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CASE REPORT

- 1 Phenotypic and cytogenetic features of an Iranian child with tetrasomy 18p syndrome: A case report
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ABOUT COVER

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Phenotypic and cytogenetic features of an Iranian child with tetrasomy 18p syndrome: A case report

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Abstract

BACKGROUND

Tetrasomy 18p is a rare chromosome abnormality disorder known to have considerable variability in clinical features and gathering data from different cases will help clinicians and researchers learn about its genotype-phenotype relationship and diagnosis.

CASE SUMMARY

Herein, we have reviewed the literature on phenotypic features of this disorder and described the phenotypic and cytogenetic features of a girl of early childhood with tetrasomy 18p for the first time from Iran. This patient showed a strong sense of smell (a unique feature not reported previously for this syndrome), had clenched hand, pes planus, forward head posture in walking and hirsutism (dysmorphic features less reported), and showed 10 clinical features that are generally observed in previously reported cases, including developmental delay/intellectual disability, triangular face, smooth philtrum, feeding difficulties, hypotonia, epicanthus, strabismus, history of constipation, growth retardation and foot anomalies. G-banding chromosome analysis from peripheral blood revealed an abnormal female karyotype with a small marker chromosome (47,XX,+mar), and oligo-array comparative genomic hybridization displayed a gain of 14Mb of the 18p arm containing 56 Online Mendelian Inheritance in Man (OMIM) genes in this patient. Overall, this patient seems to have mild phenotypes.

CONCLUSION

This Iranian tetrasomy 18p child displays a uniquely strong sense of smell, some less reported dysmorphic features and ten features generally reported.

Key Words: Tetrasomy 18p; Phenotypic features; Clinical features; Chromosome abnormality; Cytogenetic analysis; Case report

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Core Tip: Gathering data from different cases for the rare tetrasomy 18p chromosome abnormality disorder will facilitate mastering its genotype-phenotype relationship and diagnosis. This report described features for the first time for an Iranian patient. Compared to previously reported cases, this Iranian child displays a uniquely strong sense of smell, some less reported dysmorphic features and ten features generally reported. Her cytogenetic analyses revealed a small marker chromosome with a gain of 14Mb at the 18p arm. Apart from the usual clinical assessments, the non-invasive prenatal genetic testing is suggested to be used as a more accurate screening for detecting tetrasomy 18p.

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INTRODUCTION

Tetrasomy 18p is a rare chromosome abnormality occurring in approximately 1 per 180000 Live births which affects both males and females equally. In tetrasomy 18p, there is an additional iso-chromosome which is composed of two copies of chromosome 18 short arm. Thus, each cell has four copies of the short arm[1-5]. Iso-chromosome 18p is one of the most frequent iso-chromosomes in humans[3,6]. It has been suggested that maternal meiosis II nondisjunction followed by meiotic misdivision at the centromere is responsible for tetrasomy 18p. Thus, the maternal age can play a role in the formation of the iso-chromosome 18p[1,7,8].

While the clinical features of the tetrasomy 18p are variable, the main features of patients with tetrasomy 18p are growth retardation, neonatal jaundice, microcephaly, seizures, strabismus, heart defects, abnormalities in muscle tone, a history of constipation and gastroesophageal reflux, and scoliosis/kypnosis[9-15]. Because of phenotypic feature variability, gathering data from different cases will help clinicians and researchers learn about genotype-phenotype relations and diagnosis of tetrasomy 18p[16,17].

In this paper, we have reviewed the literature on phenotypic features of this disorder with the cases indexed in PubMed with the search term of “Tetrasomy 18p”, and we have described the phenotypic and cytogenetic features of a girl of early childhood with tetrasomy 18p for the first time from Iran.

CASE PRESENTATION

Chief complaints

A girl of early childhood was referred for genetic analysis due to intellectual disability, hypotonia, speech delay, strabismus, valgus deformity or feet anomaly (Figure 1A) together with pes planus (Figure 1B). The girl showed developmental delay in her infancy at which time she could not sit, made no speech-like sound, and paid no attention to surroundings.

History of present illness

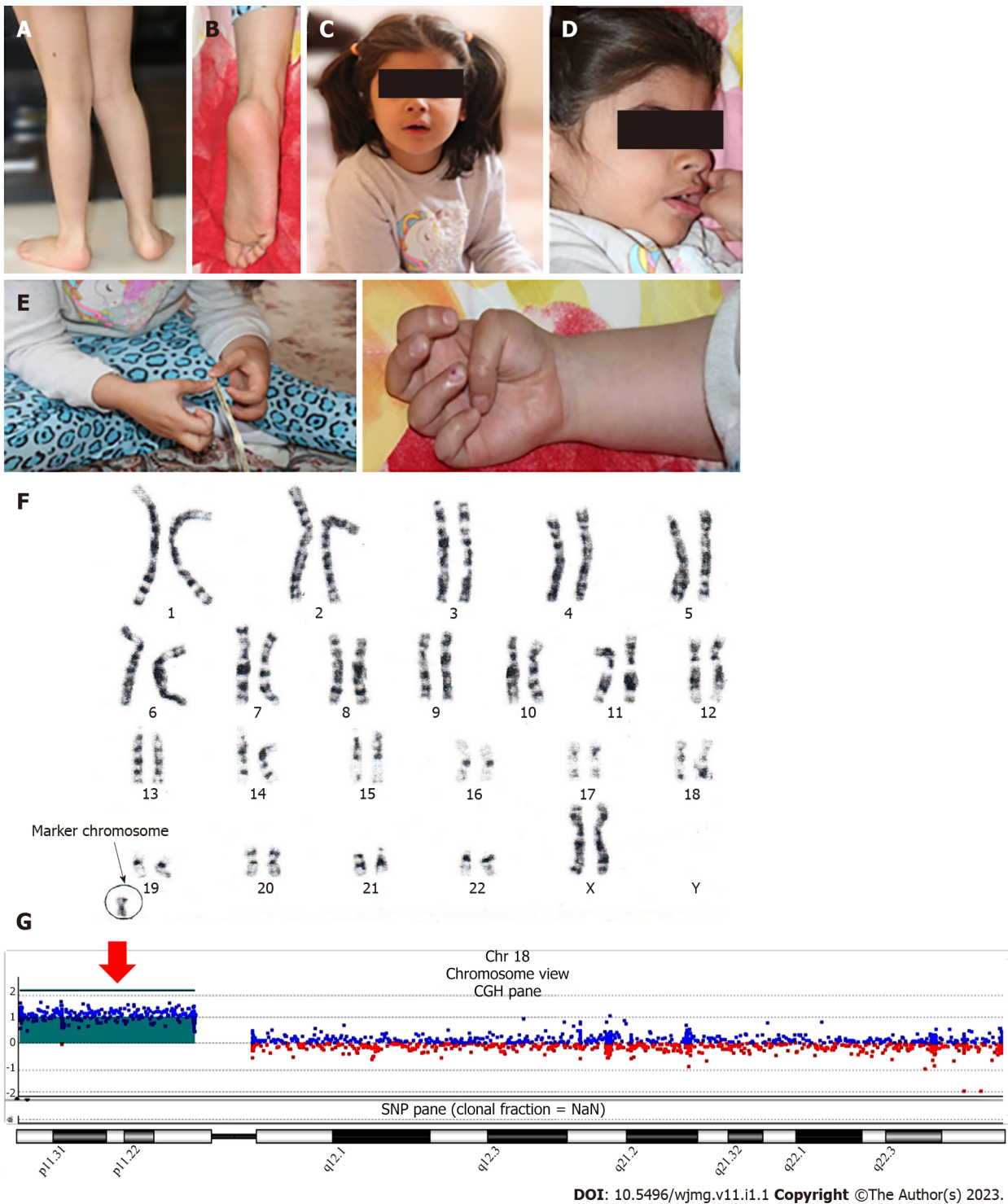
Triangular face, epicanthus and smooth philtrum are other dysmorphic features of the patient. At early childhood, evidence of strabismus can still be seen on her face (Figure 1C) although it had been partially treated by surgery at 9-months' age. Other dysmorphic features found included mild hirsutism (Figure 1D), clenched hands (Figure 1E) and forward head posture in walking.

History of past illness

At infancy, she presented with difficult breastfeeding and severe cries, she had epilepsy-like symptoms, and she developed strabismus.

Personal and family history

The patient's parents were healthy and non-consanguineous. At the time of trying to conceive, the mother was receiving antibiotic treatment for Listeriosis. At the end of the pregnancy and prior to giving birth to the baby girl, the mother had bed rest at home for one month for preventing premature birth and preterm labor. The ultrasound scans during pregnancy and screening evaluations were normal. At the time of patient's birth, the mother and the father were 30 and 29, respectively. The infant was born at 38 wk of gestation by cesarean section, weighing 3000 g with a length of 48 cm and head circumference of 32.5 cm.



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Figure 1 Phenotypic and cytogenetic features of the Iranian girl with tetrasomy 18p syndrome. A: Valgus deformity (with a valgus angle equal to or greater than 10°); B: Pes planus foot deformity with the loss of the longitudinal arch of the foot where it contacts the ground; C: The evidence of strabismus can still be seen on her face at early childhood, a condition she developed from birth and was treated by surgery at 9-month's age; D: Mild hirsutism in the face of the girl; E: Clenched hand deformity, with evidence of malformation of the hand and malpositioning of the fingers; F: G-banding chromosome analysis showing the abnormal female karyotype with a small marker chromosome (47,XX,+mar); G: Oligo-Array CGH results, showing a gain of 13.9 Mb pathogenic region on 18p11.32p11.21 from nucleotide 148963 to 14081887, which contains 56 OMIM genes.

Physical examination

Using Gilliam Autism Rating Scale (GRAS)[18] and through parental report, the probability of autism was evaluated in the patient, and her score of 38 indicated a low probability of autism. The results of her echo-cardiography evaluations were also normal.

Laboratory examinations

Peripheral blood specimens from the patient and her parents were collected for cytogenetic analyses. Twenty metaphase spreads from cultured blood lymphocytes were analyzed using G-banding technique at the resolution of 400 bands. Chromosome analysis in the patient revealed the abnormal female karyotype with a small marker chromosome (47,XX, +mar) (Figure 1F), while karyotypes of her parents were normal.

Imaging examinations

Although she had epilepsy-like symptoms at infancy, her brain ultrasonography and brain magnetic resonance imaging (MRI) evaluations showed normal results.

Further diagnostic work-up

Further investigation for copy number changes by Oligo-Array Comparative Genomic Hybridization (CGH) showed the gain of 13.9 Mb on 18p11.32p11.21 from nucleotide 148963 to 14081887 (Figure 1G). The pathogenic region was assessed and revealed that it contains 56 OMIM genes. These results are compatible with tetrasomy of 18p.

FINAL DIAGNOSIS

With the patient's medical history and combined with clinical and cytogenetic features, the final diagnosis of this Iranian girl was tetrasomy of 18p disorder.

TREATMENT

Strabismus that developed at birth was treated partially with surgery at the age of 9 mo.

OUTCOME AND FOLLOW-UP

The patient currently still shows intellectual disability.

DISCUSSION

This is the first case report of a child with tetrasomy 18p from Iran. This genetic disorder shows variable clinical characteristics. Among the 31 most reported features from tetrasomy 18p patients (listed in Table 1), our case had only 10 clinical features that are generally observed in most previously reported cases, including developmental delay/intellectual disability, triangular face, smooth philtrum, feeding difficulties, hypotonia, epicanthus, strabismus, history of constipation, growth retardation and foot anomalies. Dysmorphic features like clenched hand, pes planus, forward head posture in walking and hirsutism observed in our case are less reported in tetrasomy 18p patients. Although features such as small mouth, low-set ears, microcephaly, brain MRI variants, scoliosis/kyphosis, recurrent otitis media, jaundice, cardiac defects, and epilepsy were not seen in our patient, they have been reported in at least 25% tetrasomy 18p cases (Table 1). However, our patient showed the unique feature – having a strong sense of smell, which was not reported as a common feature for this syndrome. Overall, this patient seems to have mild phenotypes.

In 2015, 43 children with tetrasomy 18p were assessed to determine the probability of autistic behaviors, and the results demonstrated that some individuals who had mild cognitive deficits did not show characteristics associated with autism[19]. In the present study, the result of GRAS showed that the girl did not exhibit behaviors like a child with autism. However, she was educable; for example, she has learned feeding ducks and played with familiar children.

Although it has been reported that the tetrasomy 18p occurs usually de novo, some studies reported both paternal and maternal origins[6,7,16,20,21]. In our case, the karyotypes of parents were normal; so, the abnormality of chromosome 18 was not inherited.

Array CGH displayed a gain of 14Mb of the 18p arm in this patient. Among genes located in this pathogenic region, some are associated to developmental disorder phenotypes according to OMIM database (<https://www.omim.org/>), including LAMA1, GNAL, TGIF1, PIEZO2, AFG3L2 and MC2R. Another gene in the duplicated region was SMCHD1, an epigenetic regulator which has recently been found to have functions during development and developmental disorders[22,23]. Interestingly, the existence of AFG3L2, MC2R, TGIF1, SMCHD1, LAMA1 and PIEZO2 genes in the duplicated region was reported previously in tetrasomy 18p cases[9,17]. Thus, the patient's developmental disorders such as

Table 1 Comparisons of phenotypic characteristics between this Iranian child case and some reported cases of tetrasomy 18p

Clinical findings	Other reported cases	Our case	Total
Developmental delay/intellectual disability	117 of 117	Yes	(118 of 118) 100%
Triangular face	11 of 12	Yes	(12 of 13) 92%
Smooth philtrum	36 of 40	Yes	(37 of 41) 90%
Feeding difficulties	61 of 108	Yes	(62 of 118) 53%
Hypotonia	56 of 117	Yes	(57 of 118) 48%
Epicanthus	5 of 12	Yes	(6 of 13) 46%
Strabismus	44 of 99	Yes	(45 of 100) 45%
History of constipation	35 of 108	Yes	(36 of 109) 33%
Growth retardation	36 of 117	Yes	(37 of 118) 31%
Foot anomalies	21 of 93	Yes	(22 of 94) 23%
Small mouth	25 of 40	No	(25 of 41) 61%
Low-set ears	25 of 41	No	(25 of 42) 60%
Microcephaly	60 of 117	No	(60 of 118) 51%
Brain MRI variants	13 of 27	No	(13 of 28) 46%
Cryptorchidism	19 of 47	No	(19 of 48) 40%
Scoliosis/kyphosis	33 of 86	No	(33 of 87) 38%
Recurrent otitis media	38 of 108	No	(38 of 109) 35%
Jaundice	31 of 108	No	(31 of 109) 28%
Cardiac defects	26 of 107	No	(26 of 108) 25%
Seizures	24 of 117	No	(24 of 118) 20%
Respiratory distress	16 of 107	No	(16 of 108) 15%
History of gastroesophageal reflux	15 of 107	No	(15 of 108) 14%
Hearing loss	11 of 103	No	(11 of 104) 11%
Kidney defect	9 of 98	No	(9 of 99) 9%
Hernias	9 of 107	No	(9 of 108) 9%
Hypospadias	3 of 55	No	(3 of 56) 5%
Pyloric stenosis	4 of 107	No	(4 of 108) 4%
Growth hormone deficiency	4 of 96	No	(4 of 97) 4%
IgA deficiency	4 of 107	No	(4 of 108) 4%
Hypoglycemia	1 of 107	No	(1 of 108) 1%
Bradycardia	1 of 107	No	(1 of 108) 1%

Data collected from previous reports and reviews[5,6,8,11,14,20].

delayed motor development, speech delay, psychomotor retardation and hypotonia might be related to these genes. In addition, although the SMCHD1 gene has been reported to have a role in the Bosma arhinia and microphthalmia disorder where the patients have problems with the sense of smelling[22], further studies are required to establish whether and how the over-production of SMCHD1 gene or other genes in the duplicated region may contribute to the patient's phenomenon of having a very strong sense of smell.

As mentioned above, during pregnancy all screening evaluations, laboratory tests, and ultrasounds scans were normal, and no suspicious findings were found. Also, the birth weight, length and head circumference were normal. Because of the disease rarity and variety of clinical features in tetrasomy 18p, the prenatal diagnosis of this abnormality is difficult[7]. It has been demonstrated that the non-invasive prenatal testing (NIPT) can be used to detect tetrasomy 18p using cell-free DNA screening technology without any procedural risk of miscarriage[3,24,25]. Therefore, it is suggested that in addition to the

usual assessments during pregnancy, NIPT can be used as a more accurate screening test to detect tetrasomy 18p during pregnancy and prior to the birth of an abnormal baby.

CONCLUSION

To the best of our knowledge, this is the first case report of a child with tetrasomy 18p from Iran. In this study, we have reviewed the literature reported in PubMed on phenotypic features of this rare chromosome abnormality disorder and described the phenotypic and cytogenetic features of this Iranian girl of early childhood with tetrasomy 18p. This patient showed 10 clinical features that are generally observed in most previously reported cases, and she had dysmorphic features including clenched hand, pes planus, forward head posture in walking and hirsutism that are less reported in tetrasomy 18p patients. Furthermore, this patient showed the unique feature of having a strong sense of smell, which was not reported for this syndrome. G-banding chromosome analysis revealed an abnormal female karyotype with a small marker chromosome (47,XX, +mar) with karyotypes of her parents being normal, and oligo-array comparative genomic hybridization displayed a gain of 14Mb containing 56 OMIM genes of the 18p arm in this patient. Overall, while this patient seems to have mild phenotypes, further genetic work is required to define its underlying genetic defect for the unique feature of having a strong sense of smell. Based on assessments for this case and the literature, it is suggested that in addition to the usual clinical assessments during pregnancy, the non-invasive prenatal genetic testing can be used as a more accurate screening test to detect tetrasomy 18p prior to the birth of an abnormal baby.

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FOOTNOTES

Author contributions: Esmaili S and Xian CJ designed the research study; Esmaili S performed the research; Esmaili S and Xian CJ analyzed the data and wrote the manuscript; all authors have read and approved the final manuscript.

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Basic Study

Genome-wide associations, polygenic risk, and Mendelian randomization reveal limited interactions between John Henryism and cynicism

Richard R Chapleau

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Abstract

BACKGROUND

John Henryism (JH) is a strategy for dealing with chronic psychological stress characterized by high levels of physical effort and work. Cynicism is a belief that people are motivated primarily by self-interest. High scores on the JH scale and cynicism measures correlate with an increased risk of cardiovascular disease. High cynicism is also a hallmark of burnout syndrome, another known risk factor for heart disease.

AIM

To evaluate possible interactions between JH and cynicism hoping to clarify risk factors of burnout.

METHODS

We analyzed genetic and psychological data available from the Database of Genotypes and Phenotypes for genome-wide associations with these traits. We split the total available samples and used plink to perform the association studies on the discovery set ($n = 1852$, 80%) and tested for replication using the validation set ($n = 465$). We used scikit-learn to perform supervised machine learning for developing genetic risk algorithms.

RESULTS

We identified 2, 727, and 204 genetic associations for scores on the JH, cynicism and cynical distrust (CD) scales, respectively. We also found 173 associations with high cynicism, 109 with high CD, but no associations with high JH. We also produced polygenic classifiers for high cynicism using machine learning with areas under the receiver operator characteristics curve greater than 0.7.

CONCLUSION

We found significant genetic components to these traits but no evidence of an interaction. Therefore, while there may be a genetic risk, JH is not likely a burnout risk factor.

Key Words: Cynicism; Burnout syndrome; John Henryism; Genome-wide association study; Polygenic risk score; Machine-learning

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Core Tip: This study evaluates the interaction of a job-related cardiovascular disease risk factor (John Henryism) and the development of occupational burnout (specifically the cynicism and cynical distrust components). Genome-wide associations and statistical genetics revealed that while John Henryism is not a risk factor for burnout syndrome, there are independent genetic risk factors for both John Henryism and cynicism. These new results provide additional tools to industrial and occupational psychologists, as well as cardiologists, to help reduce burnout incidence.

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INTRODUCTION

First described formally by Freudenberg[1] and later expanded upon by Maslach and Jackson[2], burnout syndrome is generally considered to be a response to long-term occupational stress[3]. The most widely used definition of burnout syndrome is a 3-component syndrome comprised of emotional exhaustion, depersonalization or cynicism, and feelings of low professional efficacy or personal[4]. High levels of burnout syndrome components have been associated with increased disease prevalence including a heightened risk of cardiovascular disease[5,6], impaired cognitive function[7], increased sleep disorders[8,9], and even type II diabetes and hyperlipidemia[10,11]. Clearly, the impacts of burnout syndrome extend beyond just performance in the workplace and can adversely affect health, well-being, and quality of life.

Another response to chronic social stressors is John Henryism (JH), a coping mechanism by which an individual exerts increased effort to overcome stress[12]. As with burnout syndrome, the effects of JH affect both the individual's health and the workplace. Also, like the effects of burnout syndrome, JH can have a negative impact on cardiovascular health, hypertension, and increased rates of alcoholism[13-16]. Paradoxically, high levels of JH have been associated with lower levels of depressive symptoms in African Americans of varying socioeconomic status, suggesting a protective effect on mental health[17]. These findings suggest a nuanced interaction between JH and overall health and well-being.

The literature on the genetic contribution to burnout syndrome and JH is limited. Preliminary work regarding the heritability of cynicism (one of the three components of burnout syndrome) was reported in the late 1980's to early 1990's and revealed mixed results. One twin study of cynical hostility revealed a non-significant genetic component[18]. In contrast, three other twin studies using the Cook Medley Hostility Scale and the MMPI showed significant heritability[19-21]. The genetic heritability of JH also has limited reports, with one estimate suggesting up to 35% of the variability is explained by genetic factors[22,23]. To the best of our knowledge, there have not been any genome-wide or candidate gene studies performed to assess the genetic contribution to these traits.

Here we report the results of our study investigating the relationship between JH and cynicism. We hypothesized that JH would exert a causal influence on cynicism. We came to this hypothesis because of the nature of JH as a coping mechanism for dealing with discriminatory acts and the skepticism and negative view of others inherent to cynicism. We tested this hypothesis through the statistical approach called Mendelian randomization[24], where genetic associations with JH are considered for their independent influence on cynicism. In order to take this approach, we first performed a genome-wide association study (GWAS) to identify genetic variables for JH and cynicism independently. We also extended those GWAS results to develop polygenic risk scores (risk scores considering multiple genetic variants) for each trait and assessed if the higher genetic risk in one trait correlated to higher levels of the other trait. To our knowledge, this study is the first to report genetic associations with any of the three outcomes.

MATERIALS AND METHODS

This study was reviewed and approved by WCG IRB (Study number 1332892) for human subjects research oversight. All data were obtained from the National Institutes of Health's Database of Genotypes and Phenotypes (dbGAP). We used data from the Coronary Artery Risk Development in Young Adults (CARDIA) Study (dbGAP study accession phs000285)[25]. The CARDIA cohort study design was a longitudinal study, but we used the data in a retrospective fashion. We followed the STREGA guidelines available from the EQUATOR network[26]. The guideline table with annotations regarding how we addressed each point is available as Supplementary material.

Psychological trait definitions

JH was measured by the 12-item John Henryism Active Coping Scale, and responses were reverse-coded. JH was calculated as a mean of the responses. As our goal was to observe the effect of having above-average JH, we defined a dichotomous variable of JH as individuals with scores above the median (49) as having high JH[13,15]. Individuals with scores at or below the median were considered average or below average JH. We also performed tests using the JH score as a continuous, mean-centered variable. The JH data were obtained from dbGAP accession number phv001133534.v2.p2.c1. Cynicism and cynical distrust (CD) were measured as 12- and 8-item subscales of the Cook-Medley Hostility Scale (CMHS), respectively[27-29]. Mean-centered continuous variables and median-adjusted dichotomous variables were created for cynicism and CD in the same manner as for JH, resulting in four CMHS-derived variables (continuous cynicism, high/Low cynicism, continuous CD, and high/Low CD). The CMHS data were obtained from dbGAP accession number phv00113478.v2.p2.c1. As not all participants completed all questionnaires, missing data were filled in as the mean. Processed data were then split into a validation set (20%, $n = 623$) and the remainder were used for training (Figure 1). Statistical analyses were performed in R version 4.2.0.

Power analysis

We performed a power analysis for our study to determine if the sample sizes available in the CARDIA cohort were sufficient to identify significant genetic associations. We assumed a 10% effect allele frequency (EAF) in the control population, 20% type-II error rate (beta) and 5% type-I error rate (alpha). We used the observed case-control ratios for each condition to calculate the statistical power. We report the results of the analysis as the minimum EAF in the case population to achieve 80% power in Table 1 alongside the cohort characteristics.

Genetic data pre-processing

Microarray (Affymetrix Genome-Wide Human SNP 6.0 Array) genotype data were obtained from the CARDIA study (dbGAP accession numbers phg000092.v2 and phg000098.v2). Genetic data were pre-processed to ensure uniformity from the original plink data. Briefly, the binary plink file sets were merged and filtered for autosomal genotypes with less than 10% missing genotype calls (sample or locus), a minor allele frequencies threshold was set at 1%, and a Hardy-Weinberg equilibrium threshold of 0.0001 was used for filtering out spurious variants. After pre-processing, they were split into the training and validation sets using the sample identifiers defined in the phenotype data splitting.

Genome-wide association studies

We conducted GWAS using plink 1.9 evaluating only the total scores (not scores for the questionnaire responses) and high/Low status for each trait, totaling 6 phenotypes. We used the following command for the association: `plink --bfile <training dataset prefix> --memory 15000 --pheno <phenotype filename>.csv --all-pheno --assoc --pfilter 0.001 --adjust qq-plot --out <output filename>`. For assessing the replication of associations in continuous variables traits, we repeated the GWAS in plink restricting the input variants to only those candidates identified in the discovery phase. For dichotomous traits, the set of candidate single nucleotide polymorphisms (SNPs) identified as significantly associated with the trait ($P < 5 \times 10^{-8}$) or suggestively associated ($P < 5 \times 10^{-6}$) were then evaluated by chi-square test using the test dataset. Additionally, each analysis was repeated conditioning upon the highest associated SNP.

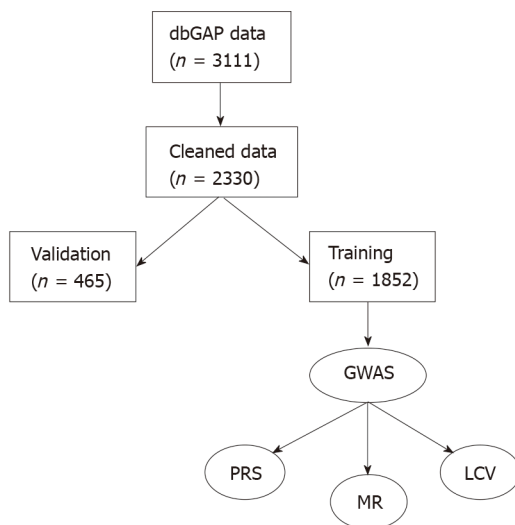
Polygenic risk score calculation

Our method of PRS development was using the machine learning package scikit-learn[30]. We used four supervised classifiers [Ridge, multi-layer perceptron, random forest, and k-nearest neighbors (KNN)] and iterated through the relevant parameter space for each classifier (e.g., number of neighbors for KNN). The classifiers were trained on the training set ($n = 1852$) and validated using the test dataset ($n = 465$). Each classifier was evaluated using the area under the receiver operator characteristics (ROC) curve (AUC) and the model with the best AUC was saved for each classifier. Finally, a ROC curve comparing the best models of each classifier was created.

Table 1 Breakdown of samples by dichotomous traits, *n* (%)

	John Henryism	Cynicism	Cynical distrust
Training high	877 (47.4)	724 (39.1)	680 (36.7)
Training not high	975	1128	1172
Case:Control ratio	1:1.1	1:1.6	1:1.7
Minimum EAF, %	14.5	15.7	16.0
Validation high	246 (52.9)	180 (38.7)	171 (36.8)
Validation not high	219	285	294

EAF: Effect allele frequency.



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Figure 1 Flow diagram for data processing. Rectangles are datasets with sample sizes in parentheses and ovals are processes. dbGAP: Database of Genotypes and Phenotypes; GWAS: Genome-wide association studies; LCV: latent causal variable; MR: Mendelian randomization; PRS: Polygenic risk score.

Mendelian randomization

We performed two-sample Mendelian randomization (2SMR) estimates using the TwoSampleMR R package[24]. We used the MR Egger regression[31], inverse variance weighted estimator[32], weighted median estimator[33], and Wald ratio estimator[34] algorithms. Instrumental variables (IV, SNPs associated with the exposure) were extracted by P value thresholds 5×10^{-6} and 5×10^{-8} . We excluded SNPs in strong linkage disequilibrium (LD) to reduce bias and used a clumping process with European samples from the 1000 Genomes Project ($r^2 < 0.001$, window size = 10000). If SNPs identified in the exposure dataset were not in the outcome dataset, proxy SNPs in LD ($r^2 > 0.9$) were used as instrumental variables. For the sensitivity analysis, we performed heterogeneity testing using Cochran's Q and I^2 analyses[35] and tested pleiotropy on the weighted median estimation results[33].

Statistical analysis

Statistical analyses for assessing variant associations were performed in R. To confirm the association of the variants identified for the high cynicism and high CD variables, we created a matrix containing the allele counts for each candidate variant. We then performed a chi-square analysis using the `chisq.test` function within base R using the allele count matrix as the input argument. The statistical analyses of the PRS estimators were performed in the scikit-learn package. As the class allocation displayed only slight imbalance (control/case group sample size ratios of 1:2.1, 1:2.5, and 1:2.7 for JH, cynicism, and CD, respectively), we used the ROC AUC for evaluating performance of the PRS classification models.

RESULTS

Characteristics of the dataset

The initial phenotype dataset consisted of 3111 samples (Figure 1). The median scores for JH, cynicism, and CD were 49, 6, and 3, respectively. Score ranges were 26 to 60 for JH, 0 to 13 for cynicism, and 0 to 8 for CD. There were 1497 (48.1%) samples with high JH scores ($JH > 49$), 1228 (39.5%) with high cynicism (> 6), and 1163 (37.4%) with high CD scores (> 3). We found minimal correlation between JH and cynicism or CD (Pearson $r = 0.159$ and 0.118 , respectively). After splitting the data into training (80%) and validation (20%) sets, the median scores were 49, 6, and 3, respectively, for the training set and 50, 5, and 3, respectively, for the validation set. The percent of samples above the population median scores was 47.1%, 40.0%, and 37.8%, respectively, for the training set and 52.3%, 37.4%, and 35.6%, respectively, for the validation set. T-tests showed that the distribution of samples in the subsets was not statistically different from the original distribution (JH training *vs* original $P = 0.52$; JH validation *vs* original $P = 0.12$; cynicism training *vs* original $P = 0.48$; cynicism validation *vs* original $P = 0.08$; CD training *vs* original $P = 0.62$; and CD validation *vs* original $P = 0.22$). Evaluating the Shapiro-Wilk normality test in R reveals that none of the six variables are normally distributed (all have $P < 2.2 \times 10^{-16}$). The Shapiro-Wilk W constants for the distributions of quantitative scores were 0.980, 0.935, and 0.970 for JH, CD, and cynicism, respectively, while the W constants for the dichotomous traits were 0.635, 0.615, and 0.622, respectively. The skewness of the three continuous traits was -0.511, 0.118, and 0.373, respectively, while the kurtosis values were 3.11, 1.01, and 2.18, respectively. From these results, we see that the data are generally positively skewed and primarily platykurtic.

The merged genetic dataset contained 2466 samples (1162 from accession phg000092 and 1441 from accession phg000098, with 137 overlapping between the two studies), of which 978 (42.1%) were male. The two datasets contained 909662 and 720622 markers, respectively. Following SNP filtering, there were 561045 variants remaining (153333 for missingness, 13410 below 1% MAF, and 144454 not passing the Hardy-Weinberg filter). 136 samples were removed for having greater than 10% missing genotype calls, creating a final dataset of 2330 samples. The total genotyping rate for the dataset was 99.4%. After splitting samples using the training and validation sets defined in the phenotype stage, there were 1852 samples in the training set, 465 samples in the validation set, and 13 (0.6%) of the samples with genetic data did not have phenotype data (Table 1).

GWAS Results

Our discovery phase GWAS analysis of 1852 samples revealed 25 candidate variants with Bonferroni-corrected P values below 5×10^{-8} associated with JH, 28926 candidates associated with cynicism, and 14134 candidate associations with CD (Figure 2). For each of the candidate variants, we performed a second GWAS on the test sample set ($n = 465$) using only the candidate SNPs identified in the first analysis. We found that 2 SNPs replicated as associated with the quantitative JH trait ($P = 0.002$), 727 replicated for association with cynicism ($P = 1.73 \times 10^{-6}$), and 204 replicated for association with CD ($P = 3.54 \times 10^{-6}$).

Similar to how we analyzed the continuous variables, we identified in the dichotomous variable analysis 3 candidate variants associated with high JH, 708 associations with high cynicism, and 17507 associations with high CD. After evaluating for replication of significance ($P < 0.05$) in the validation set of 465 samples, we found 0, 173, and 109 significantly associated variants that replicated in our test set for JH, cynicism, and CD, respectively. Of the 173 replicated variants associated with cynicism, 19 were located across 9 distinct loci (defined as within a 250000-base pair window on the same chromosome) and the other 154 were distinct variants. We also observed that 79 of the 173 high cynicism-associated variants were also associated with the quantitative trait, while none of the high CD-associated variants were present in the quantitative trait CD list. Lists of all candidate SNPs and replicated SNPs are available from the corresponding author upon reasonable request.

ML-based polygenic risk scores

We used the replicated SNPs for each condition cynicism and CD to evaluate the predictive capability for each, addressing the question of whether genetic variants associated with high JH, for example, were predictive of high cynicism. As no SNPs replicated for high JH, we used the candidate SNPs. Using the scikit-learn software package, we performed the nine tests for each input/output combination (e.g., cynicism *vs* CD, cynicism, and JH) with the four classification methods. Our results (Figure 3, Table 2) reveal that PRS algorithms based on genetic variants associated with high cynicism are predictive of high cynicism (AUC range = 0.696-0.732) and high CD (AUC range = 0.652-0.684), whereas algorithms trained on genetic markers of high JH or high CD are not predictive for any trait. These classifiers would be considered to be acceptable predictors[36] with AUC values near 0.7, these results show that cynicism and CD are genetically related, reinforcing the psychological relationship, and that JH is a distinct trait deriving from different genetic contributions.

Two-sample Mendelian randomization

Our two-sample Mendelian randomization analyses using the GWAS summary statistics previously

Table 2 Area under the curve values for ML-based polygenic risk algorithms

Input vs output pair	Ridge	MLP	RFC	KNN
JH vs JH	0.524	0.542	0.545	0.550
JH vs cynicism	0.638	0.634	0.649	0.660
JH vs CD	0.653	0.656	0.651	0.630
Cynicism vs JH	0.482	0.533	0.565	0.563
Cynicism vs cynicism	0.732	0.728	0.696	0.712
Cynicism vs CD	0.681	0.676	0.671	0.684
CD vs JH	0.526	0.545	0.546	0.563
CD vs cynicism	0.563	0.556	0.577	0.580
CD vs CD	0.516	0.516	0.526	0.533

JH: John Henryism; CD: Cynical distrust; MLP: Multi-layer perceptron; RFC: Random forest classifier; KNN: K-nearest neighbors. Bold values are the greatest area under the receiver operator characteristic curve for the input-output pairing.

described revealed significant causal relationships between all three traits (Figure 4). The odds ratios (Figure 4A) and beta coefficients (Figure 4B) for the relationships were highly similar, with odds ratios ranging from 2.0 (for the causal effect of JH on cynicism and the causal effect of CD on JH) to 3.3 (for the inverse effects), with the CD/cynicism relationship near 2.6. The mendelian randomization (MR) comparisons involved 4680 variants for the JH/cynicism relationship, 4262 variants for the JH/CD relationship, and 131185 variants for the CD/cynicism relationship. We performed pleiotropy and heterogeneity tests to assess the sensitivity of the MR estimates. We found significant pleiotropy in all cases, with the Egger intercepts significantly different from zero (Figure 4C)[37].

DISCUSSION

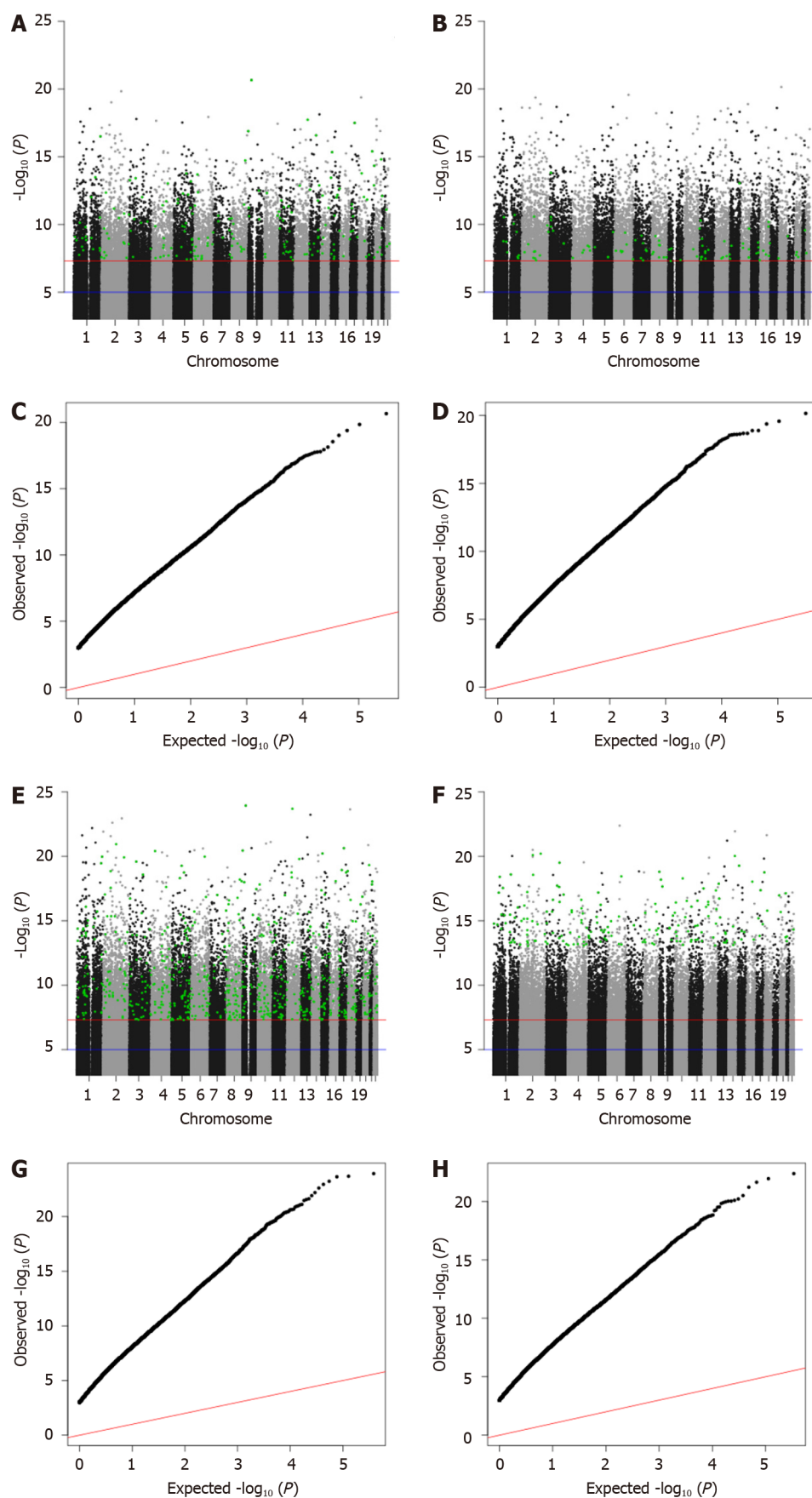
Our results from genetic analyses suggest that there is not a significant relationship between JH and cynicism. These observations are consistent with previously published literature using only psychometric approaches. Adams and co-workers[38] demonstrated that JH scores were not significantly correlated with the Cook Medley Hostility Scale, of which we used the cynicism subscale herein. In contrast, a large study investigating the effects of various psychosocial factors on chronic kidney disease found that JH and hostility exhibited inverse risk factor loading[39]. Finally, initial reports from the CARDIA study suggest a weak but significant correlation between hostility and effortful coping ($r = 0.14$, $P < 0.05$), especially among younger, less educated individuals that were more likely to consume alcohol and be smokers[40].

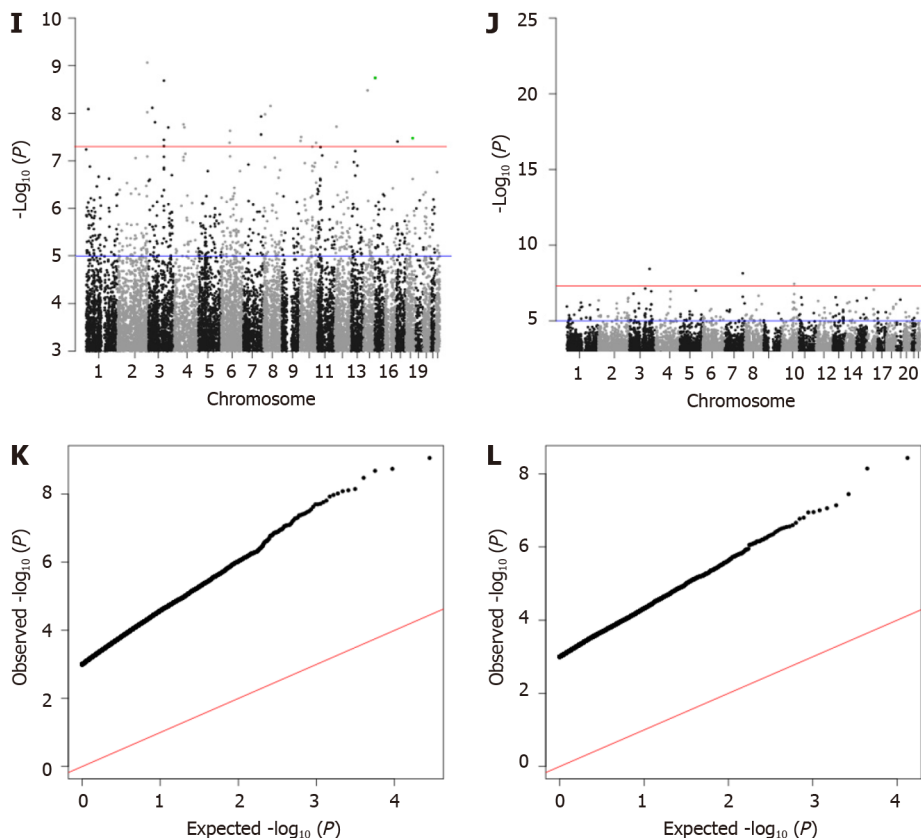
Although our findings do not substantiate a relationship between JH and cynicism, and subsequently burnout syndrome, they do suggest that there is a genetic component to both outcomes. Research among the general population has shown that hostility is an independent risk factor for cardiovascular disease[41], which has also been reinforced with evidence in population-specific work on African Americans[42]. Our results suggest that a significant predisposition to hostility and cynicism, when coupled with chronic occupational stress and hostility, could lead to elevated levels of burnout and significant long-term health decline.

Our assessment of causal effects revealed significant pleiotropy in the relationships, suggesting that any interaction which may exist is indirect. Our results do not support a causal effect, but neither do they provide sufficient evidence to refute any such relationship. Indeed, our assessment using multiple MR estimators provides limited support that there is a relationship between these traits, but that such an interaction may not be mediated by genetics. We used the MR Egger estimate to evaluate violations of the instrumental variable assumptions. We found that these assumptions are not valid in our analysis (the Egger intercepts were significantly non-zero). However, the magnitude of the impact of any deviations must be small as none of the six beta coefficients determined by the MR Egger method were outliers from the other four estimates (*i.e.*, all MR Egger estimates were within the 95% confidence interval of the mean observed beta coefficient). Therefore, it appears that using the MR Egger estimator did not introduce additional bias or increase the Type I error rate[32].

Limitations

There are three major limitations of our study. First, the pleiotropic analysis suggests that there are confounders involved in the interaction and, therefore, this pleiotropy highlights one limitation of our





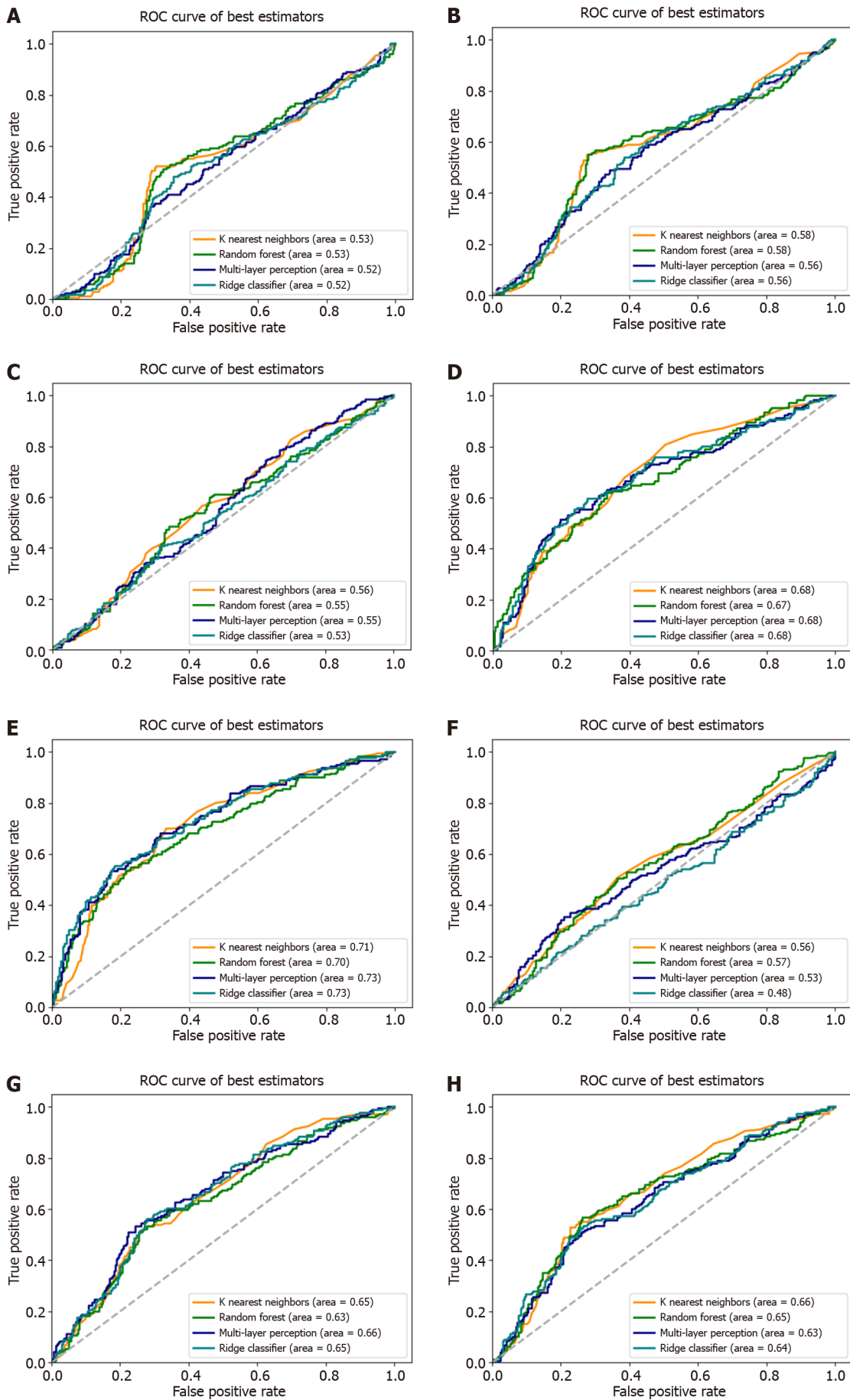
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Figure 2 Manhattan and quantile-quantile plots of genome-wide associations. A: Manhattan plot for cynical distrust score; B: Manhattan plot for high cynical distrust; C: QQ plot for cynical distrust score; D: QQ plot for high cynical distrust; E: Manhattan plot for cynicism score; F: Manhattan plot for high cynicism; G: QQ plot for cynicism score; H: QQ plot for high cynicism; I: Manhattan plot for John Henryism score; J: Manhattan plot for high John Henryism; K: QQ plot for John Henryism score; L: QQ plot for high John Henryism. Black dots = candidate associations from the training set ($n = 1852$); green dots = associations replicated in the verification set ($n = 465$); blue line = genome-wide suggestive association ($P = 5 \times 10^{-6}$); red line = genome-wide significant association ($P = 5 \times 10^{-8}$).

study in that we did not consider covariates in the initial analysis. The CARDIA study included many demographic and phenotypic datasets from which covariates could be identified. As the outcomes we measured tend to change with age[40], at least age at the time the questionnaire could be used as a future covariate. Indeed, the weak correlations reported by Albanese were largely derived from stratified data. While it was beyond the scope of our initial view of the interactions between genetics, JH, and cynicism to assess covariates, additional covariates could include gender, race, or personality, among others.

A second limitation is that the sample size is relatively small for a large-scale genomics study. Our discovery phase was comprised of only 1852 samples and our replication phase consisted of another 465 samples. While an *a priori* power analysis revealed that these sample sizes should have been powered to identify significant associations, it would be prudent to repeat these observations in larger populations to confirm which, if any, variants remain significantly associated with JH, cynicism, and/or CD. Due to this sample size limitation, the generalizability of our results should be considered alongside larger-scale studies, especially with regard to burnout syndrome, for which not much is published in the genetic literature.

Finally, there is a significant deviation from normality in all of the datasets, as determined by analyzing qq-plots (Figure 2). Considering the non-normal distribution of the data and the large sample distribution within the dataset, this deviation from normality was expected (both Cook-Medley and JH scores were previously acknowledged to have skewed distributions)[40]. What was unexpected was the relatively large number of significantly associated variants for cynicism and CD found in both the discovery and replication phases. Viewed alone, the large number of associated variants is cause for skepticism in the results. However, when taken into consideration along with the predictive ability of ML algorithms (Figure 3) developed based upon these replicated variants, the evidence for a significant genetic contribution to cynicism and CD becomes stronger.



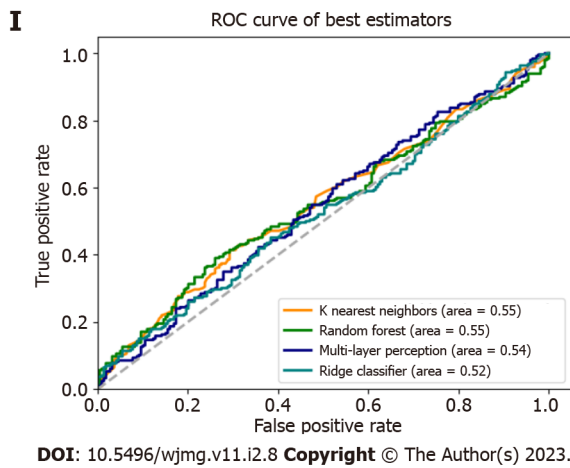


Figure 3 Receiver operator characteristics curves for genetic classification algorithms. A: High cynical distrust classifier predicting high cynical distrust; B: High cynical distrust classifier predicting high cynicism; C: High cynical distrust classifier predicting high John Henryism; D: High cynicism classifier predicting high cynical distrust; E: High cynicism classifier predicting high cynicism; F: High cynicism classifier predicting high John Henryism; G: High John Henryism classifier predicting high cynical distrust; H: High John Henryism classifier predicting high cynical distrust; I: High John Henryism classifier predicting high John Henryism. Each panel shows four predictors (orange = K-nearest neighbors, green = random forest, navy = multi-layer perceptron, teal = ridge classification).

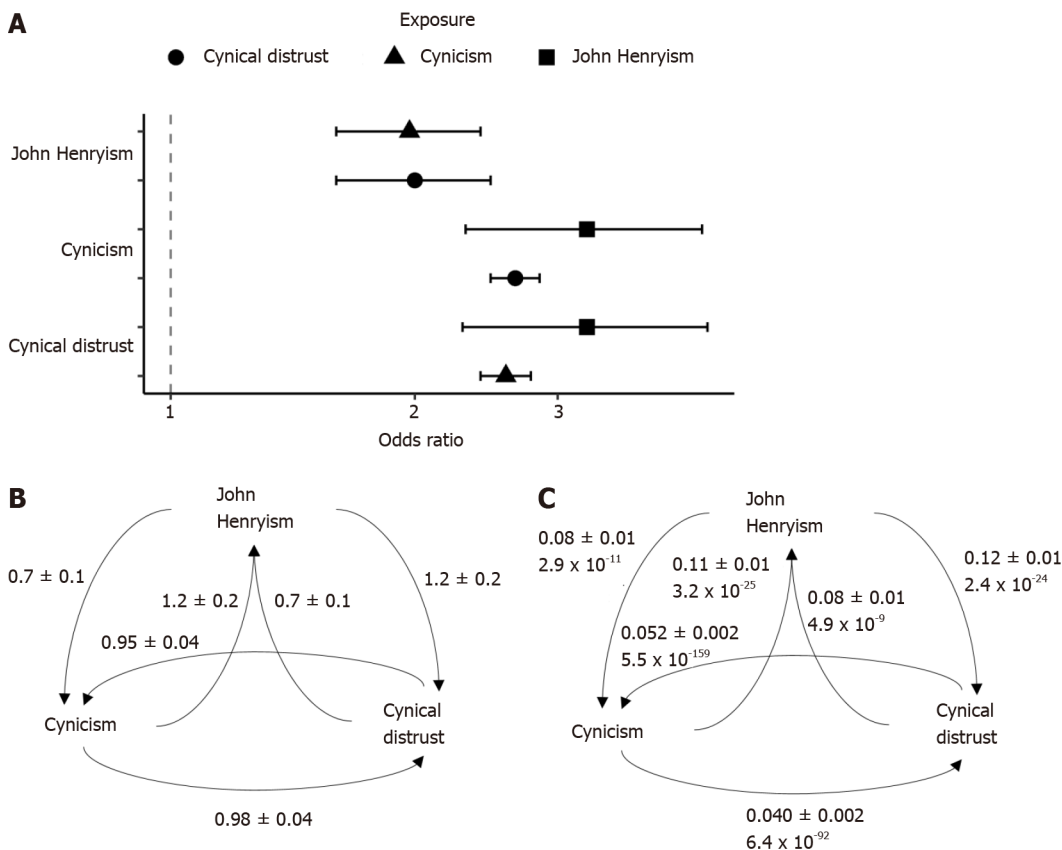


Figure 4 Results from Mendelian randomization. A: Average odds ratios and 95% confidence intervals of the causal relationships between traits as determined by Mendelian randomization (MR); B: Average beta coefficients and standard errors from four MR methods; C: Egger intercepts, standard errors, and *P* values (italic) from pleiotropy analyses.

CONCLUSION

In conclusion, our results suggest that high levels of cynicism and CD have a significant genetic component and there may some genetic component to levels of JH. This genetic component of cynicism and CD appears to have some common effects as polygenic risk scores developed to classify individuals with high scores in one trait are reasonably effective at classifying individuals with the other trait (AUC

> 0.6). Our results are insufficient to determine if this correlation is based upon causation, however, as there is a significant amount of observed pleiotropy in the MR analysis, suggesting the existence of confounding variables.

ARTICLE HIGHLIGHTS

Research background

Our hypothesis was that cynicism, a distrustful attitude that assumes people are mainly driven by self-interest, would result from John Henryism (JH), a coping strategy that involves working hard and exerting high levels of effort to deal with chronic stress. We reasoned that as JH is a way of handling discrimination, and that cynicism involves skepticism and negativity towards others, the two traits would be related. Furthermore, both JH and cynicism are linked to a higher risk of cardiovascular disease, while cynicism also often accompanies burnout syndrome, another cardiovascular risk factor.

Research motivation

Rates of burnout are increasing broadly across the globe. Burnout can cause physiological and emotional distress. Understanding the role of stress coping skills such as JH may help clarify the role of occupational stress in overall health and well-being.

Research objectives

The present study aimed to determine if JH is correlated with the cynicism component of burnout by using approaches from statistical genetics.

Research methods

Genotype and phenotype data from the “CARDIA Cohort” study were obtained from the Database of Genotypes and Phenotypes. Genome-wide association studies (GWAS) were performed in plink version 1.9 on the log-normalized JH, cynicism, and cynical distrust (CD) phenotypes as well as a binary “high/Low” trait for each phenotype using a two-stage discovery and replication approach. The GWAS results were then used to develop polygenic risk score (PRS) algorithms using supervised machine learning in scikit-learn. Significant variants identified in the discovery stage were tested for replication by performing GWAS in a second, independent set of data restricting variants only to those candidates identified in discovery (for continuous variables) or through chi-square testing for the binary variable. The performance of the PRS algorithms at classifying individuals as “high” or “low” for the phenotype was evaluated in scikit-learn using the area under the receiver operator characteristics curve.

Research results

The GWAS identified significant variant associations with JH (2), cynicism (727), or CD (204) scores and with high cynicism (173) or CD (109). PRS classifiers were successfully developed for cynicism and CD (AUC > 0.65), but not for JH. Neither of the classifiers for cynicism or CD could predict JH traits, nor could the JH classifier predict cynicism or CD.

Research conclusions

There are genetic variants associated with each trait, however JH active coping does not appear to be correlated with cynicism or CD levels.

Research perspectives

The genetic associations with these phenotypes suggest that further research could provide insight into how each trait results in health impacts such as cardiovascular disease.

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Mosaicism of a novel variant in the *ANKRD11* gene in a child with a mild KBG phenotype: A case report

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Abstract

BACKGROUND

KBG syndrome is likely underdiagnosed because of mild and non-specific features in some affected patients especially before the upper permanent central incisors eruption at about the age of 7-8 years. Somatic mosaicisms are usually recognized in the parents only after a typically affected son is diagnosed with KBG syndrome. We describe for the first time the mosaicism of a novel variant in a child with a mild KBG phenotype.

CASE SUMMARY

Our patient presented at 24 mo of age with short stature, hand abnormalities, facial dysmorphism and mild developmental delay. Pituitary hypoplasia and central hypothyroidism were also detected. By next generation sequencing (NGS) analysis we found a novel deletion in the *ANKRD11* gene (c.4880_4893del.), that

can be classified as likely pathogenic for the syndrome, with the percentage of mutated allele of 36%. We considered this finding as causative of the mild and non-specific phenotype for KBG syndrome in our patient, as previously reported in adults. A heterozygous variant in *HESX1* gene, classified as variant of uncertain significance, but suspected of causing pituitary hypoplasia and hormonal deficiency, was also found. The patient started levothyroxine and growth hormone treatment.

CONCLUSION

The increased use of NGS analysis may expand the phenotypic spectrum of KBG syndrome because it allows genetic diagnosis of somatic mosaicisms also in children.

Key Words: ANKRD11; KBG; Mosaic; HESX1; Child; Case report

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Core Tip: Somatic mosaicisms of KBG syndrome are usually recognized in the parents only after a typically affected son is diagnosed. We report for the first time the case of a somatic mosaicism for KBG syndrome diagnosed in a child with a mild and non-specific phenotype. The increased use of next generation sequencing allows a genetic diagnosis of this mosaic form in children expanding the phenotypic spectrum of the KBG syndrome.

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INTRODUCTION

“KBG” represents the initials of the last name of the first three families diagnosed with the syndrome and is a rare genetic disease (OMIM 148050). Common manifestations are macrodontia (especially of the upper central incisors), typical facial features, short stature, skeletal anomalies, hearing loss, global developmental delay, and intellectual disability[1-4]. The transmission of this disease is autosomal dominant, and is caused by either heterozygous ANKRD11 point mutations (OMIM 611192) or microdeletion in chromosome 16q24.3 including the ANKRD11 gene[5] or ANKRD11 intragenic duplication[6]. The ANKRD11 gene encodes an ankyrin repeat containing protein (ANKRD11) which is indispensable in neuron proliferation and acts as a transcriptional repressor by two transcriptional repression domains (RDs: RD1, aa 318–611; and RD2, aa 2369–2663) and promoting transcription through one activation domain (AD), aa 1851–2145[1]. Since 1975, over 200 KBG patients have been described[1].

KBG syndrome is likely underdiagnosed because of mild and non-specific features in some affected patients especially before the upper permanent central incisors eruption at about the age of 7-8 years[2, 7]. Macrodontia of the permanent upper incisors is a typical finding, making diagnosis prior to the eruption of these teeth a challenge[2]. According to the latest diagnostic criterion, KBG syndrome should be considered in a patient with cognitive delay/learning difficulties, speech delay or behavioral anomalies with at least two major criteria or one major and two minor criteria[2]. Major criteria are: (1) Macrodontia or phenotypic features of KBG in child with primary dentition; (2) height < 10th centile; (3) recurrent middle ear infections and/or hearing loss; and (4) 1st degree relative with KBG syndrome. Minor criteria are: Brachydactyly or relevant hand anomaly; epilepsy; cryptorchidism; feeding difficulties; palate abnormalities; autism; large anterior fontanelle and/or delayed closure. A phenotypic variability among KBG patients has been observed intra- and interfamilial, and between patients with the 16q24.3 microdeletion compared to those harboring *ANKRD11* gene mutations[1]. Somatic mosaicisms have been reported in the parents after a typically affected son was diagnosed with KBG syndrome, and exhibited a milder phenotype, suggesting that KBG phenotypes in adults might be dose-dependent[5-7].

Here we describe for the first time in a child a mosaicism of a novel variant in the *ANKRD11* gene. The patient had a mild KBG phenotype and the diagnosis was performed by NGS analysis, providing insights into the spectrum of mosaic mutations.

CASE PRESENTATION

Chief complaints

The proband came to our attention at the age of 24 mo, owing to postnatal growth retardation ([Supplementary Figure 1](#)).

History of present illness

The boy was born at term (41 wk) after a pregnancy achieved with egg fertilization by intracytoplasmic sperm injection. Birth weight was 3830 g (0.84 SD), length 53 cm (1.38 SD), head circumference 34 cm (-0.76 SD). Non-consanguineous parents had a normal stature (father 179 cm, mother 182 cm, mid-parent sex-adjusted target height 187 cm).

History of past illness

Not informative.

Personal and family history

Not informative.

Physical examination

His height was 89 cm (-2 SD), his weight was 12.6 Kg (-2 SD), and his head circumference was 50 cm (0 SD). Clinical examination revealed facial dysmorphisms, including tall forehead, widely spaced eyes, bushy eyebrows, left palpebral ptosis, prominent and anteverted ears, facial asymmetry. Skeletal anomalies included short fingers with fifth finger clinodactyly. He showed delay/cognitive impairment as assessed by Griffith's scale.

Laboratory examinations

Routine chemistry turned out as normal. ACTH was 16.7 pmol/L (normal range 5-25), cortisol 286.9 nmol/L (normal range 250-550), and prolactin 13.2 ug/L (normal range 4-15); insulin like growth factor 1 (IGF-1) 4.84 nmol/L (normal range 1.70-30.46), fT4 was repeatedly low: 9.8-10.2 pmol/L (normal range 12-22) with inappropriately normal TSH: 2.3-4.79 mU/L (normal range 0.2-4.5). Thyroid Ultrasound revealed an in situ and normal sized gland. An arginine stimulation test elicited a reduced peak of growth hormone (GH) peak 2.28 ng/mL and IGF-1 was 8.76 nmol/L (2.61-45.36). Considering growth retardation, psychomotor delay and dysmorphic features, clinical exome sequencing analysis was performed.

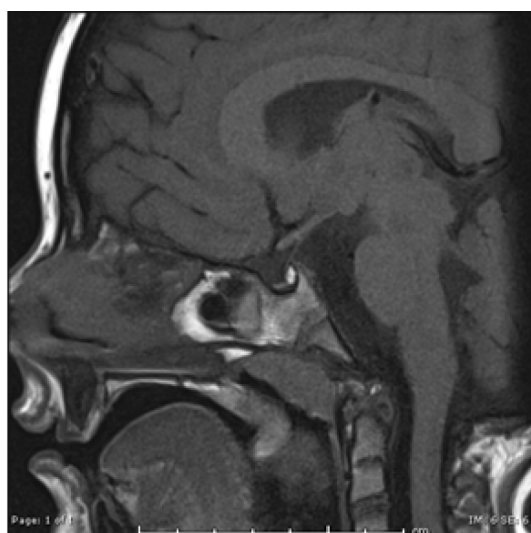
Imaging examinations

At 36 mo, bone age corresponded to 3 mo for the carpus and 12-16 mo for the phalanges, in the presence of mild malformation of the intermediate phalanx of the fifth finger ([Supplementary Figure 2](#)). He presented extra tarsal persistence of chalazion, with sub-palpebral hematomas. Pituitary magnetic resonance imaging (MRI), revealed a hypoplastic gland ([Figure 1](#)) with a normal pituitary stalk. Brain MRI excluded central nervous system abnormalities.

FINAL DIAGNOSIS

Exome sequencing analysis identified a deletion of 14 nucleotides in the *ANKRD11* gene, c.4880_4893delCCGCCCGTCGTCGTG. The deletion is a mosaic with the percentage of mutated allele of 36%. At protein level the deletion determines the introduction of a premature stop codon p.Ala1627GluTer9. The frameshift variant, not present in the father DNA, is not reported in the gnomAD database, and has never been described in literature. According to current the American College of Medical Genetics and Genomics (ACMG) guidelines[8], the variant can be classified as likely pathogenetic (class 4) for KBG syndrome.

Exome sequencing analysis also identified three variants: (1) A heterozygous variant in *HESX1* gene (c.541A>G, p.Thr181Ala, NM_003865.3), inherited from the father, who presented with normal height, TSH and fT4. This variant has been reported as heterozygous and pathogenetic in a patient with isolated growth hormone deficiency and pituitary hypoplasia[9] and in a patient with combined pituitary hormone deficiency, anterior pituitary hypoplasia, and ectopic posterior lobe[10]; in silico analysis suggests that this missense variant does not affect protein structure/function and according to current ACMG guidelines[8], the variant can be classified as variant of uncertain significance (VUS); (2) a hemizygous VUS in *ATRX* gene (c.189G>A, p.Glu63Glu, NM_000489.5); and (3) a heterozygous VUS in *NIPBL* gene (p.Ile1982Leu, p.Ile1982Leu). The latest two variants are not present in the father DNA.



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Figure 1 Pituitary magnetic resonance imaging revealed a hypoplastic gland with a normal pituitary stalk.

TREATMENT

Once the diagnosis of central hypothyroidism was confirmed, treatment with levothyroxine was started. After GH test, we started GH treatment that changed growth trajectory ([Supplementary Figure 1](#)).

OUTCOME AND FOLLOW-UP

The patient is on follow up at our outpatient clinic, he is now 7 years old, and he started GH six months ago.

DISCUSSION

KBG syndrome is a rare autosomal dominant disorder characterized by short stature, delay/cognitive impairment and distinctive craniofacial characteristics. It shows a wide spectrum of clinical phenotypes and it is likely underdiagnosed because of mild and non-specific features in some affected patients especially before the upper permanent central incisors eruption at about the age of 7-8 years. Here we present, for the first time in literature, the mosaicism (36%) of a novel variant in the *ANKRD11* gene that underlies a mosaic KBG phenotype in a child.

This finding led us to conclude that the variant was acquired at a postzygotic level, and is classifiable as likely pathogenetic for KBG syndrome.

Somatic mosaicism is usually recognized in the parents only after a typically affected son is diagnosed with KBG syndrome, because patients with somatic mosaicisms exhibited a milder phenotype. The phenotypic effect of mosaic *ANKRD11* haploinsufficiency might be dose-dependent[4] and some experiences in the literature confirm this hypothesis ([Table 1](#)).

Nevertheless, recent emerging evidence also suggests that somatic mosaicism is found in apparently healthy individuals and increases with age[11].

Our patient presented with short stature ($-2SD$ and mid-parent sex-adjusted target height of 187 cm), that is very common among patients with KBG syndrome, being found in 40%–77% of affected patients [12]. We reported typical but milder features of KBG syndrome[12]: Dysmorphic features (widely spaced eyes, bushy eyebrows, ptosis and large protruding ears), delayed bone age, hand anomalies (clinodactyly and brachydactyly), mild developmental delay and mild ocular involvement (anisotropy and left eye exodeviation). Major problems as epilepsy, intellectual disability, spinal-costal anomalies, heart defects, hearing loss, kidney abnormalities, or feeding problems, were not presented by our patient[3].

Interestingly, our patient presented with extra tarsal persistence of chalazion, with sub-palpebral hematomas; skin and hair abnormalities have been previously reported in KBG syndrome: one patient with a tendency to skin bruising, and delayed wound healing, and another with keloid scarring[3].

Primary subclinical hypothyroidism has been described in KBG syndrome[12], but our patient presented with secondary (pituitary) hypothyroidism. Our patient presented also pituitary hypoplasia,

Table 1 Cases of mosaic KBG phenotype reported in the literature

Ref.	Sex and age	Molecular analysis	% mosaicism	Phenotype	Phenotype in relative with the same non-mosaic mutation
Our case	2 yr	Deletion (c.4880_4893del.)	36	Short stature, hand abnormalities, facial dysmorphism, mild developmental delay	-
Khalifa <i>et al</i> [5], 2013	Female, 31 yr (mother)	Microdeletion 16q24.3	38	Round face, brachycephaly, macrodontia, abnormal dentition with malposition and extra teeth, brachydactyly, postaxial polydactyly, partial syndactyly between the 2 nd and 3 rd toes, short stature, learning difficulty	Female, 11 yr, Multiple congenital abnormalities including patent foramen ovale, umbilical hernia, hypospadias with chordee, penile-scrotal fusion, intestinal malrotation, chronic interstitial pulmonary disease, febrile seizure, pharyngeal dysphagia, developmental delay, dysmorphic features (round face, epicanthic folds, hypertelorism, broad arched eyebrows with synophrys, a flat nasal bridge, and a relatively small nose with a bulbous tip), brachycephaly, short neck, macrodonzia, dental malocclusion, chronic otitis media, partial syndactyly between the 2 nd and 3 rd toes, delayed bone age
Crippa <i>et al</i> [6], 2015	NA (mother)	Microduplication 16q24.3 (chr16:89,350931-89439639, hg19)	5	Mild facial dysmorphisms, similar to those of her children, and a nasal voice	Male, 17 yr. Short, stature, moderate intellectual disability, facial dysmorphisms including long triangular face, frontal bossing, arched and bushy eyebrows with slight synophrys, large and prominent ears, broad and high nasal bridge with bulbous nasal tip, anteverted nares, long philtrum, macrodontia of central incisors, and a nasal voice, brachymetacarpia, third-degree vesicoureteral reflux Female, 13 yr. Short, stature, moderate intellectual disability, facial dysmorphisms including long triangular face, frontal bossing, arched and bushy eyebrows, large and prominent ears, broad and high nasal bridge with bulbous nasal tip, anteverted nares, long philtrum, macrodontia of central incisors, and a nasal voice, brachymetacarpia, ureterocele associated with duplex pelvicalyceal district
Guo <i>et al</i> [7], 2022	Female, 30-35 yr (mother)	c.5227C>T p. (Gln1743*)	Only 2 out of 298 sequencing reads for this variant found in her blood	History of miscarriage, mild facial features, (<i>e.g.</i> , synophrys, thick eyebrow, wide nasal bridge, prominent nasal tip) with speech delays and seizures in childhood	Male, 5-10 yr. More severe phenotypic features in comparison to the mother, history of seizures and concurrent speech and motor delays, mitral valve repair at around one year of age, abdominal migraines

NA: Not available.

up to now reported as associated to KBG in only one patient[4] who presented with hypogonadotropic hypogonadism at 15 years, and GH deficiency.

Mutations in the transcription factor *HESX1* can cause several congenital pituitary defects, ranging from isolated growth hormone deficiency[9,13] to septo-optic dysplasia (SOD) with panhypopituitarism [14]; most patients carry mutations at the heterozygous state, invariably associated with reduced penetrance, and generally show a milder phenotype than the rare homozygous patients[9,15]. According to us, in our patient pituitary hypoplasia, central hypothyroidism and GH deficit might be explained by the variant in *HESX1* gene.

CONCLUSION

In conclusion, we reported for the first time in literature the case of a somatic mosaicism for KBG syndrome, diagnosed in a child with a mild and non-specific phenotype that included short stature, hand abnormalities, distinctive facial dysmorphism and mild developmental delay, in absence of macrodontia consistent with his age. A heterozygous variant in *HESX* gene, strongly suspected of causing pituitary hypoplasia and hormonal deficiency was also found.

KBG syndrome is likely underdiagnosed because of mild and non-specific features in some affected patients; mosaic forms are even more challenging. Our case underlines that the recognition of mosaicism is important not only for establishing a diagnosis, but also for assessing recurrence risk and for providing genetic counseling to the family. Our paper increases awareness of mild forms of KBG syndrome in children and underlines the importance of NGS analysis for an early genetic diagnosis of

mosaic forms.

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FOOTNOTES

Author contributions: Franceschi R, Rivieri F, Maines E, Anesi A, Soffiati M, Porretti G, and Radetti G followed the patient up; Novelli A, Ferretti D and Mucciolo M performed the genetic test; Franceschi R, Radetti G, Maines E and Mucciolo M drafted the manuscript; All authors critically reviewed and edited the manuscript, and approved the final version as submitted.

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OPINION REVIEW

- 28 Role of IL-2/IL-2 receptor in pathogenesis of autoimmune disorders: Genetic and therapeutic aspects
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Role of IL-2/IL-2 receptor in pathogenesis of autoimmune disorders: Genetic and therapeutic aspects

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Abstract

Interleukin-2 (IL-2) is an important cytokine that plays a key role in the immune response. The IL-2 receptor (IL-2R) is composed of three subunits, alpha, beta, and gamma, with the alpha subunit having the highest affinity for IL-2. Several studies reported that immune dysregulation of IL-2 may cause tissue injury as well as damage leading to the pathogenesis of various autoimmune diseases such as acute necrotizing vasculitis in systemic lupus erythematosus (SLE), inflammatory synovitis in rheumatoid arthritis (RA), salivary and lacrimal gland dysfunction in Sjogren syndrome (SS), obliterative vasculopathy fibrosis in systemic sclerosis (SSc), and inflammatory demyelination in multiple sclerosis (MS). The aim of this review paper was to examine the role of IL-2/IL-2R in various autoimmune disorders, taking into account recent advancements and discoveries, gaps in the current literature, ongoing debates, and potential avenues for future research. The focus of this review is on systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, sjogren syndrome, and multiple sclerosis, which are all linked to the malfunctioning of IL-2/IL-2R. In genetic studies, gene polymorphisms of IL-2 such as IL-2 330/T, IL-2 330/G, and rs2069763 are involved in increasing the risk of SLE. Furthermore, genetic associations of IL-2/IL-2R such as rs791588, rs2281089, rs2104286, rs11594656, and rs35285258 are significantly associated with RA susceptibility. The IL-2 polymorphism including rs2069762A, rs6822844T, rs6835457G, and rs907715T are significant connections with systemic sclerosis. In addition, rs2104286 (IL-2), rs11594656 (IL-2RA), rs35285258 (IL-2RB) gene polymorphism significant increases the risk of multiple sclerosis. In therapeutic approaches, low-dose IL-2 therapy could regulate Tfr and Tfh cells, resulting in a reduction in disease activity in the SLE patients. In addition, elevated sIL-2R levels in the peripheral blood of SLE patients could be linked to an immunoregulatory imbalance, which may contribute to the onset and progression of SLE. Consequently, sIL-2R could potentially be a target for future

SLE therapy. Moreover, Low dose-IL2 was well-tolerated, and low levels of Treg and high levels of IL-21 were associated with positive responses to Ld-IL2 suggested to be a safe and effective treatment for RA. Additionally, low-dose IL-2 treatment improves the exocrine glands' ability to secrete saliva in SS-affected mice. Whereas, Basiliximab targets the alpha chain of the IL-2 receptor suggested as a potential treatment for SSc. Also, pre-and post-treatment with Tregs, MDSCs, and IL-2 may have the potential to prevent EAE induction in patients with MS. It is suggested that further studies should be conducted on IL-2 polymorphism in Sjogren syndrome.

Key Words: IL-2; IL-2R; Pathogenesis; Autoimmune diseases; Systemic lupus erythematosus; Rheumatoid arthritis; Systemic sclerosis; Sjogren syndrome; Multiple sclerosis

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Core Tip: Immune dysregulation of Interleukin-2 (IL-2) may cause tissue injury and damage leads to pathogenesis of various autoimmune diseases The aim of this review paper was to examine the role of IL-2/IL-2R in various autoimmune disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), multiple sclerosis (MS) and Sjogren Syndrome (SS), which are all linked to malfunctioning of IL-2/IL-2R. IL-2 gene polymorphism is involved in increasing the risk of SLE, RA, SSc, and MS but no data has been available about the genetic role of IL-2 in the pathogenesis of Sjogren syndrome. In therapeutic approaches, a low dose of IL-2 therapy is found to be safe, effective, and well-tolerated in the treatment of SLE, RA, SSc, MS, and SS patients.

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INTRODUCTION

Autoimmune diseases are a significant clinical problem that affects a large number of people worldwide. These diseases arise when the immune system mistakenly attacks healthy tissues and organs in the body, leading to chronic inflammation, tissue damage, and organ dysfunction. It can affect any part of the body, including the joints, skin, blood vessels, nerves, and endocrine glands. Some common examples of autoimmune diseases include rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, systemic sclerosis, and Sjogren syndrome.

The pathogenesis of autoimmune diseases is complex and multifactorial, involving both genetic and environmental factors. While the exact mechanisms underlying autoimmune diseases are not fully understood, it is believed that they occur due to a failure of the body's immune regulatory mechanisms. This failure allows self-reactive immune cells to escape tolerance mechanisms and attack healthy tissues, leading to the development of autoimmunity[1]. Autoimmune diseases progress through three stages: Initiation, propagation, and resolution. The initiation phase involves the activation of self-reactive immune cells and their migration to target tissues. The propagation phase is characterized by the activation and expansion of these self-reactive immune cells, leading to chronic inflammation and tissue damage. The resolution phase is defined by the partial and short-term ability to restore the balance of effector and regulatory responses and the reduction of inflammation[1].

Interleukin-2 (IL-2) is an important cytokine that plays a key role in the immune response. The IL-2 receptor is composed of three subunits, alpha, beta, and gamma, with the alpha subunit (IL2RA) having the highest affinity for IL-2. The IL-2 receptor is expressed in a variety of immune cells, including T cells, B cells, and natural killer (NK) cells[2]. The most abundant type of cell that is influenced by IL-2 is T lymphocytes. IL-2 primarily functions to prompt the production of other cytokines and to increase the growth and proliferation of T lymphocytes that are specific to particular antigens, including CD4+ and CD8+ T lymphocytes. This cytokine also promotes the death of activated T lymphocytes, promotes differentiation into Th1 and Th2 subsets in CD4+ T cells, and plays a critical role in the maturation of CD4+ CD25+ Tregs. Additionally, IL-2 increases the cytotoxic activity of CD8+ cells and stimulates the growth of memory CD8+ cells[3].

Genetic variants in IL2RA and IL2RB may be associated with the development of autoimmune diseases and inflammatory diseases. For example, studies have reported that variants in these genes are associated with the risk of multiple sclerosis. However, there is limited research on the association between genetic variants in IL2RA and IL2RB and the risk of rheumatoid arthritis (RA). Further studies are needed to investigate this potential association and to better understand the role of IL-2 in the pathogenesis of RA[2].

Advancements in genotyping technology and the utilization of single nucleotide polymorphism (SNP) assays have facilitated the application of whole genome association approaches. These approaches involve identifying the correlation between genetic variants and susceptibility to various autoimmune diseases[2].

Despite advances in diagnosis and treatment, autoimmune diseases remain a significant clinical challenge due to their chronic nature, associated healthcare cost, and impact on young populations during their prime working and repro-

ductive years. Therefore, it is essential to continue to study and understand the mechanisms underlying autoimmune diseases to develop effective treatments and improve the quality of life of affected individuals[1].

The aim of this review paper was to examine the role of IL-2/IL-2R in the pathogenesis of various autoimmune disorders, taking into account recent advancements and discoveries, gaps in the current literature, ongoing debates, and potential avenues for future research. The focus of this review is on systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjogren syndrome, and multiple sclerosis, which are all linked to the malfunctioning of IL-2/IL-2R, as depicted in **Figure 1** and **Table 1**.

The authors utilized various databases, including Google Scholar, PubMed, and Science Direct, to conduct a thorough review of the relevant literature, which was completed on March 30, 2023. The search terms used included pathophysiology, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjogren syndrome, multiple sclerosis, autoimmune disorder, IL-2, and IL-2R. In addition to these search terms, the authors also searched through the references of relevant articles to identify comparable studies. Only clinical investigations published in English were included, and no time restrictions were imposed, although preference was given to more recent studies.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects the entire body, causes persistent and severe pain, and is characterized by the production of autoantibodies and inflammation in multiple organs. SLE is a complex and multifactorial disease that has an unpredictable relapsing-remitting course, resulting from various interactions between susceptibility genes, epigenetics, environmental factors, hormones, and immuno-regulatory variables. The disease can manifest with a broad range of clinical symptoms[4]. In SLE, autoantibodies that are directed against nucleic acids and their binding proteins are produced, indicating a general lack of self-tolerance. Recent research has identified over 30 genetic loci that are associated with the pathogenesis of the disease. The loss of tolerance and subsequent immunological dysregulation in SLE is caused by genetic factors in conjunction with environmental triggers and stochastic events[5].

The IL-2 gene has been linked to autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. The genetic association of rs2069763 in IL-2 was investigated in Han Chinese SLE patients and a healthy control group in Taiwan. The findings suggest that the G allele of the mutation is significantly more prevalent in SLE patients and may be linked to the genetic origin of SLE in Taiwanese individuals. The study also found that the G allele is significantly higher in SLE patients with antinuclear antibodies and significantly lower in those with discoid rash[6]. Another study investigated the impact of the IL2/IL21 region, a genetic locus associated with global autoimmunity, on the response to rituximab in individuals with SLE and other autoimmune disorders. The study found that the rs6822844 G/T polymorphism at the IL2-IL21 region was significantly associated with response to rituximab in SLE patients, but not in individuals with other autoimmune disorders. The study suggests that the IL2-IL21 region may influence the response to rituximab in SLE patients specifically[7]. Furthermore, variations in the KIAA1109-interleukin 2 (IL2)-IL21 block on chromosome 4q27 have been associated with various autoimmune disorders. The research findings confirm the link between autoimmune disorders and the KIAA1109/IL2/IL21 gene region, indicating that the 4q27 Locus may contribute to the genetic predisposition of autoimmune disorders in the Tunisian population[8]. A study investigated the association between certain SNPs in the IL-2 and interferon (IFN)- γ genes and the development of juvenile systemic lupus erythematosus (JSLE), a severe autoimmune disease with an unknown cause. The study found a negative allelic association for JSLE in IL-2 330/T and a positive allelic association for IL-2 330/G. The study suggests that IL-2 cytokine gene polymorphisms could serve as genetic risk indicators for JSLE[9].

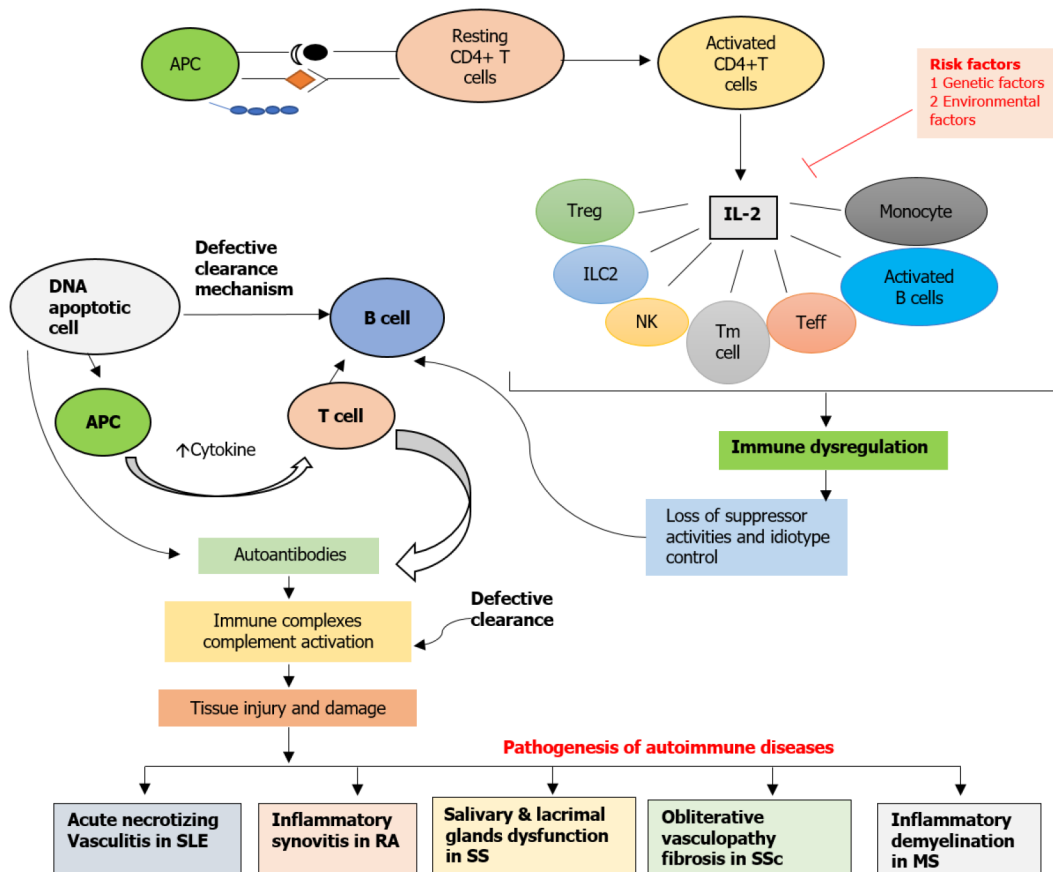
Studies have investigated the cytokine profiles of SLE patients and found that peripheral blood mononuclear cells (PBMCs) from SLE patients grow less than controls when activated with antigens and mitogens. Lupus T cells release less IL-2 compared to normal T cells and are less sensitive to IL-2 activation. However, the expression of IL-2 is higher in freshly produced SLE PBMCs compared to normal PBMCs. When stimulated optimally, lupus T cells are capable of producing normal levels of IL-2. The reduced *in vitro* IL-2 production from lupus T cells is likely due to several mechanisms, including the downregulating effects of Th2 cytokines[10]. SLE patients have notably increased levels of sIL-2R in their serum, which are strongly associated with disease activity. Additionally, a research study has revealed for the first time that sIL-2R present in the peripheral blood of SLE patients can combine with IL-2 to form a complex that reduces IL-2's biological effectiveness and detection level. This complex also hinders the development of Treg cells and disrupts peripheral tolerance. The research finding suggests that elevated sIL-2R levels in the peripheral blood of SLE patients could be linked to an immunoregulatory imbalance, which may contribute to the onset and progression of SLE. Consequently, sIL-2R could potentially be a target for future SLE therapy[11].

SLE is characterized by an imbalance between effector and regulatory CD4⁺ T cells. T cell subsets are regulated by IL-2, and SLE patients have lower levels of IL-2. In this study, researchers found that low doses of recombinant human IL-2 therapy reduced disease activity in SLE patients by selectively affecting the number of regulatory T (Treg), follicular helper T (TFH), and IL-17-producing helper T (TH17) cells, but not Th1 or Th2 cells[12]. In a clinical trial, researchers investigated the effect of low-dose IL-2 on T follicular regulatory (Tfr) and T follicular (Tfh) cells in patients with SLE. They found that low-dose IL-2 therapy could regulate Tfr and Tfh cells, resulting in a reduction in disease activity[13]. In addition, open-label clinical trials have suggested that low-dose IL-2 may be a useful treatment for SLE, but a double-blind, placebo-controlled study is necessary to properly evaluate its safety and effectiveness. Overall, low-dose IL-2 therapy may be beneficial and well-tolerated in the treatment of SLE[14].

Table 1 Role of interleukin (IL)-2/IL-2R in pathogenesis of autoimmune diseases

Autoimmune diseases	Pathogenesis	Genetics (SNP)	Therapy
Systemic lupus erythematosus	Acute necrotizing vasculitis	IL-2 330/T (IL-2); IL-2 330/G (IL-2); rs2069763 (IL-2); rs6822844 G/T; (IL-2-IL21)	Low dose IL-2, Rituximab
Rheumatoid arthritis	Inflammatory synovitis	rs791588 (IL2RA); rs2281089 (IL2RA); rs2104286 (IL2RA)	Low dose IL-2, Infliximab
Systemic sclerosis	Obliterative vasculopathy fibrosis	rs2069762A (IL-2); rs6822844T (IL-2-IL21); rs6835457G (IL-2); rs907715T (IL-2); IL2-384-G (IL-2); rs11594656 (IL-2RA); rs2104286(IL-2); rs12722495 (IL-2)	Low dose IL-2, Basiliximab
Multiple sclerosis	Inflammatory demyelination	rs2104286 (IL-2); rs11594656 (IL-2RA); rs35285258 (IL-2RB)	Tregs, MDSCs, IL-2
Sjogren syndrome	Salivary and lacrimal glands dysfunction	No data	Low dose IL-2

SNP: Single nucleotide polymorphism; IL: Interleukin; MDSCs: Myeloid-derived suppressor cells.



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Figure 1 Overview of the role of interleukin (IL)-2/IL-2 receptor in the pathogenesis of autoimmune diseases. Activation of T cells by antigen-presenting cells leads to the production of interleukin-2 (IL-2) by CD4+ T-helper cells then IL-2 interacts with IL-2 receptor expressed on several immune cells *i.e.*, Treg, ILC2 cells, natural killer, memory T cells, activated T cells, B cells, and monocytes to mediate the immune regulation. However, the function of IL-2 is disrupted by multiple risk factors such as genetic, environmental, neuroendocrine system, sex hormones, and defective clearance mechanism that are involved in the pathogenesis of acute necrotizing vasculitis in systemic lupus erythematosus, inflammatory synovitis in rheumatoid arthritis, salivary and lacrimal glands dysfunction in Sjogren syndrome, obliterative vasculopathy fibrosis in systemic sclerosis, and inflammatory demyelination in multiple sclerosis[54]. APC: Antigen presenting cells; DNA: Deoxyribonucleic acid; MS: Multiple sclerosis; NK: Natural killer; RA: Rheumatoid arthritis; IL: Interleukin; SLE: Systemic lupus erythematosus; SS: Sjogren syndrome; SSc: Systemic sclerosis.

The inadequate production of IL-2 is one of the factors that contributes to the disruption of the immune system leading to SLE. Multiple SLE models in mice have revealed a shortage of IL-2, providing valuable insights into the link between SLE and IL-2. Furthermore, research has shown that IL-15 and IL-21, which are members of the IL-2 family, are also dysregulated in SLE. Imbalanced transcriptional regulators in T cells of SLE patients lead to reduced IL-2 Levels as they

are responsible for the synthesis or suppression of IL-2 production[15]. It has been recently found that the lymphoproliferation observed in IL-2/IL-2R deficient mice is associated with a significant decrease in the number of Treg in the periphery. Similarly, individuals with SLE have a lower count of regulatory T cells. Although SLE patients do not exhibit significant lymphoproliferation as observed in knockout mice, they do have lower IL-2 levels. Nonetheless, knockout mice with IL-2/IL-2R deficiency have additional features that are present in SLE patients, such as production of autoantibodies, lymphadenopathy, and decreased Treg cells[15].

RHEUMATOID ARTHRITIS

Rheumatoid Arthritis (RA) is a medical condition characterized by long-term inflammation of the joints that eventually leads to the deterioration of bone and cartilage, causing systemic inflammation, abnormal antibody production, and persistent synovitis leading to severe disability and early death[4]. The development of RA is an imbalance in the subsets of CD4 + T lymphocytes, including T helper (Th) 17 cells, regulatory T (Treg) cells, T follicular helper (Tfh) cells, and T follicular regulatory (Tfr) cells. These cells contribute to autoimmune inflammatory responses by promoting the excessive production of cytokines and abnormal autoantibodies including anti-cyclic citrullinated peptide and rheumatoid factor [16].

The use of SNP assays and advances in genotyping technology has facilitated the application of whole genome association approaches to link genetic variants with disease susceptibility. Heritability studies have shown that genetic susceptibility plays a critical role in the progression of joint destruction in RA, estimated at 45% to 58%. Various SNPs have been identified to be associated with RA predisposition. In addition, large-scale genome-wide association studies have identified over 30 Loci that play a role in RA pathogenesis. Several genetic factors, including the human leukocyte antigen gene (*HLA*), interleukin genes (*IL23R*, *IL6*, *IL17*, and *IL12B*), autoimmune regulator gene (*AIRE*), protein tyrosine phosphatase 22 (*PTPN22*) gene, and solute carrier family 22 member 4 (*SLC22A4*), have been implicated in the pathogenesis of RA.

The cytokine IL-2, which is produced by Th1 Lymphocytes, may play a significant role in the development of RA. The research examined changes in the levels of IL-2 in the bloodstream and determined if there was a relationship between these changes and glucose metabolism abnormalities, including insulin resistance in RA patients. The study revealed that individuals with RA exhibit changes in IL-2 regulation and that there is a substantial association between IL-2 levels in the bloodstream and insulin sensitivity[17]. In another study, the connection between serum levels of IL-2 and disease activity, absolute numbers of peripheral lymphocyte subsets, autoantibodies, and cytokines in individuals with RA patients were examined. The findings demonstrated that serum levels of IL-2 were related not only to disease activity and autoantibody levels but also to the imbalance of Th17/Treg immunity in RA patients. Moreover, in individuals with active RA, there was an abnormal increase in NK cell levels, which could be due to high serum IL-2 levels[18]. The production of IL-2 and the proliferative responses of peripheral blood mononuclear cells in patients with RA and normal controls was investigated. Kitas *et al*[19] study revealed a positive relationship between proliferative responses and IL2-levels with the proportion of CD4+ cells, and an inverse relationship with Tac+ lymphocytes suggested that depressed IL-2 production in RA patients may relate to monocyte effects, but this cannot completely explain the defects observed.

IL-2, a growth factor for T lymphocytes, exerts its effects through the activation of cell surface receptors called IL-2R. These receptors are found on activated T cells, and the protein can be shed from cell membranes resulting in the detection of a soluble form of IL-2R using an enzyme-linked immunosorbent assay. This study analyzed the levels of sIL-2R in the sera of patients with RA over time. The findings indicate that the sIL-2R level in the serum of RA patients reflects the activation of underlying immunopathogenic mechanisms, making it an excellent indicator of clinical disease activity. Moreover, an increasing level of sIL-2 R may predict the exacerbation of disease activity[20].

The genetic risk factors were identified in RA patients. The results showed that a genetic variant (rs791588) in the *IL2RA* gene was associated with a decreased risk of RA, and two SNPs (rs791588 and rs2281089) were associated with a reduced risk of RA when stratified by gender or age. However, two haplotypes of *IL2RA* were associated with an increased risk of RA. These findings suggest that inherited genetic alterations at *IL2RA* may play a role in the development of RA[2]. This study aimed to investigate whether SNPs within the *IL2RA*/CD25 gene are associated with juvenile idiopathic arthritis (JIA). The *IL2RA*/CD25 gene is a susceptibility gene for autoimmune diseases due to its role in regulatory T cells. The study found strong evidence that the *IL2RA*/CD25 gene is a JIA susceptibility locus, and further investigation is necessary using both genetic and functional approaches[21]. Moreover, *IL2RA*-rs2104286 and sIL2Rα levels have been found to be associated with persistent RA. *IL2RA* gene variants have been previously shown to have protective effects against multiple sclerosis, diabetes mellitus, and RA. In addition to *HLA-SE*, *IL2RA*-rs2104286 is currently the only known genetic variant that is associated with both joint destruction and RA persistence, highlighting the importance of *IL2RA* in the development and progression of RA[22].

The study investigated various aspects of the production and function of IL-2 using mononuclear cells from synovial fluid (SF), synovial tissue, and peripheral blood (PB) of patients with RA. The findings indicated that both PB and SF rheumatoid lymphocytes produced less IFN-gamma when treated with IL-2 overnight than normal PB lymphocytes. This was inconsistent with the notable improvement in natural killer activity caused by IL-2. Removing adherent cells in synovial fluid did not correct this deficit. These abnormal behaviors of IL-2 and IFN-gamma suggest that impaired T-cell function could be a contributing factor in the immunopathogenesis of RA[23]. However, previous studies in patients with RA have produced conflicting findings regarding the relationship between sIL-2R levels and disease activity scores or other laboratory markers of inflammation. A recent study found that low sIL-2R levels might indicate a rapid response to treatment with infliximab in RA patients. However, this marker did not accurately identify patients in remission after 22

wk in their study group. Additionally, van Steenberg *et al*[22] reported that lower sIL-2R levels were associated with sustained remission without the use of disease-modifying antirheumatic drugs in RA patients[4]. The status of regulatory T cells (Tregs) in refractory RA and the potential of low-dose IL-2 therapy in restoring the decreased CD4 Tregs were investigated. Results showed that a decrease in peripheral blood CD4 Tregs in patients with refractory RA was associated with continuing disease activation, but not an increase in Th17 cells. Low-dose IL-2 therapy was found to restore decreased CD4 Tregs and promote rapid remission of patients with refractory RA without overtreatment or observed side effects, making it a potential therapeutic candidate[24]. The potential use of low-dose interleukin-2 (Ld-IL2) as a therapeutic approach to enhance the treatment of RA was investigated through a randomized, double-blind, placebo-controlled trial. The effectiveness and safety of Ld-IL2 in patients with active RA was considered. The findings showed that Ld-IL2 was well-tolerated, and low levels of Treg and high levels of IL-21 were associated with positive responses to Ld-IL2. Therefore, it can be concluded that Ld-IL2 is a safe and effective treatment for RA[25].

SJÖGREN SYNDROME

Sjögren's syndrome (SS) is a chronic autoimmune disease that is characterized by the loss of function of exocrine glands. The infiltration of exocrine glands by T and B lymphocytes is responsible for the inflammation observed in SS patients. In addition to the loss of exocrine gland function, SS patients may also experience a range of extra-glandular symptoms such as arthralgia, kidney disease, lung disease, and fatigue[26]. SS is characterized by dry mouth (xerostomia), dry eyes (xerophthalmia), and other immune-mediated symptoms. The condition can also cause dryness in the mucosal surfaces of the digestive tract, lungs, and vagina, which is referred to as "Sicca syndrome" or "Sicca Complex." Since it is a systemic illness, SS can impact various organ systems and result in a range of symptoms. These symptoms, which include fatigue, depression, anxiety, and impaired physical performance, can significantly affect a patient's quality of life and daily activities[27].

The etiology of SS, like other autoimmune diseases, is not yet fully understood. However, it is widely recognized that exposure to specific environmental factors may contribute to the dysregulation of the immune system and the onset of the disease in susceptible individuals. The interferon pathway is critical in the early stages of SS by disrupting the innate immune system's barriers[28]. Nonetheless, the adaptive immune system's role is significant in SS development, as persistent B-cell activation and the growth of Th1 and Th17 cells contribute to the disease's progression[29].

Genetic susceptibility plays a significant role in the pathophysiology of SS. Previous studies have identified several genes, including IRF5-TNPO3, STAT4, IL12A, FAM167A-BLK, DDX6-CXCR5, and TNIP1, may be involved as risk factors for SS. IRF5 and STAT4 have been recognized as susceptibility genes based on genome-wide association analysis[30]. IRF5 activates the expression of several proinflammatory factors downstream of the Toll-like receptor and type I IFN receptor, while STAT4 plays a role in autoimmune abnormalities through IFN production[31]. Women are more likely than men to develop SS, and studies have identified GTF2I and RBMS3 as the most significant susceptibility genes for pSS in women[32].

Long non-coding RNA (lncRNA) plays a role in gene expression by modifying chromosomes, DNA, transcription, and post-transcriptional modification, as well as the generation of inflammatory mediators, immune cell growth, and differentiation, which contribute to the development of autoimmunity[33]. Dysregulated lncRNAs have been linked to an increased risk of SS[34]. MicroRNAs (miRNAs) also play a role in the development of autoimmune diseases, including SS. pSS patients have decreased expression of miRNA-181a and miRNA-16 in their salivary glands[35], while the levels of miRNA-146a and miRNA-155 in their peripheral blood mononuclear cells are linked to their clinical symptoms. Epigenetic processes, such as DNA methylation and histone modification, may also contribute to the onset of SS[36].

SS is an autoimmune disorder where immune cells and IL-2 signaling are not working correctly. IL-2 is essential for the growth and maintenance of Treg cells. There is potential for the use of low doses of IL-2 to treat autoimmune diseases, but it is uncertain if it can be effective in treating SS or how it works. Research provides evidence that low-dose interleukin-2 is a promising treatment for SS in NOD mice by restoring immunological balance through promoting Treg cells and suppressing germinal center B cells and effector T cells[37]. The effects of low-dose interleukin-2 on exocrine glands in SS were not previously known. This study investigated the impact of low doses of IL-2 on the development and function of salivary glands in a mouse model of SS. Results indicate that low-dose IL-2 treatment improves the exocrine glands' ability to secrete saliva in SS-affected mice, but it does not restore the damaged structure of the glands[38].

Primary Sjögren syndrome (pSS) is a systemic autoimmune disease characterized by immune cell dysregulation, and it currently lacks effective treatment options. As a result, investigating potential therapeutic strategies is essential. One such strategy is the use of low-dose interleukin 2 (LD-IL-2), which can be studied for its safety, immunological response, and potential to treat pSS. In a randomized clinical trial, pSS patients responded positively to LD-IL-2 treatment, leading to restored immunological balance through the promotion of regulatory T cells and CD24 high CD27+ B cells[39]. A study was designed to understand the mechanism behind the increased Th17 differentiation in pSS patients and the role of IL-2 in balancing Th17 and Tregs. The findings showed that the increased Th17 generation in pSS was not dependent on Tregs, but rather due to the lack of IL-2-mediated suppression of Th17 differentiation. Therefore, this study revealed a new mechanism of immune suppression mediated by IL-2 in pSS[40].

SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is an autoimmune disease that affects multiple organs, including the skin, joints, tendons, gastrointestinal tract, lungs, heart, blood vessels, and kidneys, due to inflammation and fibrosis. It primarily affects women, with a female-to-male ratio of 4:1-10:1, depending on age and ethnicity. The disease has two clinical subtypes based on the extent of skin involvement: Diffuse cutaneous SSc (dcSSc), which affects the skin proximal to elbows and/or knees, or that affects the thorax and/or abdomen at any given time during the disease, and limited cutaneous SSc (lcSSc), which affects the skin distal to elbows and knees without the involvement of the thorax or abdomen[41].

The disease can lead to major disabilities, malnutrition, and decreased quality of life due to vascular complications, cardiopulmonary involvement, inflammatory myopathy, arthritis, and gastrointestinal tract involvement[41]. The immune response cells, inflammatory mediators, fibroblasts, and other components of the extracellular matrix, as well as endothelial damage, play a central role in the disease's numerous pathogenic pathways. This has changed the previous paradigm that the disease was predominantly fibrotic and is now viewed as a complex syndrome that requires simultaneous treatment of its various pathogenic pathways[41].

The chromosome 4q27 region that contains the IL-2 and IL-21 genes has been linked to various autoimmune diseases, as both genes are involved in immune system functions. This study aimed to investigate the role of the IL-2/IL-21 Locus in SSc. The research found that the allelic combination of rs2069762A-rs6822844T-rs6835457G-rs907715T showed a significant association with SSc and the limited cutaneous SSc subtype. Therefore, the study concluded that the IL-2/IL-21 Locus is involved in the genetic susceptibility to SSc. These findings also add to the evidence that the IL-2/IL-21 Locus is a common genetic factor in autoimmune diseases[42].

The connections were investigated between 9 SNPs in the IL10, IL1B, IL1A, IL1RN, IL2, LTA, and IL6 genes and the development of SSc, as well as the clinical subtype of SSc in patients. A total of 78 SSc patients (31 with diffuse SSc and 47 with limited SSc) and 692 healthy blood donors were genotyped for 9 SNPs: IL10 T-3575A, IL10 A-1082G, IL1B C-31T, IL1B C-511T, IL1A C-889T, IL1RN A9589T, IL2 T-384G, LTA T-91G, and IL6 G-174C. The study found that the IL1B and IL2 gene SNPs may contribute to the susceptibility to SSc. Additionally, the IL2-384-G allele may serve as an indicator for the limited phenotype of SSc[43].

IL-2A and IL-12R gene variants have been linked to SSc. IL-2 helps maintain the immune system's balance and tolerance to self, promotes B-cell immunoglobulin production, and stimulates natural killer cell proliferation and differentiation. Two studies explored the role of IL-2 in SSc. The studies found that certain IL-2 receptor alpha gene variants (rs11594656, rs2104286, and rs12722495) were associated with SSc, limited cutaneous SSc, and anticentromere antibody (ACA) positivity. However, the associations were highly dependent on ACA positivity, as removing ACA from the analysis resulted in the loss of association. Among the IL-2 RA gene variants, rs2104286 showed the strongest association with ACA positivity, and the associations with the other variants disappeared after accounting for rs2104286[44]. The minor allele of rs907715 and rs682284 are linked to SSc. The variant rs6822844 specifically affects lcSSc and ACA positivity. An allelic combination, is rs2069762*A, rs6822844*T-rs6835457G-rs907715* T is linked to both dcSSc and lcSSc. Protective for lcSSc and ACA positive is the T allele for rs6822844[42].

Basiliximab, a monoclonal antibody, targets the alpha chain (CD25) of the IL-2 receptor and has been suggested as a potential treatment for SSc. This is due to recent findings that suggest a significant role of T effector cells, specifically T-17 and T regulatory subsets, in the pathogenesis of SSc[45]. Schmidt *et al*[45] study suggested that treatment with this medication could improve skin involvement, lung fibrosis, disease progression, and mortality in systemic sclerosis. They based this on the correlation between these factors and serum levels of soluble IL-2 receptor, which could be reduced by the drug[46]. In an accessible SSc research, basiliximab medication was generally well tolerated, and the side effects that were reported were primarily modest[47]. In an open-label study on SSc, minor side-effects were mostly reported, and in general, basiliximab therapy was well tolerated[47].

Relationship between immune system and lung fibrosis in Fra-2-transgenic mice was investigated, which can serve as a model for SSc. The study examined whether an imbalance between effector and regulatory CD4+ T cells may be responsible for the lung phenotype observed in these mice. The results suggest that immunotherapies targeting the restoration of Treg cell homeostasis may be beneficial for SSc. Low-dose IL-2 injection has been suggested as a potential therapy to achieve this, which may be explored further in future studies. SSc is an autoimmune disease with significant pulmonary complications that can decrease life expectancy[48].

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a disease of the nervous system that causes chronic, progressive, and degenerative symptoms. It occurs when the immune system is not functioning properly, leading to damage of the central nervous system, including demyelination and axonal damage. In most cases, the first sign of MS is a demyelinating disease referred to as a clinically isolated syndrome. This involves focal or multifocal areas of the central nervous system, commonly affecting the optic nerve, brainstem, or spinal cord. It is caused by a disruption in the regulation of the immune system, which may be due to genetic factors and/or environmental triggers such as viral infections. The prevalence of MS is highest among individuals of Caucasian ethnicity and those of northern European descent. The disease is widespread, with approximately 2 million individuals diagnosed worldwide, and the number of cases is projected to increase due to population growth[49]. MS is prevalent in young adults and is the primary cause of non-traumatic disability in this population. MS is more common in females than males, with a ratio of 2-3:1, although there is no gender imbalance in primary progressive MS[49].

IL-2 and soluble IL-2 receptor (sIL-2R) levels in serum and CSF could serve as markers of disease activity in MS. A research study involved 63 MS patients, and the results showed that the levels of IL-2 and sIL-2R in the CSF were significantly higher in patients experiencing relapses than those in remission or the control group. Additionally, these levels correlated with disease severity scores, the number of relapses per year, and the total duration of the disease. The findings suggested that there is an association between an activated immune system and MS pathology, and measuring IL-2 and sIL-2R levels could provide a reliable indicator of disease activity in MS patients[50].

The role of CD8⁺ T cells in the development of MS has been established. IL-2R α is crucial for the function of CD8⁺ T cells, and IL2RA gene SNPs increase the risk of MS. In this study, the researchers investigated the methylation and gene expression of the IL2RA gene in isolated CD8⁺ T cells in relation to the MS-associated SNP rs2104286 and soluble IL-2R α (sIL-2R α). The results showed that rs2104286 SNP has a minor effect on CD8⁺ T cells and sIL-2R α might negatively regulate the CD8⁺ T cell response[51].

The IL-2/IL-2R pathway is crucial for T cell proliferation, survival, and regulatory T cell production. Genome-wide studies have identified polymorphisms in the IL2/IL21 genes (located in 4q27), as well as in genes encoding the IL2RA and IL2RB subunits (located in 10p15 and 22q13, respectively), that are associated with various autoimmune diseases. In a combined Spanish cohort, regression analyses indicated that two independent IL2RA association signals, namely rs2104286 and rs11594656/rs35285258, are relevant for MS susceptibility and support their common effect on autoimmune risk. Additionally, the association of IL2/IL21 requires further investigation[52].

The ability to predict the severity of MS in a newly diagnosed patient would allow for more personalized treatment strategies and improved clinical outcomes. Therefore, there is a need for prognostic biomarkers. A study was conducted to evaluate the prognostic value of intrathecal IgM synthesis, as well as cerebrospinal fluid and serum levels of IL-2, IL-6, IL-10, chitinase 3-like 2, and neurofilament heavy chains obtained shortly after the onset of the disease. The study found that the IL-2:IL-6 ratio, IL-2, and chitinase 3-like 2 (all in cerebrospinal fluid) may serve as valuable prognostic biomarkers in the early stages of multiple sclerosis[53].

This research explores the potential therapeutic and protective effects of Tregs, myeloid-derived suppressor cells (MDSCs), and IL-2 on a MS disease model. The study involved immunizing C57BL/6 mice to develop an experimental autoimmune encephalomyelitis (EAE) model, followed by investigating the effects of pre-and post-treatment of Tregs, MDSCs, and IL-2 on inflammation, demyelination in brain tissue, and the number of Treg, granulocytic-MDSC, and Th-17 cells in the spleen. The results showed that pre-and post-treatment with Tregs, MDSCs, and IL-2 prevented EAE induction, with reduced Th-17 cells and suppression of pathological properties, and no weight change in the mice. Therefore, the study concludes that pre-and post-treatment with Tregs, MDSCs, and IL-2 may have the potential to prevent EAE induction[53].

CONCLUSION

According to the genetic studies of IL-2, gene polymorphisms of IL-2 such as IL-2 330/T, IL-2 330/G, and rs2069763 are involved in triggering increased risk of SLE. Furthermore, genetic associations of IL-2/IL-2R such as rs791588, rs2281089, rs2104286, rs11594656, and rs35285258 are significantly associated with RA susceptibility. The IL-2 polymorphism including rs2069762A, rs6822844T, rs6835457G, and rs907715T are significant connections with systemic sclerosis. In addition, rs2104286 (IL-2), rs11594656 (IL-2RA), rs35285258 (IL-2RB) gene polymorphism is significantly increases the risk of multiple sclerosis. In the case of therapeutic approaches, low-dose IL-2 therapy could regulate Tfr and Tfh cells, resulting in a reduction in disease activity in SLE patients. In addition, elevated sIL-2R levels in the peripheral blood of SLE patients could be linked to an immunoregulatory imbalance, which may contribute to the onset and progression of SLE. Consequently, sIL-2R could potentially be a target for future SLE therapy. Moreover, Ld-IL2 was well-tolerated, and low levels of Treg and high levels of IL-21 were associated with positive responses to Ld-IL2 suggested to be a safe and effective treatment for RA. Additionally, low-dose IL-2 treatment improves the exocrine glands' ability to secrete saliva in SS-affected mice. Whereas, Basiliximab targets the alpha chain of the IL-2 receptor suggested as a potential treatment for SSc. Also, pre-and post-treatment with Tregs, MDSCs, and IL-2 may have the potential to prevent EAE induction in patients with MS. Further studies should be conducted on IL-2 gene polymorphisms in Sjogren syndrome.

FOOTNOTES

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- 39 Clinical utilities and end-user experience of pharmacogenomics: 39 mo of clinical implementation experience in an Australian hospital setting

Moxham R, Tjokrowidjaja A, Devery S, Smyth R, McLean A, Roberts DM, Wu KHC

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Retrospective Study

Clinical utilities and end-user experience of pharmacogenomics: 39 mo of clinical implementation experience in an Australian hospital setting

Rosalind Moxham, Andrew Tjokrowidjaja, Sophie Devery, Renee Smyth, Alison McLean, Darren M Roberts, Kathy H C Wu

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Abstract

BACKGROUND

Pharmacogenomics (PG) testing is under-utilised in Australia. Our research provides Australia-specific data on the perspectives of patients who have had PG testing and those of the clinicians involved in their care, with the aim to inform wider adoption of PG into routine clinical practice.

AIM

To investigate the frequency of actionable drug gene interactions and assess the perceived utility of PG among patients and clinicians.

METHODS

We conducted a retrospective audit of PG undertaken by 100 patients at an Australian public hospital genetics service from 2018 to 2021. *Via* electronic surveys we compared and contrasted the experience, understanding and usage of

results between these patients and their clinicians.

RESULTS

Of 100 patients who had PG, 84% were taking prescription medications, of which 67% were taking medications with actionable drug-gene interactions. Twenty-five out of 81 invited patients and 17 out of 89 invited clinicians completed the surveys. Sixty-eight percent of patients understood their PG results and 48% had medications changed following testing. Paired patient-clinician surveys showed patient-perceived utility and experience was positive, contrasting their clinicians' hesitancy on PG adoption who identified insufficient education/training, lack of clinical support, test turnaround time and cost as barriers to adoption.

CONCLUSION

Our dichotomous findings between the perspectives of our patient and clinician cohorts suggest the uptake of PG is likely to be driven by patients and clinicians need to be prepared to provide information and guidance to their patients.

Key Words: Pharmacogenomics testing; Clinical adoption; Drug gene interactions; Clinician perspectives; Patient perspectives

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Core Tip: Pharmacogenomics (PG)-guided therapy has the potential to reduce adverse drug reactions and improve clinical outcomes of patients with mental illness. Despite increasing evidence, the uptake of PG among Australian clinicians remains low. We report for the first time on the dichotomous responses between patients and their clinician counterparts in the usage of PG: Patients were generally positive and willing to use PG compared with clinicians, suggesting that the uptake of PG in Australia is likely to be driven by patients, and that clinicians need to be prepared to provide information and guidance to their patients.

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INTRODUCTION

Variabilities in an individual's genetic sequence may affect the pharmacokinetics and pharmacodynamics of a drug, leading to an altered therapeutic response and/or increased risk of adverse drug reactions (ADRs)[1]. Pharmacogenomics (PG) is the study of clinically relevant genetic biomarkers, or pharmacogenetic variants that can influence a person's response to therapeutic drugs[1]. The relationship between pharmacogenetic variants and drug response is termed drug-gene interaction (DGI). Recent advances in genomic technologies including Next Generation Sequencing, or Massively Parallel Sequencing, have led to an unprecedented capacity allowing multiple genes to be interrogated at once. This approach allows multiple genes that are involved in, not only different metabolising enzymes (pharmacokinetics) and drug receptors (pharmacodynamics) for a single drug, but also those of multiple drugs, to be analysed in a single test with a gene panel. Such pharmacogenomic testing can determine an individual's likely response to multiple, up to a few hundred, medications from a single sample, unlike the traditional pharmacogenetic test of a single variant and single DGI at a time[2].

PG, especially if done pre-emptively, has the potential to guide clinicians at point-of-care in choosing the right therapeutic drug and dosing that is tailored to each patient, with the potential promises of reduced adverse effects, improved tolerability, and enhanced therapeutic response[3].

Internationally, there is a growing interest for widespread adoption of PG among patients and its potential to improve their medication outcomes[4-6]. In contrast, studies on clinician perspectives of PG have highlighted barriers and challenges towards its implementation into clinical practice. These barriers include lack of education/training, confidence, and acceptability of PG by clinicians[7-10], as well as a lack of consensus between clinical guidelines and access to clinical decision support[11-14]. To date, there is limited literature on the perspectives of the Australian end-users on PG[15].

In Australia, only two genotypes are covered by the national Medicare scheme, namely, *HLA-B* 5701 for abacavir, and *TPMT* for thiopurine drugs. However, multigene panel PG is neither publicly-funded by Medicare nor covered by private health insurance schemes. Patient-pay testing can be requested by a specialist or a general practitioner (GP). Most clinical genetics services in Australia, which are funded by State-operated public hospitals, do not routinely offer multigene panel PG, with the exception of St Vincent's Hospital Sydney which is the setting of our investigation.

In Australia, the New South Wales Health Commission conducted an ‘Inquiry into the Management of Mental Healthcare Delivery’ in 2018 that identified under-utilisation of PG and recommended such testing as a key priority to improve clinical outcomes for mental health patients[16]. In order to see wider uptake, one needs to identify barriers to and facilitators of PG adoption in Australia, as perceived by patients who have had testing and clinicians who care for these patients. Such data, which remain limited to date, will inform an implementation science approach to promote the systematic uptake of PG into routine practice[17].

In this study we aim to: Investigate the potential impact of PG by determining the frequency of actionable DGIs in our patient cohort; assess within the same patient cohort, their experience of testing, as well as their understanding and usage of PG results and; assess among clinicians involved in the care of these patients, the experience, acceptability, understanding and perceived utility of PG (Figure 1).

MATERIALS AND METHODS

PG testing and retrospective review of results

A retrospective review was conducted on the PG reports and medication history of consecutive adult patients who were aged 18 years or older who had PG at St Vincent’s Hospital Clinical Genomics Sydney Australia between 1 August 2018 and 31 September 2021.

PG testing was conducted using a comprehensive commercial gene panel at a CLIA-certified laboratory (OneOme, Minneapolis, MN, United States), and included the following genes: *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP4F2*, *COMP*, *DPYD*, *DRD2*, *F2*, *F5*, *GRIK4*, *HLA-A*, *HLA-B*, *HTR2A*, *HTR2C*, *IFNL4*, *NUDT15*, *OPRM1*, *SLC6A4*, *SLCO1B1*, *TPMT*, *UGT1A1*, and *VKORC1*. This gene panel includes most pharmacogenes with high association evidence according to Clinical Pharmacogenetics Implementation Consortium, Dutch Pharmacogenetics Working Group and PG Knowledgebase[18].

Data collected on each patient included: age, sex, ethnicity, referring clinician and subspecialty, reason for referral, medication history, history of drug allergies and ADRs, number of high-risk and moderate-risk DGIs as shown on their PG report, number of current medications with high-risk or moderate-risk DGI.

Patient and clinician recruitment

Consecutive patients aged 18 years or older who underwent PG testing during a 39-month period between 1 August 2018 and 31 September 2021, and clinicians involved in the care of these patients, were identified and re-contacted *via* email and invited to participate in an online survey.

Survey development and distribution

Patient and clinician surveys were developed and created on the Research Electronic Data Capture (REDCap) software. The patient survey assessed patient experience including their understanding and usage of the PG results. The clinician survey assessed clinician experience including their knowledge, acceptability and perceived utility of PG, as well as barriers to and support needs for broader adoption of PG in clinical practice. Demographic data were collected from both surveys. The surveys were constructed using 5-Point Likert Agreement Scale for categorical response questions, as well as other single or multiple option and open-ended comment questions. Survey questions were constructed using simple sentences at a literacy level of 5th grade using the Flesch-Kincaid Readability Calculator[19].

Both surveys were pilot tested by a focus group of five laypeople randomly identified from investigator’s peers and five clinicians who were randomly identified through St Vincent’s Hospital network, who were representative of our intended survey respondents. Members of the focus groups were provided with written instructions on how to review and test the survey and their feedback was sought regarding clarity, comprehension, functionality of the branching logic and the duration of time taken to complete the survey. Adjustments were made to the surveys with the final versions taking 15-20 min to complete. The REDCap surveys were distributed electronically *via* email, or short message service (SMS), if emails were unavailable, to patients and clinicians respectively. Follow-up reminder emails or SMSs were sent two weeks after the initial invitation if no responses were received.

Data analysis

Descriptive statistics were used to summarise categorical data ascertained from the retrospective chart review. If applicable, correlations were made between patient’s reported medication side effects/ADR and the DGI of the implicated medication. Mean, median, and standard deviation were derived using Microsoft Excel (Microsoft Corporation, 2016, version 16.0) for continuous variables such as age and number of high/moderate risk DGIs. The survey data were exported from REDCap and analysed using a regular analysis Microsoft Excel (Microsoft Corporation, 2016, version 16.0) package. Categorical data were summarised in descriptive and numerical formats using bar graphs and tables. For the 5-Point Likert scale (*e.g.* strongly agree, agree, neither agree nor disagree, disagree, strongly disagree), data was reviewed in its original form and combined into three categories (*i.e.* agree, neutral, disagree) then analysed as categorical data.

Ethics approval was sought and received from St Vincent’s Hospital Human Research Ethics Committee (2019-ETH12892) to conduct this study.

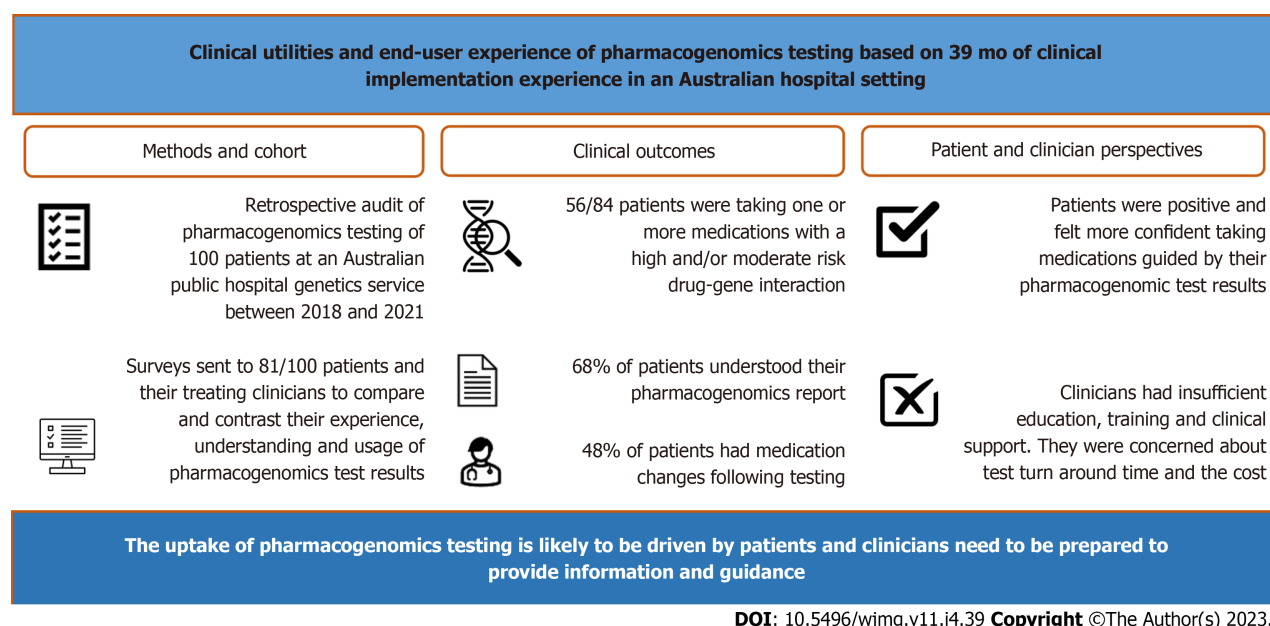


Figure 1 Graphical abstract.

RESULTS

Demographics of patients referred for PG testing

There were 100 patients identified during the audit period, consisting of 44 males and 56 females, with a mean age of 44.7 years +/- standard deviation 15.7 years (age range 18-75 years). Patients were referred by psychiatrists (39%), immunologists (15%), GPs (10%), neurologists (9%), transplant physicians (8%), and clinical pharmacologists (5%). The remaining 14% were referred by the following specialists: cardiologist, endocrinologist, gastroenterologist, haematologist, nephrologist, obstetrician, rheumatologist and thoracic physician; with each specialist category accounting for 2% of referrals. The referral indications were: to guide current pharmacotherapy (62%), previous history of ADRs (51%), history of polypharmacy (5%), and/or pre-emptive purpose to guide future prescription (5%). The demographics of the 100 patients are summarised in Table 1.

PG reports

The PG reports of the 100 patients showed that on average, patients had 11 high-risk DGIs (range: 0-49, SD: 13.3) and 55 moderate-risk DGIs (range: 1-149, SD: 28.1). Of the 84 patients who provided a medication history, 17 patients (20%) were taking medications implicated with high-risk +/- moderate-risk DGI, 39 patients (46%) were taking medications with a moderate-risk DGI; with 56 patients (66%) taking medications with an actionable DGI (either high-risk DGI or moderate-risk DGI). Of these, one patient was taking nine medications with an actionable DGI (Table 2).

Demographics of survey respondents

Of the 100 patients identified and included in the retrospective review, 81 patients were invited to participate in the patient survey, after excluding those with no contact details and those who had indicated they did not wish to be contacted for future research. Of the 81 patients invited, 25 patients (31%) responded and completed the survey. Patient survey respondents were mostly female, aged 40-49 years with a tertiary level of education. A total of 89 clinicians, including 29 subspecialist clinicians and 60 GPs, involved in the care of the same patient cohort were identified and invited to participate in the clinician survey. Of those invited, 17 clinicians (19%) responded and completed the survey. Clinician survey respondents were mostly male, practicing in psychiatry, with a medical degree attained after the year 2000, who encountered patients in the hospital setting and had referred or discussed PG with their patients in the preceding 12 mo. A summary of survey respondent demographics for both patient and clinician surveys is shown in Table 3.

Clinician knowledge and training sources of PG

Responses from clinician survey ($n = 17$) showed that clinicians have different levels of knowledge and sources of education for PG. The majority of clinicians (94.1%) knew that individuals respond differently to medications based on genetic factors. Their knowledge of how many drugs have a PG impact varied significantly. None of the clinicians had completed any formal training in PG, with the majority (52.9%) reporting not feeling confident in discussing and/or answering questions about PG with their patients. The primary sources of knowledge about PG for clinicians were medical colleagues (75%), scientific journals (62.5%), conferences and scientific meetings (25%), publicly available online resources (25%) and/or commercial providers such as pathology laboratories (18.8%).

Table 1 Demographics of 100 patients included in the retrospective review of pharmacogenomics result

Patient characteristics	
Sex	(%)
Male	44
Female	56
Age (yr)	(%)
18-29	23
30-39	12
40-49	29
50-59	17
60-69	14
> 70	5
Referring specialists	(%)
Psychiatrists	39
Immunologists	15
General practitioners	10
Neurologists	9
Transplant physicians	8
Clinical pharmacologists	5
Others ¹	14
Indications for referral	(%)
To guide pharmacotherapy	62
Previous drug reactions	51
Polypharmacy ²	5
Pre-emptive testing	5
Did not specify	5

¹Cardiologist, endocrinologist, gastroenterologist, haematologist, nephrologist, psychologist, obstetrician, rheumatologist and thoracic physician referred.

²Patient taking ≥ 5 prescription medications. Referring clinicians could list more than one indication for pharmacogenomics testing.

Patient experience: Patient respondents ($n = 25$) were generally positive in their experience with PG. The majority of patients (68%) agreed with the statement “I have a good understanding of my PG results”. Nineteen (76%) and 17 (68%) patient respondents agreed that PG has helped them understand which medications were safe, and effective, for them respectively. Likewise, 17 patients (68%) agreed with the statement “I feel more confident in a new medication chosen by my doctor according to my PG results”; and 15 patients (60%) agreed with the statement “The results of my PG test results helped explain my experiences with medications”. Nine patients (36%) agreed with the statement “My doctor was able to address my questions regarding my PG results” (Figure 2).

Clinician perspective: Clinician respondents ($n = 17$) were generally ambivalent in their perception of PG utility, with 50% reporting that PG has benefitted some of their patients, while 37.5% and 6.3% reported that PG has benefitted little, or none, of their patients, respectively; while 6.3% reported it has benefitted most of their patients. In addition, 17.7% of respondents disagreed with, and 35.3% were neutral to, the statement “PG has guided me in choosing the right medication and/or dosage for my patients”. When asked about the likelihood of utilising PG in the next 12 mo, 64.7% clinicians reported likely, 23.5% reported unlikely, and 11.8% were ambivalent about using it in the next 12 mo. Seven (41.2%) clinicians agreed that “PG can help reduce overall costs for patients”, while nine (52.9%) were neutral to the same statement. There was an equal distribution among clinicians who agreed with (31.3%), were neutral to (31.3%), or disagreed with (37.6%), the statement that PG was too expensive for their patients. Most clinicians (88.2%) agreed that they were more likely to order PG testing if Medicare covered the cost (Figure 2).

Table 2 Number of patients (*n* = 56) taking medications found to have high- and/or moderate-risk drug-gene interaction against the number of such medications, in those whose medication history was available (*n* = 84), *n* (%)

Number of medications with implicated DGI	Number of patients taking medications with high-risk +/- moderate-risk DGI implicated	Number of patients taking medications with moderate-risk DGI implicated only	Number of patients taking medications with an actionable ¹ DGI
1	5 (6)	20 (24)	25 (30)
2	7 (8)	11 (13)	18 (21)
3	3 (4)	7 (8)	10 (12)
4	1 (1)	0 (0)	1 (1)
5	0 (0)	1 (1)	1 (1)
6	0 (0)	0 (0)	0 (0)
7	0 (0)	0 (0)	0 (0)
8	0 (0)	0 (0)	0 (0)
9	1 (1)	0 (0)	1 (1)
10	0 (0)	0 (0)	0 (0)
Total	17 (20)	39 (46)	56 (66)

¹Either high-risk drug-gene interaction (DGI) or moderate-risk DGI.

DGI: Drug-gene interaction.

Table 3 Patient and clinician survey respondent demographics, *n* (%)

Patient respondents (<i>n</i> = 25)		Clinician respondents (<i>n</i> = 17)	
Sex		Sex	
Male	9 (36.0)	Male	11 (64.7)
Female	16 (64.0)	Female	6 (35.3)
Patient age (yr)		Clinician attained medical degree	
18-29	4 (16.0)	Before 1980	2 (11.8)
30-39	2 (8.0)	1981-1990	2 (11.8)
40-49	7 (28.0)	1991-2000	3 (17.6)
50-59	6 (24.0)	After 2000	10 (58.8)
60-69	5 (20.0)		
> 70	1 (4.0)		
Patient education level		Clinician specialty	
Did not finish high school	1 (4.0)	Psychiatry	5 (29.4)
High school	6 (24.0)	Clinical pharmacology	3 (17.6)
Certificate or diploma	5 (20.0)	Immunology	2 (11.8)
Bachelor's degree	11 (44.0)	Neurology	2 (11.8)
Master's degree or doctorate	2 (8.0)	General practitioner	1 (5.9)
		Other ¹	7 (41.2)

¹Rheumatology, respiratory, endocrinology, nephrology, gastroenterology.

Clinician respondents could list more than one area of specialty.

Usage and sharing of PG results

As shown in [Figure 3](#), the majority of patient respondents (92%) have shared their PG results with their health care providers; and 12 patients (48%) reported having had their medications changed over 17 clinical encounters following PG testing. Of the seventeen medication changes reported by patient respondents, nine instances (53%) of medication changes were made by their specialist, seven (41%) by their GP, and one instance (6%) was made by the patient themselves.

Clinician support needs and barriers for adoption of PG

As shown in [Figure 2](#), clinicians were ambivalent towards the adoption of PG. The majority of clinician respondents (70.6%) thought that PG requires more evidence for it to be clinically useful. The majority of respondents (70.6%) did not agree that the information generated from PG was easy to understand; the majority (52.9%) did not feel confident in discussing and answering questions about PG. When asked if support is needed before clinicians feel comfortable in requesting PG, the responses were fairly equally distributed, between agree (35.3%), neutral (23.5%), and disagree (41.2%). The majority of clinician respondents (52.9%) reported not having easy access to resources to help manage their patients; and 52.9% respondents would like support from genetic professionals to help manage patients who have undergone PG testing.

In regards to whom clinicians thought would be the most appropriate person to discuss and return PG results to patients, the responses were equally divided between: Specialist ordering the test (52.9%), and clinical geneticists/genetic counsellors (47.1%); with no responses for GPs or pharmacists.

Barriers to adoption of PG were identified from our clinician cohort whose patients have had PG testing. The single most important barrier as reported by clinician respondents includes one of: A lack of clinical decision aids (29.4%), cost factor for patients (23.5%), timeliness of results (23.5%), lack of access to information (11.8%), patient expectations (5.9%) and difficult logistics in organising testing (5.9%). If barriers were overcome, most but not all clinicians (76.5%) agreed there was value in PG for their patients, with 17.6% providing a neutral response and 5.9% disagreeing to the statement.

DISCUSSION

Despite recent advances in genomic testing, the increasing knowledge of DGIs, and the availability of dosing guidelines based on actionable DGIs[20], PG remains under-utilised in Australia. There have been several PG implementation projects undertaken in the US and Europe; such initiatives are limited in Australia[21].

Our study highlighted the potential utility of PG in identifying high- and/or moderate-risk, or actionable, DGIs that may trigger a change in prescription. Our audit showed a wide range of variabilities in the number and degree of DGIs among patients, supporting the rationale for individualised prescribing based on genetic characteristics. Additionally, our results indicated that a significant proportion of patients, 66%, were taking a medication implicated to have an actionable DGI at the time of PG testing, highlighting the potential utility of PG in guiding prescription and reducing the incidence of adverse drug effects[22].

Although our patients who have had testing were generally in favour of the utility of PG on improving their medication experience, the perception of PG among clinicians who care for these patients was however reserved with hesitancy and/or skepticism. Over half of our patient respondents (68%) reported feeling more confident in medications when their PG results were taken into consideration by their healthcare providers when prescribing. This echoes the limited literature showing that PG may increase medication adherence among patients[23]. In contrast to patient perception, and consistent with previous studies on clinician attitudes towards PG[24,25], a significant proportion of our clinician cohort was more cautious on the utility of PG and would like to see more evidence published and challenges addressed before using PG in their routine practice. Our dichotomous findings between the perspectives of our patient and clinician cohorts may suggest that the uptake of PG is likely to be driven by patients and that clinicians need to be prepared to deal with the information and provide guidance to their patients. This is a valuable finding as there are no recent studies investigating among or contrasting between the end-users in their perception and understanding of PG using the current multi-variant testing technology.

Half of the patient respondents in our study reported having their medications changed following PG, highlighting the potential impact of PG on treatment decisions. Additionally, our patient survey showed that the majority of respondents have discussed their PG results with their GPs; and of those who had medication changes, half of the respondents had their medications changed by their GPs. These findings suggest the importance of primary care engagement and involvement in the wider adoption and implementation of PG in Australia.

We identified a lack of education and training, and a lack of clinical decision aids and support as the major barriers to routine adoption of PG among our clinician cohort who was involved in the care of patients who underwent PG testing. The majority of our clinicians have not completed any formal training in PG; have expressed complexities of incorporating results in their patient care; and have expressed the need for support from trained health professionals to manage these results. Similar to initiatives in various healthcare settings in the United States[26-28], our findings could inform an interdisciplinary care model approach in Australia. Such an approach would incorporate the expertise of genetics professionals, clinical pharmacologists and pharmacists providing training and education to primary care providers, the patient's nominated specialists and/or pharmacists[29].

Other barriers to uptake of PG identified in our study include time limitations and cost of testing. The long turnaround time for results highlights the importance of testing in a pre-emptive setting so that results are available at future point-of-care. In Australia, Medicare currently covers only two DGIs (*HLA-B 5701* for abacavir, *TPMT* for thiopurine drugs).

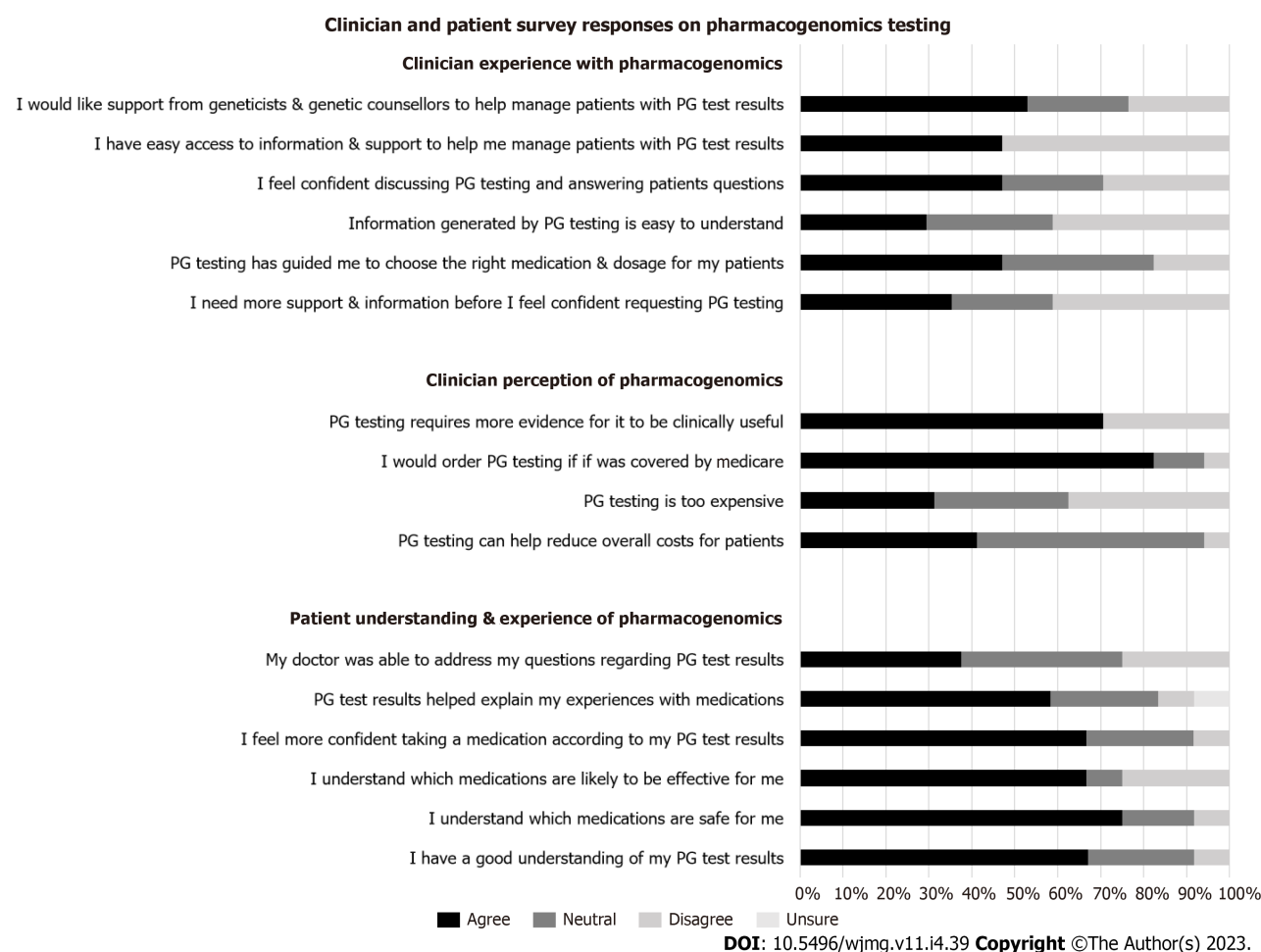


Figure 2 Patient and clinician perspectives on the utility and application of pharmacogenomics testing. PG: Pharmacogenomics.

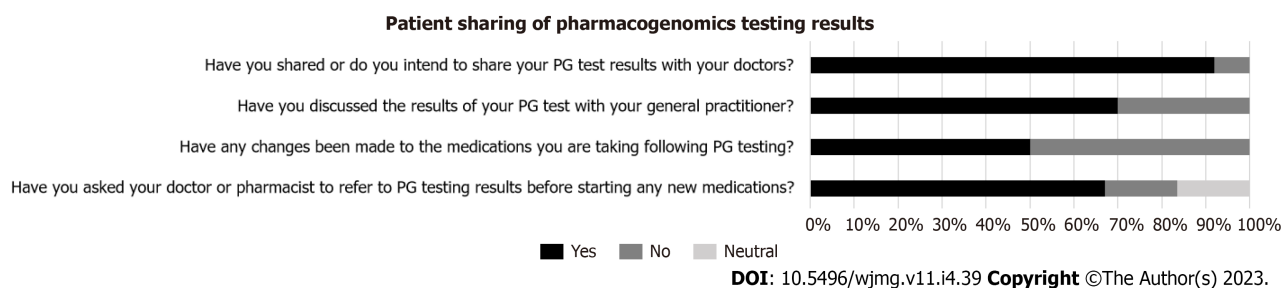


Figure 3 Sharing of pharmacogenomics testing results by patients. PG: Pharmacogenomics.

Most clinicians would like to see more PG tests covered by Medicare and reported that they are more likely to order a test if there was public funding. From the patient perspective, our patient survey did not capture cost implications as testing was undertaken at no charge to patients. To date, economic studies have suggested that PG testing can be cost-effective and that the cost of testing could be offset by its cost-savings from reduced time wastage on medication trial-and-error, enhanced therapeutic response, and mitigation of ADRs[30-32]. Future studies that explore the cost implications of PG-informed prescription should capture data on, not only individual patients, but also the overall healthcare system, to inform public funding for mainstream implementation in Australia.

The present study is limited by a lack of generalizability of our survey results, due to the small sample size, with 72% of patient respondents having a tertiary qualification or higher, and recruitment bias through a single tertiary genetics service. Similarly, our clinician respondents represent a small niche cohort who mostly work in a tertiary hospital setting. Our survey response rate of 31% from patients and 19% from clinicians in a sample size smaller than 500 can be considered sufficient to conclude estimates but is still below the average online survey response rate of 44.1% and is therefore best considered as observatory and exploratory[33]. Furthermore, our study had limited, 5.9%, survey response rate from GPs, which may be attributable to the coronavirus disease 2019 pandemic impact during the time of the survey. This makes it difficult to draw perspectives from this clinician cohort who is potentially a frontline user and adopter of

PG. As a Cronbach's Alpha Test was not performed to assess the reliability or internal consistency of the survey questions these surveys were not considered validated[34]. Finally, recall bias is an inherent limitation of survey research into past experiences, in particular, patient recall of medication changes, as well as understanding and sharing of PG results.

CONCLUSION

This study provides not only Australia-specific data, but also the perspectives of patients who have had PG and those of the clinicians involved in their care, which can inform future research into the implementation and the wider adoption of PG in Australia. Through an audit of 100 patients who have had testing, we found a significant proportion, 66%, of patients were taking a medication with an actionable DGI. Our paired end-user surveys highlighted dichotomous perspectives on PG between patients and their clinicians. Patients were generally positive and willing to use PG compared with clinicians, suggesting that patients may play a significant role in driving change and promoting the uptake of PG in clinical care.

ARTICLE HIGHLIGHTS

Research background

In Australia, the New South Wales Health Commission conducted an 'Inquiry into the Management of Mental Healthcare Delivery' in 2018 that identified under-utilisation of pharmacogenomics (PG) and recommended such testing as a key priority to improve clinical outcomes for mental health patients. In order to see wider uptake, one needs to identify barriers to and facilitators of PG adoption in Australia, as perceived by patients who have had testing and clinicians who care for these patients. Such data, which remain limited to date, will inform an implementation science approach to promote the systematic uptake of PG into routine practice.

Research motivation

PG-guided therapy has been shown to significantly enhance clinical outcomes in the field of mental health. Currently, two-thirds of patients with a major depressive disorder fail to achieve remission during the first treatment level; and many require multiple different medications sequentially on a trial-and-error basis. In addition, it has been shown that the odds of remission diminish with every additional medication trial-and-error iteration; and that a window of therapeutic opportunity for major depressive disorder appears to be within the first 2 sequential treatments. PG-guided pharmacotherapy, which aims at giving the right drug at the right dosage to the right person at the right intervention time, has the potential to enhance both patient outcomes and health cost savings in the Australian mental health system. Despite the evidence, Australia has been slow in adopting PG testing to guide therapy. To facilitate widespread clinical implementation of PG it is necessary to know and understand the attitudes and acceptability of PG, as well as the support and resource requirements, amongst potential end-users, including clinicians and patients. This information can contribute to the development of a model of care, enhance the feasibility and clinical utility of PG to ensure successful implementation of PG-guided pharmacotherapy into routine clinical care of patients with mental illness.

Research objectives

The objectives were to evaluate retrospective file data of patients participating in PG testing in Australia and to re-contact patients and their general practitioners (GPs)/clinicians to assess the impact of PG both on treatment options and patient/clinician reported outcomes.

Research methods

A retrospective audit was undertaken of the PG results of 100 patients who underwent testing as part of their routine clinical care between 1 August 2018 and 31 September 2021 at an Australian public hospital genetics service. Specifically designed and pilot-tested surveys were used to assess clinician knowledge, acceptability and perceived utility of PG in the care of patients and evaluate patients knowledge of PG results, as well as, how the PG has impacted their pharmaceutical care and overall mental and physical health. Data was analysed using descriptive statistics to summarise categorical data ascertained from the retrospective chart review where correlations were made between patient's reported medication side effects/adverse drug reaction (ADR) and the drug-gene interaction (DGI) of the implicated medication. Categorical data from clinician and patient surveys were summarised in descriptive and numerical formats with bar graphs and tables.

Research results

Through an audit of 100 patients who have had PG testing at St Vincent's Hospital Clinical Genomics Sydney, we found 67% of patients were taking a medication with an actionable DGI at the time of testing. The importance of our work is that *via* paired end-user surveys it highlighted dichotomous perspectives on PG between patients and their clinicians (psychiatrists, immunologists, neurologists, transplant physicians, clinical pharmacologists and GPs). Patients were generally in favour of the utility of PG on improving their medication experience, the perception of PG among clinicians who care for these patients was however reserved with hesitancy and/or skepticism. This suggests that the uptake of PG

is likely to be driven by patients, and clinicians need to be prepared to provide information and guidance to their patients.

Research conclusions

Our research identifies barriers to the clinical implementation of PG and suggests strategic solutions that could be put in place to support a wider adoption in routine medical practice. The majority (70%) of our patient participants have discussed their PG results with their GP; and of those who had medication changes following testing, half of the respondents had their medications changed by their GP. These findings suggest the importance of primary care involvement in the wider adoption and implementation of PG in Australia.

Research perspectives

To date, economic studies have suggested that PG testing can be cost-effective and that the cost of testing could be offset by its cost-savings from reduced time wastage on medication trial-and-error iterations, enhanced therapeutic response, and mitigation of ADRs. Future studies that explore the cost implications of PG-informed prescription should capture data on, not only individual patients, but also the overall healthcare system, to inform public funding for mainstream implementation in Australia.

FOOTNOTES

Author contributions: Moxham R and Tjokrowidjaja A recruited patients, collected and analysed data; Tjokrowidjaja A drafted the original research report and Moxham R drafted and prepared the manuscript for publication; Devery S, Smyth R and McLean A recruited patients, collected and analysed data; Roberts DM advised on clinician recruitment and data analysis; Wu KHC as the coordinating principal investigator was responsible for the study design and overall project supervision, manuscript editing and review; all authors contributed to manuscript revision, read and approved the submitted version.

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