Expanding the Diagnostic Screening of Alpha-1 Antitrypsin Deficiency: Evaluation of the AlphaID™ Test in Detecting Rare *SERPINA1* Variants

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INTRODUCTION

Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder linked to significant lung and liver disease. It is characterized by reduced serum alpha-1 antitrypsin (AAT) levels. AATD results from mutations in the *SERPINA1* gene, which encodes the AAT protein [1]. Early diagnosis is critical for effective disease management and intervention. There are over 200 known *SERPINA1* variants; however, most commercial laboratories identify only two common allelic variants (S and Z) and five associated genotypes (MS, MZ, SZ, ZZ, SS) [2].

The AlphaID™ screening program includes the AlphaID™ Confirm and the AlphaID™ Buccal test (Grifols, USA). AlphaID™ Confirm uses dried blood spot (DBS) specimens to detect *SERPINA1* variants, along with serum AAT quantification. In contrast, the AlphaID™ buccal test utilizes buccal swab (BS) specimens to screen for AATD. Both tests detect 12 additional allelic variants not identified by traditional SERPINA1-targeted genetic tests. This study aimed to assess the frequency and associated serum AAT levels of *SERPINA1* variants identified by the AlphaID™ screening program within a large, nationwide cohort and to evaluate the added diagnostic advantages of screening for the 12 additional allelic variants.

METHOD

A total of 205,632 participants enrolled in the AlphaID™ screening program, comprising 148,225 individuals in the AlphaID™ cohort and 57,407 individuals in the AlphaID™ Confirm cohort. Specimens were collected through physician-initiated submissions from across the contiguous United States. All participants provided buccal swabs or fingerstick blood (for DBS) samples for subsequent analysis. Genomic DNA was extracted from BS and DBS specimens using in-house validated laboratory protocols. Genotyping was performed utilizing an FDA-cleared AAT genotyping assay (Progenika Biopharma, Spain), which detects 14 clinically relevant allelic variants of the *SERPINA1* gene. Quantitative measurement of serum AAT levels was conducted on DBS samples using immunoturbidimetry. AAT levels were analyzed in the context of genotypic data to identify individuals with severe deficiency using the putative threshold of 57 mg/dL [3].

RESULTS

In the Confirm cohort, a total of 36 abnormal AAT genotypes were identified, with 31 genotypes exclusive to the AlphaID™ testing. In the AlphaID™ cohort, 38 abnormal genotypes were detected, 33 of which were exclusive to AlphaID™ (Table 1). Five commonly tested genotypes accounted for the majority of cases, comprising 97.6% of the Confirm cohort and 98.7% of the AlphaID™ cohort. AlphaID™ testing enabled the detection of an additional 1.3% and 2.4% of genotypes in the Confirm and AlphaID™ cohort, respectively, beyond what is typically captured by standard testing (Table 1). Notably, AlphaID™ Confirm testing identified abnormal genotypes associated with AATD in 15.0% of individuals with intermediate deficiency and 4.0% with severe deficiency.

Among these, 7.2% of intermediate and 7.7 % of severe cases would have been missed using conventional testing methods. These findings highlight the advantages of the AlphaID™ program in detecting clinically relevant, less common *SERPINA1* variants. Serum AAT concentrations were analyzed across different *SERPINA1* genotypes to evaluate genotype-phenotype relationships and to exclude samples with values below the analytical limit of quantitation (LOQ <20 mg/dL) (Table 2). The M/M genotype (n = 44,285) exhibits the highest mean A1AT concentration (165.0 mg/dL), serving as a reference for normal levels. In contrast, the deficiency-associated genotypes such as M/Z (n = 4,328), S/Z (n = 387), and Z/Z (n = 157) showed progressively reduced mean concentrations of 109.5 mg/dL, 73.5 mg/dL, and 49.6 mg/dL, respectively, consistent with established patterns of intermediate to severe AATD.

Rare genotypes involving null allele, M/M malton (n = 29) and M/QO west (n = 11) also demonstrate mean AAT levels of 95.6 mg/dL and 87.9 mg/dL, respectively, consistent with the loss-of-function effect of these alleles. Additionally, M/QO variants, Clayton, Bellingham, Granite Falls, exhibit reduced A1AT levels, with some falling in the severe deficiency range (Table 2). While these rare variants are limited by small sample sizes (n ≤ 10), their consistently low A1AT concentrations support the association with significant functional impairment. These findings underscore the importance of identifying and characterizing null alleles and rare compound heterozygous combinations, which may contribute to clinically significant AATD even in the absence of common deficiency genotypes.

CONCLUSION

The expanded genotype testing highlights the diagnostic value of the AlphaID™ Screening Program. It enables detection of both common and rare *SERPINA1* variants, many of which may be missed by conventional genotyping methods. The inclusion of rare, clinically significant compound heterozygotes and null alleles supports the utility of the AlphaID™ Screening Program in identifying individuals at risk for Alpha-1 antitrypsin deficiency. This broader detection capability supports more accurate diagnoses and promotes earlier clinical intervention.

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DISCLOSURE

TrilliumBiO partners with Grifols as the laboratory service provider for the AlphaID™ Screening Program.

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	AlphaID™		
	Genotype	Result	Frequency
Commonly Tested	M/M	127552	86.10000%
	M/S	11564	7.80000%
	M/Z	6011	4.10000%
	S/Z	408	0.30000%
	S/S	336	0.20000%
	Z/Z	307	0.20000%
Unique to AlphaID™	M/F	1003	0.70000%
	M/I	553	0.40000%
	M/P lowell	135	0.09000%
	M/M heerlen	57	0.04000%
	M/M malton	46	0.03000%
	F/Z	43	0.03000%
	F/S	42	0.03000%
	S/I	24	0.02000%
	M/M procida	22	0.01000%
	M/QO west	17	0.01000%
	Z/I	15	0.01000%
	M/QO clayton	14	0.00900%
	Z/M heerlen	10	0.00700%
	M/QO bellingham	9	0.00600%
	S/M malton	6	0.00400%
	S/P lowell	6	0.00400%
	Z/P lowell	6	0.00400%
	F/I	5	0.00300%
	S/M heerlen	5	0.00300%
	Z/M malton	5	0.00300%
	M/QO mattawa	4	0.00300%
	Z/QO west	4	0.00300%
	F/F	3	0.00200%
	Z/QO clayton	3	0.00200%
	M malton/M malton	2	0.00100%
	I/I	1	0.00070%
	M malton/M heerlen	1	0.00070%
	M/QO granite falls	1	0.00070%
	M/S iiyama	1	0.00070%
	P lowell/QO clayton	1	0.00070%
	S/M procida	1	0.00070%
	S/QO clayton	1	0.00070%
	Z/M procida	1	0.00070%
	Total	148225	100.00000%

	AlphaID™ Confirm		
	Genotype	Result	Frequency
Commonly Tested	M/M	44497	77.5000%
	M/S	6280	10.9000%
	M/Z	4328	7.5000%
	S/Z	389	0.7000%
	Z/Z	309	0.5000%
	S/S	273	0.5000%
Unique to AlphaID™ Confirm	M/F	581	1.0000%
	M/I	352	0.6000%
	M/P lowell	87	0.2000%
	M/M heerlen	58	0.1000%
	F/S	50	0.0900%
	F/Z	37	0.0600%
	S/I	30	0.0500%
	M/M malton	29	0.0500%
	Z/I	21	0.0400%
	M/QO west	11	0.0200%
	Z/M heerlen	11	0.0200%
	M/M procida	10	0.0200%
	M/QO clayton	8	0.0100%
	S/P lowell	7	0.0100%
	F/I	5	0.0090%
	S/M heerlen	5	0.0090%
	F/F	4	0.0070%
	M/QO bellingham	3	0.0050%
	Z/M malton	3	0.0050%
	Z/P lowell	3	0.0050%
	Z/QO west	3	0.0050%
	M/QO granite falls	2	0.0030%
	S/M malton	2	0.0030%
	S/QO clayton	2	0.0030%
	F/M heerlen	1	0.0020%
	F/M malton	1	0.0020%
	P lowell/P lowell	1	0.0020%
	S/QO bellingham	1	0.0020%
	S/QO granite falls	1	0.0020%
	Z/M procida	1	0.0020%
	Z/QO clayton	1	0.0020%
	Total	57407	100.000%

Table 1. Distribution of genotype results from the AlphaID™ Screening Program

Genotype	M/M	M/S	M/Z	M/F	M/I	*S/Z	S/S	Z/Z	M/P lowell	M/M heerlen	F/S	F/Z	S/I	*Z/I	M/M malton	M/M procida	M/QO west
N	44285	6274	4328	580	350	387	273	157	87	58	50	37	30	20	29	10	11
Minimum	39	43	26	83	56	24	52	20	69	50	79	54	65	43	49	66	68
Maximum	300	298	275	298	278	235	248	185	184	156	246	179	200	113	172	119	125
Mean	165.042	142.084	109.496	155.386	146.083	73.543	116.608	49.58	120.356	94.724	130.18	94.811	117.133	75.25	95.621	93.1	87.909
95% CI	164.668 to 165.416	141.212 to 142.955	108.673 to 110.319	152.626 to 158.146	142.146 to 150.020	70.979 to 76.106	112.772 to 120.444	43.480 to 55.679	114.687 to 126.026	89.382 to 100.066	121.018 to 139.342	85.680 to 103.942	104.706 to 129.561	66.837 to 83.663	85.683 to 105.559	80.795 to 105.405	76.357 to 99.461
Median	159	137	106	153	139	68	110	31	118	95	128.5	90	109	72	96	90	84
SD	40.1588	35.2129	27.606	33.8461	37.4507	25.6482	32.1908	38.6936	26.6021	20.3175	32.24	27.387	33.2612	17.9762	26.1266	17.2011	17.1957

Genotype	M/QO bellingham	M/QO clayton	S/P lowell	*Z/M heerlen	F/I	S/M heerlen	F/F	Z/P lowell	M/QO granite falls	S/M malton	S/QO clayton	F/M heerlen	F/M malton	P lowell/ P lowell	S/QO bellingham	S/QO granite falls	*Z/QO west
N	3	8	7	6	5	5	4	3	2	2	2	1	1	1	1	1	1
Minimum	68	23	62	22	91	41	135	49	72	65	41	66	112	170	72	64	126
Maximum	82	126	121	68	245	71	189	85	104	77	57	66	112	170	72	64	126
Mean	75.667	75.375	88.571	39.167	138.6	57.8	155.25	68.667	88	71	49	66	112	170	72	64	126
**95% CI	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Median	77	78.5	88	32.5	117	58	148.5	72	88	71	49	66	112	170	72	64	126
SD	7.0946	29.1496	20.2473	19.1564	60.7808	12.3167	25.8505	18.23	22.6274	8.4853	11.3137	/	/	/	/	/	/

Table 2. Serum AAT concentrations (mg/dL) across various genotypes. *Excludes samples with LOQ <20 mg/dL. **Statistical significance could not be assessed due to an insufficient sample size.