

A polygenic risk score to help discriminate primary adrenal insufficiency of different etiologies

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Abstract. Aranda-Guillén M, Røyrvik EC, Fletcher-Sandersjö S, Artaza H, Botusan IR, Grytaas MA, et al. A polygenic risk score to help discriminate primary adrenal insufficiency of different etiologies. *J Intern Med.* 2023;**294**:96–109.

Background. Autoimmune Addison's disease (AAD) is the most common cause of primary adrenal insufficiency (PAI). Despite its exceptionally high heritability, tools to estimate disease susceptibility in individual patients are lacking. We hypothesized that polygenic risk score (PRS) for AAD could help investigate PAI pathogenesis in pediatric patients.

Methods. We here constructed and evaluated a PRS for AAD in 1223 seropositive cases and 4097 controls. To test its clinical utility, we reevaluated 18 pediatric patients, whose whole genome we also sequenced. We next explored the individual PRS in more than 120 seronegative patients with idiopathic PAI.

Results. The genetic susceptibility to AAD—quantified using PRS—was on average 1.5 standard deviations (SD) higher in patients compared with healthy controls ($p < 2e - 16$), and 1.2 SD higher in the young patients compared with the old ($p = 3e - 4$). Using the novel PRS, we searched for pediatric patients with strikingly low AAD susceptibility and identified cases of monogenic PAI, previously misdiagnosed as AAD. By stratifying seronegative adult patients by autoimmune comorbidities and disease duration we could delineate subgroups of PRS suggesting various disease etiologies.

Conclusions. The PRS performed well for case-control differentiation and susceptibility estimation in individual patients. Remarkably, a PRS for AAD holds promise as a means to detect disease etiologies other than autoimmunity.

Keywords: Addison's disease, age-at-onset, autoantibodies, complex inheritance, primary adrenal insufficiency, risk scores

Introduction

In primary adrenal insufficiency (PAI), the inadequate levels of steroid hormones give rise to progressive fatigue, muscle weakness, gastrointestinal symptoms, and unintentional weight loss

[1]. Due to the insidious onset and nonspecific manifestations, many patients risk developing a life-threatening adrenal crisis before diagnosis [1–4]. To avoid a fatal outcome, all patients with PAI require prompt and lifelong substitution of

vital adrenal hormones, regardless of disease etiology.

The clinical course and the risk of passing down the disease to the next generation are dependent on the cause of disease. Monitoring and treatment are adapted to disease etiology to avoid complications, and genetic counseling may be offered for heritable forms. Clinical guidelines therefore recommend that the etiology of PAI should always be determined [5]. The most common cause is an autoimmune destruction of the adrenal glands called autoimmune Addison's disease (AAD). At presentation, about 90% of PAI cases test positive for autoantibodies against the adrenal enzyme 21-hydroxylase (*CYP21A2*), confirming the autoimmune etiology and the diagnosis of AAD [6–8]. Therefore, in most adult patients, endocrinological and serological evaluation is sufficient for an etiological diagnosis. In pediatric patients with PAI, however, genetic investigations are often advisable because a significant proportion of cases have monogenic disorders [9].

The most common form of monogenic PAI is congenital adrenal hyperplasia (CAH), a group of diseases characterized by loss-of-function mutations in enzymes involved in the synthesis of cortisol and aldosterone [10, 11]. In more than 95% of CAH cases, the underlying disease mechanism is a deficiency of the adrenal enzyme 21-hydroxylase (OMIM #201910) [4, 10]. Other forms of monogenic PAI include inborn errors of proteins essential for adrenal development and function, as well as autoimmune polyendocrine syndrome type 1 (APS-1) [11, 12].

APS-1 represents a rare cause of PAI, typically with recessive inheritance caused by loss-of-function variants in the autoimmune regulator gene (*AIRE*, OMIM #240300) [13]. Without a functional *AIRE* protein, autoreactive T cells evade apoptosis in the thymus and have potential to cause organ-specific autoimmunity later in life [14, 15]. Patients with APS-1 typically present with chronic mucocutaneous candidiasis, hypoparathyroidism, and autoimmune PAI [16]. Autoantibodies against type 1 interferon and interleukin-22 are hallmarks of the disease, and many have autoantibodies against 21-hydroxylase [17].

Whereas monogenic disorders are diagnosed using targeted next-generation sequencing panels, AAD commonly has a complex inheritance, and no

simple genetic test is available for confirmation. Nevertheless, AAD has a strong heritability (0.97 [95% CI 0.88–0.99]), demonstrating that genetic variation determines disease risk to a high degree [18]. Common AAD risk variants discovered in association studies collectively make a significant contribution to AAD susceptibility [19, 20]. Summarizing the effects of common genetic variation to construct a polygenic risk score (PRS) has proven useful in identifying individuals with increased disease risk—for some diseases, a risk equivalent to that conferred by monogenic mutations [21]. To systematically dissect PAI inheritance, we constructed and validated a PRS for AAD in two nationwide patient registries and used it in parallel with whole genome sequence (WGS) analysis in patients with early-onset PAI.

Results

Derivation of a PRS for autoimmune Addison's disease

First, we tried two different approaches to develop PRSs for AAD: clumping and thresholding, and the Bayesian LDpred2 software. We studied 1223 seropositive cases with AAD and 4097 healthy controls [19], of which 72% were used for training and tuning the risk scores. The subsets of study participants selected for training ($n = 763$ cases and 2557 controls), tuning ($n = 120$ cases and 380 controls), and validation ($n = 340$ cases and 1160 controls) were identical for both approaches, and throughout the study (Fig. 1A). APS-1 patients were carefully excluded from all subsets using cytokine autoantibody screening, clinical information, and *AIRE* gene sequencing. For the clumping and thresholding method, the effects of human leukocyte antigen (HLA) alleles and amino acids were modeled separately using stepwise regression, as previously described [19]. Both approaches were optimized to discriminate cases and controls in the tuning set, and their performance was evaluated in the independent validation set. As the results of both methods were highly similar, we chose to proceed with the PRS from clumping and thresholding in subsequent analyses (PRS14_{AAD}). The final PRS consisted of 5 single nucleotide polymorphisms (SNPs) and 9 HLA alleles/amino acids (Table S1–S5, Fig. S1).

Validation and performance of the PRS

The performance of the PRS was evaluated in an independent validation set of 340 cases and 1160 controls. The capacity to discriminate cases from

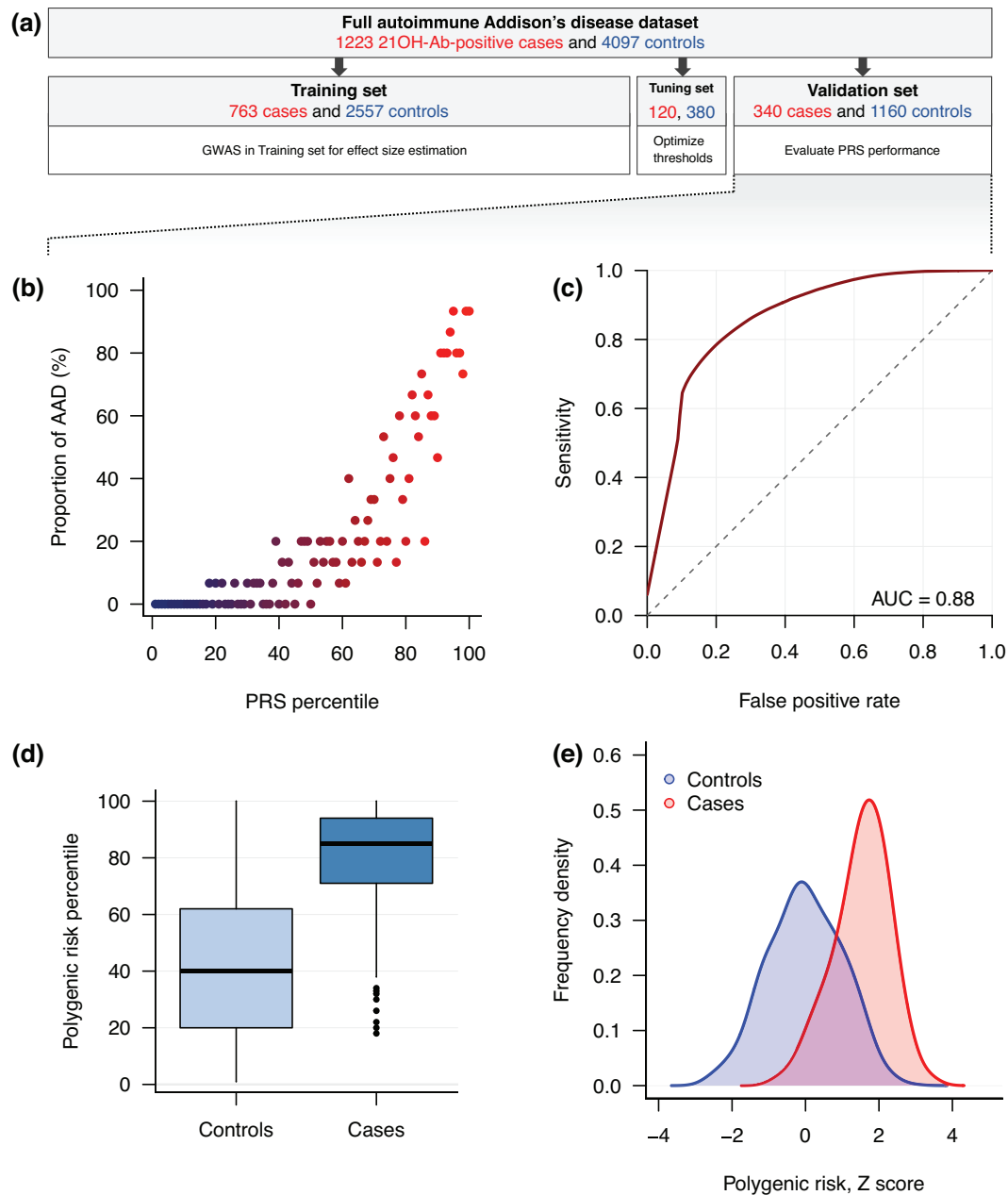


Fig. 1 Development and evaluation of the polygenic risk score (PRS) for autoimmune Addison's disease (AAD): (A) The full sample dataset was subdivided so that the effect size of every SNP was estimated using a GWAS performed on the training set (left box), the most optimal PRS model was determined on the tuning set (middle box), and the predictive ability of the PRS_{14AAD} was evaluated in the validation set, independent of both the training and the tuning data (right box); (B) proportion of AAD cases in 100 groups of the validation set ($n = 1500$) defined by PRS_{14AAD} percentiles; (C) receiver operating characteristic curve describing the link between sensitivity and specificity for PRS_{14AAD}; (D) polygenic risk percentile among cases and controls in the validation set. Single data points fall outside of the range defined by the whiskers, that is, 1.5 times the interquartile range; (E) frequency distribution of polygenic risk score (PRS) in the validation set. The x axis represents polygenic risk scores, standardized so that healthy controls have a mean of 0 and a standard deviation of 1. Blue and red highlight the control and case distributions, respectively.

Table 1. Impact of polygenic risk score (PRS) on the risk of autoimmune Addison's disease (AAD)

AAD centiles ^a	Control centiles ^a	PRS Z-score ^b	Sensitivity (%)	Specificity (%)	FDR (%)	Youden index ^c	PPV (%)	NPV (%)
1	33.2	-0.4	99	33	69	32	30	99
5	54.9	0.08	95	54	61	49	38	97
10	66.9	0.4	89	66	55	56	44	95
20.6	80.3	0.9	79	80	45	59	54	93
25	84.2	1.1	74	84	41	58	58	91
50	94.7	1.6	49	94	27	44	73	86
75	98.9	2.1	25	98	13	23	86	81
90	99.7	2.3	9.7	99	8	9	91	79
95	99.8	2.6	5	99	10	4	89	78
99	100	3.1	0.88	100	0	0.88	100	77

^aCentiles based on the AAD cases. The prediction accuracy for each centile was evaluated in terms of sensitivity, specificity, false discovery rate (FDR), positive predictive value (PPV), and negative predictive value (NPV) in the validation set.

^bPRS normalized to mean 0 and standard deviation 1 in the healthy controls.

^cThe Youden index was defined as $j = \text{sensitivity} + \text{specificity} - 1$. The maximum Youden index marks the PRS Z-score at which a cutoff would maximize case-control differentiation.

controls reached an area under the receiver operating characteristic curve (AUC) of 0.88. Across the 1160 healthy controls, the PRS was approximately normally distributed, so we scaled all values to a mean of 0 and a standard deviation (SD) of 1 to facilitate interpretation (Fig. 1B–E). We found that the odds ratio for AAD per 1 SD of the PRS was 6.4 (95% CI 5.2–8.0) (Fig. S2), and the average PRS in cases was 1.5 SDs above the average in healthy controls ($p < 2e - 16$). In other words, 96% of cases were above the 50th percentile of the healthy controls, whereas only 5% of the healthy controls had a genetic predisposition at or above the average case (Table 1). At the best achievable balance between sensitivity and specificity, 79% of cases and 80% of controls were correctly classified.

Genetic background of PAI in children

We next sought to comprehensively study the genetic background of PAI with early onset. To this end, we selected cases diagnosed before age 12 ($n = 18$) and quantified their genetic susceptibility to AAD at the individual level using the PRS14_{AAD}. To also screen for rare pathogenic variants as potential causes of monogenic PAI, we sequenced the whole genome (WGS) of the selected cases. In order to facilitate variant interpretation and detection of de novo variants, we sequenced trios whenever possible. All together, we sequenced index cases and their close relatives from 18 families, in total, 35 subjects.

Most patients with early-onset PAI had a high PRS14_{AAD}, at or above the median for cases with confirmed AAD (Fig. 2). Only two cases were below the median for healthy controls, a level at which merely 5% of AAD cases are found. Strikingly, WGS identified pathogenic variants that fully explained the phenotype in the two patients with low susceptibility to AAD (patients 5 and 10).

Patient 5 was a 52-year-old female diagnosed with PAI at 11 years of age, with neither autoimmune comorbidities nor autoantibodies against 21-hydroxylase when included in the Addison registry. We found that she had an unexpectedly low PRS for pediatric AAD and could uncover a homozygote pathogenic variant in the *CYP11A1* gene (c.1076C>T, p.A359V) known to cause congenital adrenal insufficiency with recessive inheritance (OMIM #613743) [22]. Patient 10 was a 29-year-old male who presented with PAI at age 1 year and with primary hypogonadism at puberty. He tested negative for 21-hydroxylase autoantibodies, was never tested genetically, and was later presumed to have AAD. Besides his low PRS, we found that he was hemizygous for a pathogenic insertion in exon 1 of the *NROB1* gene (c.577insT, p.T193NfsTer12), causing a frameshift and a premature termination. Loss of function of *NROB1* is linked to adrenal hypoplasia congenita with X-linked inheritance (OMIM #300473) [23–26]. In the remaining 16 cases, we could not detect any

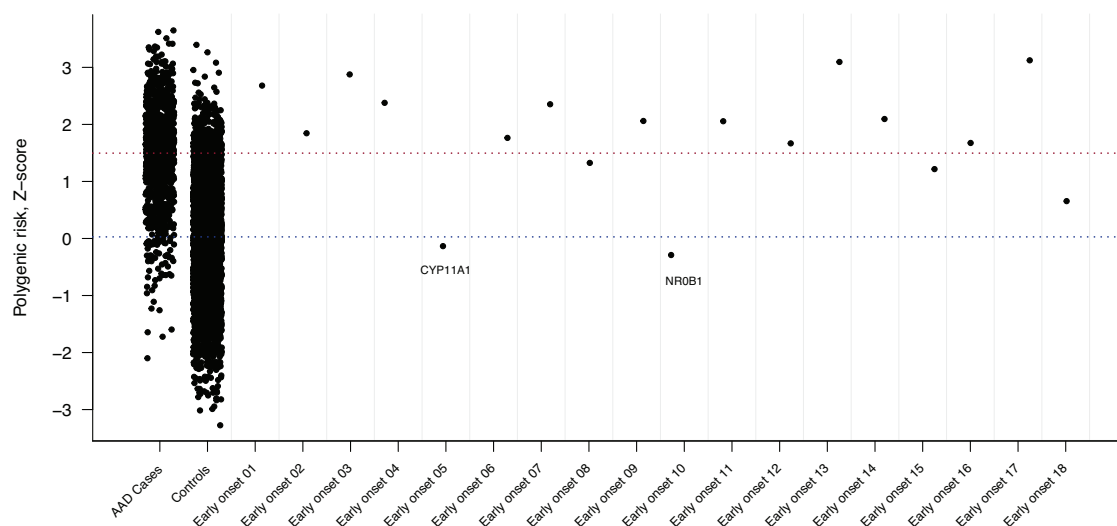


Fig. 2 Polygenic risk scores in children with primary adrenal insufficiency (PAI) uncover monogenic cases. Polygenic risk for index cases from 18 families with early-onset PAI. The blue horizontal line marks the median $PRS14_{AAD}$ in healthy controls ($n = 4097$). The red line marks the median $PRS14_{AAD}$ in autoimmune Addison's disease ($n = 1223$).

pathogenic or likely pathogenic variants connected to PAI (Table S6).

In four of the patients, we detected heterozygous variants of uncertain significance in genes that cause PAI with recessive inheritance: *PEX10* and *PEX26*, associated with peroxisome biogenesis disorder; *POLE*, associated with IMAGE-I syndrome; and *TXNRD2*, associated with glucocorticoid deficiency. A targeted search for compound heterozygous variants yielded no additional findings. Potential dominant effects on adrenal function have not been previously reported in any of these genes. A detailed discussion of the variants of uncertain significance is included in the Supporting Information (Note I).

Age of onset is linked to $PRS14_{AAD}$

For cases of AAD with 21-hydroxylase autoantibodies, the age of disease onset ranged from 6 to 82 years (Fig. 3A). Regressing $PRS14_{AAD}$ on the age of onset demonstrated a significant negative trend (Beta = -3.8 (95% CI -4.7 to -2.8), $p = 2e - 14$, $R^2 = 0.048$). For every SD increase of $PRS14_{AAD}$, the average age of onset decreased more than 3 years, albeit with large individual differences (Fig. 3B). In other words, AAD can start at any time in life, and a linear model is not informative for the age of onset at the individual level. However, the collective burden of known risk alleles explained up to 20 years of difference in average age of onset. The

PRS differed significantly between AAD cases with early (≤ 11 years, $n = 29$) and late onset (≥ 70 years, $n = 18$) (difference in means = 1.2 SD, $p = 3e - 4$) (Fig. 3C).

PRS in patients with Addison's disease lacking 21-hydroxylase autoantibodies

The levels of 21-hydroxylase autoantibodies have been shown to decrease with disease duration, defined here as time from diagnosis to serum sampling [7, 8, 27]. Decreasing titers have therefore been discussed as an explanation for the fact that about 14%–17% of cases tested negative at registration in the Addison's disease biobanks [7, 8, 27]. The $PRS14_{AAD}$, in contrast, remains constant over an individual's lifetime. We therefore calculated the $PRS14_{AAD}$ in 21-hydroxylase autoantibody-negative (21OH-Ab-negative) patients ($n = 209$). If the 21OH-Ab-negative were a random subset of 21OH-Ab-positive cases, their distribution of $PRS14_{AAD}$ would be nearly identical. However, we found that the average of $PRS14_{AAD}$ in 21OH-Ab-negative cases was lower (difference in means = 0.74 SDs, $p = 6e - 14$) and the variance higher, even though patients with APS-1 and confirmed non-autoimmune cases were excluded from the calculation (Fig. S3).

To better understand this finding, we stratified the 21OH-Ab-negative group by concomitant autoimmune diseases (Fig. 4A,B). Cases without

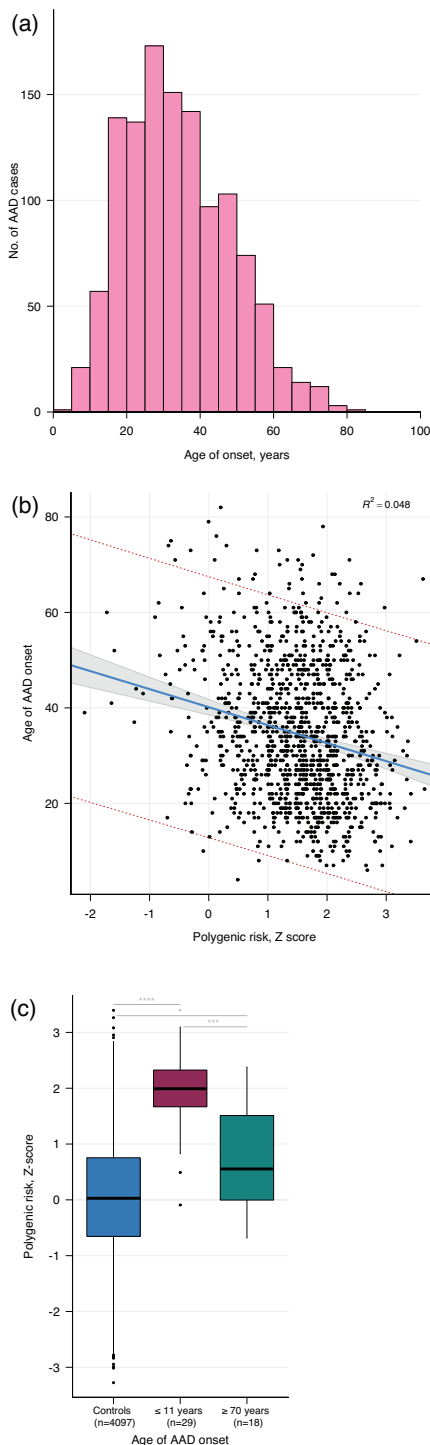


Fig. 3 Correlation between age of onset and $PRS14_{AAD}$: (A) histogram of the age of disease onset in autoimmune Addison's disease (AAD) ($n = 1210$); (B) scatterplot of $PRS14_{AAD}$ and age of AAD onset. Each data point represents a case.

comorbidity ($n = 124$) showed a wide distribution centered between 21OH-Ab-positive cases and controls, and so did the cases with hypothyroidism ($n = 52$). In contrast, patients with type 1 diabetes ($n = 18$)—although 21OH-Ab-negative—had a $PRS14_{AAD}$ at the level of the seropositive AAD cases.

By instead stratifying the 21OH-Ab-negative cases by their disease duration, we could study the effect of potential seroconversion across the spectrum of unchanging $PRS14_{AAD}$ (Fig. 4C,D). The average $PRS14_{AAD}$ of 21OH-Ab-negative patients with disease duration of 5 years or less ($n = 16$) differed significantly from 21OH-Ab-negatives screened for autoantibodies 20 years or more after diagnosis (difference in means = 1 SD, $p = 0.016$). The latter ($n = 69$) had on average the same $PRS14_{AAD}$ as seropositive cases ($p = 0.6$). On the other hand, in patients sampled no more than 5 years after diagnosis, a negative 21OH autoantibody test result was associated with a lower genetic predisposition to AAD (difference in means = 1.2 SD, $p = 0.004$). Hence, the 21OH-Ab-negative cases are not merely a subset of 21OH-Ab-positive cases that over time lost their autoantibodies.

PRS14_{AAD} in autoimmune polyendocrine syndrome type 1 and type 2

By definition, patients with APS-2 have at least two of the three autoimmune manifestations AAD, type 1 diabetes, and autoimmune thyroid disease. As opposed to APS-1, APS-2 manifestations show complex inheritance patterns, and it is believed that APS-1 and APS-2 cause autoimmune adrenal destruction through different genetic mechanisms. To evaluate the contribution of common AAD risk alleles in APS-1 and APS-2 pathogenesis, we compared the $PRS14_{AAD}$ of affected patients with healthy controls (Fig. 5, Fig. S4). For all APS-1 patients, we confirmed the diagnosis with biallelic pathogenic variants in the causal gene *AIRE*. As expected, we found no evidence to support that patients with APS-1 had polygenic predisposition to AAD higher than the healthy controls ($p = 0.8$), although the number of patients with APS-1 was

The linear model is displayed as a blue line with its 95% confidence interval shaded in gray. The 95% prediction interval is given by red dashed lines. (C) The polygenic risk scores significantly differed between the youngest and oldest AAD cases (difference in means = 1.2 SD, $p = 3e - 4$).

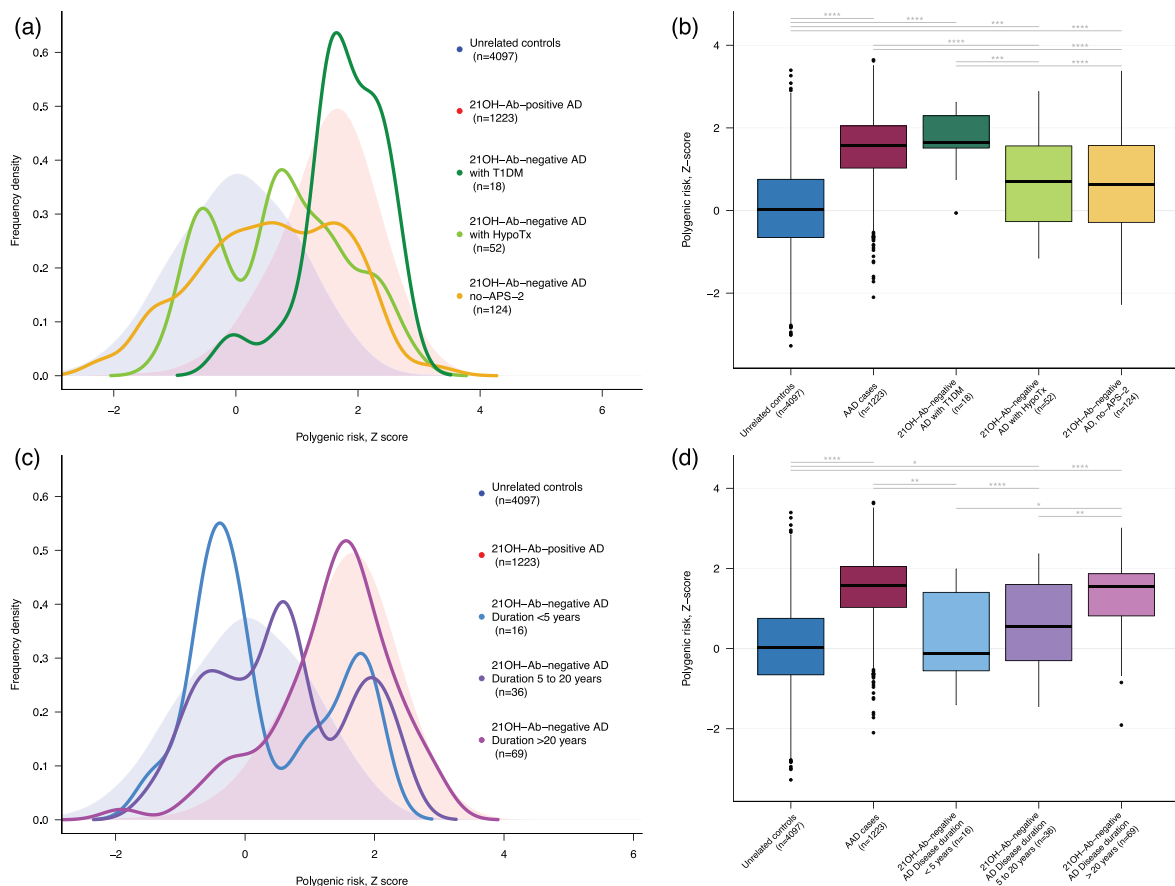


Fig. 4 Polygenic risk scores in seronegative patients stratified by comorbidities and disease duration. Frequency distribution of PRS₁₄_{AAD} in the full dataset (blue and red shades), as well as in cases testing negative for 21-hydroxylase autoantibodies (colored lines). Monogenic and confirmed non-autoimmune cases were excluded from the analysis. In panels A and B, cases are stratified by autoimmune comorbidity. In panel C and D, cases are stratified by the time from disease onset to serum sampling. PRS₁₄_{AAD} was standardized so that healthy controls have a mean of 0 and a standard deviation of 1. Games–Howell post hoc test was used to detect significant differences between groups. 21OH-Ab-negative, 21-hydroxylase autoantibody negative; 21OH-Ab-positive, 21-hydroxylase autoantibody positive; APS-2, autoimmune polyendocrine syndrome type 2; HypoTx, hypothyroidism; T1DM, type 1 diabetes mellitus.

too small to detect differences smaller than 0.7 SDs with 80% power ($n = 15$). In-line with the high penetrance of the syndrome, biallelic loss-of-function mutations in *AIRE* are sufficient to cause APS-1, including PAI. In contrast, patients with APS-2 had an increased burden of risk alleles compared to controls (difference in means = 1.4 SD, $p = 2e - 13$).

We next investigated whether a PRS₁₄_{AAD} could distinguish between cases with isolated AAD ($n = 444$) and cases with APS-2 ($n = 662$). No statistical difference was observed between the two ($p = 0.2$), which may reflect that the genetic risk

score was constructed regardless of autoimmune comorbidity, and that cases with isolated AAD may still acquire additional manifestations of autoimmunity. Cases with isolated AAD were on average 3.4 years younger ($p = 1e - 4$), suggesting that age—rather than genetic determinants—is the main difference between the groups (Fig. S5).

Discussion

We report the construction and validation of the first PRS for AAD. Based on 1223 21OH-Ab-positive cases and 4097 controls, PRS₁₄_{AAD} is useful for estimating the risk of AAD at the individual

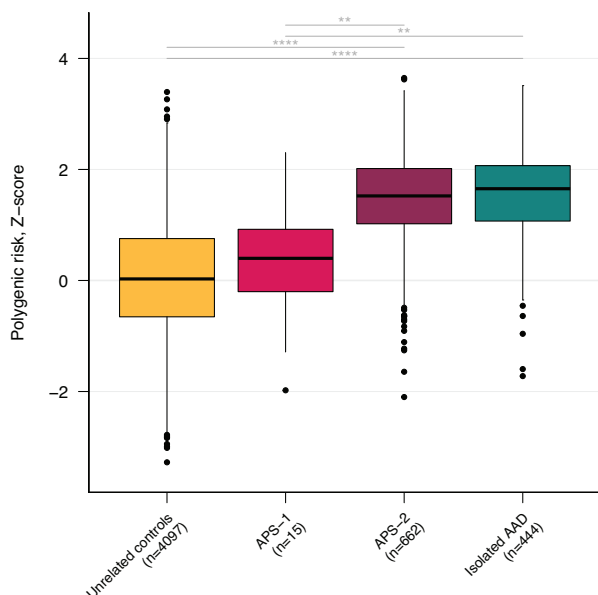


Fig. 5 Polygenic risk scores in autoimmune polyendocrine syndrome (APS) type 1, type 2, and isolated autoimmune Addison's disease (AAD). Distribution of PRS_{14AAD} in AAD, APS-1, and unrelated controls. AAD cases were divided into cases with APS-2 and isolated AAD for comparison. Cases with APS-1 were confirmed by *AIRE* gene sequencing and cases with isolated AAD had no known autoimmune comorbidity. Significant differences between groups were detected by Games–Howell post hoc test.

level. As a proof of its utility, we identified outlier individuals with lower-than-expected risk scores that could be diagnosed with monogenic PAI using WGS.

The PRS_{14AAD} performed well with a case-control discrimination of 0.88 AUC. No PRS from previous studies of AAD was available for reference, but in type 1 diabetes and autoimmune thyroid disease, the case-control discrimination has ranged from 0.70 to 0.93 [28–31] and 0.63 to 0.71 [32, 33], respectively. Hence, despite our relatively small sample size, PRS_{14AAD} is comparable to that developed for type 1 diabetes and autoimmune thyroid disease. All three diseases have been associated with HLA risk haplotypes with large effects on disease risk [34–37].

PRSs for autoimmune diseases that share risk alleles will be correlated [19]. AAD and type 1 diabetes, for instance, share their major HLA risk alleles—DQB1*0201 and DQB1*0302—both of which make an important contribution to PRS_{14AAD} [34]. Ide-

ally, the association of PRS_{14AAD} with type 1 diabetes should also be tested in a cohort of patients with type 1 diabetes and no adrenal insufficiency, and similarly for autoimmune thyroid diseases. In this study, in which all patients have AAD, we saw no difference in PRS_{14AAD} in patients also diagnosed with APS-2.

Monogenic PAI is typically congenital, whereas AAD seldom affects children. Still, non-classic forms of monogenic PAI with milder phenotypes may have late debut [38]. Several cases of congenital PAI presenting at age 5–15 years have been described, linked to *NROB1*, *CYP11A1*, *MC2R*, *AAAS*, and *TXNRD2* [39]. Using WGS, we diagnosed two pediatric cases with loss-of-function variants in *NROB1* and *CYP11A1*. In both cases, the molecular diagnosis was previously unknown and their clinical presentation misconceived as AAD. Our index patient who was found to be homozygous for *CYP11A1* p.A359V presented clinically at 11 years of age and exemplifies that monogenic PAI can remain undiagnosed or misinterpreted for many years. We conclude that the age of onset cannot reliably discriminate between AAD and monogenic PAI, and that genetic analyses are warranted before diagnosing AAD by exclusion.

When PRS_{14AAD} was applied to estimate the genetic susceptibility to AAD in pediatric cases, we found higher scores than the average AAD patient, in line with previous findings [40]. For instance, the two youngest AAD cases, presented with PAI at 6 years of age, had autoantibodies against 21-hydroxylase, PRS_{14AAD} at +3.1 and +2.7 SDs, and no pathogenic variants detectable by WGS. Strikingly, the two exceptions with notably low risk scores were patients 5 and 10 for whom a monogenic disorder was detected by WGS. Taken together, the genetic predisposition to AAD can be estimated at the individual level and used to identify patients with other disease etiologies that might benefit from genetic counseling. Coexistence of Mendelian PAI and AAD is also a possibility, although exceptionally rare [41].

Not much is known about disease etiology in patients lacking autoantibodies against 21-hydroxylase, and where no disease cause has been found. Our results confirm that 21OH-Ab-negative patients—although diagnosed with PAI and sometimes presumed to be autoimmune—may constitute a group with mixed disease etiology. We found that two decades after a PAI diagnosis, a

negative serum sample had no implications on the genetic predisposition in the tested individuals. In contrast, in 21OH-Ab-negative patients sampled no more than 5 years after diagnosis, a negative autoantibody test result was associated with a lower genetic predisposition than expected from AAD cases. We speculate that this is not because a low PRS predisposes the patient to an early loss of autoantibodies, but rather because the patients were likely never seropositive. All 21OH-Ab-negative PAI patients with concomitant type 1 diabetes had high PRS14_{AAD}, in line with the shared HLA risk haplotypes between AAD and autoimmune diabetes. However, patients with hypothyroidism had risk scores overlapping to a greater extent with the healthy controls. Hypothyroidism is relatively common in the general population, and misdiagnosis has been reported among AAD patients [42]. Further studies are required to definitively answer whether a subset of 21OH-Ab-negative cases has a different disease etiology.

PRSs have been used to estimate an individual's genetic susceptibility to diseases [43]. However, for a disease as rare as AAD the pretest probability is so low that even an excellent PRS predictor would be useless as a screening tool in the general population (Table S7) [44, 45]. PRS may still be utilized to estimate individual risk in populations in which the incidence is expected to be higher, such as among siblings or children of patients. There is currently no treatment available to prevent AAD. Nevertheless, in studies of early immunologic interventions it will be important to identify those with most to gain from preventive measures.

If implemented clinically, the strength of the PRS14_{AAD} would lie in its negative predictive value for AAD. We believe that the PRS would be a useful complement in the recommended investigation of disease cause for patients with PAI, especially for patients with a negative or borderline result for 21-hydroxylase autoantibodies. In patients with negative or borderline results, a low PRS indicates a low probability of AAD and may warrant next-generation sequencing of genes associated with adrenal function or development to seek other disease causes. PRS could also be valuable in patients tested for autoantibodies years after diagnosis. Delayed testing of autoantibodies has a substantial risk of false negatives, whereas the PRS remains constant throughout an individual's lifetime.

Altogether, 30%–40% of heritability for AAD has been attributed to common genetic variation, enabling the derivation of a PRS predictor [19]. We caution that cryptic relatedness and residual population stratification may inflate the prediction accuracy of PRSs. Because we had access to individual-level genotypes for all datasets, close relatedness and sample overlap could be eliminated, reducing the risk of type 1 errors. The study was based solely on subjects with European ancestry as determined by genotype data. The advantage of ancestral homogeneity is that spurious associations due to confounding links between ancestry and disease status are avoided. The downside is that the transferability of the results across populations may be impaired. First, the associated variants included in the PRS are not necessarily causal variants, and their predictive accuracy in other populations will depend on the extent to which associated SNPs and the causal SNPs are inherited together in a population of interest. Second, even if the PRSs were constructed exclusively of causal SNPs, differences in allele frequencies could still alter its accuracy. In this regard, given the correlation of allele frequencies, we would expect an acceptable performance of the PRS14_{AAD} only in populations with European ancestry. Finally, the causal variants may vary between ancestries, and environmental factors may be population specific. These limitations do not invalidate the PRS predictions in the study cohort, but when applied in a population of different ancestry or subject to another environmental load, the same accuracy cannot be taken for granted.

Conclusions

The calculation of individual-level PRS14_{AAD} demonstrated that the youngest patients have a strong enrichment of risk alleles, and helped to correctly diagnose the two cases with low PRS14_{AAD} with a Mendelian form of PAI. The stratification of autoantibody-negative cases by PRS suggested mixed disease etiologies and further demonstrated that PRS14_{AAD} can contribute to the investigation of PAI etiology. The average age of AAD onset differed up to 20 years depending on PRS14_{AAD}. Given that future studies may uncover more of the genetic background of AAD, the predictive performance of a PRS will improve even further. Additional research will be needed to validate individual risk predictions outside the Scandinavian population used to derive and test our PRS14_{AAD}.

Materials and methods

Subjects

Genotypes, clinical information and serological results were extracted from the Swedish and Norwegian Addison registries [7, 8], as previously described [19]. In short, PAI was defined as low serum cortisol with a compensatory increase in plasma adrenocorticotrophic hormone. AAD was defined as PAI with (1) autoantibodies against 21-hydroxylase, the adrenal-specific enzyme that is the major autoantigen in AAD; and (2) the absence of APS-1—that is, presence or history of hypoparathyroidism, mucocutaneous candidiasis, autoantibodies against cytokines (interferon- α , interferon- ω , and/or interleukin 22), or biallelic/dominant *AIRE* gene mutations. Hence, patients with a *suspected* APS-1 diagnosis were excluded from the AAD cases using clinical, serological, and/or genetic criteria. In contrast, for the separate subgroup analysis of patients with *confirmed* APS-1, we required biallelic pathogenic variants in *AIRE*. *AIRE* gene variants from array genotyping, PCR, targeted sequencing, and Sanger sequencing were available from previous studies [19, 46, 47]. These studies have aimed to target exons and exon-intron boundaries, as well as the deletion of exons 1–8 associated with APS-1. Healthy controls were recruited from blood donors across Sweden and Norway to match the national coverage of both registries.

All study subjects gave their informed consent. Swedish Ethical Review Authority (dnr 2008/296-31/2), as well as the local ethics committee in Western Norway (biobank 2013-1504, project 2017-624), approved the study, which was performed in compliance with the Declaration of Helsinki.

Genotyping

Samples were genotyped by the Human Genomics Facility at Erasmus MC (Rotterdam, the Netherlands) using the Illumina Infinium Global Screening Array 1.0, as previously reported [19]. After imputation and quality control, 7.1 million genetic markers for 5320 unrelated subjects remained for analysis. Phasing was performed in-house to leverage available pedigree information (SHAPEIT version 2.r837) [48], and imputation using the Sanger Imputation Service (PBWT) and the Haplotype Reference Consortium release 1.1 [49].

Derivation of the PRSs

Our dataset comprised 1223 AAD cases and 4097 controls that we divided into a training set (763 cases, 2557 controls); a tuning set (120 cases, 380 controls); and a validation set (340 cases, 1160 controls). We allocated 2000 subjects for tuning and validation, in-line with recommendations [50], leaving 62% of samples for training the model. All subsets had the same proportion of cases and controls.

We constructed and compared two genome-wide scores: (1) a score built with the software PRSice-2 v.2.1.11 [51, 52] (clumping and thresholding) with subsequent manual inclusion of HLA risk scores, and (2) a score built using LDpred2 [53, 54]. Both are methods for constructing PRSs that take into account the correlation of SNPs in order to avoid double counting of genetic effects in prediction [55].

Clumping and thresholding

We first estimated effect sizes of risk alleles in a genome-wide case-control association analysis using logistic regression exclusively in the training set. Sex and the first five principal components were included as covariates. Associations with alleles and amino acids in the HLA region were estimated using stepwise logistic regression, as previously described [19]. Second, we used PRSice-2 in the tuning set [51, 56, 57] to select a set of SNPs to include in the PRS using clumping and thresholding. Clumping refers to a procedure in which the most significant of two correlated SNPs ($r^2 > 0.1$)—in a window of size 250 kbp—is kept, whereas the rest is discarded [58]. Thresholding refers to the application of a hard cutoff for association p -values for inclusion of SNPs in the score. We tested a grid of 18 thresholds and selected the model with the best case-control discrimination in the tuning set, defined as the simplest model to reach the maximum AUC (p -value $\leq 1e - 4$ for HLA risk and p -value $\leq 1e - 7$ for non-HLA region, Table S1). Finally, the predictive performance of the optimized PRS was evaluated in the validation set.

LDpred2

The algorithm LDpred2 is a Bayesian method that derives genetic risk scores from genome-wide association study (GWAS) summary statistics and a linkage disequilibrium (LD) reference matrix [53, 59, 60]. We used the same datasets and summary

statistics as for clumping and thresholding. To test multiple PRSs with different combinations of SNP-heritability (h^2) and the proportion of causal SNPs (p), we ran the LDpred2-grid algorithm with default settings, using an LD reference panel built from the tuning and validation sets. LDpred2 automatically adjusts the effect sizes obtained from the summary statistics according to h^2 and p . The best PRS model—identified by maximum AUC in the tuning set—was achieved when h^2 and p were estimated to 0.44 and 0.0046, respectively.

Statistical analyses

Case-control association in the training set was estimated using logistic regression on allele dosages, with the first five principal components and sex as covariates. Welch's ANOVA test followed by the Games–Howell post hoc test was used to compare the mean PRS among groups, and the Mann–Whitney U test when comparing only two groups. Significant p -values were represented with asterisks (* <0.05 , ** $<1e - 3$, *** $<1e - 4$, **** $<1e - 5$). Association of age of AAD onset and PRS14_{AAD} was performed with a linear regression (lm) in R v4.1.1.

Whole genome sequencing

Patients with early disease onset were recruited for whole-genome sequencing ($n = 18$). Whenever possible, their parents were offered participation in WGS to facilitate detection of de novo variants. Approximately 2.2 μg DNA was extracted from each sample of peripheral blood. We used a PCR-free library preparation, paired-end Illumina TruSeq 150 bp. Sequencing was performed with Illumina HiSeq X at the National Genomics Infrastructure at the Science for Life Laboratory Stockholm (Sweden). DNA from additional relatives was extracted (QIAGEN QIAamp DNA Blood Midi Kit CAT#51183) and subsequently sequenced with NovaSeq 6000 using the same library preparation. Both sequencing methods achieved 30X median read depth across the genome, and more than 90% of the genome was covered at least 15X. Mean quality across all bases was 35 (Phred Score). Bioinformatic analysis of the sequencing data was performed at the Clinical Genomics Stockholm platform at the Science for Life Laboratory using the Mutation Identification Pipeline (v.8.2.1) [61, 62]. Sequencing reads were aligned to GRCh37 before variant calling, variant recalibration, and functional annotation of variants with VEP, SnpEff, ANNOVAR, and GENMOD.

Variant interpretation

Variants from WGS were uploaded into the graphical user interface Scout v.4.12 [63] for prioritization. In short, variants were ranked according to deleteriousness, variant quality, allele frequency, gene intolerance prediction, conservation rate, and inheritance mode [61]. Variants with an allele frequency higher than 2% in the population database gnomAD v2.1.1 [64] and noncoding variants beyond intron-exon boundaries were disregarded. Remaining variants were assessed using criteria from the American College of Medical Genetics and Genomics [65]. First, variants in genes known to cause PAI were screened (Table S6, Note I) and, subsequently, potential de novo variants were investigated.

Author contributions

Maribel Aranda-Guillén, Eystein S. Husebye, Olle Kämpe, Sophie Bensing, Stefan Johansson, and Daniel Eriksson conceived and designed the study. Maribel Aranda-Guillén, Sara Fletcher-Sandersjö, Ileana Ruxandra Botusan, Marianne A. Grytaas, Åsa Hallgren, Lars Breivik, Anders P. Jørgensen, Elinor Vogt, Eystein S. Husebye, Olle Kämpe, Anette S. Bøe Wolff, Sophie Bensing, and members of the Swedish and Norwegian Addison Study groups characterized the patients. Maribel Aranda-Guillén, Ellen Christine Røyrvik, Sara Fletcher-Sandersjö, Haydee Artaza, Ileana Ruxandra Botusan, Maria Pettersson, Anna Lindstrand, Eystein S. Husebye, Olle Kämpe, Anette S. Bøe Wolff, Sophie Bensing, Stefan Johansson, Daniel Eriksson acquired, processed, analyzed, and interpreted data. Maribel Aranda-Guillén and Daniel Eriksson drafted the manuscript. All authors critically revised the manuscript for important intellectual content.

Acknowledgements

We sincerely thank the numerous clinicians and technicians who contributed to patient inclusion and sample collection, as well as all study participants. We also thank C. Wong for critical review of the manuscript. This study was funded by Swedish Research Council, the Knut and Alice Wallenberg Foundation, the Torsten and Ragnar Söderberg Foundations, the Novo Nordisk Foundation, the Swedish Society for Medical Research, the KG Jebsen Foundation, the Research Council of Norway, the Health Authorities of Western Norway, and the ALF-agreement between the Swedish

government and the county councils. We would like to acknowledge support from the National Genomics Infrastructure and Clinical Genomics Stockholm, both at the Science for Life Laboratory for providing excellent assistance with massive parallel sequencing and expertise and service with sequencing analysis. We would also like to acknowledge the computational resources provided by the Swedish National Infrastructure for Computing (SNIC) through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX), under projects sens2017513 and sens2018526.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1: PRS optimization results in tuning set.

Table S2: Loci included in the PRS from GWAS of the training set (763 21-hydroxylase autoantibody positive cases and 2557 controls).

Table S3: The PRS14_{AAD} components and weights.

Table S4: Population allele frequencies, non-HLA risk alleles.

Table S5: Population allele frequencies, HLA risk alleles.

Table S6: List of variants found in genes associated with monogenic PAI.

Table S7: Impact of PRS on the risk of autoimmune Addison's disease in the general population.

Figure S1: Population allele frequencies, non-HLA risk alleles.

Figure S2: Odds ratio (OR) for autoimmune Addison's disease (AAD) in polygenic risk score (PRS) quintiles of the validation set (340 cases and 1160 controls).

Figure S3: PRS in 21-hydroxylase autoantibody negative patients with primary adrenal insufficiency.

Figure S4: Frequency distribution of genetic risk scores in APS-1, APS-2 and isolated AAD.

Figure S5: Age of onset and disease duration comparison between patients suffering from APS-2 and isolated AAD. ■