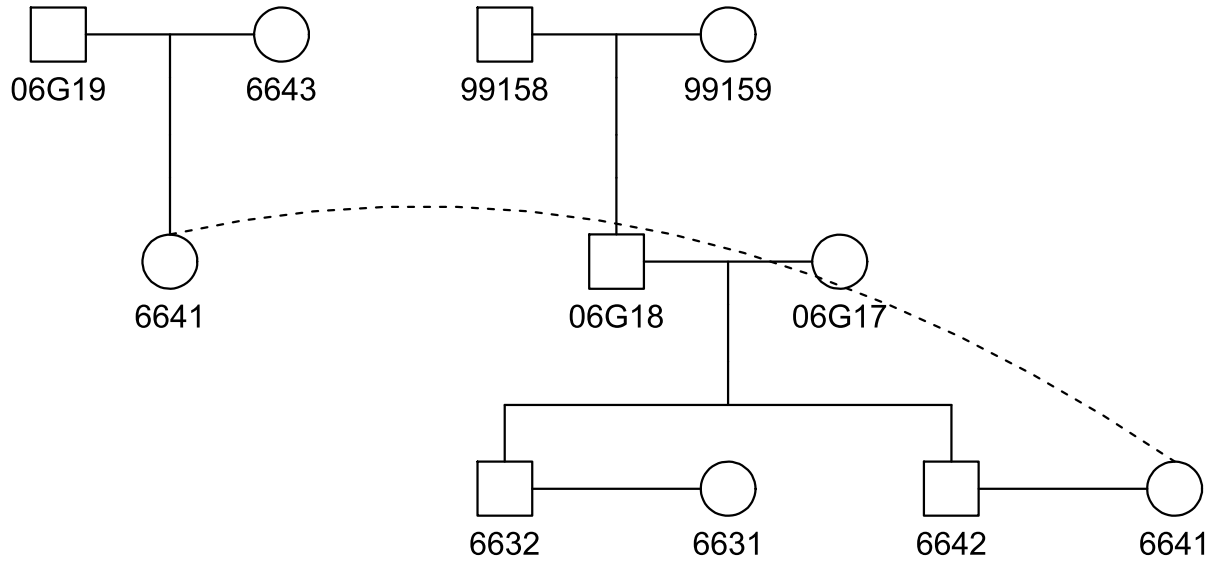
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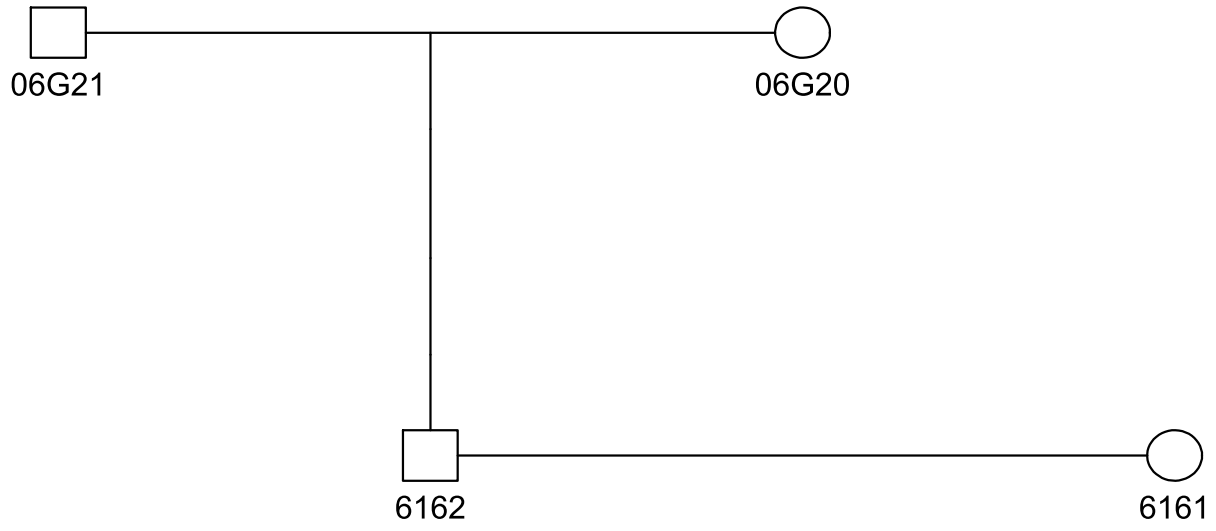
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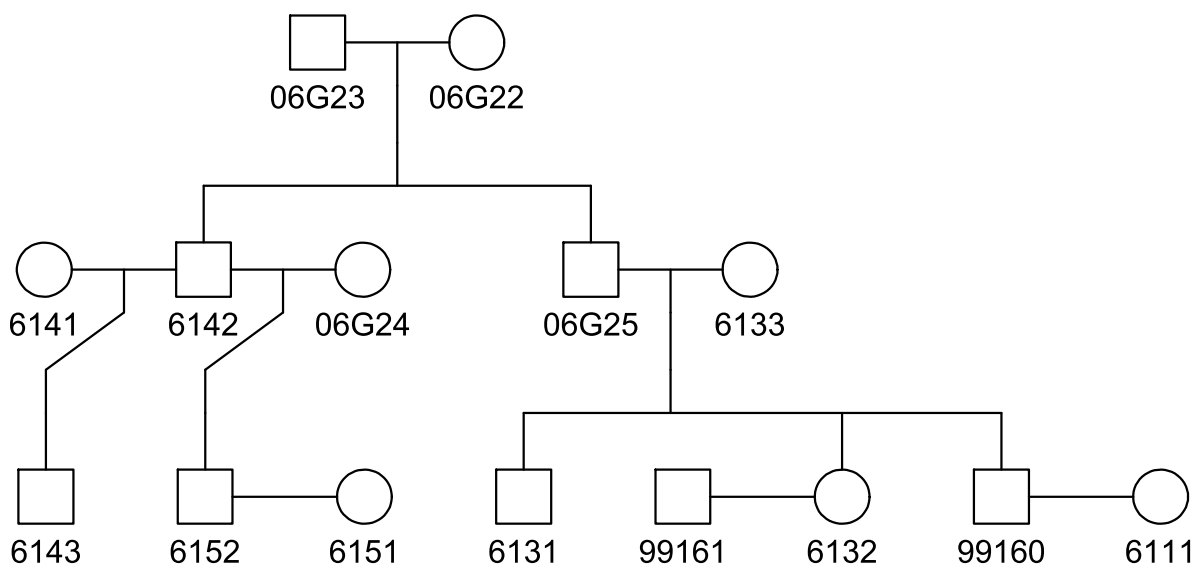
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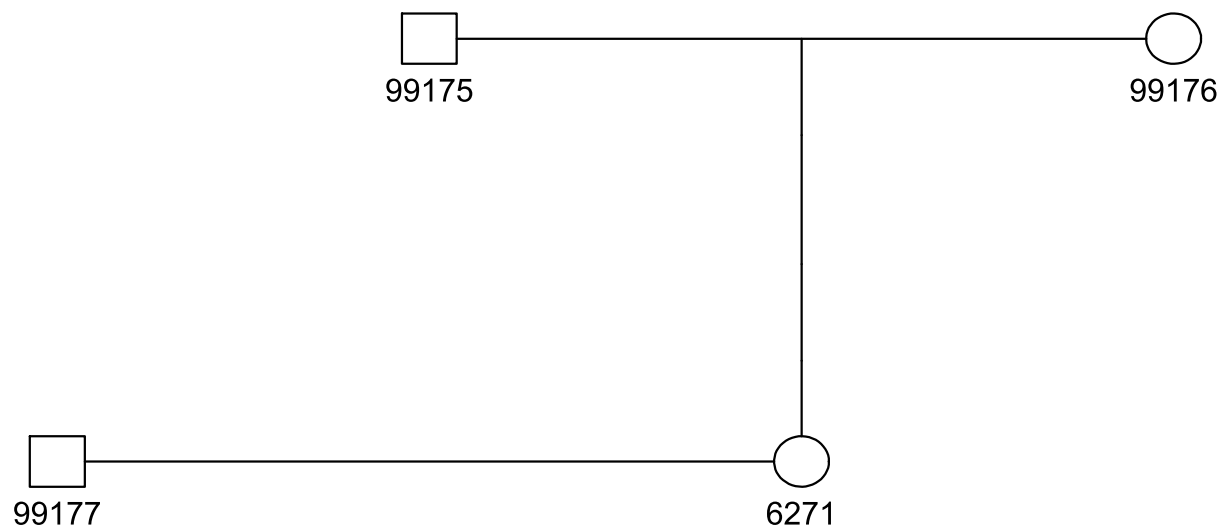
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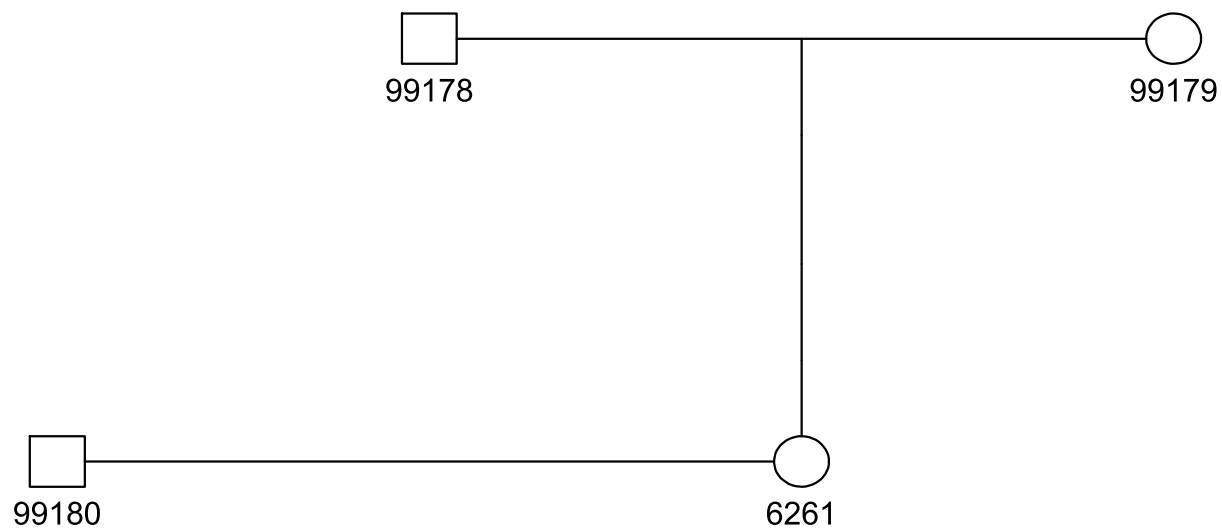
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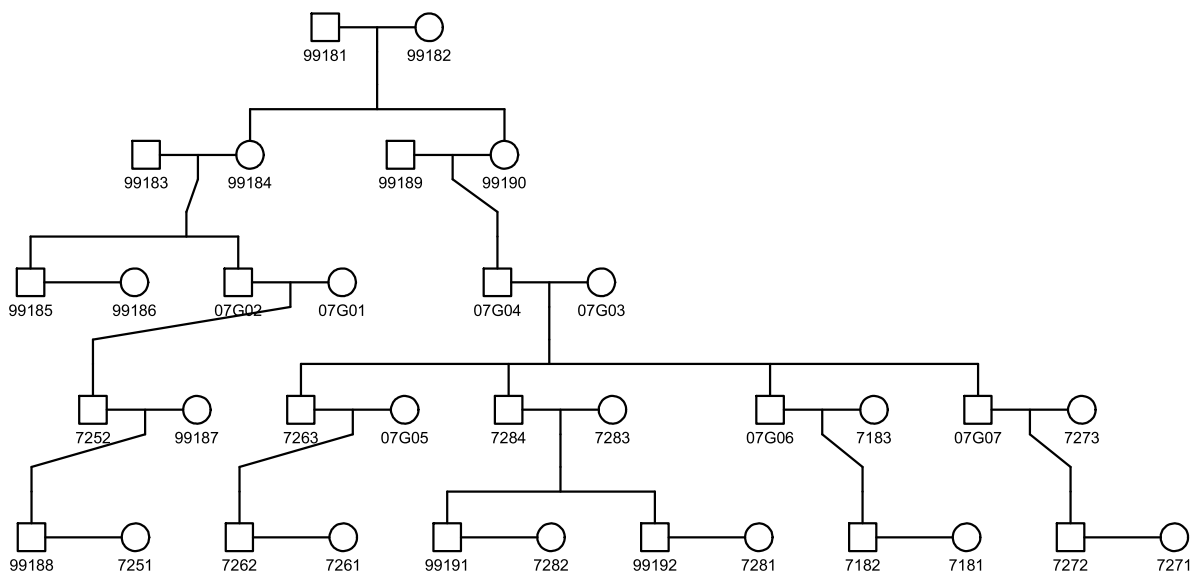
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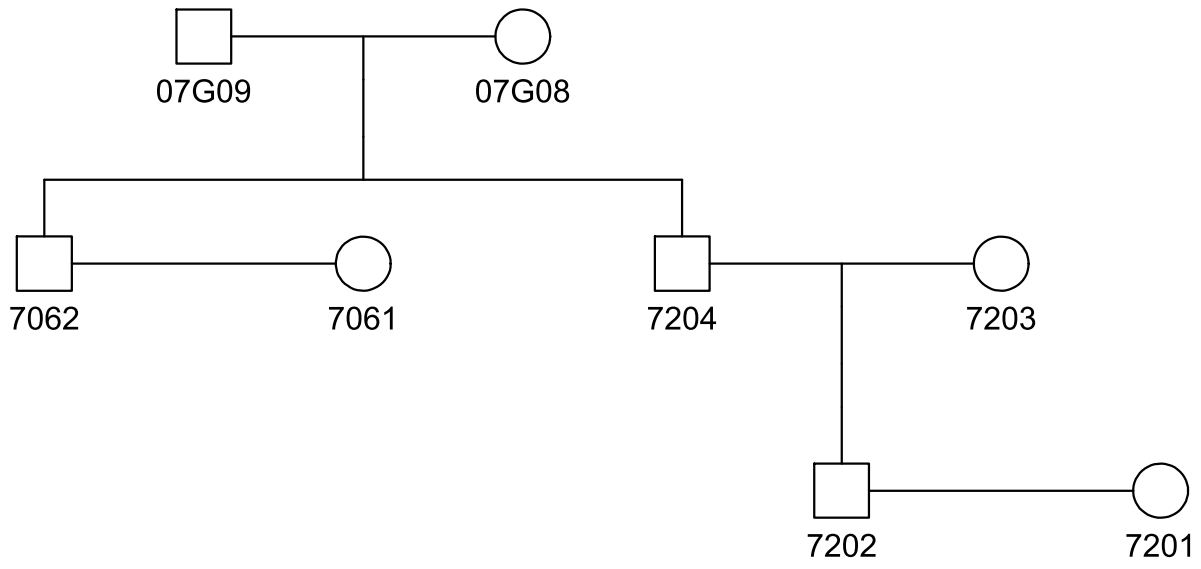
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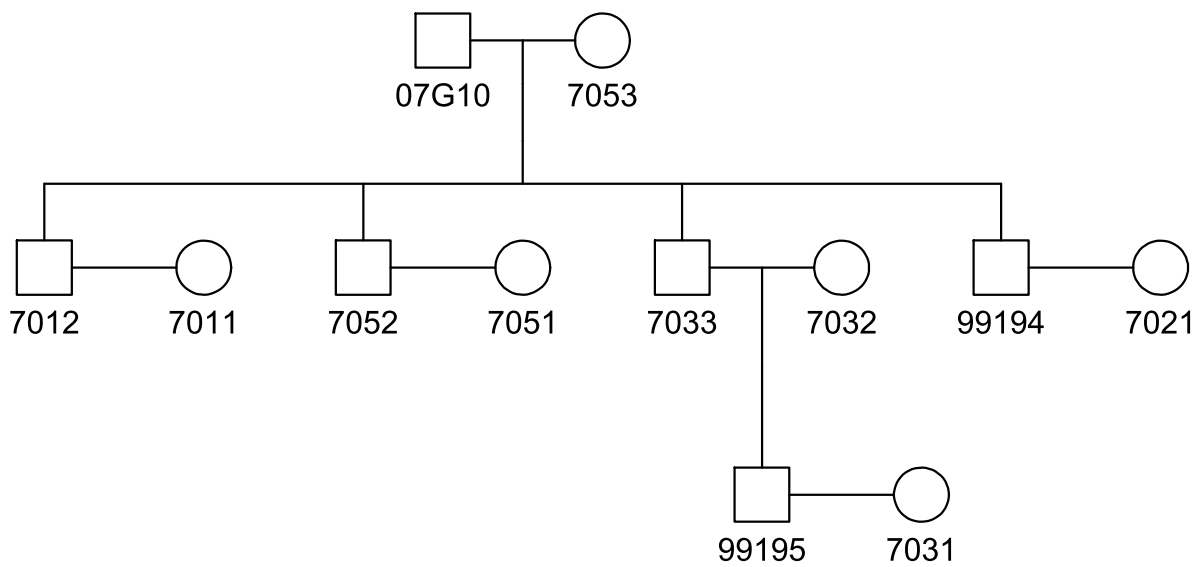
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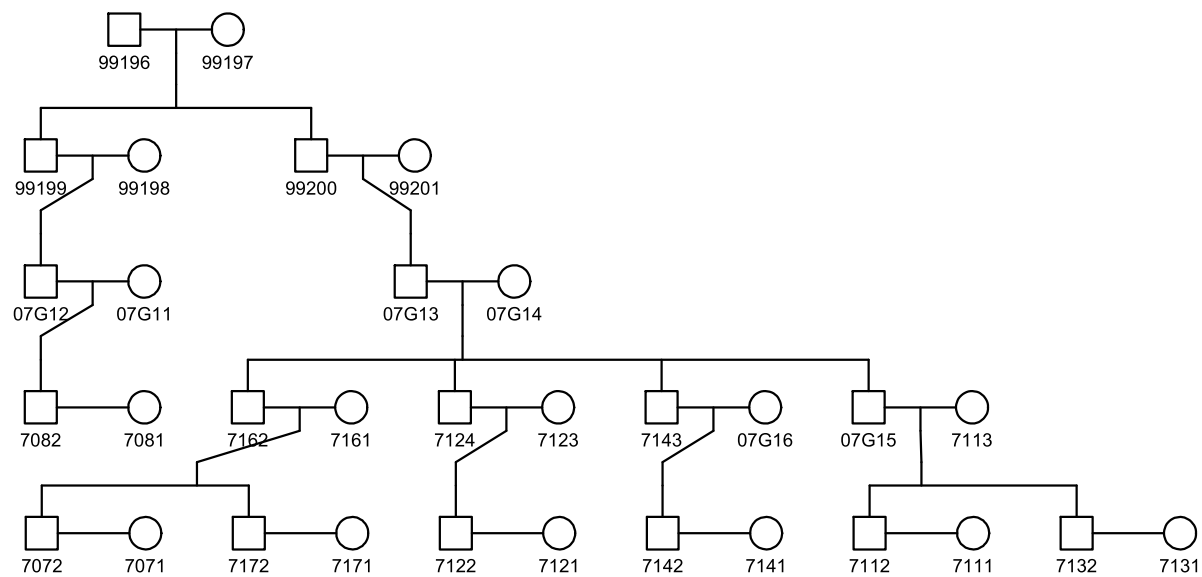
07D02



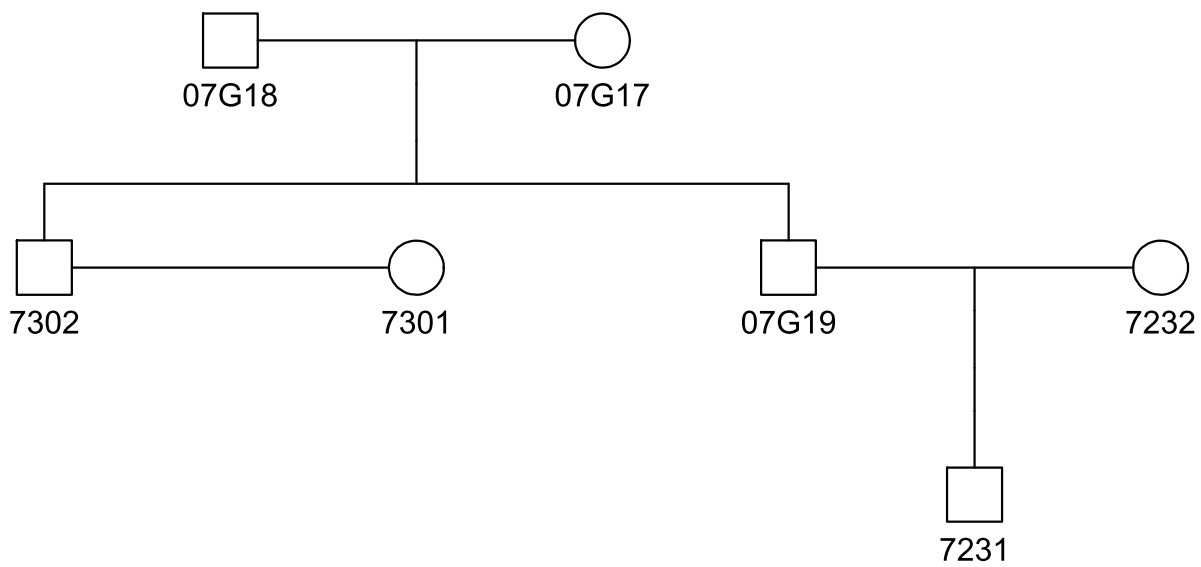
07D03



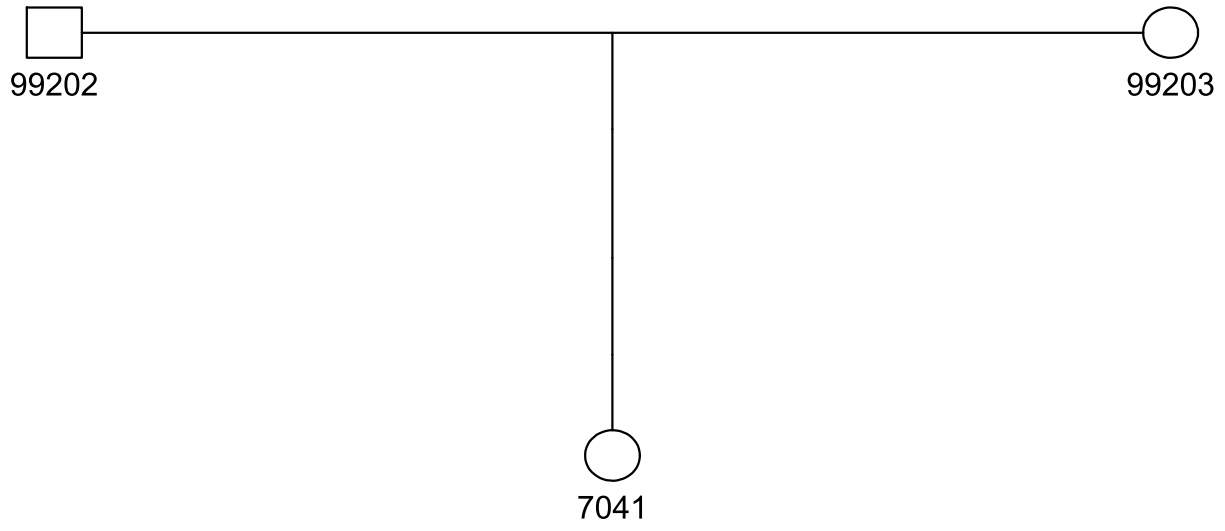
07D04



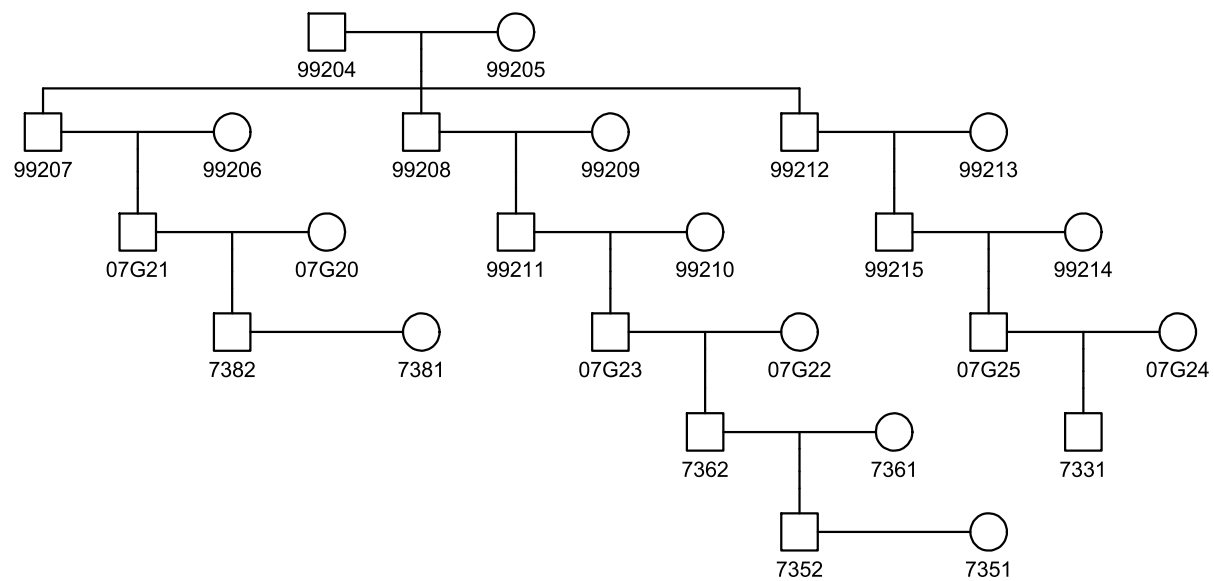
07D05



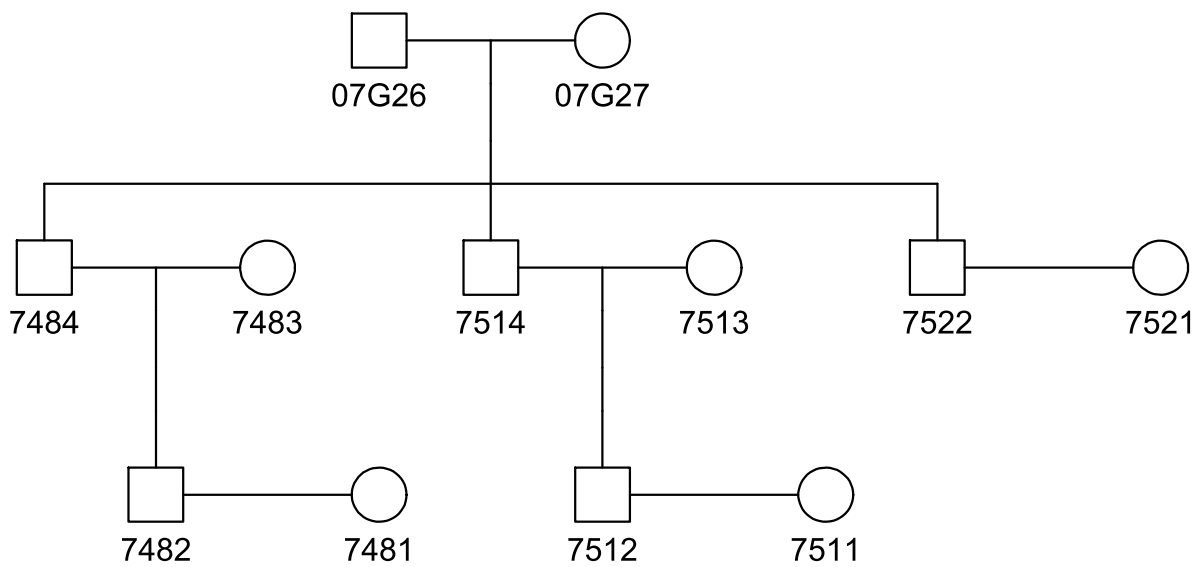
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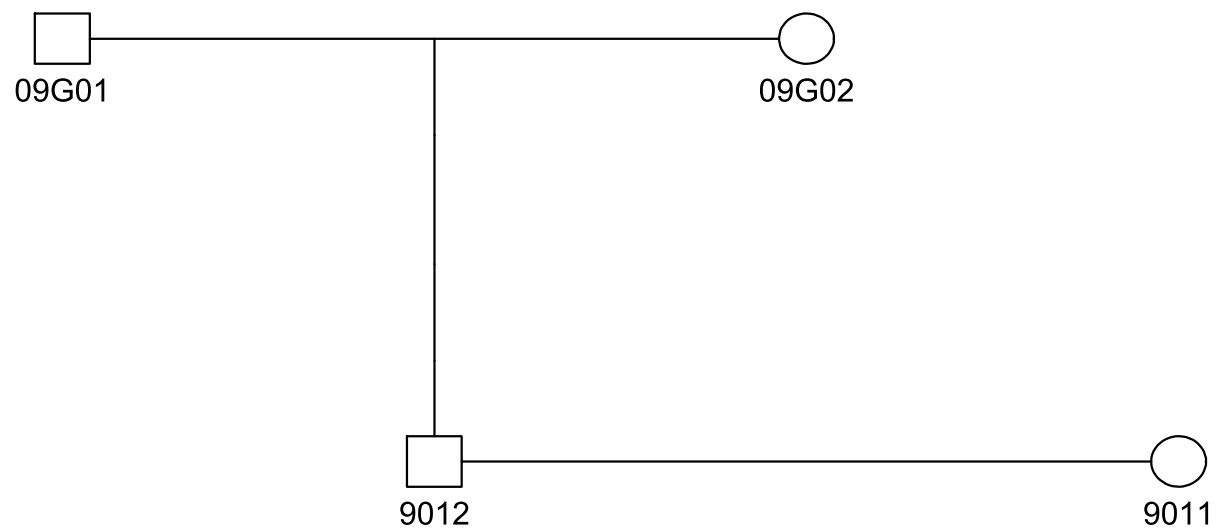
07D07



07D08

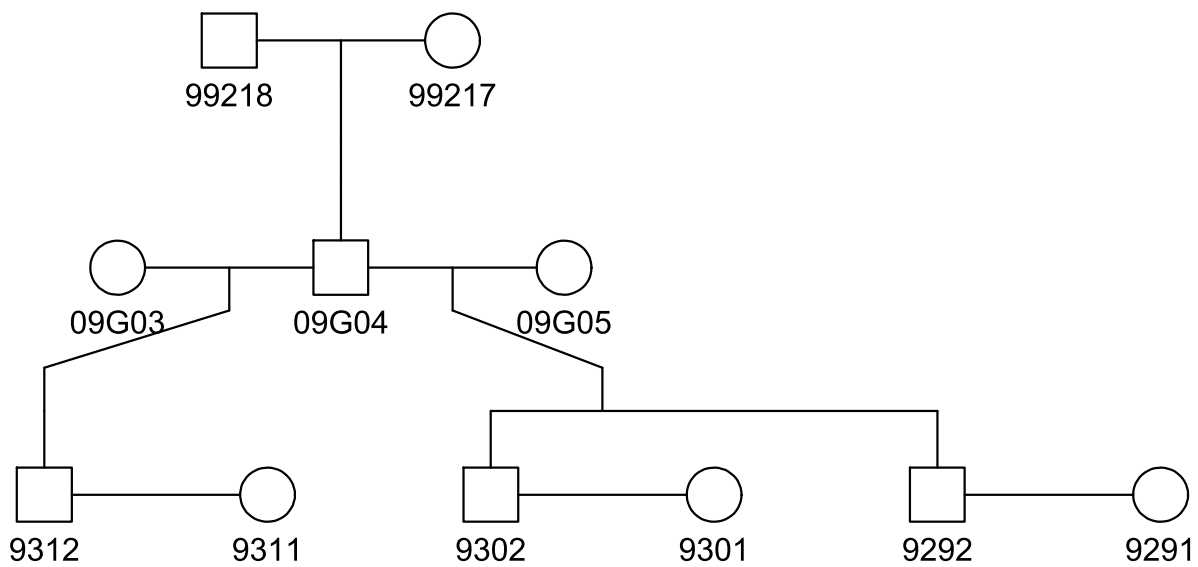


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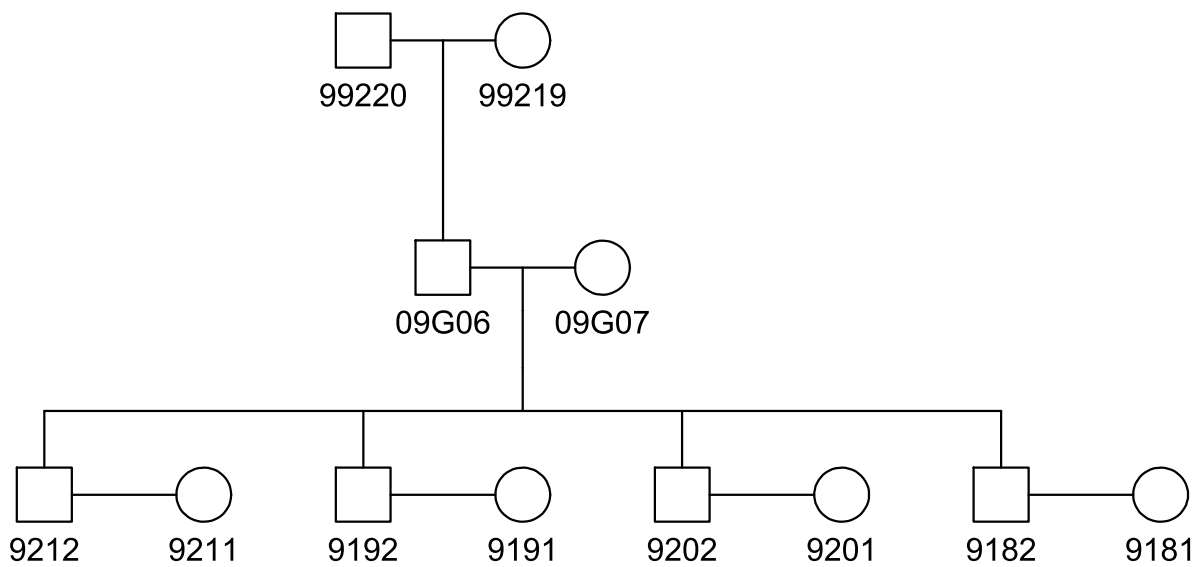


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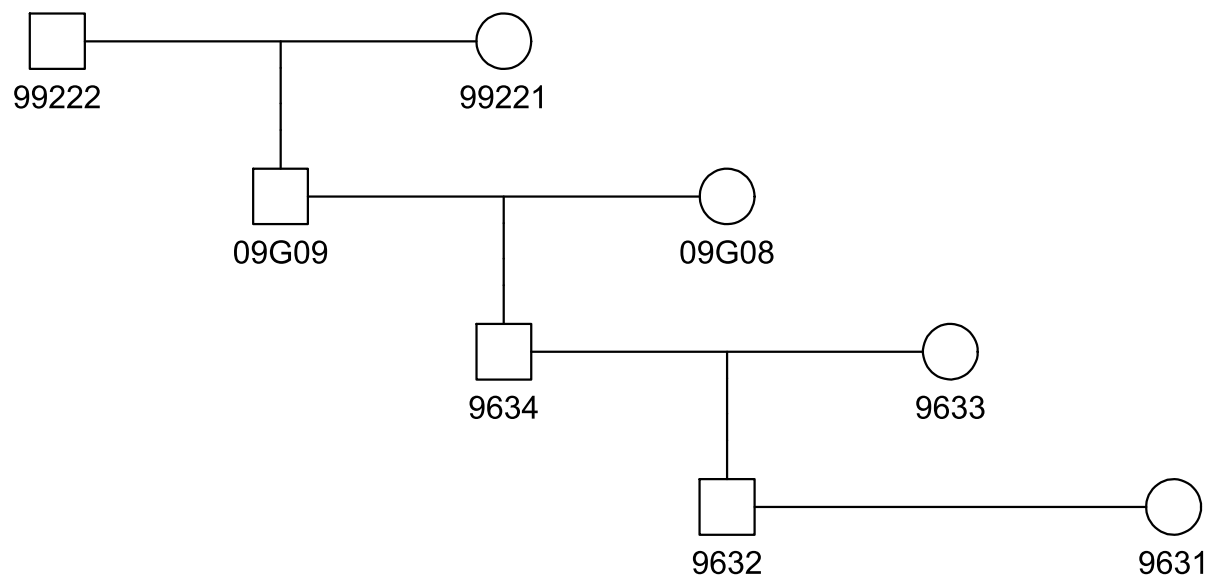
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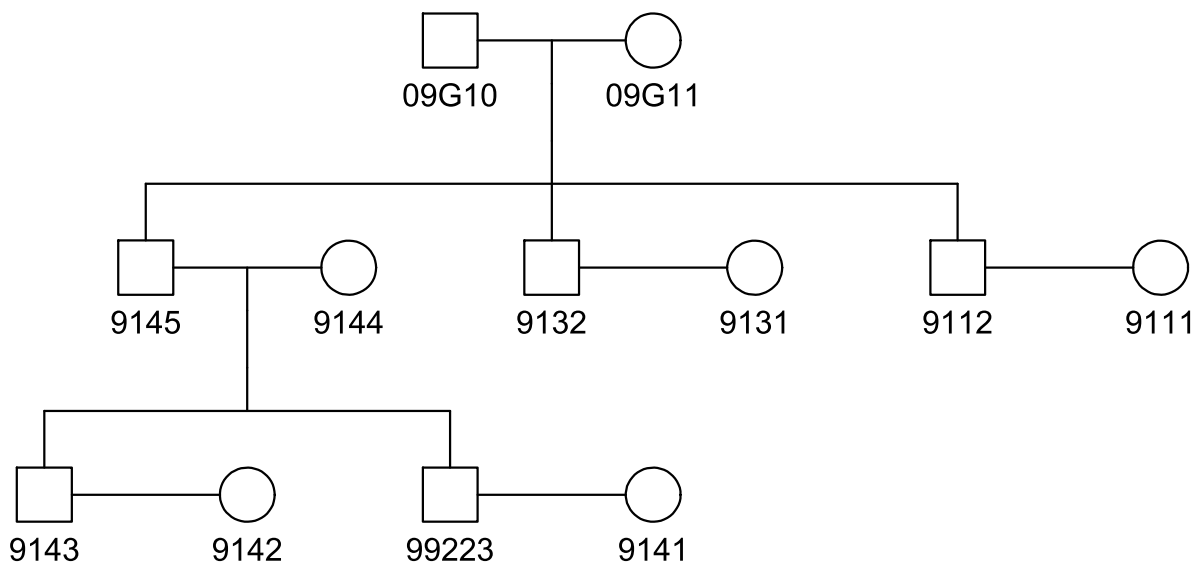
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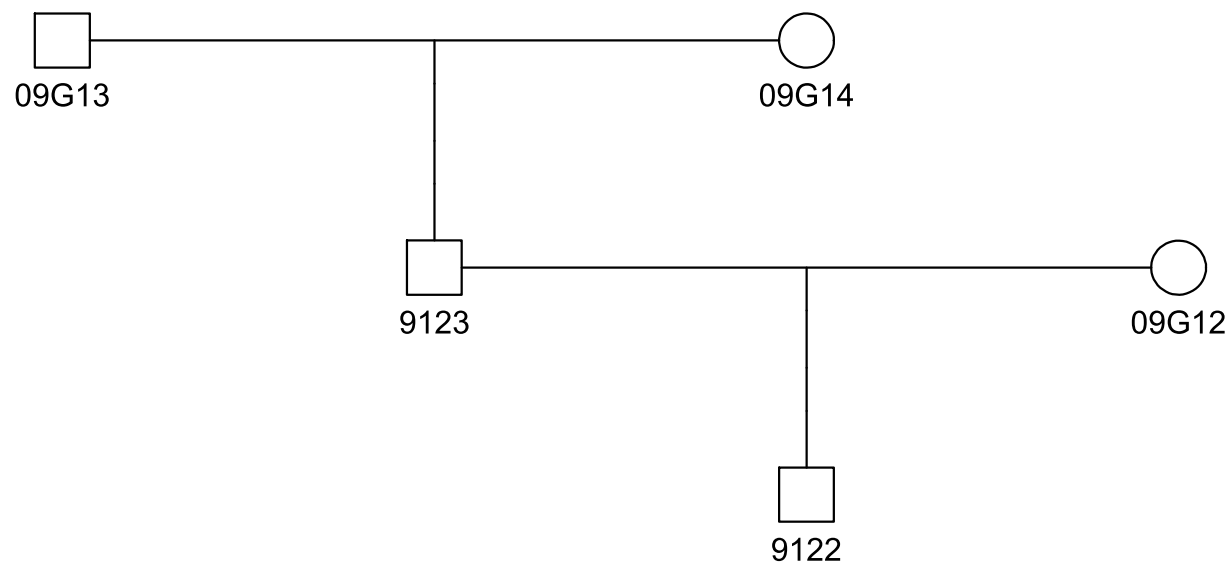
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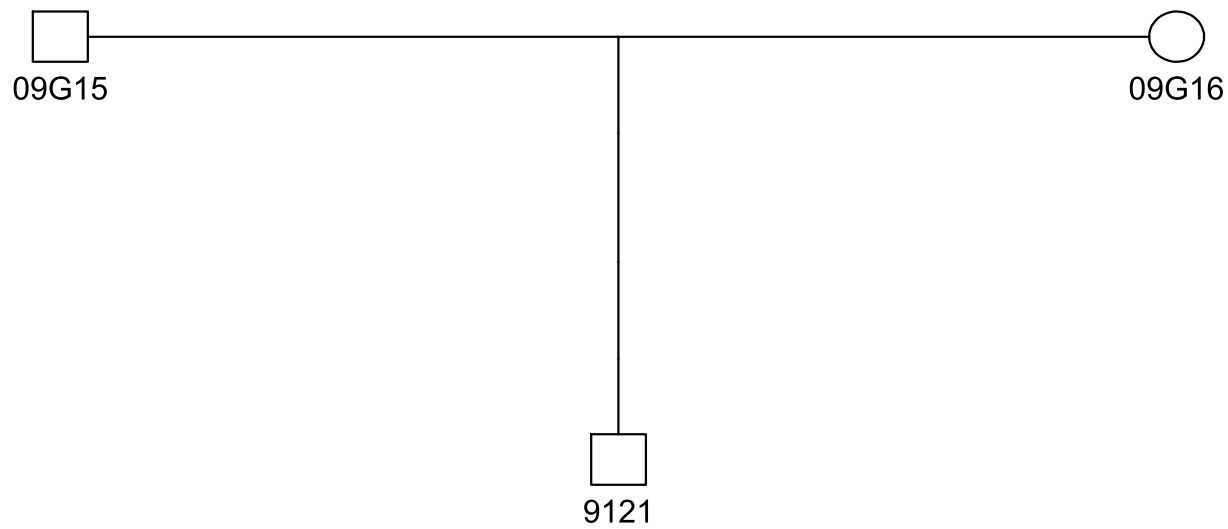
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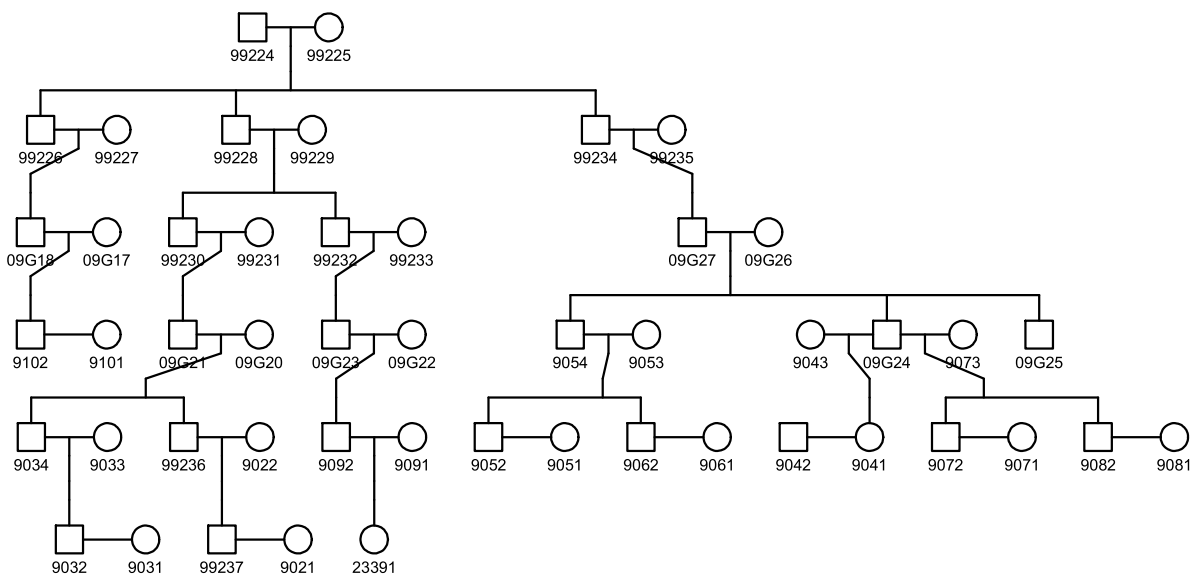
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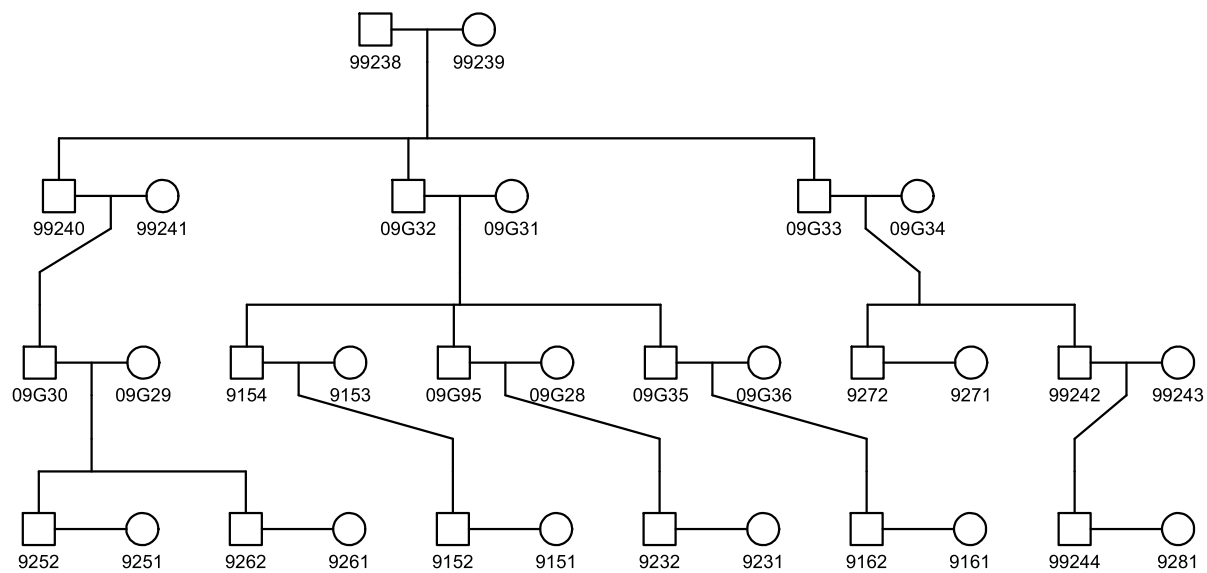
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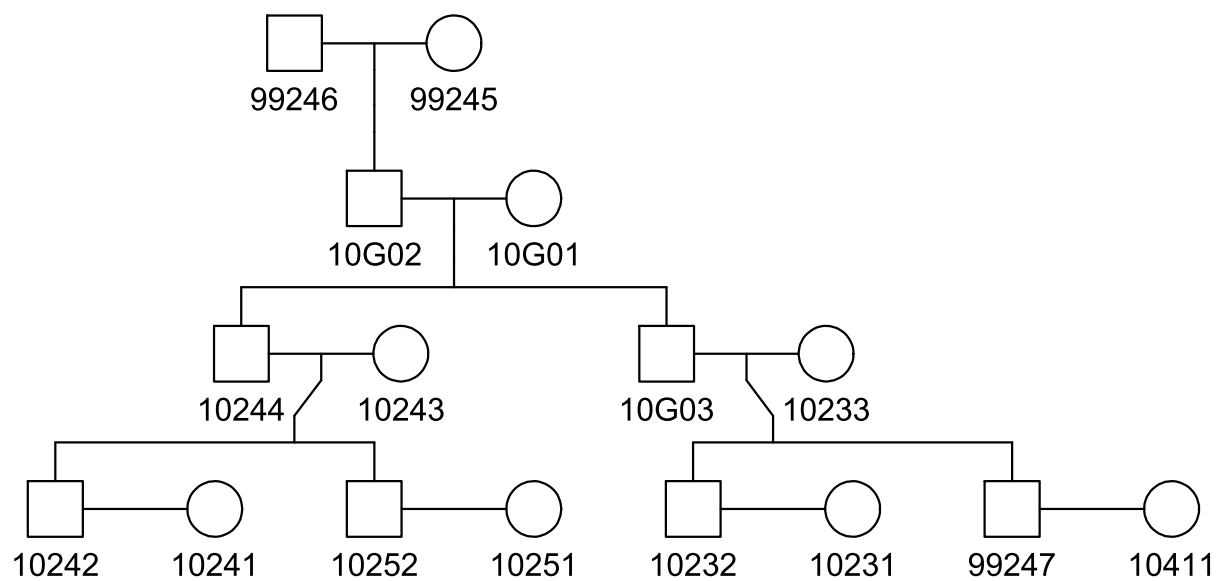
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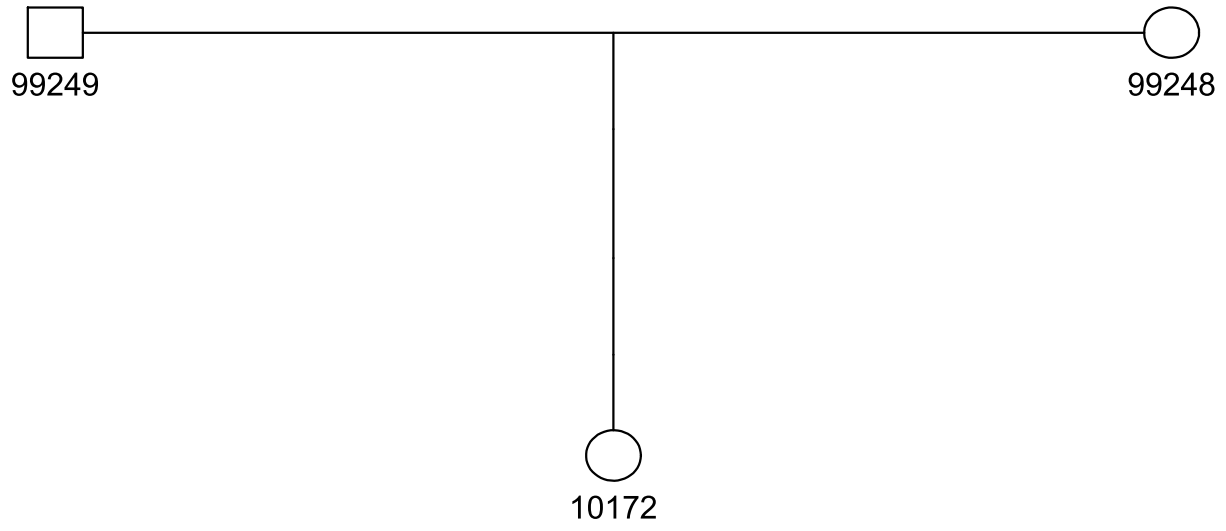
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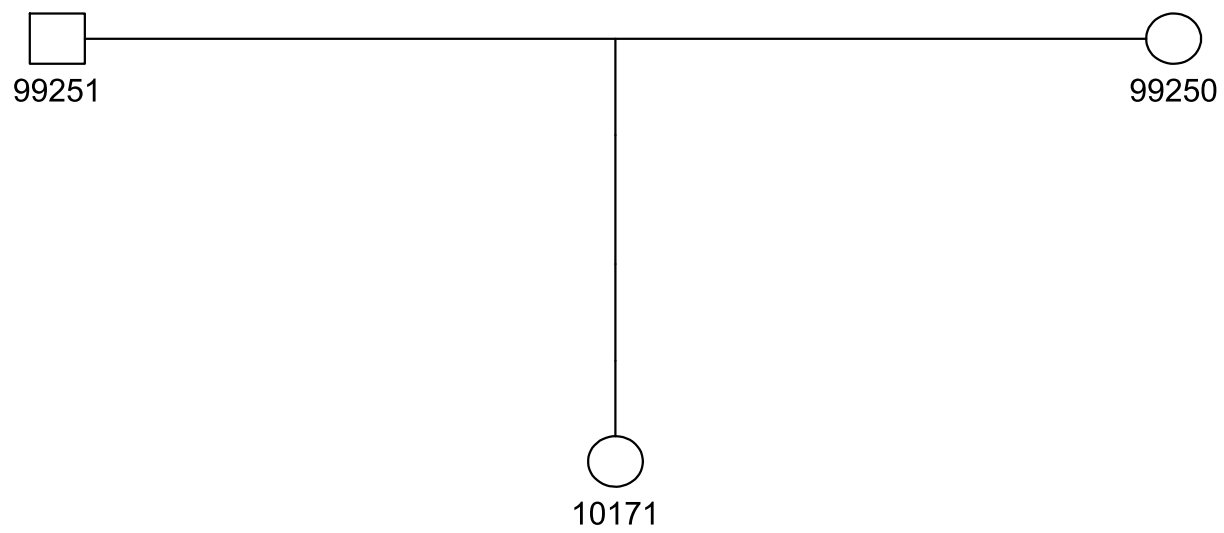
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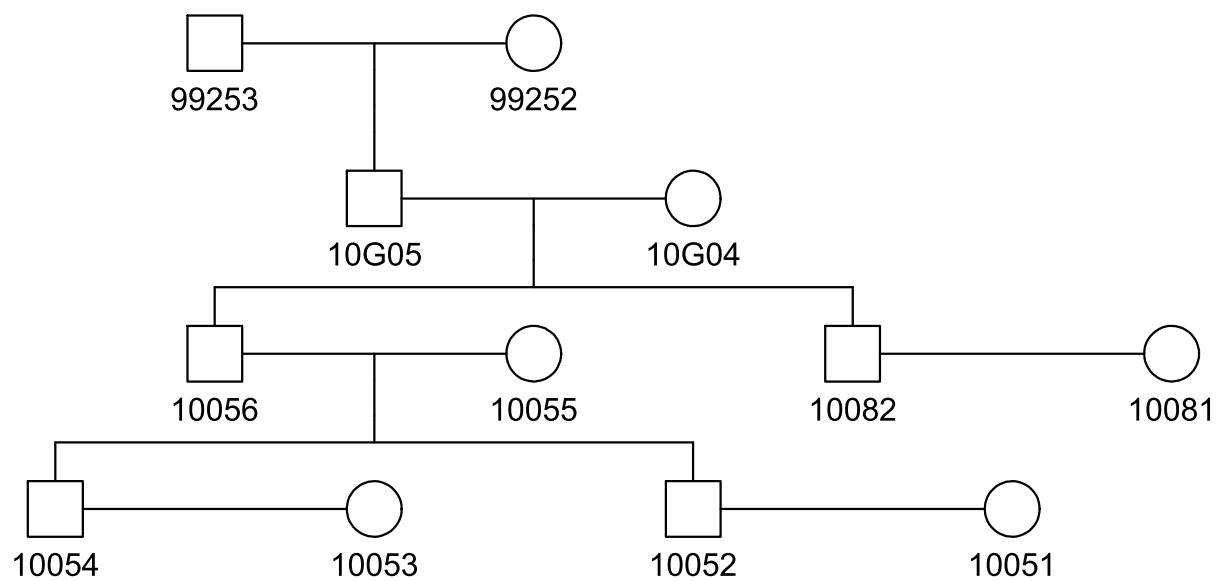
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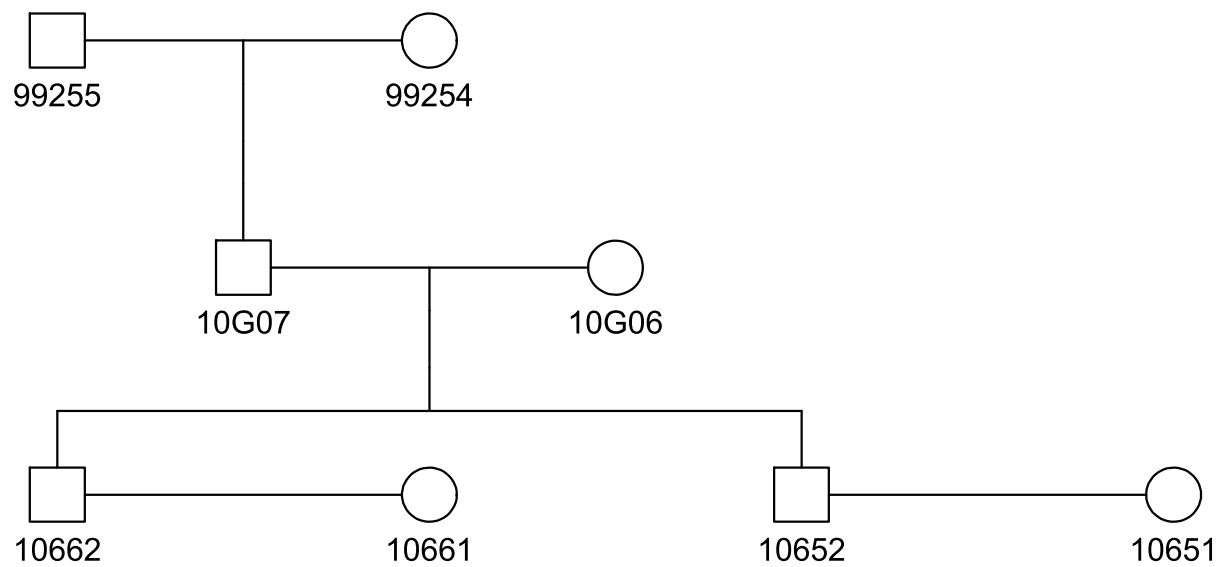
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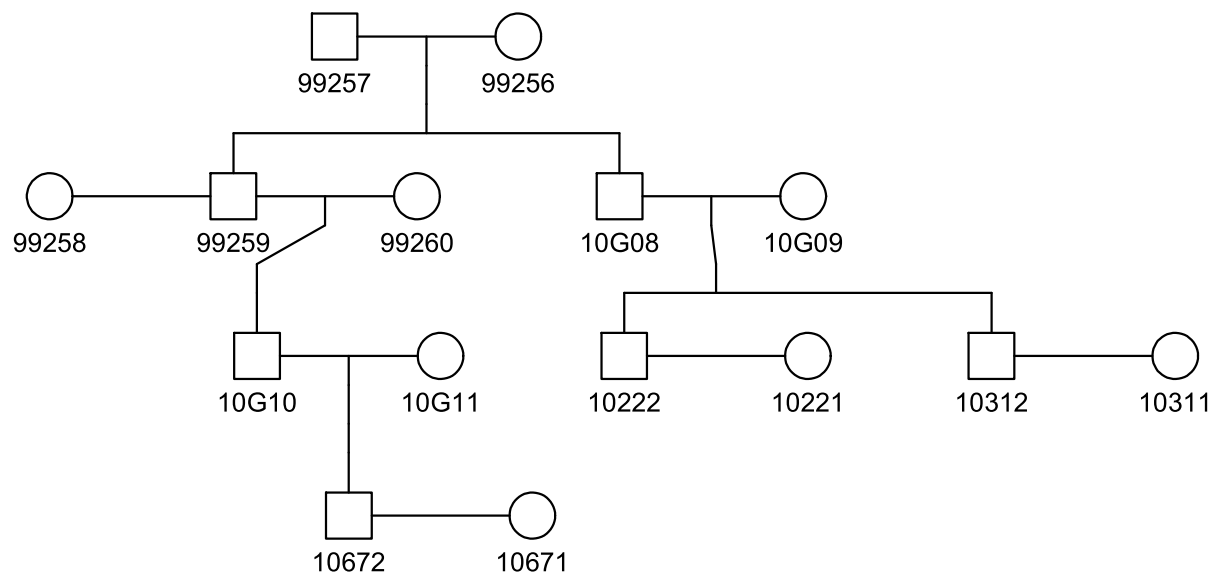
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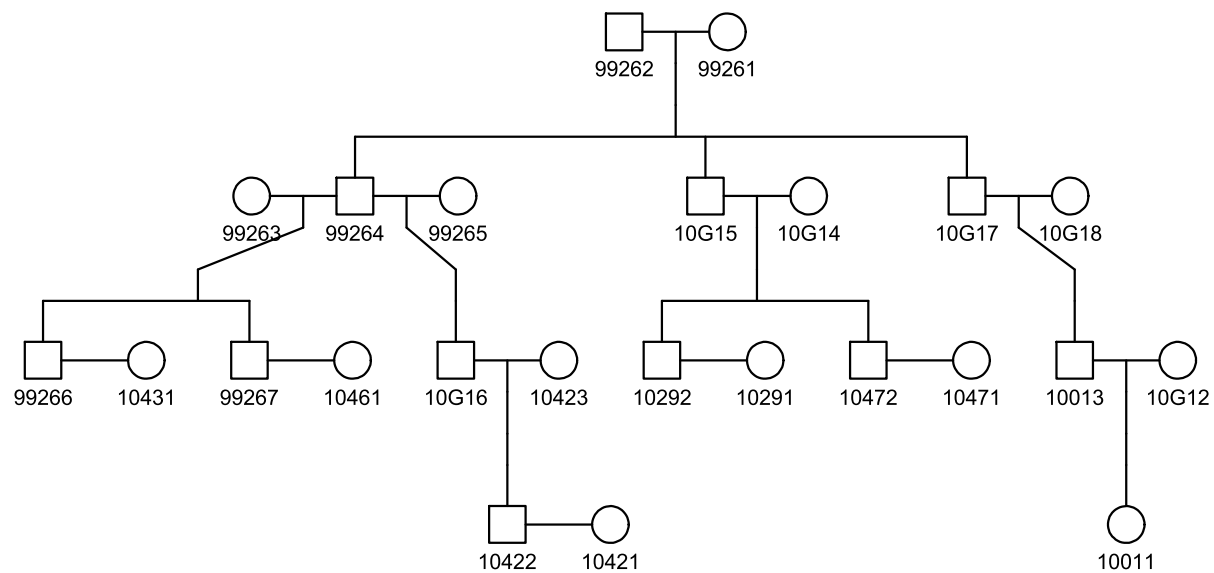
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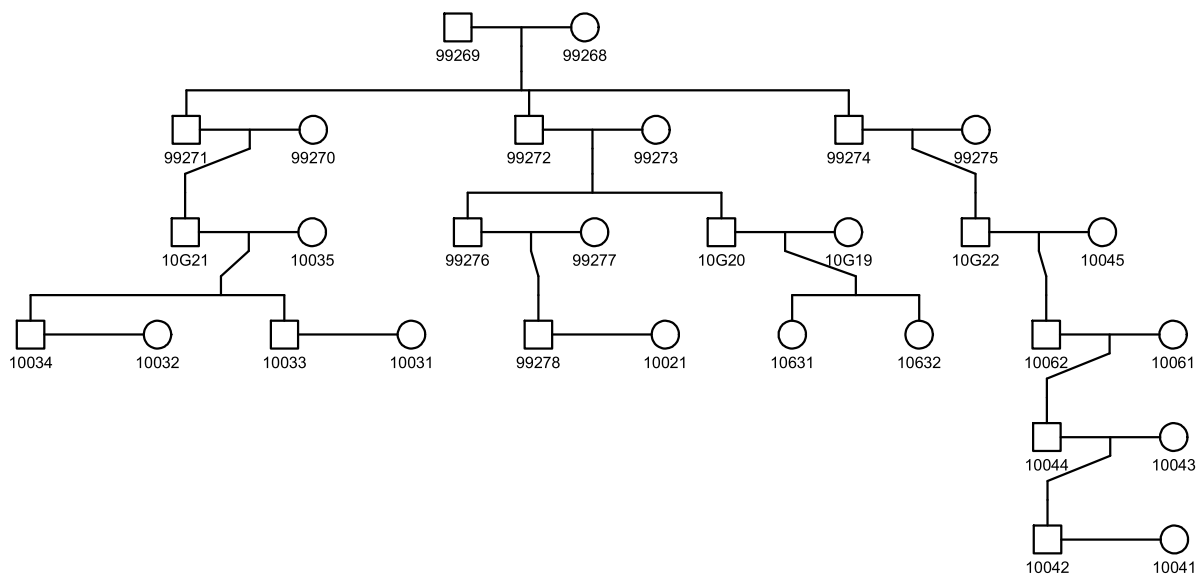
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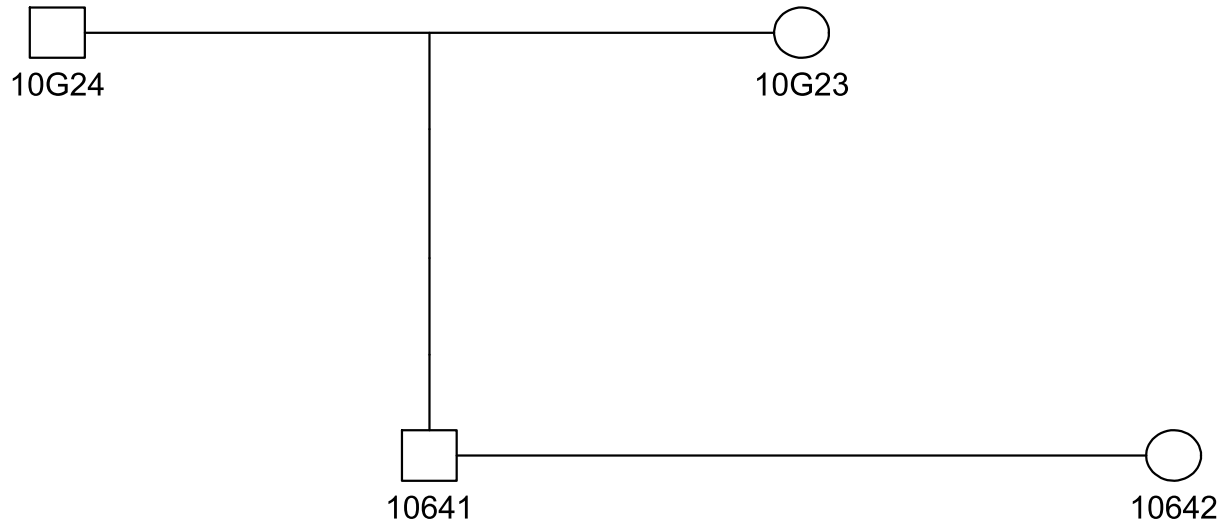
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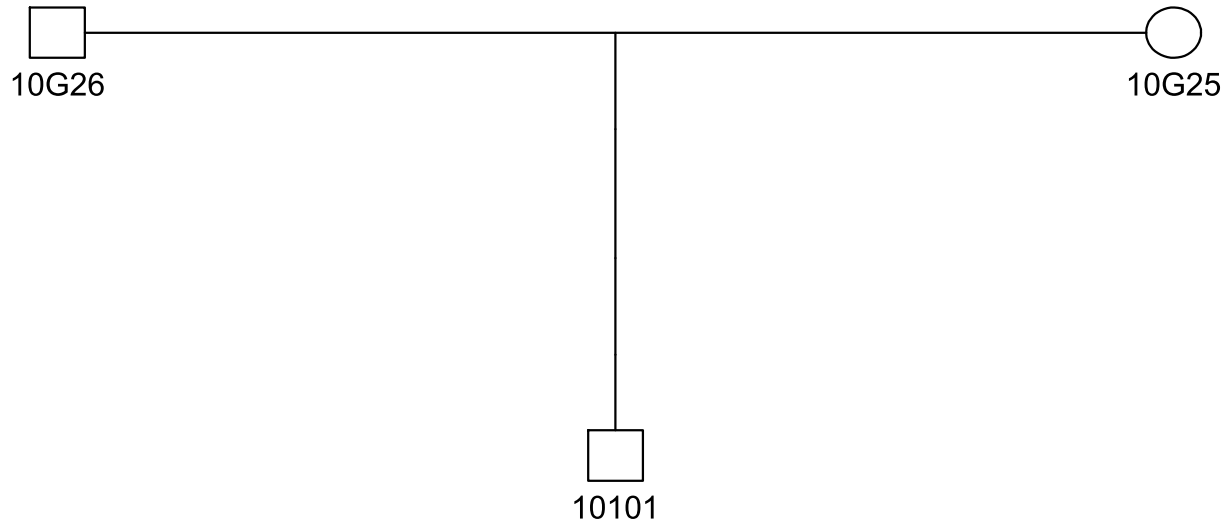
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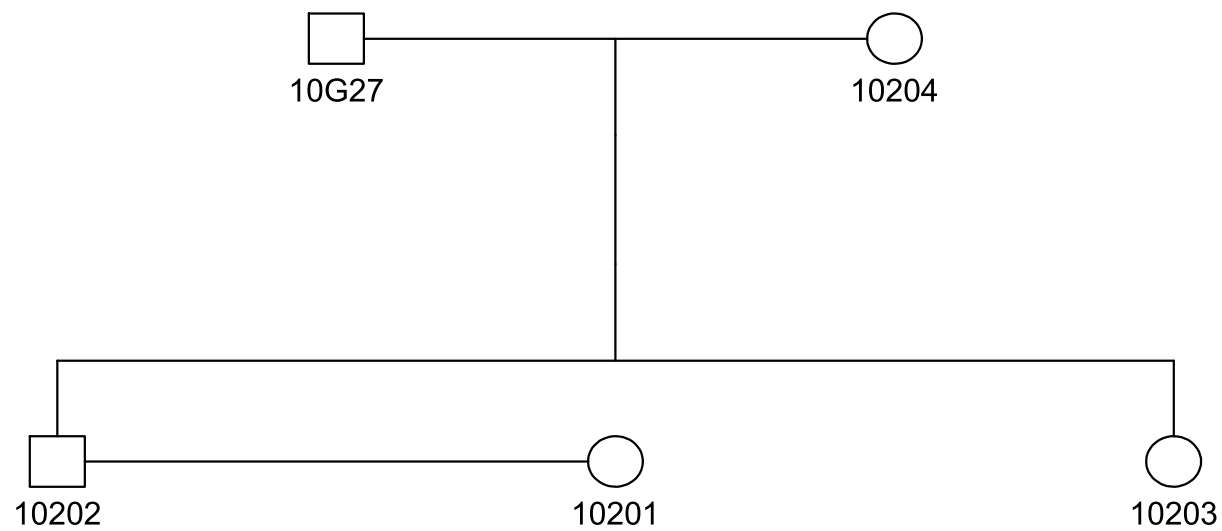
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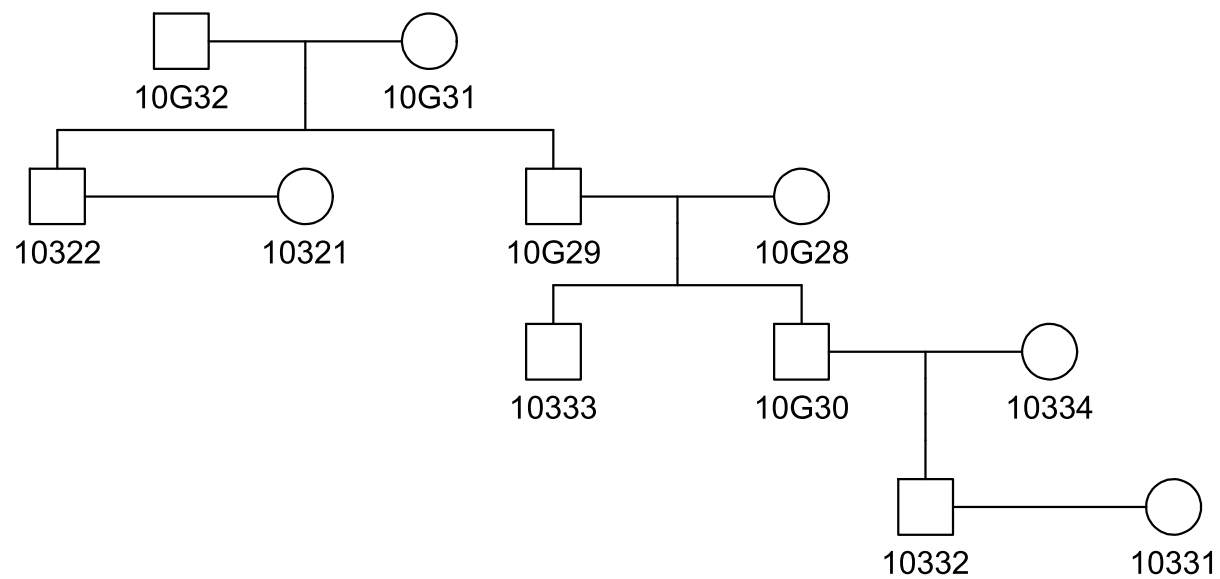
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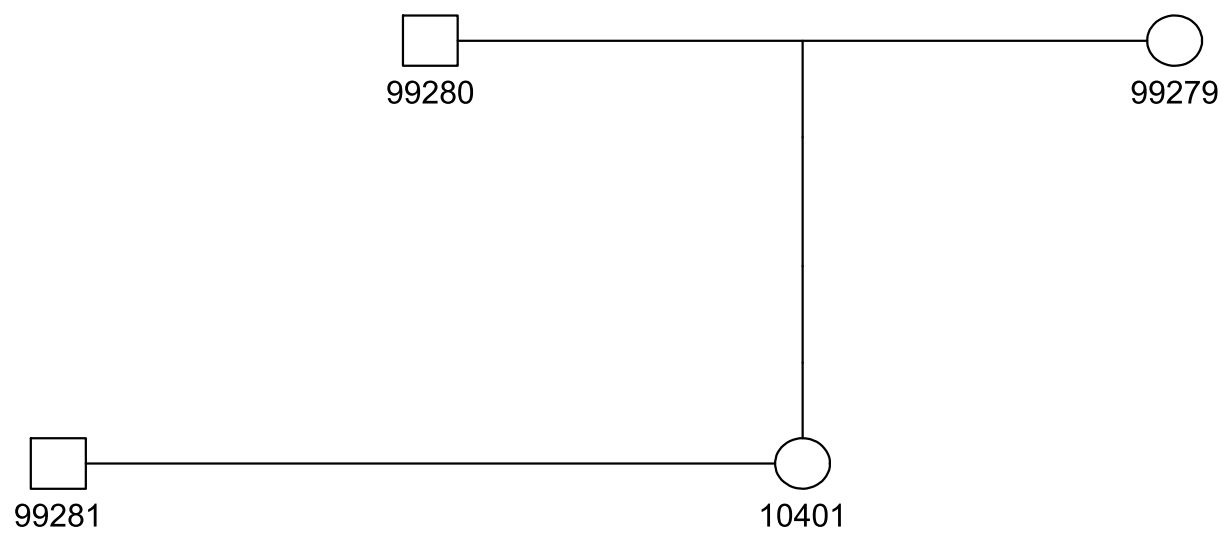
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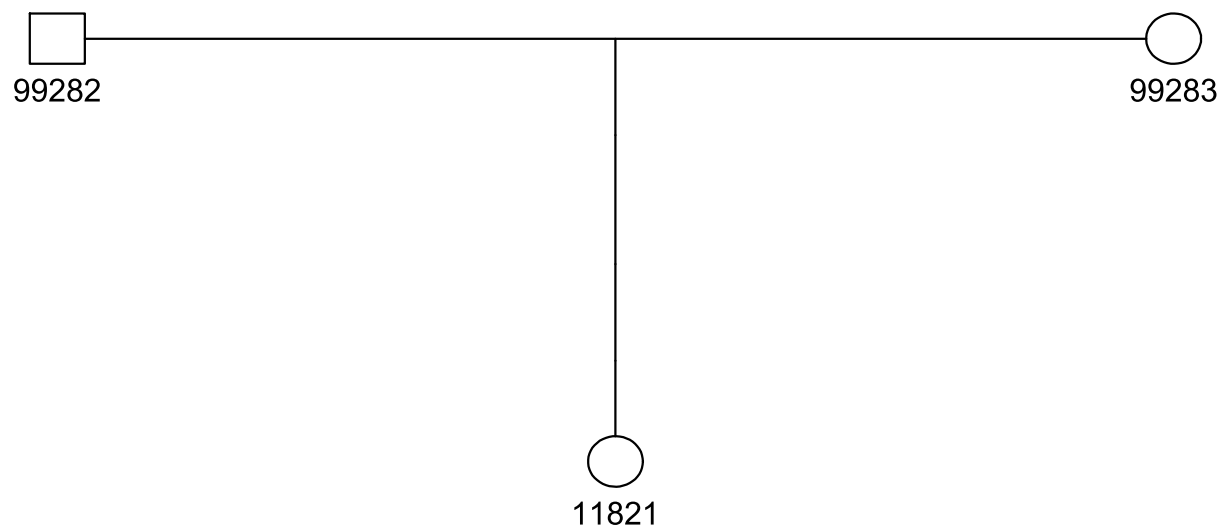
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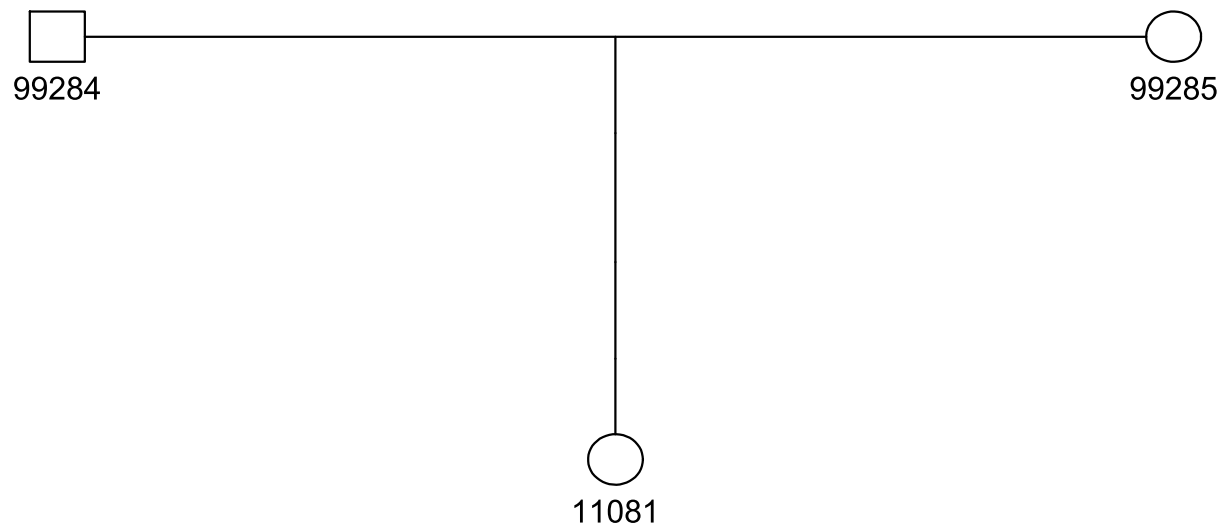
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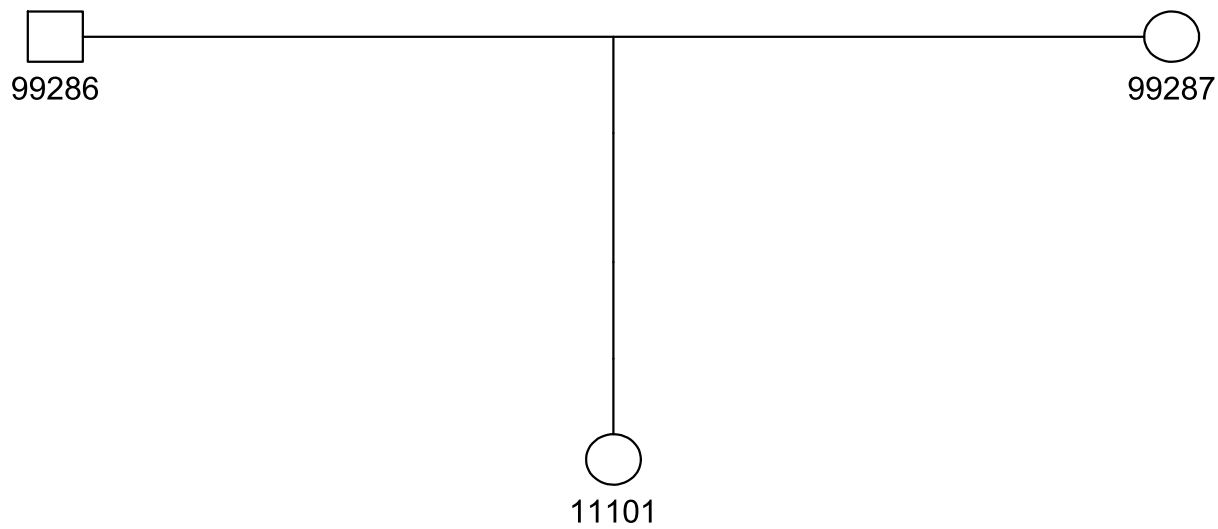
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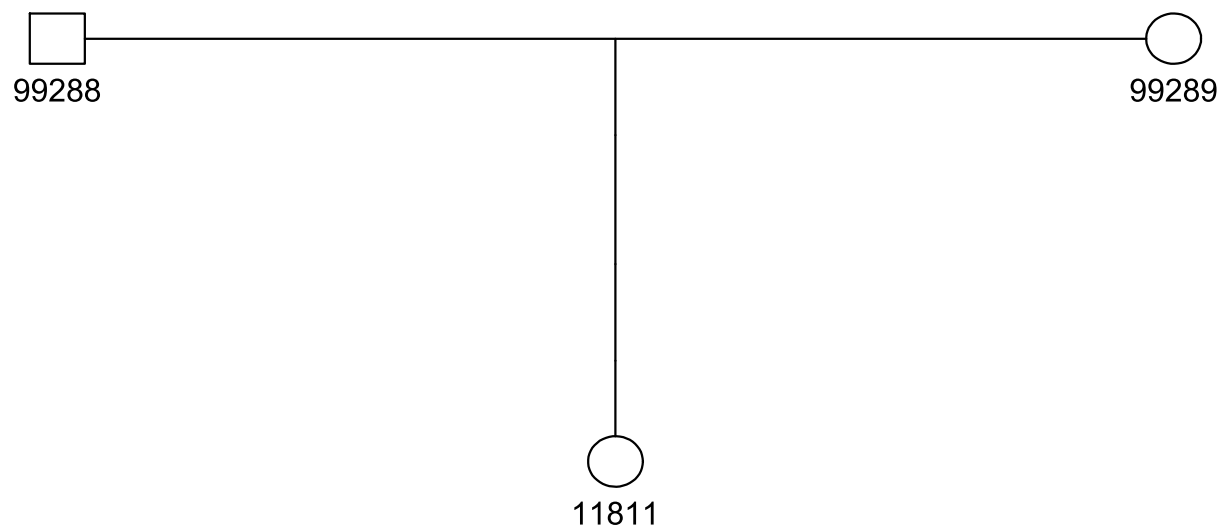
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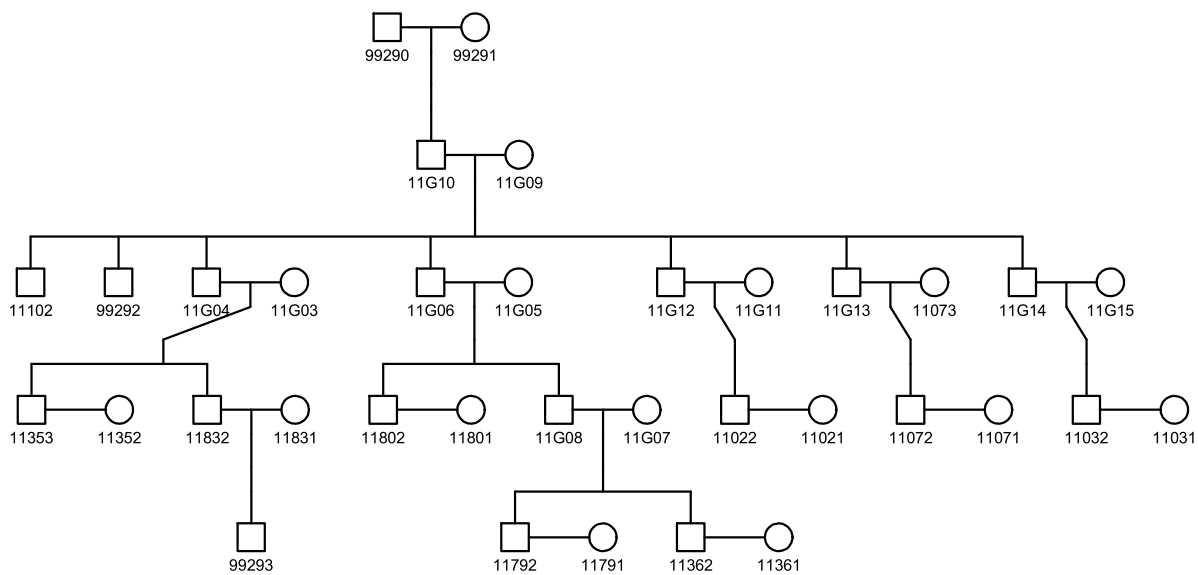
11R03



11R04

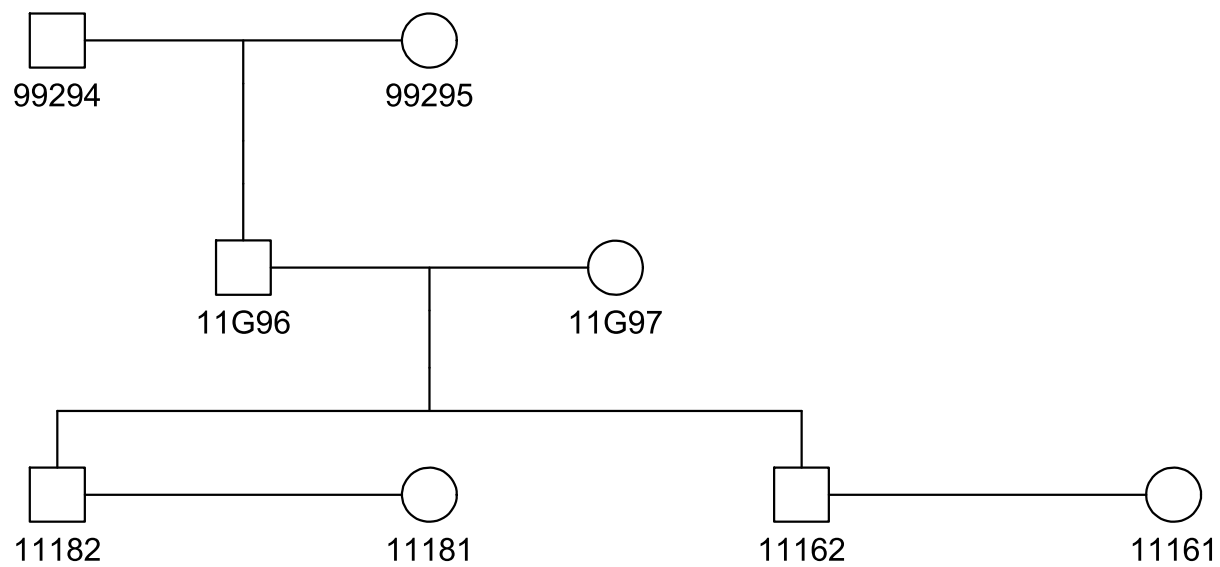


11R05

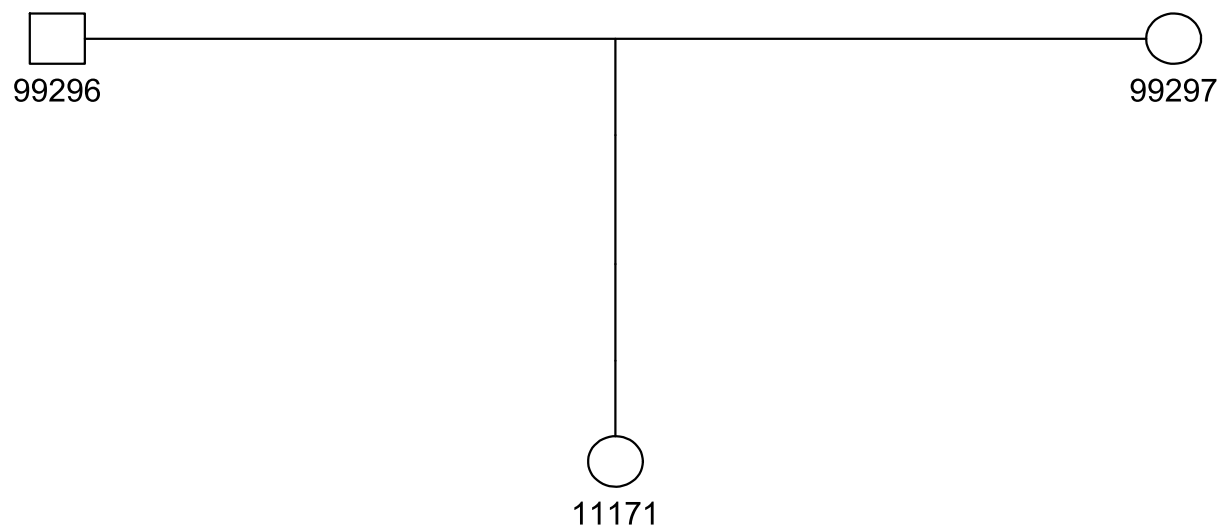


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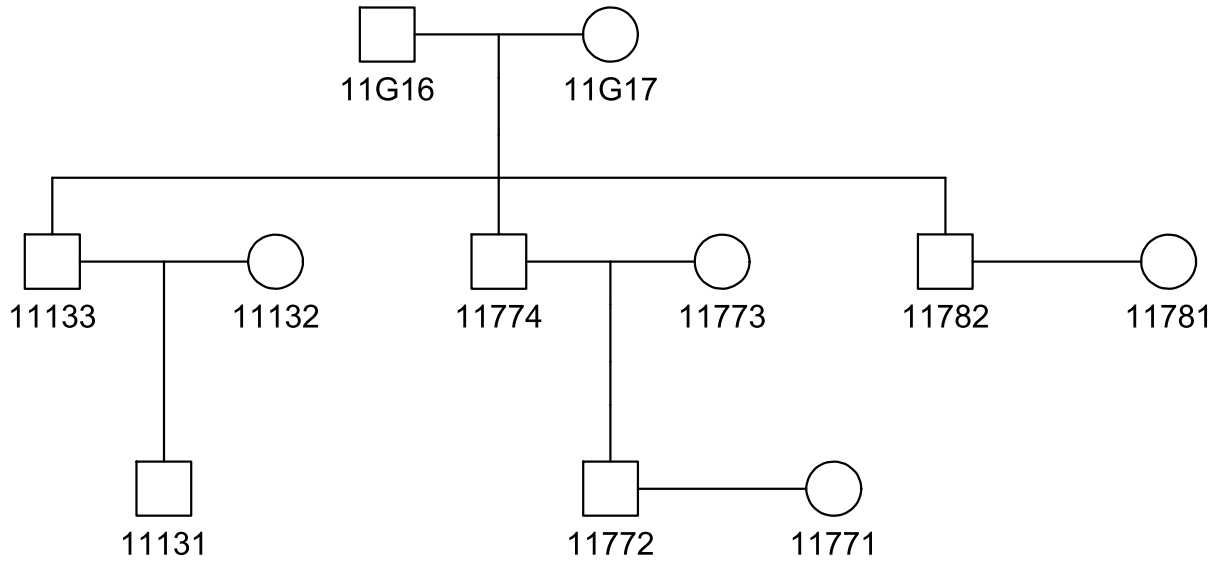
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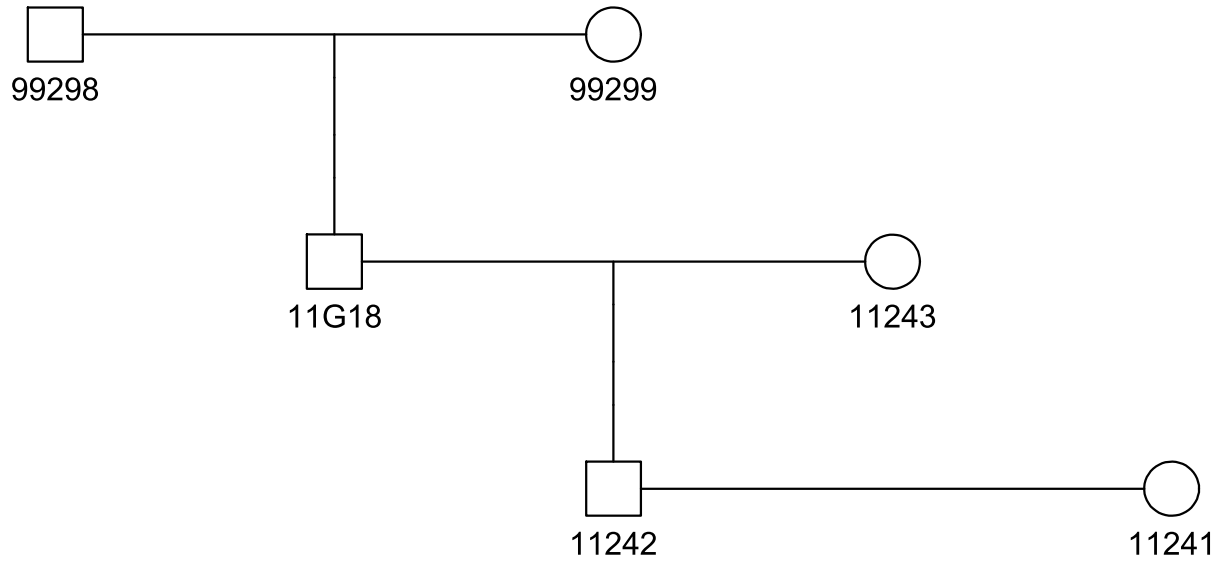
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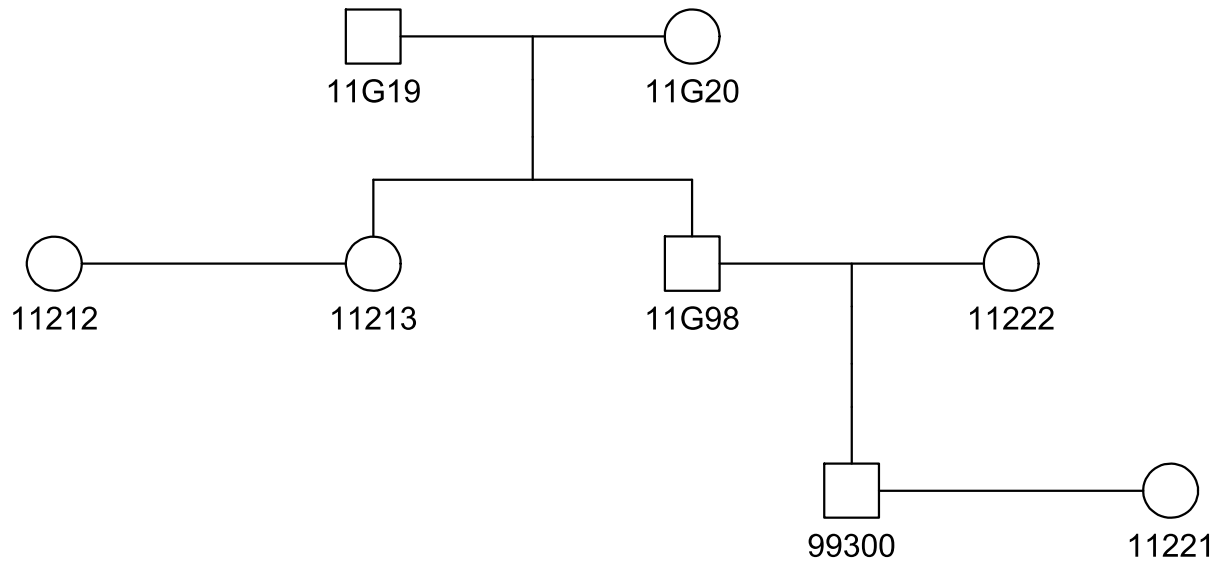
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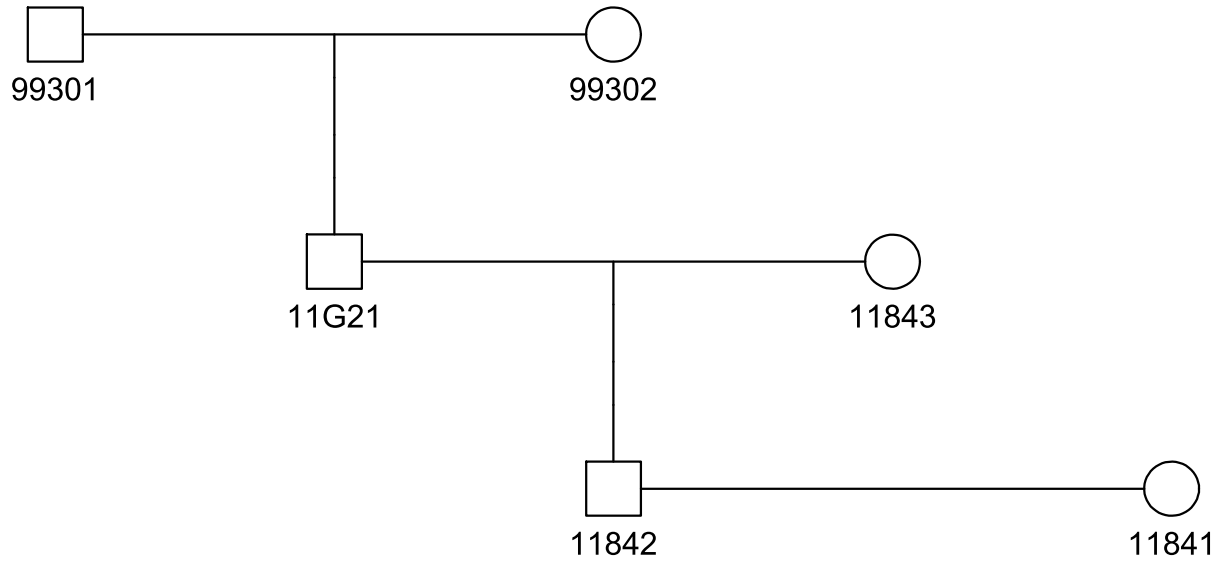
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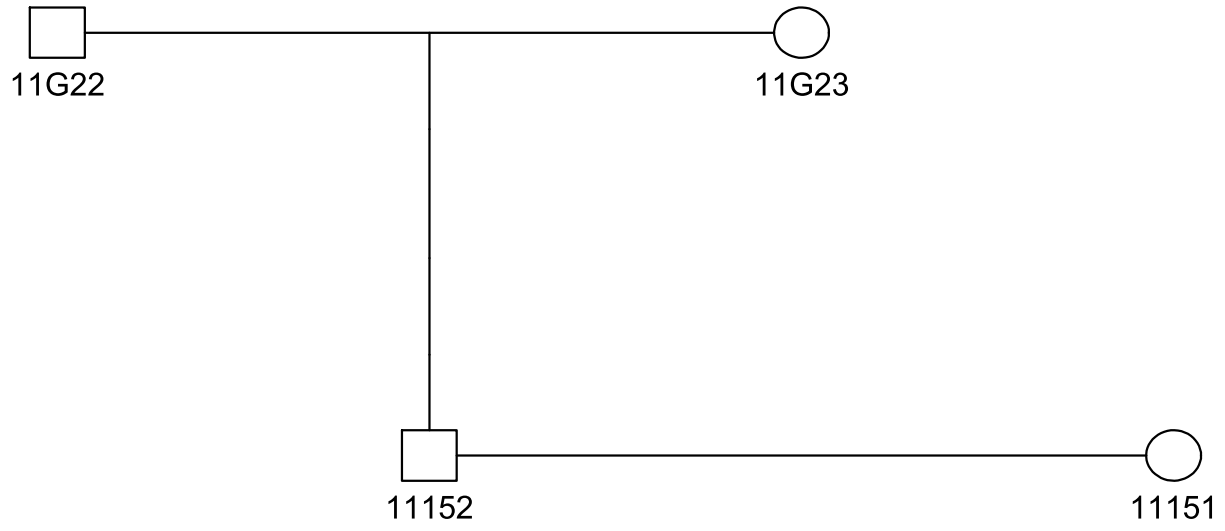
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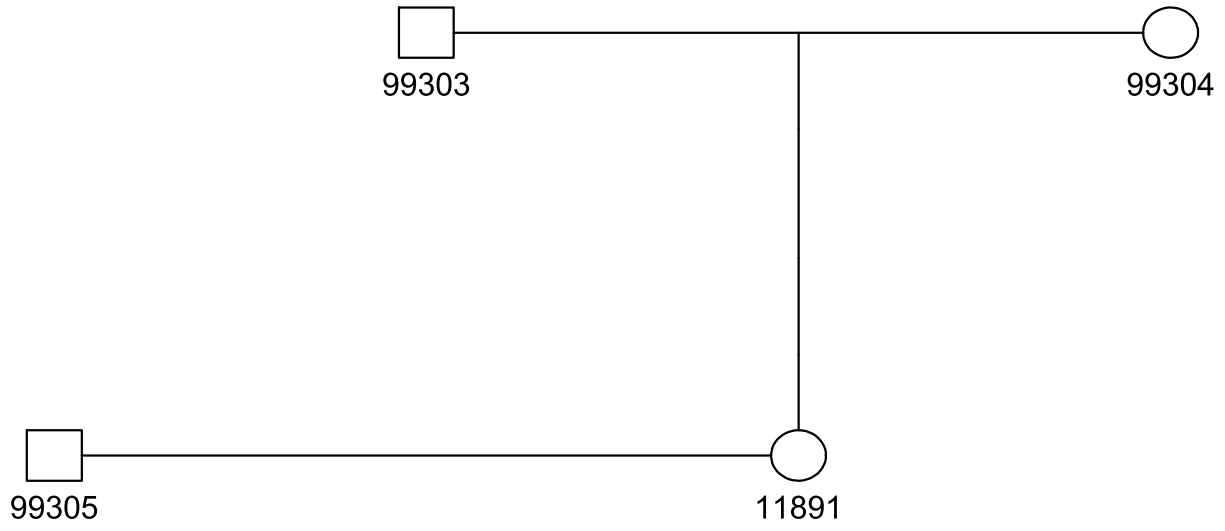
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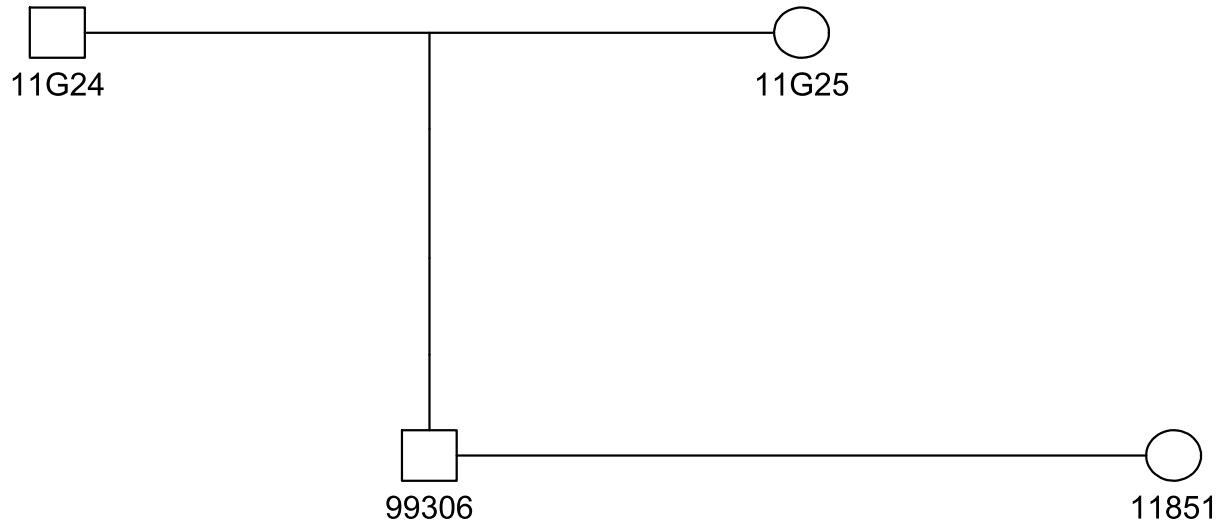
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11R13

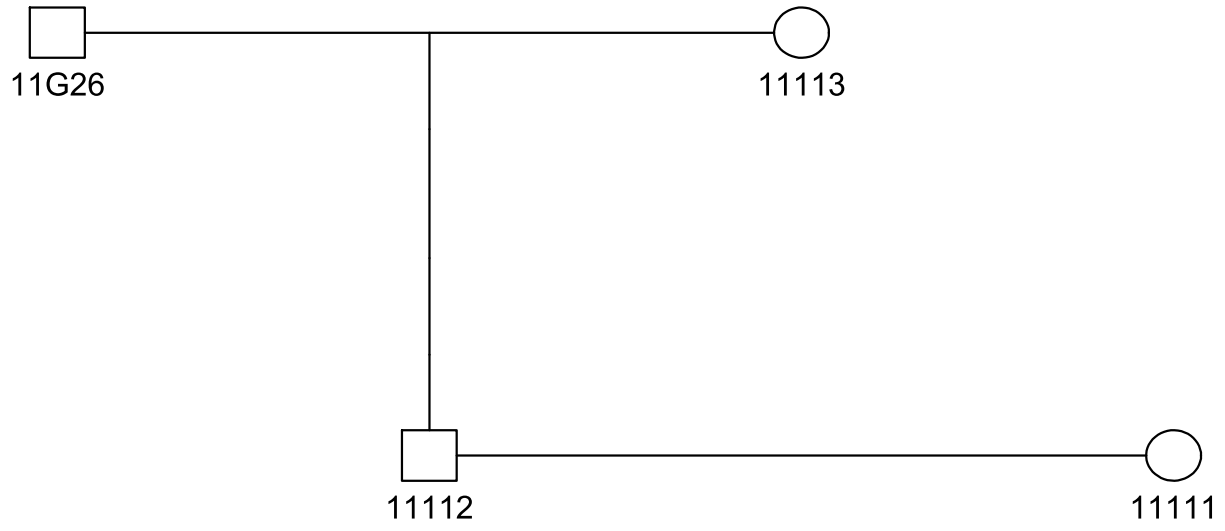


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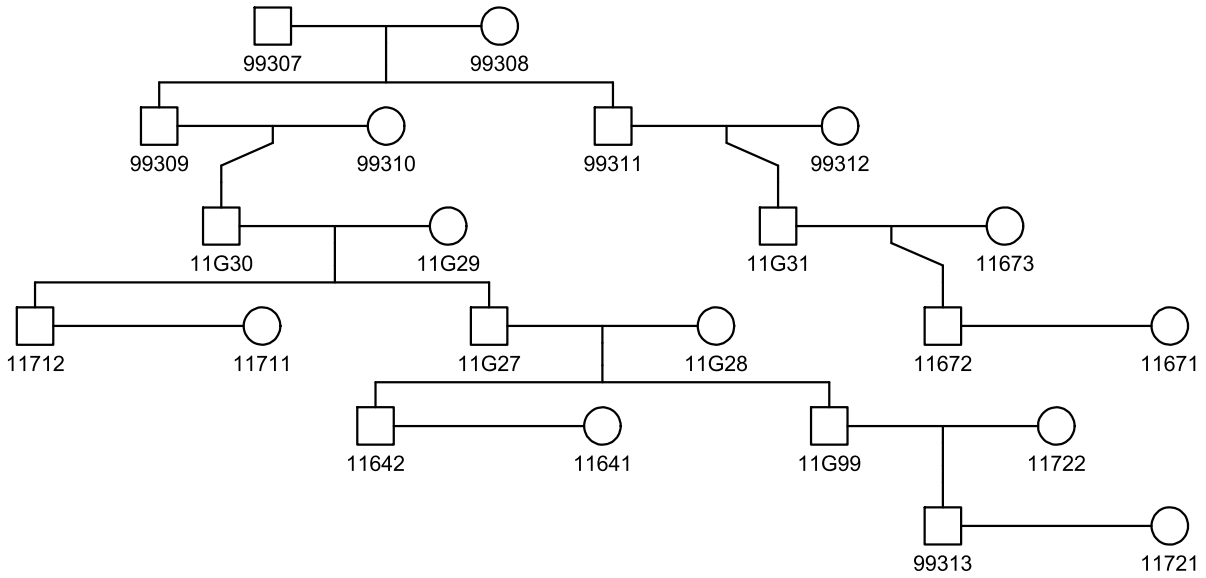


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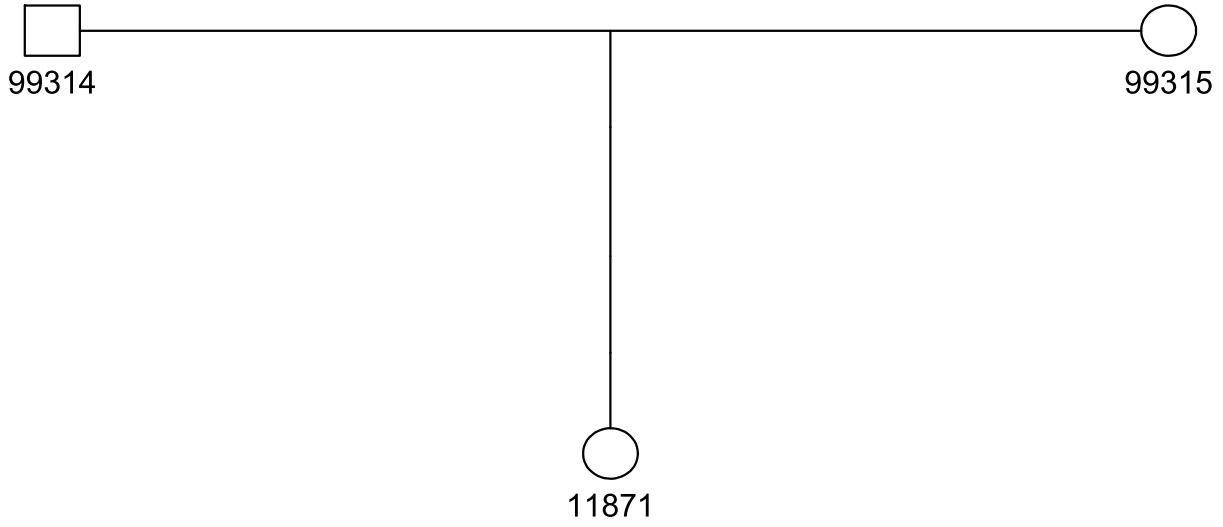
11R15



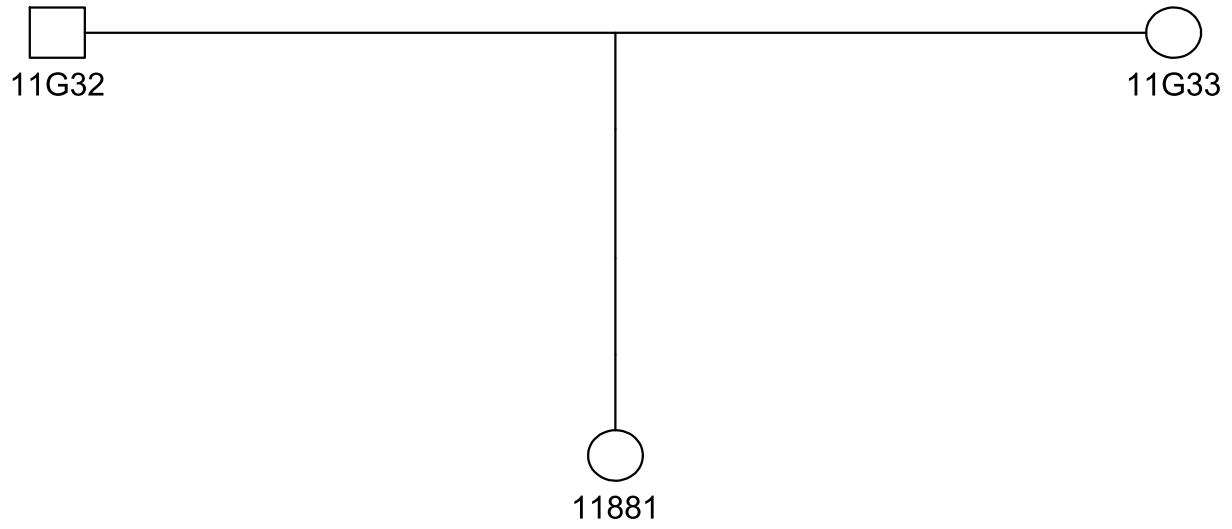
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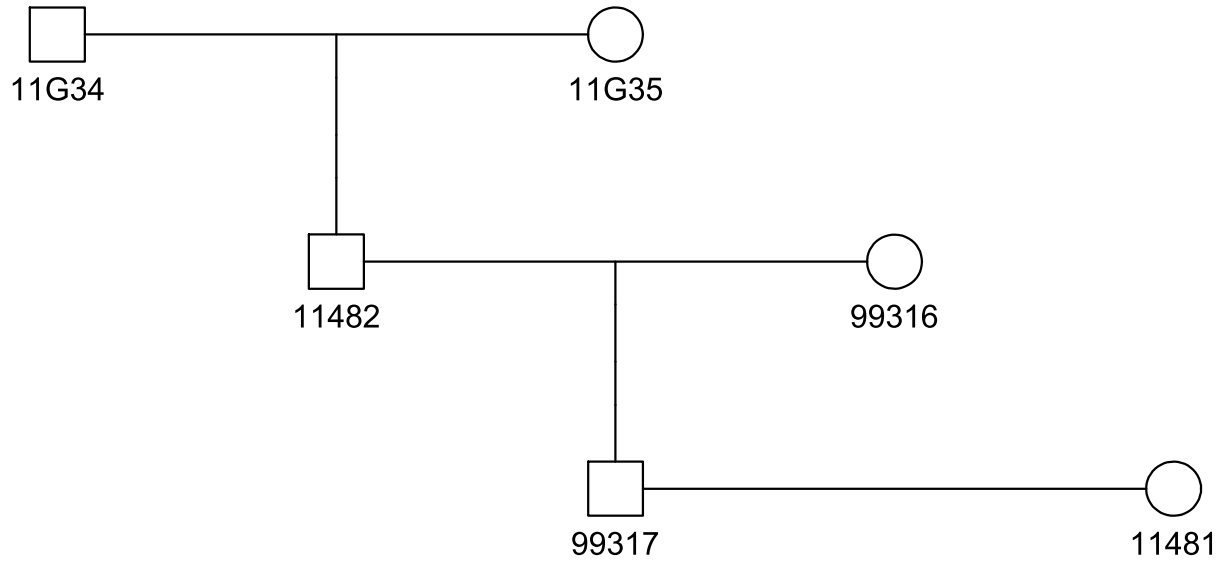
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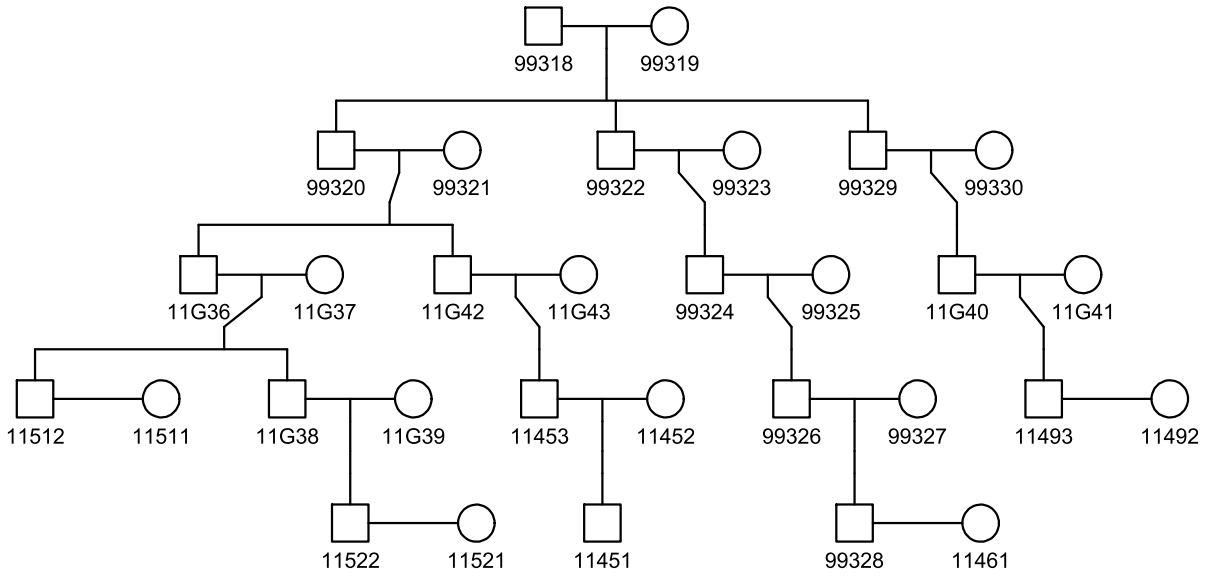
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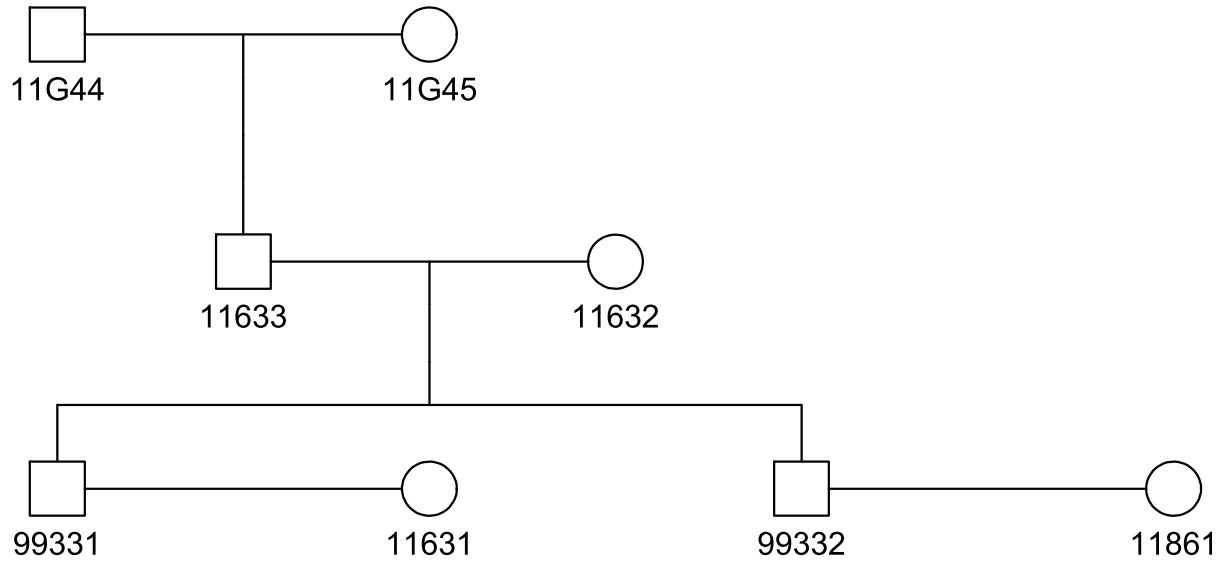
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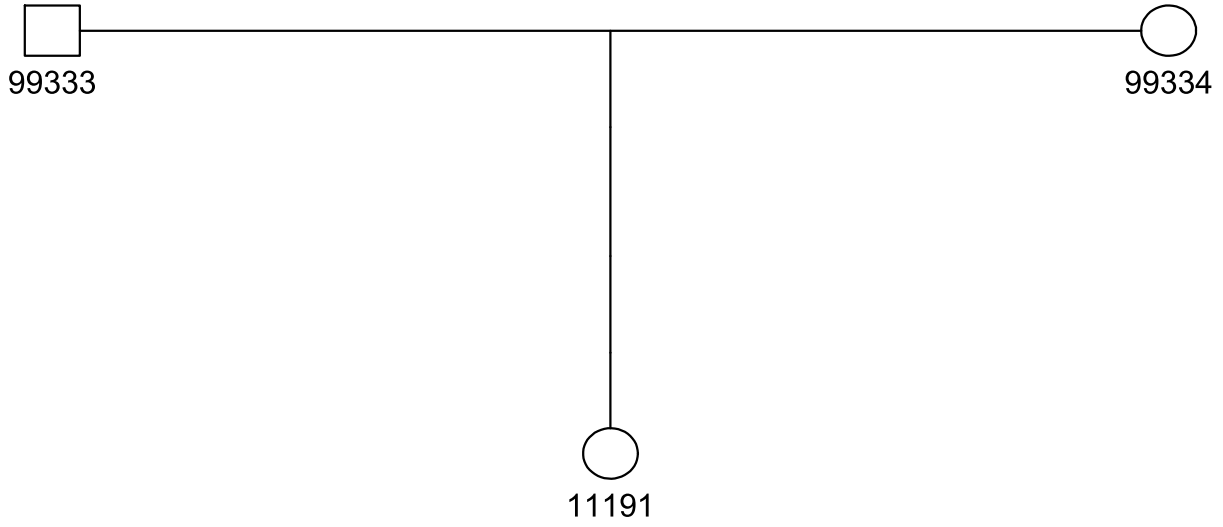
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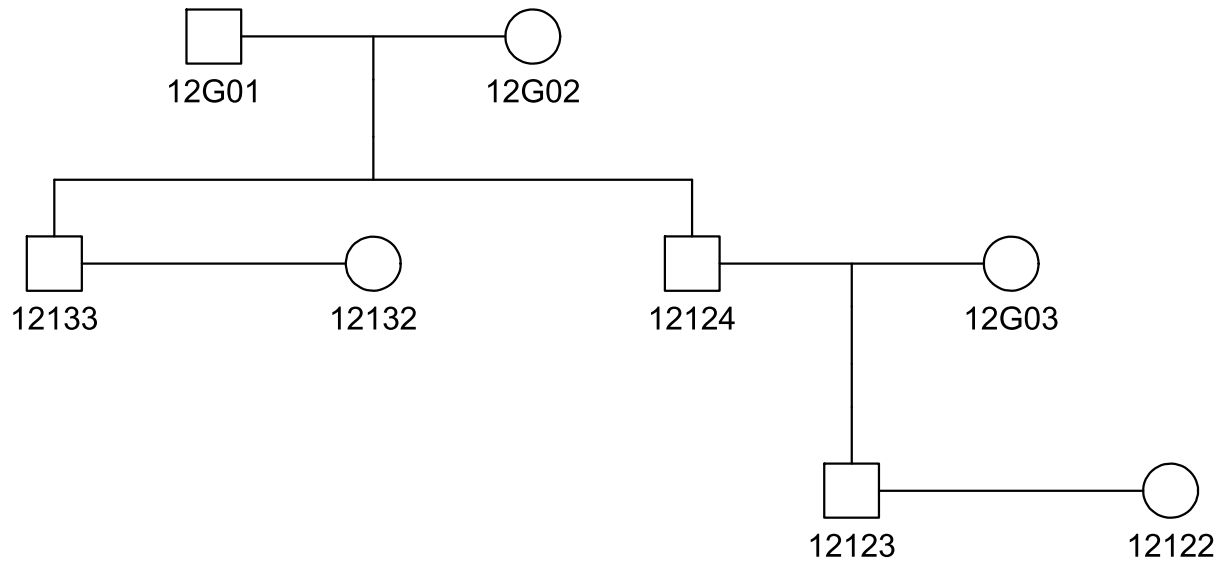
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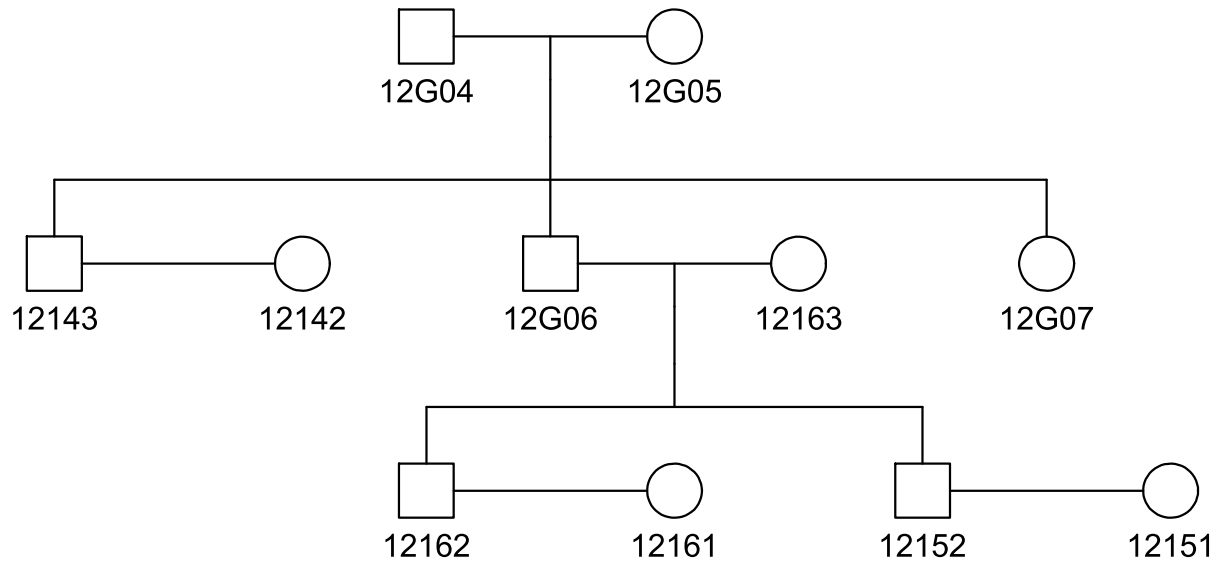
11R22



12H01

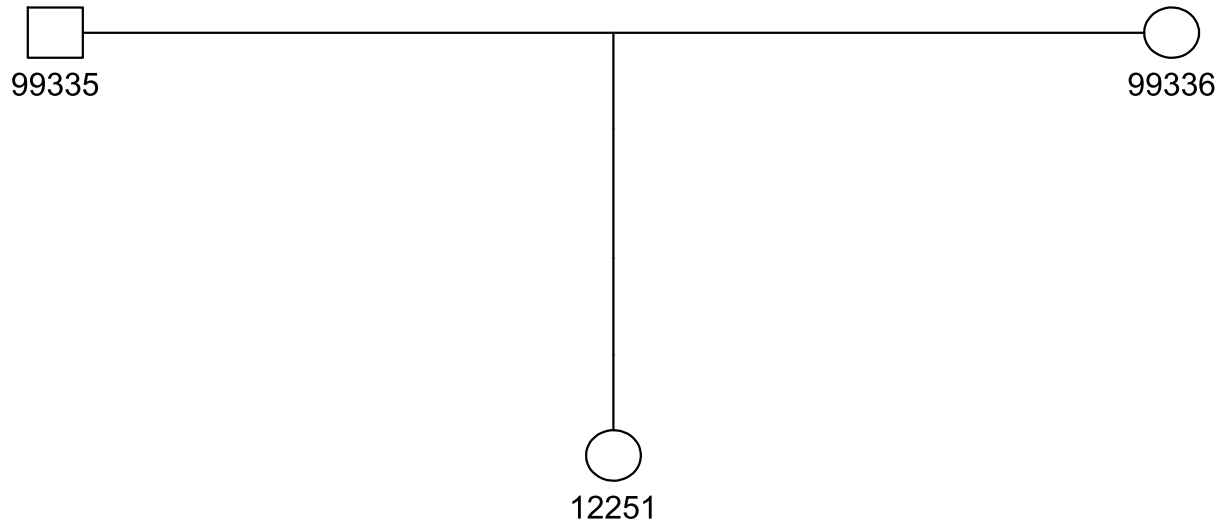


12H02

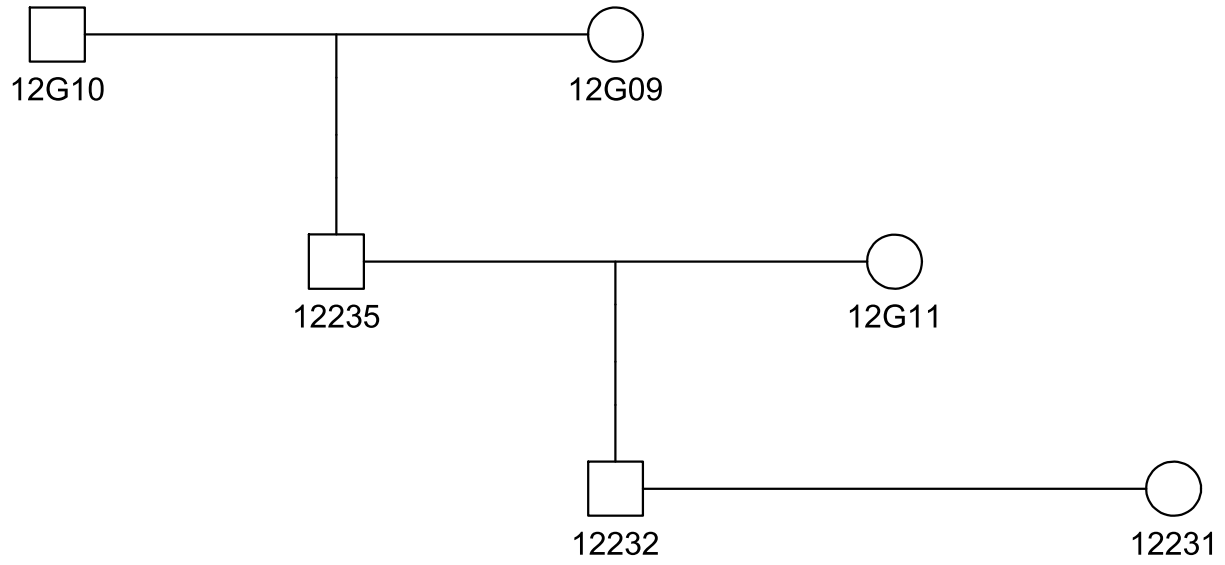


Did not plot the following people: 12171 12G08

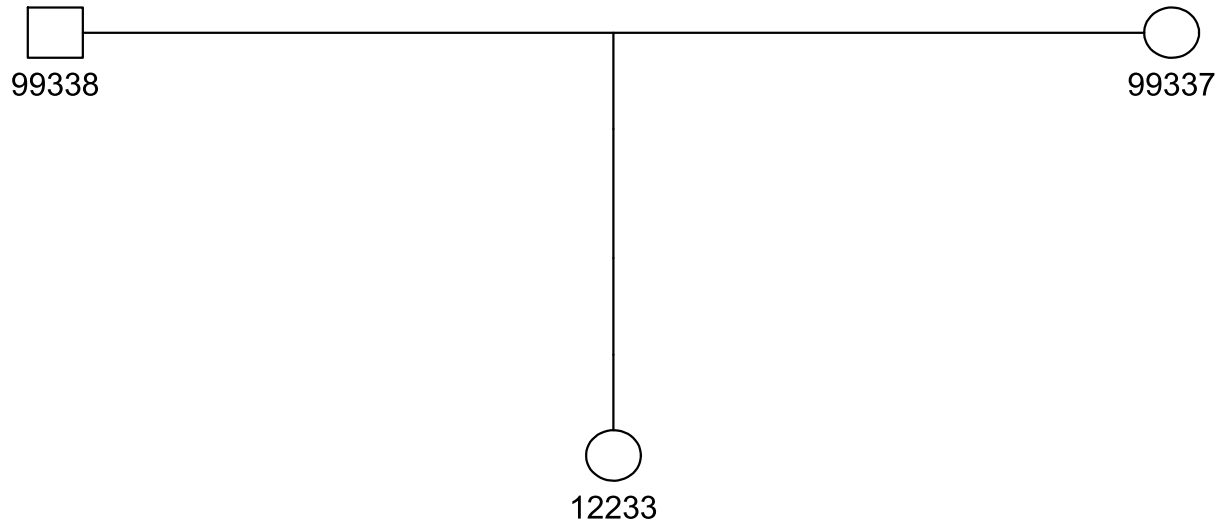
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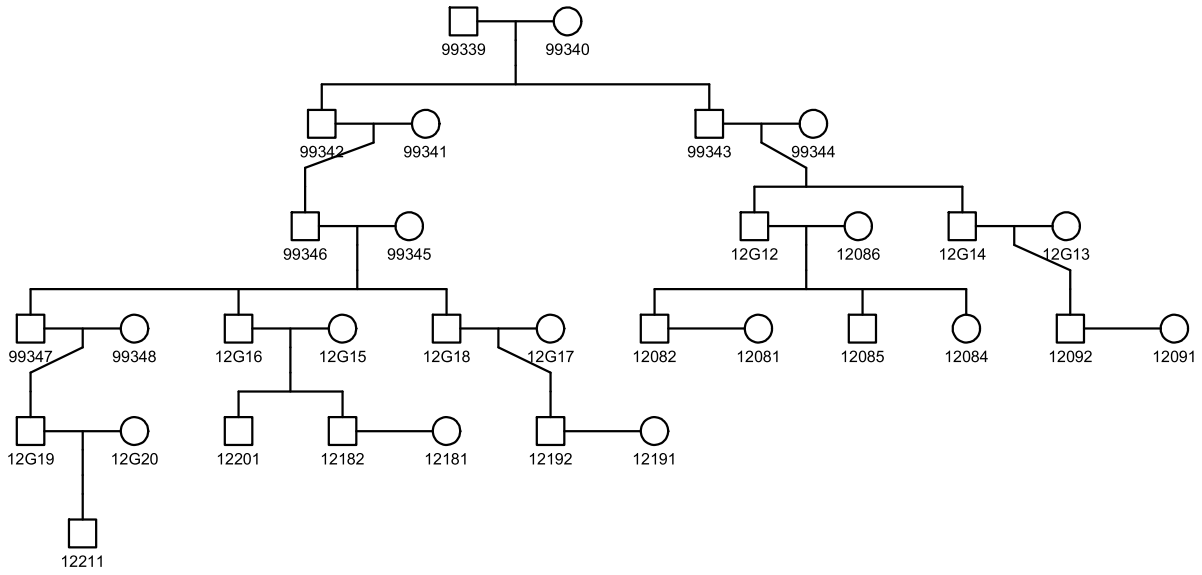
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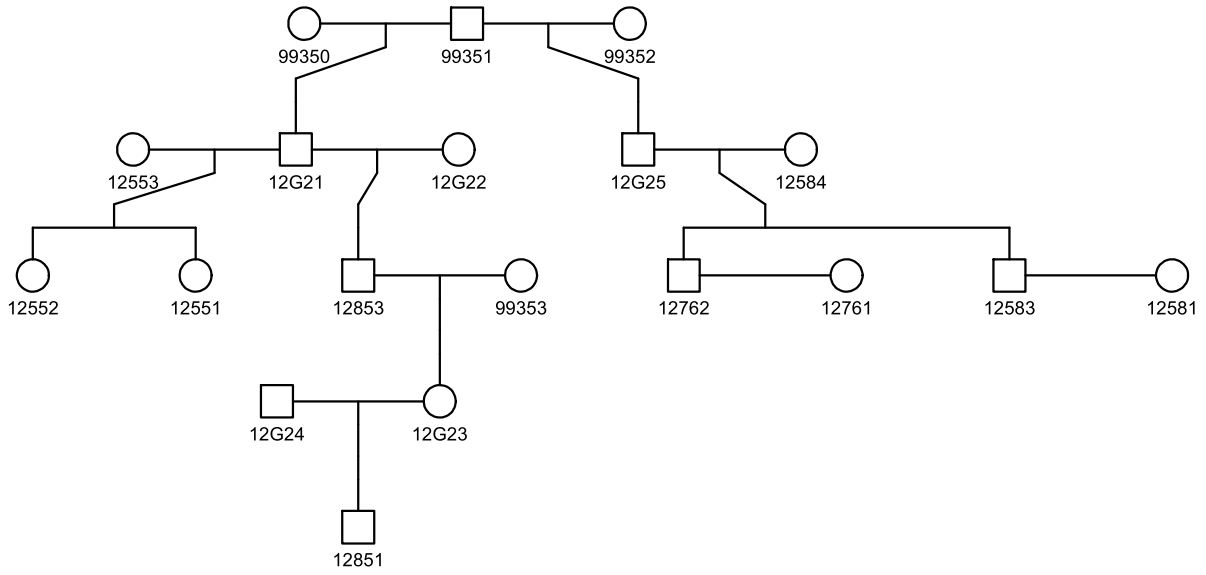
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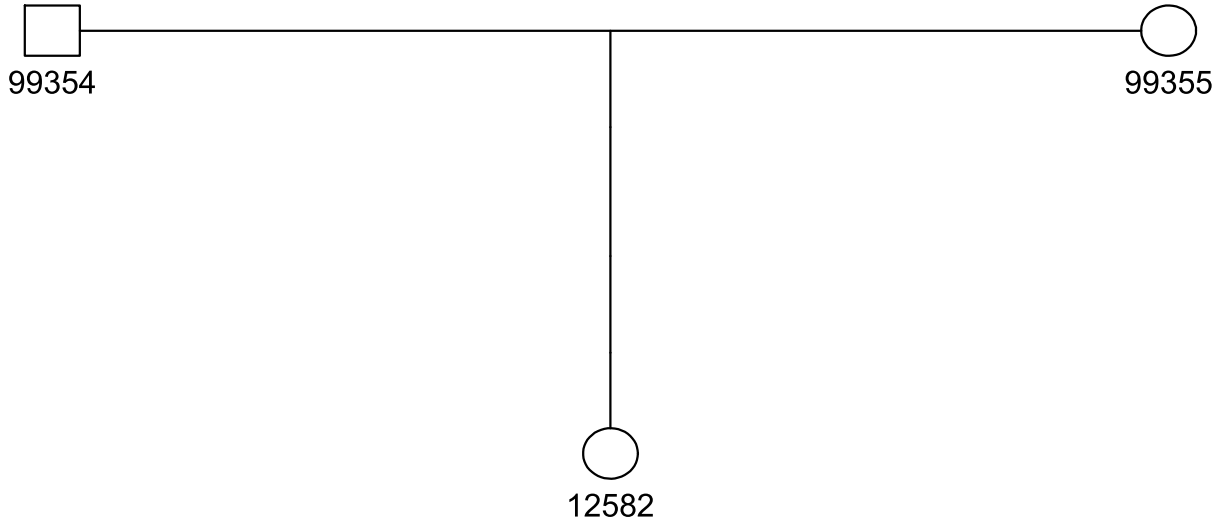
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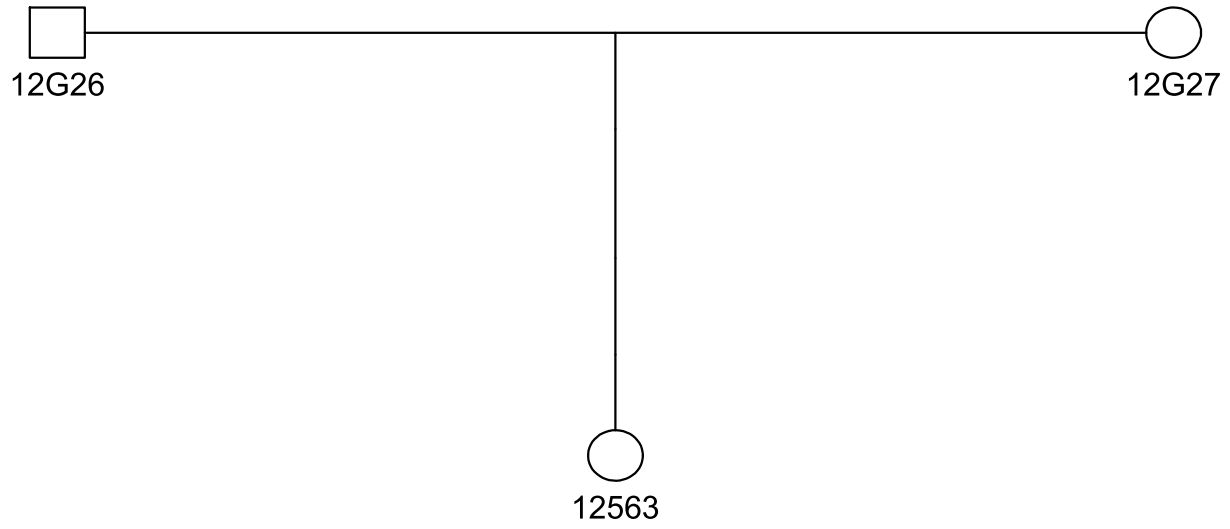
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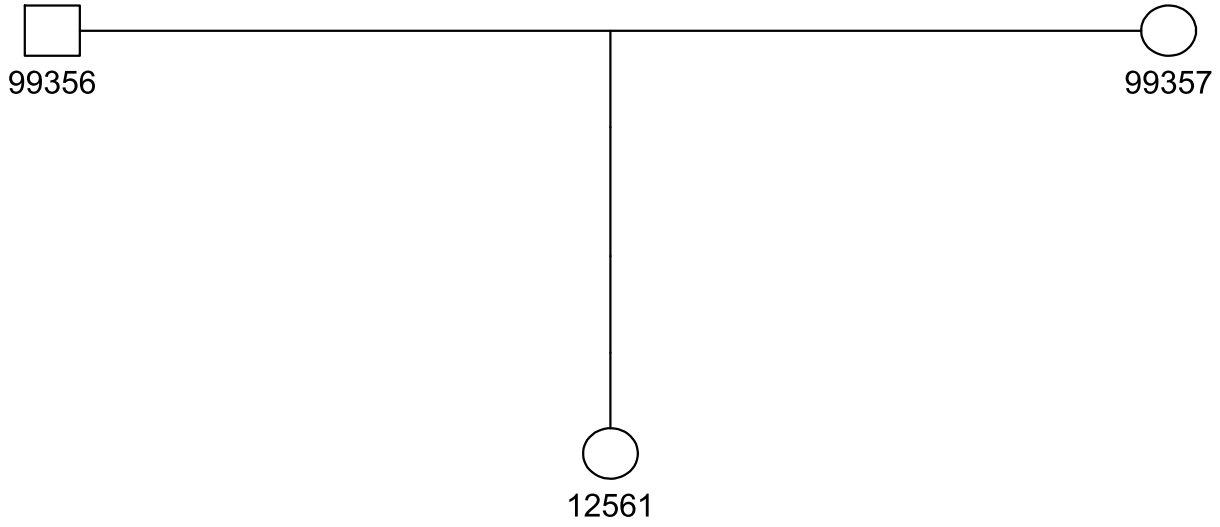
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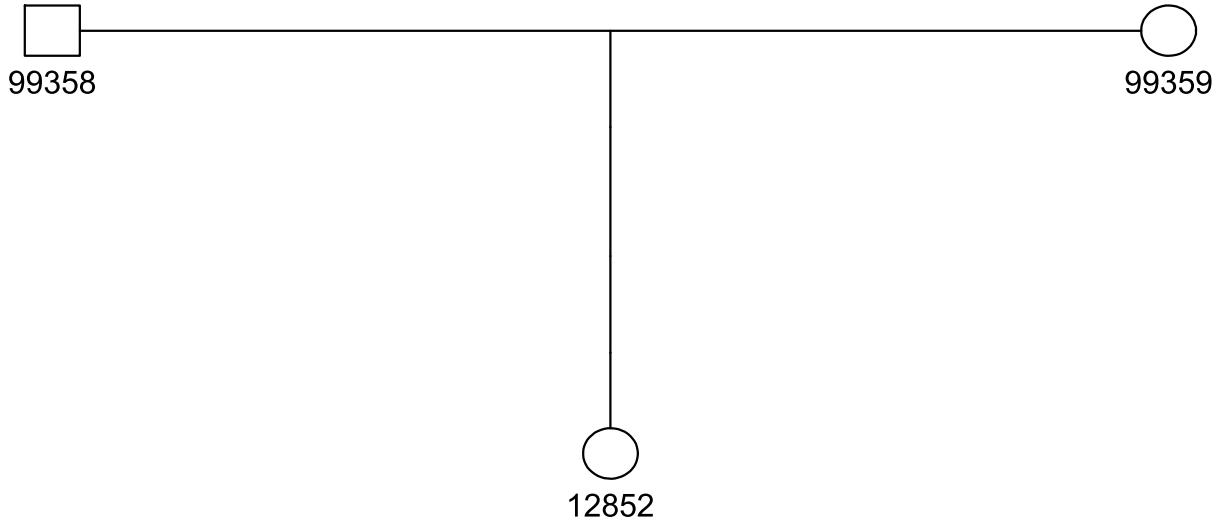
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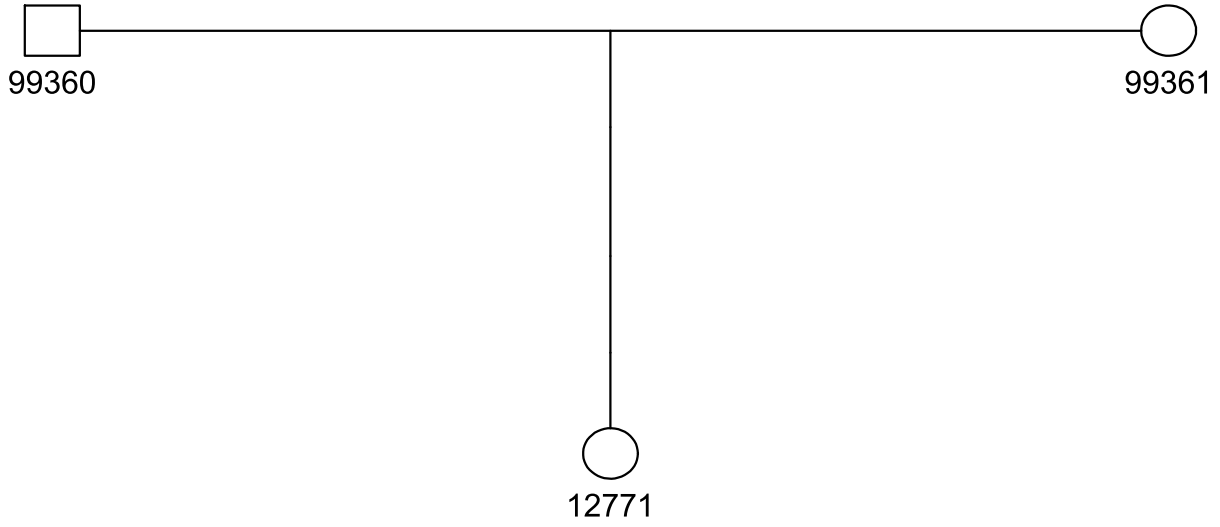
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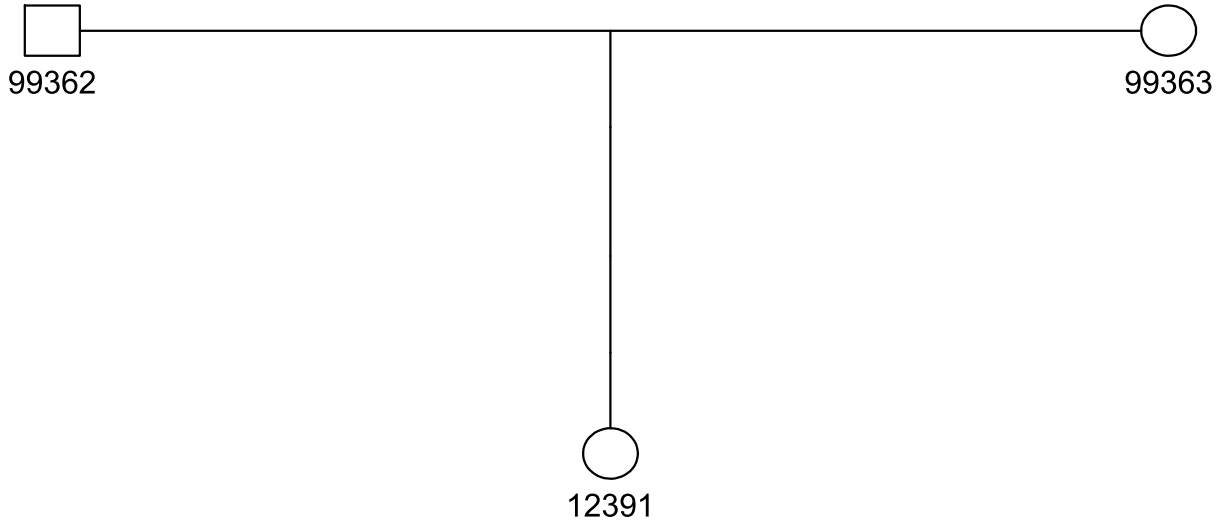
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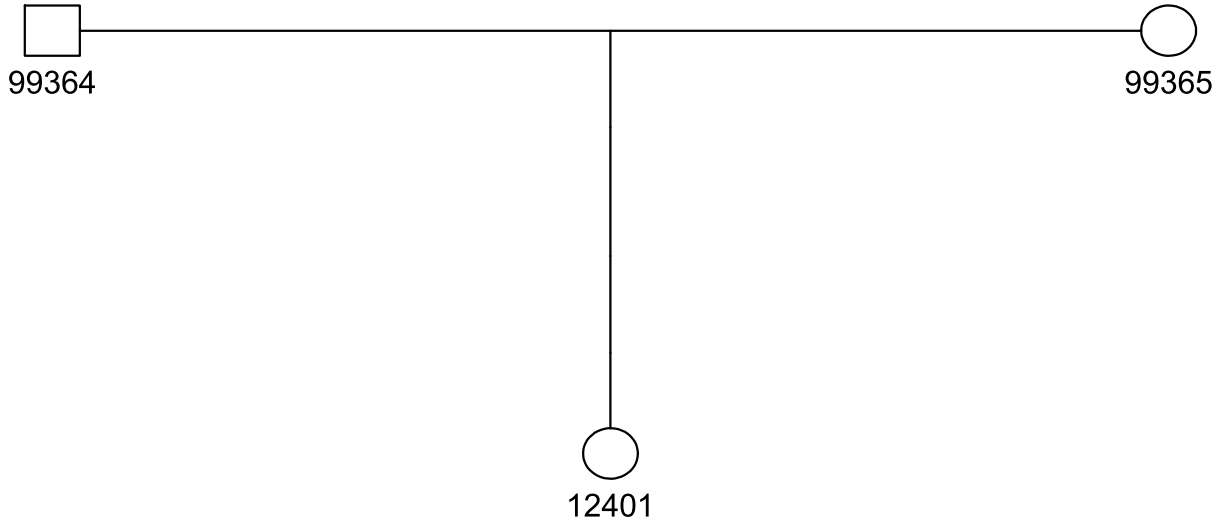
12H12



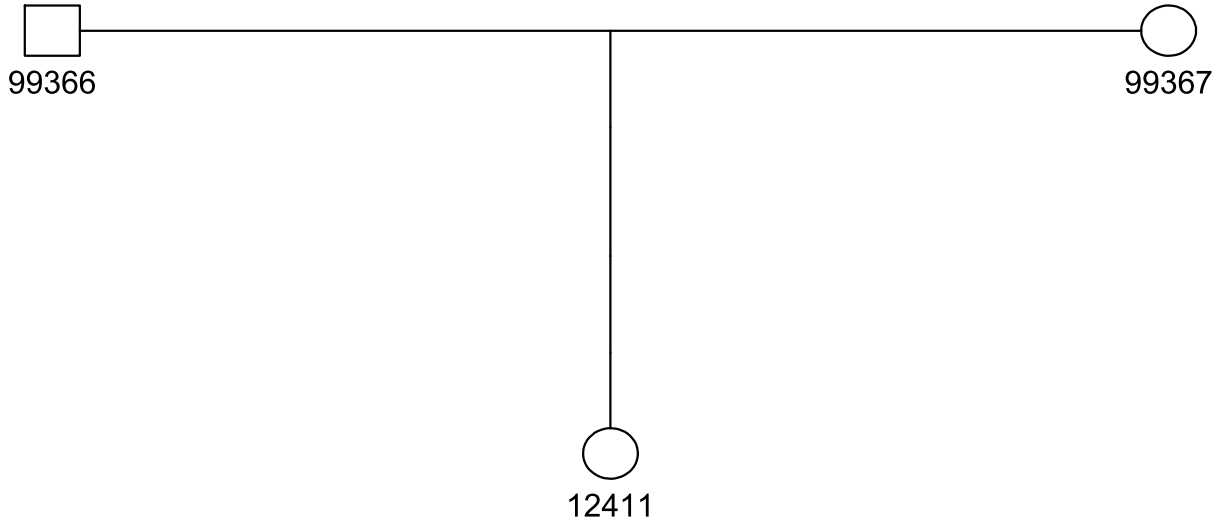
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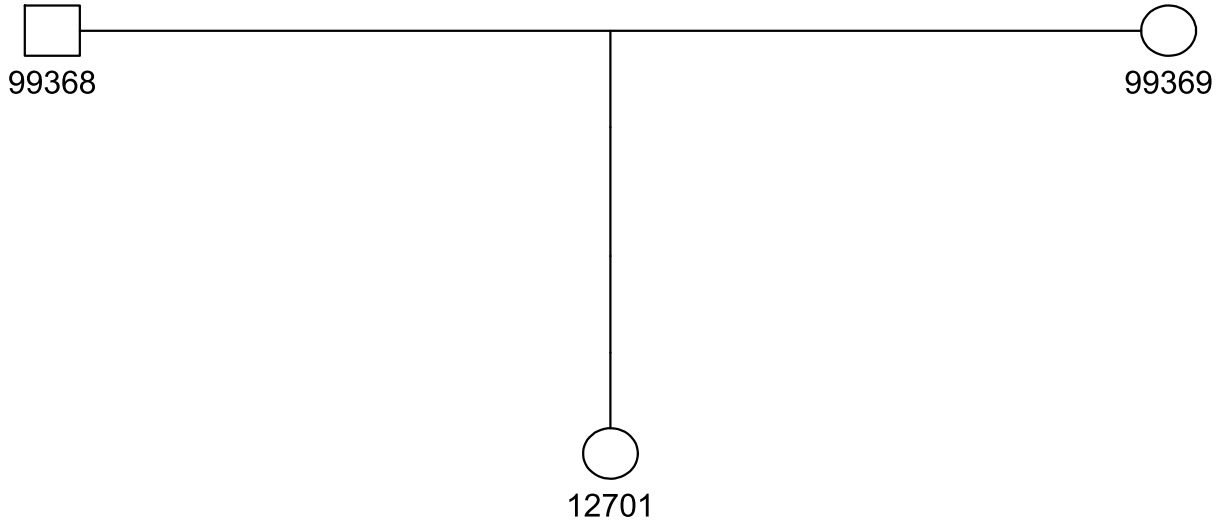
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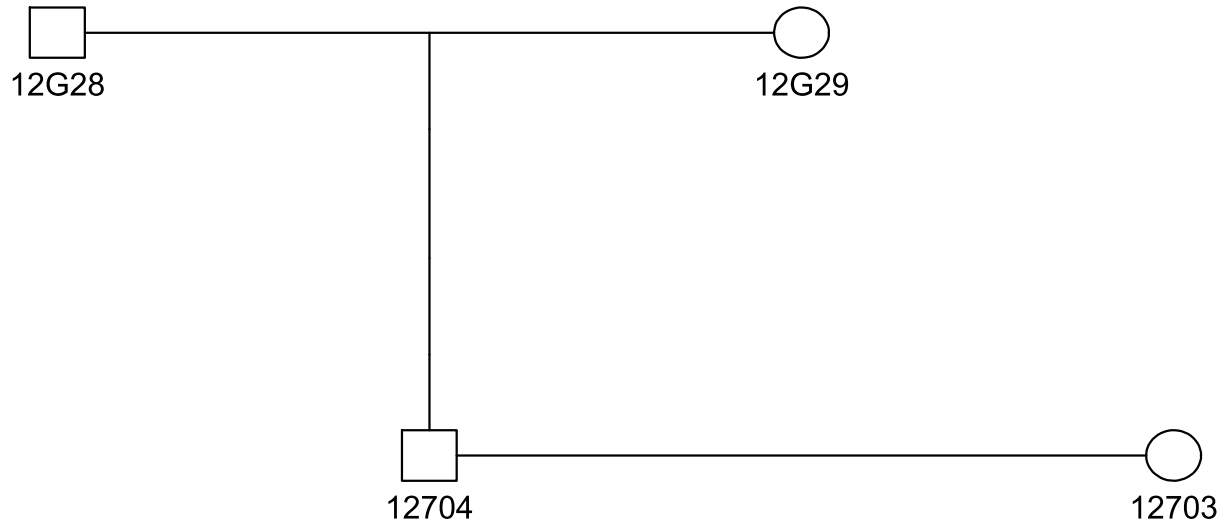
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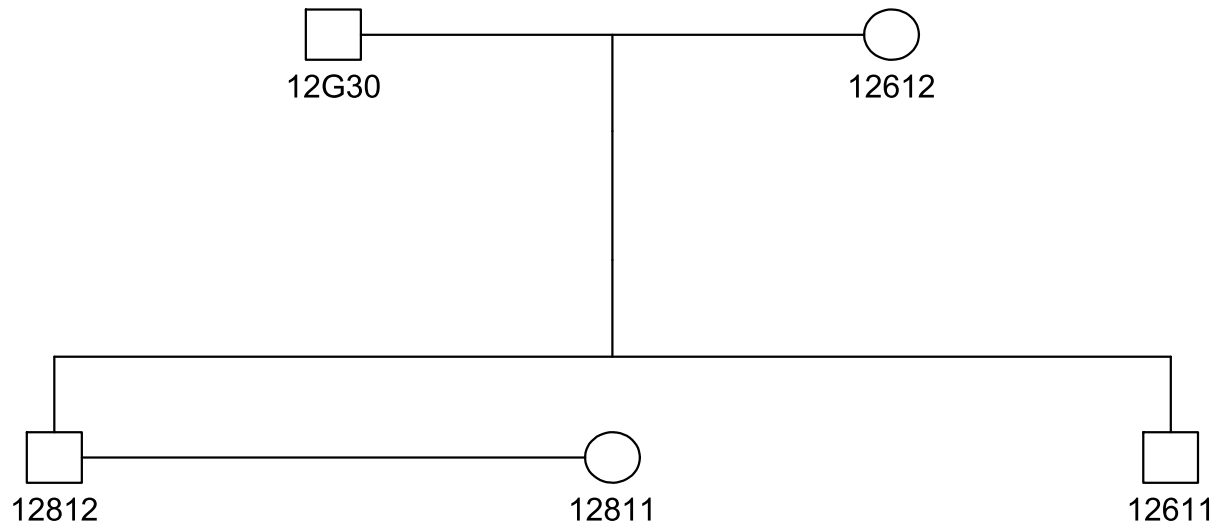
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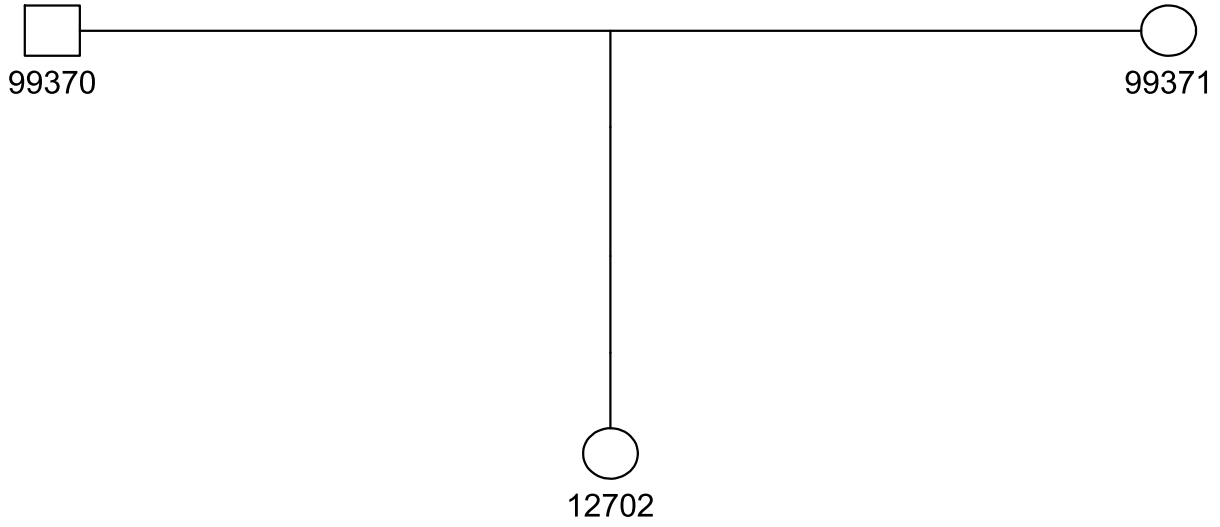
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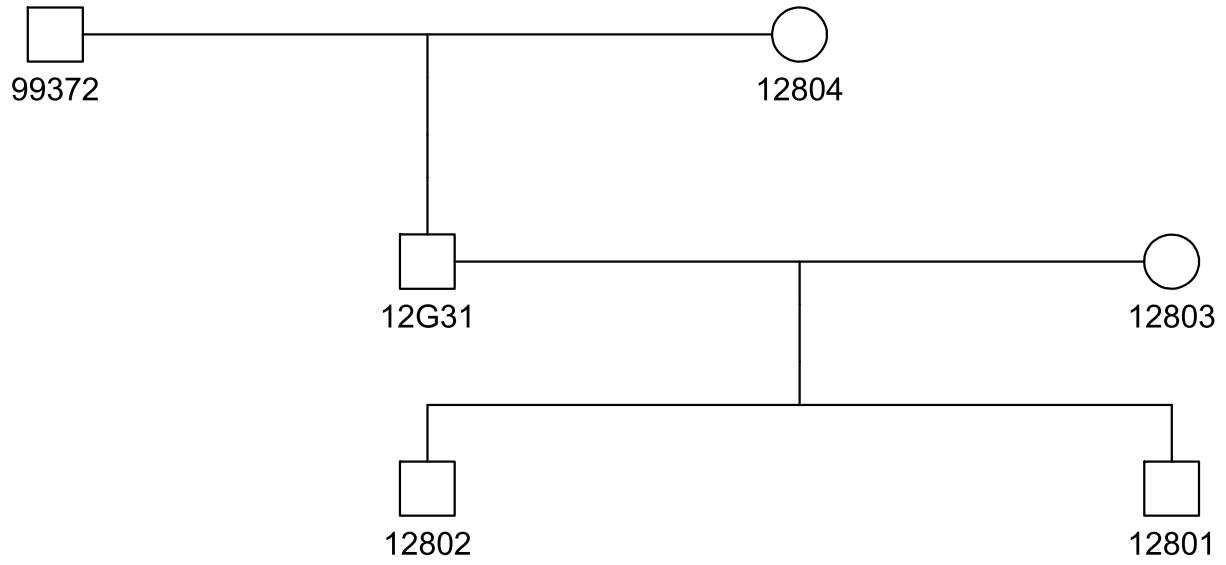
12H18



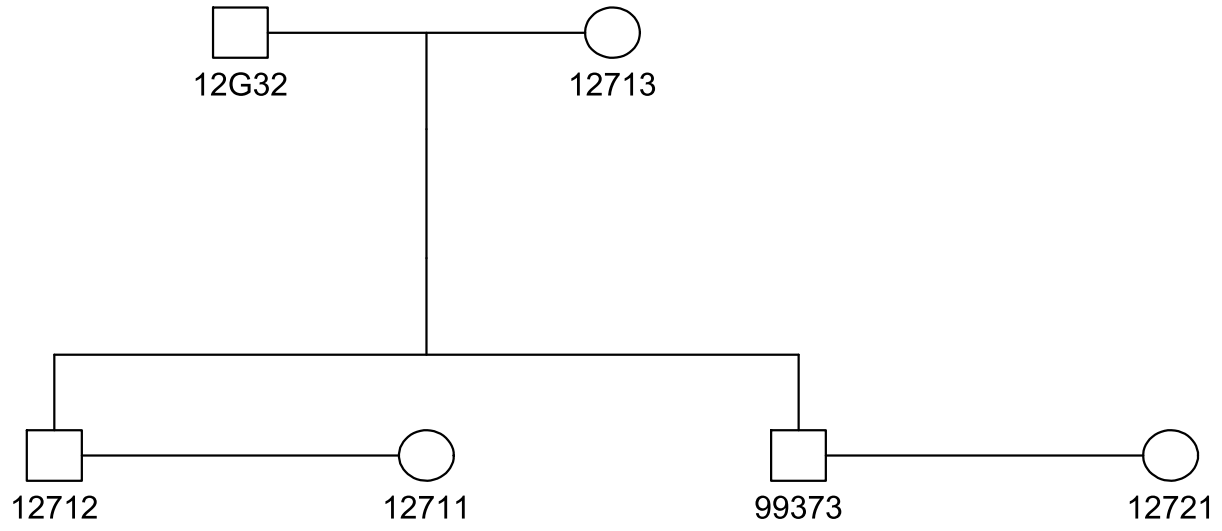
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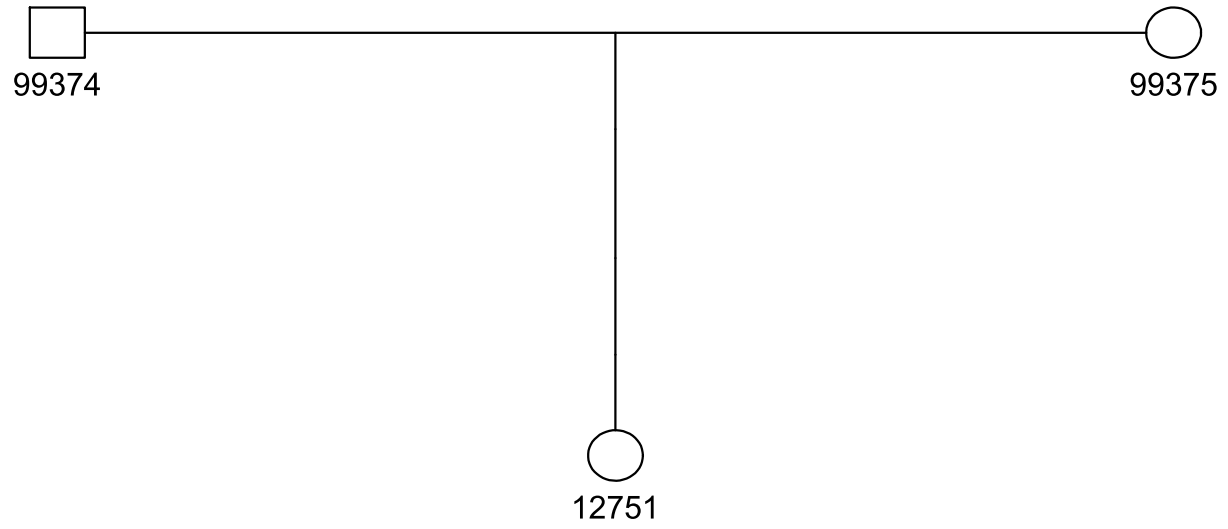
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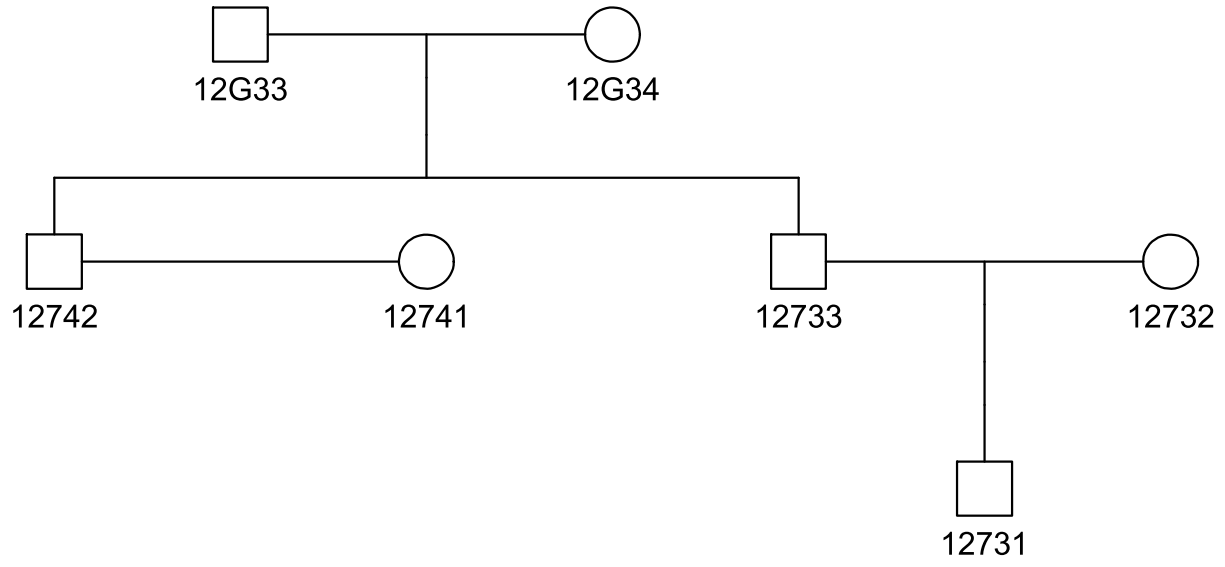
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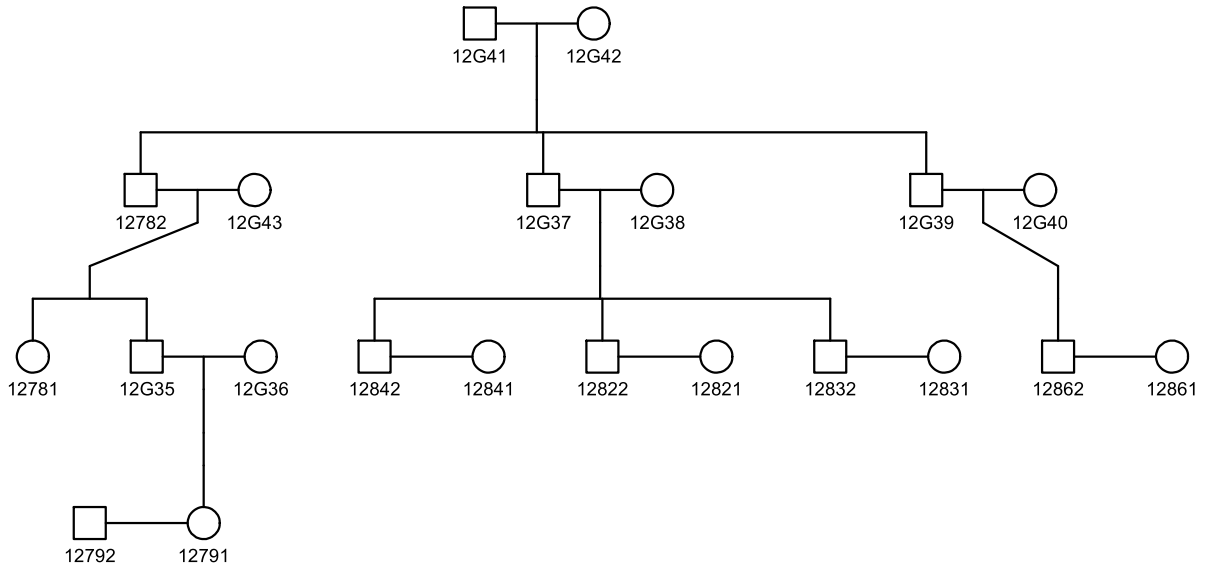
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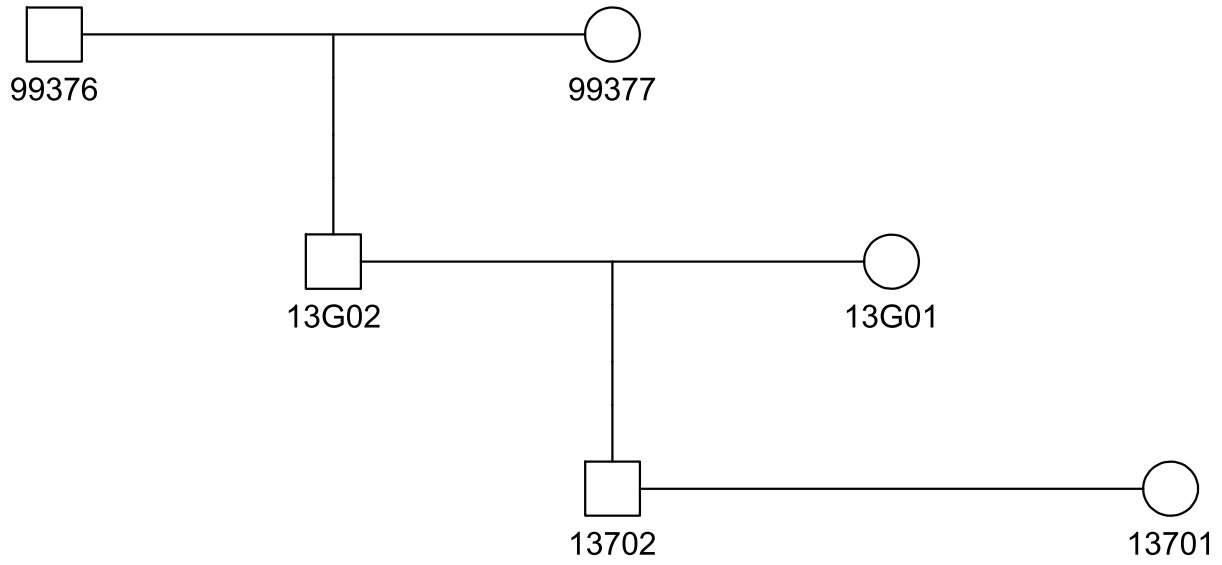
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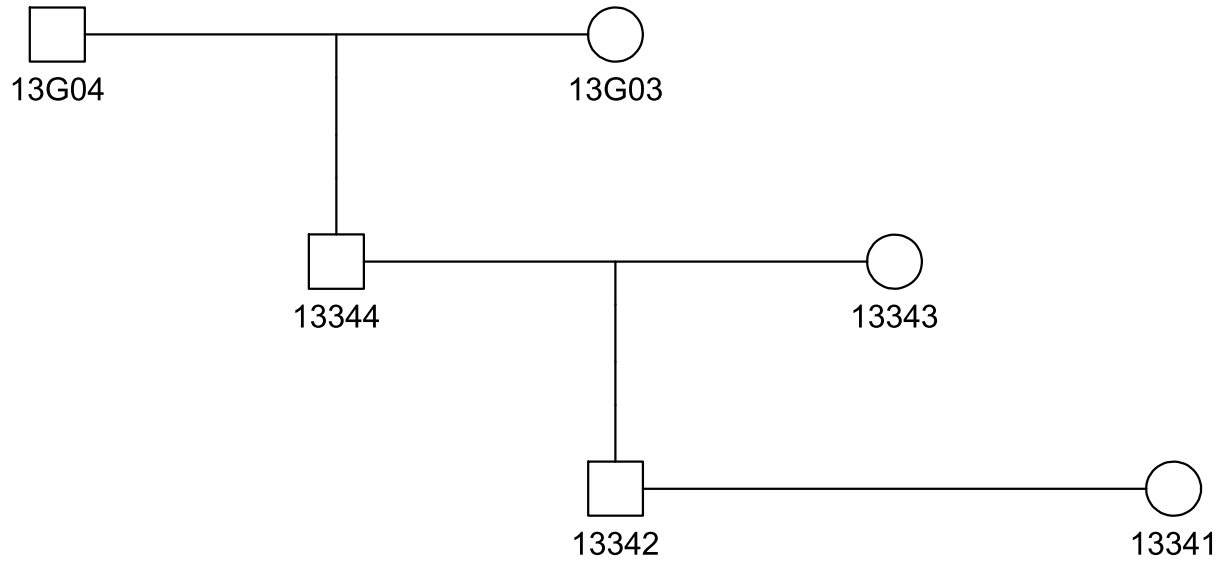
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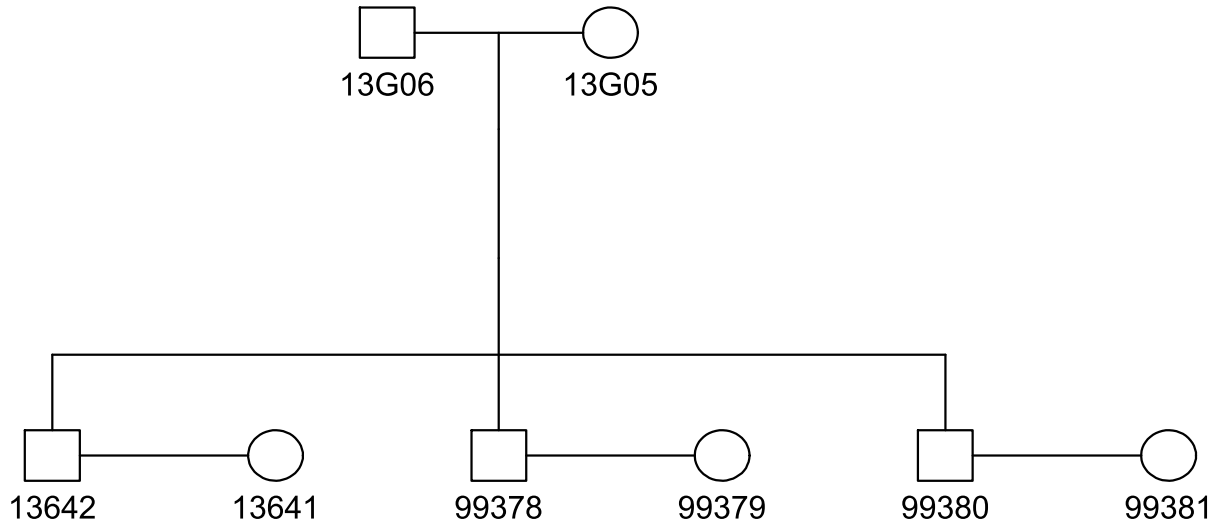
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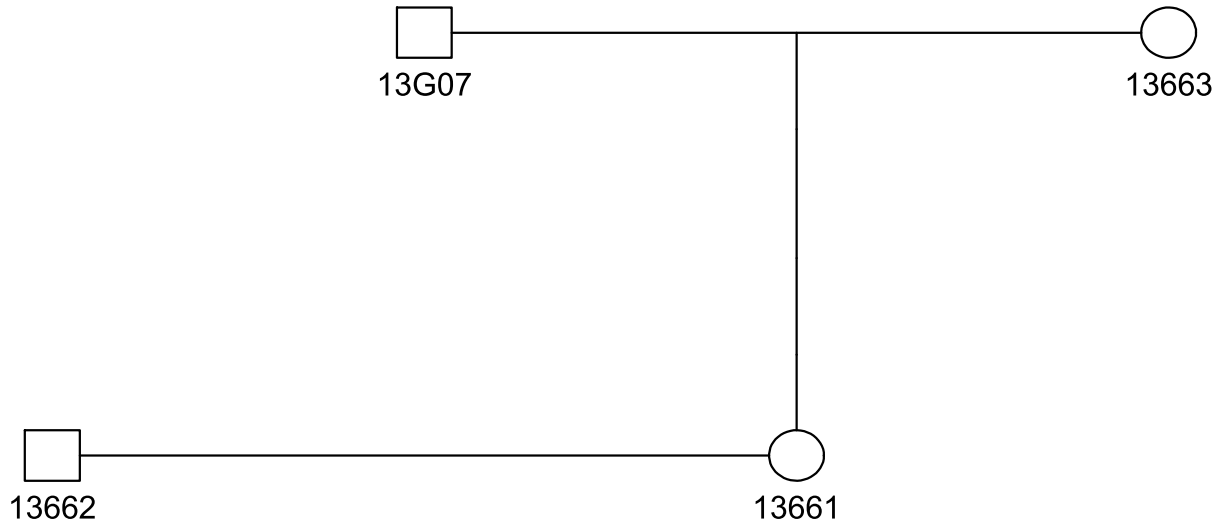
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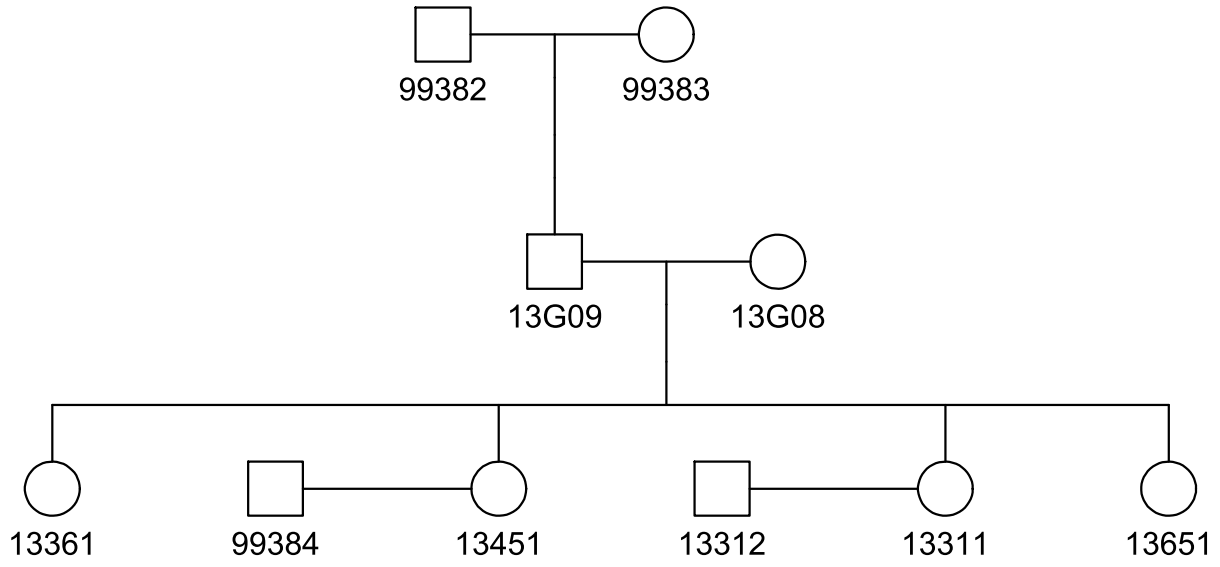
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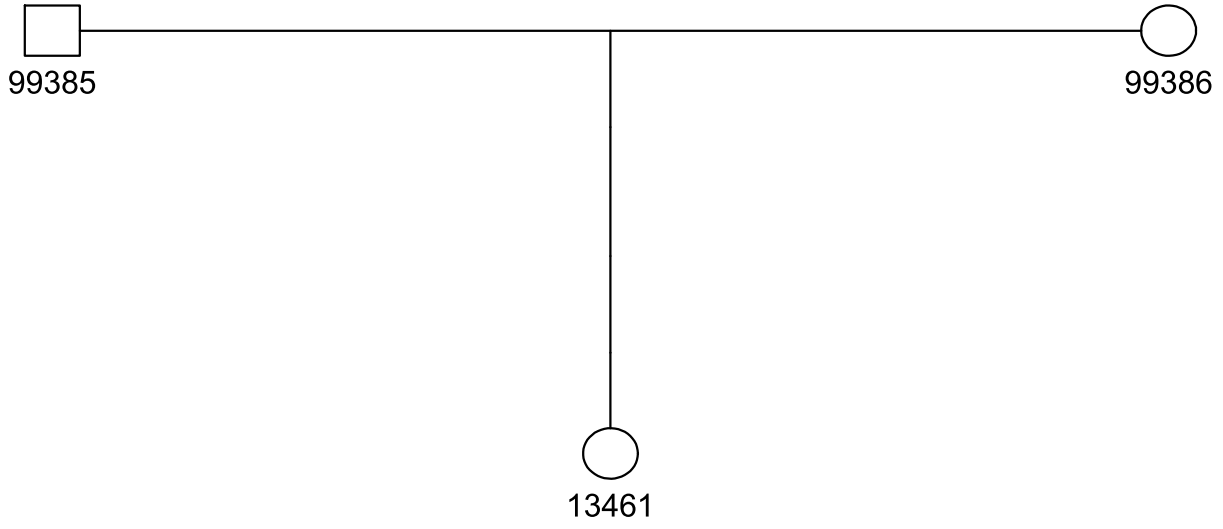
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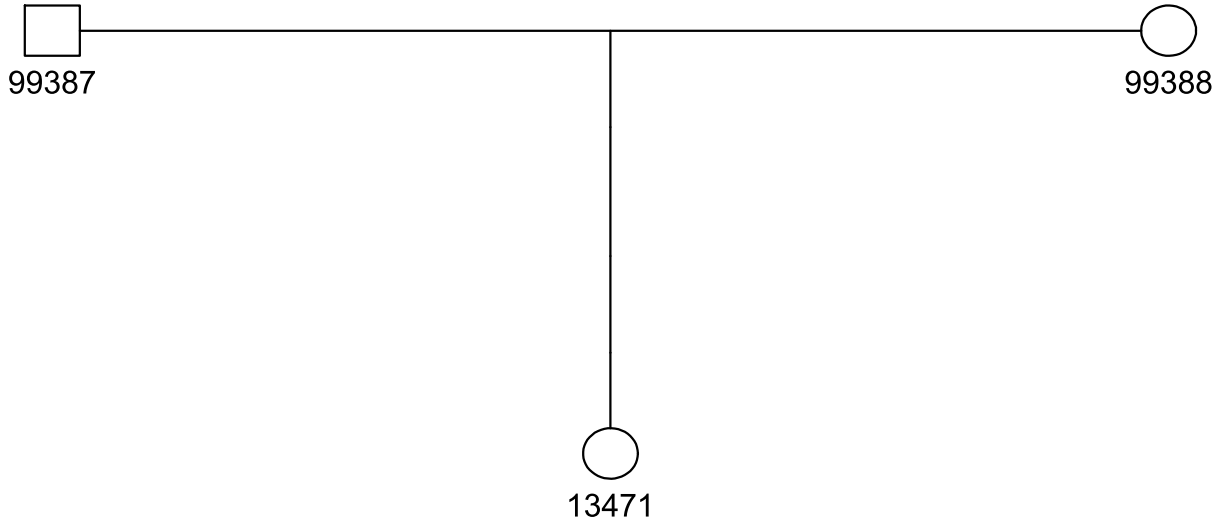
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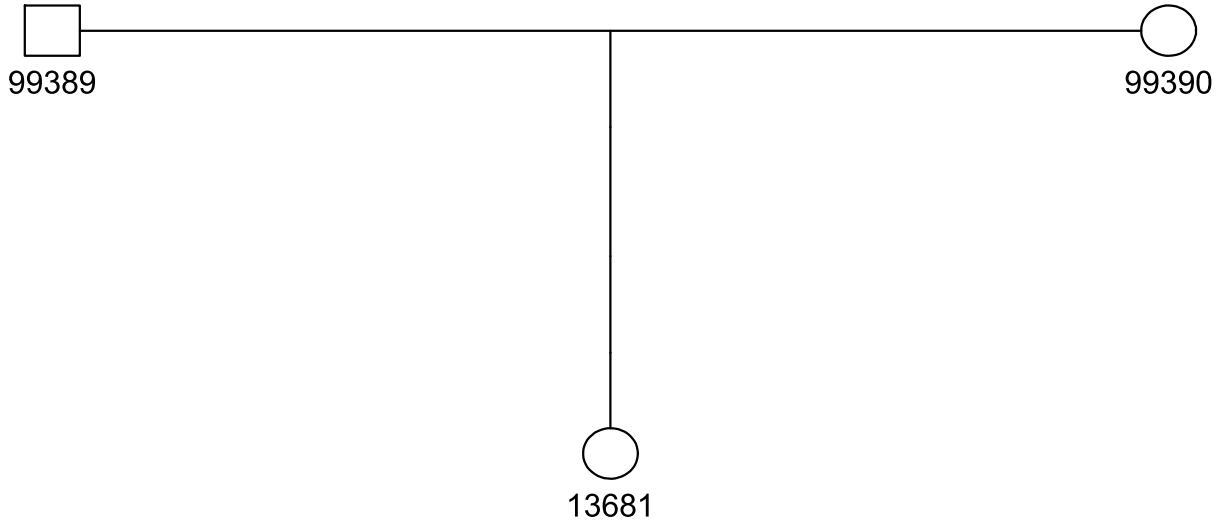
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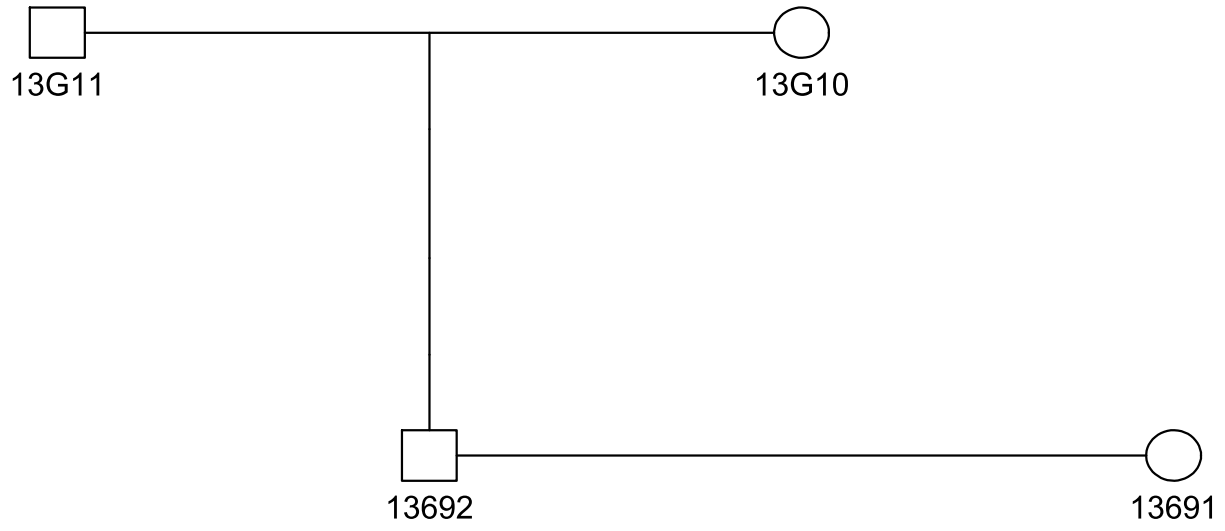
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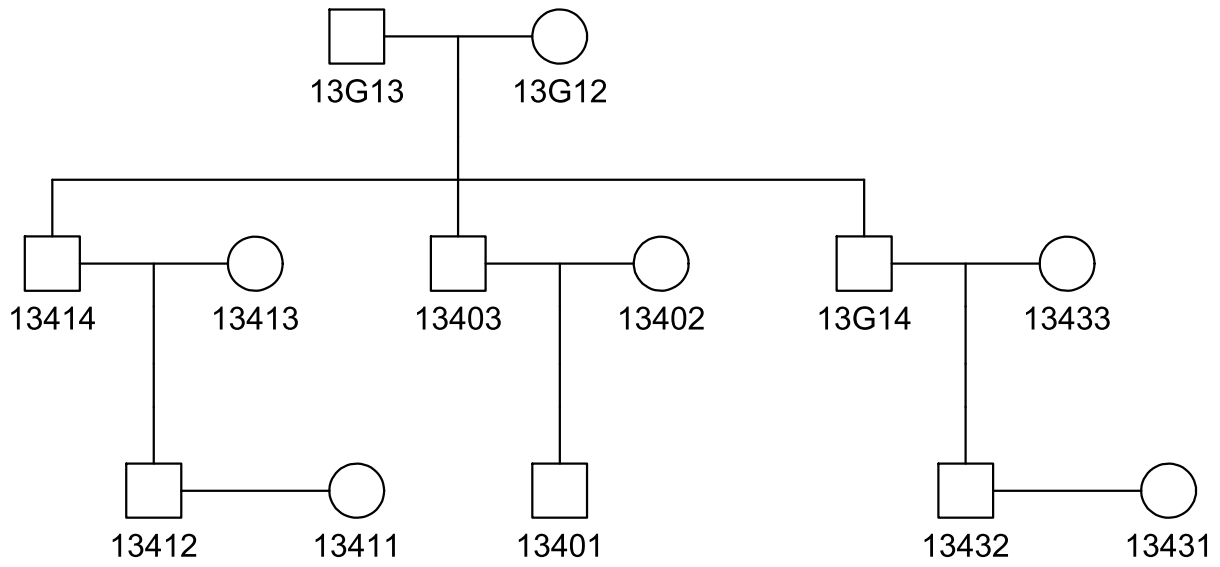
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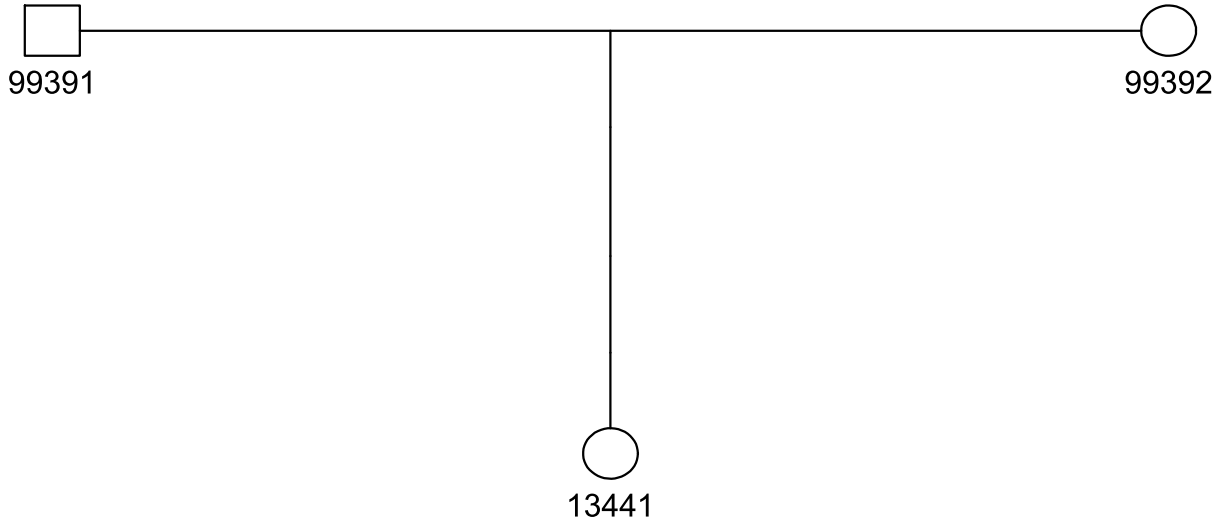
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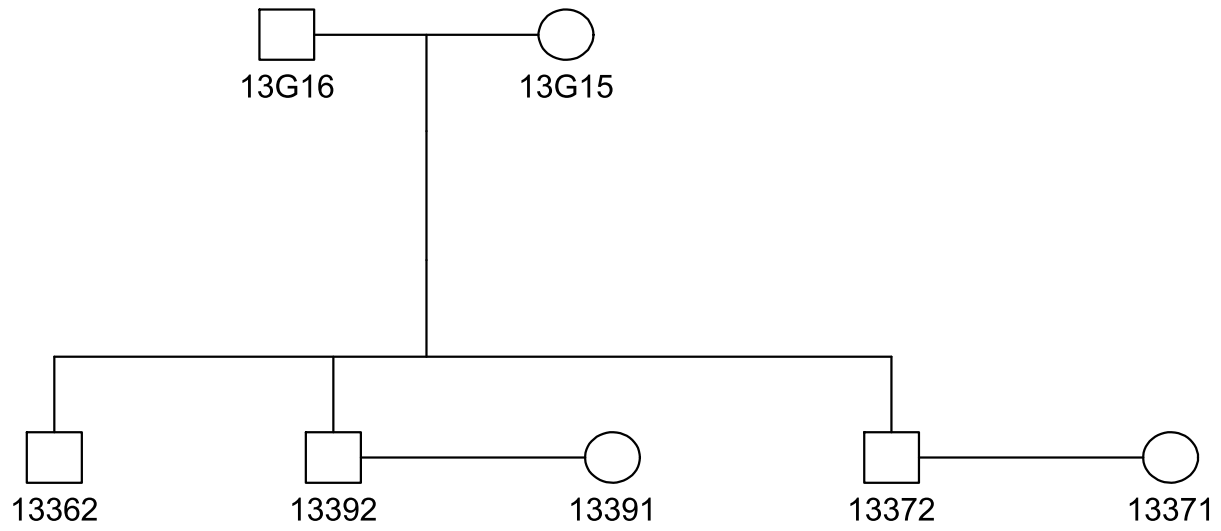
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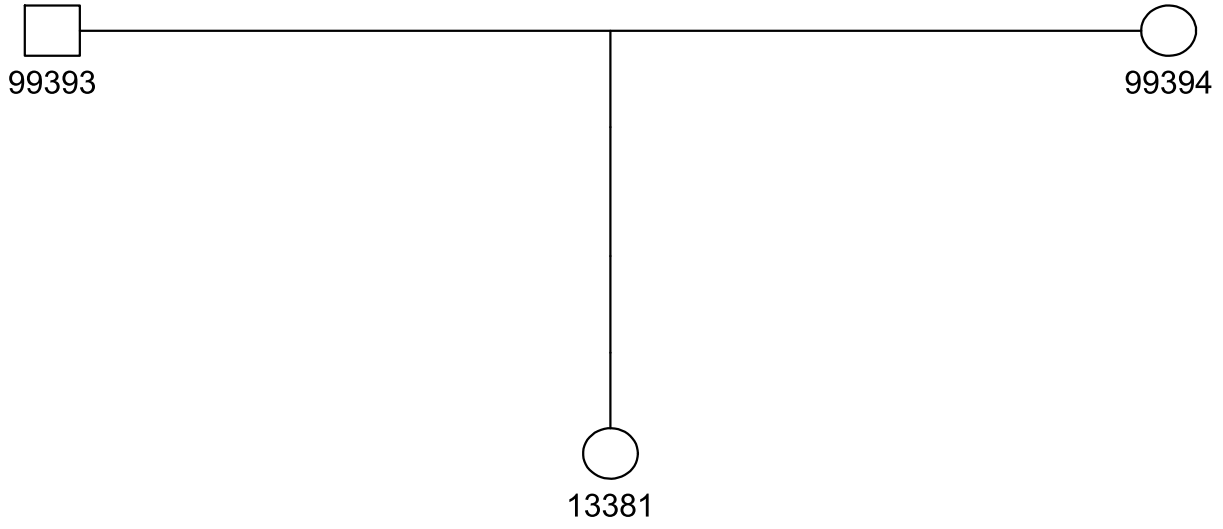
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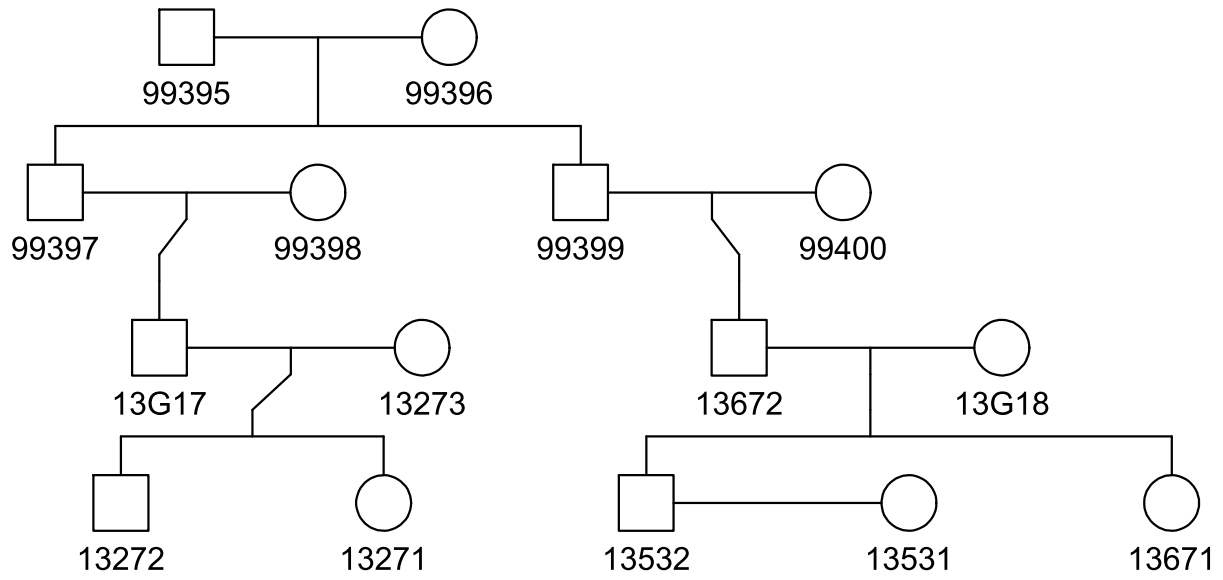
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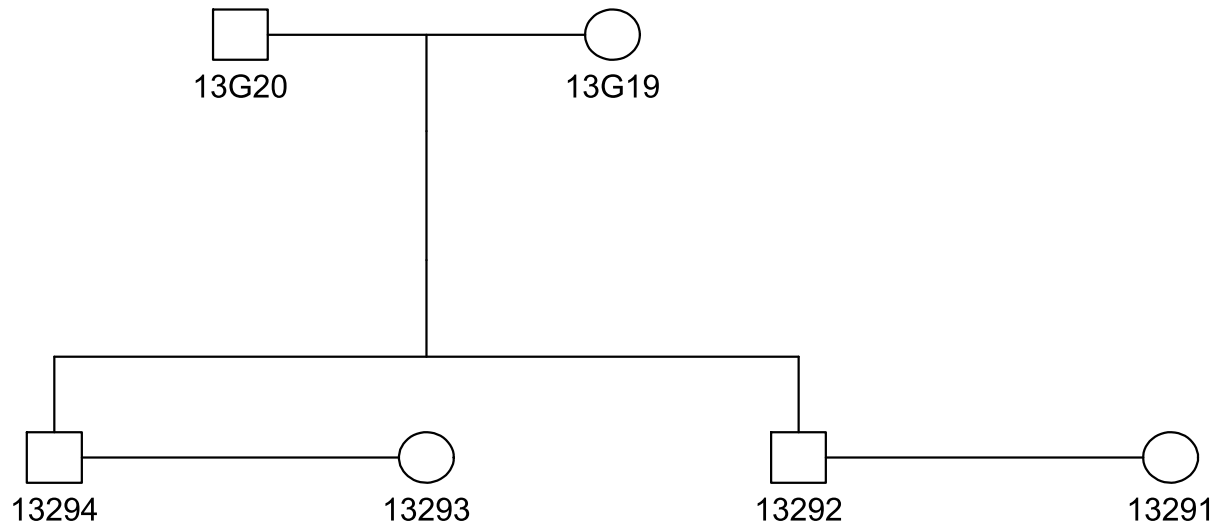
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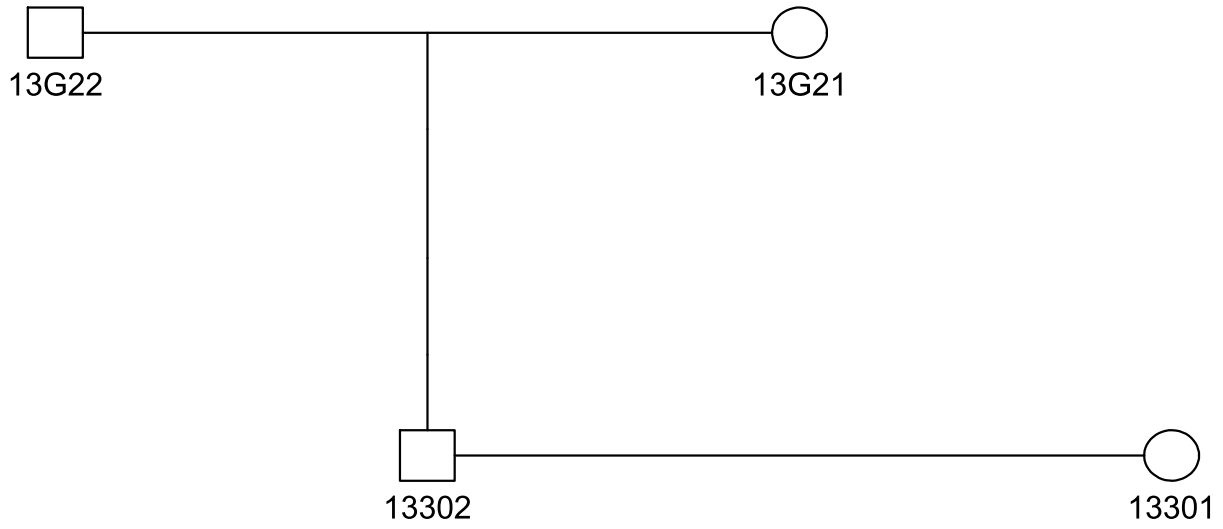
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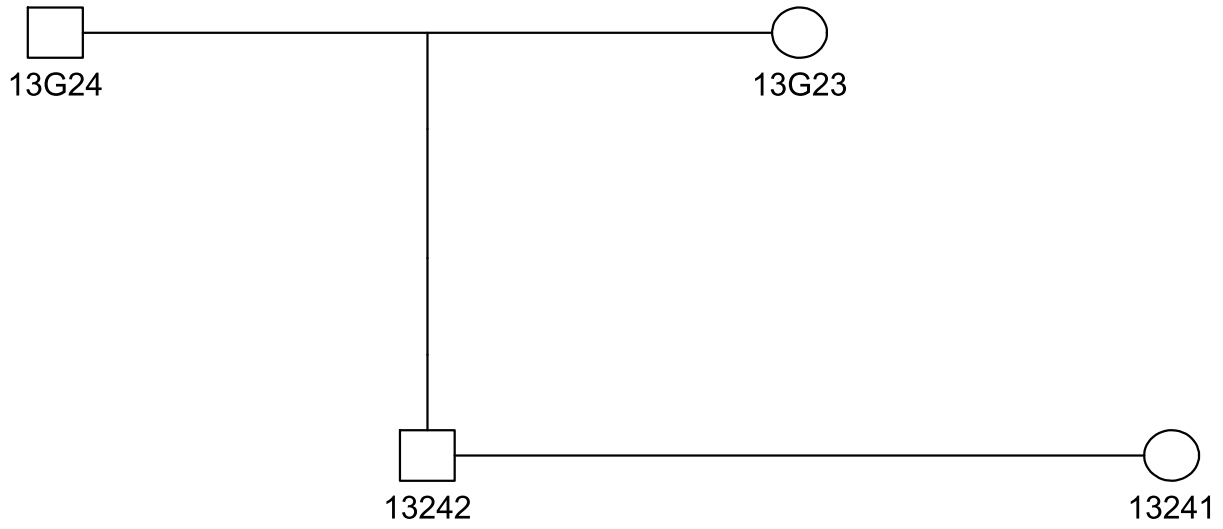
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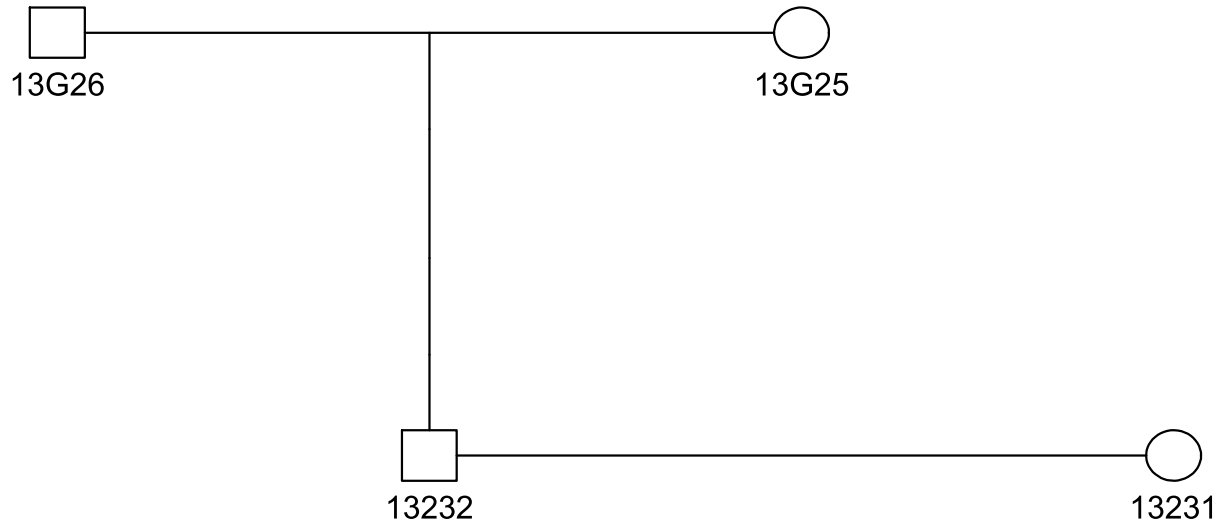
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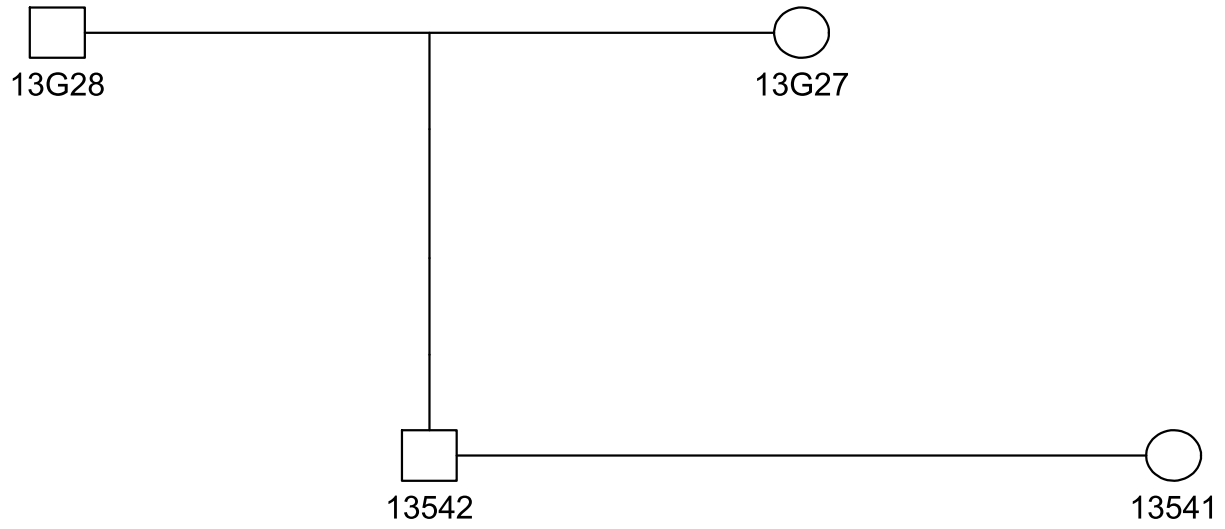
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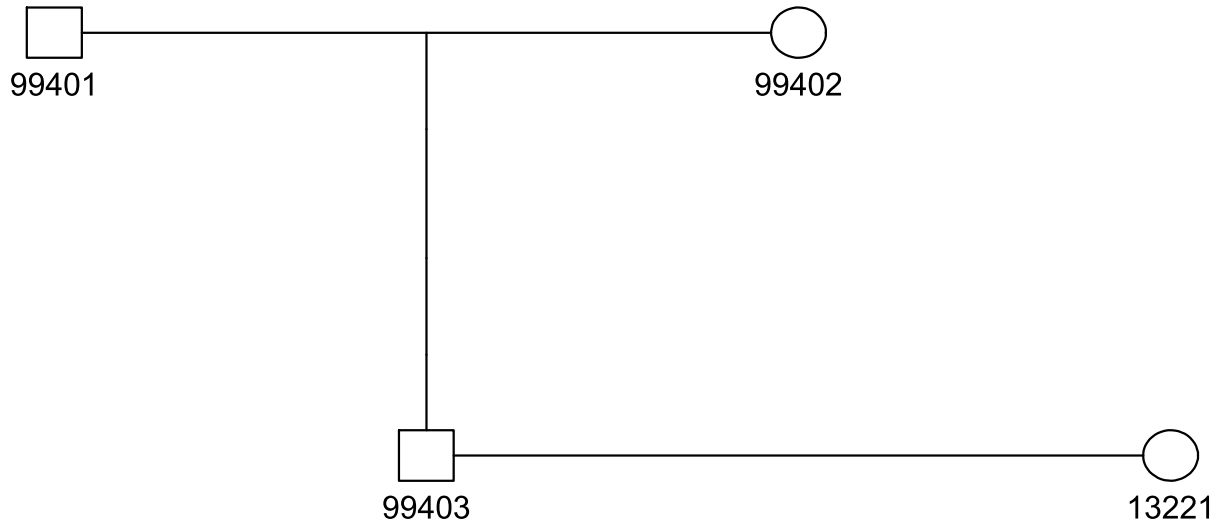
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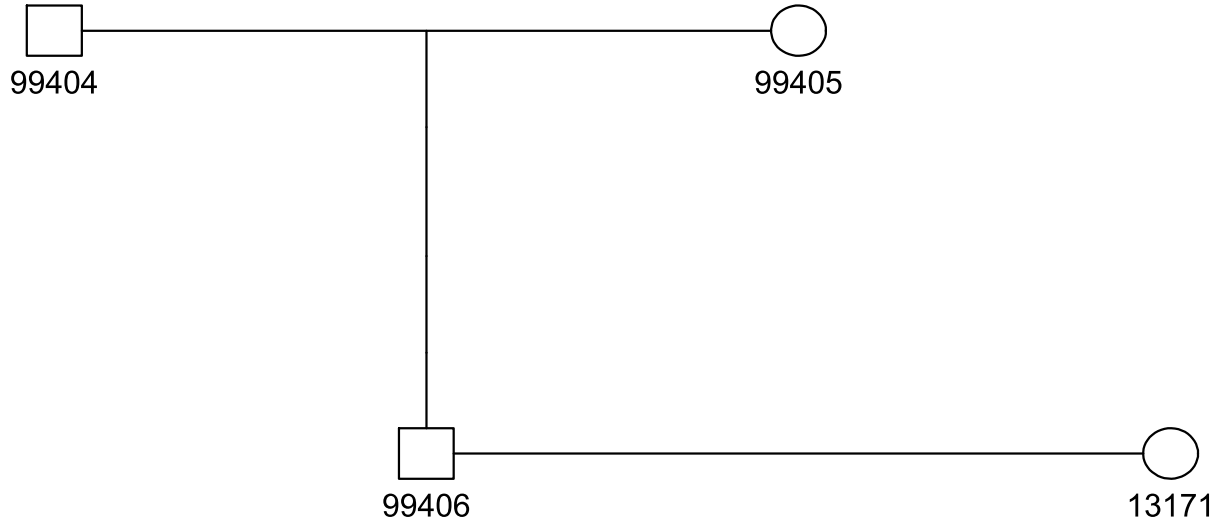
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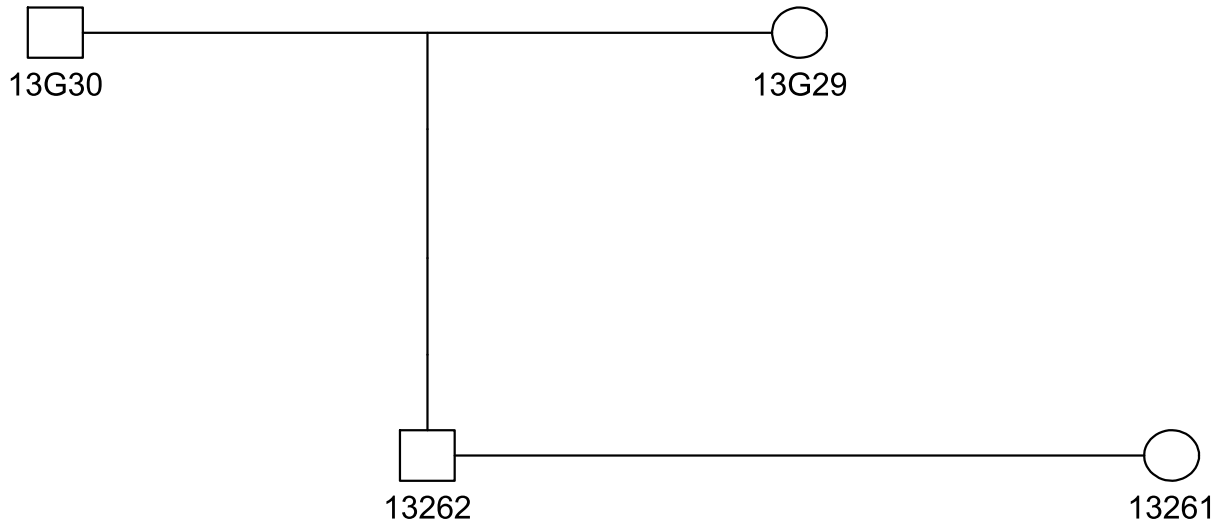
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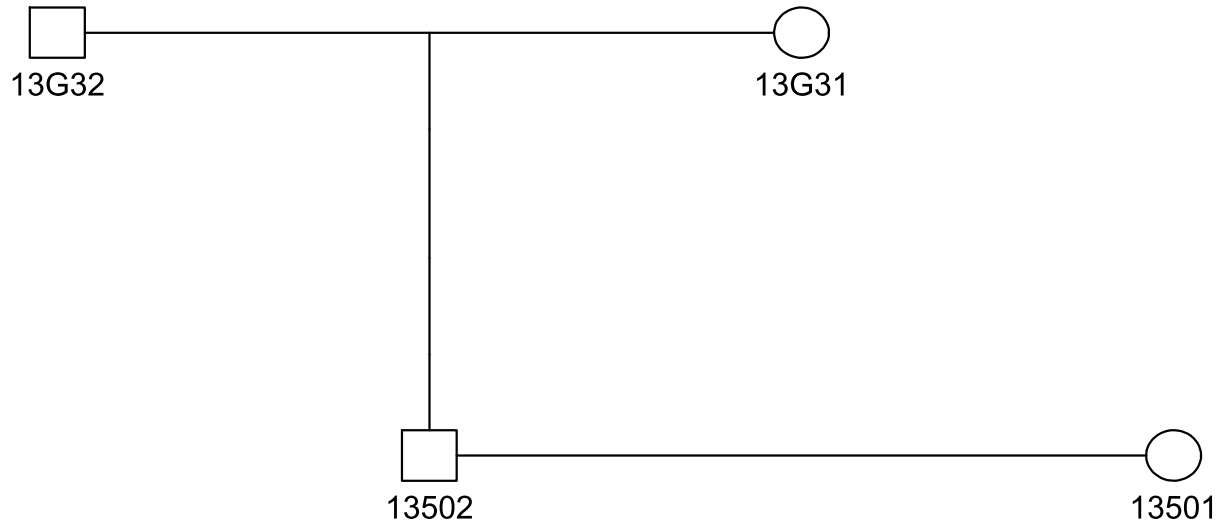
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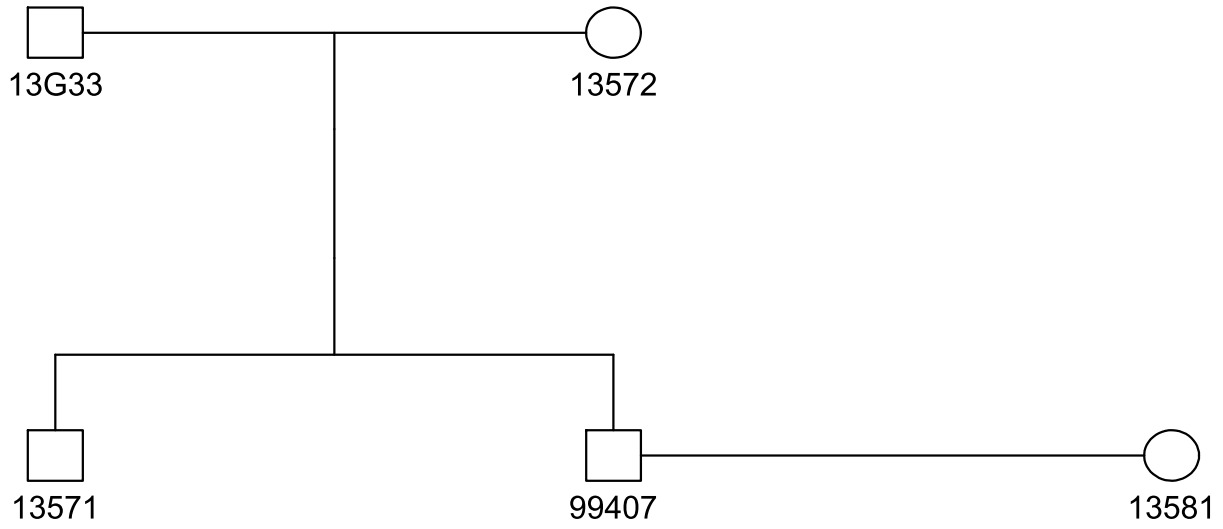
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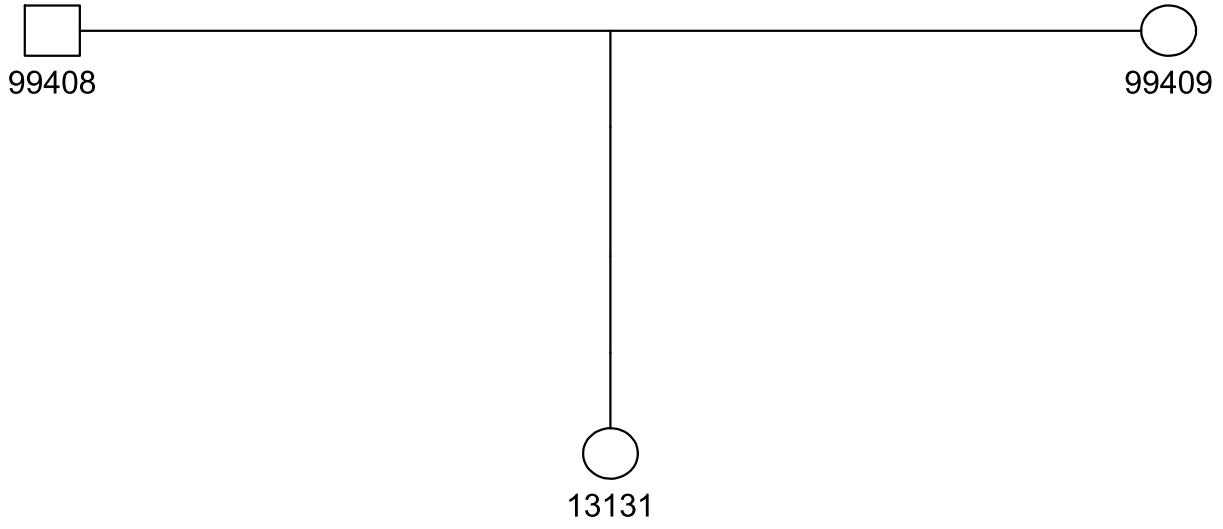
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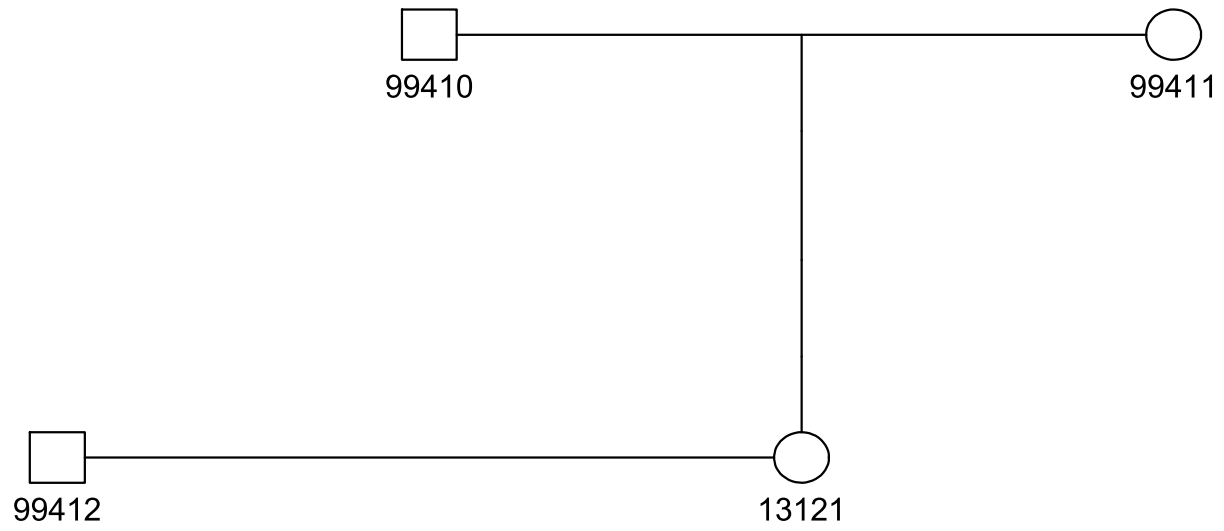
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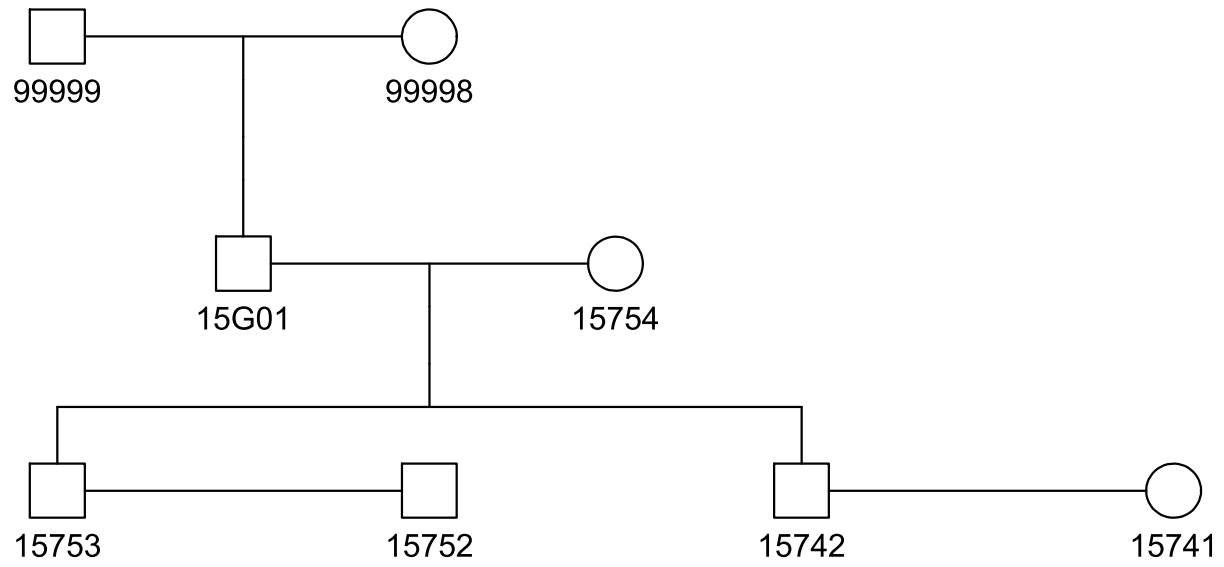
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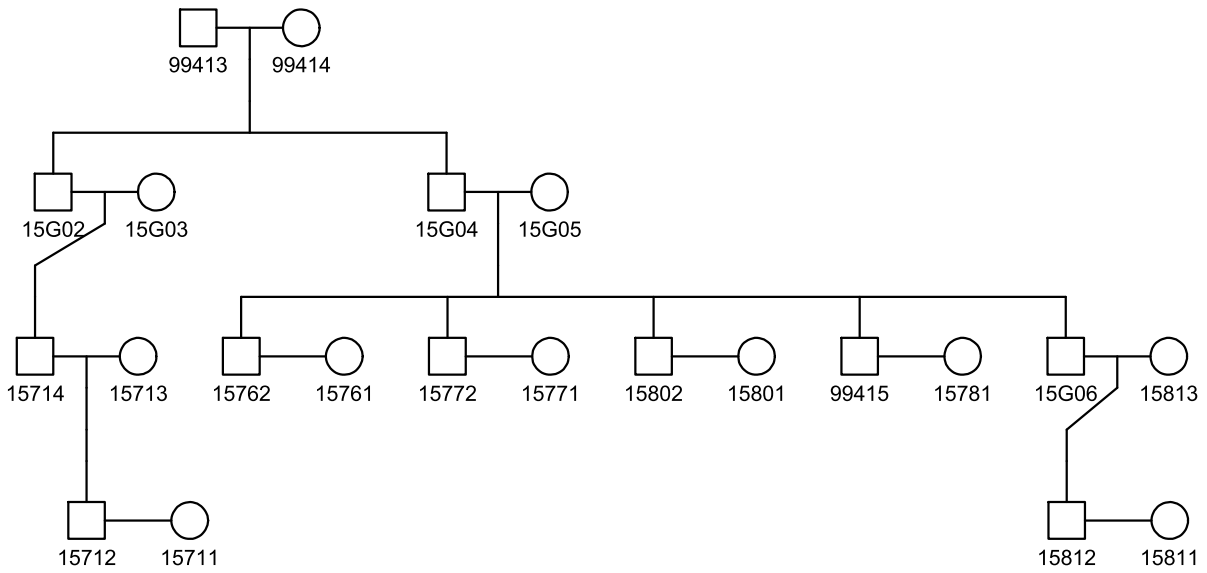


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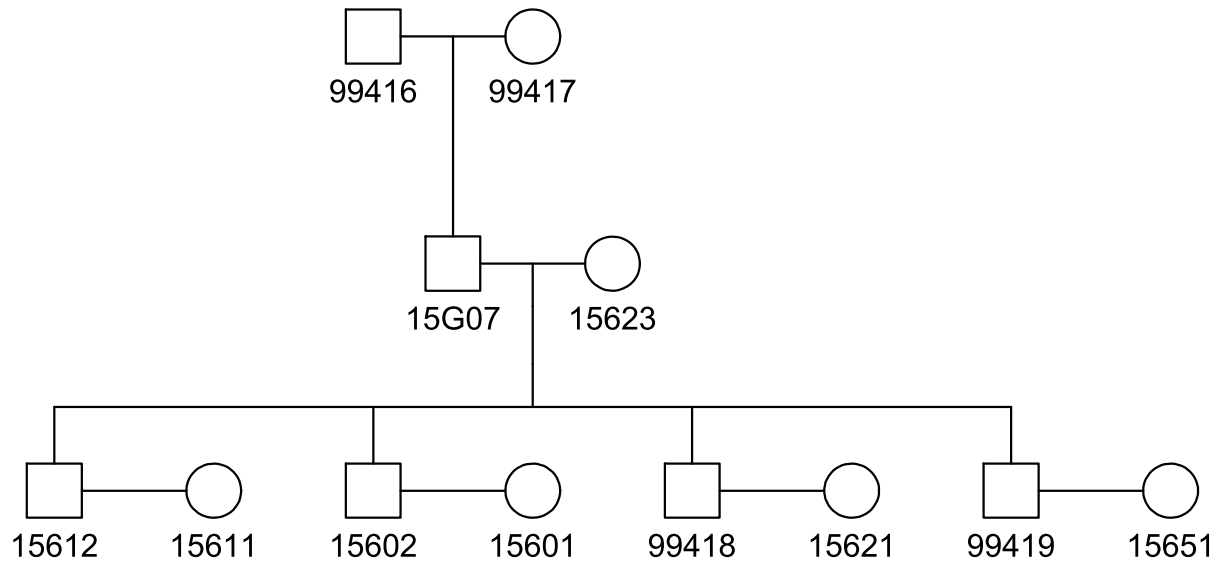


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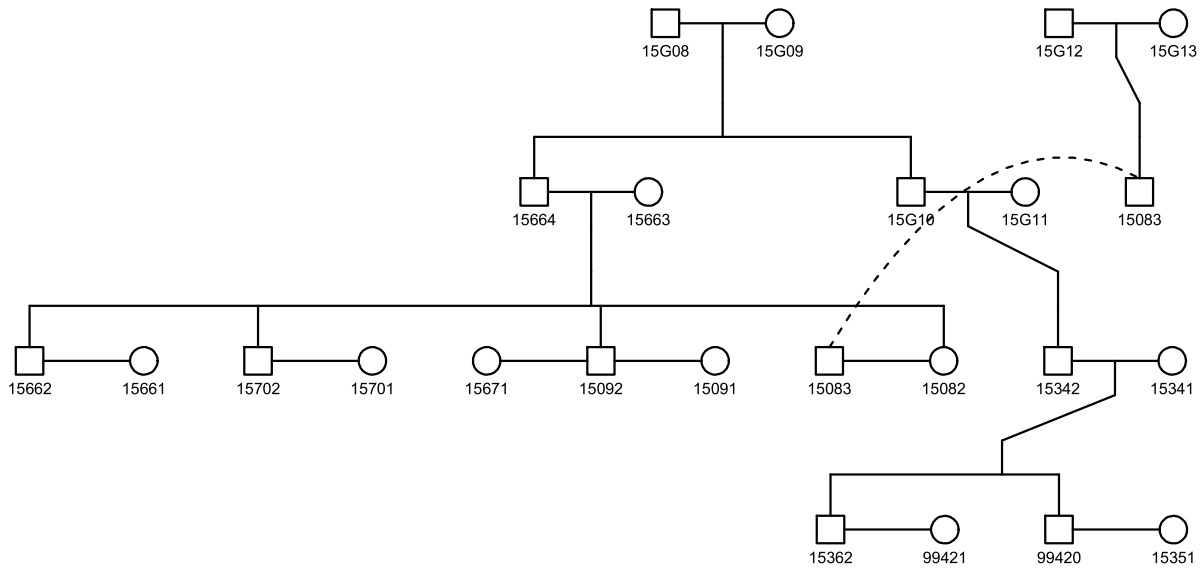
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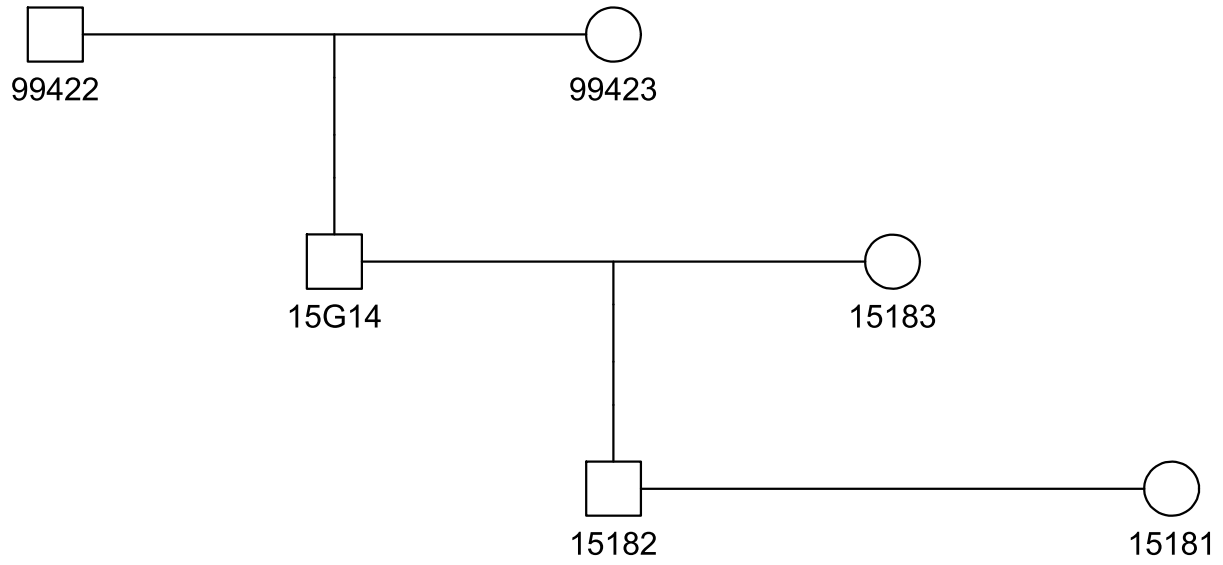


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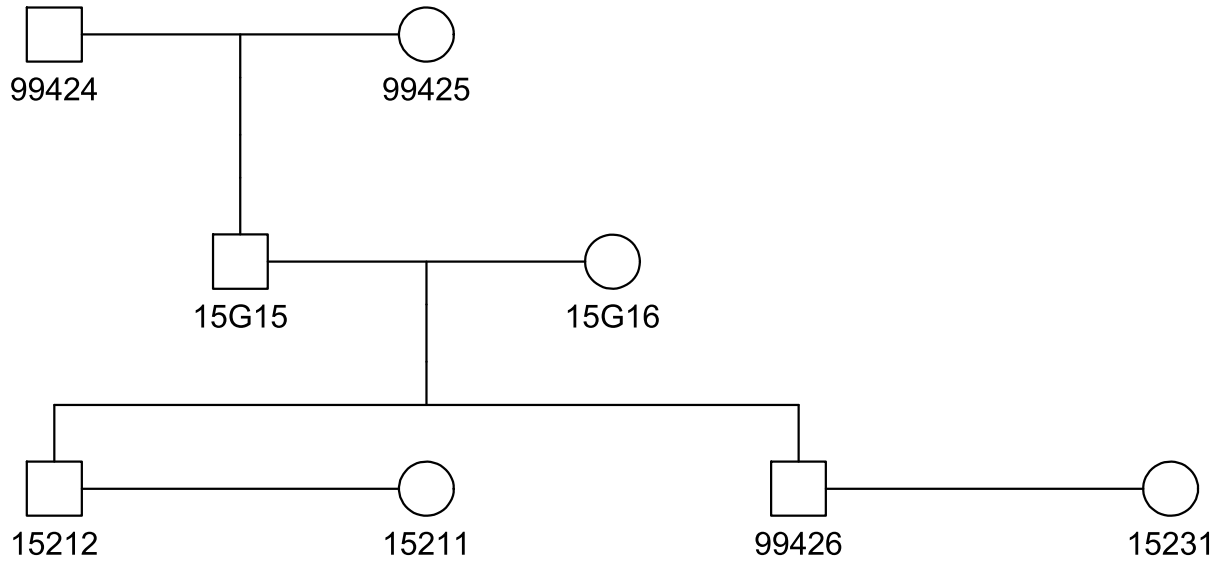


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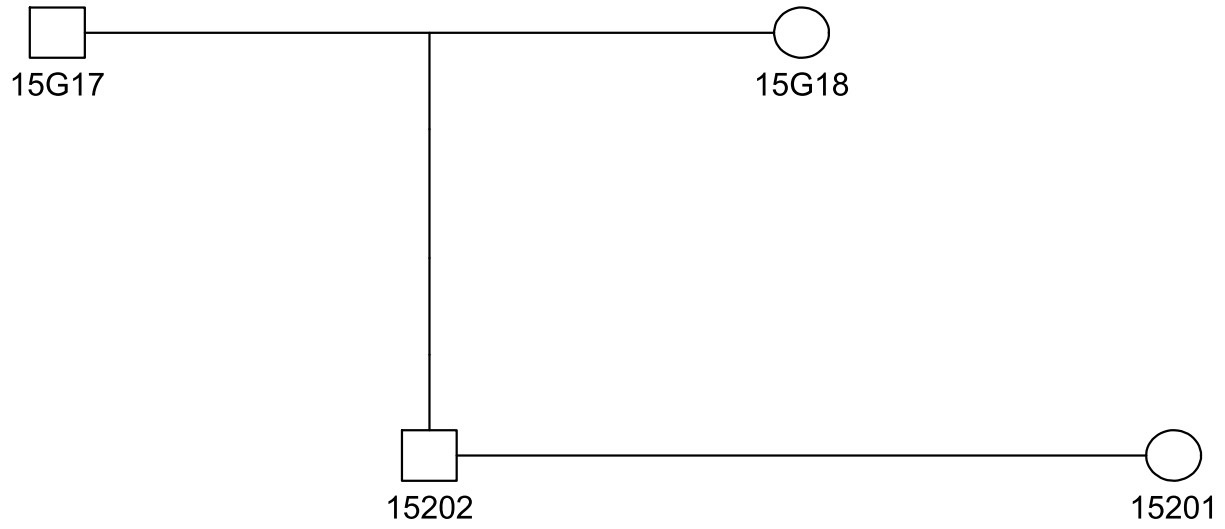
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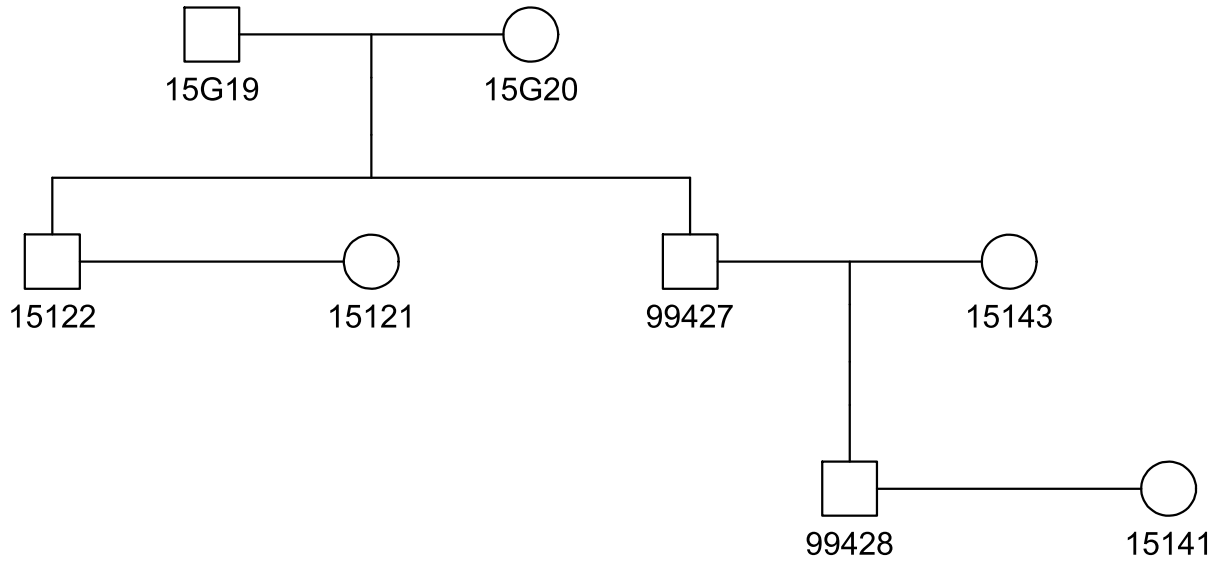
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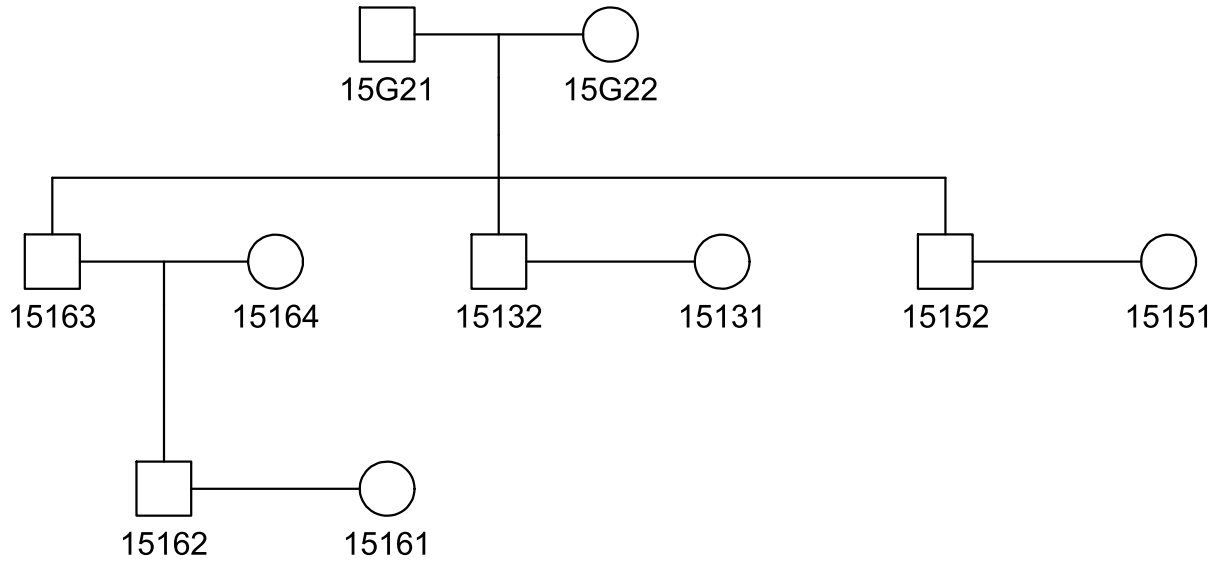
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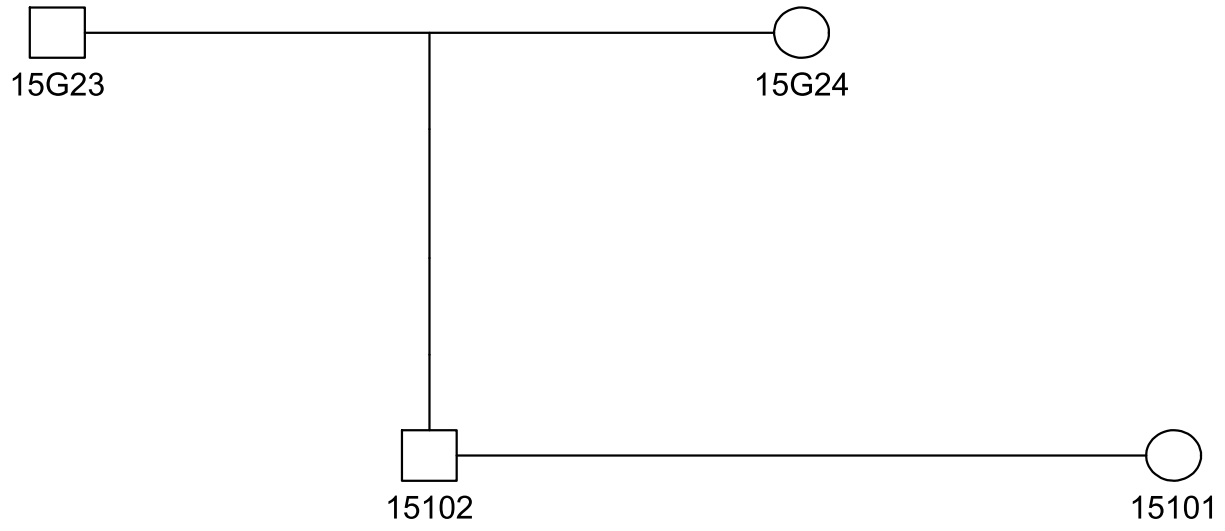
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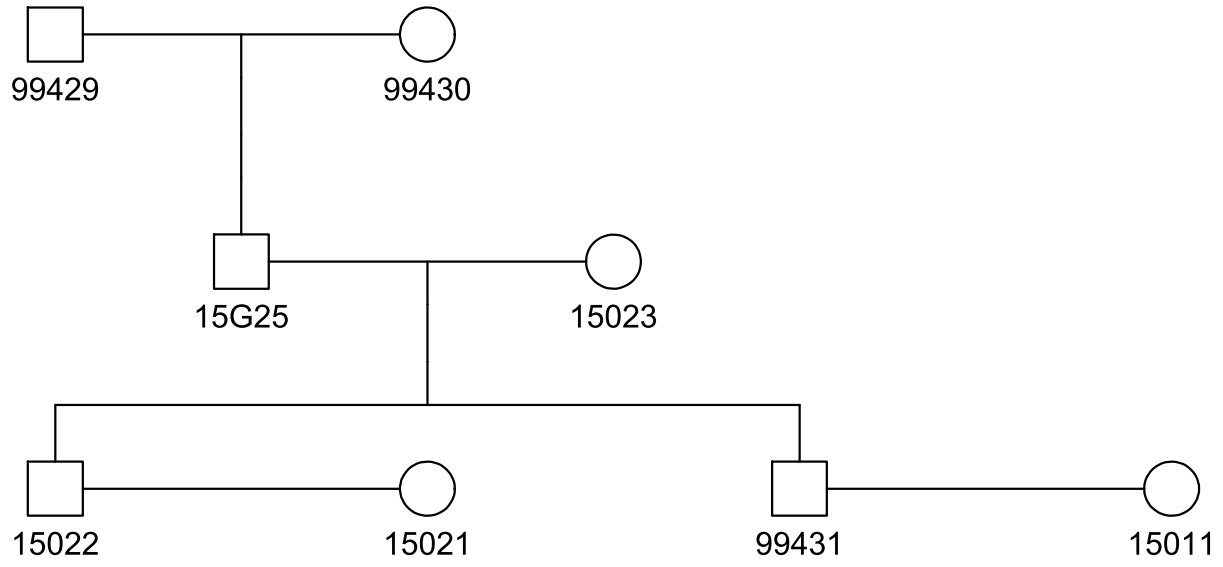
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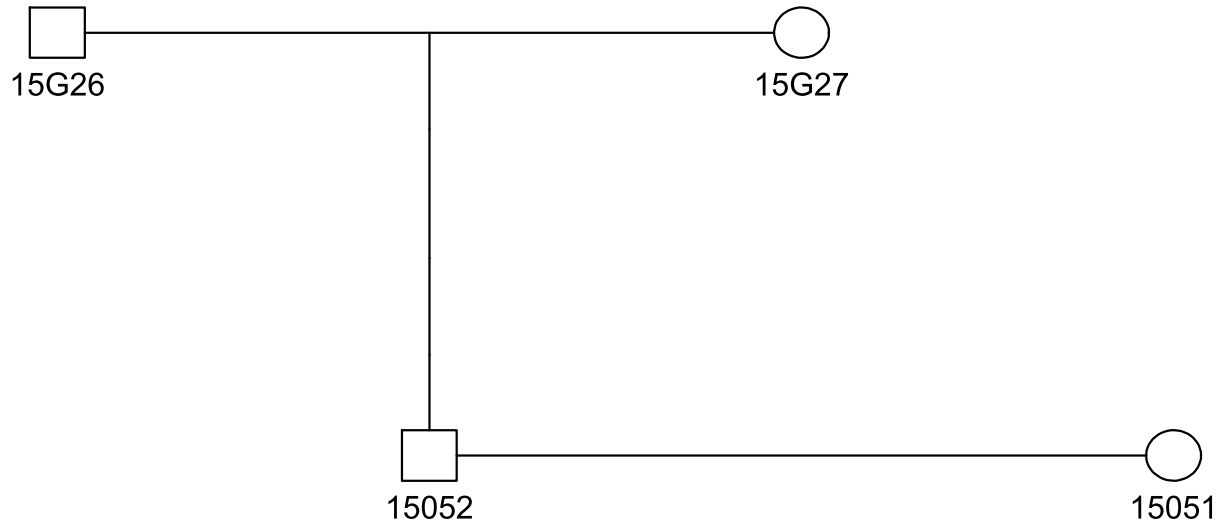
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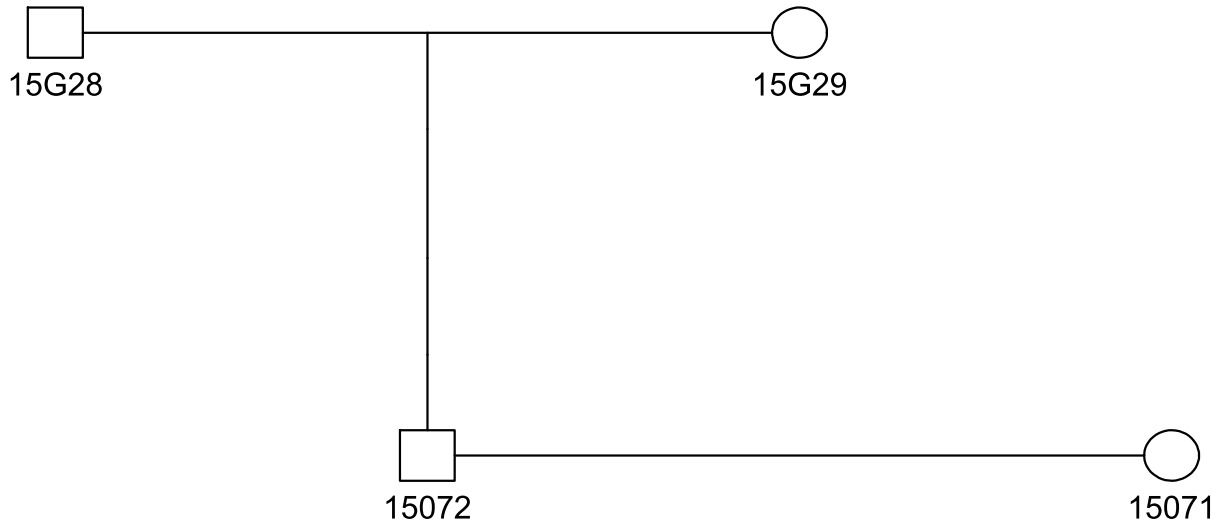
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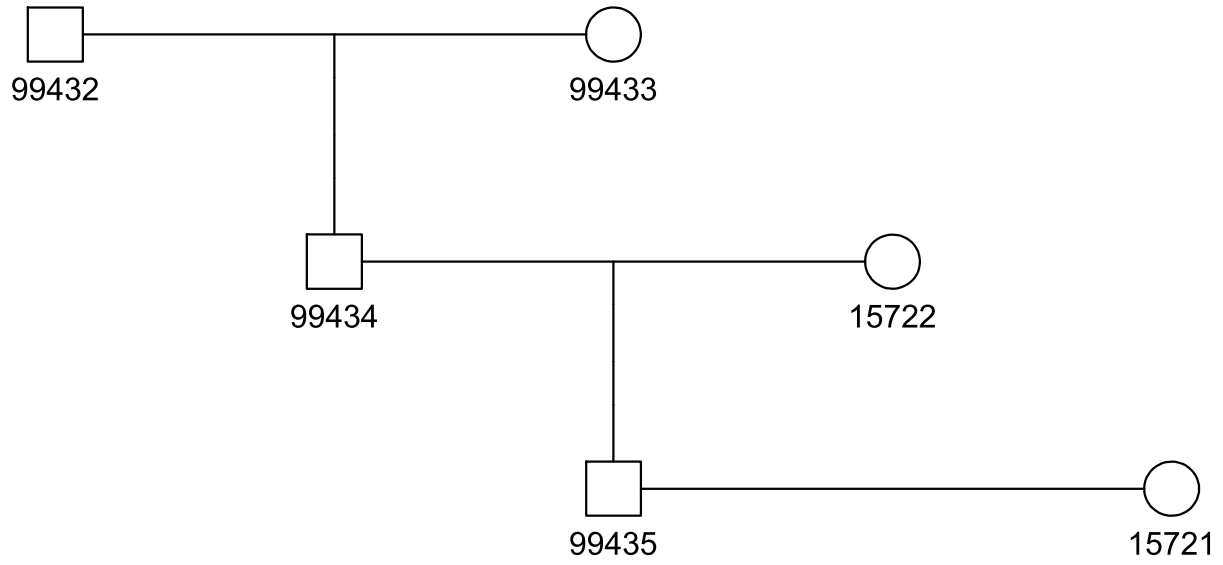
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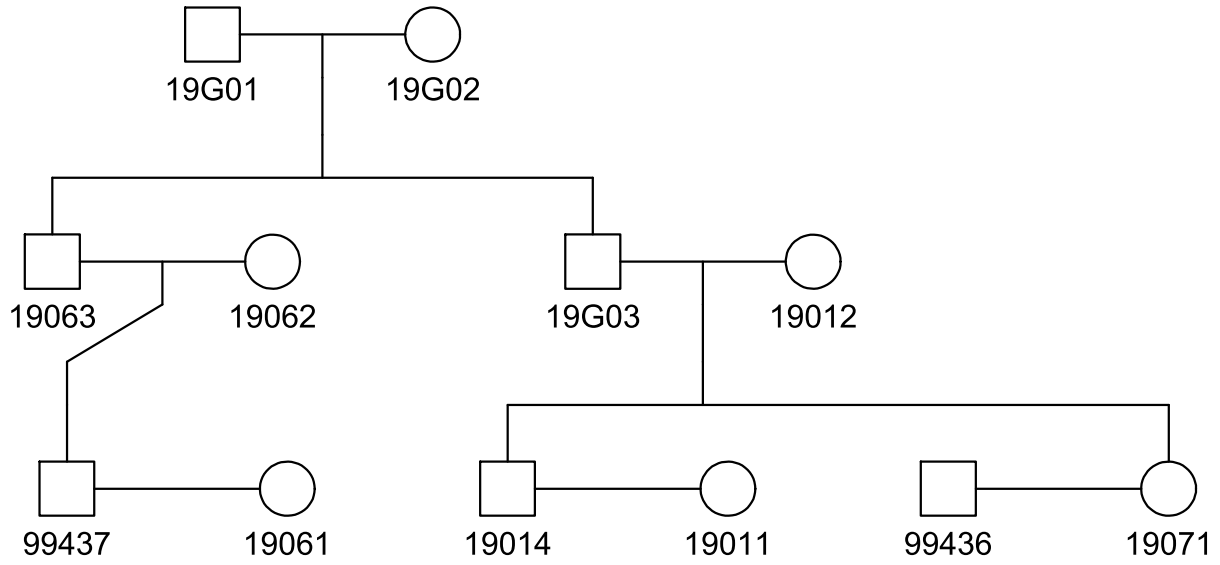
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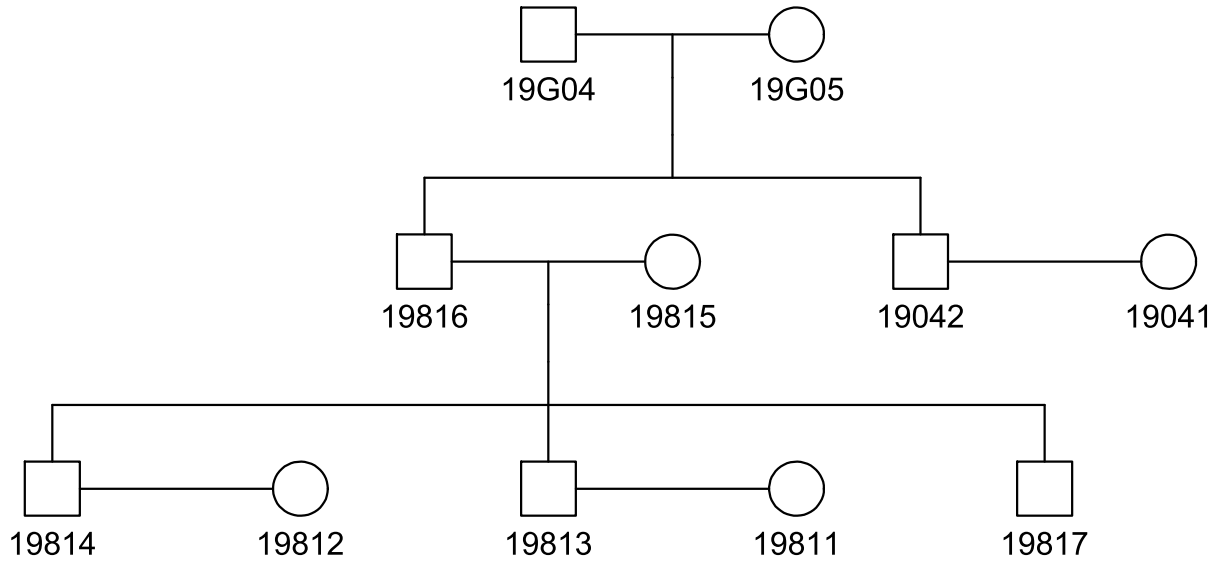
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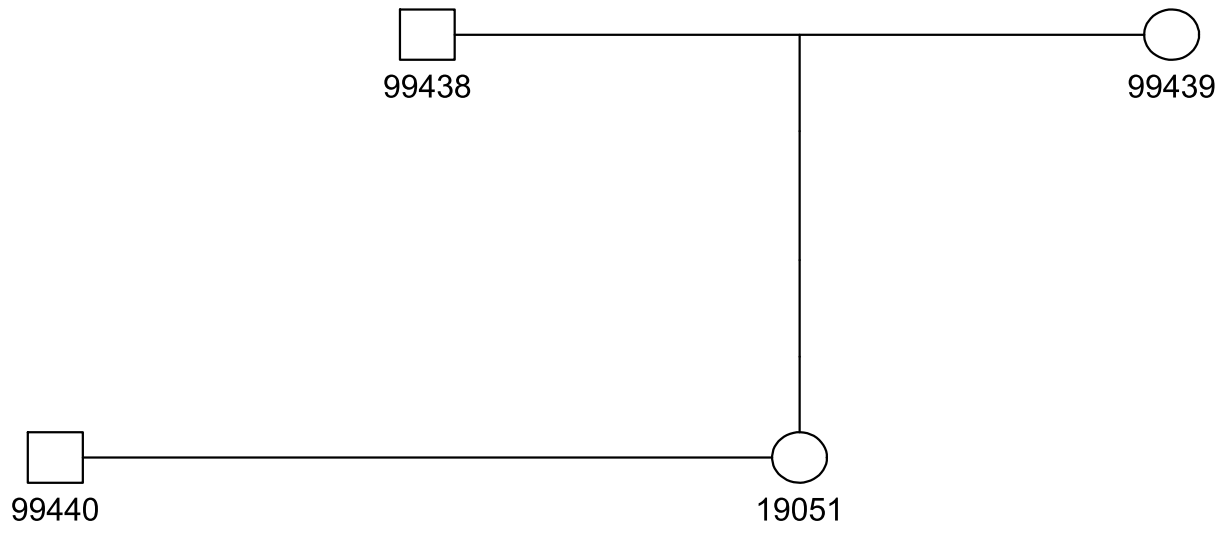
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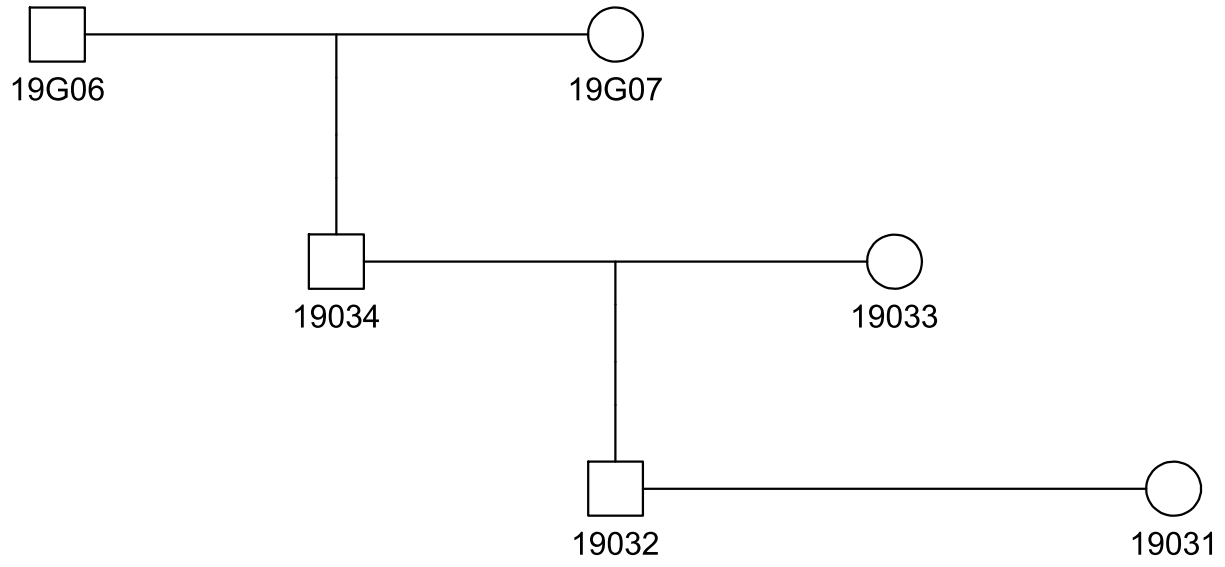
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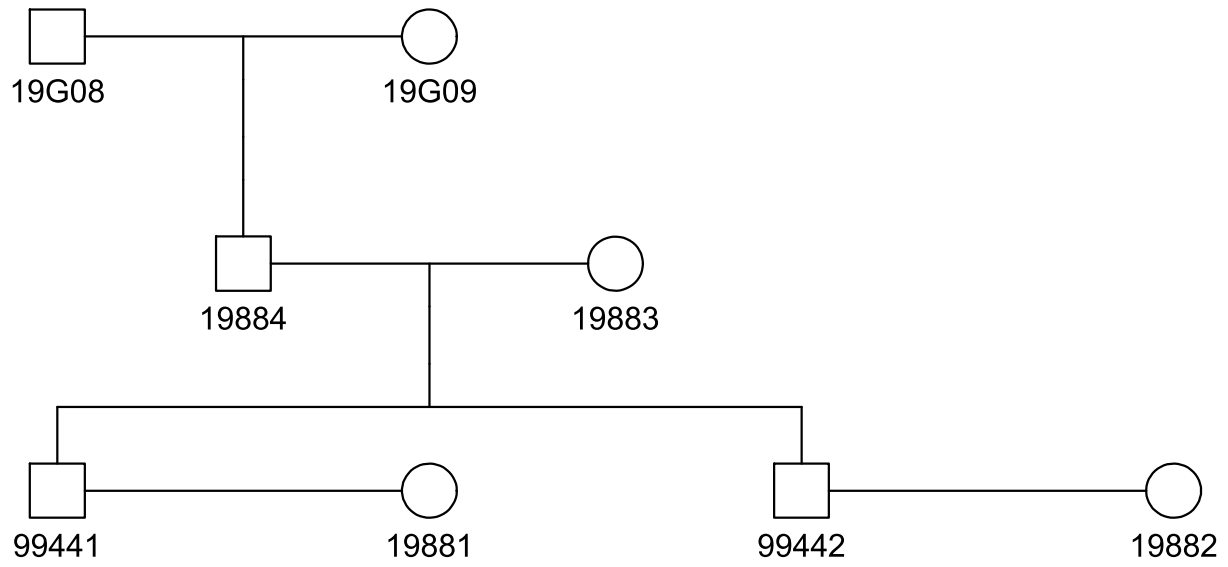
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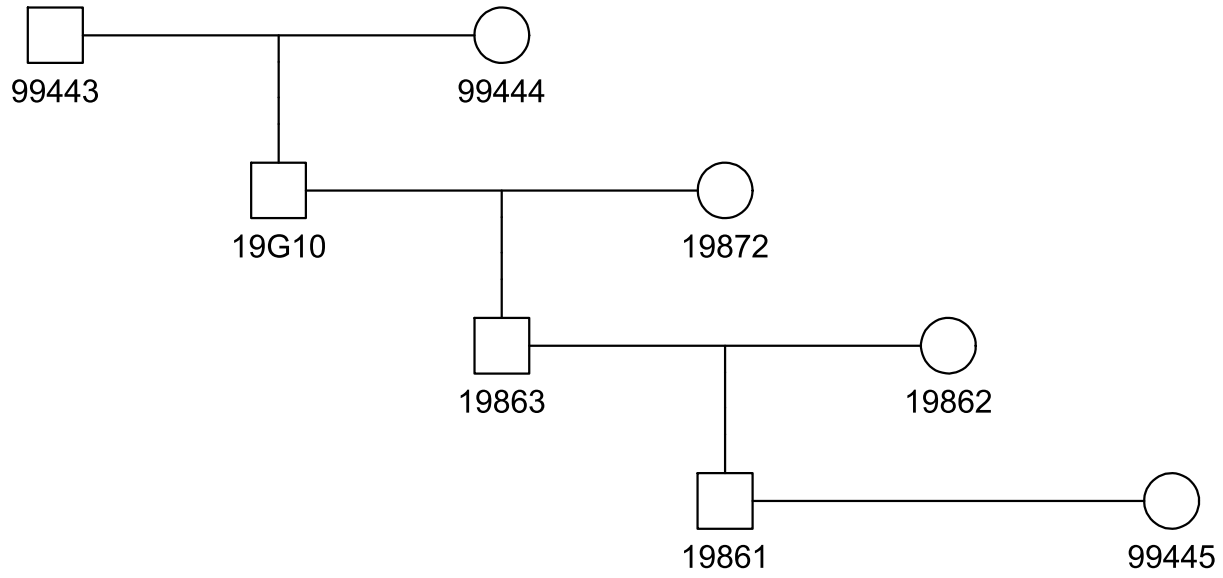
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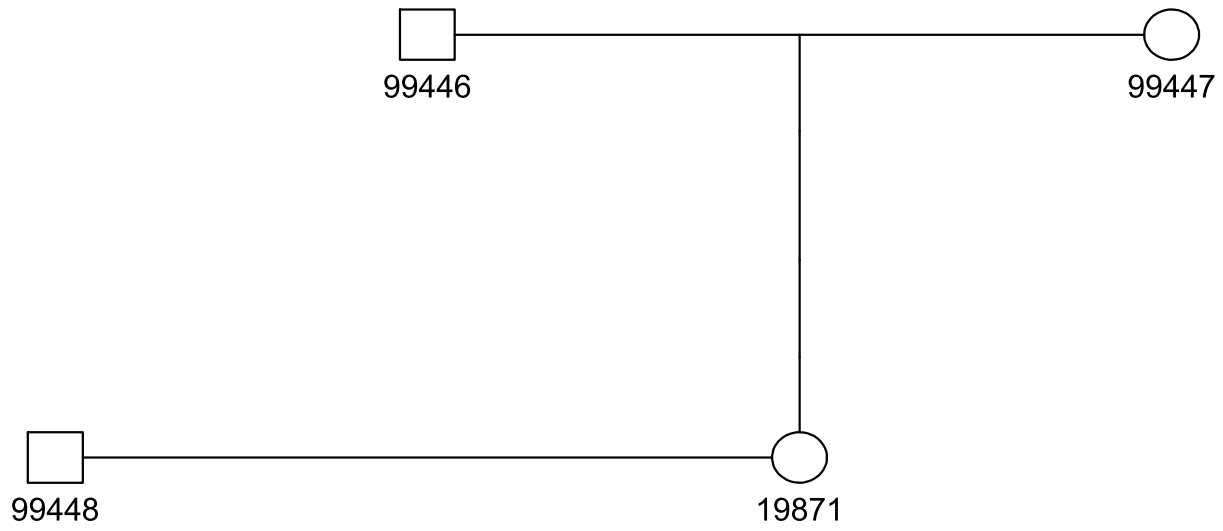
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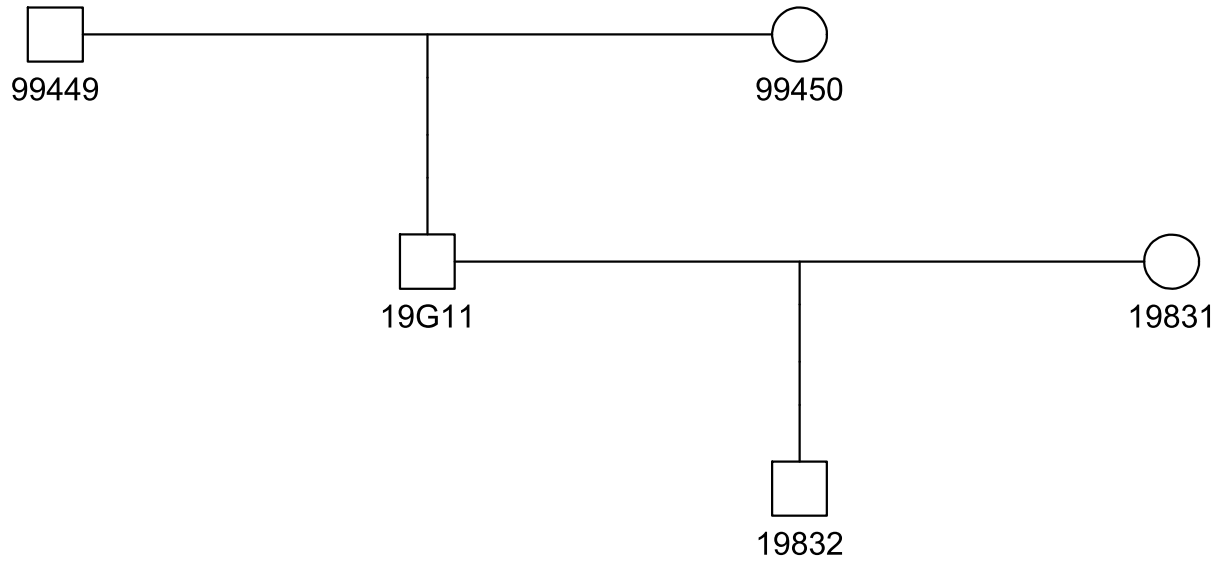
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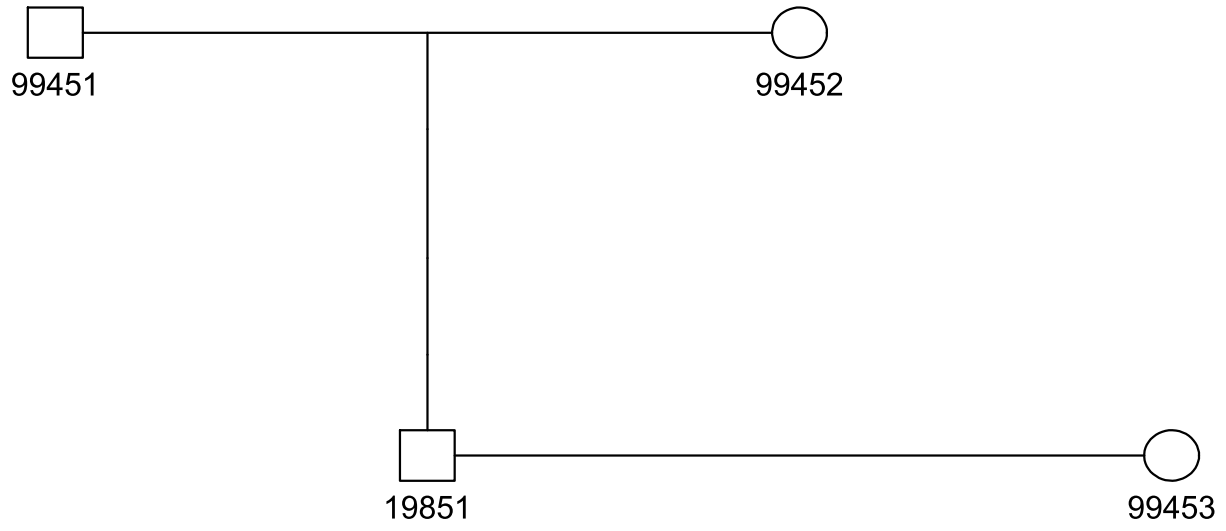
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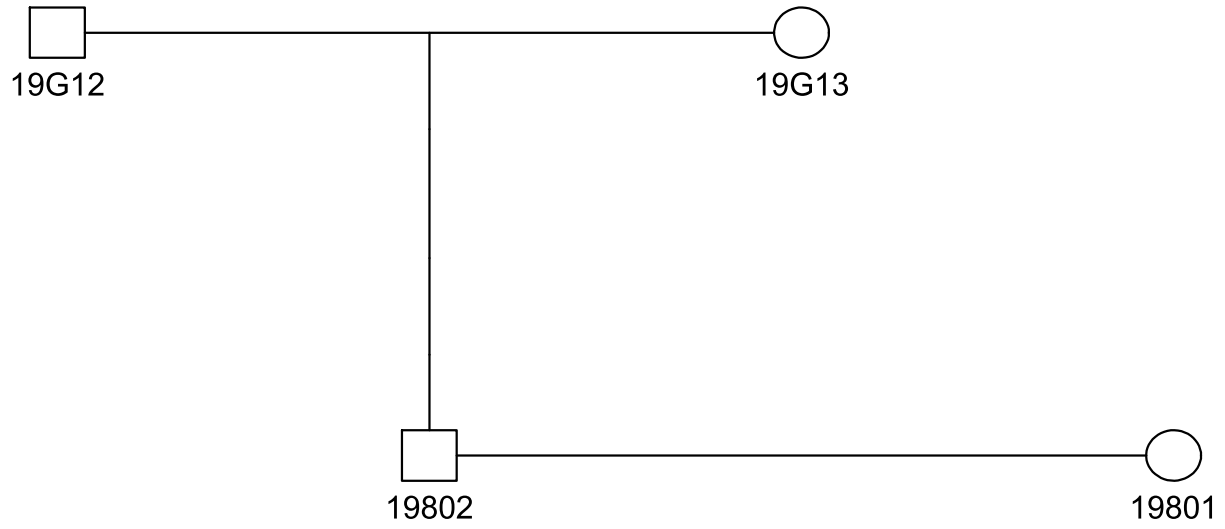
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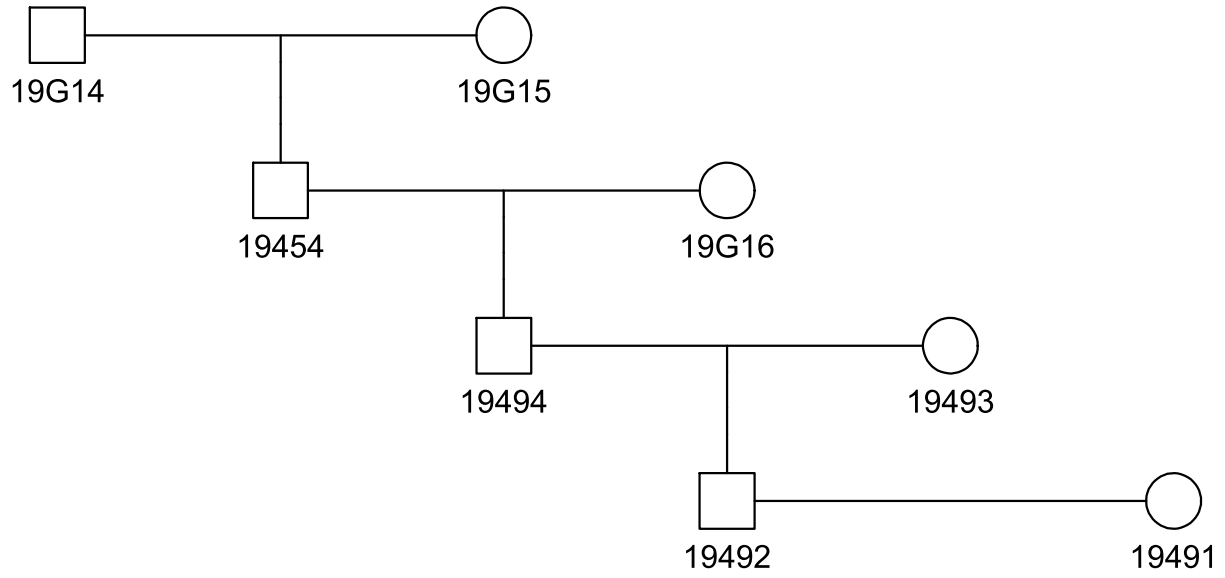
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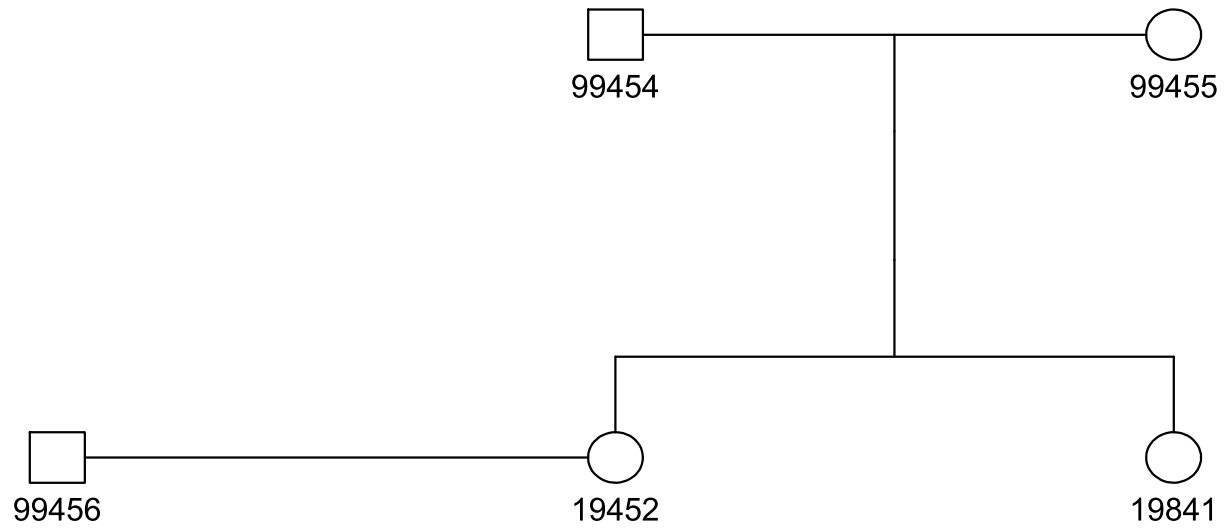
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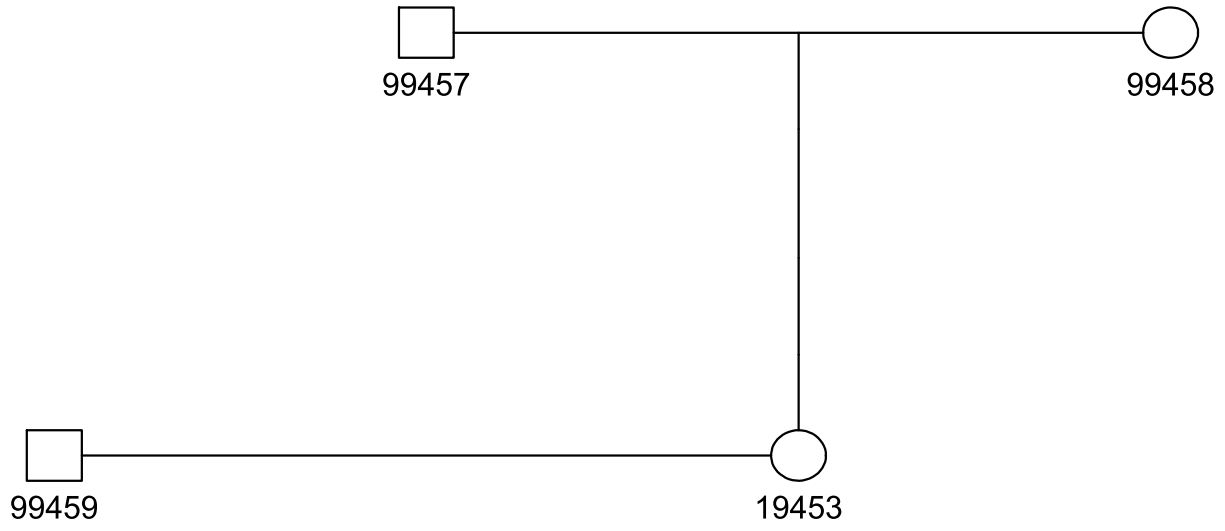
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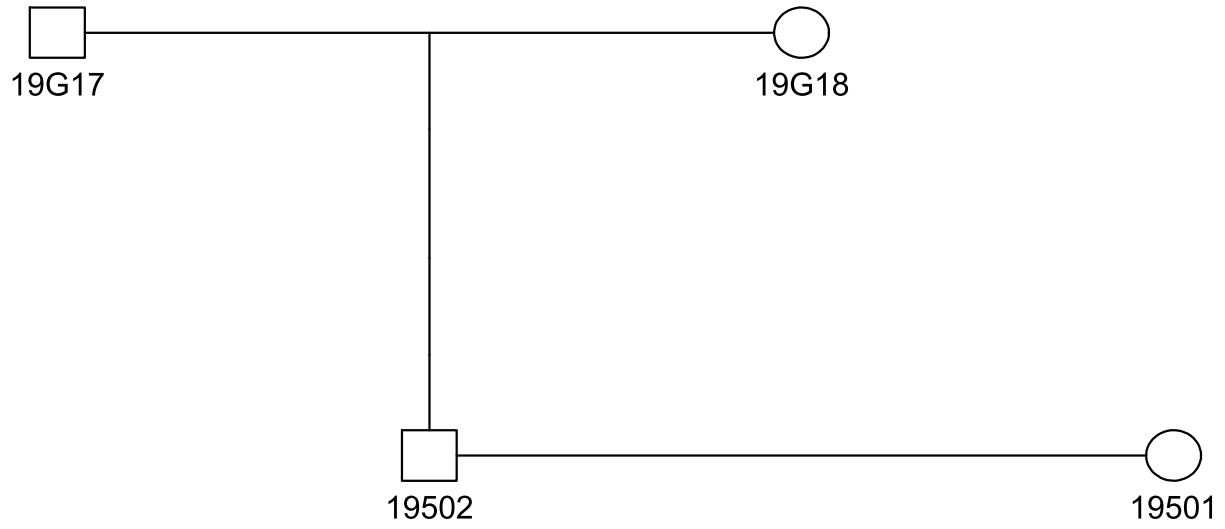
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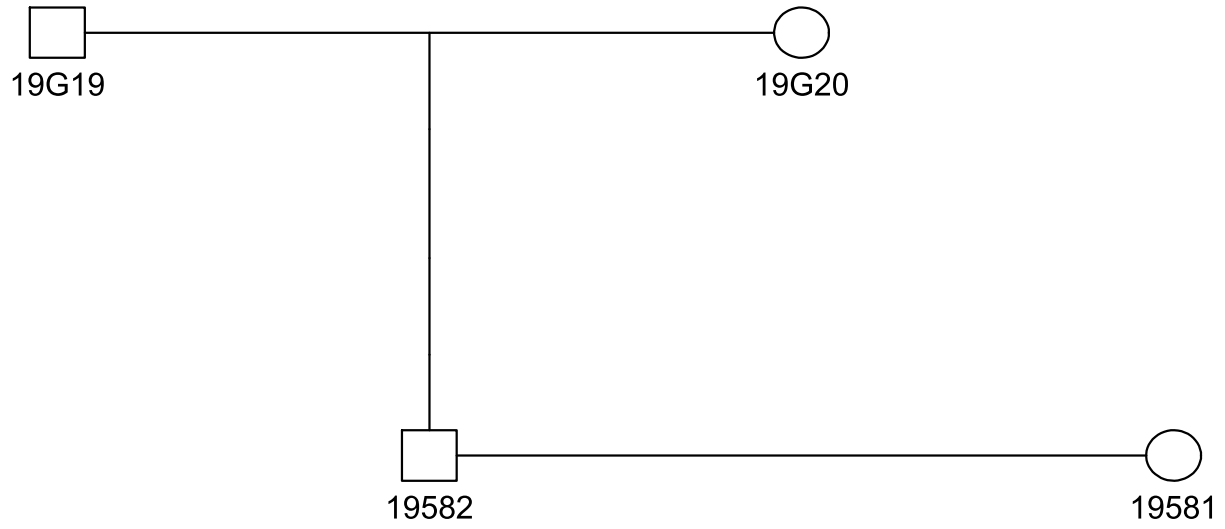
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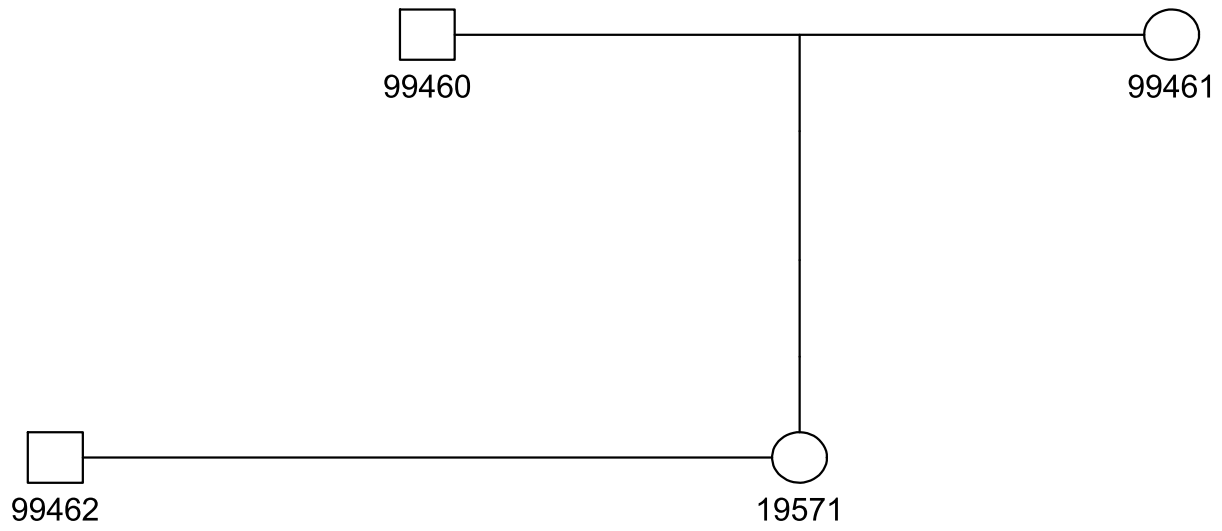
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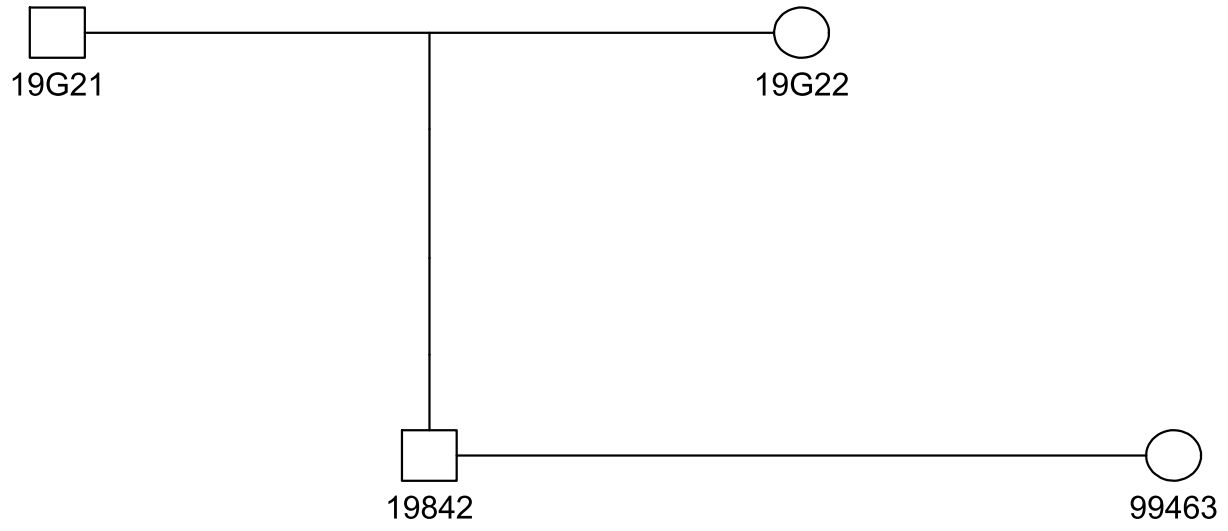
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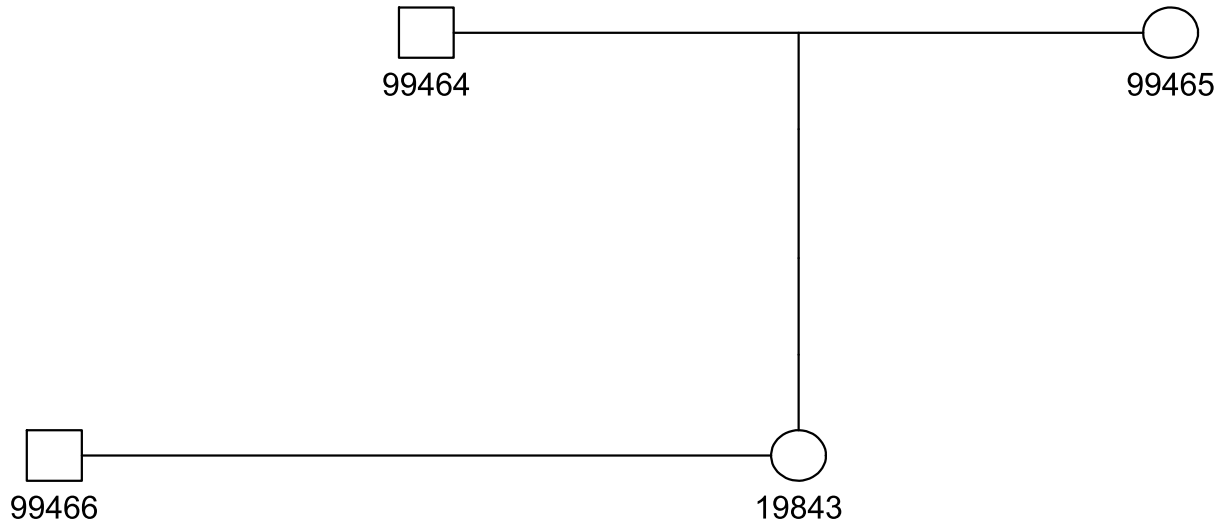
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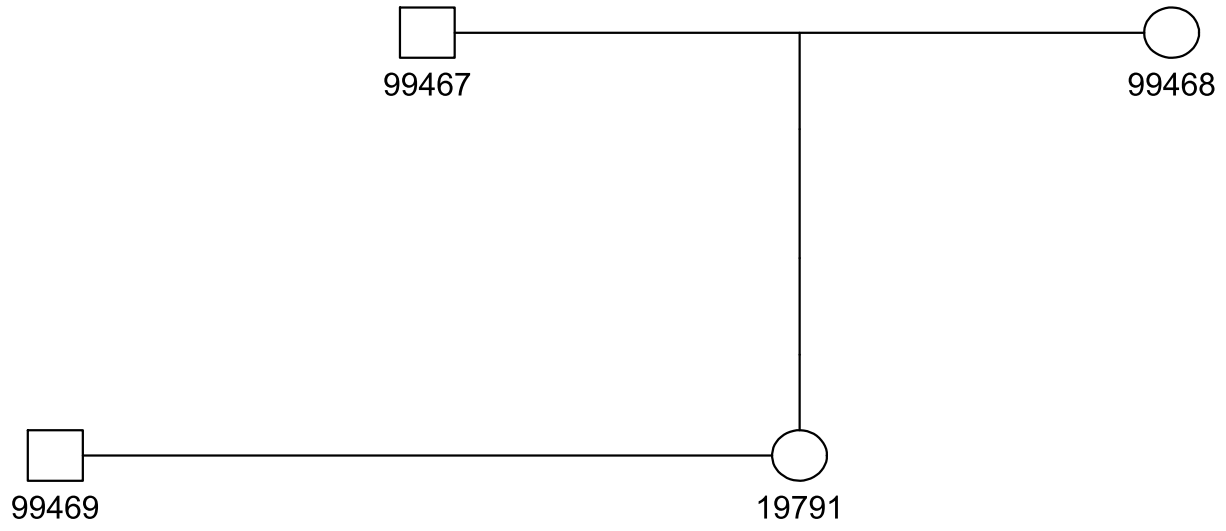
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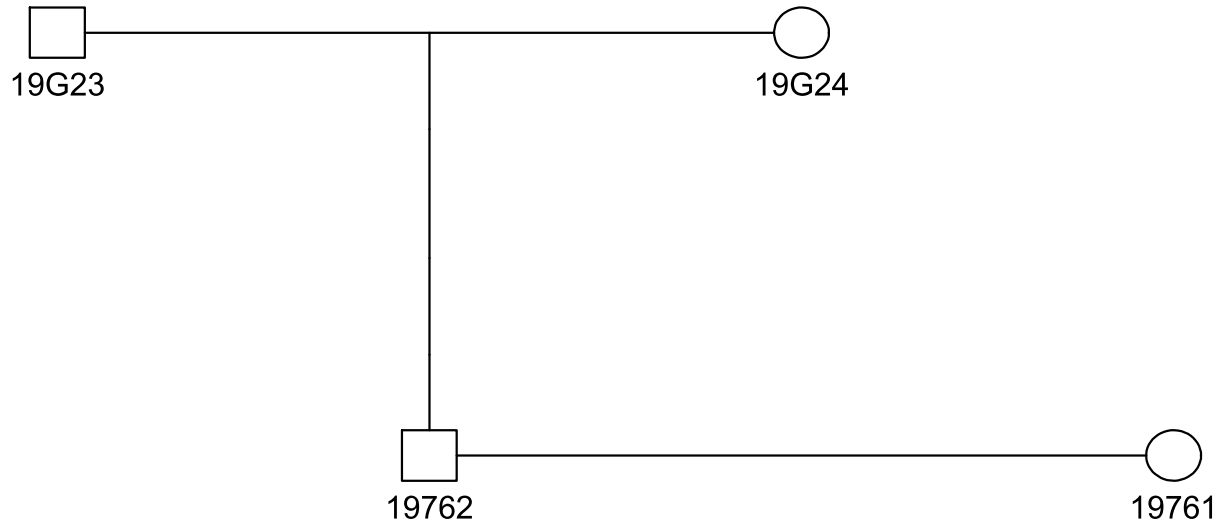
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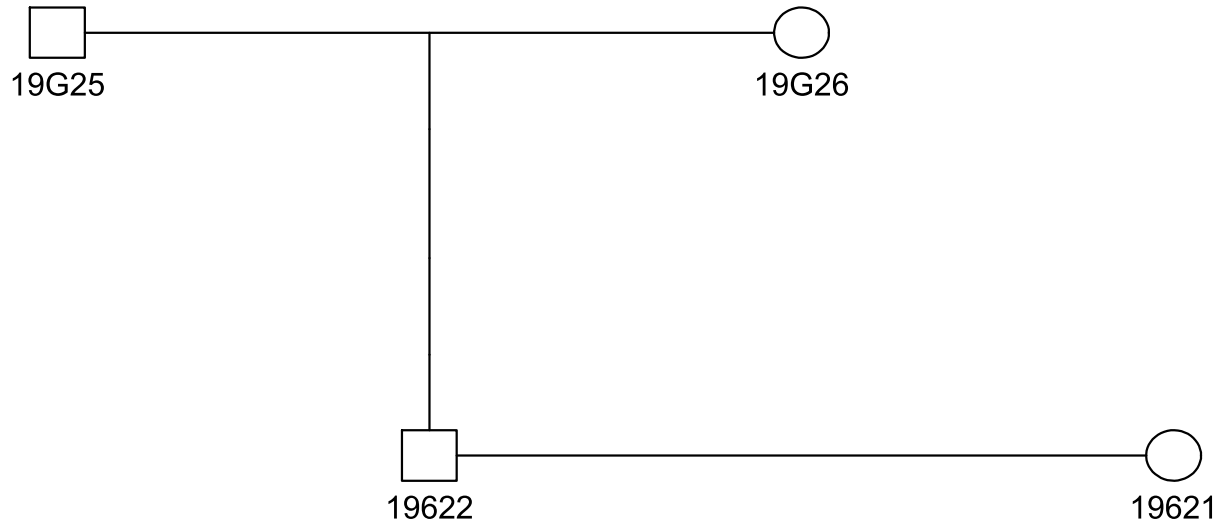
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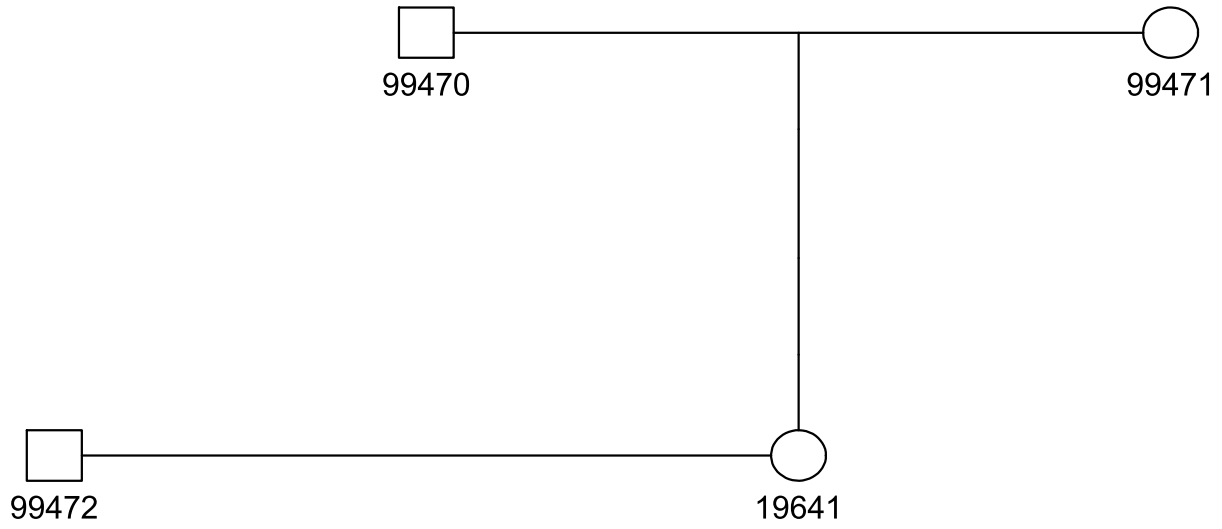
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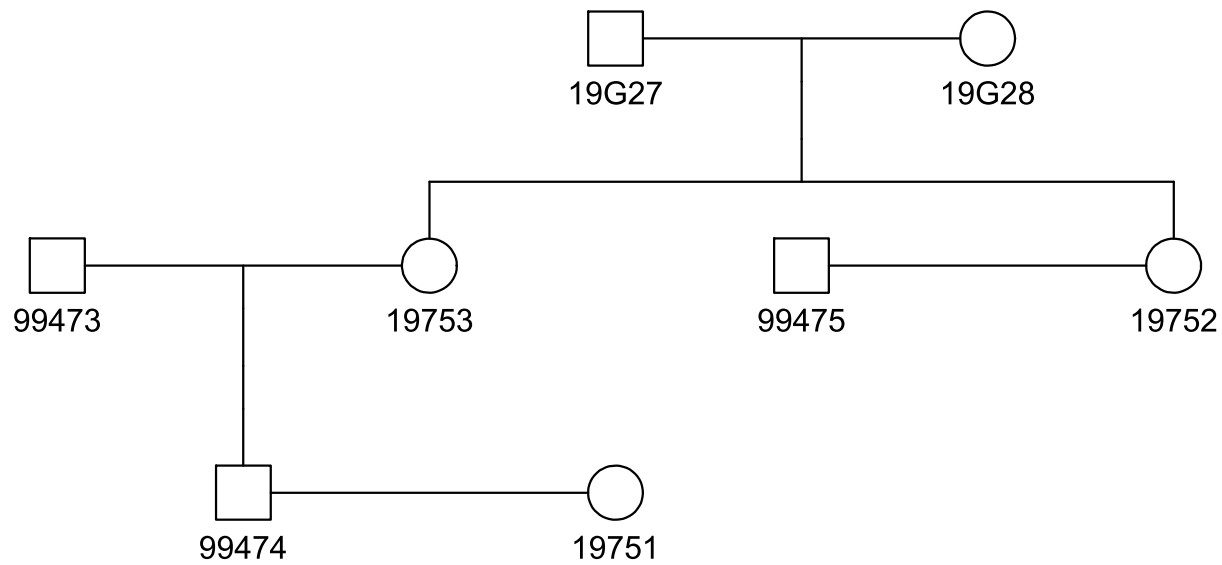
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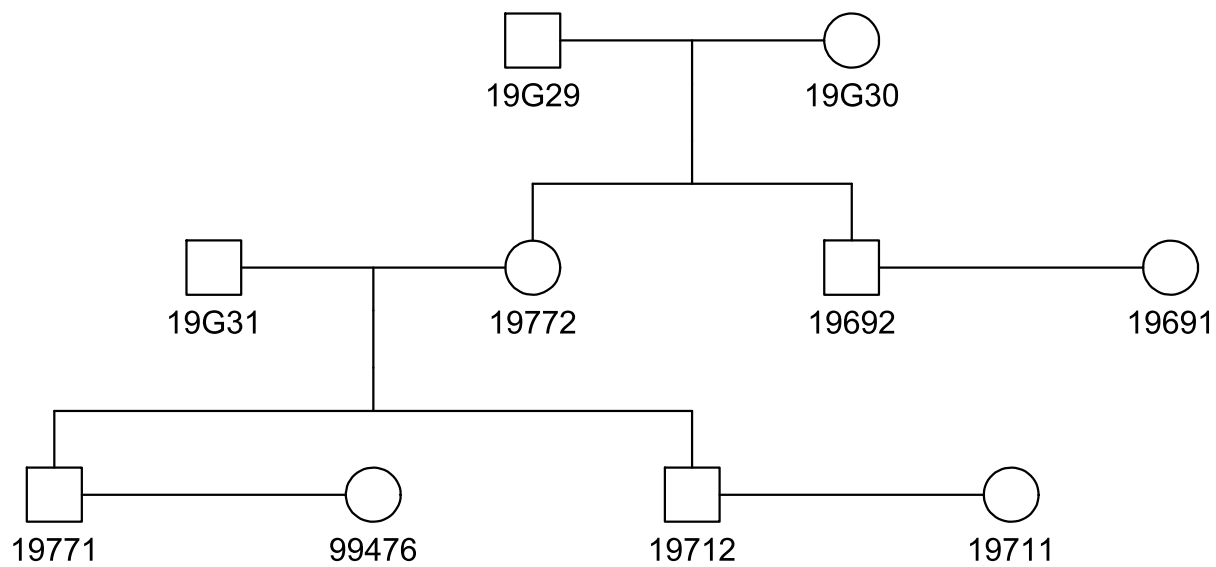
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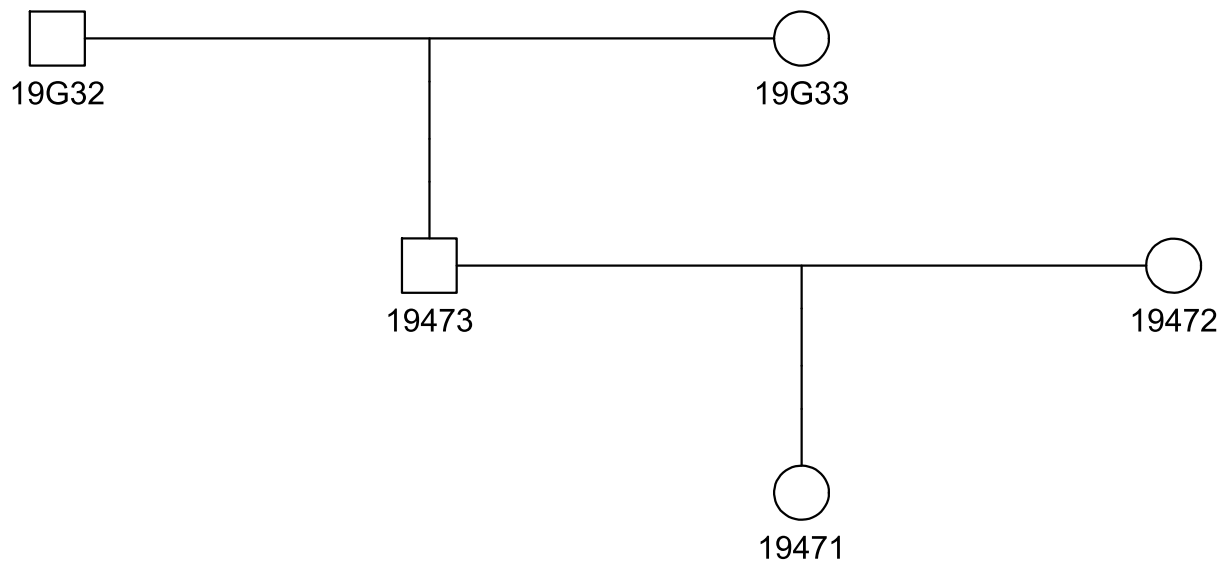
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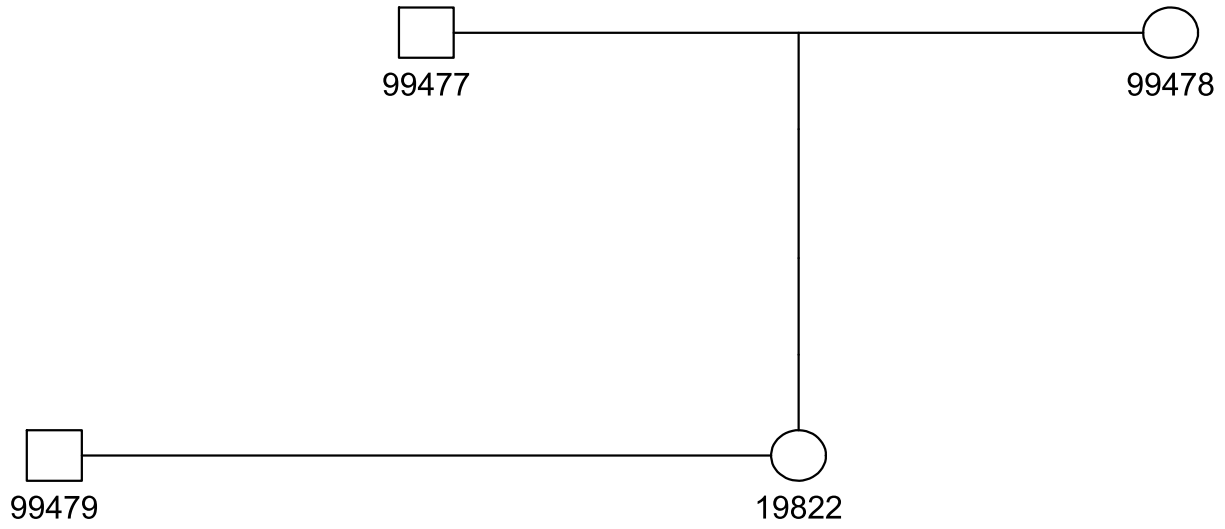
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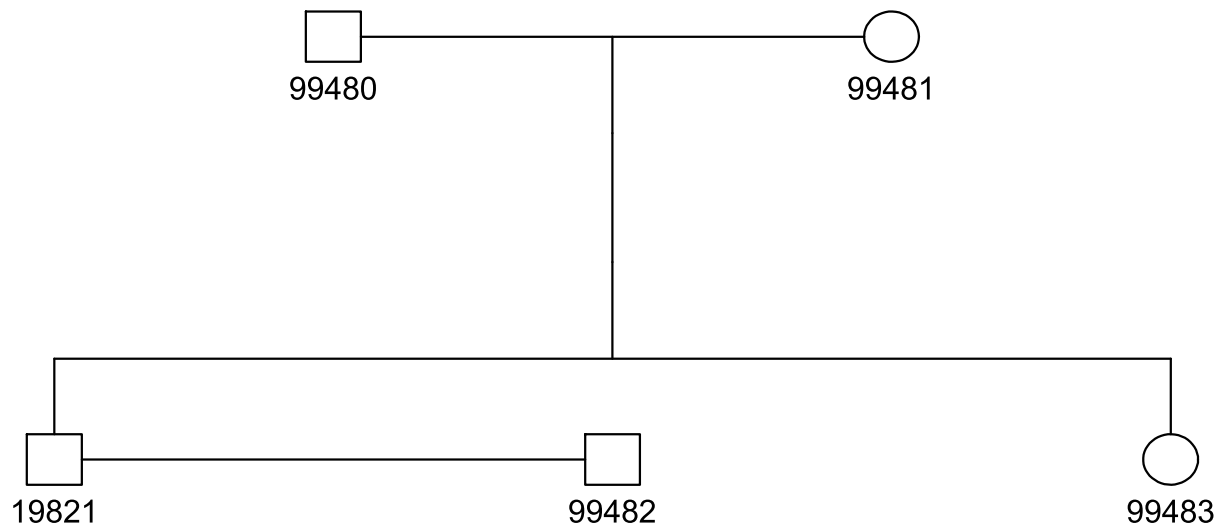
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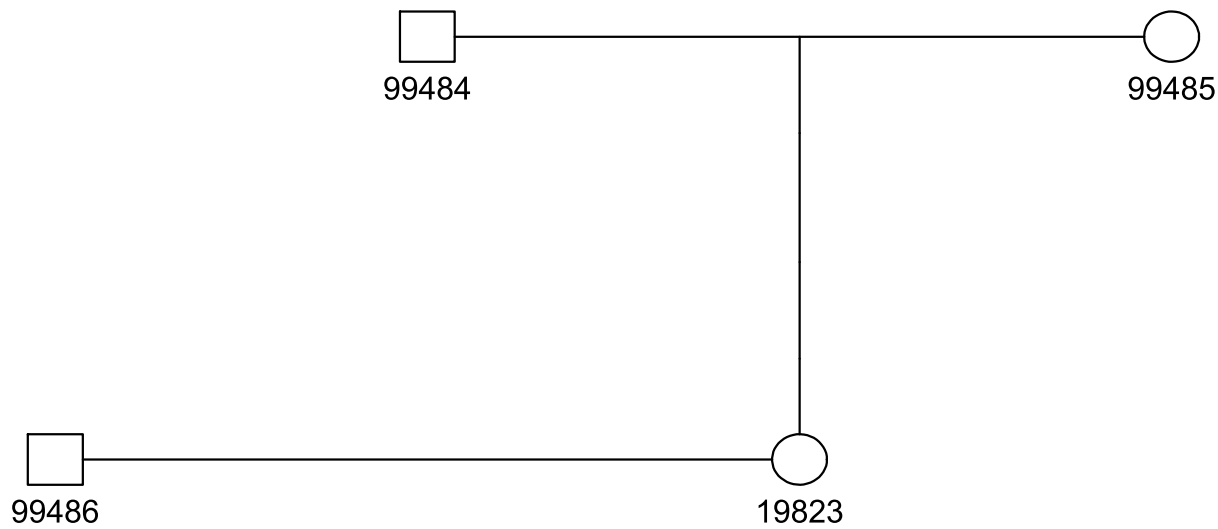
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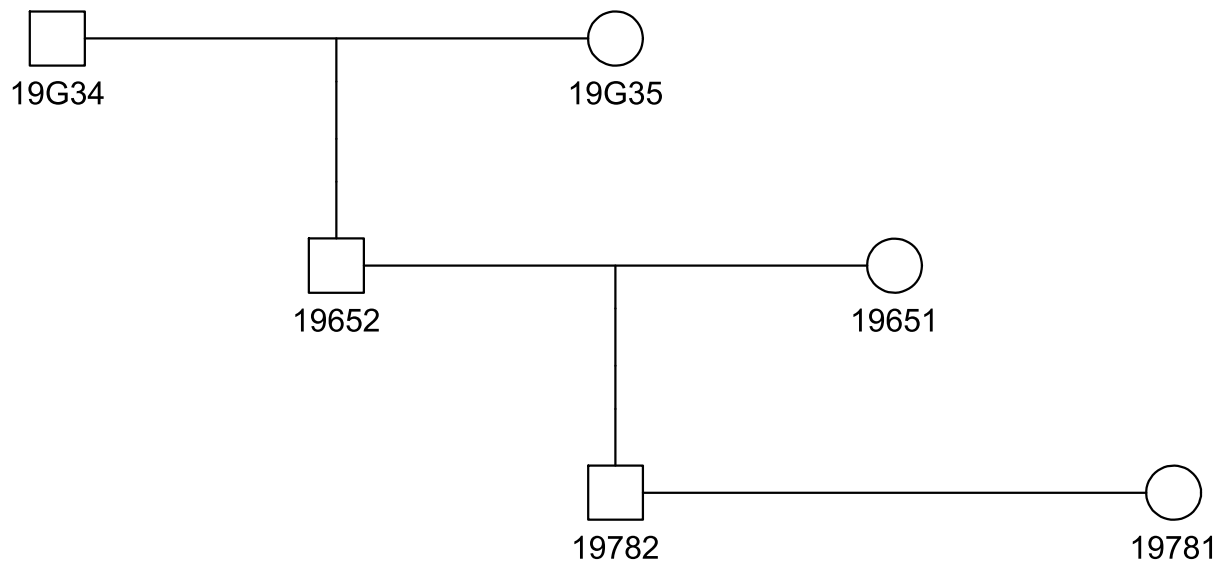
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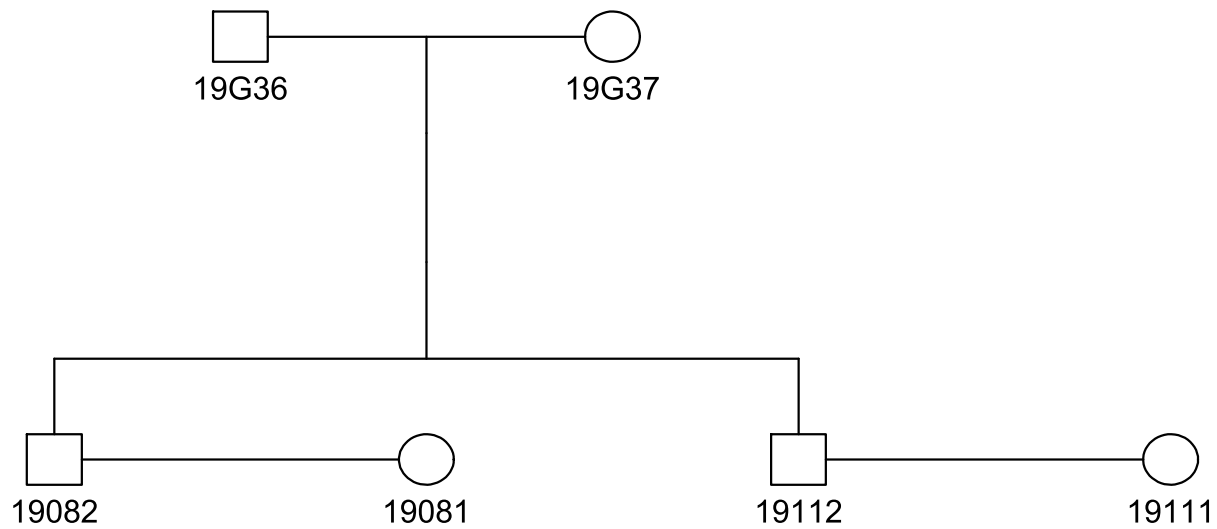
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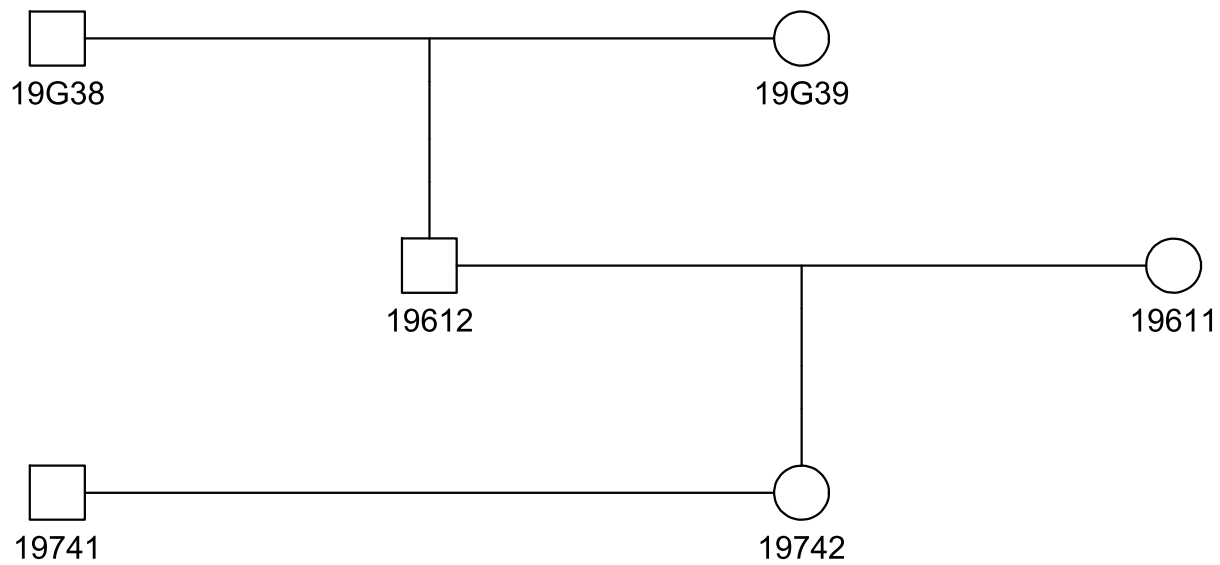
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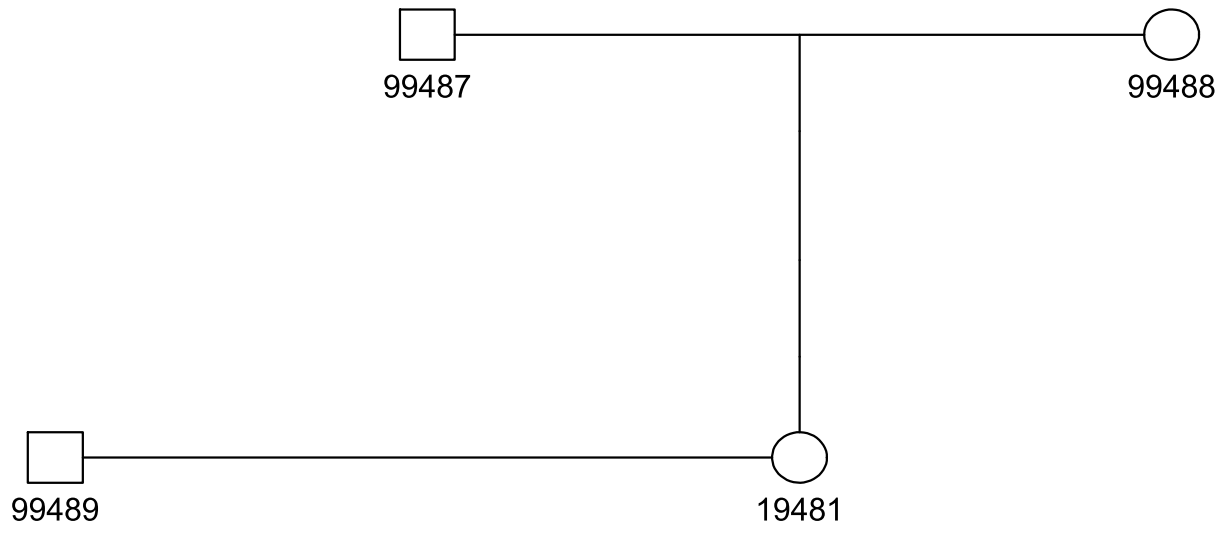
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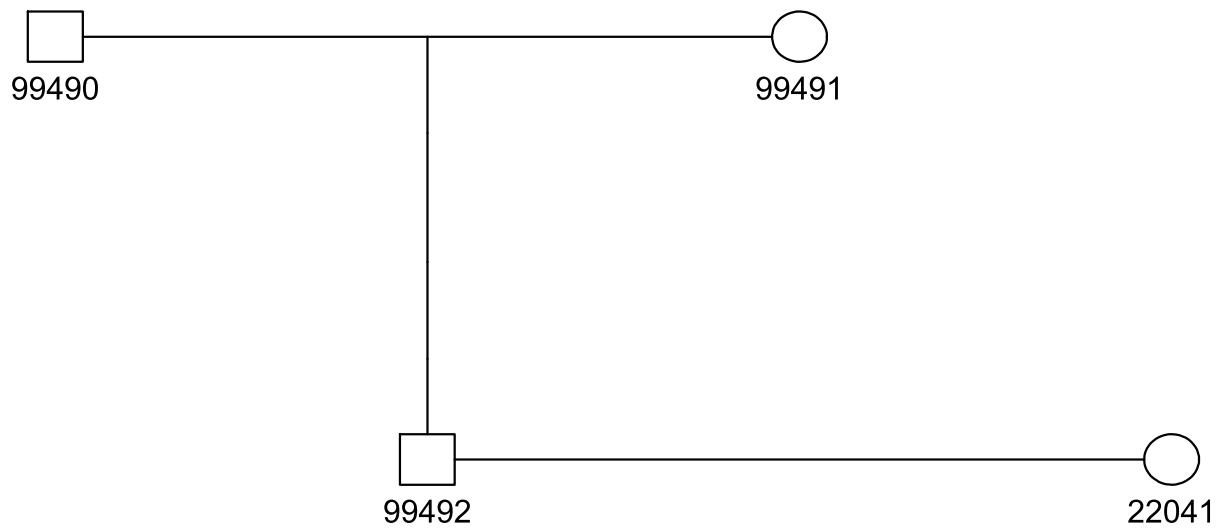
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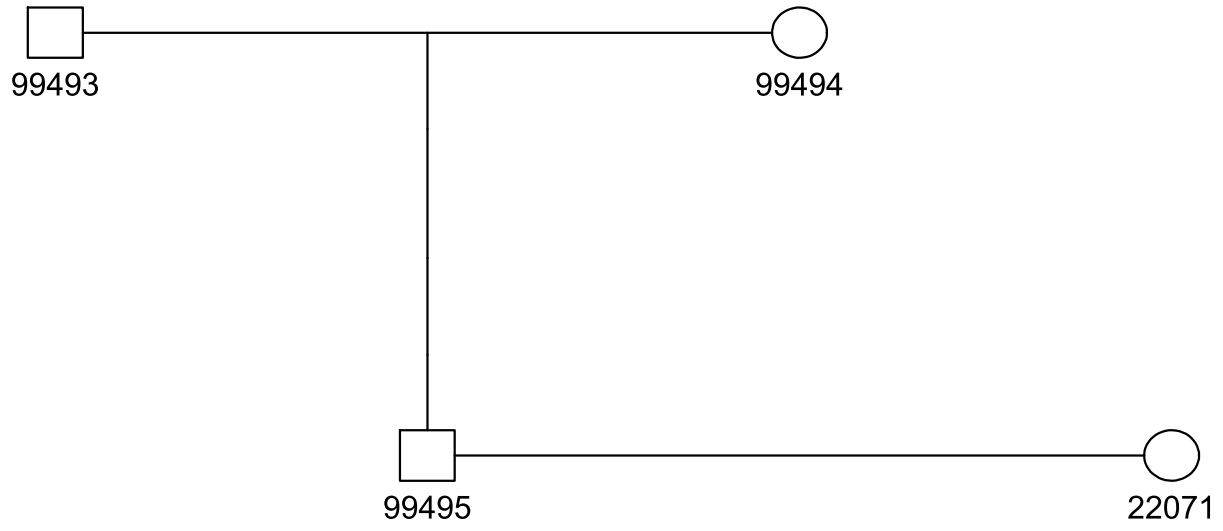
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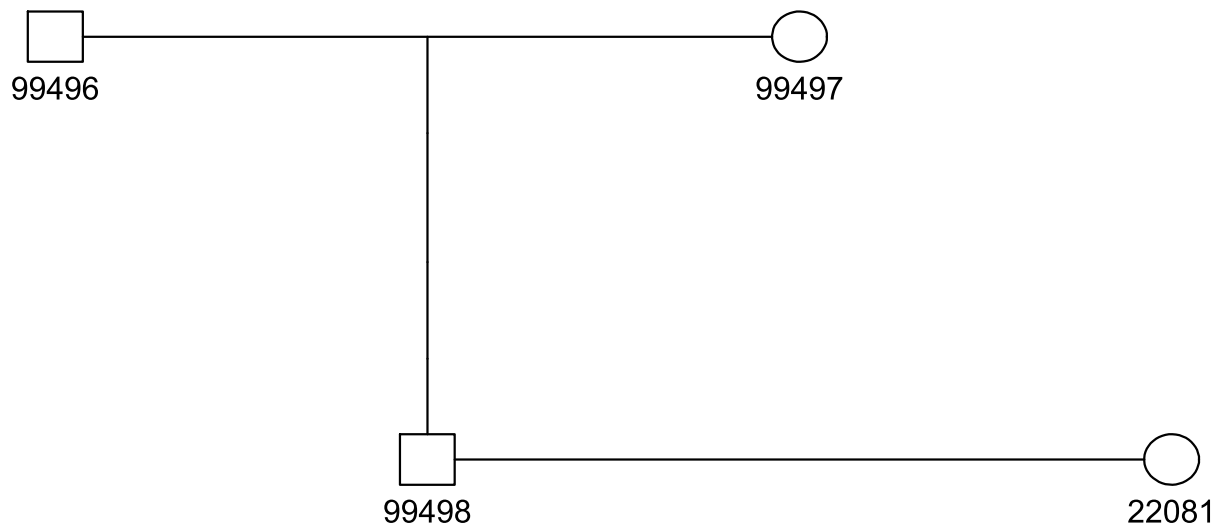
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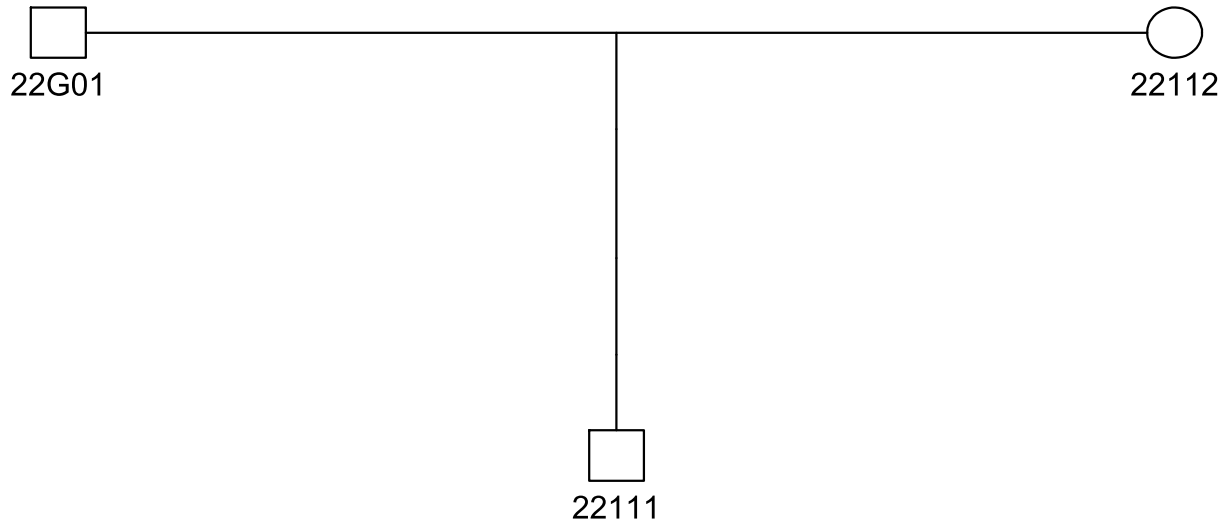
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22L03

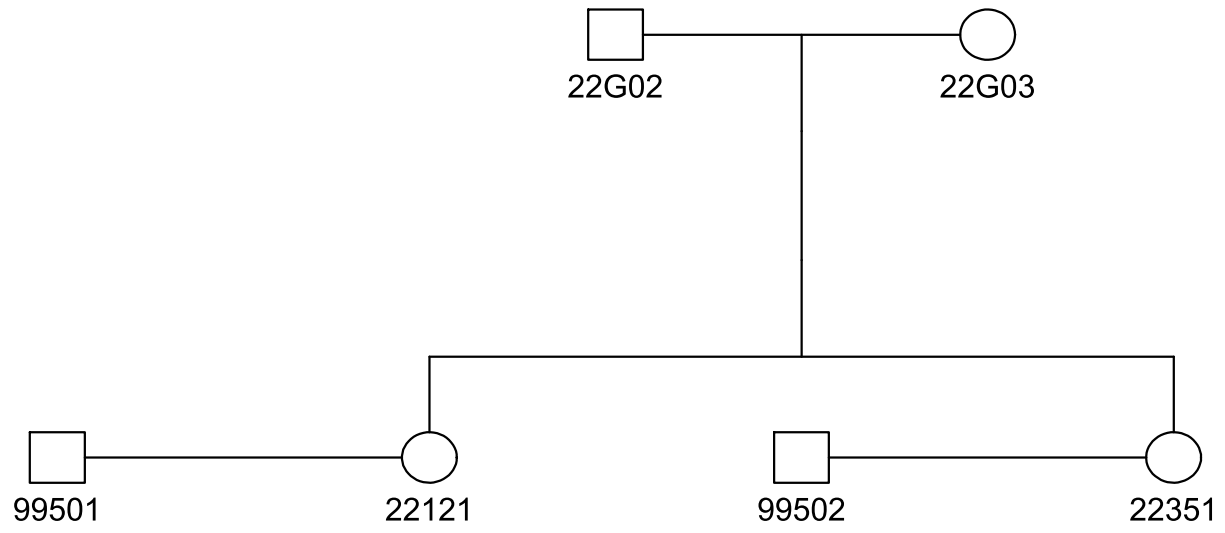


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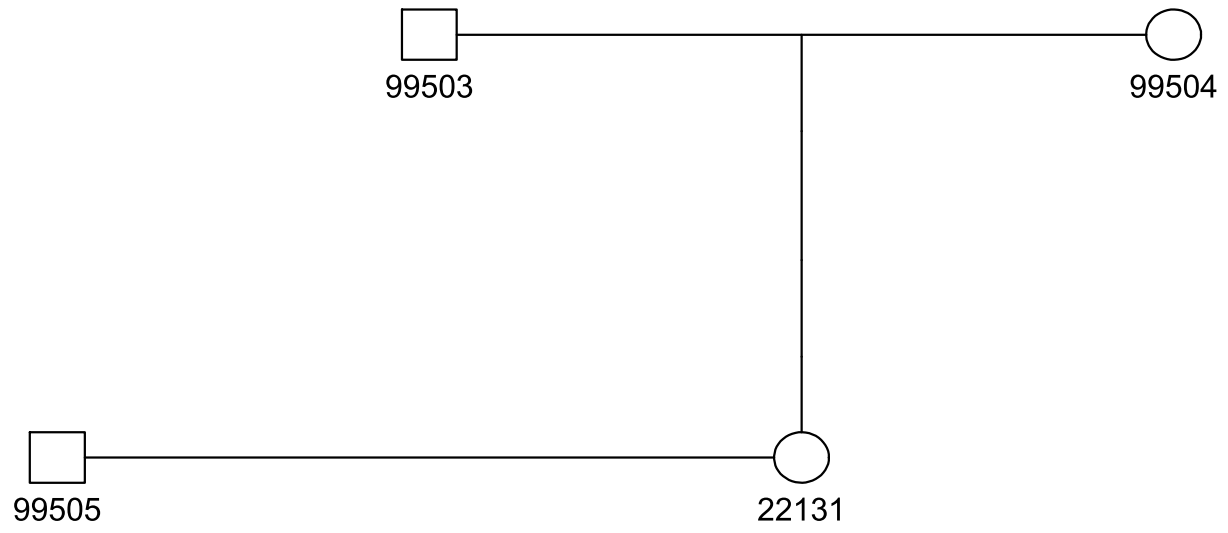


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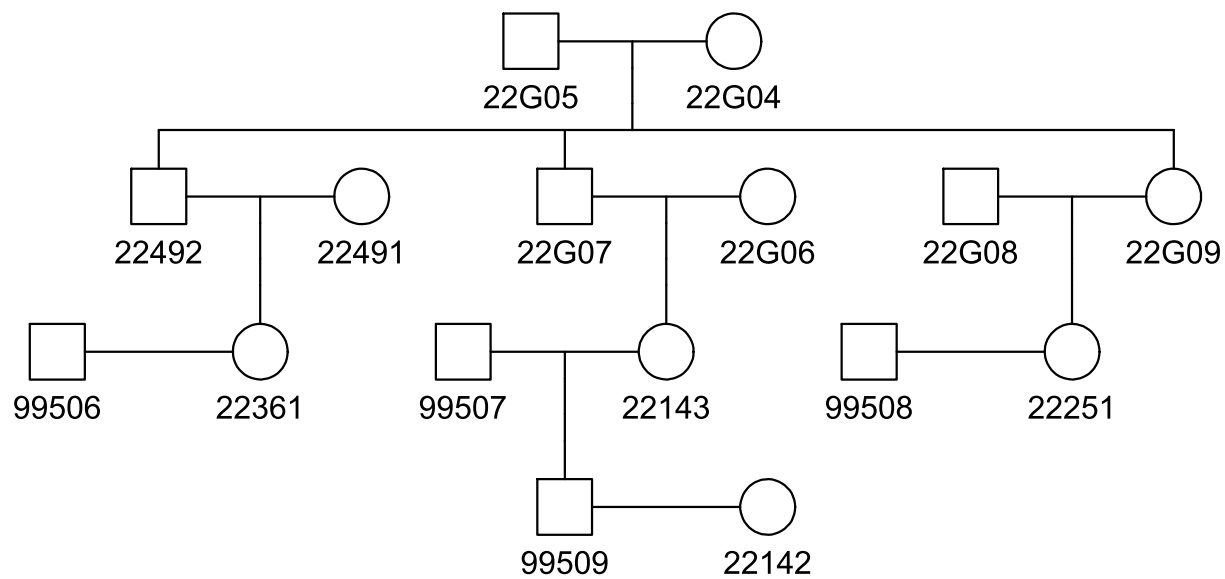
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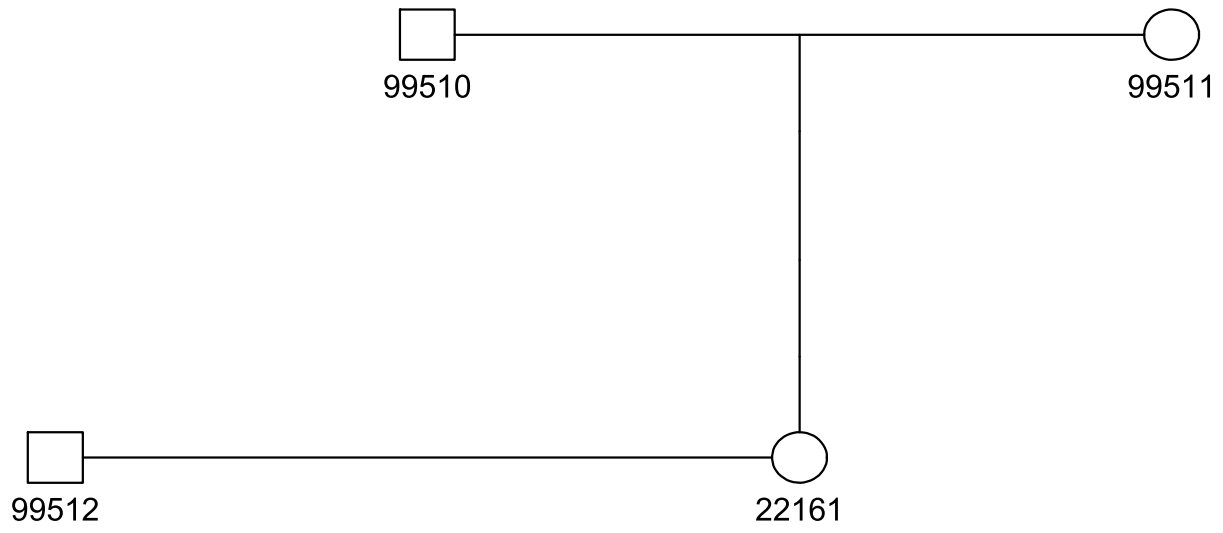
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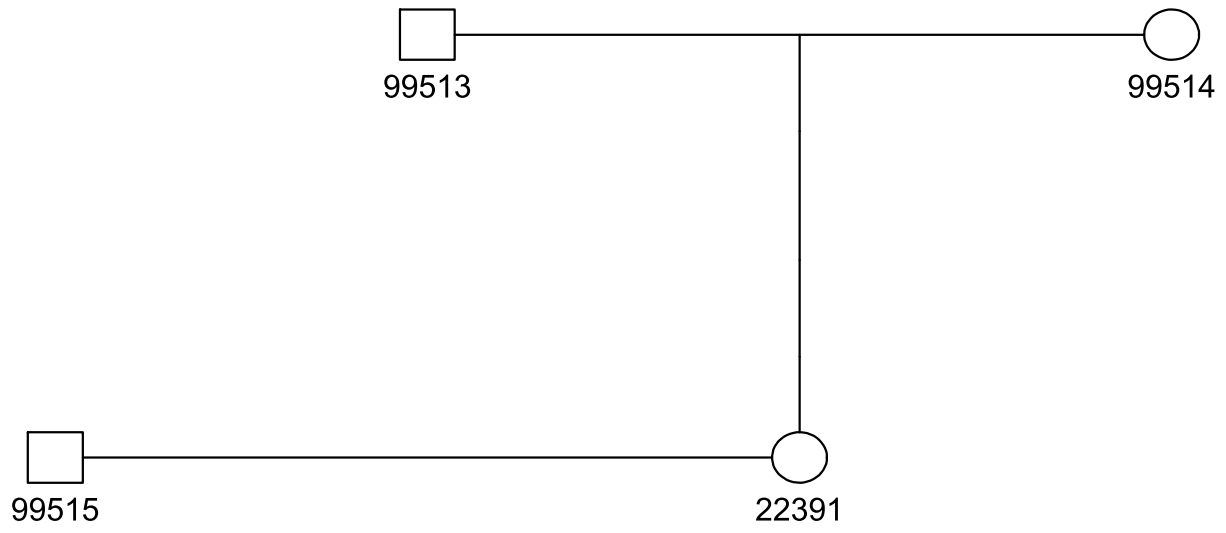
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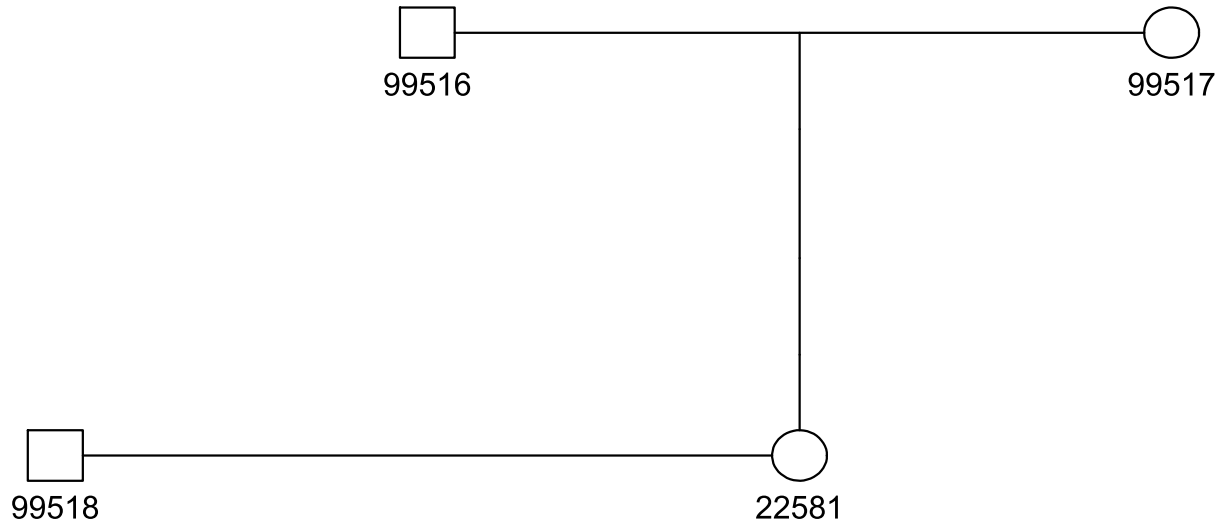
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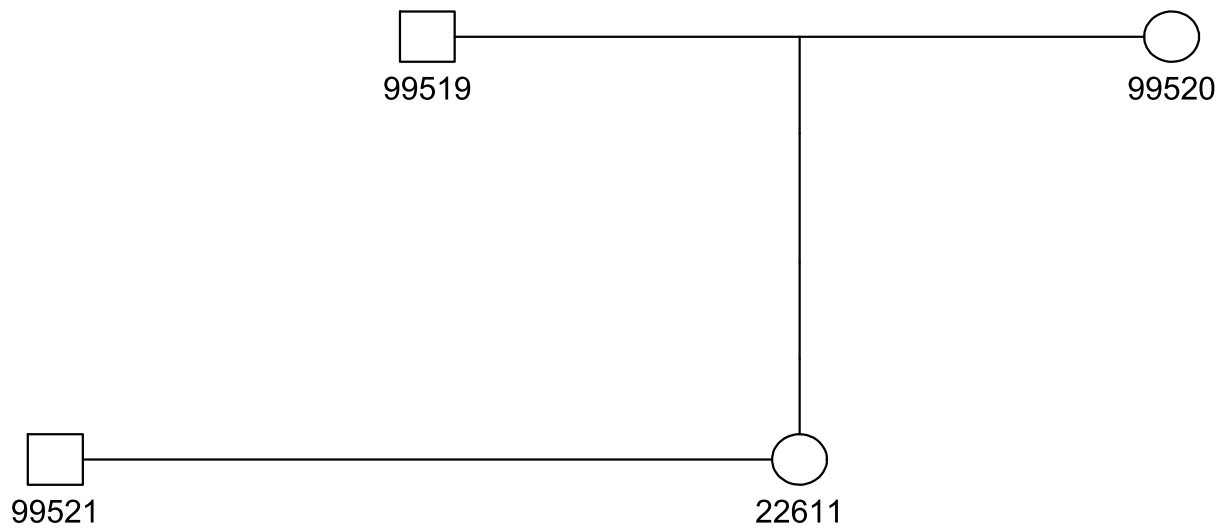
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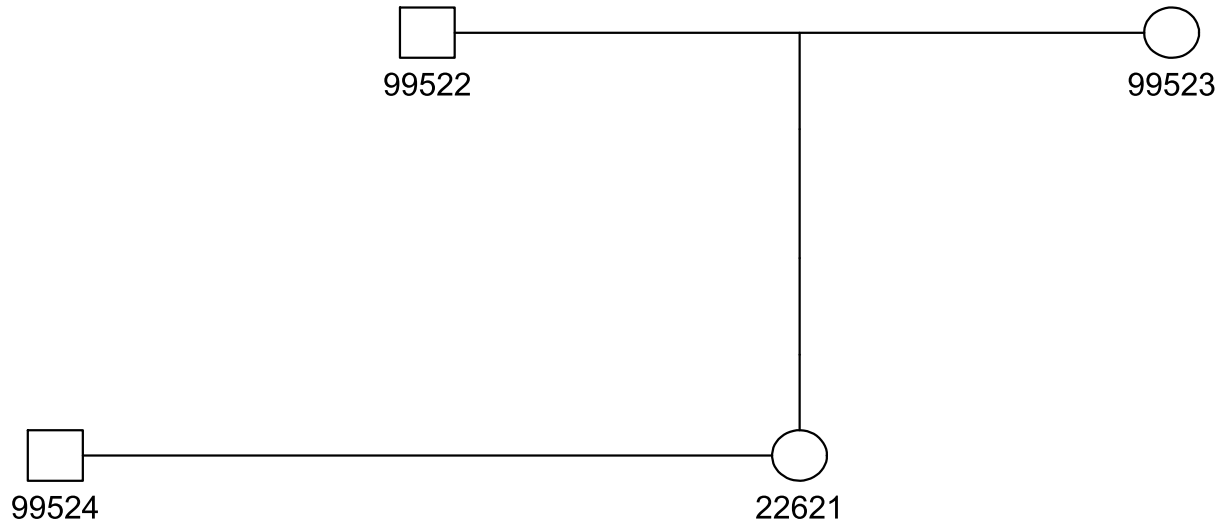
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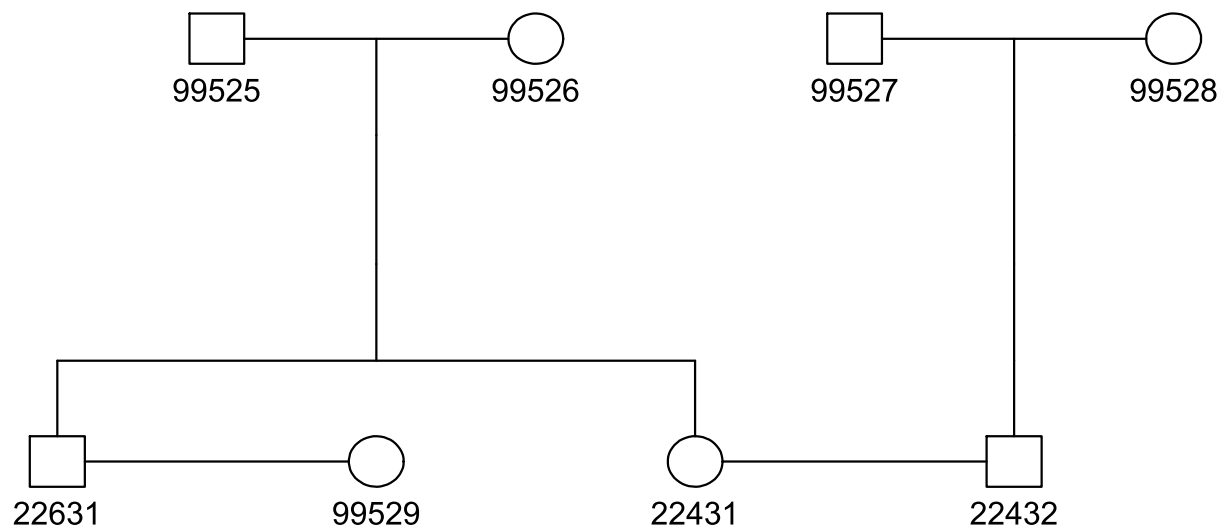
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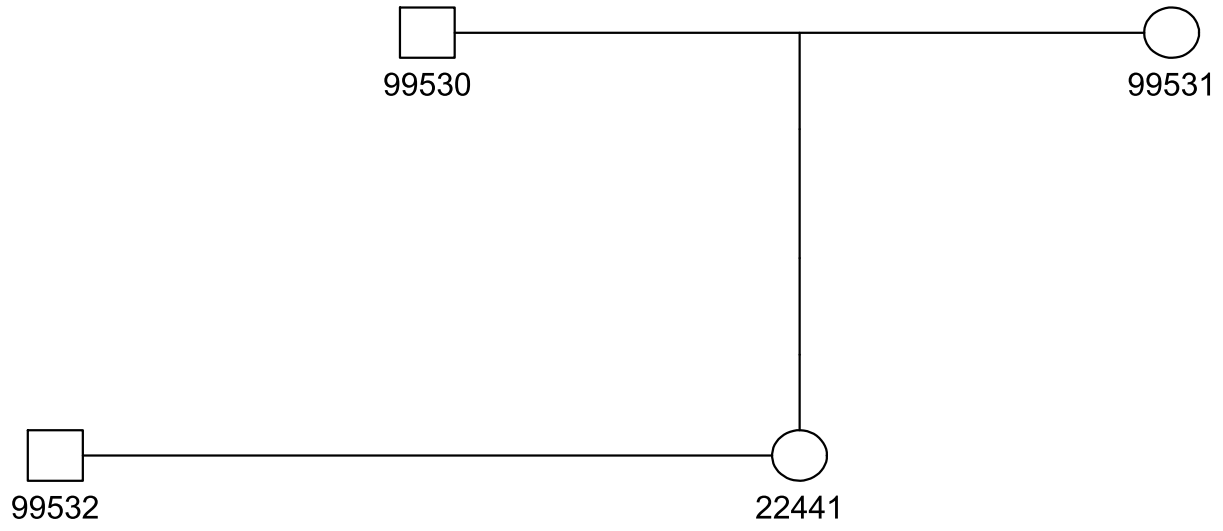
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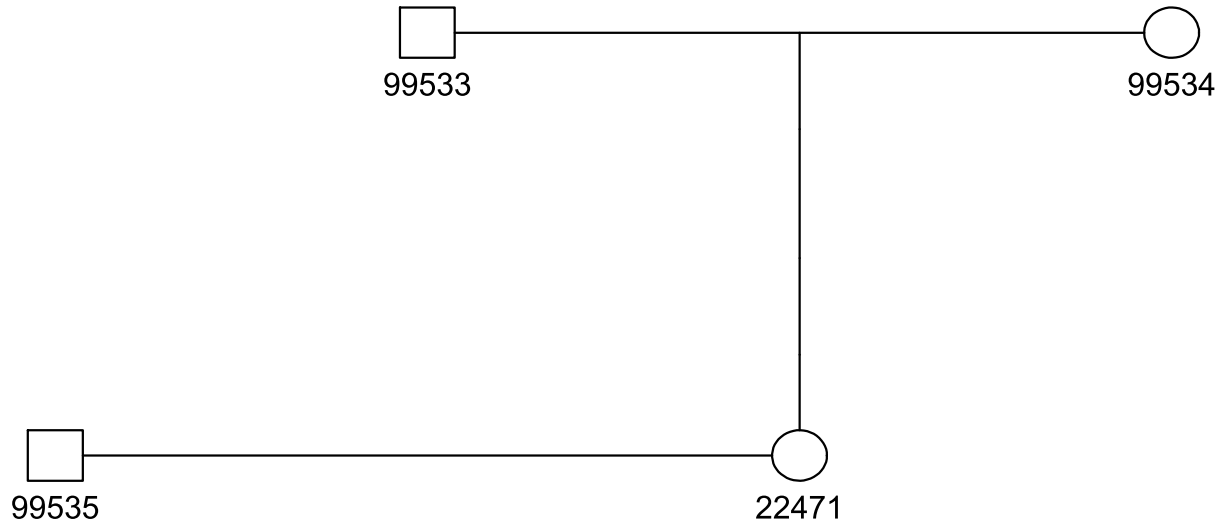
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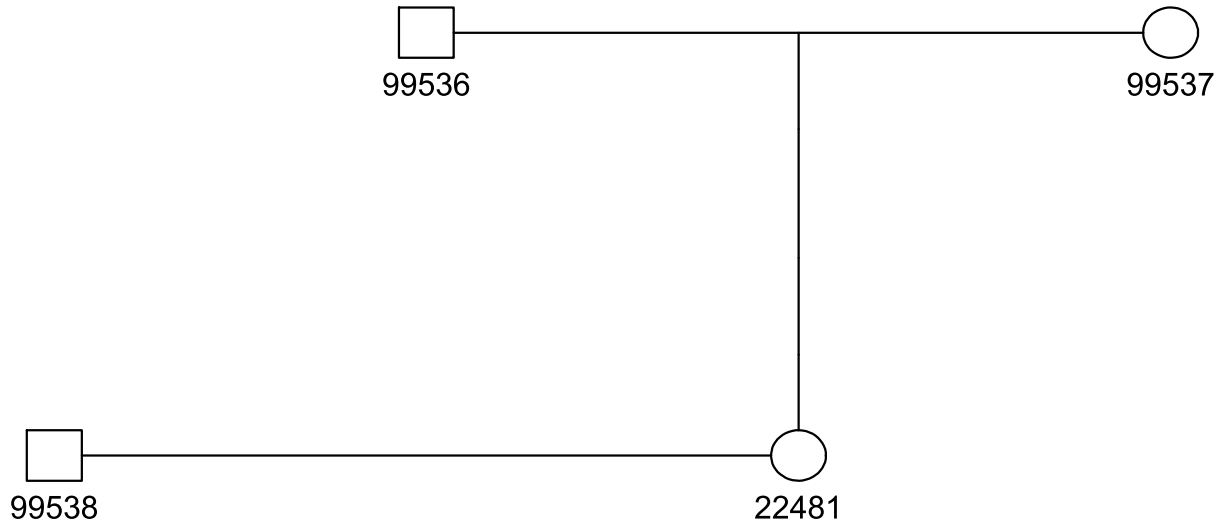
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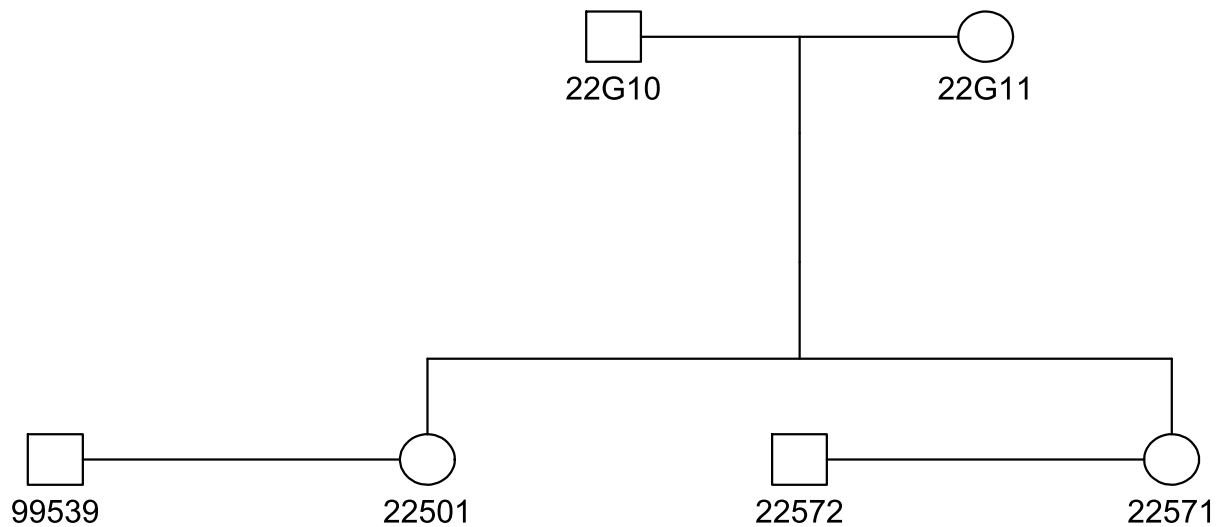
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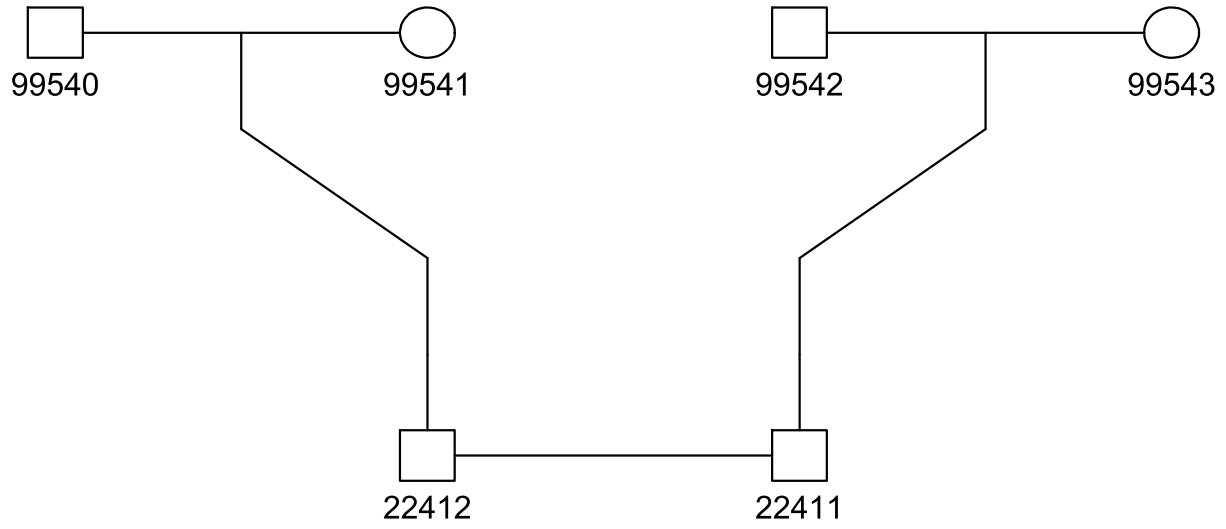
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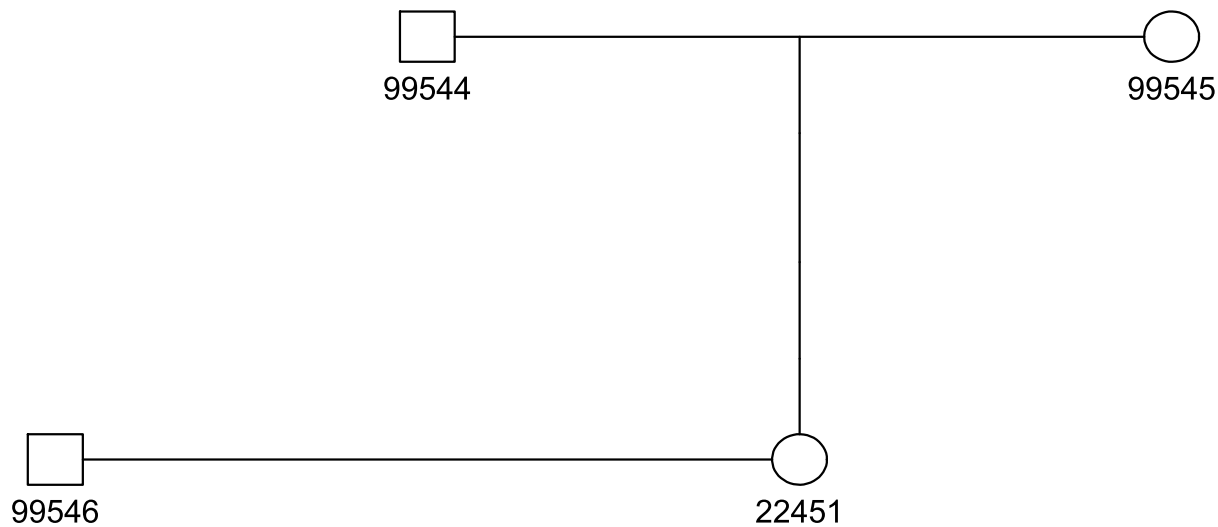
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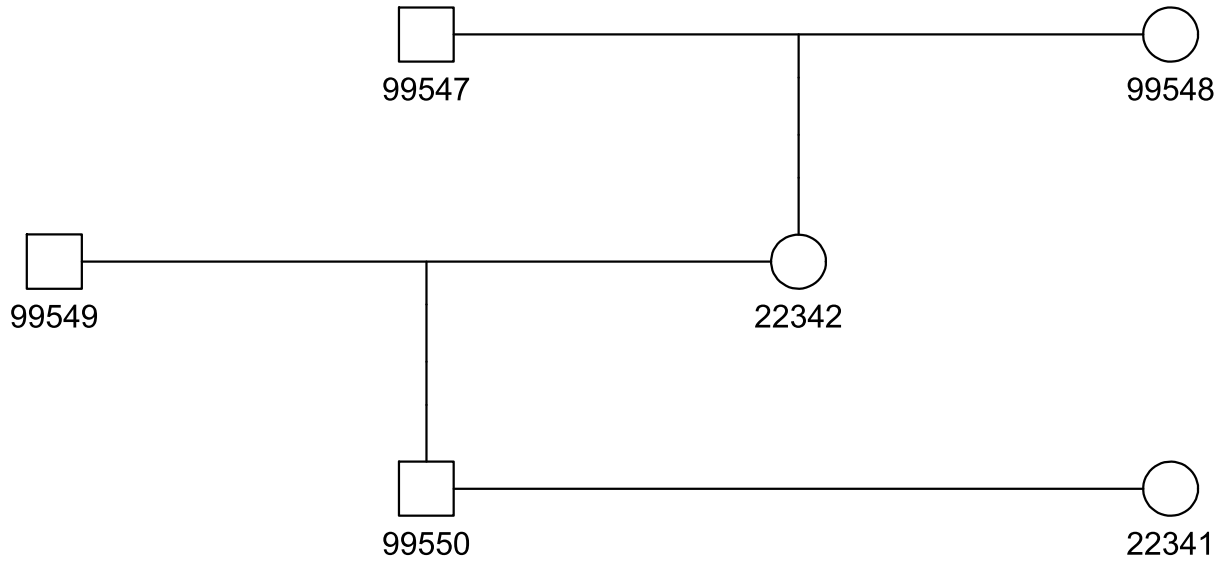
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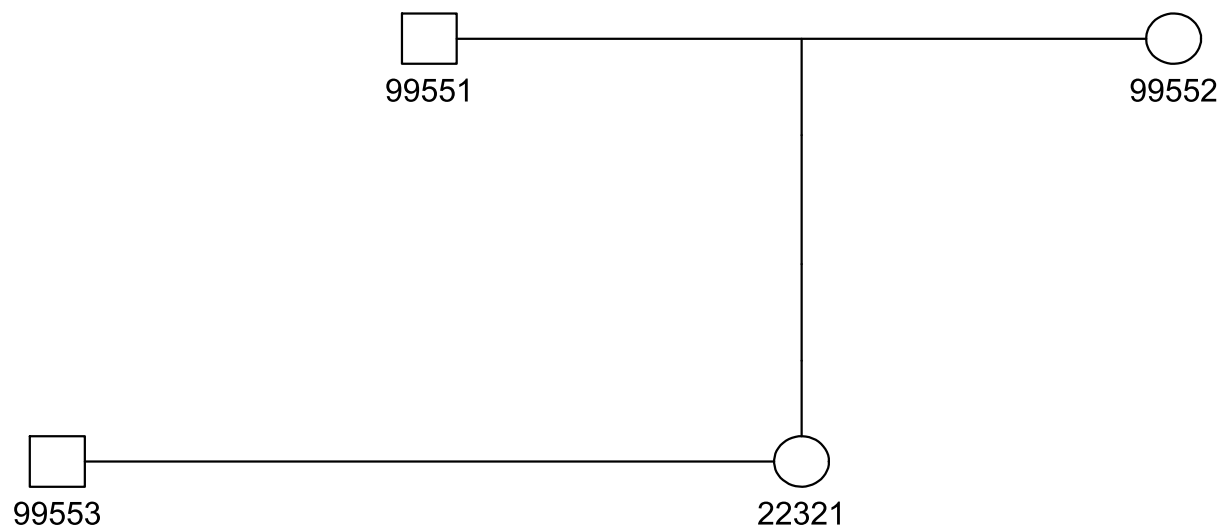
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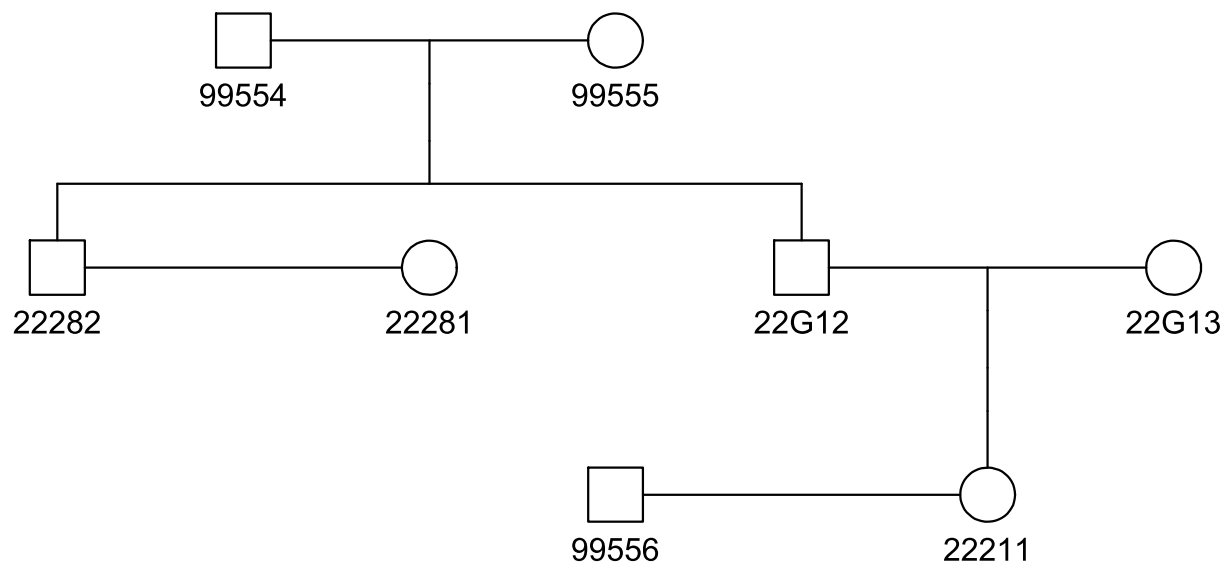
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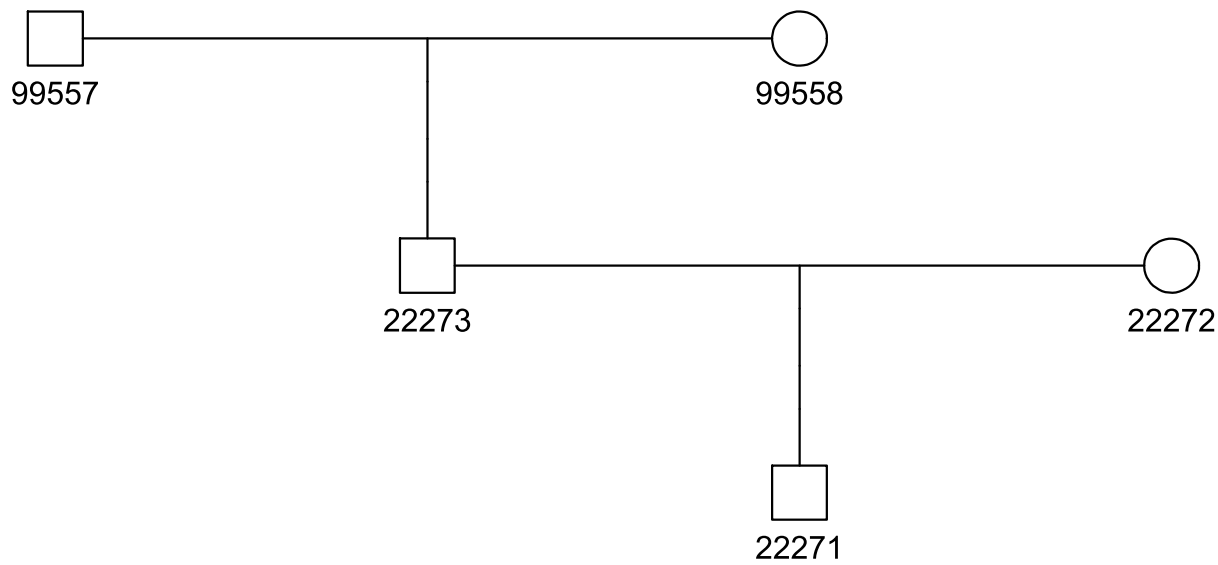
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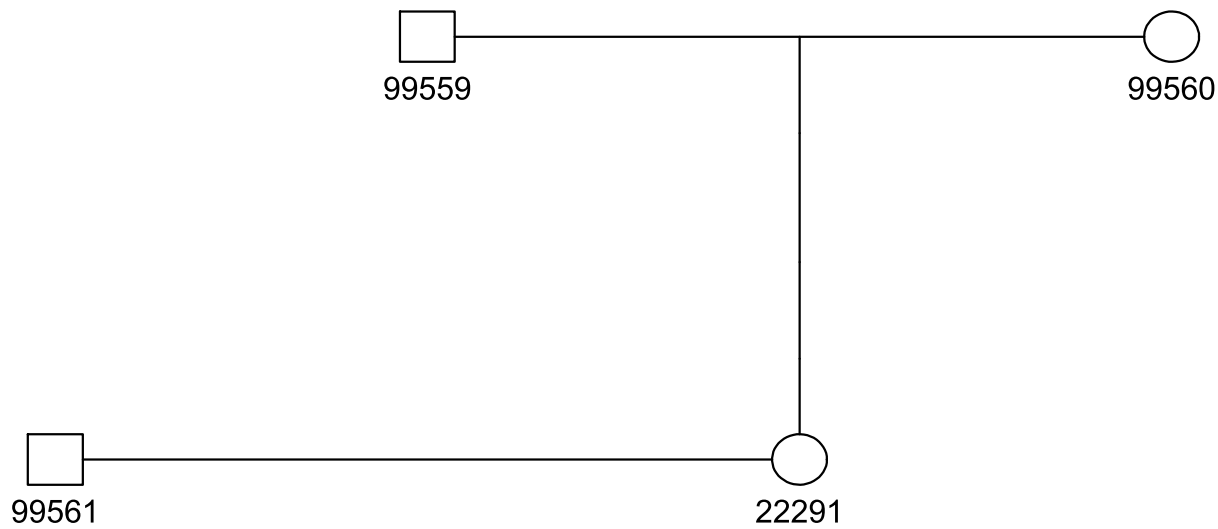
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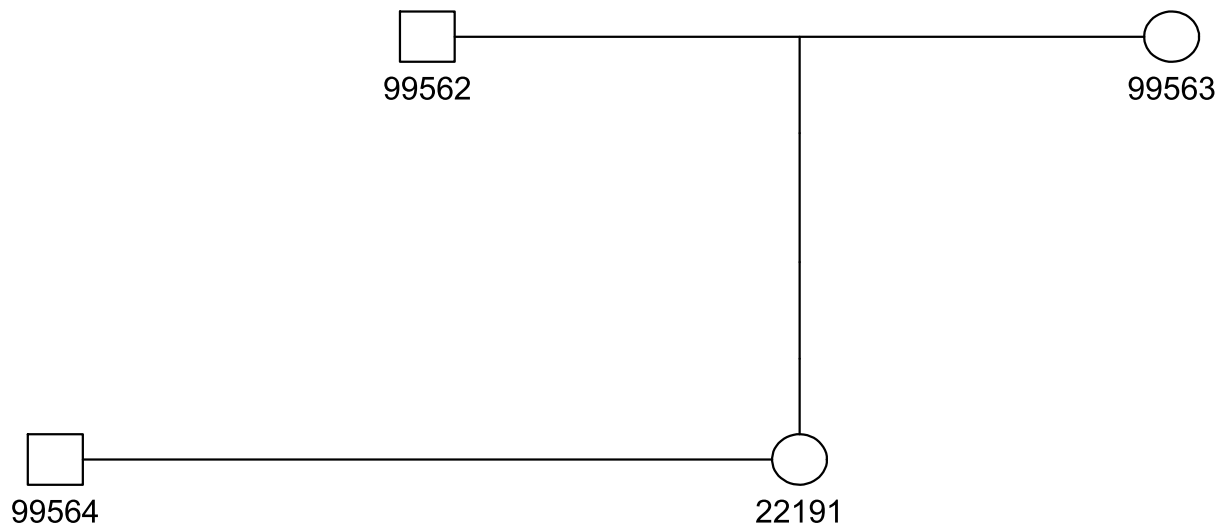
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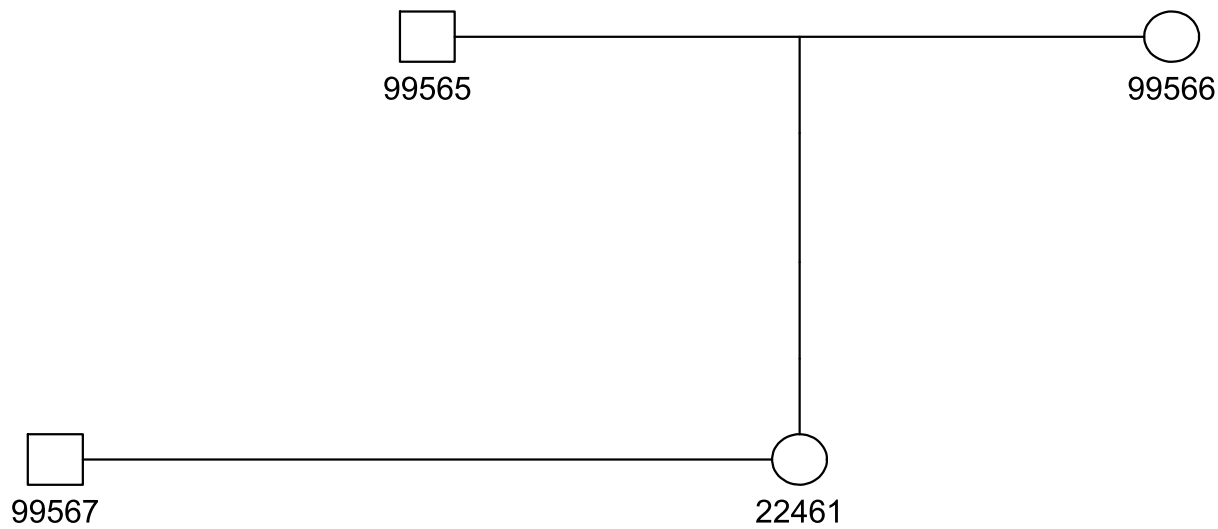
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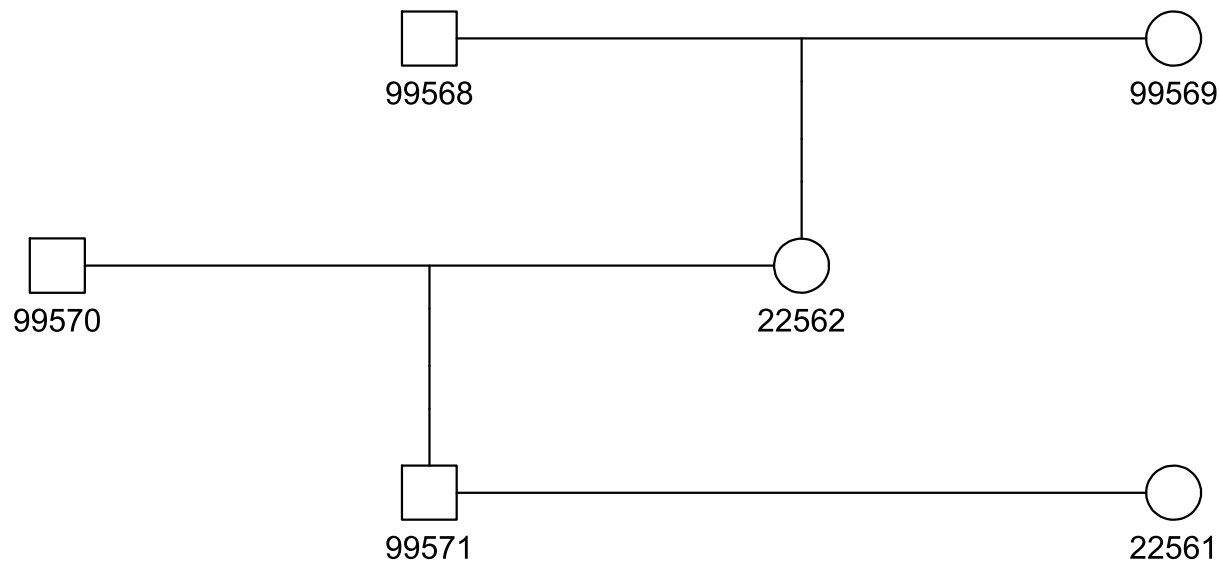
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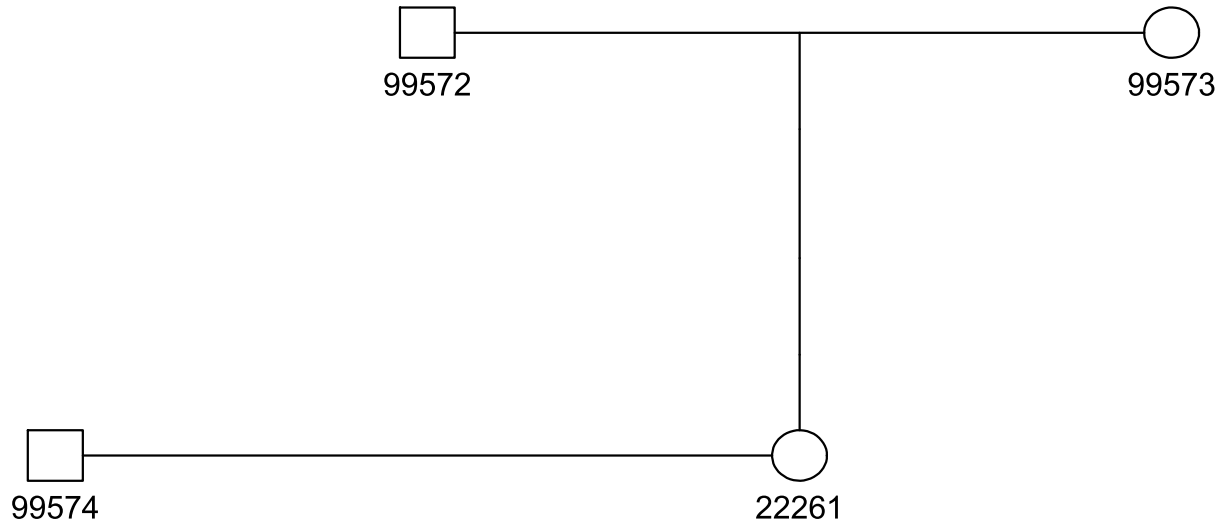
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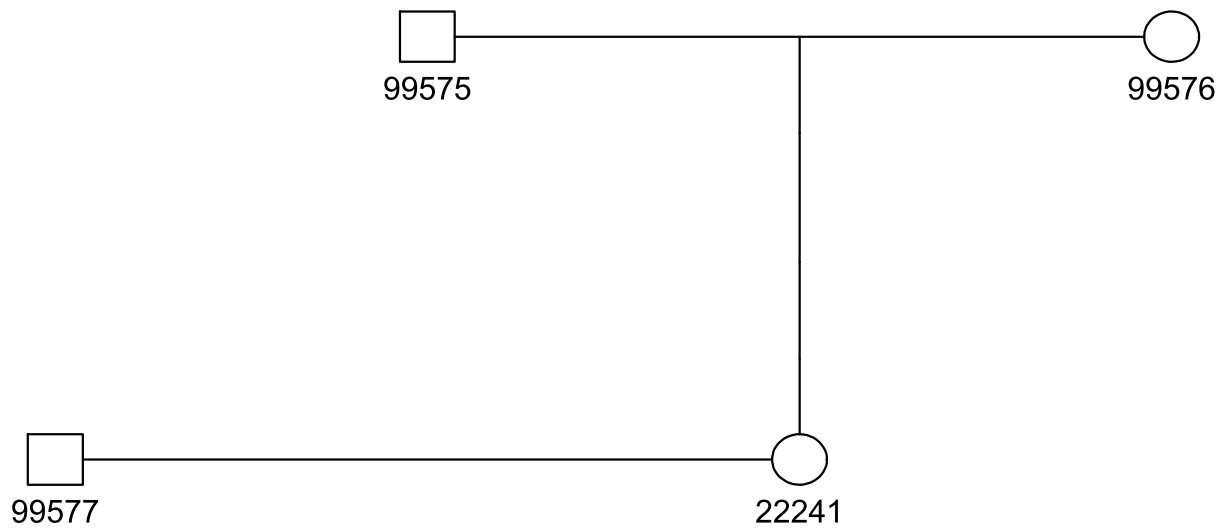
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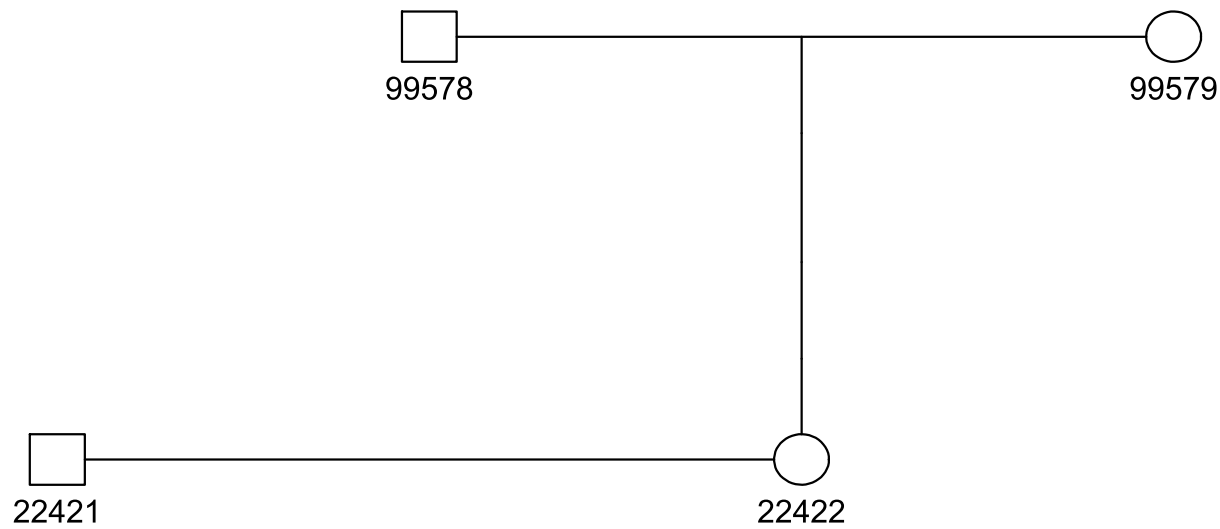
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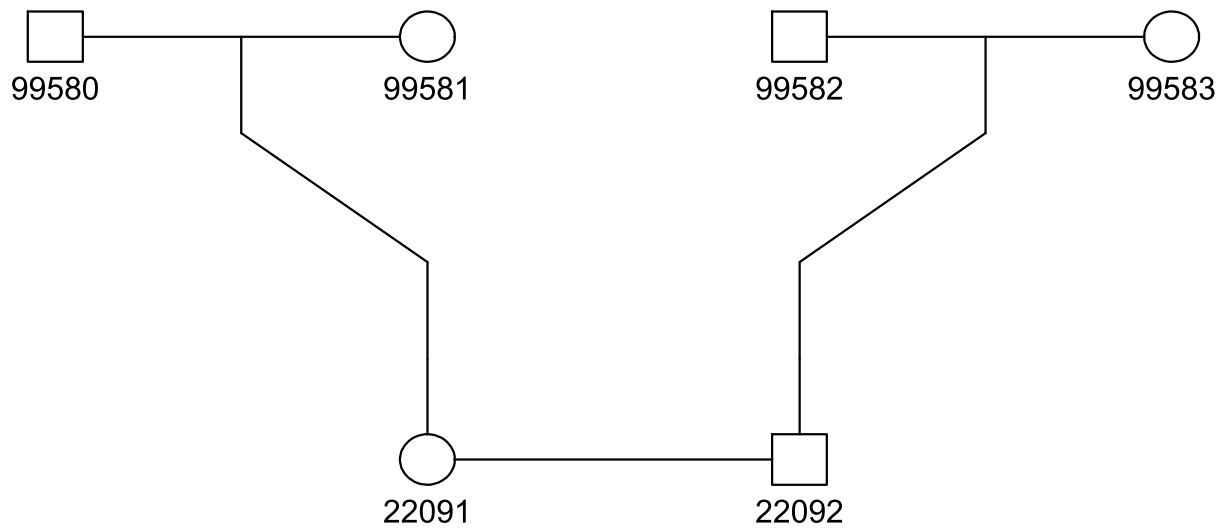
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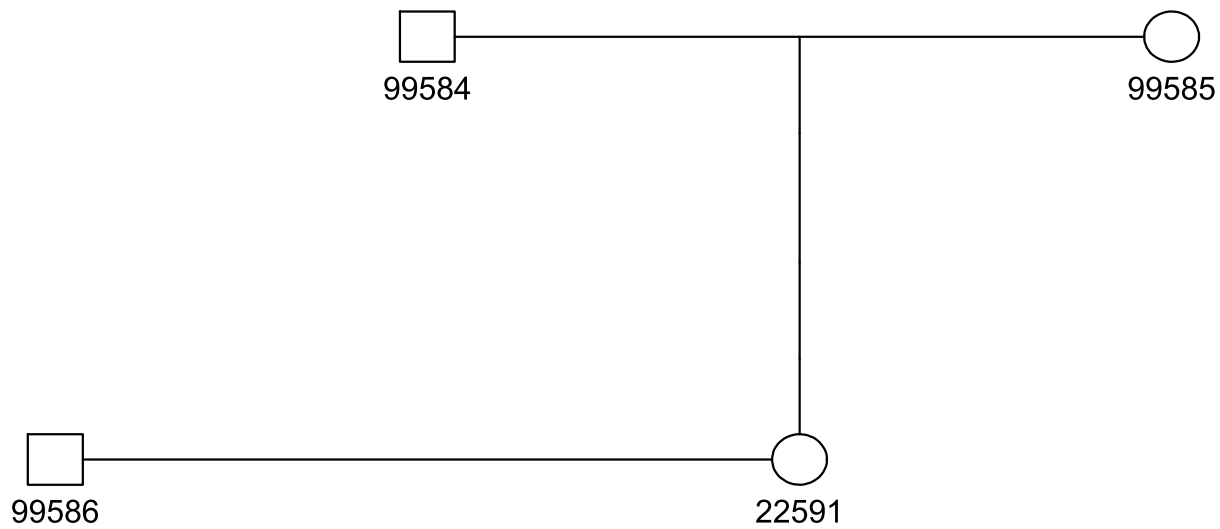
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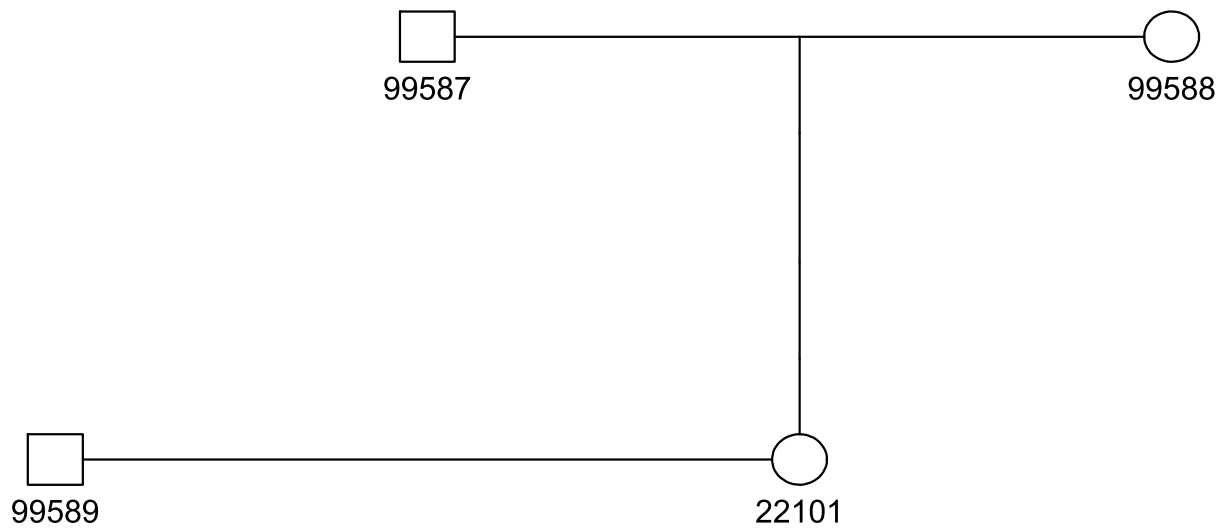
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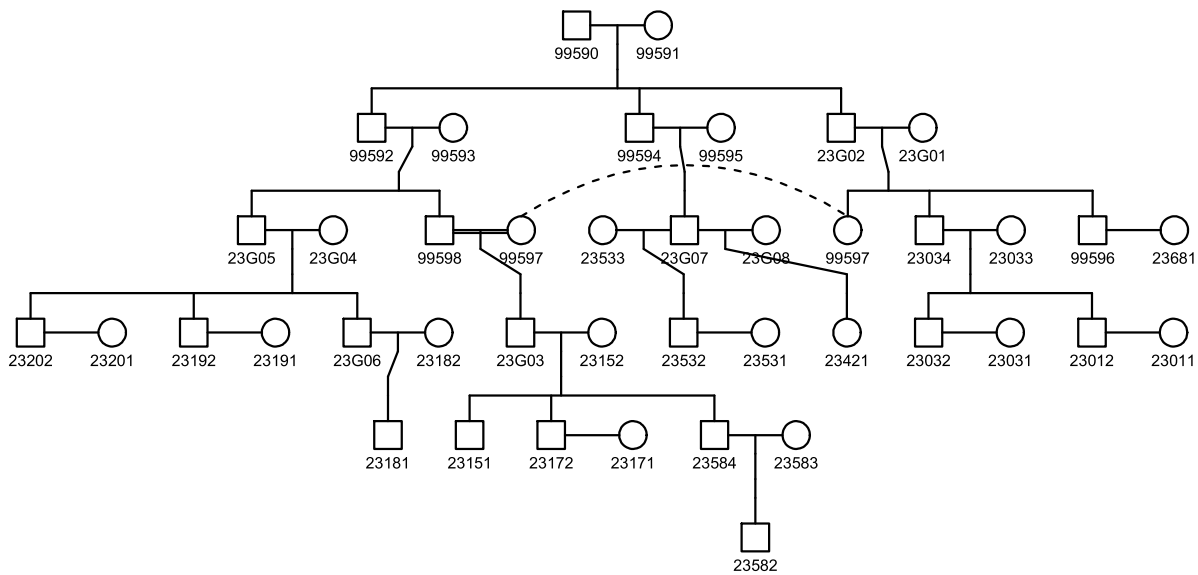
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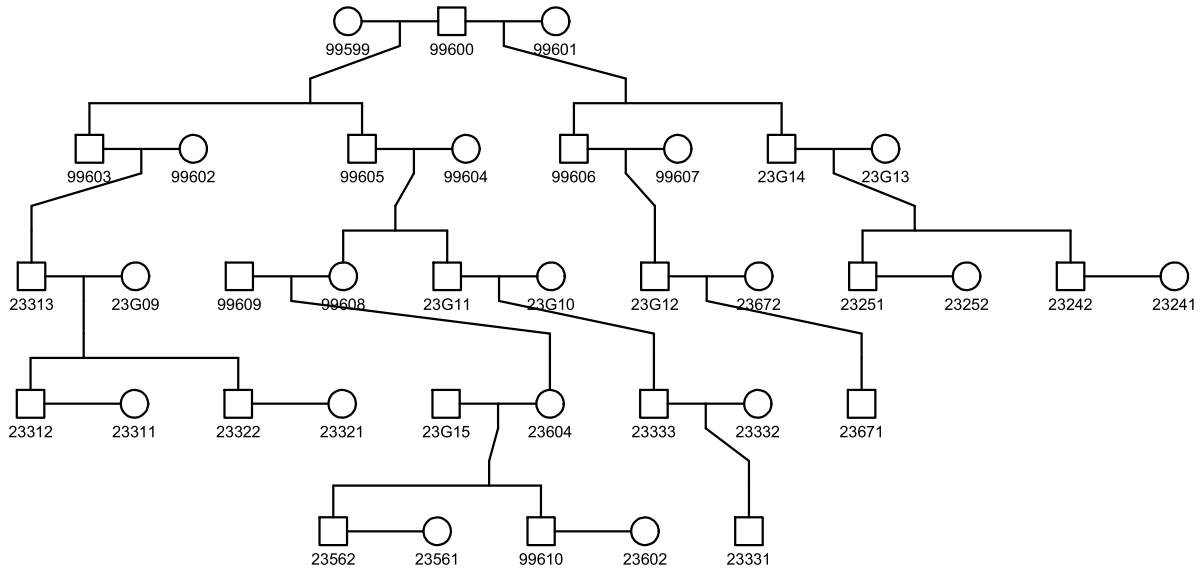
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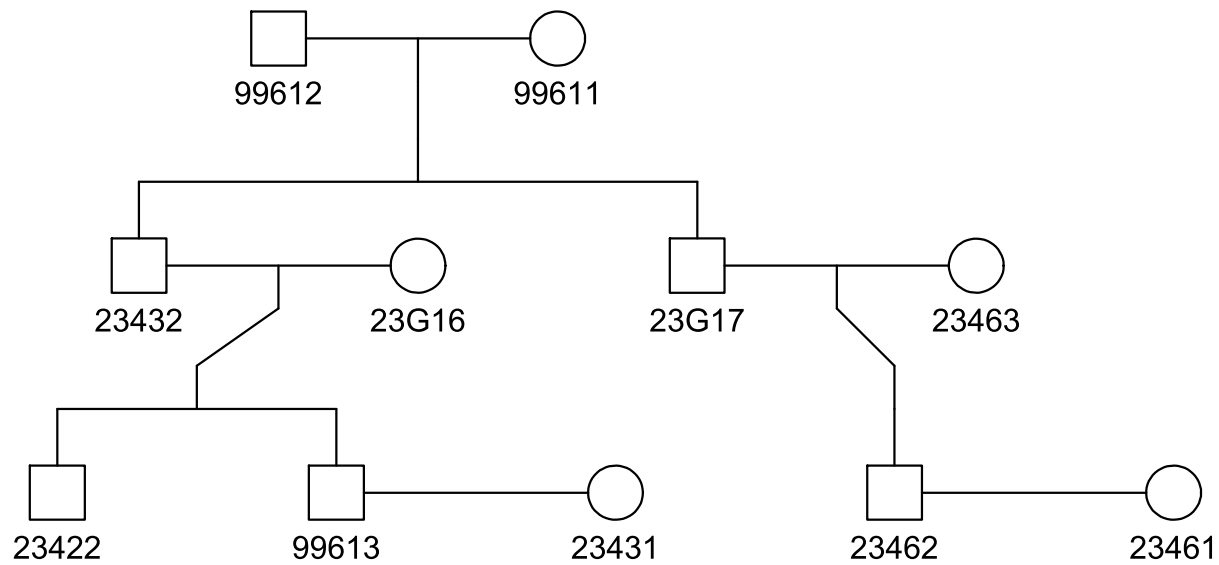
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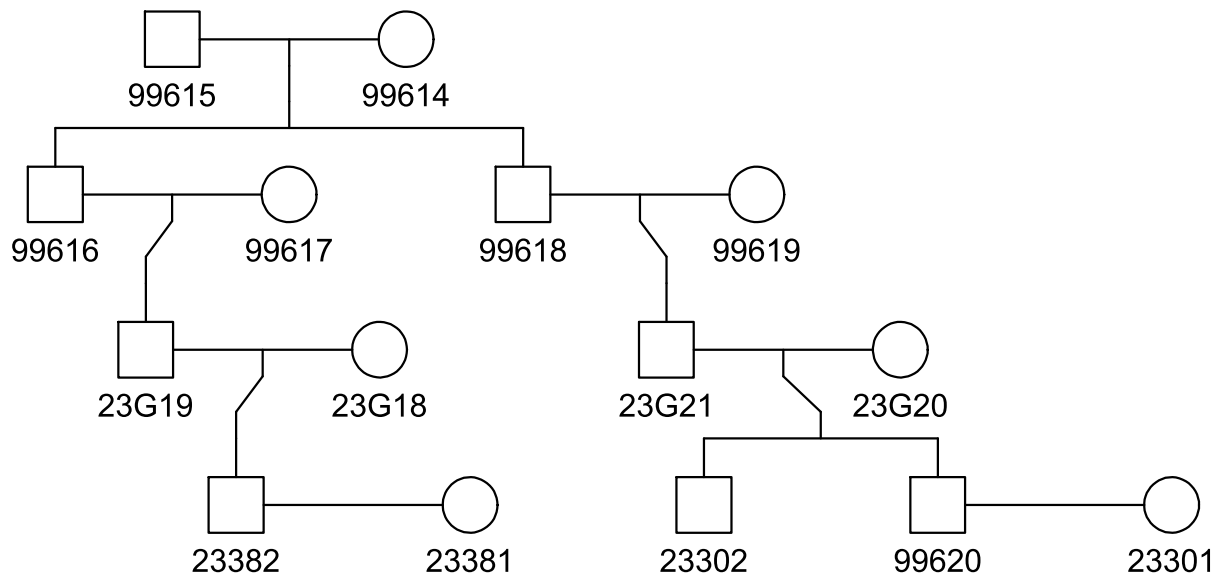
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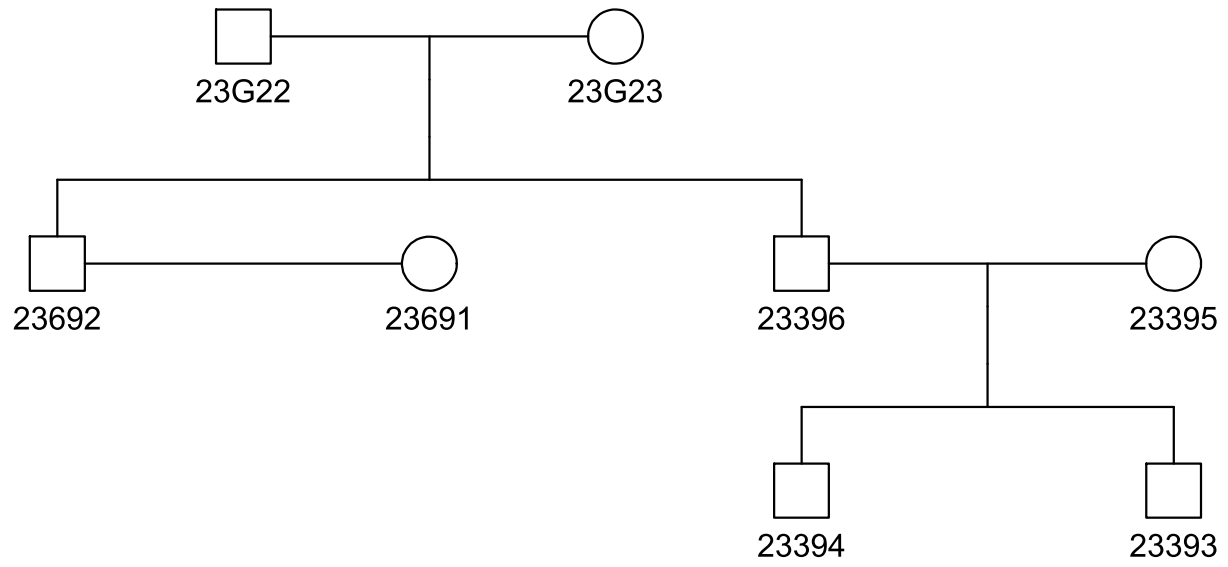
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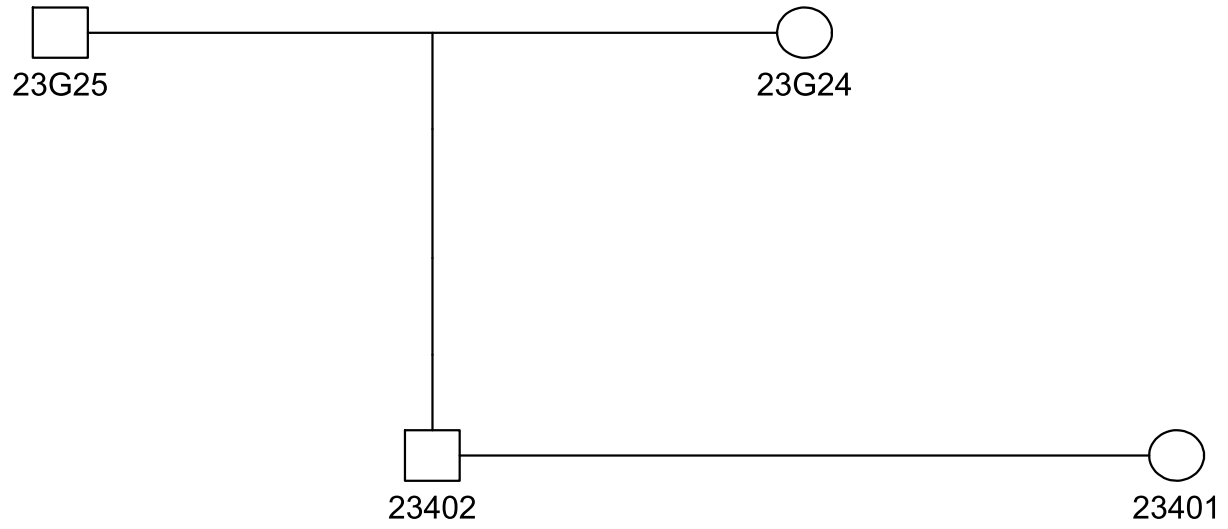


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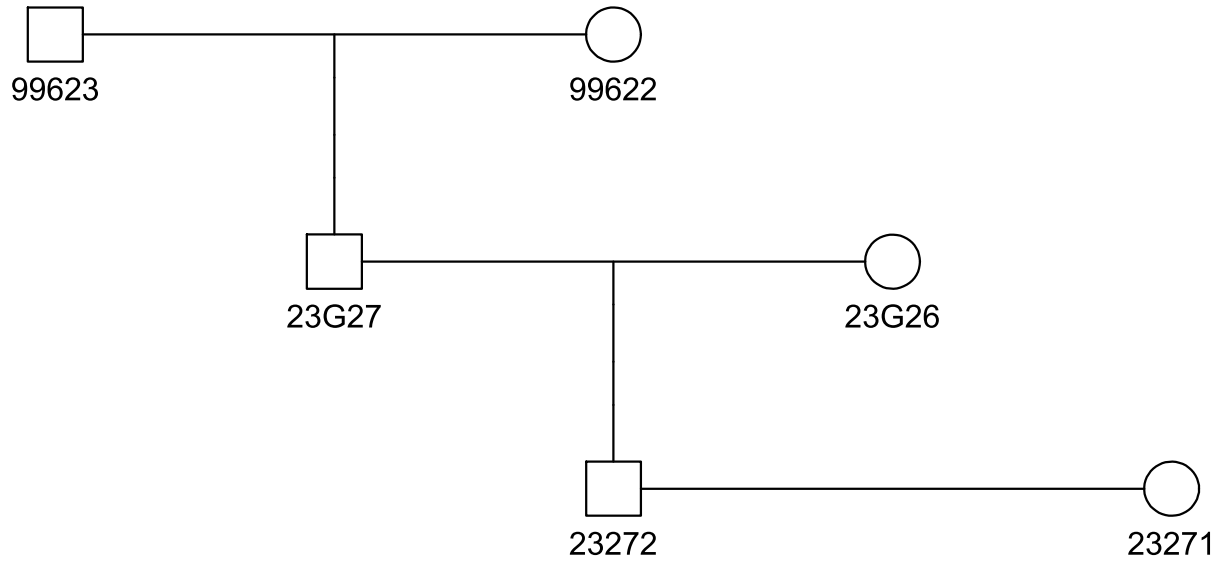


Did not plot the following people: 23392

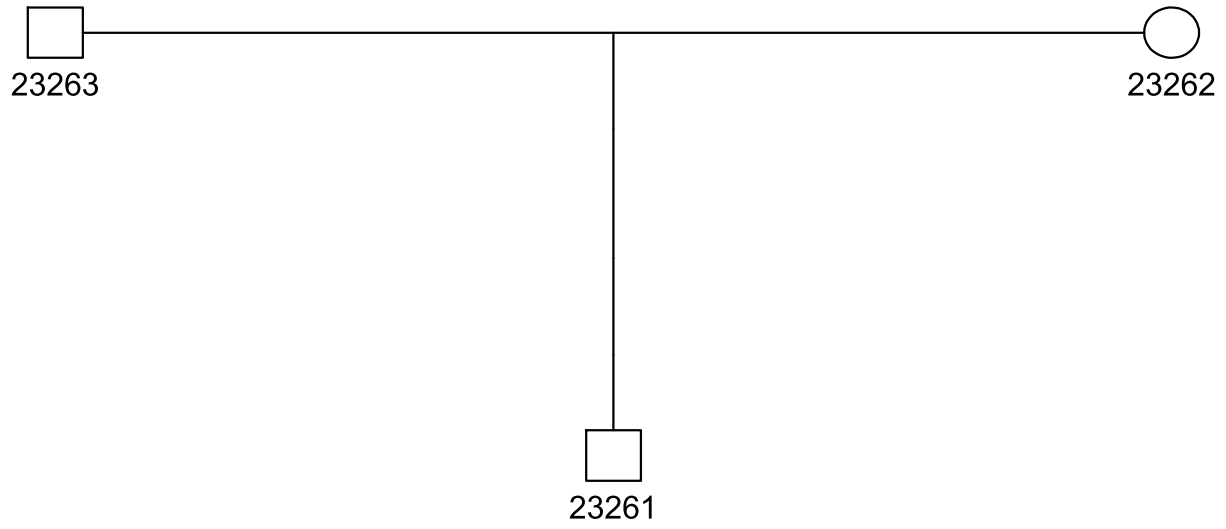
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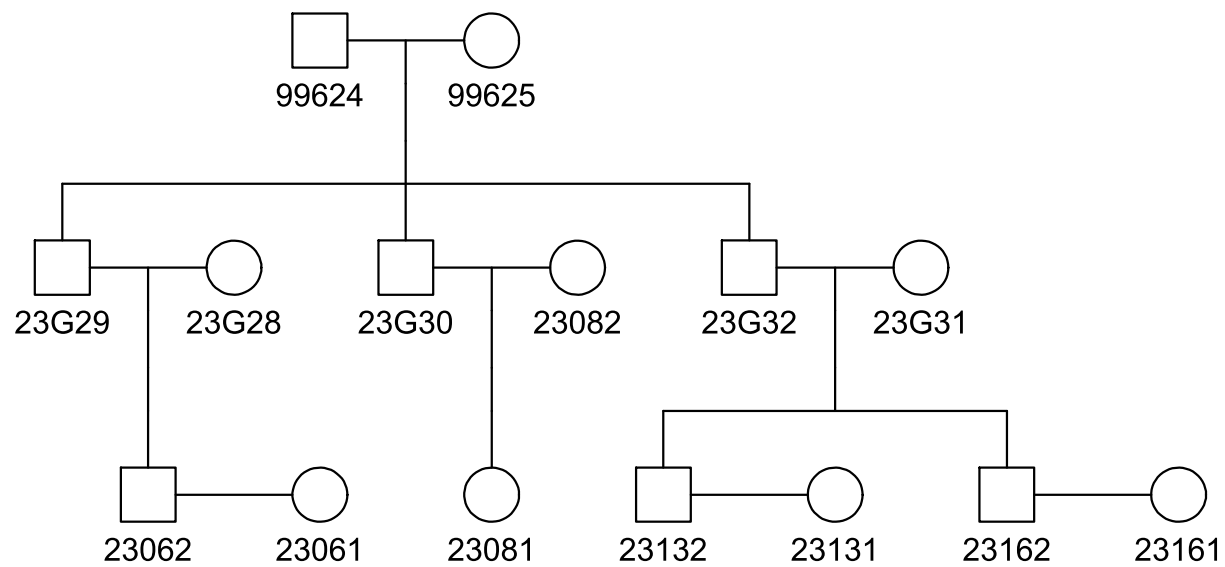
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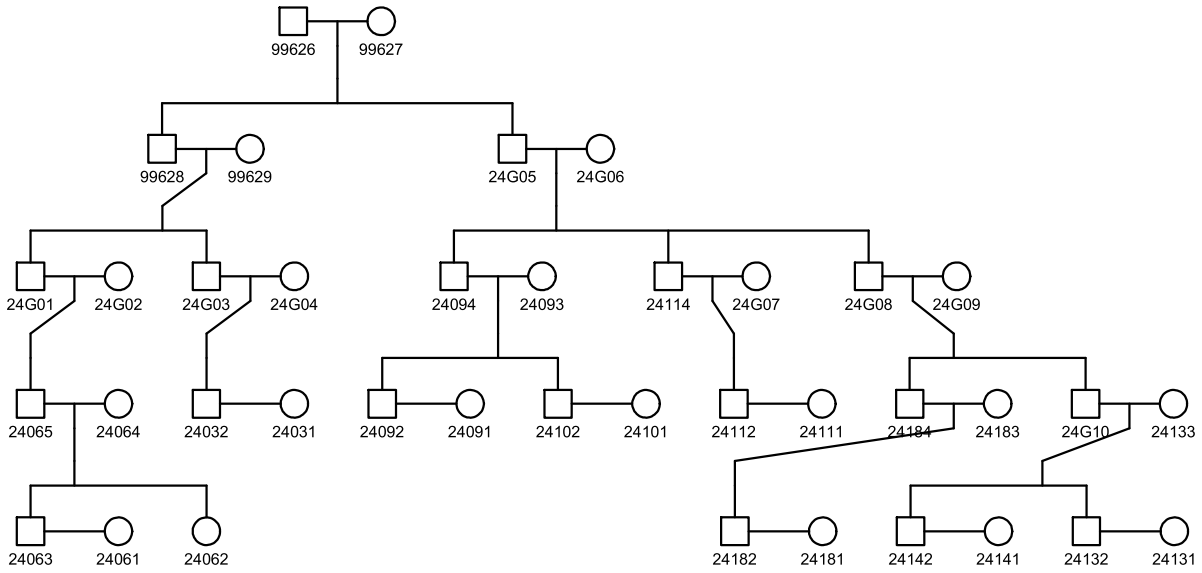
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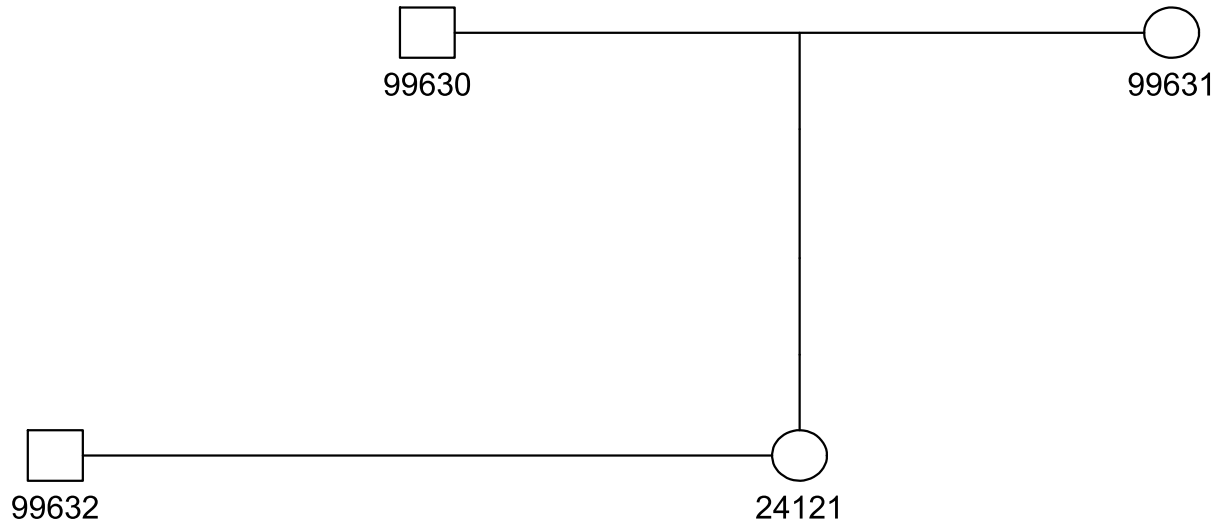
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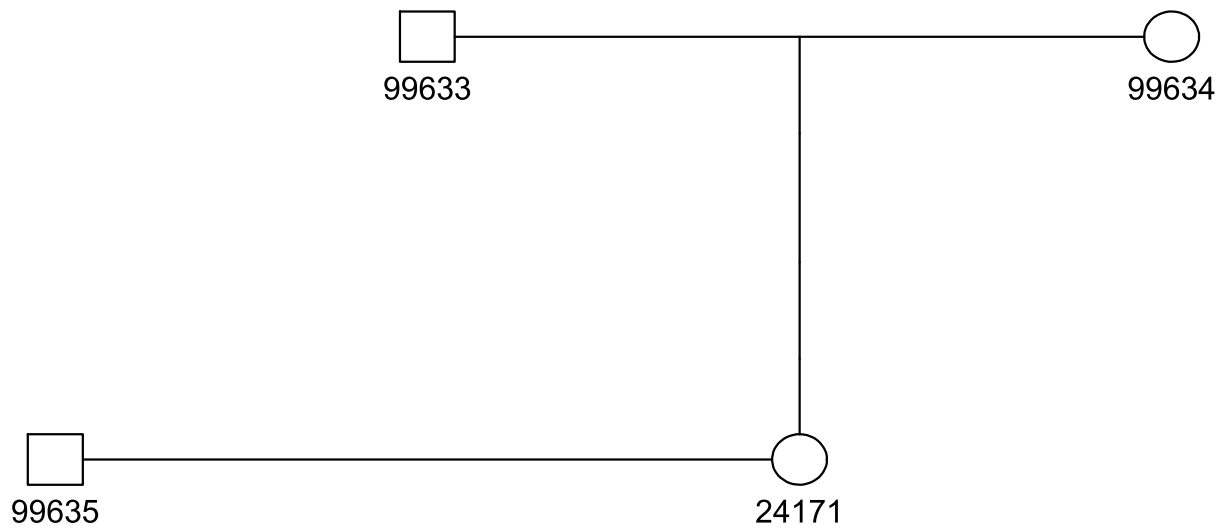
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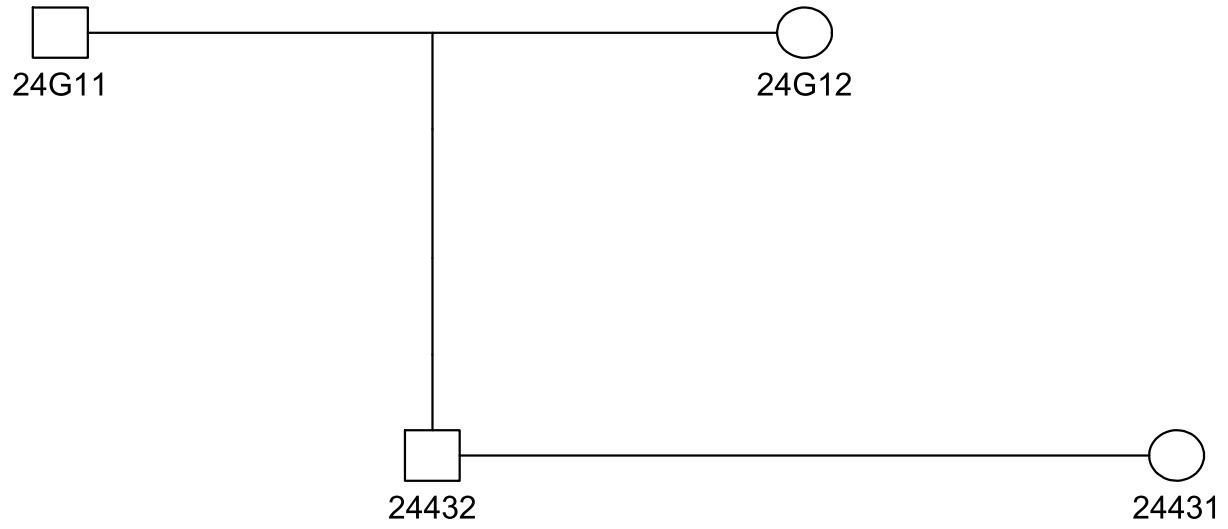
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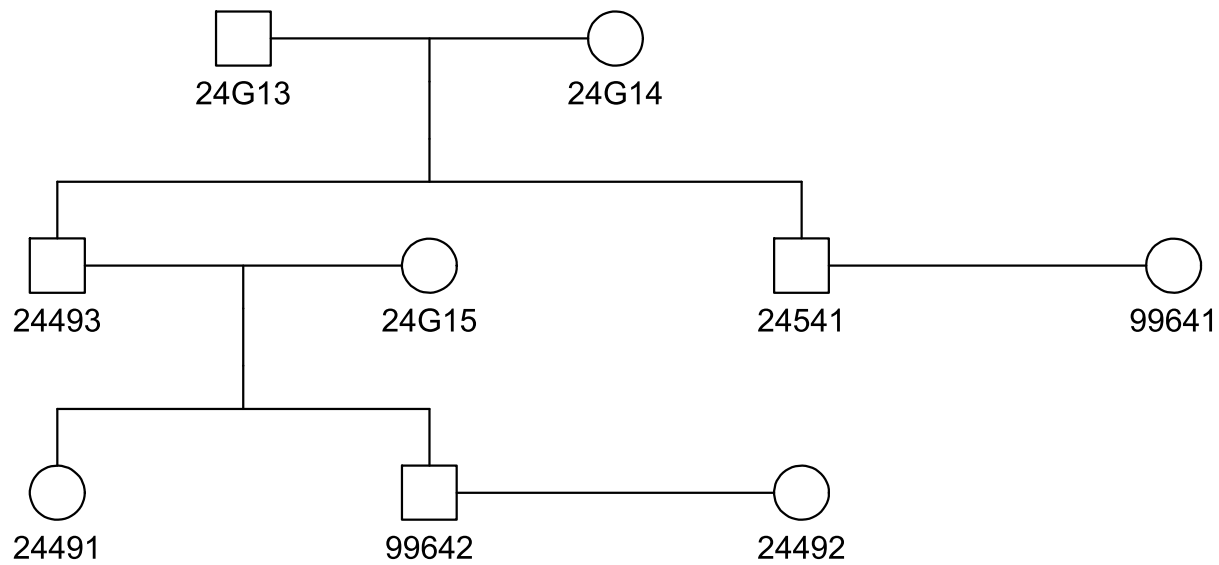
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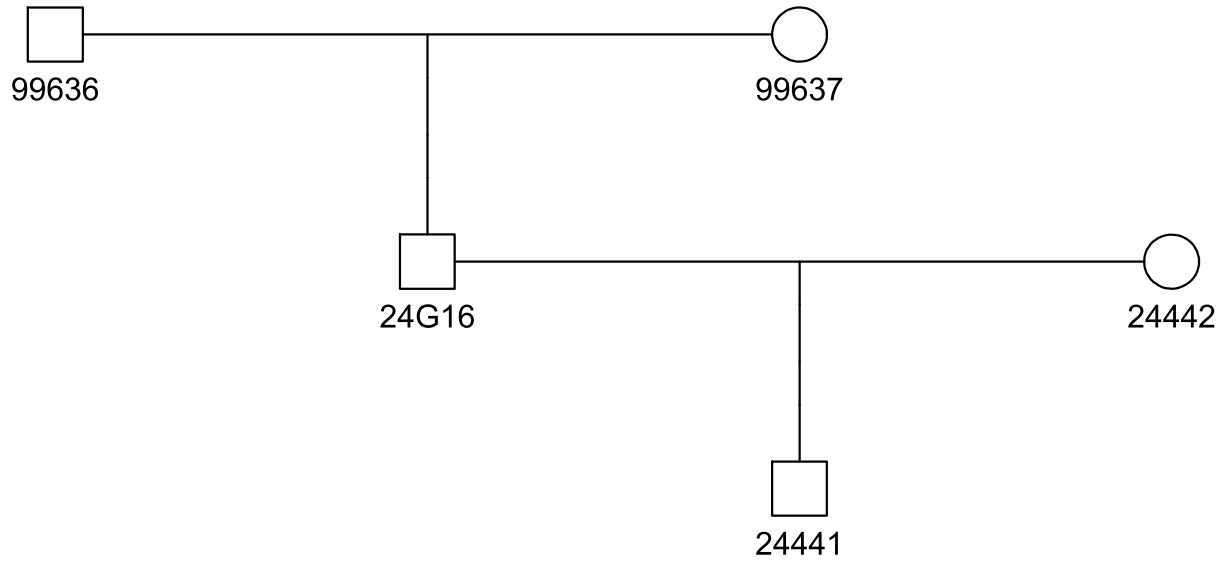
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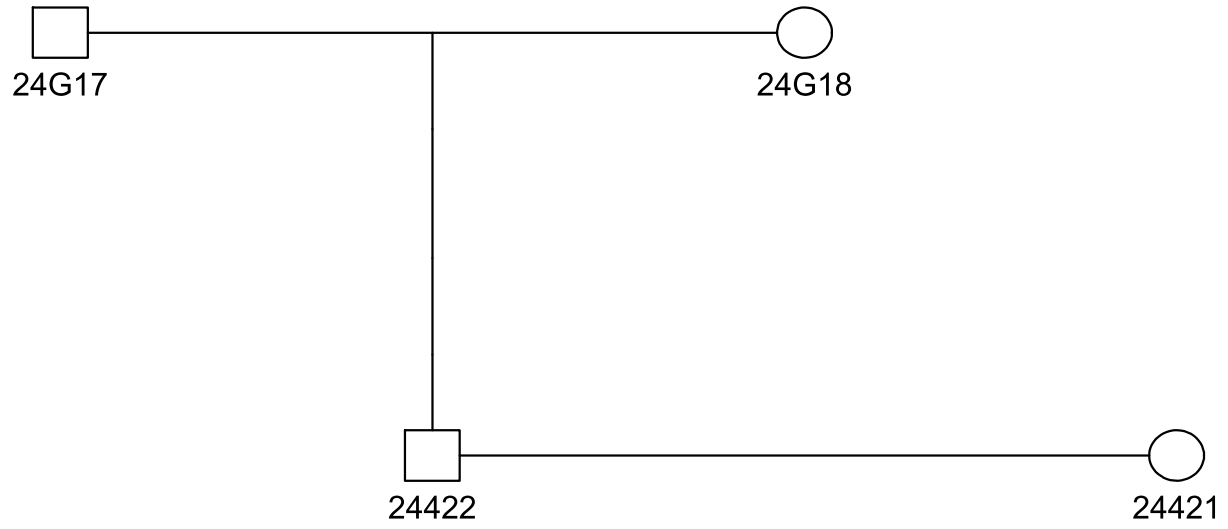
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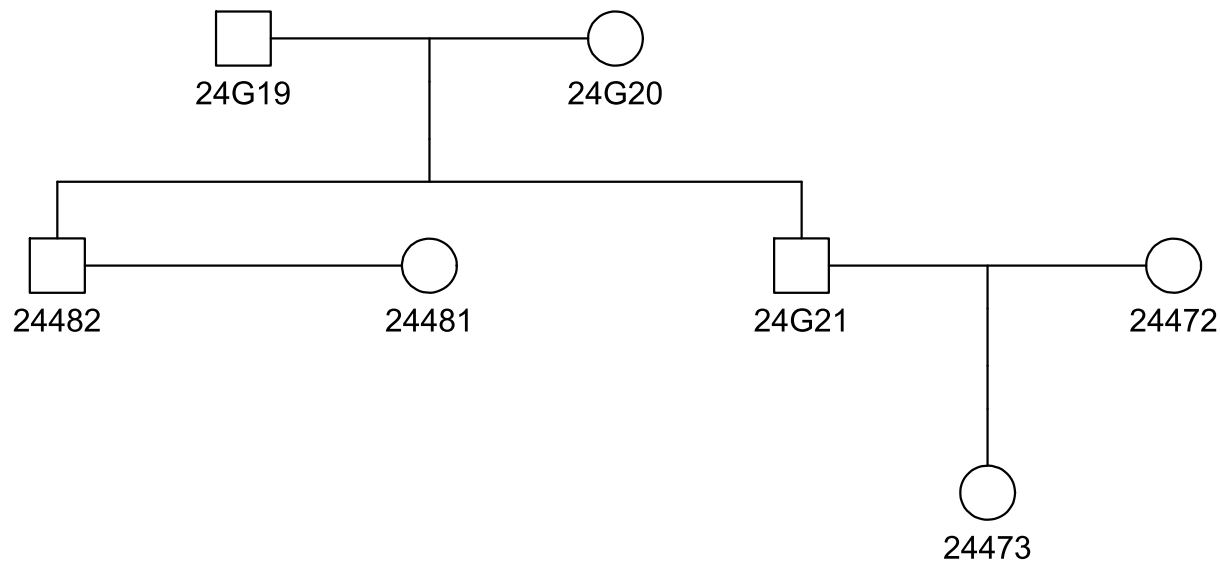
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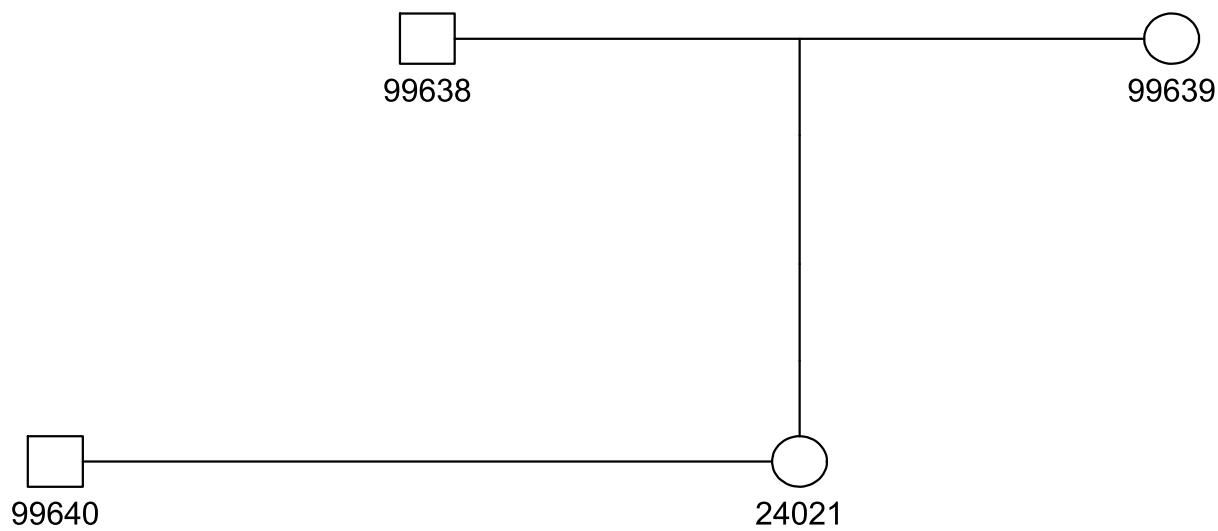
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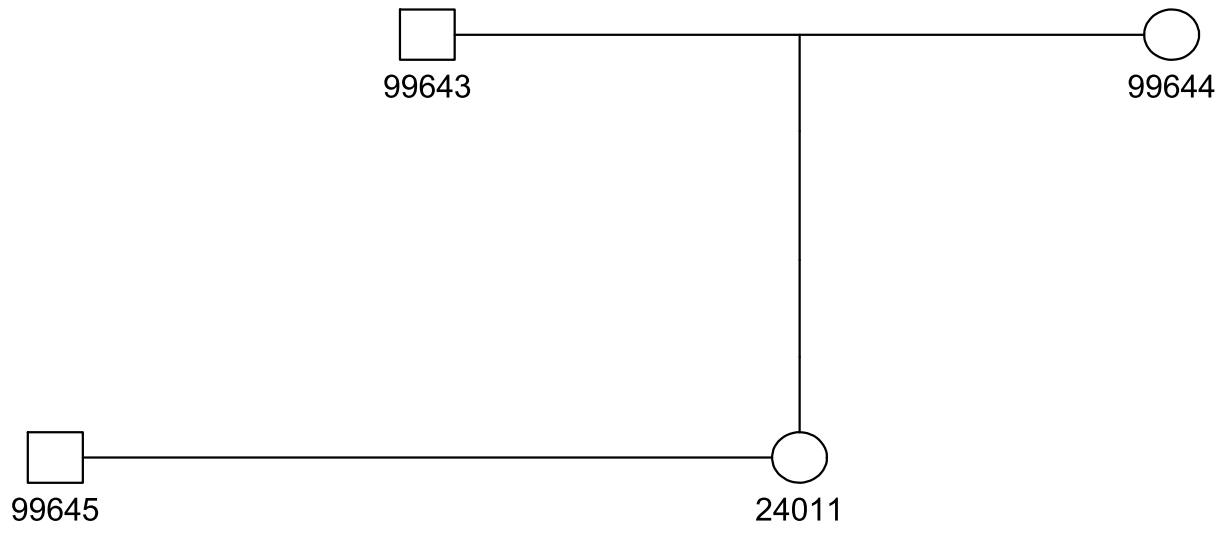
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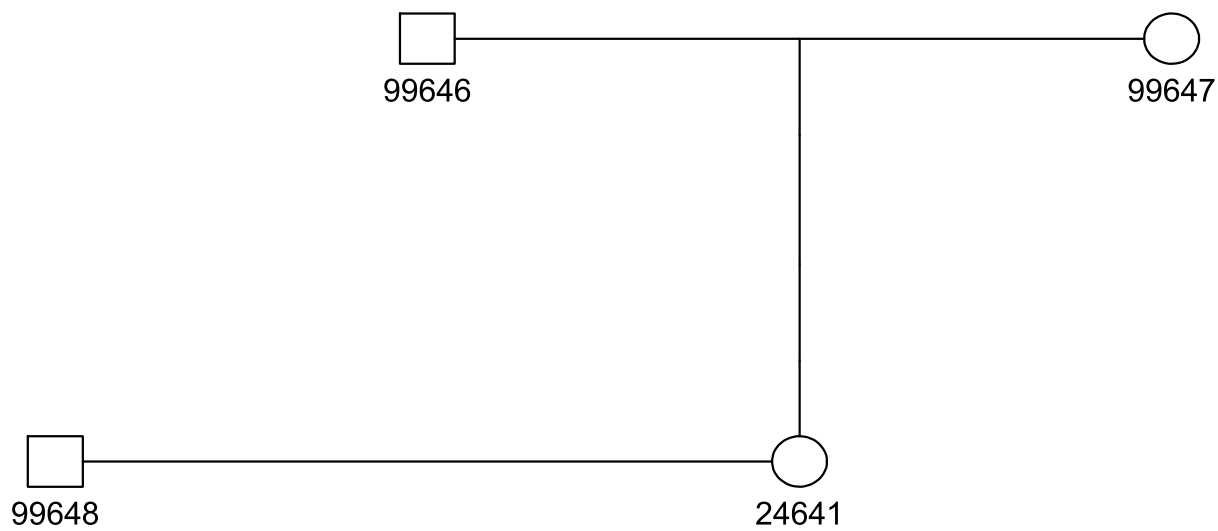
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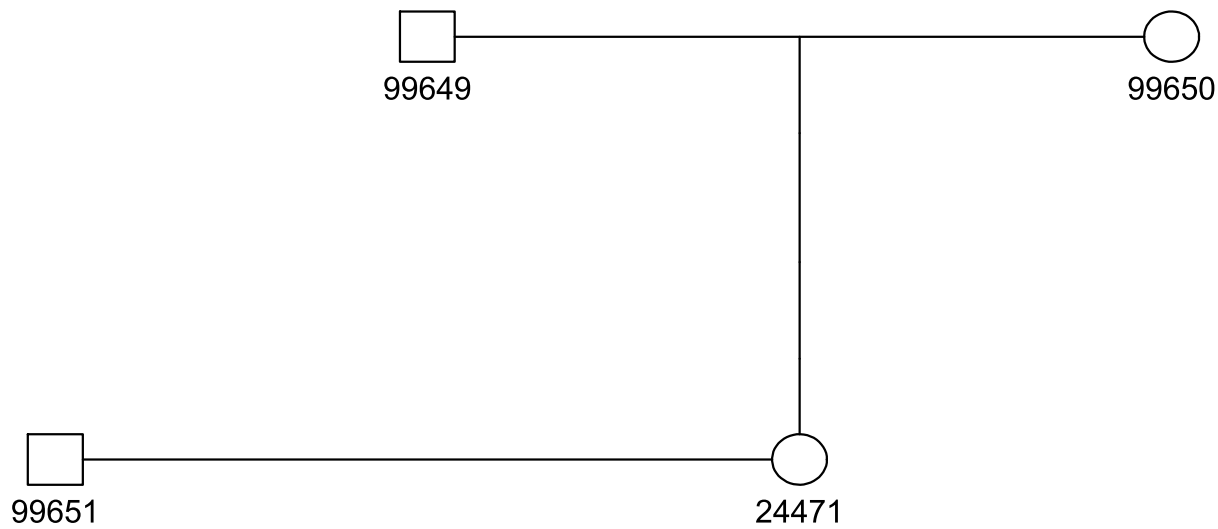
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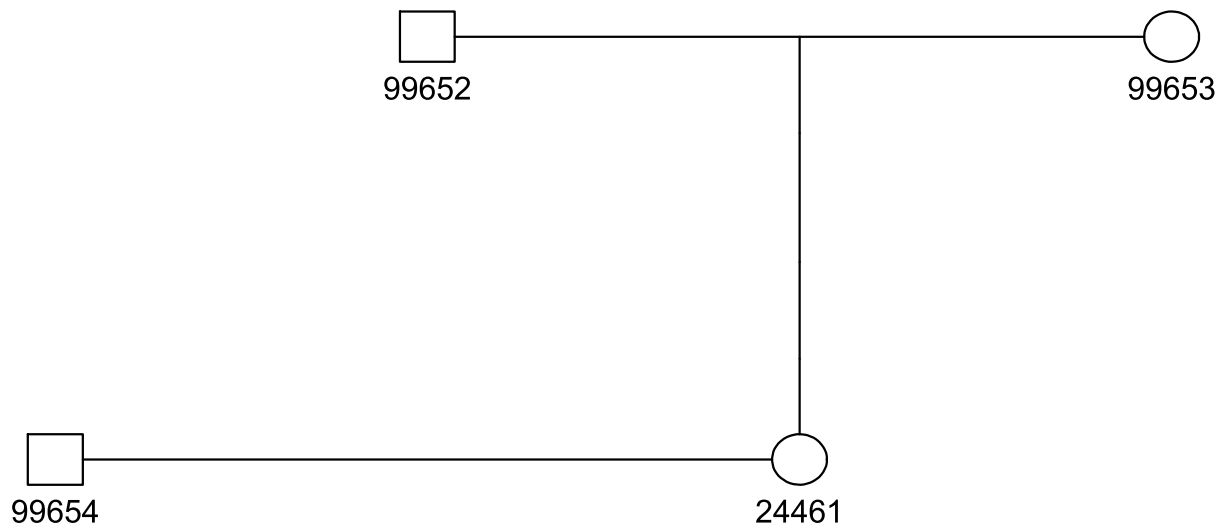
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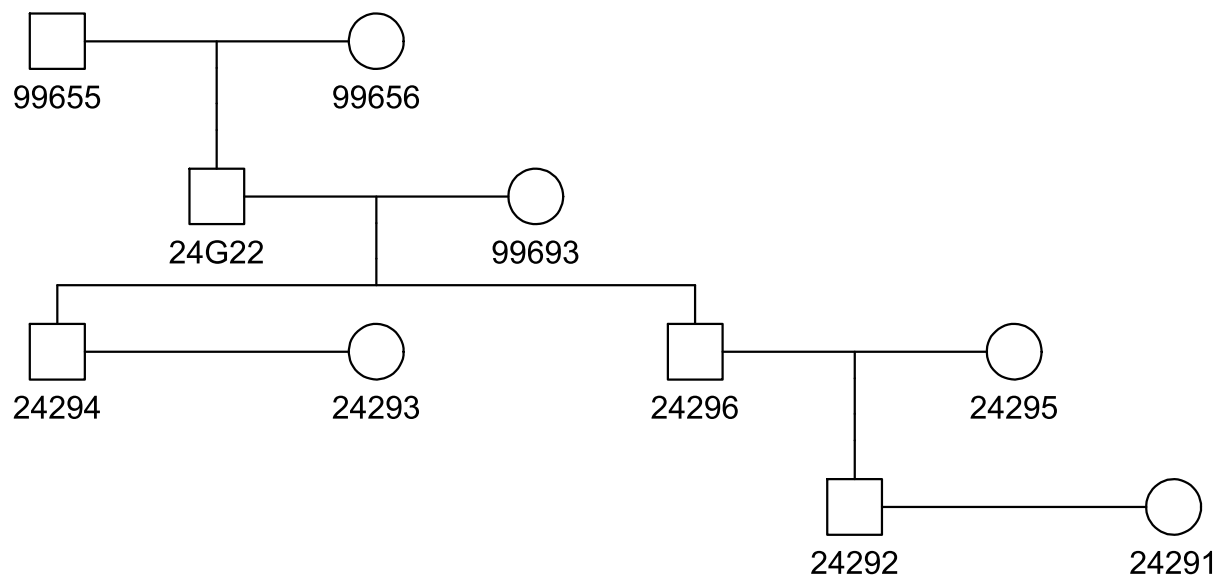
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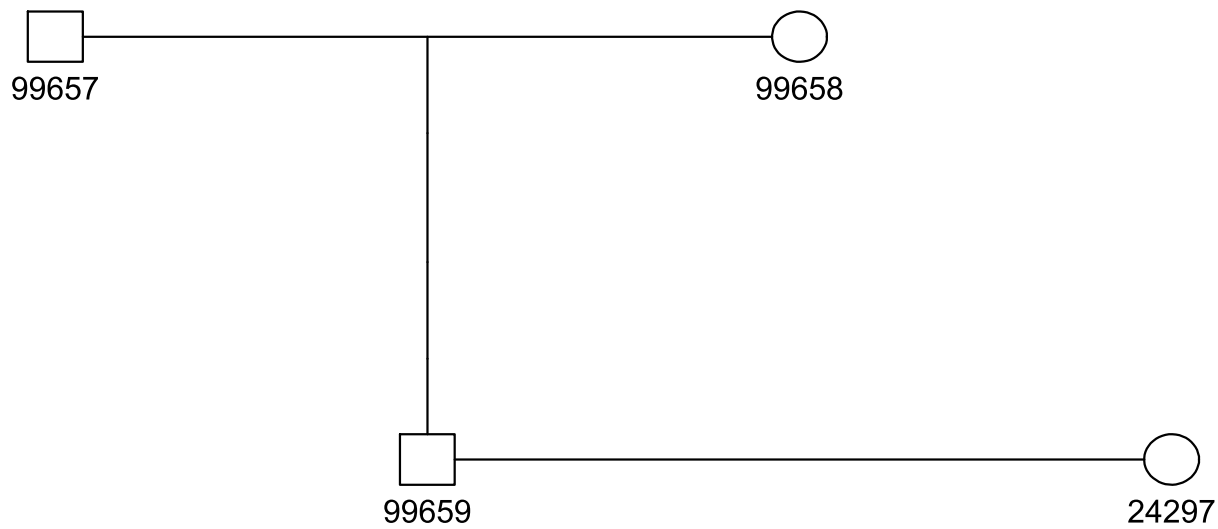
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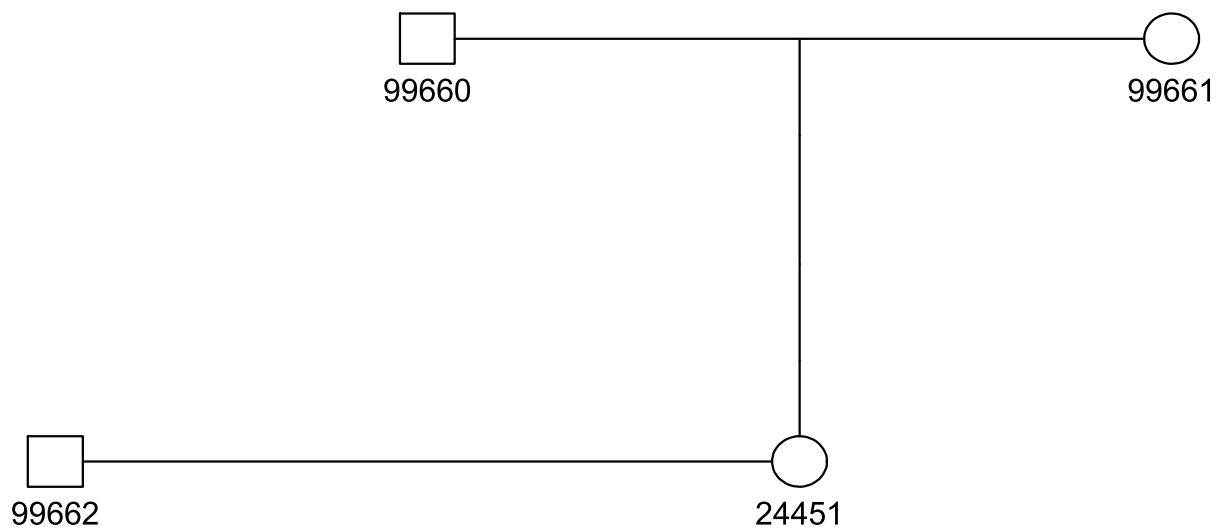
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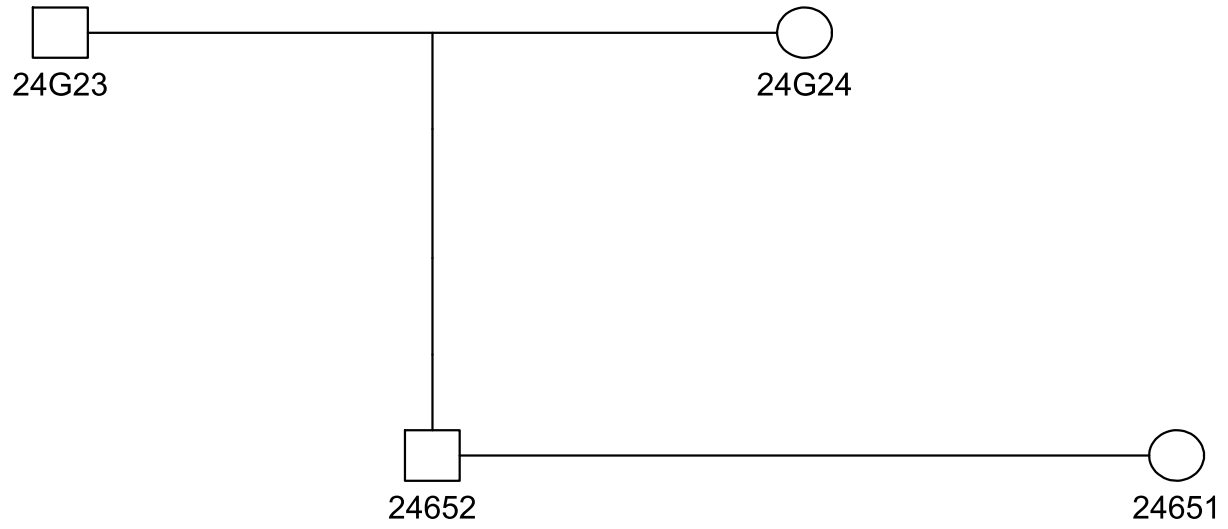
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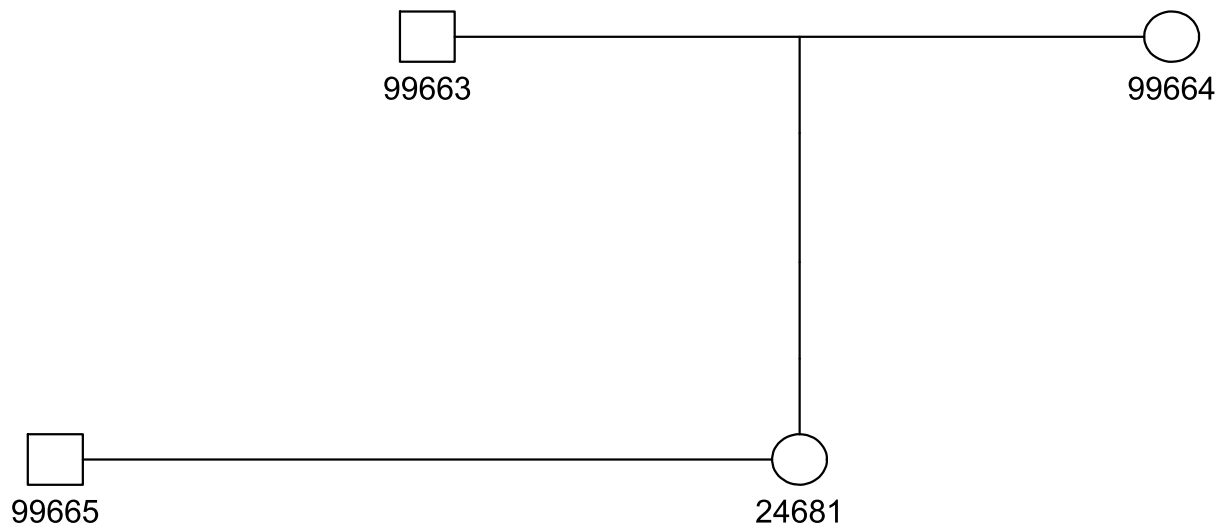
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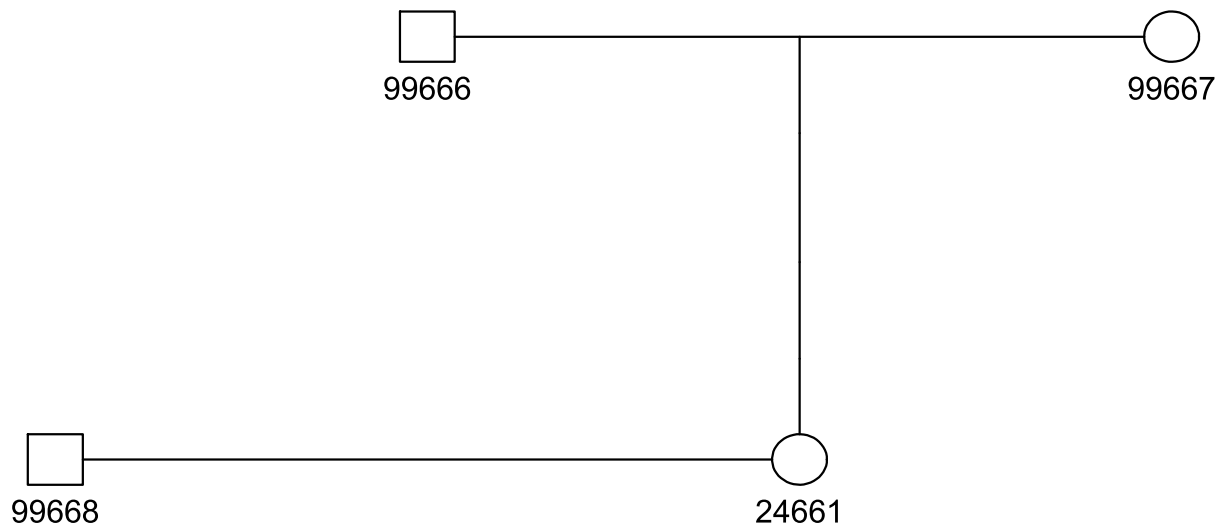
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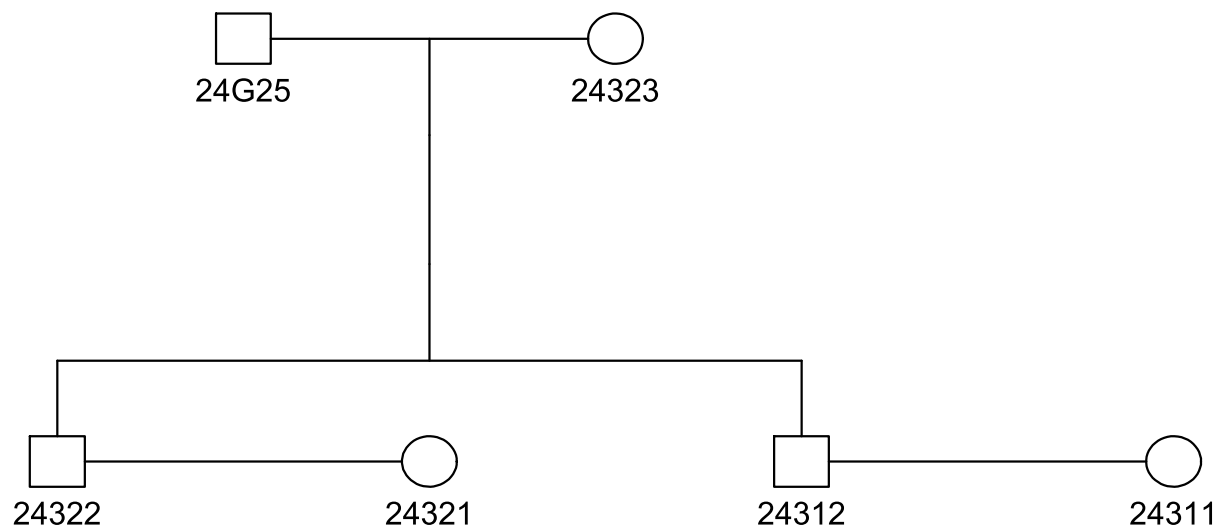
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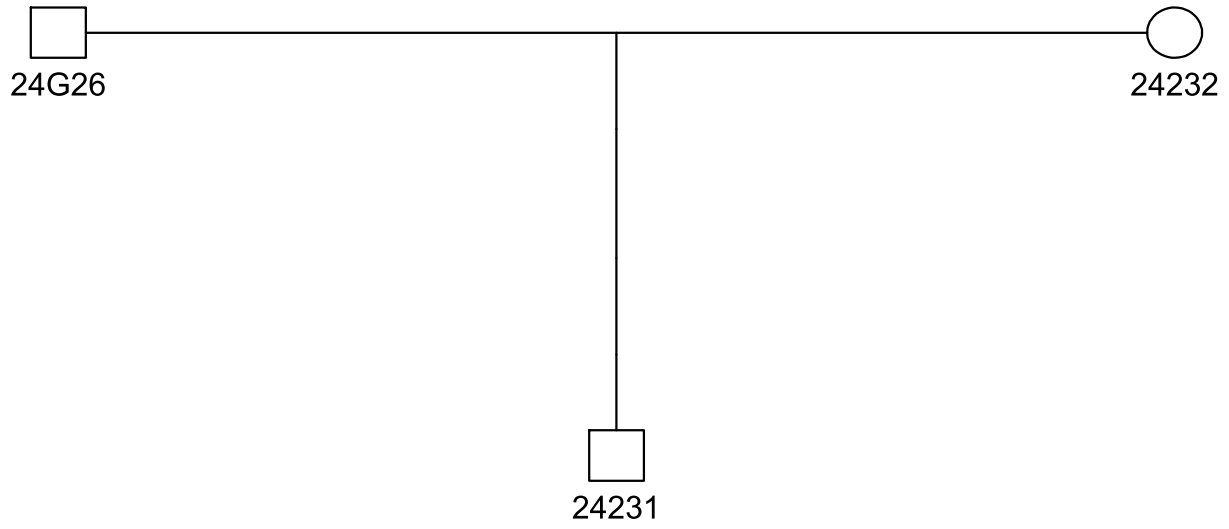
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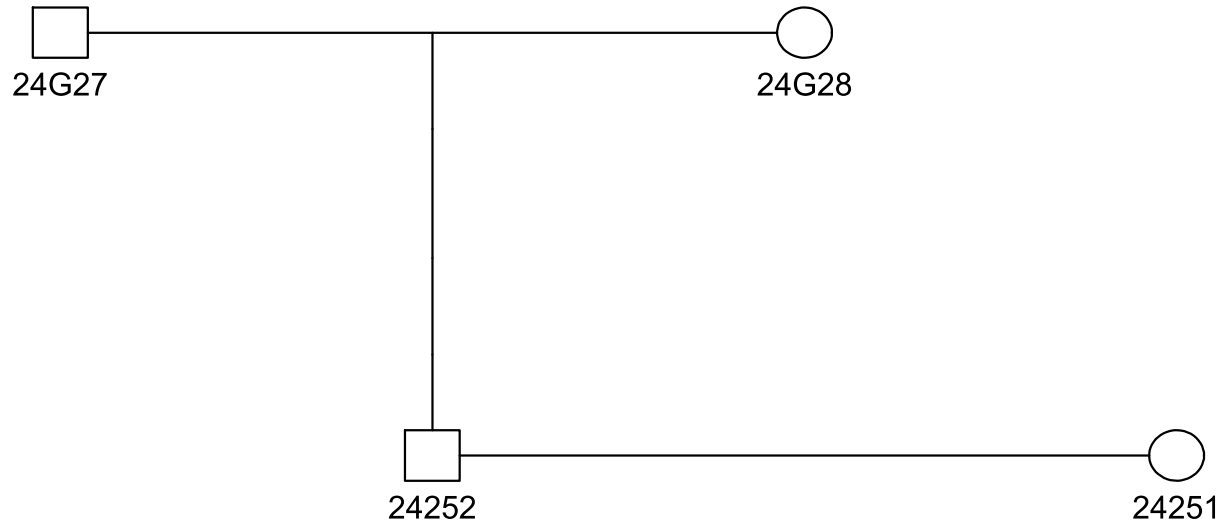
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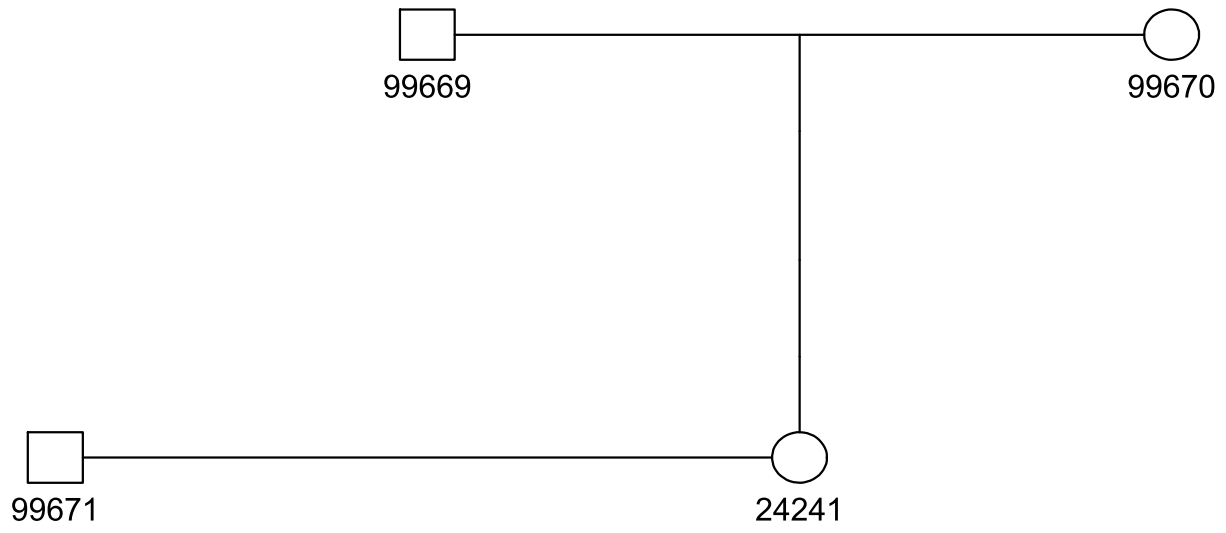
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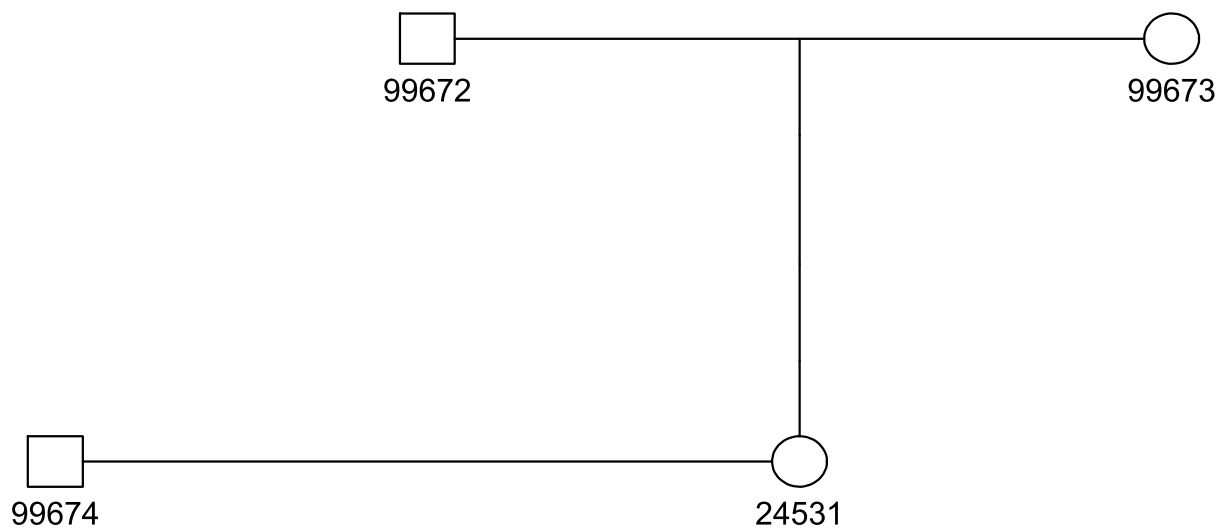
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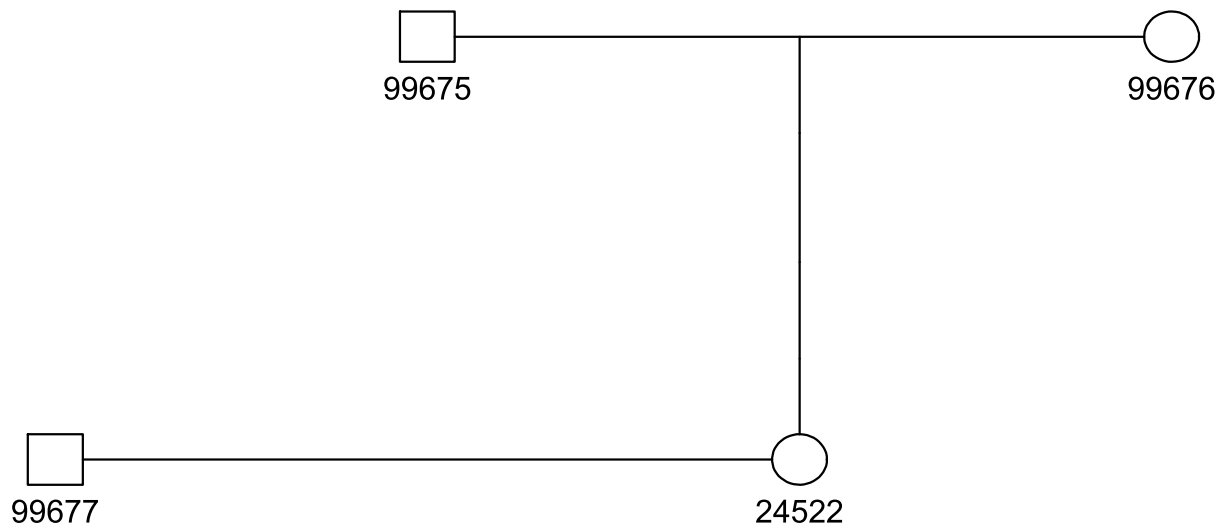
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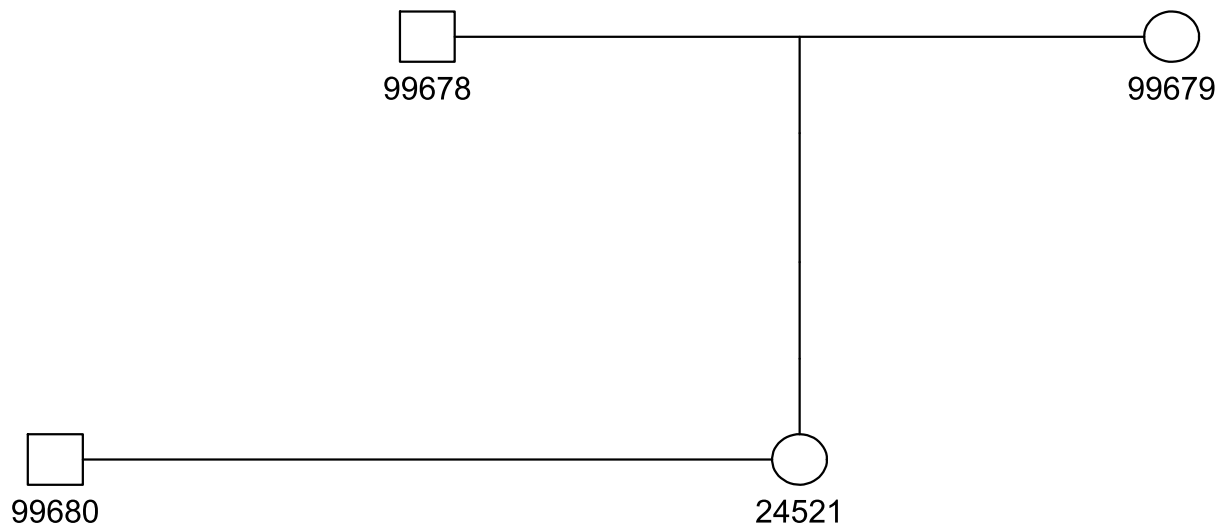
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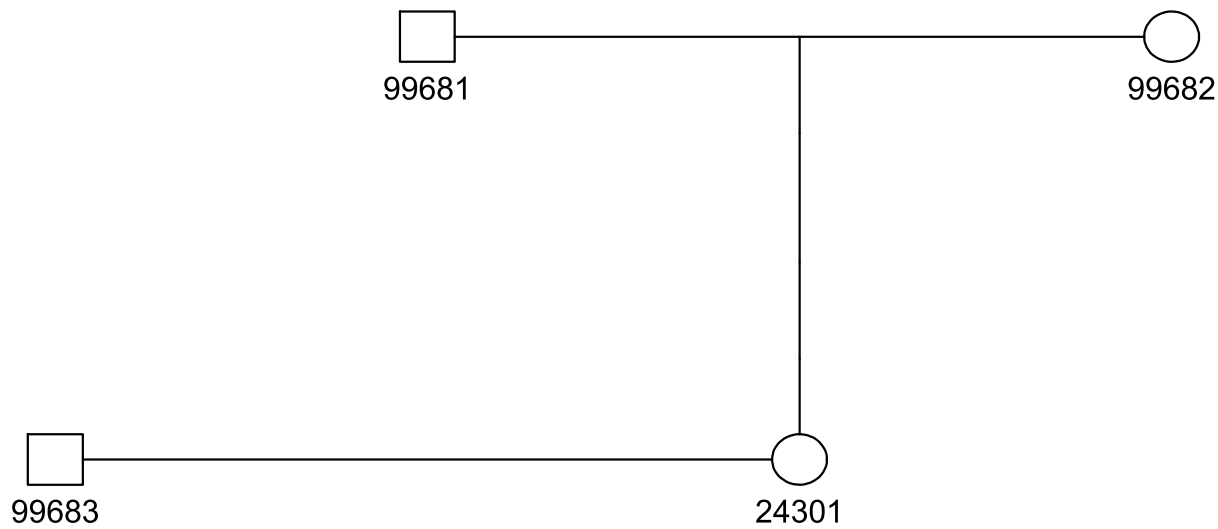
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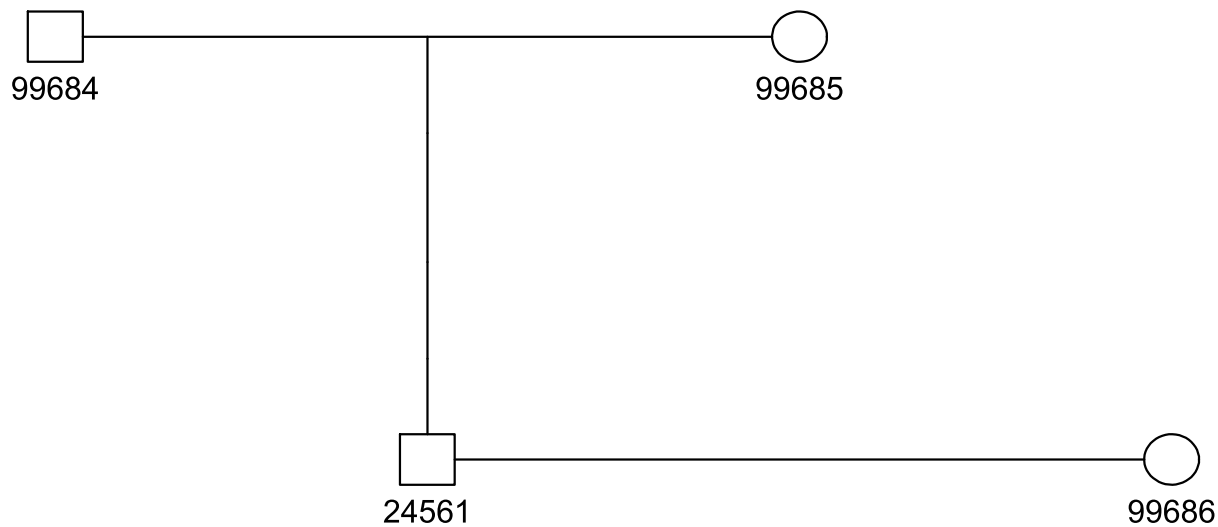
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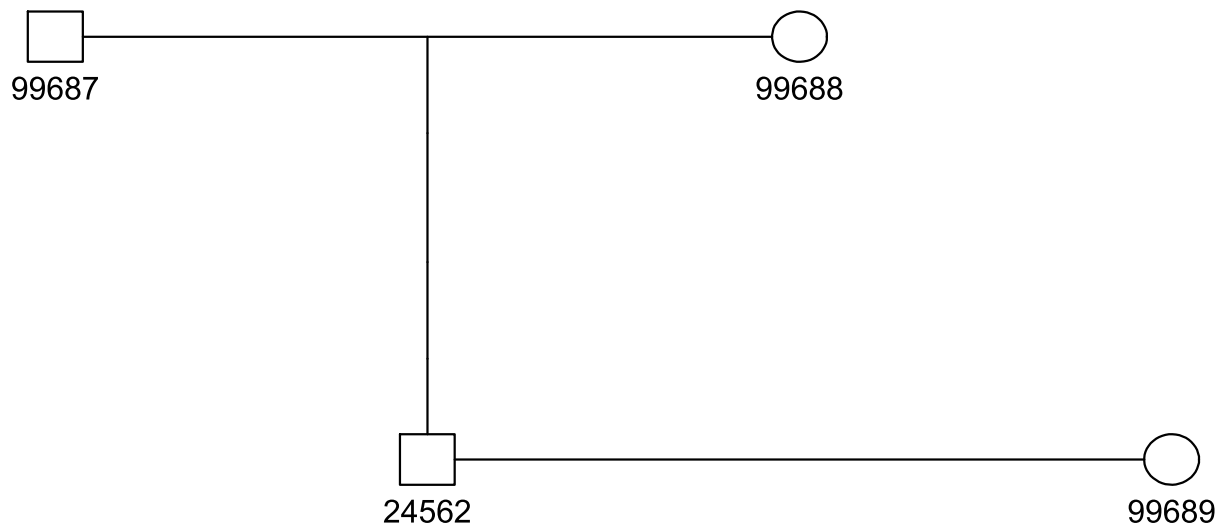
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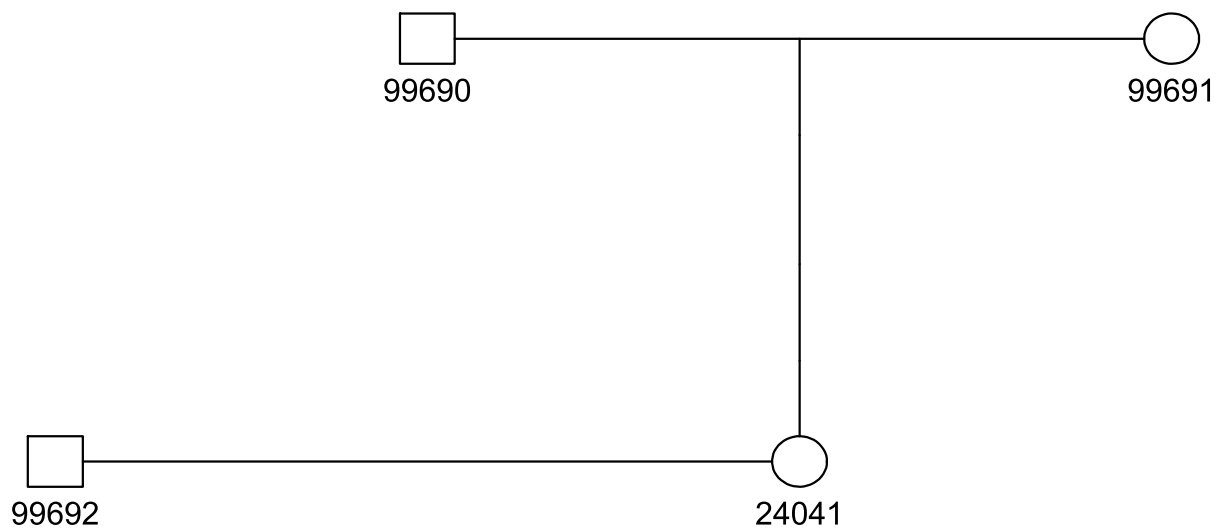
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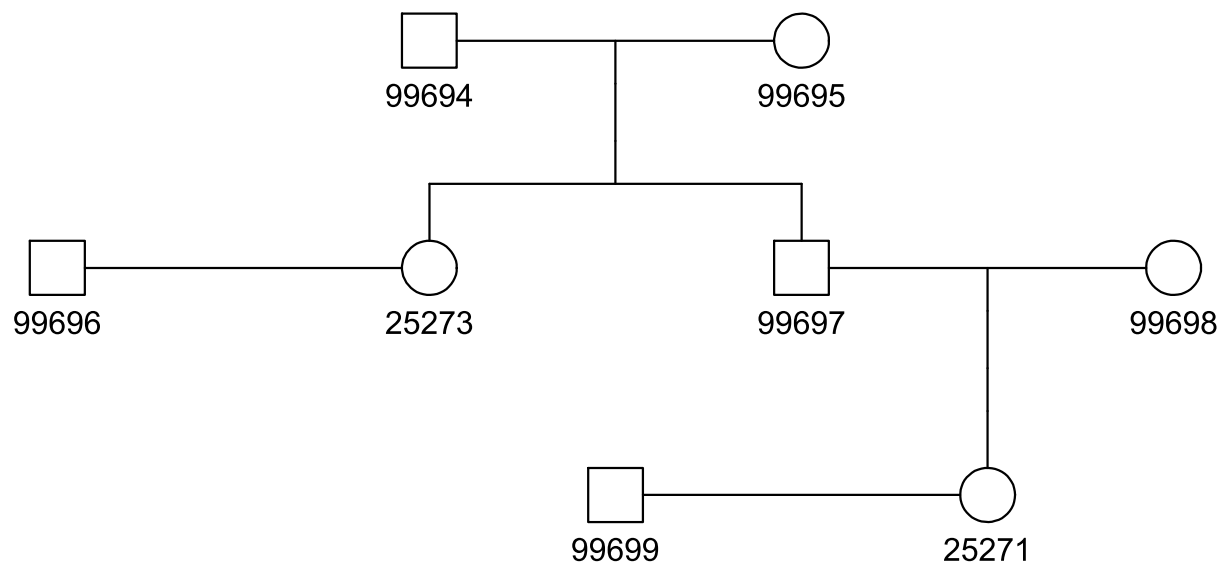
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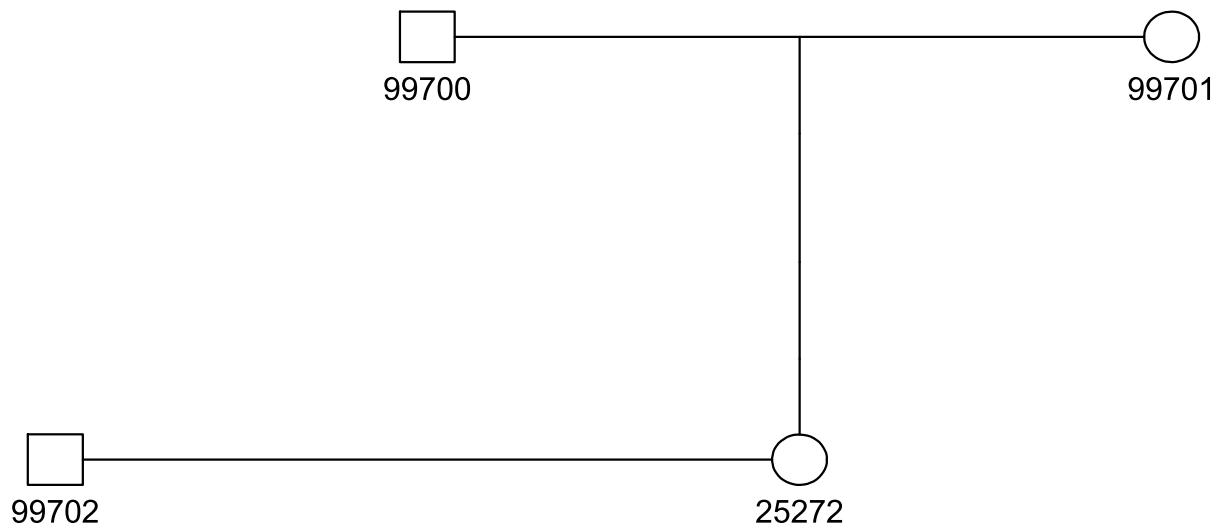
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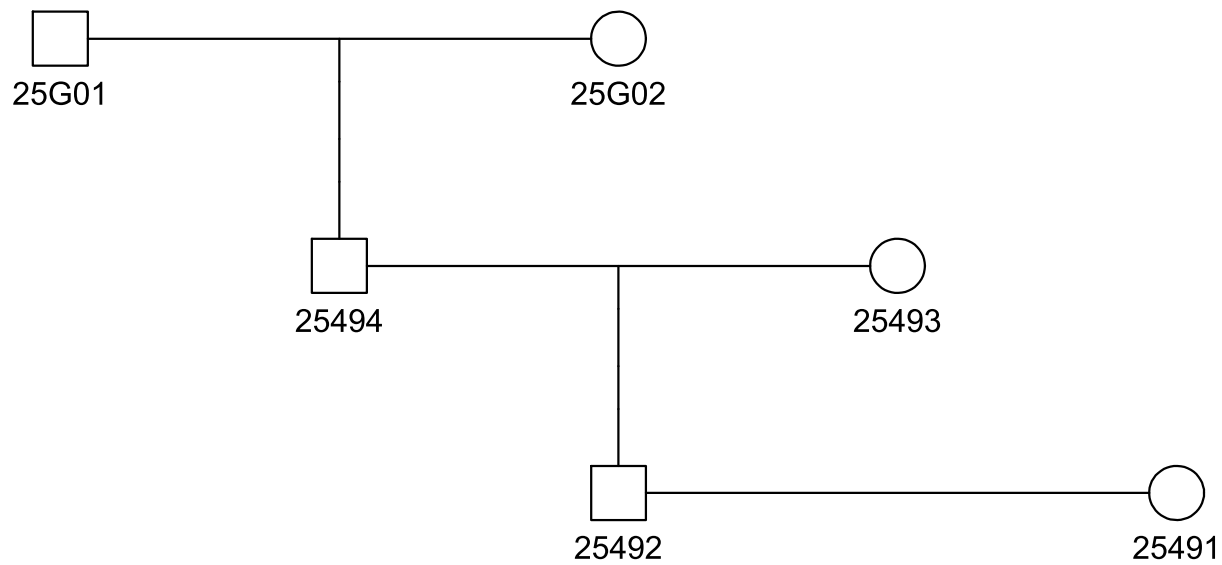
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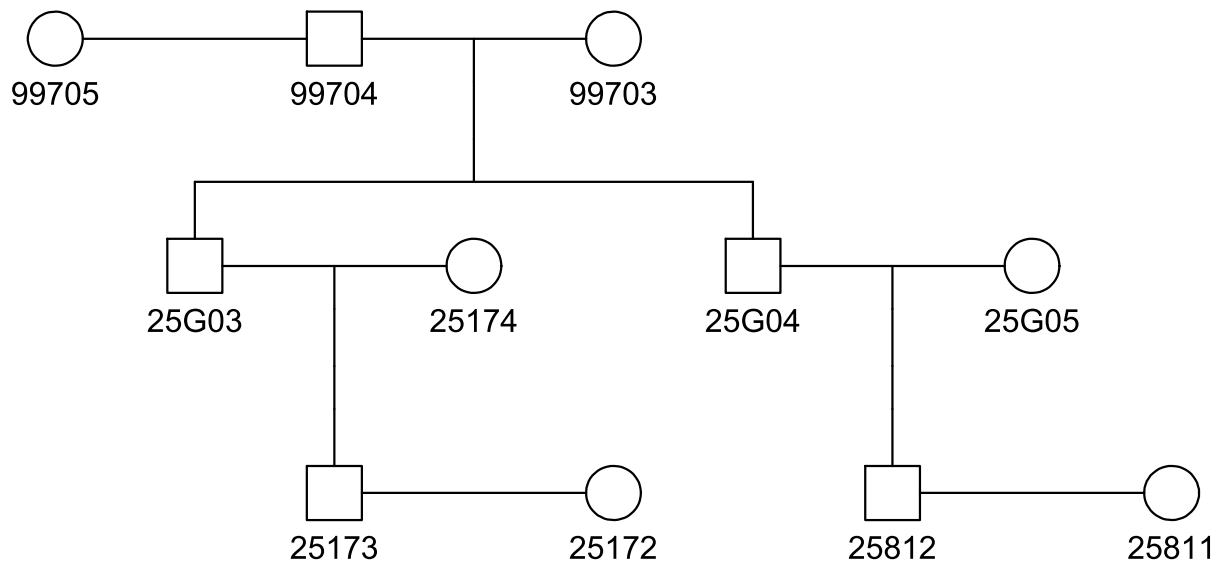
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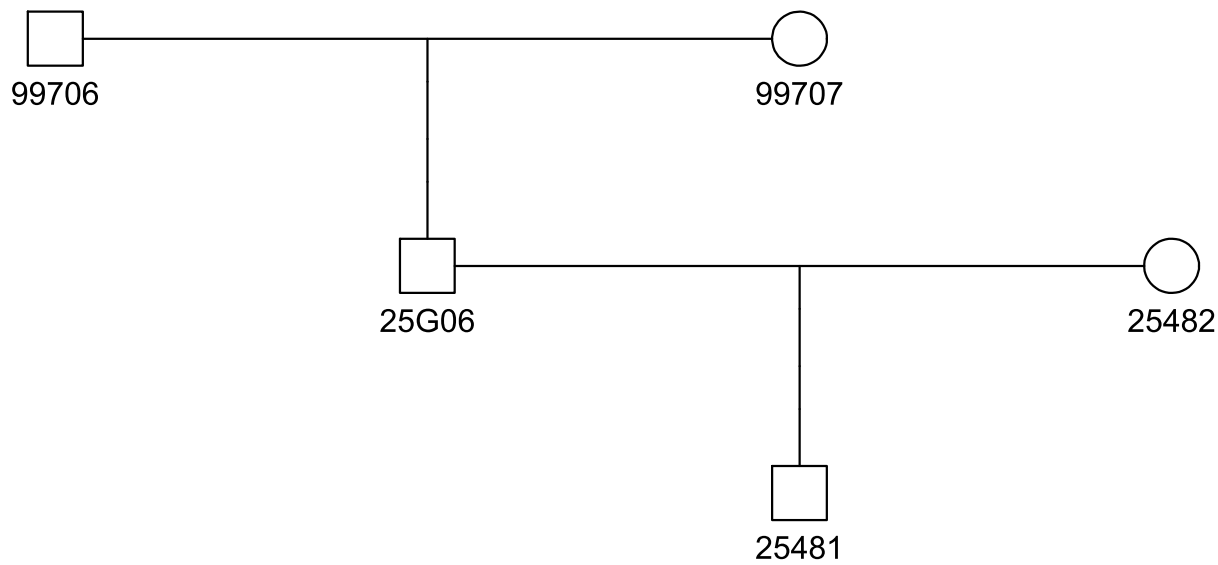
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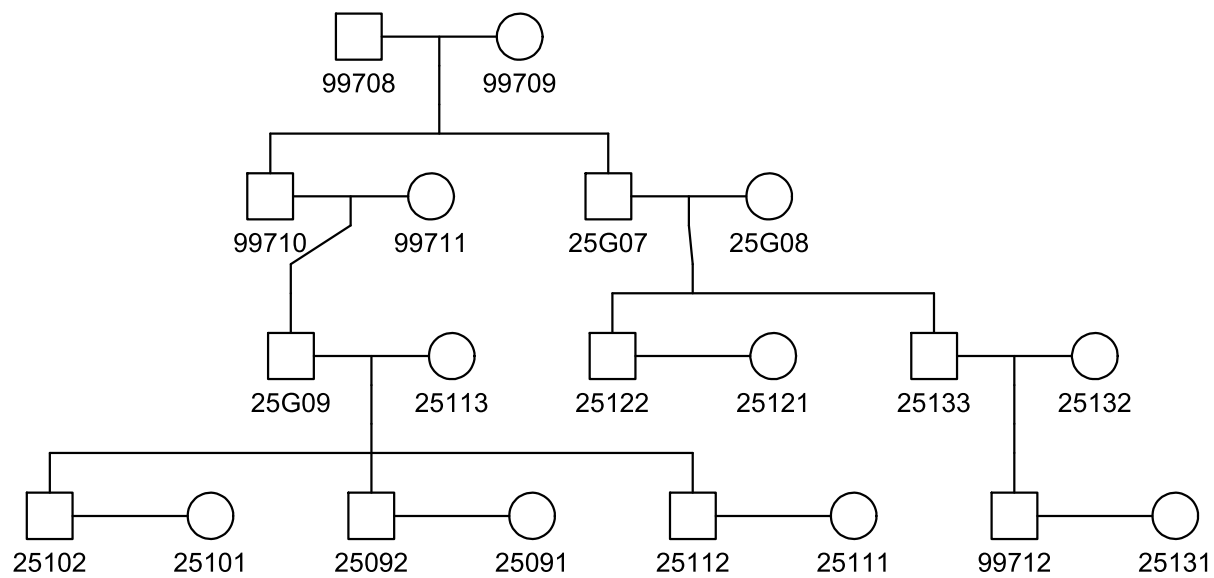
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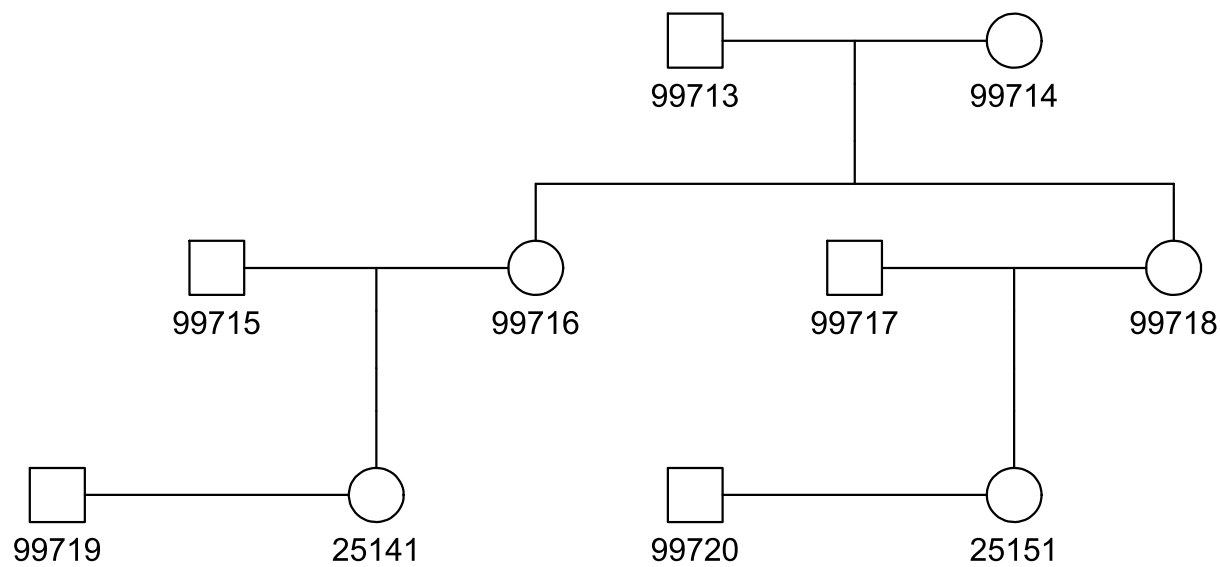
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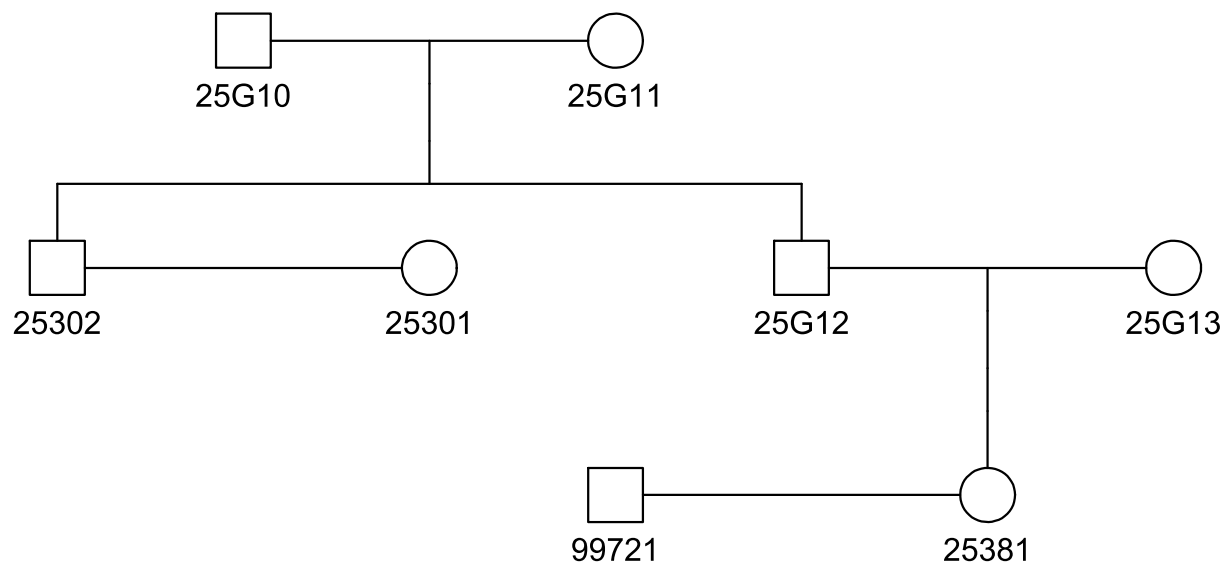
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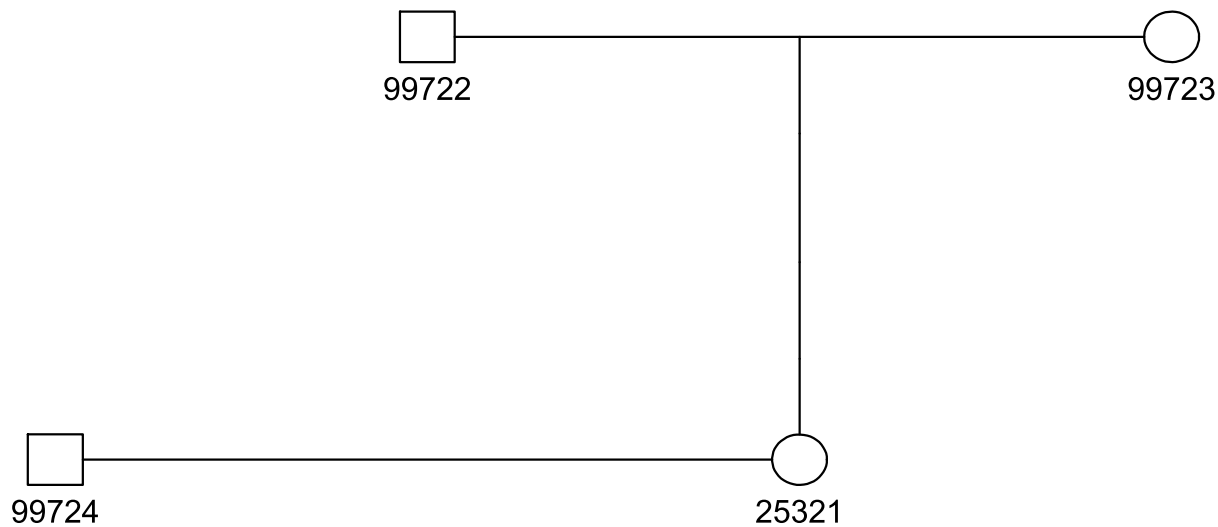
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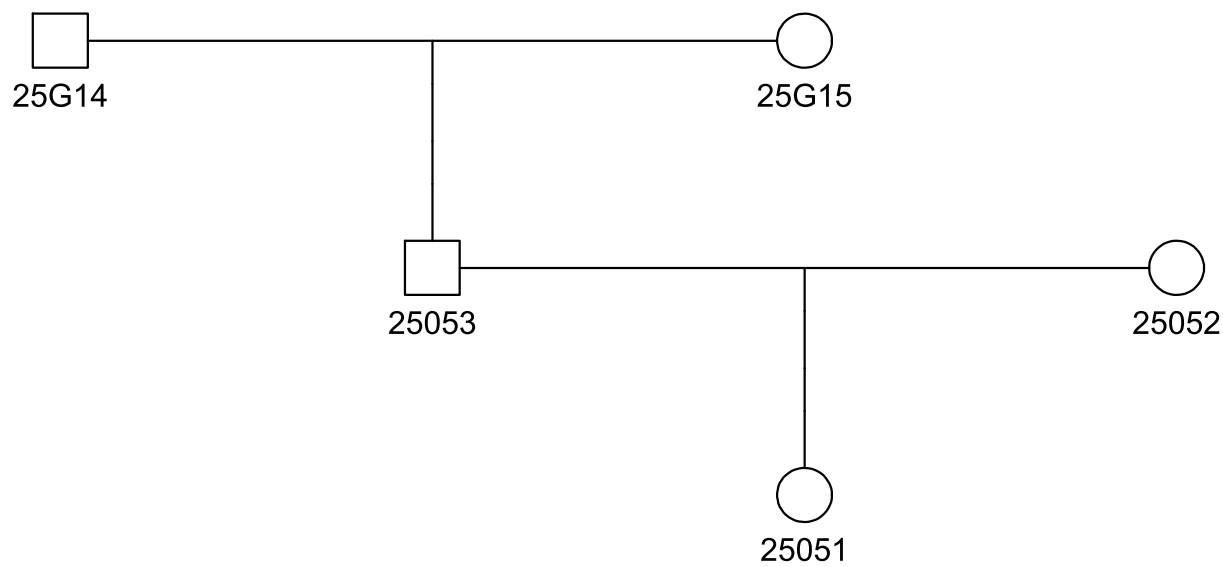
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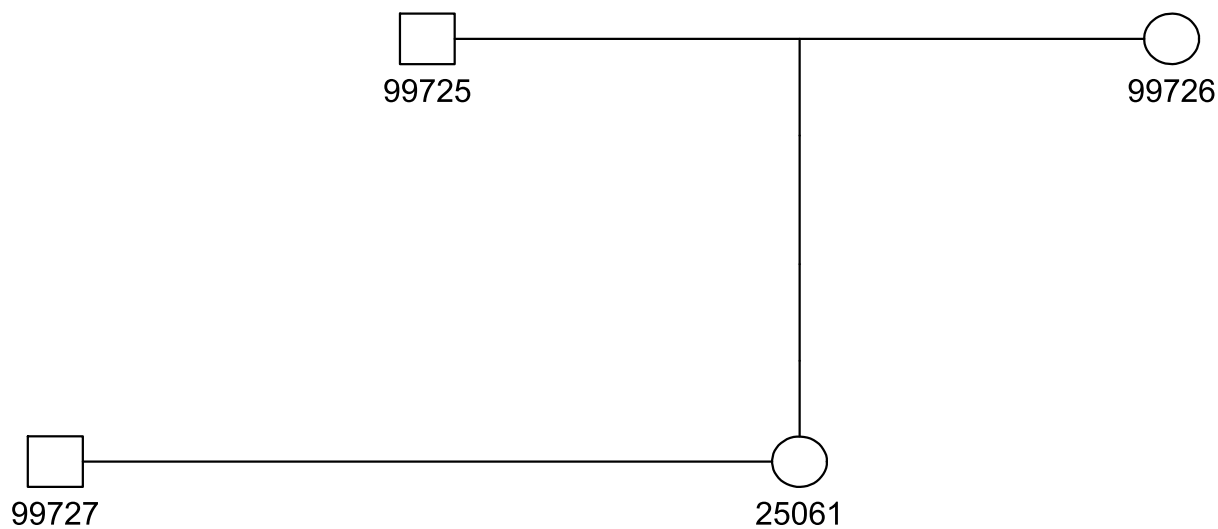
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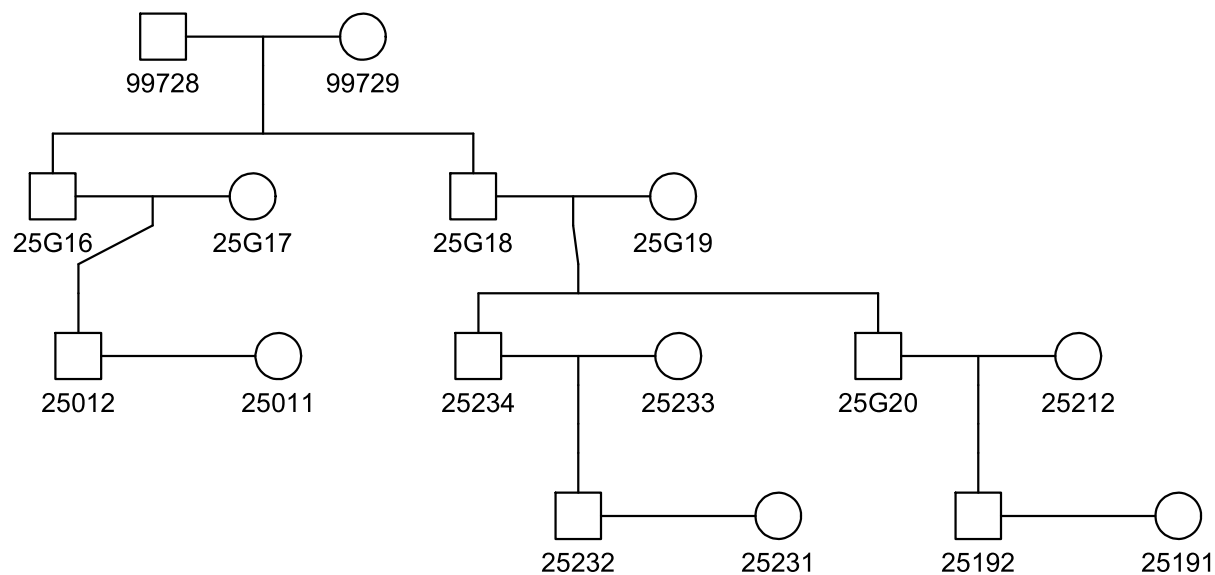
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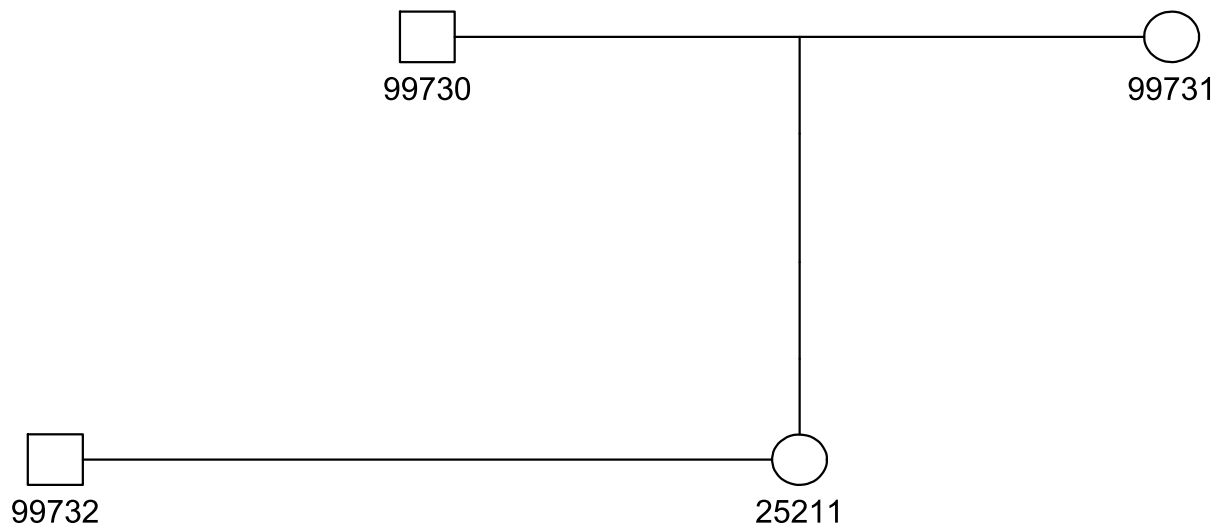
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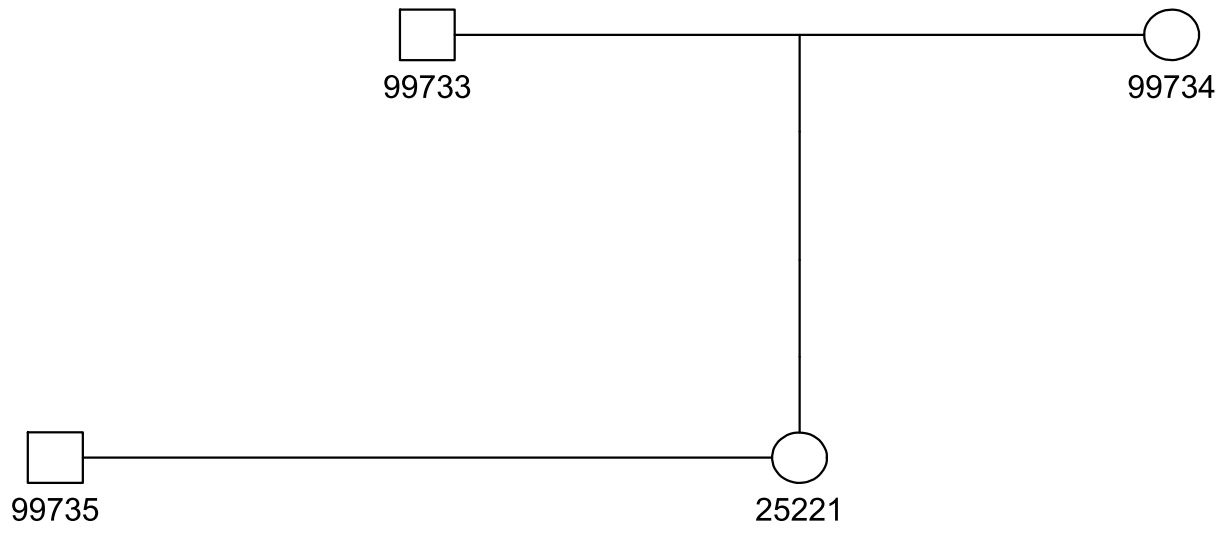
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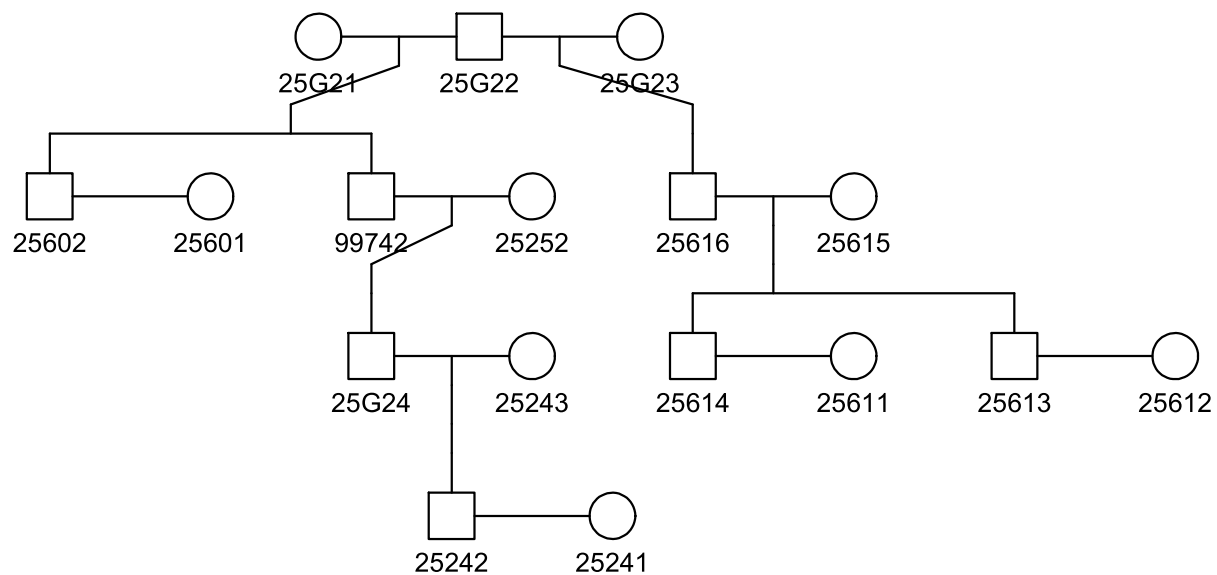
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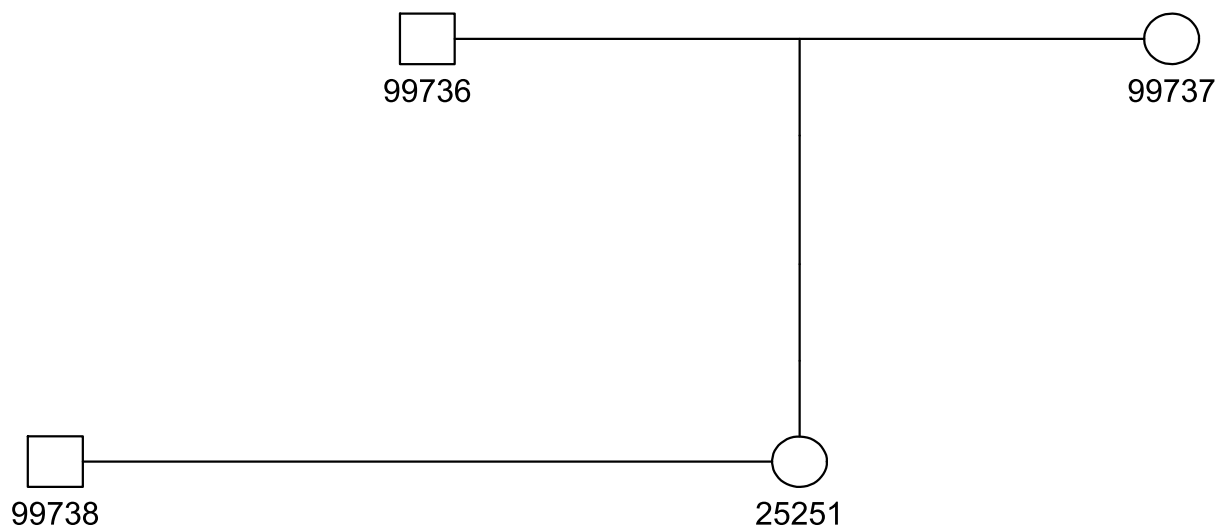
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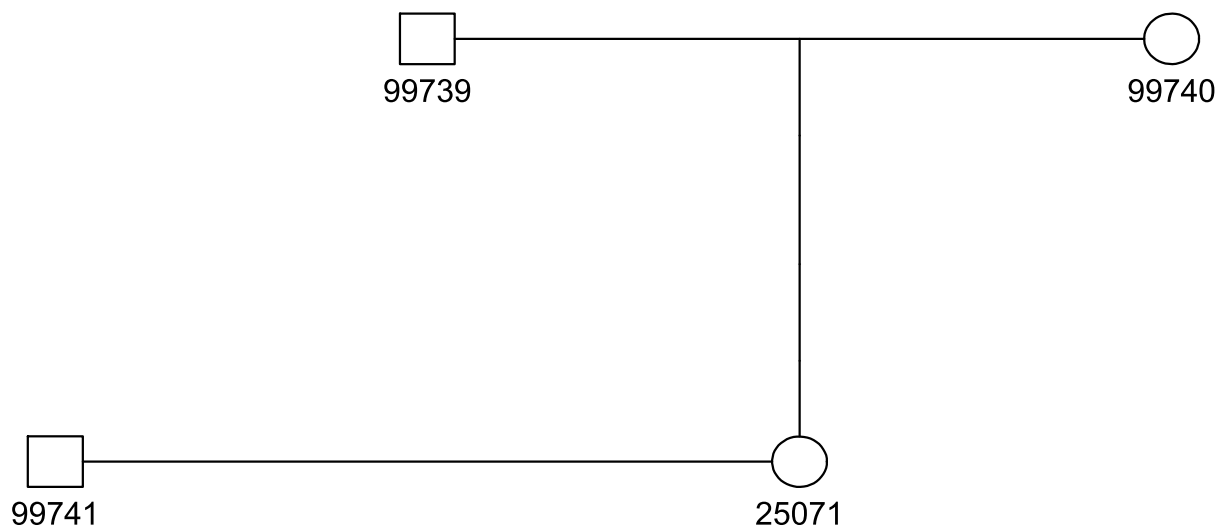
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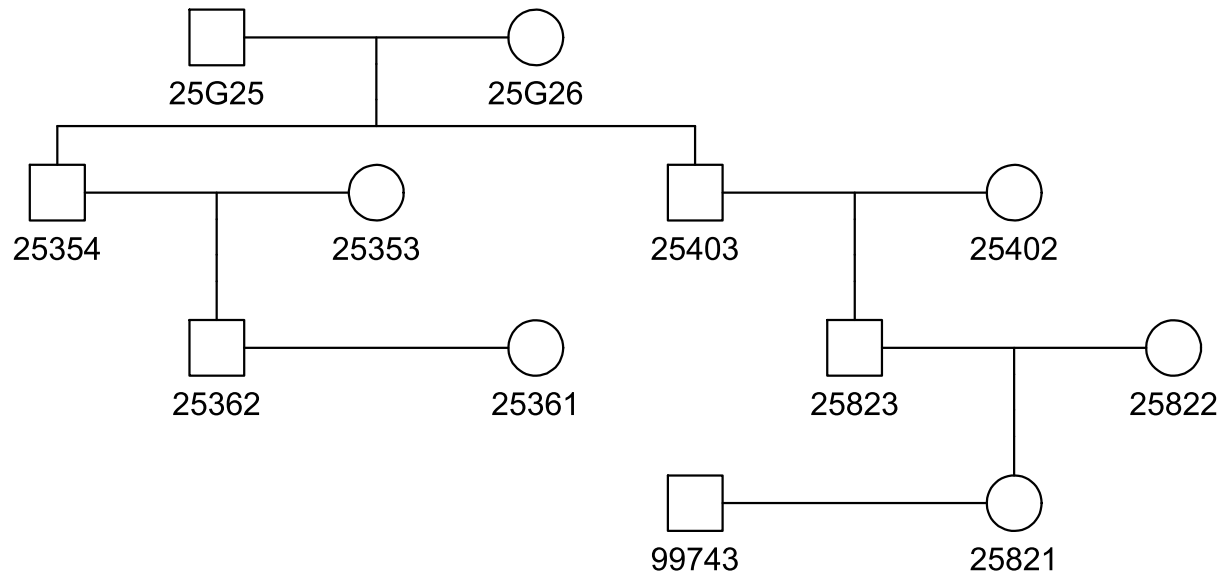
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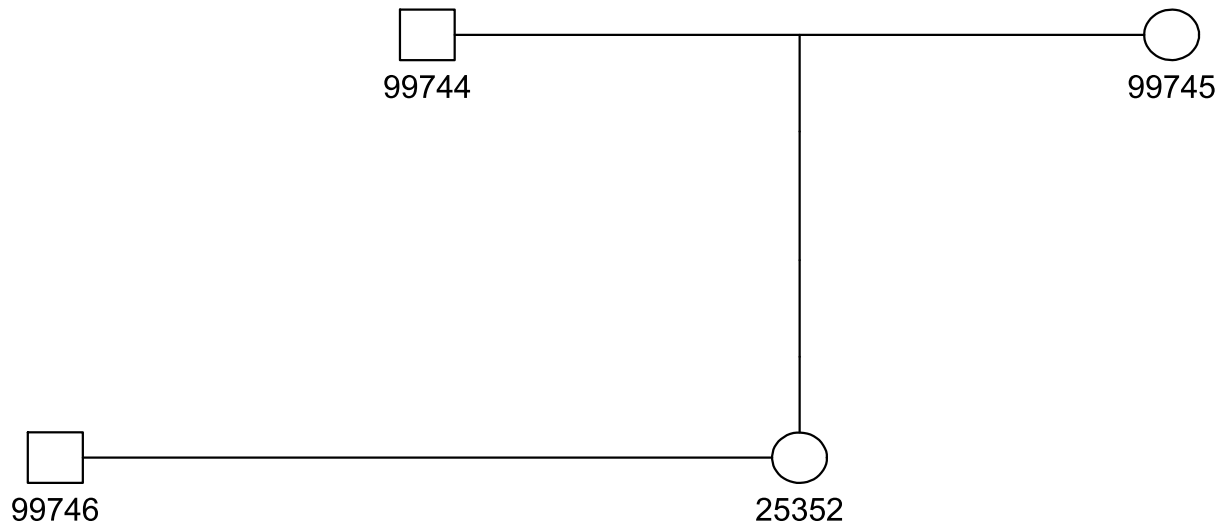
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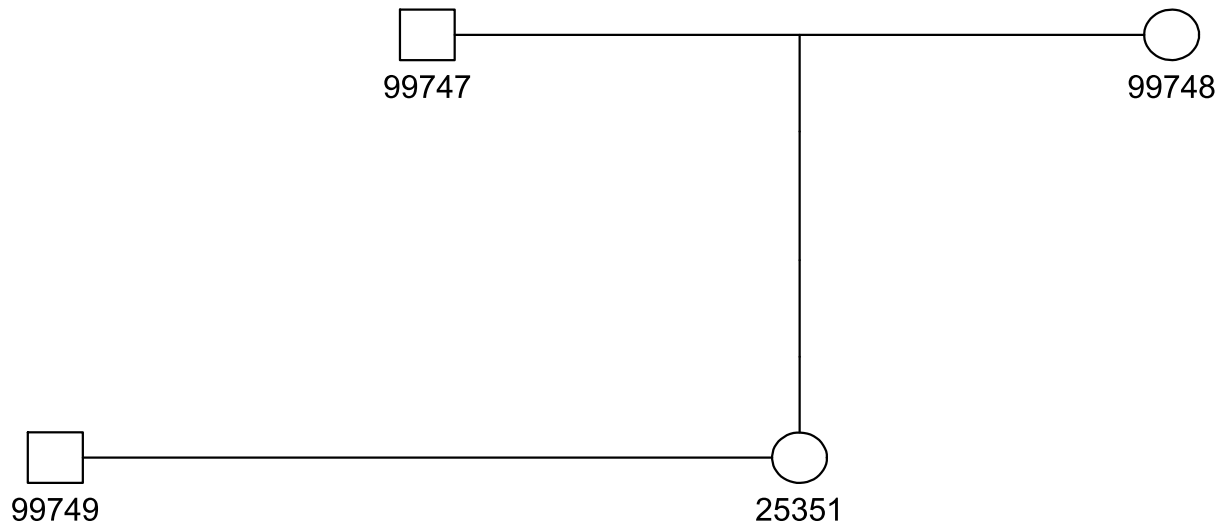
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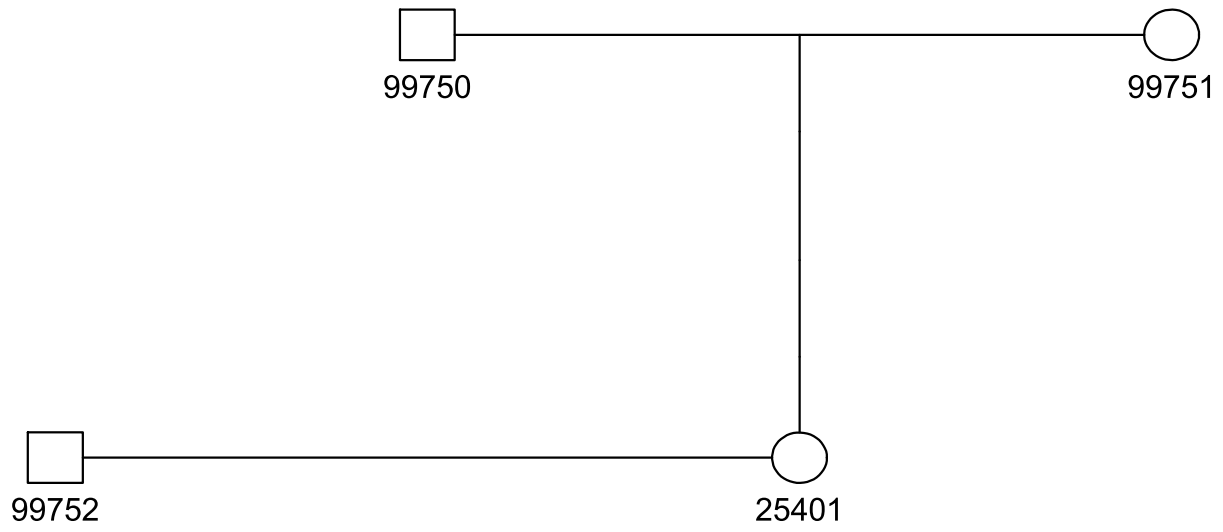
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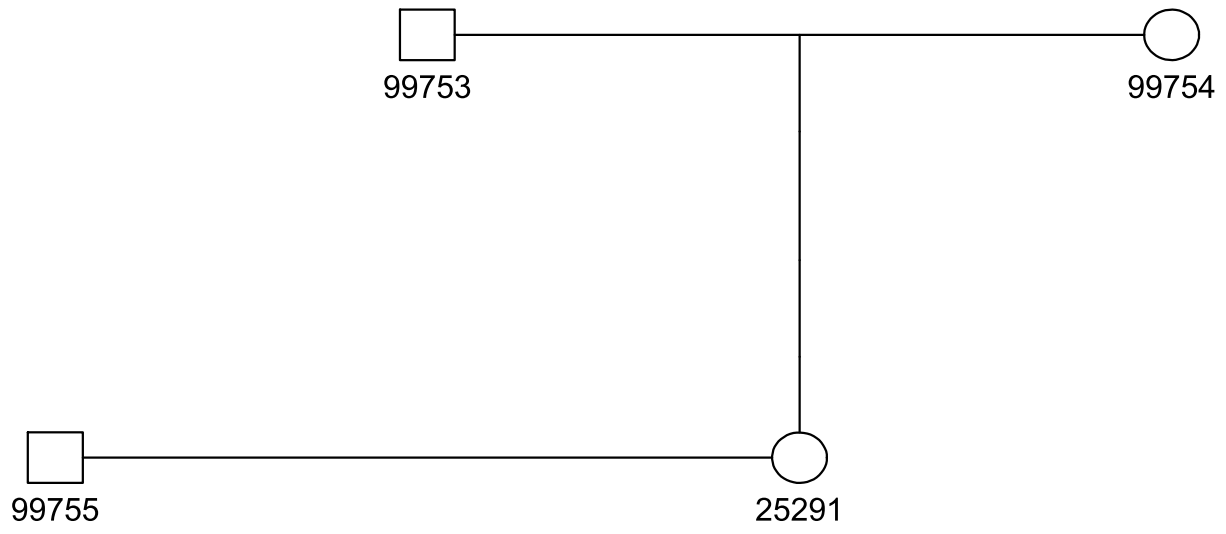
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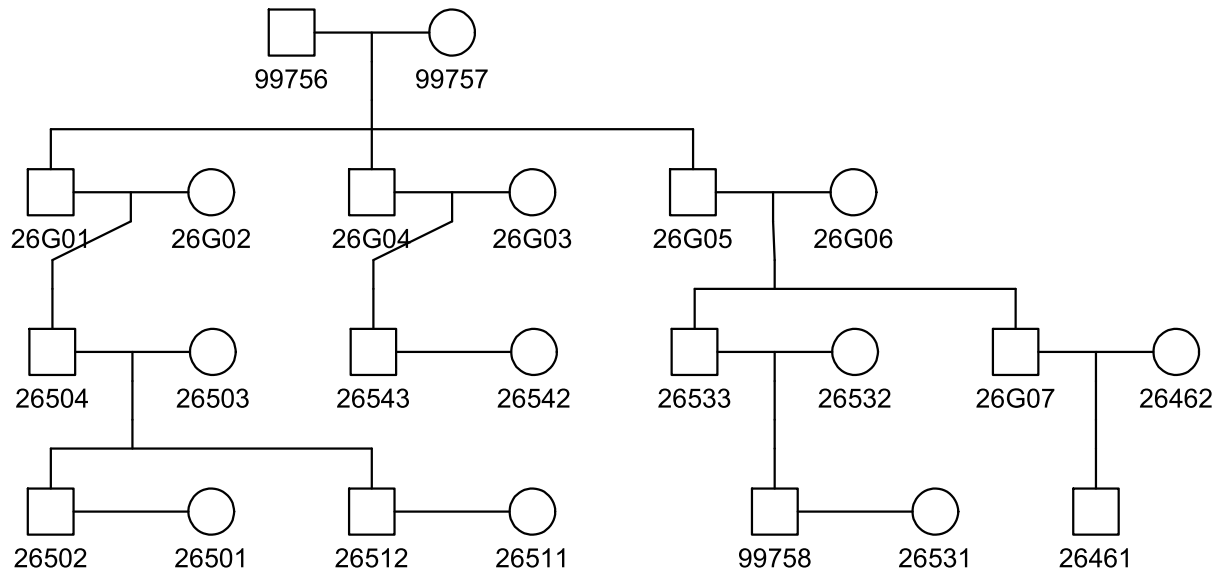
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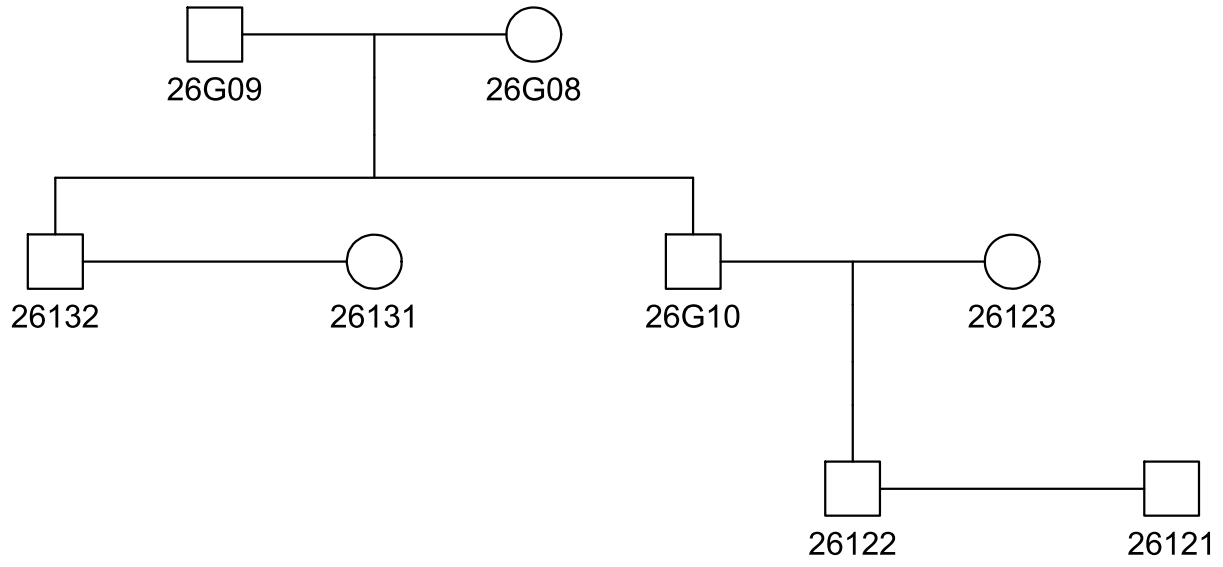
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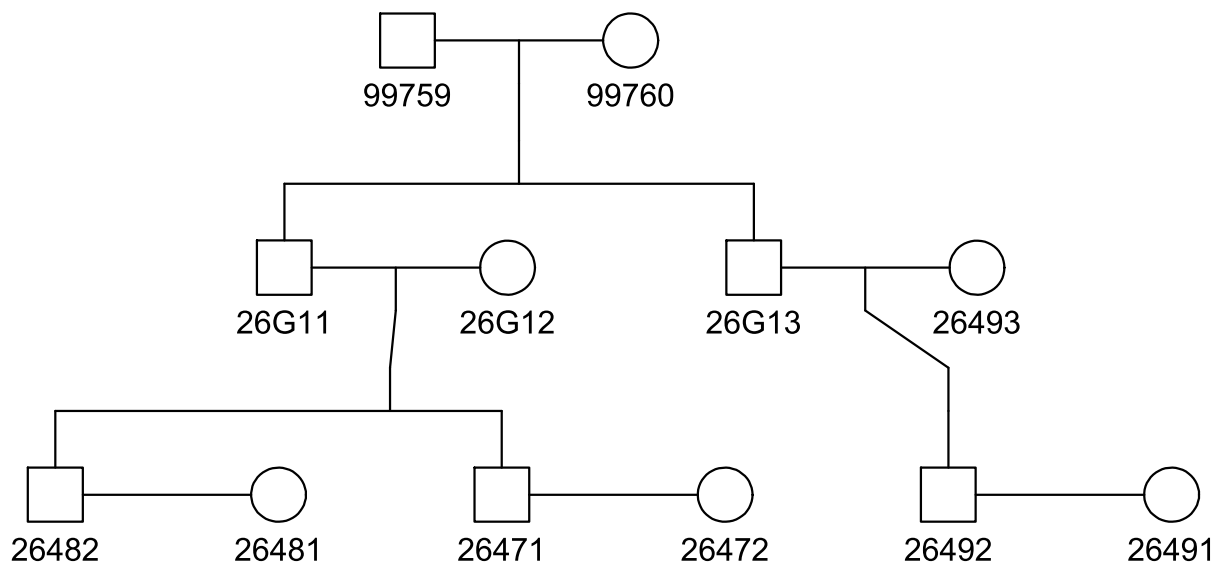
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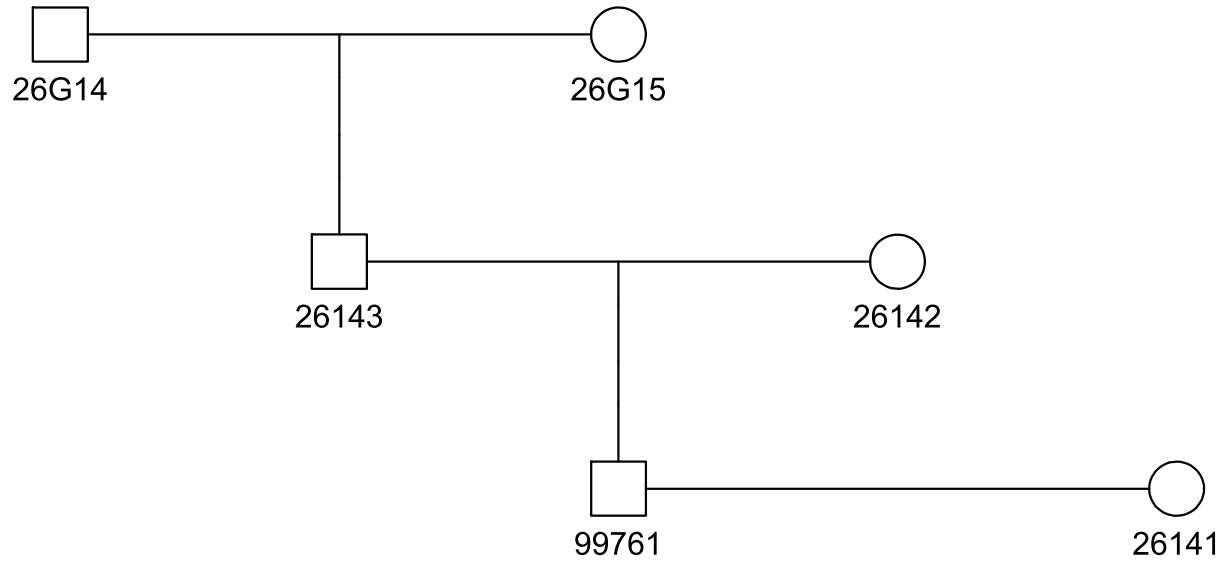
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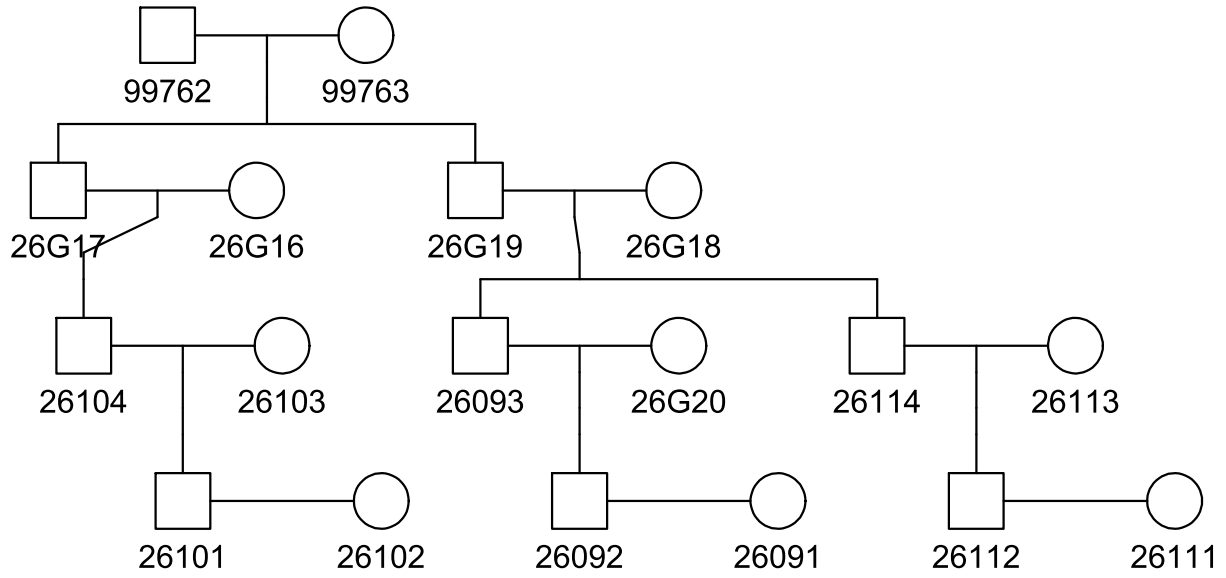
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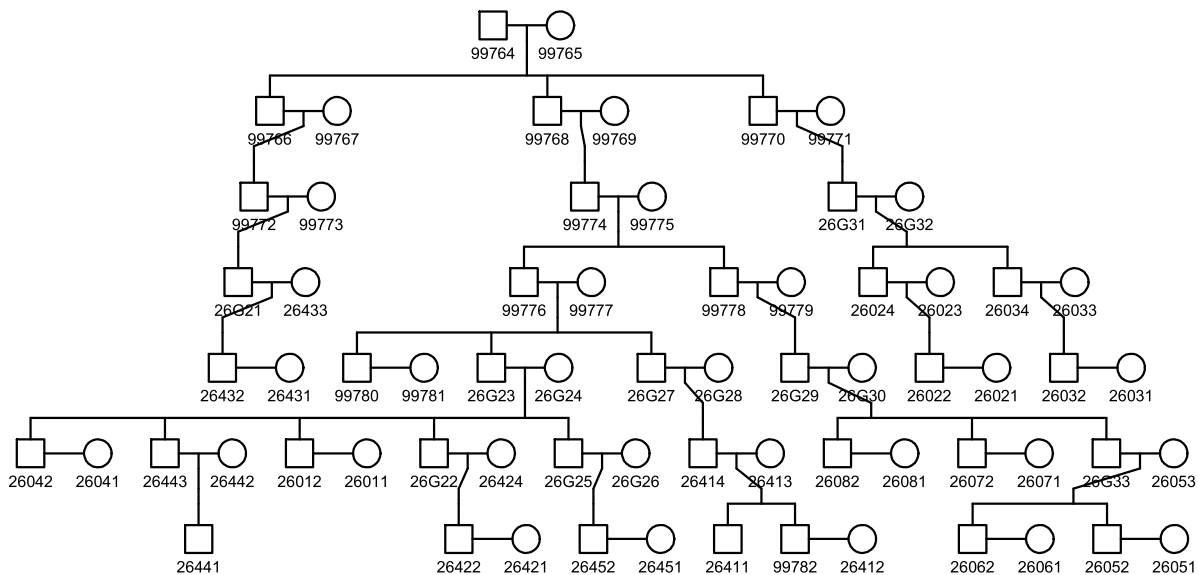
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26P05



26P06



Did not plot the following people: 26423

26P07

