Normal ranges and genetic variants of antithrombin, protein C and protein S in the general Chinese population. Results of the *Chinese Hemostasis Investigation on Natural Anticoagulants* Study I Group

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Supplementary data

Detailed description of the statistical analysis

Normal-based centile curves are given by $C_p = \mu_T \pm u_p\sigma_T$, where T is continuous age expressed in years, μ_T and σ_T indicate the corresponding values of mean and standard deviation (SD) at age T, and up the corresponding centile of the standard Gaussian distribution. Initially, a smooth running line of the measurement of interest Y [protein activities (PC, PS and AT) and APC-R] against age T depending on sex provides an impression of the shape of the mean curve and also examines the need to model each curve separately by sex.

To reduce positive skewness and heteroscedasticity of the measurement of interest, Y, an initial Box-Cox transformation is applied with natural logarithmic transformation being a particular case. A fractional polynomial of degree m is then fitted by least squares regression of the measurement of interest against age to create a suitable function for μ_T with the powers as numbers chosen from the set {–2, –1, –0.5, 0, 0.5, 1, 2, 3}, where T^0 denotes the natural logarithm of T. An appropriate fractional polynomial is selected by comparing the difference in deviance between models having degrees m and m+1 using a χ^2 variate on 2 degrees of freedom (DF). The fitted values from the regression give the estimated mean curve and the 'scaled absolute residuals' σ_T. If the scaled absolute residuals appear to show no trend with age, the SD is estimated as that of the residual of the measurement of interest from the regression on age, otherwise fractional polynomial regression analysis is performed to estimate an appropriate curve in the same way as for the mean. The model fit is assessed by calculating the standard deviation scores (Z-score) as $Z\_score=\frac{Y-\mu_T}{\sigma_T}$. The ordered Z-scores are plotted in order to provide a graphical check of normality (QQ-plots). If normality is accepted, no further modeling is required. Estimated centile and reference intervals are calculated by substituting the fitted curves of the mean and standard deviation into equation of C_p. When the variable being modeled, Y, is initially Box-Cox transformed, centiles curves on the original scale are obtained by applying a back-transformation to the calculated curves, $C_{original\ scale} = \left[1+\theta C_p(t)\right]^{\frac{1}{\theta}}$ with θ being the parameter of the Box-Cox transformation. Each curve is derived according to sex, except the curve for APC-R since no difference was seen.

Online Supplementary Table S1. Equations for estimating reference values for PC activity (A for men and B for women), PS activity (C for men and D for women), AT activity (E for men and F for women) and APC-R for all (G).

A	$_{\ln(Y)} = 1.876171 + 1.258097\ age^{0.5} - 0.21849\ age^{0.5}\ \ln(age)$ $\sigma_{\ln(Y)} = 0.1725551$	**B**	$_B = 7.306372 + 0.0000214\ age^3 - 0.00000465\ age^3\ \ln(age)$ $\sigma_B = 0.4339694$ $\theta = 0.19063$
C	$_B = 35.22298 + 0.0018746\ age^2 - 0.0000367\ age^3$ $\sigma_B = 5.107575$ $\theta = 0.7014403$	**D**	$_B = 29.1174 + 45.35696\ age^{-0.5} - 34.78935\ age^{-0.5}\ \ln(age)$ $\sigma_B = 1.9457$ $\theta = 0.4898306$
E	$_B = 76972.75 - 0.0455784\ age^3$ $\sigma_B = 16398.51$ $\theta = 2.585277$	**F**	$_B = 4755.6 + 3274359\ age^{-2} - 1144520\ age^{-2}\ \ln(age)$ $\sigma_B = 3962.369 - 1564.426\ age^{0.5} + 282.8304\ age^{0.5}\ \ln(age)$ $\theta = 1.913772$
G	$_B = 2.657075 - 692.7044\ age^{-2} + 465.2072\ age^{-2}\ \ln(age) - 79.80275\ age^{-2}\ \ln^2(age)$ $\sigma_B = -0.1343263 + 0.3371941\ age^{-0.5} + 0.0294884\ \ln(age)$ $\theta = -0.295014$		

(A) needs a natural logarithmic transformation of the measurement and all others a Box-Cox transformation for which θ are given.

Online Supplementary Table S2. Reference values for PC, PS, AT activities (%) and APC-R (sec) according to age and sex.

2A: PC activity in men.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	62	66	74	82	92	103	115	129	138
20-25	68	72	81	90	101	114	126	142	151
25-30	72	77	86	96	107	121	134	151	160
30-35	75	79	89	99	111	125	139	156	166
35-40	76	81	91	101	113	127	141	159	169
40-45	76	81	91	102	114	128	142	160	170
45-50	76	81	91	101	114	128	142	159	170
50-55	75	80	90	100	112	126	140	158	168
55-60	74	79	88	98	110	124	138	155	165
60-65	72	77	86	96	108	121	134	151	161
65-70	70	75	84	93	105	118	131	147	156
70-75	68	72	81	90	101	114	127	142	152
75-80	66	70	78	87	98	110	122	137	146

2B: PC activity in women.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	64	69	78	87	99	112	124	139	148
20-25	65	70	79	89	100	113	126	141	150
25-30	66	71	81	91	102	115	128	144	153
30-35	68	73	83	93	105	118	131	147	156
35-40	70	75	85	95	107	121	134	150	160
40-45	72	77	87	98	110	124	138	154	164
45-50	74	79	90	100	113	127	141	158	168
50-55	76	81	92	103	116	130	145	162	172
55-60	78	83	94	105	119	133	148	165	175
60-65	79	85	96	107	121	136	150	168	178
65-70	80	86	97	108	122	137	152	170	180
70-75	80	86	98	109	122	137	152	170	181
75-80	80	85	97	108	121	136	151	169	179
80-85	78	84	95	106	119	134	148	166	176

2C: PS activity in men.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	60	66	79	90	104	118	131	146	154
20-25	60	67	79	91	105	119	132	147	155
25-30	61	67	80	91	105	119	132	147	156
30-35	61	67	80	92	105	119	132	148	156
35-40	61	67	80	92	105	119	132	148	156
40-45	60	67	79	91	105	119	132	147	155
45-50	60	66	78	90	104	118	131	146	154
50-55	58	64	77	88	102	116	129	144	152
55-60	56	62	75	86	99	113	126	141	149
60-65	53	59	71	83	96	110	122	137	145
65-70	50	56	67	79	92	105	118	132	141
70-75	45	51	63	74	86	100	112	127	135
75-80	40	46	57	68	80	93	105	119	127

2D: PS activity in women.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	49	54	64	75	87	100	113	128	137
20-25	46	51	62	72	84	97	110	125	133
25-30	46	51	61	71	83	96	109	124	132
30-35	46	51	62	72	84	97	109	124	133
35-40	47	52	63	73	85	98	111	126	134
40-45	48	53	64	74	86	99	112	127	136
45-50	49	54	65	75	88	101	114	129	138
50-55	50	55	66	77	89	102	115	131	139
55-60	51	57	67	78	90	104	117	133	141
60-65	52	58	69	79	92	106	119	134	143
65-70	53	59	70	81	93	107	120	136	145
70-75	54	60	71	82	95	109	122	138	147
75-80	55	61	72	83	96	110	124	140	149
80-85	56	62	74	84	98	112	125	141	150

2E: AT activity in men.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	86	91	99	105	112	118	123	128	131
20-25	86	91	99	105	112	118	123	128	131
25-30	85	90	98	105	112	118	123	128	131
30-35	85	90	98	105	111	117	122	128	130
35-40	84	89	97	104	111	117	122	127	130
40-45	83	88	97	103	110	116	121	127	129
45-50	82	87	96	102	109	116	121	126	129
50-55	80	86	94	101	108	115	120	125	128
55-60	78	84	93	100	107	113	119	124	127
60-65	75	81	91	98	106	112	117	123	126
65-70	72	79	89	96	104	110	116	122	125
70-75	68	75	86	94	102	108	114	120	123
75-80	63	71	82	91	99	106	112	118	121

2F: AT activity in women.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	90	95	103	110	117	124	130	136	139
20-25	84	88	96	103	110	116	122	128	131
25-30	84	88	95	102	108	114	119	125	128
30-35	85	89	96	102	108	114	119	125	128
35-40	85	90	97	103	109	115	120	126	128
40-45	86	90	97	103	110	116	121	127	130
45-50	86	90	97	104	110	117	122	128	131
50-55	85	90	97	104	111	118	123	129	133
55-60	84	89	97	104	112	119	125	131	134
60-65	82	88	97	104	112	120	126	133	136
65-70	81	86	96	104	113	121	127	134	138
70-75	79	85	96	104	113	121	128	136	140
75-80	76	83	95	104	113	122	130	138	142
80-85	74	81	94	104	114	123	131	139	144

2G: APC-R in all subjects.

Age (years)	Percentile								
	1st	2.5th	10th	25th	50th	75th	90th	97.5th	99th
15-20	107.5	112.5	122.4	132.3	144.6	158.4	172.3	189.7	200.1
20-25	115.8	120.8	131.0	141.0	153.4	167.2	181.0	198.3	208.5
25-30	116.3	121.2	131.1	140.8	152.7	166.0	179.3	195.8	205.5
30-35	115.6	120.5	130.2	139.7	151.5	164.5	177.5	193.6	203.1
35-40	115.1	119.9	129.6	139.2	150.9	163.9	176.9	193.1	202.6
40-45	114.7	119.6	129.4	139.1	151.0	164.2	177.4	193.8	203.5
45-50	114.6	119.5	129.5	139.4	151.5	165.0	178.5	195.4	205.3
50-55	114.5	119.6	129.7	139.8	152.2	166.1	180.1	197.5	207.8
55-60	114.5	119.6	130.1	140.4	153.2	167.5	181.9	199.9	210.6
60-65	114.5	119.7	130.4	141.0	154.2	168.9	183.8	202.5	213.7
65-70	114.4	119.8	130.8	141.7	155.2	170.4	185.8	205.2	216.8
70-75	114.4	119.9	131.1	142.3	156.2	171.9	187.8	208.0	220.1
75-80	114.3	120.0	131.4	142.9	157.2	173.4	189.9	210.8	223.3
80-85	114.2	120.0	131.7	143.4	158.1	174.8	191.8	213.5	226.5

Online Supplementary Table S3. Normal ranges of AT, PC and PS in different populations (large series).

3A: Antithrombin

References	Population		Method		Mean ± 2SD		Median 2.5th / 97.5th percentile	
	Country	Number	Technique	Units	Men	Women	Men	Women
22, 23	Scotland	9669	activity	IU/dL	106.4 84-129 105.6 (83.2-128)	105 .1 83-127		
24	England	1381	activity	IU/mL			1.10 0.72/1.65 0.85/1.37	1.07 0.75/1.69
26	Nether-lands	(474)	activity	U/mL	Lower limit of normal : 0.80			
27	Italy	4000	activity	IU/dL	92.0 72.4 – 111.6		72.0/ 76.7 – 109.4/112.0	
28	Scotland	1564	activity	IU/dL			99 (83-121) 96 (72-120)	100 (82-117) 105 (84-119)
20, 32	Japan	4517	activity	%	106.1 81.5 – 130.7			
Our study	China	3493	activity	%	109 89 - 129 109 89 - 129	109 89 - 129	110 88 - 132 110 89 - 131	110 89 - 131

All AT activity assays were carried out using the same type of technique: amidolytic assay and chromogenic substrate, but the equipment and reagents differed between studies. Reference 23: most cases of inherited deficiencies had AT activities between 40% and 50%, i.e much lower than mean – 2SD. Reference 26 (number of patients between parentheses): this study was not designed to establish normal ranges but to analyze the risk of thrombosis associated with PC deficiency. 474 controls were included in this study and AT activity was also assayed but the authors did not give details and/or references about the number of healthy people included for the previous establishment of the lower limit of normal AT values. Reference 27: the 2.5th- 97.5th percentiles were calculated using a non-parametric method. Reference 28: expression of the results: lowest value = 5th percentile and highest value= 95th percentile.

3B: Protein C

Refe-rences	Population		Method		Mean ± 2SD		Median 2.5th / 97.5th percentile	
	Country	Number	Technique	Units	Men	Women	Men	Women
21	USA	5422	antigen	µg/mL (%)			2.82/5.65 (70)	
24	England	1381	activity	u/mL	1.07 0.72/ 1.38	1.01 0.74/ 1.49		
					0.72/1.47			
26	Nether-lands	(474)	activity	U/mL	Lower limit of normal : 0.67			
25	Scotland	9648	activity	iu/mL	1.00 0.68/1.34			
27	Italy	4000	activity	IU/dL	93.5 46.5/140.5		57.0-69.8 / 123.0-148.4	
28	Scotland	1564	activity	IU/dL			99 (68/148) to 108 (66/175)	98 (64/155) to 117 (59/172)
20, 32	Japan	4517	activity	%	122.4 (80.2-164.6) N. lower limit : 59.3% and AT/PC>3SD			
Our study	China	3493	activity	%	111 71/151	109 67/150	109 76/142	108 74/142
					110 70/150		110.18 69.4/150.9	

All PC activity assays were carried out using the same type of technique: amidolytic assay and chromogenic substrate, but the equipment and reagents differed between studies. Reference 26 (number of patients between parentheses): this study was not designed to establish normal ranges but to analyze the risk of thrombosis associated with PC deficiency; 474 controls were included in this study but the authors did not give details and/or references about the number of healthy people included for the previous establishment of the lower limit of normal PC values. Reference 27: the 2.5th- 97.5th percentile were calculated using a non-parametric method. Reference 28: expression of the results: lowest value = 5th percentile and highest value= 95th percentile. References 20, 32: the authors considered two criteria for defining the N. (normal) lower limit of PC activity and identifying people with suspected deficiency: i) PC activity level > 3SD (59.3%) and ii) AT activity/PC activity ratio > 3 SD (1.27).

3C: Protein S

References	Population		Method		Mean ± 2SD		Median 2.5th / 97.5th percentile	
	Country	Number	Technique	Units	Men	Women	Men	Women
26	Nether-lands	(474)	antigen ELISA	U/mL	Lower limit of N (mean-1.96 SD) **TPS**: 0.67; **FPS**: 0.57			
28	Scotland	1564	activity	%			113 (83 /149) to 117 (81/152)	92 (47/128) to 112 (69 /149)
29	Scotland	3788	antigen ELISA	%			**TPS** 108 72/164	**TPS** : « **hormone** » 87 (57/142) « **non hormone** » 96 (68/142)
							FPS : 115 68/178	**FPS** : 96 54/155
20, 31	Japan	2688	activity	%	92.6 49.8/ 135.4	82.9 47.4/ 118.5		
					87.4 45.8/129			
Our study	China	3493	activity	%	104 61/145	87 49/127	103 66/140	86 53/119
					95 53/137		94 56/132	

All PS activity assays were carried out using the same type of technique: amidolytic assay and chromogenic substrate, but the equipment and reagents differed between studies except for both Japanese studies (20, 31) and the Chinese study. Reference 28: expression of the results: lowest value = 5th percentile and highest value= 95th percentile. Reference 29: "hormone": women taking oral contraceptive "non hormone": women not taking oral contraception. Oral contraception had a lowering effect on total protein S (TPS) but not on free protein S (FPS).