Online supplementary file 3: Additional information relating to pass data processing

1.1. Classification of genetic diagnoses

Genetic testing records for indexes and those relatives who received a test included the date a patients' test was requested, the date the result was made available, whether the diagnosis was deemed positive, VUS, or negative, and what genetic mutation was detected (if any). A patients' genetic diagnosis was defined by the corresponding genetic mutation classification used by the services as of 15/04/2020. In cases where multiple mutations were recorded, the patient was classified according to the most severe mutation on record. The proportion of patients reclassified from VUS to a monogenic or non-monogenic diagnosis informed the historical rates of reclassification in VUS cases. Reclassification-related notes made in PASS and comments compiled specifically for this project were used to inform instances of reclassification. Reclassifications from an initial non-VUS diagnosis were extremely rare and deemed exceptional cases; it was assumed that all reclassifications to pathogenic or non-pathogenic variants were only from initial VUS diagnoses. In special cases where an FH mutation was recorded as having been downgraded, the updated diagnosis was applied but the patient was assumed not to have been reclassified. Note that genetic classifications recorded in PASS may have been overwritten without a record made in the system notes, meaning the number of historic reclassifications compiled may be an underestimate. In addition, reclassification rates calculated in this analysis are unlikely to reflect those observed currently in practice given the changing landscape in genetic segregation.

1.2. Patient characteristics

Patient characteristics considered in the analysis of Welsh and Wessex index and relative cases included: age, gender, LDLC, FH status, and a relatives' degree from their index case. A patients' age was informed by rounding down the number of years between the date of genetic testing and the midpoint of their year of birth. Ages were banded into <10, 10-17, 18-39, 40-59 and 60+ year groups to align with the cost-effectiveness model. LDL-C was taken as the highest raw LDL-C level recorded prior to genetic testing. The availability of TC, TG and HDL-C measurements enabled the Friedwald equation to obtain missing LDL-C measurements (17). A patients' FH status was determined by their diagnosis according to current genetic mutation classifications **Error! Reference source not found.**with mutations tabulated by affected gene (*LDLR*, *PCSK9*, *APOB* and *APOE*). A relatives' relationship to their index case was categorised by degree. First-degree relatives consisted of parents, full-siblings or children of an index case (sharing approximately 50% of the genes). Data on the

specific relationship between a relative and their index case beyond the first degree were not available, meaning all further degrees of separation were pooled together and categorised as ≥2nd degree relatives.

1.3. CV and LLT history

CV history was defined as having had any record of angina, MI/ACS, PVD, CABG, PTCA or TIA prior to genetic testing. Individuals were only deemed to have had no CV history if a complete record existed of not having experienced any CV-related events or procedures prior to cascade testing. CV history was indeterminable in cases where: 1) relatives without evidence of a prior event had missing data in any single CV-related variable; or 2) relatives had non-dated CV events and no other evidence of prior CV history. Events recorded as "unknown" were set to missing. Any event-related comments recorded by FH nurses in PASS were assessed and incorporated accordingly.

LLT history was defined in accordance to three data fields: 1) evidence of statins use prior to testing compiled by a specialist FH nurse specifically for this analysis; 2) treatment data recorded in PASS; and 3) service notes of relatives having been "on treatment" prior to testing. All cases and non-cases of LLT history denoted from 1) and 3) were combined into a composite variable with the addition that any records of treatments prescribed within PASS at or before a genetic test date were treated as a history of LLT. Any entry categorised as "unknown" was treated as missing.

1.4. Area status

Health board and LSOA data for each service were tabulated and examined; health boards and LSOA boundaries deemed to potentially fall outside of each respective services' remit were reviewed by specialist FH nurses and categorised accordingly. Area status for Wessex relatives was primarily recorded in relatives' method of contact, and as a result area status for Wessex relatives was informed by a combination of method of contact, health board, and LSOA code data. Area statuses in relative cases with an "unknown" health board were treated as missing. No relative cases from "unknown" health boards had evidence of being contacted by the service and consequently not included in any non-area analyses (Figure 1). Relatives without evidence of area were not dropped from the analysis as it was assumed relatives who completed the cascade without area data noted in PASS were within-area.

1.5. Age-adjusted welsh genetic testing criteria scores

To increase the sample of age-adjusted scores in the Welsh analysis set, inferred age-adjusted scores were also calculated. Inferred scores added the presumed differential between non age-adjusted and age-adjusted cholesterol-related scores onto the observed

overall non-age adjusted scores. The highest untreated LDL-C on record was used including adjustments for recorded prior LLT in accordance with Welsh correction factors (7). Age-adjustments were applied to LDL scores using the formula used to adjust LDLC to calculate the age-adjusted Welsh score [ref]:

$$LDL_{ageadjusted} = LDL_{unadjusted} + 0.042 * (52 - \text{Age}) \quad (1)$$

1.6. Method of contact

All recorded contact methods were tabulated and sorted into each relevant category. Any method of contact which appeared unclear was clarified by a relevant specialist FH nurse and categorised accordingly. Since the Wessex service does not conduct direct methods of contact, direct contact and indirect contact was only comparable in the Welsh analysis set.

2. Additional results

Table 4.1: Logistic regression used to calculate the probability of successfully completing the cascade in Wales

Logistic regression estimating the probability of being successfully cascaded - Wales

	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Female	1.534	0.145	4.53	0.000	1.275	1.845	***
1st degree	1.552	0.150	4.55	0.000	1.284	1.875	***
MOC							
Indirect	1.000						
Direct	2.113	0.260	6.08	0.000	1.661	2.689	***
Other	3.531	0.553	8.05	0.000	2.597	4.801	***
Paediatric	2.651	0.367	7.04	0.000	2.020	3.477	***
Unknown	0.535	0.146	-2.29	0.022	0.314	0.914	**
Constant	0.486	0.060	-5.88	0.000	0.382	0.618	***

Mean dependent var	0.596	SD dependent var	0.491
Pseudo r-squared	0.055	Number of obs	2011
Chi-square	149.860	Prob > chi2	0.000
Akaike crit. (AIC)	2577.036	Bayesian crit. (BIC)	2616.281

*** p<0.01, ** p<0.05, * p<0.1

MOC: Method of contact; St.Err: Standard error SD: Standard deviation; Sig: Significance

Table 4.2: Logistic regression used to calculate the probability of successfully completing the cascade in Wessex

Logistic regression estimating the probability of being successfully cascaded - Wessex

cascade	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
female	1.735	0.240	3.99	0.000	1.323	2.274	***
1st degree	0.851	0.126	-1.09	0.275	0.638	1.137	
MOC							
Indirect	1.000						
Paediatric	5.830	0.991	10.37	0.000	4.178	8.136	***
Unknown	15.333	11.508	3.64	0.000	3.522	66.757	***
Constant	0.515	0.075	-4.56	0.000	0.388	0.685	***

Mean dependent var	0.499	SD dependent var	0.500
Pseudo r-squared	0.115	Number of obs	1000
Chi-square	159.420	Prob > chi2	0.000
Akaike crit. (AIC)	1236.871	Bayesian crit. (BIC)	1261.409

*** p<0.01, ** p<0.05, * p<0.1

MOC: Method of contact; St.Err: Standard error SD: Standard deviation; Sig: Significance

Table 4.3: Logistic regression interacting contact and relative degree in Wales

Logistic regression estimating the probability of being successfully cascaded - Wales

cascade	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
female	1.420	0.172	2.89	0.004	1.120	1.801	***
1st degree	1.387	0.266	1.70	0.089	0.952	2.020	*
direct contact*	2.467	0.499	4.46	0.000	1.660	3.668	***
1st degree & direct (interaction)	0.774	0.197	-1.01	0.314	0.470	1.275	
Constant	0.545	0.089	-3.71	0.000	0.395	0.751	***

Mean dependent var	0.553	SD dependent var	0.497
Pseudo r-squared	0.031	Number of obs	1155
Chi-square	49.937	Prob > chi2	0.000
Akaike crit. (AIC)	1548.110	Bayesian crit. (BIC)	1573.369

*** p<0.01, ** p<0.05, * p<0.1

St.Err: Standard error SD: Standard deviation; Sig: Significance

*Direct contact relative to indirect contact

Table 4.4: Logistic regression interacting contact and gender in Wales

Logistic regression estimating the probability of being successfully cascaded - Wales

cascade	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
female	1.702	0.318	2.85	0.004	1.180	2.454	***
1st degree	1.198	0.150	1.44	0.149	0.937	1.532	
direct contact*	2.467	0.442	5.04	0.000	1.736	3.504	***
female & direct (interaction)	0.748	0.184	-1.18	0.236	0.462	1.210	
Constant	0.540	0.084	-3.95	0.000	0.398	0.733	***

Mean dependent var	0.554	SD dependent var	0.497
Pseudo r-squared	0.033	Number of obs	1158
Chi-square	51.848	Prob > chi2	0.000
Akaike crit. (AIC)	1550.177	Bayesian crit. (BIC)	1575.450

*** *p<0.01, ** p<0.05, * p<0.1*

St.Err: Standard error SD: Standard deviation; Sig: Significance

*Direct contact relative to indirect contact

Table 4.5: Sensitivity and specificity of alternative thresholds on the welsh genotype score for diagnosing FH and non-FH in those Welsh indexes eligible for testing

	Welsh genotype scoring criteria							
	Unadjusted (n=2,531)		Age-adjusted (n=1005)		Maximum observed* (n=1005)		Inferred age-adjusted (n=2,295)	
Threshold	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
≥6	100.00%	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%	0.00%
≥7	80.99%	38.10%	88.56%	21.24%	90.23%	25.34%	88.80%	25.43%
≥8	70.89%	56.10%	80.07%	42.92%	81.11%	46.45%	79.43%	45.86%
≥9	64.55%	68.91%	69.28%	58.15%	70.68%	59.88%	67.01%	61.53%
≥10	53.07%	79.91%	55.56%	75.32%	57.33%	75.62%	53.97%	75.96%
≥11	41.19%	89.14%	45.75%	82.40%	46.25%	83.49%	46.03%	85.40%
≥12	36.44%	93.12%	39.87%	90.13%	40.72%	89.44%	39.71%	90.98%
≥13	30.69%	96.22%	33.33%	95.28%	34.53%	95.59%	33.20%	95.57%
≥14	24.16%	97.71%	26.14%	97.85%	26.06%	97.89%	26.68%	97.21%
≥15	19.60%	98.38%	21.57%	98.93%	22.15%	98.85%	20.77%	98.03%
≥16	11.88%	98.99%	13.73%	99.36%	14.66%	99.42%	12.22%	98.69%
≥17	6.34%	99.33%	7.84%	99.36%	7.82%	99.42%	6.72%	99.10%
≥18	3.37%	99.66%	3.59%	99.36%	4.56%	99.42%	3.46%	99.43%
≥19	1.98%	99.73%	2.94%	99.36%	2.93%	99.42%	2.24%	99.59%
≥20	1.19%	100.00%	1.63%	100.00%	1.63%	100.00%	1.22%	100.00%
>20	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%

*Maximum unadjusted or age-adjusted result reported in the Welsh PASS system