Clonal hematopoiesis of indeterminate potential and diabetic kidney disease: a nested case-control study

Supplementary Material

Supplementary tables and figures

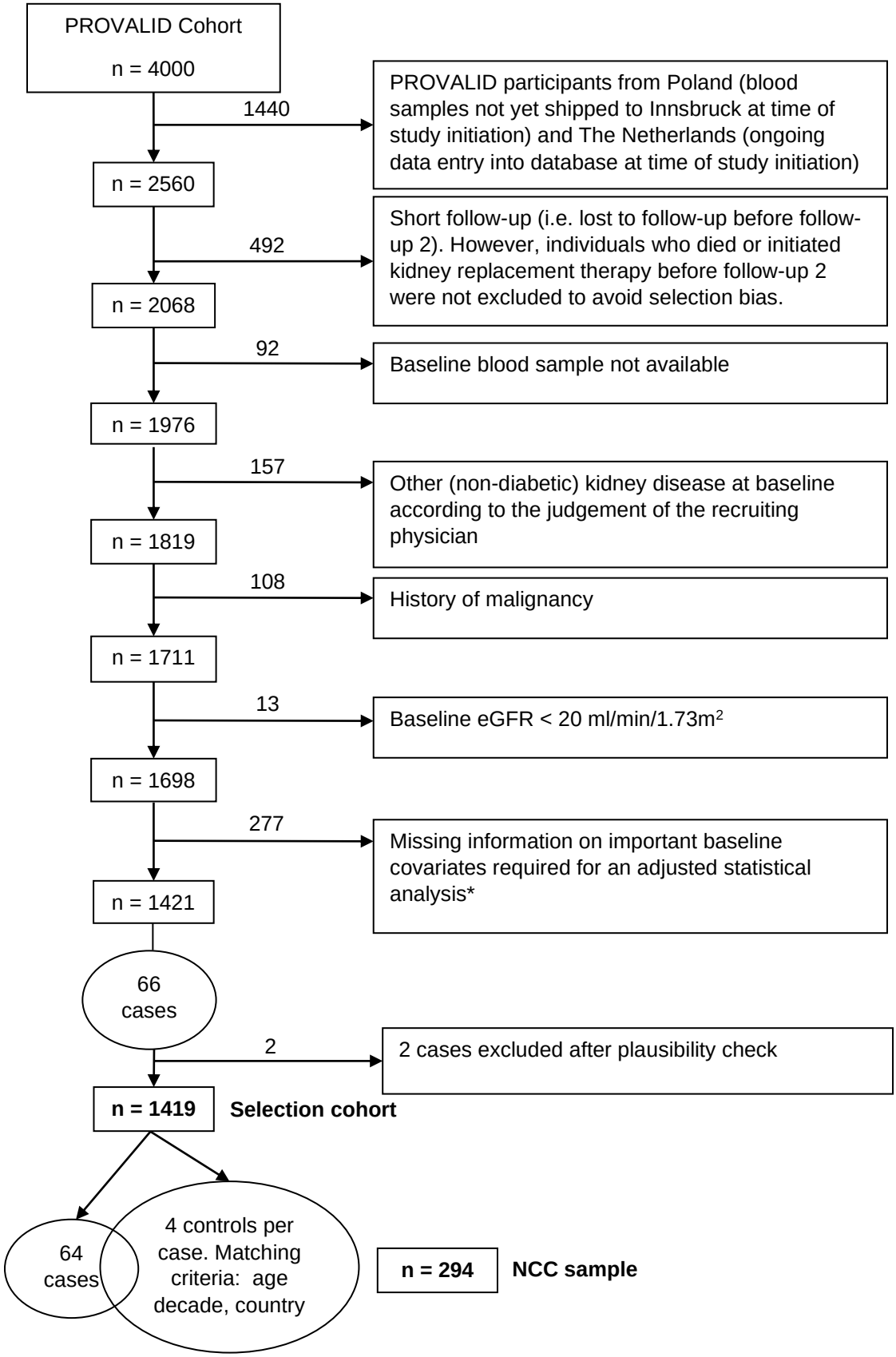
Table of content:

Supplementary Table S1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
– Incident or prevalent T2DM (inclusion criterion of PROVALID)	– Active malignancy (exclusion criterion of PROVALID)
– Age > 18 years (inclusion criterion of PROVALID)	– Any history of malignancy at baseline
– Available baseline peripheral blood samples	– Other (non-diabetic) kidney disease at baseline according to the judgement of the recruiting physician
– Complete information on important baseline covariates* required for the adjusted statistical analysis	– Baseline eGFR < 20 ml/min/1.73m^2
	– Short follow-up (i.e. lost to follow-up before follow-up 2). However, individuals who died or initiated kidney replacement therapy before follow-up 2 were not excluded to avoid selection bias.

*BL covariates: date of visit, country, gender, age, year of T2DM diagnosis, smoking, systolic and diastolic blood pressure, HbA1C, serum cholesterol (total), serum cholesterol (LDL), serum triglycerides, serum cholesterol (HDL), hemoglobin, body weight, height, eGFR, albuminuria, C-reactive protein (CRP), history of heart failure stage III or IV, history of coronary artery disease (any angina, myocardial infarction, coronary intervention), history of peripheral artery disease (claudicatio, amputation, etc.), history of cerebrovascular disease (stroke, transient ischemic attack, prolonged reversible ischemic neurological deficit), history of diabetic retinopathy. T2DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate.

Supplementary Figure S1: Selection of the study population



*BL covariates: date of visit, country, gender, age, year of T2DM diagnosis, smoking, systolic and diastolic blood pressure, HbA1C, serum cholesterol (total), serum cholesterol (LDL), serum triglycerides, serum cholesterol (HDL), hemoglobin, body weight, height, eGFR, albuminuria, C-reactive protein (CRP), history of heart failure stage III or IV, history of coronary artery disease (any angina, myocardial infarction, coronary intervention), history of peripheral artery disease (claudicatio, amputation, etc.), history of cerebrovascular disease (stroke, transient ischemic attack, prolonged reversible ischemic neurological deficit), history of diabetic retinopathy T2DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate.

Supplementary Table S2: Components of the composite endpoint

Component	**Number**
– Decline in eGFR of ≥ 40% from baseline to < 60 ml/min/1,73m^2	25*
– Progression to macroalbuminuria including a 30% increase in the mean UACR from baseline	32*
– Initiation of kidney replacement therapy	7
– Death from kidney failure	2

* both events occurred simultaneously in two patients. UACR, urine albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

Supplementary Table S3: Next generation sequencing panel

Genes	Chromosomes	Exons	NM-Nr.
ABL1	9	4-6	NM_007313.3
ASXL1	20	13	NM_015338.5
ATRX	X	8, 10-11,17-31	NM_000489.5
BCOR	X	2-15	NM_001123385.1
BCORL1	X	1-12	NM_021946.4
BRAF	7	15	NM_004333
CBL	11	8-9	NM_005188.3
CBLC	19	9, 11	NM_012116.4
CDKN2A	9	1-3	NM_000077.4
CEBPA	19	1	NM_004364.4
CUX1	7	1-24	NM_001202543
DNMT3A	2	2-23	NM_022552.4
ETV6	12	1-8	NM_001987
EZH2	7	2-20	NM_004456.4
FBXW7	4	9-11	NM_033632
FLT3	13	13-15, 20	NM_004119.2
GATA2	3	2-6	NM_032638.4
GNAS	20	8-10	NM_000516.4
IDH1	2	4	NM_005896.3
IDH2	15	4	NM_002168.3
IKZF1	7	2-8	NM_006060.6
JAK2	9	12,14	NM_004972
JAK3	19	13	NM_00215
KDM6A	X	1-29	NM_021140
KIT	4	2, 8-11, 13, 17	NM_000222.2
KRAS	12	2-3	NM_033360.3
MPL	1	10	NM_005373.2
MYD88	3	3-5	NM_002468
NOTCH1	9	26-28, 34	NM_017617
NPM1	5	11	NM_002520.6
NRAS	1	2-3	NM_002524.4
PHF6	X	2-10	NM_032458.2
PTEN	10	5, 7	NM_000314.8
PTPN11	12	3, 13	NM_002834.4
RAD21	8	2-14	NM_006265
RUNX1	21	2-9	NM_001754.4
SF3B1	2	13-16	NM_012433.3

SMC1A	X	2, 11, 16-17	NM_006306.4
SMC3	10	10,13, 19, 23, 25, 28	NM_005445.4
SRSF2	17	1	NM_003016
STAG2	X	3-35	NM_001042749
TET2	4	3-11	NM_001127208.2
TP53	17	2-11	NM_000546.5
U2AF1	21	2, 6	NM_006758.2
WT1	11	7, 9	NM_024426.4
ZRSR2	X	1-11	NM_005089.3

Supplementary Table S4: Multiplex cytokine assay

Cytokines LEGENDplex Human Inflammation Panel 1
IL-1β
IFN-α2
IFN-γ
TNF-α
MCP-1
IL-6
IL-8
IL-10
IL-12p70
IL-17A
IL-18
IL-23
IL-33

Supplementary Table S5: Principal component analysis

Cytokine name	Weight in the first principal component
IL-1β [pg/ml]	0.059662112
IFN-α2 [pg/ml]	0.005544896
IFN-γ [pg/ml]	0.003802583
TNF-α [pg/ml]	0.020426208
MCP-1 [pg/ml]	0.195629598
IL-6 [pg/ml]	0.024016677
IL-8 [pg/ml]	0.172703265
IL-10 [pg/ml]	0.014054166
IL-12p70 [pg/ml]	0.005796645
IL-17A [pg/ml]	0.003693312
IL-18 [pg/ml]	0.962251449
IL-23 [pg/ml]	0.005871354
IL-33 [pg/ml]	0.033117263

Supplementary Table S6: List of identified CHIP-associated variants

Gene	Chromosome	Exon	Locus	Variant (cDNA)	Variant (Protein)	VAF (%)	Variant Reads	Total Coverage
ABL1	9	E4	133738393	c.851_852delinsTT	p.Ser284Ile	2.0	70	3500
ASXL1	20	E13	31022288	c.1773C>A	p.Tyr591Ter	2.6	73	2808
ASXL1	20	E13	31022287	c.1772dupA	p.Tyr591Ter	13	429	3300
ASXL1	20	E13	31022900	c.2385_2394delCTGGGAAAGT	p.Trp796Metfs*19	9.9	463	4677
ASXL1	20	E13	31022883	c.2368_2371delGAGT	p.Glu790Leufs*27	37	2694	7281
ASXL1	20	E13	31023632	c.3117delA	p.Gln1039Hisfs*8	6.6	145	2197
ATRX	X	E21	76874432	c.5290T>A	p.Phe1764Ile	2.3	418	18174
BCOR	X	E10	39921499	c.4321C>G	p.Gln1441Glu	2.7	15	556
BCOR	X	E4	39932954	c.1645G>A	p.Gly549Ser	9.8	744	7592
BCOR	X	E9	39922093	c.4079A>G	p.Lys1360Arg	2.3	20	870
BCORL1	X	E3	129149742	c.2994dupC	p.Thr999Hisfs*7	7.8	177	2269
BCORL1	X	E3	129147736	c.988C>A	p.Leu330Ile	2	18	900
BCORL1	X	E3	129147709	c.961G>A	p.Val321Met	2.4	24	1000
BCORL1	X	E3	129147730	c.982T>A	p.Leu328Met	2.5	15	600
BCORL1	X	E3	129147217	c.469G>A	p.Ala157Thr	11	674	6127
BCORL1	X	E3	129149473	c.2725C>T	p.Arg909Trp	7.2	382	5306
BCORL1	X	E6	129159337	c.4061G>A	p.Arg1354Gln	41	1630	3976
BCORL1	X	E3	129149919	c.3171T>A	p.Asp1057Glu	2	50	2500
BCORL1	X	E3	129147916	c.1168T>C	p.Phe390Leu	2.1	48	2286
BCORL1	X	E3	129147695	c.947delT	p.Leu316Profs*102	2.2	16	727
BRAF	7	E15	140453154	c.1781A>G	p.Asp594Gly	12	1553	12942
CBL	11	E8	119148991	c.1211G>A	p.Cys404Tyr	16	1492	9325
CEBPA	19	E1	33792728	c.558_593delGCCGCCGCCCTCGCACCCGCACCCGCACCCGCCGCC	p.Pro187_Pro198del	9.8	17	173
CEBPA	19	E1	33792754	c.564_566dupGCC	p.Pro189dup	26	23	88
CEBPA	19	E1	33792727	c.556_594delCCGCCGCCGCCCTCGCACCCGCACCCGCACCCGCCGCCC	p.Pro186_Pro198del	2.1	17	810
CEBPA	19	E1	33792714	c.530_607delGCCTCTTCCCTTACCAGCCGCCGCCGCCGC CGCCGCCCTCGCACCCGCACCCGCAC	p.Gly177_Ala202del	17	16	94

				CCGCCGCCCGCGCACCTGGCCG				
CEBPA	19	E1	33792728	c.558_593delGCCGCCGCCCTCGCACCCGCACCCGCACCCGCCGCC	p.Pro187_Pro198del	4.8	16	333
CUX1	7	E6	101747699	c.523A>G	p.Lys175Glu	9.3	17	183
DNMT3A	2	E16	25466767	c.1936G>A	p.Gly646Arg	2.1	19	905
DNMT3A	2	E16	25466800	c.1903C>T	p.Arg635Trp	3	18	600
DNMT3A	2	E6	25497918	c.531G>C	p.Glu177Asp	2.3	16	696
DNMT3A	2	E20	25462006	c.2401A>G	p.Met801Val	9.8	307	3133
DNMT3A	2	E21	25459805	c.2478G>A	p.Lys826=	2.1	57	2714
DNMT3A	2	E16	25466816	c.1870_1886dupCCACCTGTCCCAGCTGA	p.Glu629Aspfs*28	4.8	46	958
DNMT3A	2	E17	25464493	c.2020A>G	p.Met674Val	7.9	16	203
DNMT3A	2	E13	25468187	c.1485_1489delCTCCT	p.Ile495Metfs*49	8.6	249	2895
DNMT3A	2	E23	25457242	c.2645G>A	p.Arg882His	9	329	3656
DNMT3A	2	E15	25467205	c.1670G>A	p.Cys557Tyr	16	519	3244
DNMT3A	2	E8	25470557	c.917G>A	p.Trp306Ter	4.9	490	10000
DNMT3A	2	E19	25463289	c.2204A>T	p.Tyr735Phe	17	705	4147
DNMT3A	2	E23	25457288	c.2599delG	p.Val867Tyrfs*14	4.5	106	2356
DNMT3A	2	E16	25466799	c.1904G>A	p.Arg635Gln	14	133	950
DNMT3A	2	E18	25463562	c.2120G>A	p.Gly707Asp	2.6	78	3000
DNMT3A	2	E19	25463287	c.2206C>T	p.Arg736Cys	2.5	98	3920
DNMT3A	2	E16	25466770	c.1933A>G	p.Thr645Ala	4.4	47	1068
DNMT3A	2	E18	25463599	c.2083A>T	p.Ile695Phe	5	163	3260
DNMT3A	2	E19	25463181	c.2312G>A	p.Arg771Gln	19	332	1747
DNMT3A	2	E23	25457243	c.2644C>T	p.Arg882Cys	4.2	114	2714
DNMT3A	2	E21	25459806	c.2477A>G	p.Lys826Arg	3.6	111	3083
DNMT3A	2	E23	25457242	c.2645G>A	p.Arg882His	7.5	244	3253
DNMT3A	2	E9	25469976	c.1066C>T	p.Gln356Ter	3	30	1000
DNMT3A	2	E23	25457250	c.2637C>G	p.Asn879Lys	2.6	88	3385
DNMT3A	2	E13	25468141	c.1522_1535delCTCTTCGTTGGAGG	p.Leu508Asnfs*33	7.3	218	2986
DNMT3A	2	E15	25467119	c.1756T>A	p.Cys586Ser	2.1	56	2667
DNMT3A	2	E10	25469642	c.1126G>C	p.Ala376Pro	5	37	740

DNMT3A	2	E18	25463540	c.2140_2141dupTC	p.Ile715Profs*65	3	100	3333
DNMT3A	2	E8	25470497	c.977G>A	p.Arg326His	5.4	158	2926
DNMT3A	2	E19	25463184	c.2309C>T	p.Ser770Leu	7.4	259	3500
DNMT3A	2	E15	25467033	c.1841_1842delAC	p.Asp614Alafs*4	7.4	205	2770
DNMT3A	2	E19	25463289	c.2204A>G	p.Tyr735Cys	20	1209	6045
DNMT3A	2	E23	25457168	c.2719G>T	p.Glu907Ter	14	203	1450
DNMT3A	2	E8	25470498	c.976C>A	p.Arg326Ser	3.3	83	2515
DNMT3A	2	E23	25457243	c.2644C>T	p.Arg882Cys	2.2	85	3864
DNMT3A	2	E6	25497897	c.500_552delGGGGCCGGCTGCGGGGTGGCTTGGGCTGGGAGTCCAGCCTCCGTCAGCGGCCC	p.Arg167Hisfs*31	2.4	26	1083
DNMT3A	2	E10	25469605	c.1163delA	p.His388Profs*19	26	492	1892
DNMT3A	2	E23	25457243	c.2644C>T	p.Arg882Cys	28	1263	4511
DNMT3A	2	E11	25469044	c.1414delG	p.Asp472Metfs*179	3.9	114	2923
DNMT3A	2	E19	25463277	c.2216A>C	p.His739Pro	7.7	512	6649
DNMT3A	2	E21	25459809	c.2470_2473dupATAG	p.Ala825Aspfs*31	5	171	3420
DNMT3A	2	E16	25466800	c.1903C>T	p.Arg635Trp	5.4	42	778
DNMT3A	2	E9	25469968	c.1074delG	p.Tyr359Thrfs*48	8.9	150	1685
DNMT3A	2	E19	25463186	c.2307C>G	p.Ile769Met	9.3	386	4151
DNMT3A	2	E20	25462030	c.2377T>G	p.Tyr793Asp	2.7	142	5259
DNMT3A	2	E19	25463181	c.2312G>A	p.Arg771Gln	35	1191	3403
DNMT3A	2	E17	25464568	c.1945G>C	p.Val649Leu	17	31	182
DNMT3A	2	E9	25470011	c.1031T>C	p.Leu344Pro	3.1	65	2097
DNMT3A	2	E14	25467496	c.1580A>C	p.Gln527Pro	2.3	110	4783
EZH2	7	E2	148544341	c.50A>G	p.Lys17Arg	3.9	20	513
GNAS	20	E8	57484421	c.602G>A	p.Arg201His	13	49	377
IDH2	15	E4	90631934	c.419G>A	p.Arg140Gln	12	996	8300
KDM6A	X	E24	44945224	c.3548A>G	p.Lys1183Arg	2.3	26	1130
KDM6A	X	E17	44929161	c.2261T>C	p.Met754Thr	2	19	950
KDM6A	X	E1	44732865	c.68A>G	p.Lys23Arg	4.6	20	435
KRAS	12	E2	25398284	c.35G>T	p.Gly12Val	41	4340	10585

MYD88	3	E3	38182056	c.680A>G	p.Lys227Arg	2.2	18	818
NOTCH1	9	E26	139399222	c.4921C>T	p.Pro1641Ser	3.3	17	515
NOTCH1	9	E34	139390795	c.7396A>G	p.Thr2466Ala	3.1	20	645
PHF6	X	E2	133511703	c.56T>G	p.Phe19Cys	6.5	16	246
PHF6	X	E10	133559253	c.991G>A	p.Gly331Arg	4.1	151	3683
PHF6	X	E7	133547860	c.593G>A	p.Arg198Lys	2.6	108	4154
RAD21	8	E7	117868887	c.812C>G	p.Ser271Ter	2.1	119	5667
SMC1A	X	E17	53423482	c.2618T>C	p.Leu873Pro	2.2	21	955
SMC3	10	E28	112363035	c.3569A>G	p.Lys1190Arg	2.3	26	1130
SRSF2	17	E1	74732959	c.284C>A	p.Pro95His	11	136	1236
SRSF2	17	E1	74732959	c.284C>T	p.Pro95Leu	3.2	18	563
STAG2	X	E28	123215311	c.2857C>G	p.Arg953Gly	4.3	149	3465
TET2	4	E10	106193779	c.4241A>G	p.Gln1414Arg	5.5	370	6727
TET2	4	E11	106196930	c.5263G>T	p.Glu1755Ter	8.6	1172	13628
TET2	4	E8	106182971	c.4010dupA	p.Tyr1337Ter	5.3	203	3830
TET2	4	E3	106155427	c.328A>T	p.Lys110Ter	8.2	839	10232
TET2	4	E9	106190818	c.4096C>T	p.Arg1366Cys	12	398	3317
TET2	4	E3	106155675	c.576C>G	p.Tyr192Ter	11	1097	9973
TET2	4	E3	106158506	c.3407dupT	p.Glu1137Argfs*5	2.3	141	6130
TET2	4	E11	106196611	c.4944delC	p.Tyr1649Ilefs*46	16	2258	14113
TET2	4	E3	106156723	c.1624A>T	p.Lys542Ter	2.4	61	2542
TET2	4	E9	106190826	c.4104_4110delCTCAGGG	p.Phe1368Leufs*78	4.1	202	4927
TET2	4	E9	106190849	c.4127A>G	p.Asp1376Gly	15	705	4700
TET2	4	E7	106180784	c.3812dupG	p.Cys1271Trpfs*29	4.6	268	5826
TET2	4	E6	106164916	c.3784C>T	p.Arg1262Trp	2.3	83	3609
TET2	4	E11	106196213	c.4546C>T	p.Arg1516Ter	2.9	279	9621
TET2	4	E3	106155291	c.192_193insG	p.Cys65Valfs*8	3.9	160	4103
TET2	4	E9	106190819	c.4097G>A	p.Arg1366His	5.3	254	4792
TET2	4	E3	106157953	c.2854delC	p.Leu952Ter	3.2	447	13969
TET2	4	E3	106158372	c.3273delA	p.Pro1092Glnfs*14	5.4	531	9833
TP53	17	E5	7578446	c.484A>T	p.Ile162Phe	4	81	2025

TP53	17	E7	7577538	c.743G>A	p.Arg248Gln	3.8	489	12868
TP53	17	E4	7579448	c.213_239delCCCCGTGGCCCCTGCACCAGCAGCTCC	p.Pro72_Pro80del	2.3	38	1652
TP53	17	E4	7579394	c.293C>A	p.Pro98His	4	31	775
TP53	17	E4	7579350	c.337T>A	p.Phe113Ile	24	329	1371
TP53	17	E10	7574030	c.997C>G	p.Arg333Gly	3.8	15	395
TP53	17	E6	7578265	c.584T>C	p.Ile195Thr	2.3	57	2478
TP53	17	E5	7578395	c.535C>G	p.His179Asp	2.1	33	1571
U2AF1	21	E2	44524456	c.101C>T	p.Ser34Phe	4.8	995	20729
ZRSR2	X	E11	15841177	c.1261C>T	p.Arg421Ter	4.4	201	4568
ZRSR2	X	E11	15841259	c.1332_1343dupGAGCCGGAGCCG	p.Ser445_Arg448dup	88	382	434
ZRSR2	X	E4	15821882	c.275A>G	p.Lys92Arg	2.3	23	1000
ZRSR2	X	E4	15821888	c.281A>G	p.Glu94Gly	2.1	20	952

Data on intra- and inter-assay variation

Intra-assay variation (measured in duplicates)

Analyte	Sample	Mean (pg/ml)	SD	%CV
hIL-1β	Sample 1 (Patient A)	7.74	1.25	4.29
	Sample 2 (Patient B)	2.89	0.00	0.00
	Sample 3 (Patient C)	567.79	32.33	13.45
hIFN-α2	Sample 1	9.18	0.26	1.99
	Sample 2	3.64	0.30	2.77
	Sample 3	12.74	0.35	2.48
hIFN-γ	Sample 1	7.84	0.00	0.00
	Sample 2	7.28	0.56	6.73
	Sample 3	8.40	0.56	6.15
hTNF-α	Sample 1	11.69	2.43	11.79
	Sample 2	10.47	1.22	6.15
	Sample 3	3.33	0.00	0.00
hMCP-1	Sample 1	147.16	9.30	13.00
	Sample 2	468.73	20.24	13.52
	Sample 3	80.22	5.33	10.88
hIL-6	Sample 1	17.26	1.12	6.73
	Sample 2	10.03	1.31	9.43
	Sample 3	7.33	0.00	0.00
hIL-8	Sample 1	12.34	3.64	12.86
	Sample 2	11.61	2.91	10.48
	Sample 3	13.06	0.00	0.00
hIL-10	Sample 1	11.47	2.14	15.29
	Sample 2	2.55	0.96	14.14
	Sample 3	4.92	0.14	1.43
hIL-12p70	Sample 1	6.64	1.00	12.86
	Sample 2	1.54	0.10	10.88
	Sample 3	2.25	0.14	3.01
hIL-17A	Sample 1	1.81	0.30	8.84
	Sample 2	0.69	0.25	11.79
	Sample 3	0.58	0.03	1.52
hIL-18	Sample 1	411.30	19.91	8.77
	Sample 2	381.33	13.53	6.36
	Sample 3	226.62	27.63	19.64
hIL-23	Sample 1	9.03	0.81	4.56
	Sample 2	17.36	0.39	1.70
	Sample 3	3.00	0.40	3.01
hIL-33	Sample 1	55.17	1.67	2.24
	Sample 2	29.52	0.00	0.00
	Sample 3	79.38	6.26	7.25

Overall intra-assay variation depicted as median of %CV of each analyte

Analyte	%CV
hIL-1β	7.44
hIFN-α2	4.04
hIFN-γ	7.44
hTNF-α	8.32
hMCP-1	13.31
hIL-6	8.32
hIL-8	11.7
hIL-10	7.86
hIL-12p70	6.15
hIL-17A	8.84
hIL-18	11.9
hIL-23	3.82
hIL-33	5.62

Inter-assay variation (measured in triplicates)

Analyte	Sample	Mean (pg/ml)	SD	%CV
hIL-1β	Sample 1 (Patient D)	2.89	0.73	25.29
	Sample 2 (Patient E)	14.59	1.93	13.22
	Sample 3 (Patient F)	19.04	3.09	16.25
hIFN-α2	Sample 1	0.59	0.11	18.62
	Sample 2	0.45	0.03	6.29
	Sample 3	0.63	0.23	36.42
hIFN-γ	Sample 1	2.93	1.16	39.78
	Sample 2	3.88	0.18	4.55
	Sample 3	3.26	0.70	21.51
hTNF-α	Sample 1	1.51	0.11	7.25
	Sample 2	1.51	0.11	7.25
	Sample 3	2.07	0.89	43.10
hMCP-1	Sample 1	165.00	33.07	20.04
	Sample 2	251.66	60.74	24.14
	Sample 3	80.95	10.79	13.33
hIL-6	Sample 1	5.10	0.58	11.29
	Sample 2	4.15	1.14	27.51
	Sample 3	10.42	1.43	13.75
hIL-8	Sample 1	3.37	0.51	15.01
	Sample 2	3.37	0.51	15.01
	Sample 3	4.44	1.00	22.43

hIL-10	Sample 1	2.38	0.62	26.17
	Sample 2	1.22	0.12	9.73
	Sample 3	1.62	0.18	11.42
hIL-12p70	Sample 1	0.84	0.15	18.14
	Sample 2	0.95	0.11	11.97
	Sample 3	1.94	0.54	27.66
hIL-17A	Sample 1	0.48	0.03	5.32
	Sample 2	0.48	0.03	5.32
	Sample 3	0.62	0.22	35.40
hIL-18	Sample 1	34.73	1.12	3.21
	Sample 2	36.98	1.14	3.08
	Sample 3	89.12	6.87	7.71
hIL-23	Sample 1	1.09	0.14	13.12
	Sample 2	1.09	0.14	13.12
	Sample 3	1.19	0.00	0.00
hIL-33	Sample 1	3.93	0.89	22.75
	Sample 2	6.48	0.57	8.73
	Sample 3	2.52	0.35	14.03

Inter-assay variation (across 3 samples) depicted as median of %CV of each analyte

Analyte	**%CV**
hIL-1β	16.25
hIFN-α2	18.62
hIFN-γ	21.51
hTNF-α	7.25
hMCP-1	20.04
hIL-6	13.75
hIL-8	15.01
hIL-10	11.42
hIL-12p70	18.14
hIL-17A	5.32
hIL-18	3.21
hIL-23	13.12
hIL-33	14.03

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3, 4
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 5,6
Methods			
Study design	4	Present key elements of study design early in the paper	5, 6, 8, 9, 10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5, Supplement page no. 2, 3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5, 6, 7, 8, 9, 10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6,7 ,8
Bias	9	Describe any efforts to address potential sources of bias	9, 10
Study size	10	Explain how the study size was arrived at	5, 6, Supplement

			page no. 2, 3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 9, 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	5, 9, Supplement page no. 2, 3
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5, 9
		(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Supplement page no. 3
		(b) Give reasons for non-participation at each stage	Supplement page no. 3
		(c) Consider use of a flow diagram	Supplement page no. 3
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10, 27, 28
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	6, Supplement page no. 4
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	10
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10, 11, 12
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15,16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12, 13, 14, 15
Generalisability	21	Discuss the generalisability (external validity) of the study results	12, 13, 15
Other information			

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16, 17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.