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Who to Enroll in Parkinson Disease Prevention Trials?

The Case for Genetically At-Risk Cohorts

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Abstract

Therapies that prevent the occurrence of Parkinson disease (PD) (primary prevention) or mitigate the progression of symptoms in those with early disease (secondary prevention) are a critical unmet need in disease management. Despite great promise, PD prevention trials have not yet demonstrated success. Initiation of treatment too late in the disease course and the heterogeneity of disease are obstacles that may have contributed to the failure. Genetically stratified groups offer many advantages to primary and secondary prevention trials. In addition to their ease of identification, they decrease disease heterogeneity on several levels. Particularly, they comprise a phenotypically and pathologically enriched group with defined clinical features, pathogenic mechanisms and associated proteins that may serve as specific trial endpoints, therapeutic targets and biomarkers for disease state, and pharmacodynamic and pharmacokinetic status. However, challenges arise from genetic variant heterogeneity, from reduced penetrance whereby many carriers will not develop PD, and in recruiting a population that will meet the desired outcome in the proposed study duration. In this review, we discussed the opportunities afforded by the enrollment of genetically stratified cohorts (i.e., leucine-rich repeat kinase 2 and glucocerebrosidase 1) into prevention trials with a primary focus on primary prevention trials. We also outlined challenges surrounding the enrollment of these cohorts and offered suggestions to leverage their many advantages.

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Glossary

AAO = age at onset; **AJ** = Ashkenazi Jewish; **DaT** = dopamine active transporter; **DLB** = dementia with LB; ***GBA1*** = glucocerebrosidase 1; **GD** = Gaucher disease; **iPD** = idiopathic PD; **LB** = Lewy body; ***LRRK2*** = leucine-rich repeat kinase 2; **MSD** = Movement Disorder Society; **NSAID** = nonsteroidal anti-inflammatory drug; **PD** = Parkinson disease; **PRS** = polygenic risk score; **RBD** = REM behavior disorder; **SNCA** = α-synuclein.

While effective symptomatic therapies form the cornerstone of Parkinson disease (PD) treatment (tertiary prevention), trials for disease-modifying therapies that slow or halt progression in those with pathologically evident disease (secondary prevention) have failed.[1] Treatment may have been initiated too late in the disease course and/or tested in a group too heterogeneous.[2,3] Thus, for successful primary (to prevent the occurrence of disease) and secondary prevention, both reduction in variability and an earlier intervention are needed.

PD risk variant carriers who have not yet developed disease, particularly leucine-rich repeat kinase 2 (*LRRK2*), glucocerebrosidase 1 (*GBA1*), and α-synuclein (*SNCA*), provide the opportunity to decrease heterogeneity and can be identified before disease onset.[1–4] They offer the potential for enriched cohorts with similar phenotypes and pathogenic mechanisms and which may improve the ability to discern drug effects. They have defined proteins and pathways that inform specific therapeutic targets and corresponding biomarkers of drug and pathway engagement. Through reduction in variability, there is also greater power at similar sample sizes.

However, there are conceptual and practical challenges that arise with enrolling genetic cohorts into primary prevention trials. Pragmatic concerns relate to variant heterogeneity[5,6] and difficulties in recruiting a sufficiently large population that will meet the desired outcome in a reasonable study duration.[3] This is amplified by the fact that most risk variant carriers will never progress to PD.[7–9] For example, up to 90% of *GBA1* risk variant carriers and 70% of *LRRK2*-G2019S variant carriers will not go on to develop clinically manifest PD. Although for some genes such as *SNCA*, most carriers will develop PD because of its high penetrance, *SNCA* mutations are rare, accounting for less than 0.1% of all PD, and age at onset (AAO) varies, making it difficult to ascertain sufficient at-risk carriers who might phenoconvert to PD or another surrogate outcome in the duration of a trial.[4] Thus, we focused on *LRRK2* and *GBA1* variants—both moderately frequent genes with a potentially large enough sample of prodromal patients that might facilitate a primary prevention trial. We elaborated on the advantages and the challenges these cohorts presented and offered suggestions to optimize future studies. We advocated for collecting samples at trial onset that may allow for eventual post hoc stratification by genetics or biomarker subgroups and discussed considerations in limiting trial enrollment to specific genetic variant types and the need to consider safety and ethical concerns. We proposed this as a framework to be built upon as scientific knowledge and technology advances in this field.

A Focus on *LRRK2* and *GBA* Variant Carriers

LRRK2

Pathogenic *LRRK2* variants account for 5%–6% of familial and 1%–2% of sporadic PD in North American and European populations.[10] Missense variants vary in frequency between populations, with the G2019S variant by far the most frequent pathogenic variant in Western populations. Penetrance of the G2019S variant is incomplete and population dependent.[9] It is estimated that 26% of Ashkenazi Jewish (AJ) carriers and 43% of non-Ashkenazi mixed Europeans develop PD by 80 years of age.[10] The overall carrier frequency of G2019S is estimated at 0.48%[7,11] and approximately 2% in AJ groups where there is a population founder.[10] However, there is still limited information on the frequency and types of pathogenic variants in populations of Latino and African ancestry.[11,12] G2019S carriers interested in research have been identified through family members of affected patients, direct-to-consumer genetic testing, and more than 300 have participated in initiatives thus far.[13] Because of reduced penetrance, many carriers will not be prime candidates for primary prevention trials that are designed to assess phenoconversion to PD.

Recruitment of at-risk individuals with more penetrant variants such as I2020T is limited by the low frequency of carriers.[6] Conversely, the G2385R variant, which is present in up to 10% of East Asian populations, but of lower penetrance, represents one of the largest genetically defined at-risk PD cohorts in the world.[14] PD in G2385R carriers more closely approximates idiopathic PD (iPD) than G2019S-related disease. This presents an interesting conundrum for trial consideration. While the higher frequency of carriers has the advantage of easier enrollment, the potential lower specificity and greater variability of etiology associated with lower penetrance require much larger sample size to demonstrate risk reduction. In addition, there may be a greater contribution of other factors to disease etiology, thereby reducing the benefits of genetic homogeneity.[15] Thus, we will focus further discussion on the G2019S variant.

GBA1

GBA1 variants are present in 5%–10% of PD worldwide and approximately 20% in the AJ[4,16] While homozygous or compound heterozygous variants lead to Gaucher disease (GD), a multisystem lysosomal storage disorder, both biallelic and monoallelic carriers have an increased PD risk.[4,17] *GBA1* variants are classified into 3 groups based on the severity of their phenotypic effects: severe, mild, and risk variant. Severe variants are also termed neuronopathic (i.e., L444P), and mild

are considered non-neuronopathic (i.e., N370S). Risk variants (i.e., E236K and T369M) do not cause GD in the biallelic state but have been associated with decreased glucocerebrosidase activity and are found at an increased frequency in both PD and dementia with Lewy bodies (DLB).[17,18] However, they are associated with a much lower risk of developing PD. The distribution of the variant type varies depending on the population, with the N370S variant most prevalent in AJ groups and L444P and N370S most frequent of the mild and severe variants in White mixed European samples.[18] While the overall gene frequency is greater than *LRRK2*, the penetrance of *GBA* is lower and depends on mutation severity, with an overall estimated 10%–19% of mild and severe variant carriers developing PD by 80 years of age.[8] More than 400 unaffected carriers have participated in observational studies thus far.[13] As with *LRRK2*, selection of at-risk groups will need to consider the breadth of variants included, and most *GBA1* risk variant carriers will never develop PD.[11]

Targets and Potential Therapeutics

Both *GBA* and *LRRK2* cohorts comprise a well-studied population with defined biology and pathophysiologic targets potentially amenable to specific pharmacologic manipulation .[1–4] In addition, there are postulated shared pathogenic pathways with iPD,[3] and thus, once disease modification has been achieved in genetic cohorts, it may be appropriate for other at-risk groups, increasing the disease population that may benefit from a particular therapeutic.

LRRK2

The *LRRK2* gene encodes LRRK2, a multidomain protein containing a kinase, GTPase, and protein-protein interaction domains.[2,7] Pathogenic variants are believed to lead to a toxic gain-of-function mechanism resulting in an increased kinase activity generated either directly, by variants in the kinase MAPKKK-like kinase domain (G2019S and I2020T), or indirectly, by variants in the Rab-like ROC (R1441G/C/H) or the adjoining C-terminal of Ras COR domain (Y1699C/G).[2,7] LRRK2 plays an important role in vesicular trafficking and degradation of SNCA and the inflammatory response. Other pathways implicated in LRRK2-mediated neurodegeneration include those related to lysosomal, endosomal, mitochondrial, and autophagosomal functions.

Although not currently being tested in nonmanifesting *LRRK2* carriers, major *LRRK2*-specific therapeutic approaches include blocking LRRK2 kinase activity with selective small molecule inhibitors and brain-specific modulation through intrathecal injection of antisense oligonucleotides (Table).[1,2,4,19] Potential concerns have been raised regarding the safety of systemic kinase inhibition because *LRRK2* is not only expressed in the brain but also in immune cells and several other peripheral organs, and kinase inhibitors can vary in selectively.[19,20] Other future considerations include molecules that specifically inhibit variant *LRRK2*, targeting the *LRRK2* Rab-like ROC domain, and more generalized inhibition of reactive oxygen species.[1]

While both the effect size and risk profiles are likely greater for agents that directly affect kinase activity or expression, there are fewer ethical and adherence concerns with lower risk interventions for *LRRK2*-PD primary prevention. A form of vitamin B_{12}, 5′deoxyadenosylcobalamin, has been identified as a novel potential modulator of LRRK2 activity—although it is unclear whether physiologically tenable levels will be achieved.[21] Epidemiologic studies also suggest promise for other strategies such as regular nonsteroidal anti-inflammatory drug (NSAID) usage,[22] higher daily caffeine intake,[23] elevation of antioxidants such as urate,[24] and anti-tumor necrosis factor therapy.[25] Finally, while not specific to *LRRK2*, observational data suggest that certain dietary and lifestyle interventions, such as stress management, vitamin D supplementation, regular high-intensity aerobic exercise, and the Mediterranean diet, may be neuroprotective.[15] Commensurate with the lower effect sizes of lower-risk interventions, such trials would require larger sample sizes, but may be amendable to innovative trial designs such as remote/virtual trials, which can enroll larger cohorts.[26]

GBA1

Building on years of GD research, the *GBA* pathway is well-studied, with potential pharmacologics for intervention and related biomarkers to demonstrate both pharmacoengagement and disease effect. The *GBA1* gene encodes glucocerebrosidase (GCase), a lysosomal enzyme that facilitates the processing of glycosphingolipids.[16] GCase dysfunction leads to the accumulation of the downstream substrates: sphingolipid, glucosylceramide, and glucosylsphingosine.[27] Peripheral replacement of GCase and substrate reduction therapies form the cornerstone of GD treatment, and it has been postulated that glucosylsphingosine accumulation in the brain may also mediate PD.[16] Although the pathophysiology of *GBA*-PD is still being elucidated, at its core is the dysregulation of brain cellular GCase. GCase reduction or dysfunction is postulated to impair lysosomal protein degradation, which has been postulated to lead to toxic accumulation and aggregation of SNCA in a bidirectional feedback loop that promotes further SNCA aggregation. It also reduces mitochondrial function, leads to stress of the endoplasmic reticulum, and activates microglia causing neuroinflammation.

Accordingly, there are a plethora of *GBA*-specific pharmacologic approaches (though none being tested in nonmanifesting carriers). These include, but are not limited to, increasing gene expression, gene editing, activating mutant GCase, chaperoning misfolded proteins, targeting SNCA aggregation, and inhibiting substrate accumulation (Table).[1,2,4]

Potential for *LRRK2*-Specific and *GBA*-Specific Biomarkers

Biomarkers can serve multiple roles including aiding to stratify groups during study enrollment, tracking target and pathway engagement, and determining subgroups in post hoc analysis.[28,29] The elucidation of the *LRRK2*-related and *GBA1*-related PD models has led to the identification of

Table Gene-Specific Prevention Strategies, Emerging Disease-Modifying Therapeutics, and Ongoing Investigations

Gene-specific target/mechanism for prevention	Therapeutic class/agents	Current investigations in NMC/PD	Recruited participants/ targeted population
LRRK2			
Decrease LRRK2 kinase (CNS selective)	Antisense oligonucleotides (intrathecal injection)	BIIB094: NCT03976349(94)	*LRRK2*-PD and iPD
	PROteolysis-Targeted Chimera		
Decrease LRRK2 kinase (systemic)	Small molecule kinase inhibitors AdoCbl	DNL201: NCT03710707	*LRRK2*-PD and iPD
Decrease inflammation	NSAIDs, anti-TNF therapy		
Increase urate	Inosine		
Mixed mechanisms	Exercise[a]	SPARX3: NCT 042884436	*LRRK2*-NMC
GBA			
Increase normal glucocerebrosidase (GCase)[b]	Intrathecal recombinant DNA	PR001 (NCT04127578)	*GBA*-PD
Increase GCase activity/ function	GCase activators	LTI-291 (NL 7061:NTR7299)	*GBA*-PD
	Novel chaperone/GCase activator (i.e., Ambroxol)	Registry (NCT 04388969)	*GBA*-PD and GD patients
		Aim-PD (NCT 029441822)	GBA-PD and iPD
Substrate reduction	Glucosylceramide synthase inhibition	[Terminated: Venglustat GZ/SAR402671]	
SNCA			
Decrease *SNCA* expression	siRNA		
	Epigenetic editing (CRISPR-Cas9)		
Enhance extracellular *SNCA* clearance	Active immunization	PD01A, PD03A (NCT 02267434, NCT02267434)	iPD
	Passive immunization	BIIB204 (NCT 03318523), PRX002/ R07046015 (NCT 03100149)	
	SNCA aggregation inhibition	(NPT200-11 planned NCT02606682)	
	SNCA degradation enhancers		

Abbreviations: AdoCbl = 5′deoxyadenosylcobalamin; BBB = blood-brain barrier; *GBA* = glucocerebrosidase A; GCase = glucocerebrosidase enzyme; iPD = idiopathic PD; *LRRK2* = leucine-rich repeat kinase 2; NSAID = nonsteroidal anti-inflammatory drug; PD = Parkinson disease; *SNCA* = α-synuclein; TNF = tumor necrosis factor.
[a] Not *LRRK2* specific.
[b] Enzyme replacement does not cross BBB.

several promising biomarkers that may facilitate prevention trials.

Fluid and Tissue Biomarkers

LRRK2

Some of the highest *LRRK2* levels can be found in the blood (Gtexportal.org). LRRK2 and phospho-LRRK2 rapidly reduce in the blood in response to LRRK2 kinase inhibitors, providing a strong target engagement opportunity for LRRK2 inhibitors.[29,30] LRRK2 protein and autophosphorylated LRRK2 protein have been found to be upregulated in both *LRRK2* variant carriers and iPD, and urinary autophosphorylated LRRK2 and phospho-Rab decrease with kinase inhibitor treatment in nonhuman primates. Urinary basic metabolic panel levels depend on LRRK2 kinase activity, and isoforms were found to be higher in G2019S carriers with and without PD when compared with nonmutant PD, with the highest levels in disease-manifesting carriers. Finally, LRRK2 protein has been detected in CSF, and autophosphorylated LRRK2 protein may be elevated in CSF. Both total LRRK2 protein and autophosphorylated LRRK2 protein diminish with LRRK2 inhibitor treatment in CSF from nonhuman primates.[30] Thus, between blood, urine, and CSF, a comprehensive vantage of target engagement is possible.

GBA

As with *LRRK2*, there are several promising biochemical outcome measures that demonstrate pharmacodynamic engagement with the GCase-sphingolipid pathway. Of primary interest is

GCase and substrate and product glycosphingolipid levels.[27,31] Measures related to SNCA are also relevant as supported by the inverse correlation between serum GCase activity and SNCA in *GBA*-related PD.[3] CSF GCase and SNCA levels have been used as biomarkers for drug engagement with the GCase/SNCA pathway in the investigation of Ambroxol, a proposed pharmacologic chaperone of variant GCase.[1,2,4] Severe variants exhibit the lowest levels of CSF SNCA, which has been correlated with impaired cognitive function and REM behavior disorder (RBD).[27] While synuclein imaging is still under development, SNCA oligomers in CSF, skin,[32] and other tissues,[28] including a novel method using real-time quaking-induced conversion, demonstrate promise in identifying individuals with early SNCA deposition in *GBA* trials.[19]

Phenotypic Biomarkers

Both *GBA*-related PD and G2019S variant *LRRK2*-PD (referred to subsequently as *LRRK2*-PD) are phenotypically similar to iPD, and individual cases may not be clinically distinguishable.[33,34] However, there are some relevant group differences that may help guide specificity in trial outcome and design.

LRRK2

Unlike RBD cohorts, which may develop to a range of outcomes from PD to DLB to multiple systems atrophy, G2019S variants are predominantly associated with PD.[34,35] While similar to iPD, G2019S-associated PD typically has an earlier AAO, usually 50s–60s, and generally exhibits a less severe phenotype with mildly slower motor progression.[10,34] Early gait involvement is a predominant feature, offering an opportunity for an early biomarker and suggesting gait instability and falls as relevant outcome measures.[3,10] Nonmotor features, especially RBD and cognitive decline, are less frequent in this group.[10,35] Generally, olfactory impairment is less severe, although there are better and worse performing olfactory subgroups, which may guide in the selection of at-risk individuals for post hoc stratification.[36]

While mutation type helps reduce disease heterogeneity, even among G2019S carriers, phenotypic and pathologic diversity remains.[5,6] Most *LRRK2*-PD is associated with nigral degeneration and Lewy body (LB) formation, but up to 30% of cases have isolated nigral degeneration without LBs.[3] This is particularly relevant because the presence of LBs has been correlated with nonmotor symptoms, including cognitive impairment, anxiety, and dysautonomia, whereas their absence with a predominantly motor phenotype.[5] Unfortunately, there are currently limited means of separating this group in vivo without biopsy, but potential biomarker proxies include SNCA, as discussed. While these promising methods are currently limited by substantial interindividual and technical variability,[28] they have the future potential to stratify subtypes of G2019S disease based on pathophysiology. Regardless, if the therapeutic intervention is upstream of the LB pathology and there is a shared mechanism for the onset of disease, then agents for *LRRK2* could work in both pathologic outcomes.

Subtle preclinical findings such as parkinsonian motor signs, gait abnormalities, arm swing asymmetry, and spiral drawing variability have been demonstrated in nonmanifesting *LRRK2* carriers (*LRRK2*-NMC) and may imply a distinct *LRRK2*-PD prodrome that can be used to identify at-risk patients.[37,38] Various imaging modalities in *LRRK2*-NMC suggest viable compensatory network changes and imply early synaptic plasticity possibly amendable to disease modification.[35,39]

GBA

Compared with *LRRK2* and iPD, *GBA*-related PD tends to have a more rapid progression with more prevalent motor and nonmotor features, especially hyposmia, cognitive impairment, hallucinations, depression, RBD, and dysautonomia.[3,16] Severe *GBA* variants are associated with a shorter survival and an earlier, more extreme phenotypic expression of motor and nonmotor features.[17] However, severe phenotypic features can occur with mild variants, typically later in disease course.[40]

Cognitive impairment in *GBA*-related PD demonstrates the greatest deficits in working memory, executive function, and visuospatial processing[16,40] and may be seen before the onset of motor symptoms, thus representing another prospective genotype-specific clinical endpoint. *GBA*-related PD could also represent a cohort for secondary prevention trials because agents to prevent dementia in PD are sorely needed. For such studies, the ability to predict the course of cognitive decline is useful, and mutation status may serve to stratify patients because severe mutations have the most rapid cognitive decline. A hypothetical power estimate for a 3-year trial on a therapy to halt cognitive decline in PD showed a 25-fold reduction of sample size when restricting enrollment to severe *GBA1* variants rather than all-comers with PD.[41]

Although some *GBA1* risk variant carriers develop PD and others DLB, both are disorders of similar SNCA aggregation, akin to iPD, though with even more widespread cortical LB involvement.[3] Thus, an advantage of *GBA1* risk variant cohorts is improved neuropathologic homogeneity. Although the mechanism by which carriers develop different phenotypes on the spectrum of PD to PD-dementia to DLB needs further elucidation, it is assumed that putative agents would treat the entire *GBA* phenotypic spectrum. Because biological sex may affect metabolism, glycosphingolipid expression, and possibly phenotypic expression, trial randomization schema incorporating sex might be considered (Steffany Bennett, PhD, oral communication 5/13/22).

As in *LRRK2*-PD, evidence suggests there may be a distinct prodrome of *GBA*-related PD. Retrospectively, patients with *GBA*-related PD report a shorter and more severe prodromal phase than those with iPD.[35] Studies in nonmanifesting *GBA* carriers (*GBA*-NMC) report early depression, RBD, subtle motor signs, autonomic dysfunction, olfactory loss, and varying degrees of cognitive impairment, primarily in verbal

and executive domains, although some studies indicate that cognitive features may be very mild.[35,40,42,43] It is unclear whether the difference in studies may be attributable to the severity of mutation or other factors.

Additional Challenges and Future Directions for Genetic Cohorts

Despite the tremendous advantages of enrolling *LRRK2* and *GBA1* cohorts into primary prevention trials, there are several interrelated obstacles to overcome. The primary challenge relates to incomplete penetrance, which affects the ability to recruit a sufficient sample size of carriers at a high risk for developing trial outcomes. As discussed, allocation schema that take into account modifiers of phenotypic expression and biomarkers of potential pathologic correlates could help improve loss of power due to pathologic and clinical heterogeneity. Other challenges include genetic and variant heterogeneity. Care must be taken so that carriers are recruited in a manner that allows them the ability to participate without having to know the genetic status. Finally, gene-specific therapies may not be generalizable to iPD or even to all *GBA*-related and *LRRK2*-PD because the etiology of PD results from a complex interplay of several genetic factors, environmental exposures, and advancing age.

Challenge: Genetic Heterogeneity (Additional Genetic and Epigenetic Factors)

Because *LRRK2* and *GBA1* are mild-to-moderately frequent mutations with reduced penetrance, it is a challenge to identify those who will develop PD and/or surrogate outcomes. Known genetic modifiers for earlier AAO or development of PD include the influence of constructed polygenic risk scores (PRSs) for *LRRK2* in or near *CORO1C* and for *GBA1* in *SNCA* and cathepsin B, among others.[9,44] Simulated trial designs for *GBA1* cohorts suggest that genetic variance may affect trial outcomes and confounds therapeutic effects in genetically unmatched trials.[45]

Solution 1: Incorporate Biomarkers Into Trial Design, Using a Multipronged Approach With Genetic Information Available in the Development of Trial and for Post Hoc Analysis

Other yet to be discovered genetic and epigenetic modifiers such as age, sex, ethnicity, and environmental factors are likely at play, affecting disease onset and development.[9,15,44] Parsing these may aid in identifying enriched cohorts of at-risk individuals and understanding and reducing heterogeneity. Therefore, additional DNA samples should be considered in all trials in genetic cohorts regardless of predominant variant[2] Information can be used during study design to limit studies to include those with certain modifiers that predict a greater risk and, thus, oversample risk groups in the recruitment schema. It can also be used to allow balanced allocation schema such that individuals are randomized to study arms based on genetic factors. Known genetic information can also be used post hoc for stratification, and/or as new genetic and epigenetic modifiers emerge, having genetic data will facilitate post hoc analyses. In addition, the application of PRSs, which aggregate the effects of multiple protective and risk genetic factors, has the potential to facilitate enrollment decisions.

Solution 2: Overenrollment of Family Members Could Also Increase the Likelihood of Additional Genetic Modifiers and Improve Enrollment

As anticipated, because most cases with PD do not have a known attributable genetic variant, family members of affected patients with PD could not be included in prevention trials in most populations. However, enrolling carriers of known risk variants who additionally have a family history of PD may help address the challenge of reduced penetrance because a positive family history is a known risk factor for the development of PD and specifically is associated with an increased penetrance of *GBA1* mutations.[8] In addition, positive prodromal PD markers may be more frequent, even in variant family members, as seen with abnormal transcranial sonography in nonaffected family members of patients with *LRRK2*-PD.[46] Thus, a family history of PD may be used to identify the highest risk individuals to be prioritized, and oversampling could increase the likelihood of including additional modifiers of disease. Family members may also be highly motivated to participate in trials both for their own health and for the potential benefit of others because they have personally experienced the effect of disease and may be compelling advocates for research.

Challenge: Mutational Heterogeneity in *GBA1*

While we have discussed that for *LRRK2*, it might be most advantageous to decrease heterogeneity by focusing on the more frequent and moderately penetrant variant, G2019S, the decision is more challenging for *GBA*-related PD. Penetrance and disease risk depend on variant severity with severe variant carriers having a 13- to 15-fold increased risk of PD compared with a 2- to 4-fold risk associated with mild variants and an earlier AAO and greater age-related penetrance in many, but not all, studies.[8,17,27]

Solution: Consider Restricting Studies to Classes or Specific *GBA1* Variants

The tremendous variant heterogeneity in *GBA1* may be reduced by focusing on a specific variant class (severe, mild, and variant) and type. Furthermore, clinical trial endpoints may be selected based on variant class because severe variants are associated with a more rapid disease course and earlier AAO by 5 years.[17]

However, restricting enrollment to severe variant carriers may improve trial efficiency at the cost of decreasing recruitment because there are fewer eligible participants. Similarly, not all agents will work on all *GBA1* variants and the specific variant; not only the class must be considered.[2] Therapies may differ in their efficacy depending on residual enzyme activity and underlying mechanism. GCase activity can be helpful for stratification and generally correlates to variant severity.[3] In addition to class, variant type also affects GCase function differently, with some reducing GCase activity, affecting endoplasmic reticulum quality control or trafficking to the lysosome through Saposin C and LIMP2.[16,27] Therefore, depending on the particular therapeutic and desired outcome, *GBA1* trials will need to account for variant type and class.

Challenge: Identifying Sufficient Carriers Enriched to Be At Risk for Outcome in the Time Frame of a Clinical Trial

While older age is associated with greater penetrance, and thus likelihood to develop outcome, the optimal time in disease course to begin a trial in genetic cohorts is unknown. Elderly *LRRK2* and *GBA* risk variant carriers can still phenoconvert much later than the typical AAO, which may suggest protective genetic modifiers in some carriers.[47] Although biomarkers may serve to improve study design and efficacy, they may not be enough to individually stratify at-risk groups as seen with plasma biochemical markers that delineate GD (i.e., chitotriosidase, glucosylsphingosine, and GCase), which are not indicative of an increased risk of PD in *GBA*-NMC.[31]

While there is evidence to support a possible genotype-specific prodrome, there is a challenge in determining which markers are inherent to trait only (i.e., associated with the gene variant and not necessarily the development of PD) and which are associated with trait and disease state. Furthermore, there is uncertainty regarding proximity to disease onset. Despite tremendous progress, it is still not clear where genetic factors fall on the PD causal model. While gait and arm swing alterations were noted in *LRRK2*-NMC, gait measures did differ between individuals with a higher probability of prodromal PD per the Movement Disorder Society (MDS) Prodromal Research Criteria for PD, suggesting a state effect and supporting the utility of these markers in delineating carriers at the highest risk for phenoconversion.[38] Similarly, *LRRK2*-NMC may have several types of imaging abnormalities, but it is unknown whether these are a manifestation of baseline developmental upregulation and compensation independent of disease state.[35] While data in both LRRK2-NMC and *GBA*-NMC demonstrate increased striatal binding ratios on dopamine active transporter (DaT) scans, predating subtle motor signs,[33] further ongoing study is needed to discern the trajectory of progression of DAT-SPECT imaging abnormalities.

Solution 1: Develop Risk Profiles by Combining Markers

One strategy is combining individual biomarkers with genetic and epigenetic information to develop risk profiles that can be used to stratify risk and determine proximity to phenoconversion. While epigenetic modifiers have not been definitively demonstrated, a family history in a first-degree relative may increase the likelihood of identifying at-risk carriers. In addition, gene-environment interactors discussed as candidate interventions for *LRRK2*, such as urate levels, and NSAID and caffeine use[22-24] might be included in risk profiles. Furthermore, high urate levels and abnormal DaT imaging may have compounding effects in *LRRK2*-NMC,[24] suggesting a higher risk and possibly closer proximity to phenoconversion. In addition, RBD and hyposmia are uncommon among G2019S carriers, but due to their high predictive value for phenoconversion their presence may indicate a higher risk.[19,35]

In addition, the MDS Research Criteria for Prodromal PD could be applied to *LRRK2*-NMC to address the hurdle of incomplete penetrance to exclude carriers from prevention trials who are unlikely to phenoconvert in a certain time window because it demonstrated a high specificity, sensitivity, and negative predictive value for the diagnosis of PD within 5 years in healthy G2019S carriers.[48] However, the criterion does not determine proximity to phenoconversion. One solution to address this challenge is to apply PRS and the MDS Research Criteria for Prodromal PD to perform screening for the highest risk carriers and begin enrollment a few years before the median AAO of *LRRK2*-PD.

Clustering clinical and historical data in *GBA*-NMC with biofluid and imaging profiles has the potential to discern preclinical/prodromal PD. For example, nonmotor symptoms such as olfaction and RBD may cluster with serum SNCA to denote potential patients on the trajectory for decline.[49] RBD, which is common in *GBA*-PD and in itself has a high positive predictive value for the development of disease, also has been associated with cognitive impairment.[35] Therefore, in secondary prevention trials aiming to prevent cognitive decline in *GBA*-PD, RBD may be an important potential eligibility criterion.

Solution 2: Consider Alternative Trial Designs

Adaptive trial designs may also help offset the limitation of a smaller sample size from genetic cohort studies. Trials that allow for inclusion or exclusion based on drug engagement or response might help to improve efficiency. Basket trials may reduce sample sizes by reusing controls. Platform trials that permit the testing of multiple therapies simultaneously may be advantageous because successful neuroprotection may require simultaneous application of multiple agents acting

on different targets. Platform trials have demonstrated success in cystic fibrosis when testing was limited to genetic cohorts and have been implemented by the DIAN-TU Next Generation Alzheimer's disease prevention study (ClinicalTrials.gov NCT01760005). There are additional ethical concerns that arise with requirement for knowledge of genetic status from NMCs, and designs to allow participants to participate without requiring knowledge of their own genetic status would be ideal. Modeling after the Trial of Parkinson's And Zoledronic acid, piloting virtual, home-based trial designs is another innovative approach of attaining sufficient sample size for prevention trials and has the additional advantages of convenience, efficiency, and cost-effectiveness.[26] Remote trials might be feasible to demonstrate even small effects for lower-risk interventions (i.e., exercise, caffeine, and NSAIDs) when applied to a very large cohort.

Conclusion

In sum, genetic cohorts provide a tremendous future for PD prevention trials through improving precision, decreasing disease heterogeneity, and providing promising drug targets and associated biomarkers. Our greatest challenge comes in increasing sample and effect size by identifying larger cohorts and defining more deeply enriched clusters at risk for the proximal development of a modifiable outcome while offering safe and ethical trials. Additional biomarkers, including genetic ones, may improve randomization schema, and post hoc stratification and analysis are needed. While we have emphasized shared deleterious factors as an increasing risk, there are also questions regarding whether genetic factors might be protective. For example, care should be taken in the enrollment of individuals with both *GBA1* and *LRRK2* mutations[50] because it will be important for therapies not to disrupt compensatory plasticity. Applying alternative trial designs and defining risk profiles to enroll more deeply enriched at-risk groups may help leverage the many advantages of genetic cohorts to prevent the next wave of PD. Although current options for implementing some of the strategies we outlined in this article are limited, there is a rapidly growing interest in PD prevention, and clinical capabilities are quickly evolving so much so that such approaches are more foreseeable now than ever before. A continued prospective study interrogating mechanism, biomarkers, and clinical course is warranted, and research participant engagement is fundamental to trial success.

Study Funding

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Rachel Saunders-Pullman, MD, MPH	Department of Neurology, Mount Sinai Beth Israel Medical Center; Department of Neurology, Icahn School of Medicine at Mount Sinai, New York	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and study concept or design

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Who to Enroll in Parkinson Disease Prevention Trials?: The Case for Genetically At-Risk Cohorts

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