

Protocol 4: DNA isolation from frozen tissue with DNeasy procedure

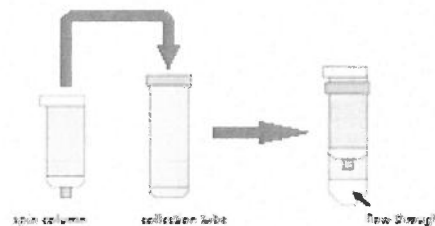
Best results are obtained with fresh material or material that has been immediately frozen and stored at -80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. In order to obtain optimum DNA yield and quality, it is important not to overload the DNeasy procedure, as this can lead to significantly lower yields than expected. Maximum amount of starting material of animal tissue is 25 mg. Cut off, if possible, contaminating normal tissue and pieces like necrosis. DNA isolation is performed with DNeasy Tissue Kit from Qiagen, art.nr. 69504. stored at RT. Buffers ATL and AL may form precipitates upon storage. If a precipitate has formed in either buffer, incubate at 55°C until the precipitate has fully dissolved. Buffers AW1 and AW2 are supplied as concentrates. Before using for the first time, add the appropriate amounts of abs. ethanol as indicated on the bottles.

Solutions:

- Buffers ATL, AL, AW1, AW2, AE -> buffers DNeasy-kit
- Prot k (10 mg/ml)
- RNAase A (10 mg/ml)
- EtOH_{abs}

Material:

- Eppendorf tubes (1,5 and 2,0 ml)
- DNeasy spin column, kit
- Thermal incubator, 55°C/70°C



Protocol:

- Cut up to 25 mg tissue and transfer it to a clean, sterile 2ml tube
- To lyse add 360 µl of buffer ATL
- Add 40 µl proteinase k and mix by vortexing
- Incubate at 55°C until the tissue is completely lysed; depending on type of tissue, usually 1-3 hours. Can be done overnight at 37°C.
- Optional: add 4 µl of RNase A, mix by vortexing and incubate for 2 min at room temperature
- Vortex for 15 seconds
- Add 400 µl of buffer AL to the sample, mix thoroughly by vortexing and incubate at 70°C
- Add 400 µl of EtOH_{abs} to the sample to bind the DNA to the DNeasy membrane and mix well
- Pipet 600 µl of the mixture into the DNeasy spin column
- Centrifuge 1 min at 8.000 rpm
- Discard flow-through
- Pipet remaining mixture (max 600 µl) into the column
- Centrifuge 1 min at 8.000 rpm
- Discard flow-through and collection tube
- Place the DNeasy spin column in a new 2 ml collection tube
- Add 500 µl of AW1 to the column
- Centrifuge 1 min at 8.000 rpm
- Discard flow-through and collection tube
- Place the DNeasy spin column in a new 2 ml collection tube
- Add 500 µl of AW2 to the column
- Centrifuge 3 min at full speed
- Discard flow-through and collection tube
- Place the DNeasy spin column in a new 2 ml collection tube
- Pipet 200 µl AE directly onto the DNeasy membrane
- Incubate at room temperature for 1 min
- Centrifuge for 1 min at 8.000 rpm to elute
- Pipet 100 µl of buffer AE directly onto the DNeasy membrane
- Incubate at room temperature for 1 min
- Centrifuge for 1 min at 8.000 rpm to elute
- Store DNA at 4°C or at -20°C if stored for a long time

Appendix 3: optimization DNA isolation from FFPE material

Because a substantial amount of 117 samples did not give results in the genotyping-SNP assays when using protocol 1 for DNA isolation, the integrity of the DNA was checked with an integrity PCR.

The principle of this PCR is as follows. In a mastermix 8 primers (4 pairs) are added. These primers produce products of different sizes: 100 basepairs (bp), 200 bp, 300 bp and 400 bp. If the integrity of the DNA is good all 4 products are made and can be visualized on a 1,5% agarose gel. If the quality of the DNA decreases, less bands are visible on the agarose gel. These results give an indication of the quality of the DNA.

The overall results of these PCR were that samples produced, no product, 100 bp and 200 bp in comparison with the pBI samples which show all 4 products. In figure A4-1 the results of pBI samples are shown on a 1,5% agarose gel.

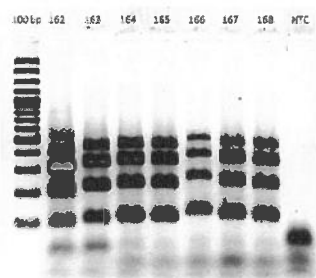


Figure A4-1: Integrity PCR; sample 162 until 168 are pBI samples showing all 4 product sizes.

The integrity of the samples, which did not give any results in the genotyping-SNP assays, were tested using this integrity PCR. In figure A4-2 these results are shown. Half of the samples did not give satisfactory results: 11 samples gave three or more bands, 12 samples gave zero to two bands.

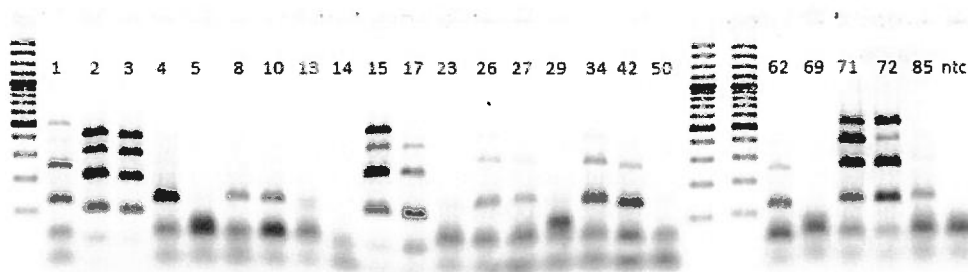


Figure A4-2: Integrity PCR

The conclusion was that the integrity of the DNA was not good enough to make products over 200 bp in size. This is probably because during fixation with formalin crosslinks are made and cannot be reversed with the current method. A new method was needed. After searching PubMed the following article was found.

Shi SR, Cote RJ, Wu L, Liu C, Datar R, Shi Y, Liu D, Lim H, Taylor CR. DNA extraction from archival formalin-fixed, paraffin-embedded tissue sections based on the antigen retrieval principle: heating under the influence of pH. J Histochem Cytochem 2002;50:1005-11.

This article used an isolation buffer which could reverse the crosslinks in the DNA with the help of a high pH and high temperature. The results of this article are shown in Figure A4-3.

In the article they looked at the yield, Figure A4-3: Figure 1, which was highest at pH 9. In Figure A4-3: Figure two a gel electrophoresis of PCR products is shown (152 bp, 347 bp and 541 bp). Actin the biggest product is produced when isolated at 100 °C and 120 °C with all different pH buffers. When lowering temperature only a buffer with a pH of 10 or higher shows a product. In Figure A4-3: Figure 3 the results of the "real time" PCR show that with a temperature of 120 °C and a pH of 11 most amplifiable DNA is produced.

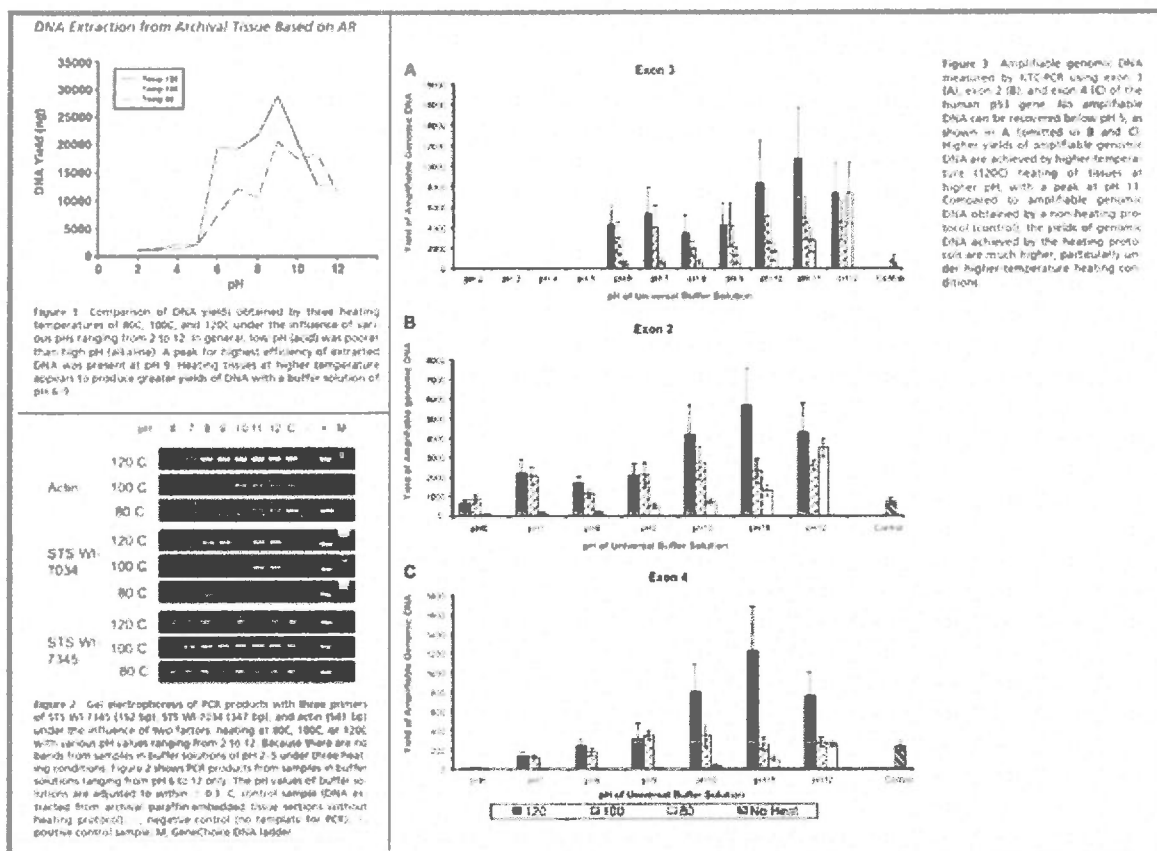


Figure A4-3 shows three figures shown in: DNA extraction from archival formalin-fixed, paraffin-embedded tissue sections based on the antigen retrieval principle: heating under the influence of pH.

With the results shown in Figure A4-3 an isolation buffer with a pH of 10 is made called universal buffer. At first this buffer was added to the tissue and the Eppendorf tube were put in the autoclave to reach a temperature of 120 °C. These results are shown in Figure A4-4.

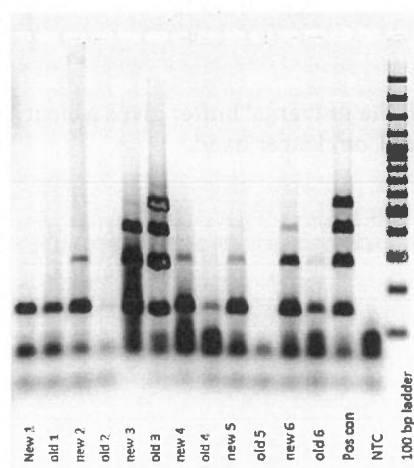


Figure A4-4: integrity PCR; 6 different samples (numbered 1 till 6) were used with protocol 1 (old) and with the new protocol, with the isolation step using the universal buffer pH 10 at 120 °C. 4 of the 6 samples show improvement using the new protocol. Sample 1 shows no improvement. Sample 1 says equal. Sample 1: 4, Sample 2: 8, sample 3: 9. Sample 4:122, sample 5: 149, sample 6: 160.

Due to technical issues during this step. As an alternative it was chosen to use a heat block and a temperature of 100 °C could be reached.

For further optimization four conditions were tested. This was performed on one sample. Although pBI was available it did not give any results. For this experiment DNA from FFPE material was isolated using these four conditions .

1. Protocol 1, lysis buffer + prot K o.n. 52°C
2. New protocol, universal buffer, o.n. -20 °C
3. New protocol, universal buffer, o.n. 52°C lysisbuffer + prot K
4. New protocol, universal buffer, o.n. 52°C prot K

The results are shown in Figure A4-5. For the positive control a pBI sample is used shown A4-1.

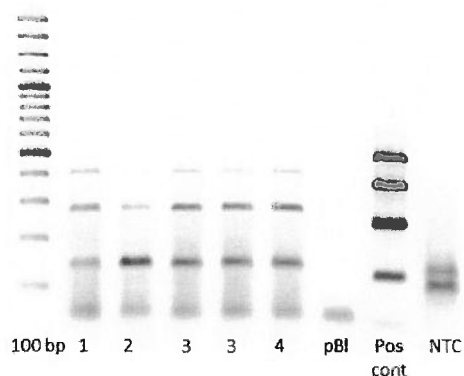


Figure A4-5: 4 isolation conditions and old pBI sample. Without o.n. step (2) less stronger band is shown. 1, 3 and 4 show similar results. pBI gave no bands.

From these results it was concluded that an o.n. incubation with prot K improved DNA quality. In a next step two conditions were tested on two samples (no 23 and 50) which showed zero product sizes in the quality control PCR This was done because the first condition showed already three 3 bands in the previously used sample. The conditions chosen were:

1. New protocol, universal buffer, o.n. -20 °C
2. New protocol, universal buffer, o.n. 52°C prot K

These results are shown in Figure A4-6. Showing that isolating with the universal buffer gives a clear improvement. And adding an o.n. step with prot K gives an additional, or clearer band.

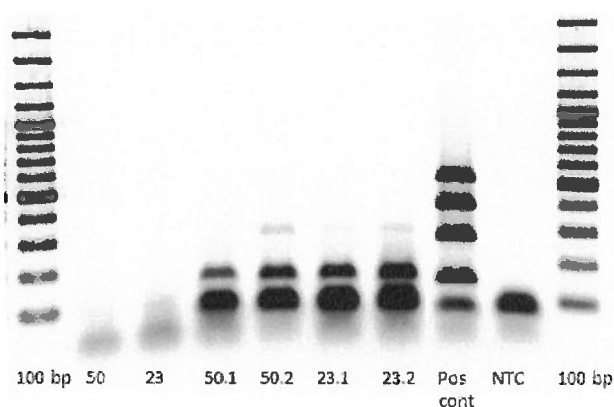


Figure A4-6: sample 50 and 23, isolated with protocol 1, 50.1 and 23.1 were isolated with universal buffer, 50.2 and 23.2 were isolated with universal buffer and adding prot K o.n. 52°C.

From 21 more samples DNA was isolated using the universal buffer with an o.n. step at 52 °C with prot K added. The DNA samples isolated with the old isolation (protocol 1) and the new protocol, were analyzed with the genotyping-SNP assays. If the DNA isolated using the old method already gave a result in the genotyping assay, it was not analyzed again on the DNA isolated with the new protocol from the same sample (indicated in grey). When a sample did not give a result, even after isolation with the new protocol, a red cross is placed in the table. When a sample gave an improvement in comparison with the old protocol the genotype is shown in green. When both samples were amplified, the sample isolated with this new protocol the amplification curve shifted towards left. Clearly an improvement can be seen using the new isolation method, this was further used as the standard isolation method for all FFPE samples (Appendix 2, protocol 2). See Table A4-1.

Isolation protocol using universal buffer on isolated samples

Protocol 2 optimized results on FFPE material. Seeing these improved results there might be a possibility that this isolation buffer could also improve the DNA quality of DNA isolated with isolation protocol 1. This was tested on five samples, using the four conditions given below. The results clearly show no improvement, see Figure A4-7. It is possible that the universal buffer with a high pH degraded the DNA.

1. Protocol 1
2. sample isolated with protocol 1, adding universal buffer o.n. at 52 °C
3. sample isolated with protocol 1, adding universal buffer 100 °C o.n. at 52 °C
4. protocol 2

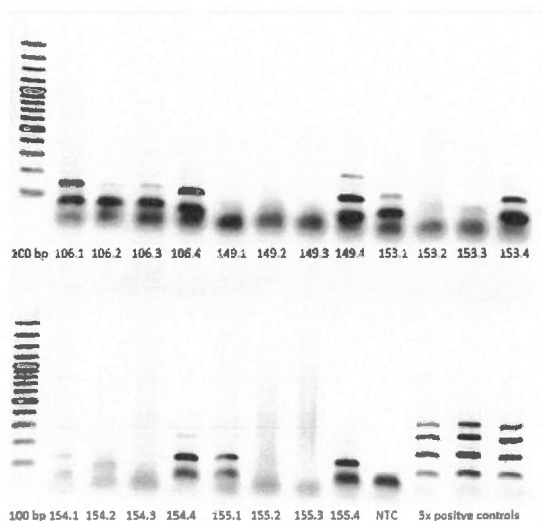


Figure A4-7: five different samples, four conditions. Condition two and three lose bands in comparison with condition one. Condition four improves is four of the five cases.

Sample No	ATFTP	SPRY4	BAK1	HPGDS	PPMIE	RFVDS	TEX14	MAD1L1	UCK2	CEMPE	TERT	TERT/CLPTMIL	DMRT1	KITLG	KITLG (p53 bs)	result per sample
10	old	CC	AG	AA	AA	CC	TT	CC	GG	CC	X	AG	CT	GG	GG	5/9
23	new	CC	AG	GG	AG	CC	TT	CC	CC	CC	AC	AG	X	GG	GG	9/9
29	old	CC	GG	X	AG	CT	TT	CT	X	CC	CC	GG	AA	GG	AG	8/14
38	new	CC	GG	X	AG	CT	TT	CT	X	CC	CC	GG	AA	GG	AG	11/14
38	old	CT	GG	AA	AG	CC	GT	TT	AG	TT	AC	GG	TT	AG	AG	1/1
41	new	CC	AA	X	GG	CC	X	X	X	X	AC	X	CT	GG	GG	10/17
41	old	CC	AA	X	GG	CC	TT	CT	GG	CC	AC	AG	CT	GG	GG	16/17
42	old	X	AG	X	AG	X	X	CT	AG	X	CC	AG	CT	GG	GG	11/9
43	new	CC	CT	AG	GG	CT	GT	TT	X	TT	TT	AG	X	GG	GG	8/9
43	old	CT	AG	X	GG	CC	TT	CT	X	X	CC	AG	TT	GG	GG	4/17
47	new	CT	AG	X	AG	X	CT	CC	AG	CT	CC	GG	CT	AG	AG	14/17
47	old	CT	AG	X	AG	CT	GT	CC	AG	CT	CC	GG	CT	AG	AG	0/6
48	new	CT	AA	X	GG	CC	TT	CT	AG	TT	AC	AG	CT	GG	GG	6/6
48	old	CT	AA	X	GG	CC	TT	CT	AG	TT	AC	AG	CT	GG	GG	4/5
50	new	CT	AA	X	GG	CC	X	X	X	X	X	X	CC	X	X	4/5
50	old	CT	AA	X	GG	CC	X	X	X	X	X	X	CC	X	X	11/17
53	new	CT	AG	X	GG	X	X	CT	X	X	X	X	AA	GG	GG	8/17
53	old	CT	AG	X	GG	X	X	CT	X	X	X	X	AA	GG	GG	11/17
54	new	TT	AA	X	GG	X	X	CT	X	X	X	X	AA	AG	GG	8/17
54	old	CC	AA	AG	AA	CC	TT	TT	AG	CT	AC	GG	CT	GG	GG	0/2
58	new	X	X	X	GG	X	X	X	X	X	X	X	X	X	X	2/2
58	old	X	X	X	GG	X	X	X	X	X	X	X	X	X	X	0/17
59	new	CT	AG	X	GG	X	X	CT	X	X	X	X	AA	GG	GG	8/17
59	old	X	GG	X	GG	X	X	CT	X	X	X	X	AA	GG	GG	11/17
60	new	CT	AG	X	GG	CC	GT	CT	X	X	AC	GG	TT	GG	GG	13/17
60	old	X	X	X	GG	CC	X	CT	X	X	X	X	X	X	X	0/17
61	new	CT	AA	X	GG	CC	TT	CT	X	X	CC	GG	TT	GG	GG	14/17
61	old	X	X	X	GG	CC	X	CT	X	X	X	X	CT	X	X	0/16
62	new	TT	GG	X	GG	CC	TT	CT	GG	CC	AC	AG	TT	GG	GG	11/16
62	old	TT	GG	X	GG	CC	TT	CT	GG	CC	AC	AG	TT	GG	GG	9/10
64	new	TT	GG	AG	GG	TT	GT	TT	AA	CT	AA	GG	CT	GG	GG	10/10
64	old	TT	X	GG	GG	TT	GT	TT	AA	CT	AA	GG	CT	GG	GG	0/1
74	new	X	AG	X	X	X	X	X	X	X	AC	X	X	X	X	1/1
74	old	CC	AA	X	GG	X	X	CT	X	X	X	X	TT	GG	GG	3/17
153	new	old	X	X	X	X	X	X	X	X	X	X	CT	X	X	8/17
153	old	X	X	X	X	X	X	X	X	X	X	X	CT	X	X	1/17
154	new	CT	AG	X	GG	CC	TT	TT	AG	CT	AA	GG	CT	GG	GG	16/17
154	old	CT	AG	AA	AG	CT	X	TT	AA	CC	AG	GG	CT	GG	GG	9/17
155	new	CT	AG	X	GG	CT	GT	TT	AA	CC	AC	GG	CC	GG	GG	17/17
155	old	CT	AG	X	GG	CT	X	TT	X	X	CC	GG	CC	GG	GG	9/17
160	new	CT	AG	AA	AG	CT	TT	CC	GG	CT	CC	GG	CT	GG	GG	17/17
160	old	TT	AG	X	AG	X	GT	X	X	X	X	AG	X	X	X	7/17
Result per assay	old	10/17	0/18	1/19	5/14	5/18	2/17	1/16	1/15	1/16	4/16	7/16	7/13	6/15	4/18	5/14
new	17/17	19/19	5/18	15/19	14/14	14/18	12/17	16/16	6/15	7/16	12/16	12/16	13/13	17/17	15/15	14/14

Table A4-1: overview protocol 1 (old) vs protocol 2 (new), protocol 2 shows a clear improvement in comparison with protocol 1.

Appendix 4: Taqman Genotyping Assay protocol

Before pipetting a plate setup is made. Depending on the amount of samples and assays a setup will be made using the guidelines below.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Guidelines plate setup:

- 96-wells plate sorted by row, bottom right is reserved for the no-template control (NTC)
- Positive controls have to be positioned directly after the samples. (with 2 assays on a plate, first all samples are pipetted, after which the positive controls are added (assay 1 AA, BB, AB and then assay 2 AA, BB, AB)
- The NTC has to be always bottom right; so with two assays NTC 1 on H11 and NTC 2 on H12.
- With 2 assays on a plate use the same number of samples for both assays. Use the assay with the most samples designing a run, if the other assay has less samples leave wells open. This is necessary for the software to be able to run and analyze the results.

When the number of samples is known the amount of mastermix has to be calculated using the scheme below. Remember, make always 10% more mastermix then needed.

Scheme for pipetting the mastermix, per well 13 µl mastermix is pipetted.

Mastermix:			
1x		Number of samples	X
7,5 µL	PCR mastermix	...	µL
0,375 µL	SNP assay	...	µL
5,125 µL	dH2O	...	µL

The mastermix will be pipetted in a 96-wells plate using 13 µl per well, then 2 µl sample is added, with a concentration of 25 ng/µl. After pipetting a plate, the plate will be run on a AB 7500 Fast Real-Time PCR machine (ThermoFisher Scientific) according to the following program .

Temperature	time	Cycle
60.0°C	1 min	1
95.0°C	10 min	1
95.0°C	15 sec	45
60.0°C	1 min	
60.0°C	1 min	1

Appendix 5: Information Genotyping-SNP assays, life technologies

	Nearby gene	SNP ID	Ordernumber
1	ATF7IP	rs2900333	C__715253_10
2	SPRY 4	rs4624820	C_27983854_10
3	BAK 1	rs210138	C__940463_20
4	CENPE	rs4699052	C_29753239_10
5	HPGDS	rs17021463	C_34194662_10
6	RFWD3	rs4888262	C__504433_10
7	PPM1E	rs7221274	C_11624231_10
8	TEX 14	rs9905704	C_11624260_10
9	MAD1L1	rs12699477	C__1837468_10
10	UCK2	rs4657482	C_27958871_10
11	TERT	rs2736100	C__1844009_10
12	TERT/CLPTM1L	rs4635969	C__1844186_10
13	DMRT1	rs7040024	C_31947497_10
14	DMRT1	rs755383	C__1690720_10
15	KITLG	rs995030	C__8716323_20
16	KITLG	rs1508595	C__8716619_10
17	KITLG P53	rs4590952	C_27945700_20

Appendix 6: raw data odd ratio rs4590952

In total this genotyping-SNP assay gave result for 644 patients in the TGCT group and for 230 patients in the 1000 genomes phase 3 EUR population. The allele frequency is shown below for both groups.

allele frequency TGCT group

	A	G
AA	14	0
AG	113	113
GG	0	1048
total	127	1161

allele frequency 1000 genomes EUR group

	A	G
AA	14	0
AG	66	66
GG	0	314
total	80	380

With this allele frequencies the OR is calculated in SPSS, the 2X2 table is shown below

2x2 table to calculate the OR

		TGCT		Total
		no	yes	
allele	A	94	127	221
	G	380	1161	1541
Total		474	1288	1762

cross tabulation to calculate Chi-square, included CT

		allele		
		A	G	Total
TGCT	NS	59	523	582
	SE	60	704	764
Total		119	1227	1346

In Table 9 the data of the Chi-square test is shown, CT excluded. The p-value of the Chi-square test is 0.792 (2-sided). So in both analyses there is no significant difference in allele frequencies between SE and NS.

cross tabulation to calculate Chi-square, excluded CT

		allele		
		A	G	Total
TGCT	NS	38	472	510
	SE	60	704	764
Total		98	1176	1274

Appendix 7: Patient data

Sample No	Karyotype	material	Diagnosis, Mut Found	pre- cursor/tumor/ zijdigheid	Age diagnosis
1	46XY	pBL			13
2	46XY	Frozen		Li en Re: GB	16
3	46XY	FFPE		GB, DG	14
4	46XY	FFPE		Re GB, iTE, mTE Li: YST	9 and 11
5	46XX	FFPE			
6	46XX	pBL			4
7	46XY	pBL			3 months
8	46XY	FFPE	17B-HSD R80Q and 325+4		7
9	46XY	pBL			18
10	46XY	FFPE		GB, DG	18
11	46XY	pBL	AR 521del13, AIS		18
12	46XY	pBL	MAP3K1 Ex 10	GB, DG	19
13	46XY	FFPE		GB	19
14	46XY	FFPE	CAIS, no mut found		
15	46XY	pBL			3
15	46XY	pBL	773		
16	46XY	pBL	AR F891L (T3035A), AIS	GCNIS	18
17	46XY	FFPE		GB (remnants), SE/DG	36
18	46XX	pBL		YST	
19	46XY	FFPE	17 B-HSD IVS3+4A>T		3
20	46XY	FFPE	CAIS Q591X (C2133T)		16
21	46XY	FFPE	PAIS, no AR mutation		14
22	46XY	FFPE	PAIS, no AR mutation		23
23	46XY	FFPE	AIS		3
24	46XY	FFPE	CAIS, 2206 G to A, R615H		15
25	46XY	Frozen	AIS		?
26	46XY	pBL	DMRT1 mut	Re: GB	14
27	46XY	FFPE	17B-HSD def		15
28	46XY	FFPE	AIS A756T		10
29	46XY	FFPE	SF1 del first 2 coding exons		13
30	46XY	pBL			8m
31	46XY	pBL			2
32	46XY	FFPE Chelex	46XY, CAIS stop codon exon1	GCNIS	14
33	46XY	FFPE	Frasier	GCNIS, itSE, SE	21

Sample No	Karyotype	material	Diagnosis, Mut Found	pre- cursor/tumor/ zijdigheid	Age diagnosis
34	46XY	FFPE	SRY deletion, Swyer	Li: zeer klein GB	2
35	46XY	pBL	SRY W70L	Li: klein GB	23
36	46XX	pBL			2
37	46XX	pBL	SRY + TSPY transloc		2
38	46XY	pBL			1
39	46XX	pBL			3
40	46XX	pBL			4
41	46XY	FFPE	Swyer	Re: GB	17
42	46XY	FFPE	Swyer	Li: GB	16
43	46XY	FFPE		GB	23
44	46XX	pBL			37
45	46XY	FFPE	CAIS W741R		18
46	46XX	pBL			41
47	46XY	FFPE	PAIS		?
48	46XY	FFPE			14
49	46XY	pBL		GB, GCNIS, itSE, SE	26
50	46XY	FFPE			5
51	46XY	pBL		pre-GCNIS	1
52	46XY	pBL	CAIS		20
53	46XY	FFPE			
54	46XY	FFPE	CAIS		15
55	46XY	pBL			0
56	46XY	pBL	46XY female (suspision Swyer)		16
57	46XY	pBL	SRY + WT1 mutation	Li en Re: GB, DG	13
58	46XY	FFPE	PAIS		
59	46XY	FFPE	Denys-Drash syndroom		17
60	46XY	FFPE	microdeletion SF1		6
61	46XY	FFPE	minimal AIS L722F		11
62	46XY	FFPE	AIS		13
63	46XX	pBL		Re: DG, YST	
64	46XY	FFPE	PAIS		53
65	46XY	pBL	DHH mutation		
67	46XY	pBL			
68	46XY	pBL			
69	46XY	pBL	SR2 mutation		
70	46XY	pBL			?
71	46XY	pBL			

Sample No	Karyotype	material	Diagnosis, Mut Found	pre- cursor/tumor/ zijdigheid	Age diagnosis
72	46XY	pBL	Del downstream SOX9		14
73	46XY	FFPE			
74	46XY	FFPE			1
75	46XY	FFPE			17
76	46XY	FFPE			11
77	46XY	FFPE			13
78	46XY	pBL	CAIS		16
79	46XY	FFPE	CAIS		9
80	46XY	FFPE	CAIS		
81	46XY	FFPE	CAIS		6
82	46XY	FFPE	CAIS		
83	46XY	pBL	CAIS		17
84	46XY	FFPE	CAIS	Pre-GCNIS, GCNIS	15
85	46XY	FFPE	CAIS	GCNIS	15
86	46XY	FFPE	SOX9		
87	46XY	FFPE			
88	46XY	FFPE			3
89	46XY	FFPE			
90	46XY	FFPE			
91	46XY	FFPE			
92	46XY	FFPE	CAIS		
93	46XY	FFPE	CAIS		21
94	46XY	FFPE	CAIS		27
95	46XY	FFPE			16 months
96	46XY	FFPE			
97	46XY	FFPE	CAIS		15
98	46XY	FFPE	SRY	GB	3
99	46XY	FFPE	PAIS R486H fam. of 120		
100	46XY	pBL		SE	38
101	46XY	FFPE			23
102	46XY	FFPE	17B-HSD deficiency		7
103	46XY	FFPE			
104	46XY	FFPE	CAIS		
105	46XY	FFPE			11
106	46XY	FFPE			5
107	46XY	FFPE			2 weeks
108	46XX	pBL			10

Sample No	Karyotype	material	Diagnosis, Mut Found	pre- cursor/tumor/ zijdigheid	Age diagnosis
109	46XY	pBL			11
110	46XY	FFPE		Pre-GCNIS, GCNIS	3 months
111	46XY	pBL			2
112	46XY	FFPE			1
113	46XY	FFPE	DDS, WT1 R362X mutation, Wilm's tumor		2
114	46XY	FFPE	5a-reductase deficiency		19
115	46XY	pBL			?
116	46XY	FFPE	AIS		1
117	46XY	FFPE	CAIS Alternative spliced mRNA (mother heterozygous)		0
118	46XY	FFPE	CAIS R846C		0
119	46XY	FFPE	CAIS Q59X		2
120	46XY	FFPE	AIS		15
121	46XY	FFPE	PAIS	GCNIS	21
122	46XY	FFPE	AIS		21
123	46XX	pBL	46XX DSD, Ovotesticulair		3
124	46XY	FFPE	CAIS	GCNIS	35
125	46XY	pBL	CAIS hemizygous mutation c.796dup,p.Asp266GlyfsX39 mutation in exon 1		22
126	46XX	pBL			11
127	46XX	pBL	father 126		41
128	46XX	pBL	mother 126		42
129	46XY	FFPE	CAIS		18
130	46XY	FFPE	Suspect PAIS		
131	46XY	FFPE	Suspect PAIS		
132	46XY	FFPE	Gonadal Dysgenesis.		
133	46XY	FFPE	CAIS		16
134	46XY	pBL			0
135	46XY	pBL	father 134		31
136	46XY	pBL	mother 134		
137	46XY	FFPE	NR5A1 (SF1) mutation: unclassified variant, exon 2: M 1 T, 2T>C		9
138	46XX	pBL			
139	46XY	FFPE	orchidectomy	GB	
140	46XY	pBL	unilaterale cryptorchism and hypospadias. AIS		

Sample No	Karyotype	material	Diagnosis, Mut Found	pre- cursor/tumor/ zijdigheid	Age diagnosis
141	46XX	FFPE			
142	46XY	FFPE	CAIS intron 1 pos -2 A>G,		16
143	46XY	FFPE	PAIS L759M		15
144	46XY	FFPE	PAIS L759M		14
145	46XY	FFPE	CYP17 mutatie	possible pre-GCNIS	
146	46XY	FFPE			
147	46XY	FFPE	CAIS R822X		
148	46XY	FFPE	AIS, No gene defect		
149	46XY	FFPE	LHR W491*+insertion exon1		
150	46XY	FFPE	17B-HSD def 325+4 see 153, sister		
151	46XY	FFPE	CAIS L871R		
152	46XY	FFPE	CAIS L735F		
153	46XY	FFPE	17 B-HSD def 325+4 Zie ook 150, zus		
154	46XY	FFPE	17B-HSD def IVS3+4		
155	46XY	FFPE	17B-HSD def no mutation		
156	46XX	FFPE	sister 157, parents 158, 159		
157	46XY	FFPE	sister 156, parents 158, 159		
158	46XX	FFPE	Mother of 156, 157		
159	46XY	FFPE	Father of 156, 157		
160	46XX	FFPE			
161	46XY	FFPE	46XY DSD, SF1 mutation		
162	46XY	pBL	grandfather patient 167 and 168 (fathers side)		
163	46XX	pBL	grandmother patient 167 and 168 (mothers side)		
164	46XY	pBL	Grandfather patient 167 and 168 (mothers side)		
165	46XY	pBL	Father of 167 and 168		
166	46XX	pBL	Mother of 167 and 168		
167	46XX	pBL	sister 168		
168	46XX	pBL	sister 167		
169	46XY	FFPE	AIS		
170	46XY	FFPE	AIS		
171	46XY	FFPE + pBL	AIS		
172	46XY	FFPE + pBL	AIS		

Sample No	Karyotype	material	Diagnosis, Mut Found	pre- cursor/tumor/ zijdigheid	Age diagnosis
173	46XY	FFPE	46XY DSD, suspect SF1 mutation		
174	46XY	FFPE + pBL	AIS		
175	46XY	FFPE	17BHSD R80D		
176	46XY	FFPE	AIS		
177	46XY	pBL	46XY, AIS, AR mutation Pro 892 Ser.		
178	46XY	FFPE			
179	46XX	FFPE + pBL			
180	46XY	FFPE + pBL			
181	46XY	FFPE			
182	46XX	FFPE + pBL			
183	46XY	FFPE			
184	46XY	FFPE			
185	46 XY	FFPE			
186	46XX	FFPE			
187	46XY	FFPE			
188	46XX	FFPE			
189	46XY	FFPE		GB	
190	46XY	FFPE		GB, pre-GCNIS/GCNIS	
191	46XY		CAIS	re,li GCNIS	

Appendix 8: Genotyping data

Total cohort, risk alleles in red

Genotype	CT	AG	GT	AT	CT	AG	GT	CT	AG	CT	AG	CT	AG	CT	AG	CT	AG	CT	AG
Risk allele	C	A	T	T	C	A	T	C	A	T	A	T	A	T	A	T	A	T	A
OR per allele	1.22	1.47	1.53	1.19	1.26	1.27	1.27	1.31	1.31	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Nearby gene	ATF7IP	SPRY4	BAK1	HPGDS	PPM1E	REFW3	TEX14	MAD1L1	UCK2	CENPE	TER1	TER1	TER1	TER1	TER1	TER1	TER1	TER1	TER1
SNP ID	rs2900333	rs4624820	rs210138	rs17021463	rs7221274	rs4888262	rs9905704	rs12899477	rs4657482	rs4639052	rs2736100	rs4635969	rs755383	rs7040024	rs995030	rs1308595	rs4590952	rs4590952	rs4590952
1	TT	AG	AG	GG	AA	CC	TT	TT	AG	CC	CC	GG	TT	AA	GG	AG	AG	AG	AG
2	CT	AG	AA	GT	GG	CT	GT	CT	AA	CC	CC	AG	TT	AA	GG	GG	GG	GG	GG
3	CC	AG	AA	GT	GG	CT	GT	CT	GG	TT	CC	AG	TT	AA	GG	AG	AG	AG	AG
4	CC	AG	AA	TT	AA	CT	TT	TT	GG	TT	CT	AA	TT	AA	GG	GG	GG	GG	GG
5	CC	AG	AA	GT	AA	CT	TT	TT	GG	CC	CC	GG	TT	AA	AG	AG	AG	AG	AG
6	CC	AG	AA	GG	AG	CT	TT	TT	AG	TT	CC	GG	CC	AC	GG	GG	GG	GG	GG
7	CT	AG	AA	GT	AA	TT	TT	TT	AG	CC	AC	GG	CT	CC	GG	GG	GG	GG	GG
8	CT	AG	AG	GG	AG	CT	GT	TT	GG	TT	AC	AG	CC	CC	GG	GG	GG	GG	GG
9	CC	AG	AG	GG	AA	CC	TT	TT	AA	CC	AC	GG	CC	AC	GG	AG	AG	AG	AG
10	CC	AG	AA	GG	AA	CC	TT	CC	GG	CC	AC	AG	CT	AA	GG	GG	GG	GG	GG
11	CT	AA	AA	GT	AG	CC	GT	TT	AG	CC	CC	GG	TT	AA	GG	GG	GG	GG	GG
12	CC	GG	AA	TT	AA	CT	TT	TT	AA	CC	CC	GG	CT	AA	GG	AG	AG	AG	AG
13	CC	GG	AA	TT	AA	CT	TT	TT	AA	CC	CC	GG	CT	AA	GG	AG	AG	AG	AG
15	CT	AG	AG	GG	AA	TT	GT	CC	AA	CT	AA	GG	CT	AA	AG	AG	AG	AG	AG
16	CC	AA	AA	GT	GG	CT	TT	TT	AG	CC	AC	AG	CT	AA	GG	GG	GG	GG	GG
17	CC	AG	AA	GG	AG	CT	GT	TT	GG	CT	CC	AG	CT	AC	GG	GG	GG	GG	GG
18	CT	AA	AA	GT	AG	CT	GT	CT	GG	CC	CC	GG	CT	AC	GG	GG	GG	GG	GG
19	CT	GG	AA	GT	AA	TT	TT	TT	GG	CC	CC	GG	CT	AA	GG	GG	GG	GG	GG
20	CT	GG	AA	GG	AA	CC	TT	CT	GG	TT	AA	GG	TT	AA	GG	AG	AG	AG	AG
21	CT	GG	GG	TT	AG	TT	TT	CT	AG	TT	AA	GG	TT	AA	GG	GG	GG	GG	GG
22	CT	AA	AG	GG	AA	CT	TT	TT	AA	CT	AC	AG	CT	AC	GG	GG	GG	GG	GG
23	CC	AA	GG	GG	GG	CC	GG	TT	AA	CC	AA	GG	CC	AC	GG	GG	GG	GG	GG
24	CC	AG	AG	GT	AA	CC	TT	TT	AA	CC	AA	GG	CC	AC	GG	GG	GG	GG	GG
25	CC	GG	AG	GG	AG	CT	GT	CC	GG	CT	AC	GG	CT	AC	GG	GG	GG	GG	GG
26	CT	AG	AA	GT	AG	TT	GT	CT	AG	CT	CC	AG	TT	AC	GG	GG	GG	GG	GG
27	CT	AG	AG	TT	GG	CC	GT	TT	AG	CC	CC	GG	TT	AA	GG	GG	GG	GG	GG
28	CT	GG	AA	GT	AA	CT	TT	TT	AG	CT	AC	GG	TT	AA	GG	GG	GG	GG	GG
29	CC	GG	AG	GG	AG	CT	TT	CT	AG	CT	CC	GG	TT	AA	GG	AG	AG	AG	AG
30	CC	GG	AG	GT	AA	CT	TT	CT	AG	CT	AA	AG	CT	AA	AG	AG	AG	AG	AG
31	CT	AG	AA	GT	AA	CT	TT	TT	AG	CT	AA	AG	TT	AA	GG	GG	GG	GG	GG
32	CC	AG	AG	TT	AG	TT	GT	CT	AG	CC	CC	AG	CT	AA	GG	GG	GG	GG	GG
33	CC	AG	AA	GG	AG	CT	TT	TT	GG	TT	AC	GG	TT	AA	GG	GG	GG	GG	GG
34	CC	AA	AA	GG	AA	CT	TT	TT	GG	CT	AC	AG	TT	AA	AG	AG	AG	AG	AG
35	TT	GG	AA	GG	GG	CC	GG	TT	AG	CT	CC	GG	CT	AA	AG	AG	AG	AG	AG
36	CT	AG	AG	GT	AG	CT	GT	CT	AG	CT	AA	AG	CT	AA	AG	AG	AG	AG	AG
37	CT	GG	AA	GG	AG	CT	GT	CT	AG	CT	AA	AG	CT	AA	AG	AG	AG	AG	AG
38	CT	GG	AA	GT	AA	CT	GT	TT	AG	CT	AC	GG	TT	AA	AG	GG	GG	GG	GG
39	CT	AG	AA	GT	AA	CT	TT	TT	GG	CT	AC	GG	TT	AA	GG	GG	GG	GG	GG
40	CT	GG	GG	GT	AA	CT	TT	CT	GG	CC	AA	GG	CT	AC	GG	GG	GG	GG	GG
41	CC	AA	AA	GT	GG	CT	TT	CT	GG	CC	AC	AG	CT	AC	GG	GG	GG	GG	GG
42	CC	AG	AG	GT	AG	CT	GT	TT	AG	CC	CC	GG	CT	AA	GG	GG	GG	GG	GG
43	CT	AG	AG	GT	GG	CC	TT	CT	AG	CT	CC	AG	CT	AA	GG	GG	GG	GG	GG
44	CT	GG	AA	GT	AA	CT	TT	TT	AA	CC	AC	GG	CC	CC	AG	AG	AG	AG	AG
45	CC	AG	AA	GT	AA	CT	TT	TT	AG	CC	AA	GG	CT	AA	GG	GG	GG	GG	GG
46	CC	AG	GG	GG	GG	CC	GT	CC	AA	CT	CC	AG	CT	AA	GG	GG	GG	GG	GG
47	TT	AG	AA	GT	AG	CT	GT	CC	AG	CT	CC	GG	CT	AC	AG	AG	AG	AG	AG

AIS cohort, risk alleles in red

[illegible]

Appendix 9: Chi-square test, total 46-XY DSD cohort

rs2900333

Crosstab

			Affected		Total
			no	yes	
rs2900333	0	Count	13	4	17
		% within Affected	12,7%	13,3%	12,9%
	1,23	Count	49	8	57
		% within Affected	48,0%	26,7%	43,2%
	2,46	Count	40	18	58
		% within Affected	39,2%	60,0%	43,9%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4,738 ^a	2	,094

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 3,86.

rs4624820

Crosstab

			Affected		Total
			no	yes	
rs4624820	0	Count	23	5	28
		% within Affected	22,5%	16,7%	21,2%
	1,47	Count	53	17	70
		% within Affected	52,0%	56,7%	53,0%
	2,94	Count	26	8	34
		% within Affected	25,5%	26,7%	25,8%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	,487 ^a	2	,784

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 6,36.

rs210138

Crosstab

			Affected		Total
			no	yes	
rs210138	0	Count	55	25	80
		% within Affected	53,9%	83,3%	60,6%
	1,53	Count	37	5	42
		% within Affected	36,3%	16,7%	31,8%
	3,06	Count	10	0	10
		% within Affected	9,8%	0,0%	7,6%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9,051 ^a	2	,011

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 2,27.

rs1702146

Crosstab

			Affected		Total
			no	yes	
rs17021463	0	Count	33	10	43
		% within Affected	32,4%	33,3%	32,6%
	1,19	Count	54	13	67
		% within Affected	52,9%	43,3%	50,8%
	2,38	Count	15	7	22
		% within Affected	14,7%	23,3%	16,7%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,464 ^a	2	,481

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,00.

rs7221274

Crosstab

			Affected		Total
			no	yes	
rs7221274	0	Count	19	7	26
		% within Affected	18,6%	23,3%	19,7%
	1,20	Count	45	10	55
		% within Affected	44,1%	33,3%	41,7%
	2,40	Count	38	13	51
		% within Affected	37,3%	43,3%	38,6%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,129 ^a	2	,569

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,91.

rs4888262

Crosstab

			Affected		Total
			no	yes	
rs4888262	0	Count	23	5	28
		% within Affected	22,5%	16,7%	21,2%
	1,26	Count	49	13	62
		% within Affected	48,0%	43,3%	47,0%
	2,52	Count	30	12	42
		% within Affected	29,4%	40,0%	31,8%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,304 ^a	2	,521

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 6,36.

rs9905704

Crosstab

			Affected		Total
			no	yes	
rs9905704	0	Count	10	3	13
		% within Affected	9,8%	10,0%	9,8%
	1,27	Count	44	8	52
		% within Affected	43,1%	26,7%	39,4%
	2,54	Count	48	19	67
		% within Affected	47,1%	63,3%	50,8%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,807 ^a	2	,246

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 2,95.

rs12699477

Crosstab

			Affected		Total
			no	yes	
rs12699477	0	Count	46	13	59
		% within Affected	45,1%	43,3%	44,7%
	1,21	Count	46	13	59
		% within Affected	45,1%	43,3%	44,7%
	2,42	Count	10	4	14
		% within Affected	9,8%	13,3%	10,6%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	,305 ^a	2	,859

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 3,18.

rs4657482

Crosstab

			Affected		Total
			no	yes	
rs4657482	0	Count	39	10	49
		% within Affected	38,2%	33,3%	37,1%
	1,31	Count	52	12	64
		% within Affected	51,0%	40,0%	48,5%
	2,62	Count	11	8	19
		% within Affected	10,8%	26,7%	14,4%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4,789 ^a	2	,091

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 4,32.

rs4699052

Crosstab

			Affected		Total
			no	yes	
rs4699052	0	Count	16	7	23
		% within Affected	15,7%	23,3%	17,4%
	1,27	Count	49	14	63
		% within Affected	48,0%	46,7%	47,7%
	2,54	Count	37	9	46
		% within Affected	36,3%	30,0%	34,8%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,049 ^a	2	,592

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,23.

rs2736100

Crosstab

			Affected		Total
			no	yes	
rs2736100	0	Count	27	11	38
		% within Affected	26,5%	36,7%	28,8%
	1,35	Count	55	13	68
		% within Affected	53,9%	43,3%	51,5%
	2,70	Count	20	6	26
		% within Affected	19,6%	20,0%	19,7%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,343 ^a	2	,511

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,91.

rs4635969

Crosstab

			Affected		Total
			no	yes	
rs4635969	0	Count	72	15	87
		% within Affected	70,6%	50,0%	65,9%
	1,56	Count	28	12	40
		% within Affected	27,5%	40,0%	30,3%
	3,12	Count	2	3	5
		% within Affected	2,0%	10,0%	3,8%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6,651 ^a	2	,036

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is 1,14.

rs755383

Crosstab

			Affected		Total
			no	yes	
rs755383	0	Count	15	2	17
		% within Affected	14,7%	6,7%	12,9%
	1,49	Count	48	19	67
		% within Affected	47,1%	63,3%	50,8%
	2,98	Count	39	9	48
		% within Affected	38,2%	30,0%	36,4%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,805 ^a	2	,246

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 3,86.

rs7040024

Crosstab

			Affected		Total
			no	yes	
rs7040024	0	Count	8	4	12
		% within Affected	7,8%	13,3%	9,1%
	1,70	Count	39	9	48
		% within Affected	38,2%	30,0%	36,4%
	3,40	Count	55	17	72
		% within Affected	53,9%	56,7%	54,5%
Total	Count		102	30	132
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,233 ^a	2	,540

a. 1 cells (16,7%) have expected count less than 5. The minimum expected count is 2,73.

rs995030

Crosstab

			Affected		Total
			no	yes	
rs995030	0	Count	3	0	3
		% within Affected	2,9%	0,0%	2,3%
	2,38	Count	37	1	38
		% within Affected	36,3%	3,3%	28,8%
	4,76	Count	62	29	91
		% within Affected	60,8%	96,7%	68,9%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13,950 ^a	2	,001

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is ,68.

rs1508595

Crosstab

			Affected		Total
			no	yes	
rs1508595	0	Count	3	2	5
		% within Affected	2,9%	6,7%	3,8%
	2,56	Count	35	4	39
		% within Affected	34,3%	13,3%	29,5%
	5,12	Count	64	24	88
		% within Affected	62,7%	80,0%	66,7%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5,338 ^a	2	,069

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is 1,14.

rs4590952

Crosstab

			Affected		Total
			no	yes	
rs4590952	0	Count	3	1	4
		% within Affected	2,9%	3,3%	3,0%
	2,46	Count	42	6	48
		% within Affected	41,2%	20,0%	36,4%
	4,82	Count	57	23	80
		% within Affected	55,9%	76,7%	60,6%
Total		Count	102	30	132
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4,523 ^a	2	,104

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is ,91.

Appendix 10: Chi-square test, AIS cohort

rs2900333

Crosstab

			Affected		Total
			no	yes	
rs2900333	0	Count	8	0	8
		% within Affected	17,4%	0,0%	15,1%
	1,23	Count	20	2	22
		% within Affected	43,5%	28,6%	41,5%
	2,46	Count	18	5	23
		% within Affected	39,1%	71,4%	43,4%
Total	Count	46	7	53	
	% within Affected	100,0%	100,0%	100,0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3,003 ^a	2	,223

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,06.

rs4624820

Crosstab

			Affected		Total
			no	yes	
rs4624820	0	Count	13	0	13
		% within Affected	28,3%	0,0%	24,5%
	1,47	Count	25	3	28
		% within Affected	54,3%	42,9%	52,8%
	2,94	Count	8	4	12
		% within Affected	17,4%	57,1%	22,6%
Total	Count	46	7	53	
	% within Affected	100,0%	100,0%	100,0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6,370 ^a	2	,041

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,58.

rs210138

Crosstab

			Affected		Total
			no	yes	
rs210138	0	Count	27	6	33
		% within Affected	58,7%	85,7%	62,3%
	1,53	Count	13	1	14
		% within Affected	28,3%	14,3%	26,4%
	3,06	Count	6	0	6
		% within Affected	13,0%	0,0%	11,3%
	Total	Count	46	7	53
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,075 ^a	2	,354

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is ,79.

rs17021463

Crosstab

			Affected		Total
			no	yes	
rs17021463	0	Count	14	1	15
		% within Affected	30,4%	14,3%	28,3%
	1,19	Count	25	2	27
		% within Affected	54,3%	28,6%	50,9%
	2,38	Count	7	4	11
		% within Affected	15,2%	57,1%	20,8%
	Total	Count	46	7	53
		% within Affected	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6,498 ^a	2	,039

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,45.

rs7221274

Crosstab

			Affected		Total
			no	yes	
rs7221274	0	Count	12	1	13
		% within Affected	26,1%	14,3%	24,5%
	1	Count	22	2	24
		% within Affected	47,8%	28,6%	45,3%
	2	Count	12	4	16
		% within Affected	26,1%	57,1%	30,2%
Total	Count	46	7	53	
	% within Affected	100,0%	100,0%	100,0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,783 ^a	2	,249

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,72.

rs9905704

Crosstab

			Affected		Total
			no	yes	
rs9905704	0	Count	8	1	9
		% within Affected	17,4%	14,3%	17,0%
	1	Count	22	2	24
		% within Affected	47,8%	28,6%	45,3%
	2	Count	16	4	20
		% within Affected	34,8%	57,1%	37,7%
Total	Count	46	7	53	
	% within Affected	100,0%	100,0%	100,0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,337 ^a	2	,513

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,19.

rs4888262

Crosstab

			Affected		Total
			no	yes	
rs4888262	0	Count	8	4	12
		% within Affected	17,4%	57,1%	22,6%
	1	Count	22	1	23
		% within Affected	47,8%	14,3%	43,4%
	2	Count	16	2	18
		% within Affected	34,8%	28,6%	34,0%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5,884 ^a	2	,053

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,58.

rs4657482

Crosstab

			Affected		Total
			no	yes	
rs4657482	0	Count	17	2	19
		% within Affected	37,0%	28,6%	35,8%
	1	Count	24	2	26
		% within Affected	52,2%	28,6%	49,1%
	2	Count	5	3	8
		% within Affected	10,9%	42,9%	15,1%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4,927 ^a	2	,085

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,06.

rs12699477

Crosstab

			Affected		Total
			no	yes	
rs12699477	0	Count	20	3	23
		% within Affected	43,5%	42,9%	43,4%
	1	Count	19	2	21
		% within Affected	41,3%	28,6%	39,6%
	2	Count	7	2	9
		% within Affected	15,2%	28,6%	17,0%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	,887 ^a	2	,642

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,19.

rs4699052

Crosstab

			Affected		Total
			no	yes	
rs4699052	0	Count	9	0	9
		% within Affected	19,6%	0,0%	17,0%
	1	Count	19	1	20
		% within Affected	41,3%	14,3%	37,7%
	2	Count	18	6	24
		% within Affected	39,1%	85,7%	45,3%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5,456 ^a	2	,065

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,19.

rs2736100

Crosstab

			Affected		Total
			no	yes	
rs2736100	0	Count	9	1	10
		% within Affected	19,6%	14,3%	18,9%
	1	Count	25	2	27
		% within Affected	54,3%	28,6%	50,9%
	2	Count	12	4	16
		% within Affected	26,1%	57,1%	30,2%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,823 ^a	2	,244

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,32.

rs4635969

Crosstab

			Affected		Total
			no	yes	
rs4635969	0	Count	37	1	38
		% within Affected	80,4%	14,3%	71,7%
	1	Count	8	4	12
		% within Affected	17,4%	57,1%	22,6%
	2	Count	1	2	3
		% within Affected	2,2%	28,6%	5,7%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15,427 ^a	2	,000

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is ,40.

rs755383

Crosstab

			Affected		Total
			no	yes	
rs755383	0	Count	9	0	9
		% within Affected	19,6%	0,0%	17,0%
	1	Count	24	6	30
		% within Affected	52,2%	85,7%	56,6%
	2	Count	13	1	14
		% within Affected	28,3%	14,3%	26,4%
Total	Count	46	7	53	
	% within Affected	100,0%	100,0%	100,0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3,026 ^a	2	,220

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,19.

rs7040024

Crosstab

			Affected		Total
			no	yes	
rs7040024	0	Count	4	0	4
		% within Affected	8,7%	0,0%	7,5%
	1	Count	21	2	23
		% within Affected	45,7%	28,6%	43,4%
	2	Count	21	5	26
		% within Affected	45,7%	71,4%	49,1%
Total	Count	46	7	53	
	% within Affected	100,0%	100,0%	100,0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,840 ^a	2	,399

a. 4 cells (66,7%) have expected count less than 5. The minimum expected count is ,53.

rs995030

Crosstab

			Affected		Total
			no	yes	
rs995030	0	Count	2	0	2
		% within Affected	4,3%	0,0%	3,8%
	1	Count	11	1	12
		% within Affected	23,9%	14,3%	22,6%
	2	Count	33	6	39
		% within Affected	71,7%	85,7%	73,6%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	,714 ^a	2	,700

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is ,26.

rs1508595

Crosstab

			Affected		Total
			no	yes	
rs1508595	0	Count	2	2	4
		% within Affected	4,3%	28,6%	7,5%
	1	Count	10	1	11
		% within Affected	21,7%	14,3%	20,8%
	2	Count	34	4	38
		% within Affected	73,9%	57,1%	71,7%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5,125 ^a	2	,077

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is ,53.

rs4590952

Crosstab

			Affected		Total
			no	yes	
rs4590952	0	Count	2	1	3
		% within Affected	4,3%	14,3%	5,7%
	1	Count	12	2	14
		% within Affected	26,1%	28,6%	26,4%
	2	Count	32	4	36
		% within Affected	69,6%	57,1%	67,9%
Total	Count		46	7	53
	% within Affected		100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1,212 ^a	2	,545

a. 4 cells (66,7%) have expected count less than 5. The minimum expected count is ,40.