Appendix B: Biological Systems and Gene Function

1 Neurotransmitter Pathway Gene Functions

1.1 Glutamatergic System Genes

GRIN2A (Glutamate Ionotropic Receptor NMDA Type Subunit 2A)

- **Function**: NMDA receptor subunit; critical for synaptic plasticity and learning
- **Enrichments**: ADHD=1546.0, Autism=1195.7, Shared=1359.6
- **Literature**: Associated with intellectual disability, epilepsy, autism
- **Mechanism**: Loss-of-function mutations impair NMDA-mediated excitatory neurotransmission

GRM5 (Glutamate Metabotropic Receptor 5)

- **Function**: G-protein coupled mGluR5 receptor; modulates synaptic transmission
- **Enrichments**: ADHD=743.8, Autism=1759.9, Shared=1144.2
- **Literature**: mGluR theory of autism; clinical trials of mGluR5 antagonists
- **Mechanism**: Excessive mGluR5 signaling → exaggerated protein synthesis

GRIA1 (Glutamate Ionotropic Receptor AMPA Type Subunit 1)

- **Function**: AMPA receptor subunit; mediates fast excitatory transmission
- **Enrichments**: ADHD=773.2, Autism=1154.4, Shared=944.7
- **Literature**: Implicated in intellectual disability and autism

- **Mechanism**: Altered AMPA receptor trafficking affects synaptic strength

GRIN2B (Glutamate Ionotropic Receptor NMDA Type Subunit 2B)

- **Function**: NMDA receptor subunit; critical for early brain development
- **Enrichments**: ADHD=374.9, Autism=883.9, Shared=575.7
- **Literature**: De novo mutations in autism and schizophrenia
- **Mechanism**: Developmentally regulated; early expression critical for circuit formation

1.2 GABAergic System Genes

GABRB2 (GABA A Receptor Subunit Beta 2)

- **Function**: GABAA receptor subunit; mediates inhibitory neurotransmission
- **Enrichments**: ADHD=587.0, Autism=1017.5, Shared=772.9
- **Novel finding**: Higher cross-disorder signal than GABRB3 (147 vs. 2 significant SNPs)
- **Literature**: Limited prior focus compared to GABRB3
- **Mechanism**: GABAA receptor composition affects inhibitory tone

GABRB3 (GABA A Receptor Subunit Beta 3)

- **Function**: GABAA receptor subunit; part of 15q11-q13 autism locus
- **Enrichments**: ADHD=407.7, Autism=761.9, Shared=557.4
- **Literature**: Extensively studied; maternal duplications in autism
- **Mechanism**: Reduced GABAA signaling → decreased inhibition

GABRB1 (GABA A Receptor Subunit Beta 1)

- **Function**: GABAA receptor subunit; widespread CNS expression
- **Enrichments**: ADHD=694.4, Autism=466.8, Shared=569.4
- **Literature**: Less studied than GABRB2/3 in neurodevelopmental disorders
- **Mechanism**: Contributes to inhibitory receptor diversity

1.3 Serotonergic System Genes

TPH2 (Tryptophan Hydroxylase 2)

- **Function**: Rate-limiting enzyme for brain serotonin synthesis
- **Enrichments**: ADHD=192.4, Autism=239.2, Shared=214.5
- **Literature**: Associated with aggression, autism, mood disorders
- **Mechanism**: Reduced TPH2 function → decreased serotonin synthesis
- **Trans-diagnostic**: Significant across all 11 cross-disorder comparisons

1.4 Dopaminergic System Genes

COMT (Catechol-O-Methyltransferase)

- **Function**: Degrades catecholamines (dopamine, norepinephrine)
- **Enrichments**: ADHD=156.1, Autism=194.5, Shared=174.3
- **Literature**: Val158Met polymorphism affects dopamine clearance
- **Mechanism**: Lower COMT activity → increased prefrontal dopamine

DDC (DOPA Decarboxylase)

- **Function**: Converts L-DOPA to dopamine
- **Enrichments**: ADHD=177.0, Autism=382.8, Shared=260.3
- **Mechanism**: Critical for dopamine biosynthesis

DRD2 (Dopamine Receptor D2)

- **Function**: D2-class dopamine receptor; target of antipsychotics
- **Enrichments**: ADHD=152.9, Autism=99.9, Shared=123.6
- **Literature**: Implicated in ADHD; stimulant medications increase dopamine
- **Mechanism**: Reduced D2 signaling associated with ADHD symptoms

DRD5 (Dopamine Receptor D5)

- **Function**: D1-class dopamine receptor; high affinity for dopamine

- **Enrichments**: ADHD=200.4, Autism=267.6, Shared=231.5
- **Literature**: Polymorphisms associated with ADHD
- **Mechanism**: Modulates prefrontal cortex function

2 Pathway-Level Biology

2.1 Excitatory/Inhibitory Balance

E/I Ratio Hypothesis (Rubenstein & Merzenich 2003):

$$\text{E/I ratio} = \frac{\text{Glutamatergic excitation}}{\text{GABAergic inhibition}} \tag{1}$$

Predictions:

- Increased E/I $\rightarrow$ hyperexcitability, seizures, sensory hypersensitivity
- Decreased E/I $\rightarrow$ reduced plasticity, cognitive impairment

Our findings support:

- Glutamatergic pattern (highest enrichment) $\rightarrow$ excessive excitation
- GABAergic pattern (high enrichment) $\rightarrow$ reduced inhibition
- Combined effect: Elevated E/I ratio in AuDHD

Evidence from our data:

- Glutamatergic mean enrichment (1006) > GABAergic (633) in absolute terms
- Both patterns show >97% cross-disorder replication
- Suggests E/I imbalance is core shared feature

2.2 Monoamine Modulation

Dopamine: Attention, motivation, reward processing

- Primary ADHD neurotransmitter
- Dopaminergic pattern shows only 59% replication $\rightarrow$ ADHD-specific

- Lower shared enrichment (197) than glutamate/GABA patterns

Serotonin: Mood, aggression, social behavior

- TPH2 pattern shows 100% replication across disorders
- Moderate enrichment (215)
- Trans-diagnostic role (aggression, mood dysregulation)

2.3 Pathway Interactions

Neurotransmitter systems do not operate in isolation:

Glutamate-GABA reciprocity:

- Excitatory-inhibitory neurons form local circuits
- Glutamate can drive feedforward inhibition via GABAergic interneurons
- Disruption of either system affects E/I balance

Monoamine modulation of E/I:

- Dopamine modulates prefrontal glutamate release
- Serotonin regulates GABAergic interneuron activity
- Complex interactions make prediction difficult

3 Developmental Trajectories

3.1 Temporal Dynamics

Gene expression and function change across development:

Early development (prenatal-early postnatal):

- GRIN2B expression peaks early; critical for synapse formation
- GABAergic genes establish inhibitory circuits
- Disruption → permanent circuit alterations

Childhood:

- GRIN2A expression increases; replaces GRIN2B in mature synapses
- Dopaminergic system matures; relevant for ADHD symptom emergence
- Critical period for intervention

Adolescence:

- Prefrontal cortex maturation
- Serotonergic system changes (mood, aggression regulation)
- ADHD symptoms may persist or remit

3.2 Critical Periods

Hypothesis: Genetic variants in glutamatergic/GABAergic genes exert maximal effects during critical periods when:

1. Gene expression is highest
2. Circuits are being established (high plasticity)
3. Compensatory mechanisms not yet developed

Implication: Timing of intervention may matter more for glutamate/GABA patterns than dopamine patterns.

4 Clinical Translation Framework

4.1 Why These Findings Do NOT Translate to Clinic (Yet)

4.1.1 Gene Enrichment $\neq$ Treatment Target

Common misconception: "High genetic signal $\rightarrow$ good drug target"

Reality: Genetic enrichment indicates:

- Common variants near gene associated with disorder risk
- Effect sizes typically small (OR $\approx$ 1.05-1.2)
- Does NOT indicate:
  - Direction of effect (gain vs. loss of function)

  - Druggability of target
  - Therapeutic window
  - Off-target effects

4.1.2 No Patient Stratification

Critical limitation: Gene patterns describe *genes*, not *patients*.

Cannot answer:

- Which patients have glutamatergic vs. dopaminergic etiology?
- How to stratify for treatment selection?
- What biomarkers predict response?

Would require:

- Individual-level genetic profiling
- Validation in independent cohorts
- Clinical trial data
- FDA approval process

4.1.3 Direction of Effect Unknown

Problem: GWAS identifies association, not mechanism.

Example - GRIN2A:

- High genetic enrichment
- Could indicate:
  - Too much NMDA receptor activity → need antagonist
  - Too little NMDA receptor activity → need agonist
  - Altered trafficking/localization → need modulator

Resolution: Functional studies, expression data, animal models required.

4.1.4 Clinical Trial Failures

Cautionary example - mGluR5:

- GRM5 shows highest enrichment (1144.2)
- Strong biological rationale (mGluR theory of autism)
- Clinical trial of mavoglurant (mGluR5 antagonist) $\rightarrow$ **FAILED**
- Negative results in fragile X syndrome and autism

Lesson: Genetic signal $\neq$ successful therapeutic intervention.

4.2 What IS Scientifically Justified

4.2.1 Hypothesis Generation

Valid use: Prioritize genes for basic research.

Examples:

- Investigate GABRB2 (understudied relative to signal)
- Study GRIN2A mutations in patient-derived neurons
- Test GRM5 modulators in animal models

4.2.2 Biological Insights

Valid use: Understand shared vs. disorder-specific mechanisms.

Examples:

- Glutamate/GABA shared AuDHD biology
- Dopamine ADHD-specific biology
- TPH2 trans-diagnostic aggression/mood

4.2.3 Research Stratification

Valid use: Design studies testing pathway-specific hypotheses.

Examples:

- MRI studies: glutamate spectroscopy in AuDHD vs. controls
- Animal models: GRIN2A knockouts + GABRB2 variants
- Drug repurposing: test GABAergic modulators in mouse models

5 Evolutionary Perspective

5.1 Why Are Neurodevelopmental Risk Variants Common?

Paradox: If ADHD/autism reduce fitness, why do risk variants persist at high frequency?

Hypotheses:

1. Balancing selection:

- Heterozygotes have advantage
- Example: Glutamate receptor variants may balance excitability vs. stability

2. Ancestral neutrality:

- Variants were neutral in ancestral environments
- Modern environments (education, social demands) reveal costs

3. Pleiotropic benefits:

- Variants increase risk but also confer advantages
- Example: Dopamine variants may enhance novelty-seeking (adaptive in some contexts)

4. Mutation-selection balance:

- Continuous generation of new variants
- Selection not strong enough to eliminate

5.2 Cross-Species Conservation

Gene conservation scores:

- GRIN2A, GRM5, GABRB genes: Highly conserved across vertebrates
- Dopamine receptor genes: Less conserved; mammalian-specific isoforms

Interpretation:

- Glutamate/GABA systems: Ancient, fundamental to nervous system
- Dopamine system: More recent elaboration; species-specific variations

6 Multi-Omic Integration

6.1 What Additional Data Would Help

1. **Gene expression**:

- Brain region-specific expression (GTEx, Allen Brain Atlas)
- Developmental time courses (BrainSpan)
- Single-cell RNA-seq (cell type specificity)

2. **Protein-protein interactions**:

- Physical interactions (BioGRID, STRING)
- Functional pathways (KEGG, Reactome)
- Post-synaptic density complexes

3. **Chromatin accessibility**:

- ATAC-seq, ChIP-seq data
- Identify regulatory variants
- Tissue/cell-type specificity

4. **Clinical phenotypes**:

- Symptom dimension scores
- Comorbidity patterns
- Treatment response data

5. **Imaging genetics**:

- Structural MRI (cortical thickness, volumes)
- Functional MRI (connectivity, activation)
- MR spectroscopy (glutamate, GABA levels)

6.2 Integration Approaches

Network-based methods:

- Build gene networks from multi-omic data
- Identify modules enriched for ADHD/autism risk
- Test if glutamatergic/GABAergic genes form coherent modules

Machine learning:

- Train classifiers on genetic + clinical data
- Test if gene patterns predict:
  - ADHD vs. autism vs. comorbid
  - Treatment response
  - Developmental trajectories

Mendelian randomization:

- Use genetic variants as instruments
- Test causal effects:
  - Does GRIN2A expression causally affect ADHD risk?
  - Mediation by intermediate phenotypes (e.g., cortical excitability)?

7 Ethical Considerations

7.1 Genetic Testing for ADHD/Autism

Current state: NOT clinically useful.

Why not:

- Polygenic architecture (thousands of variants, small effects)
- Predictive value low (area under ROC curve < 0.6)
- No actionable information for treatment

Future scenario: If genetic stratification becomes validated:

- **Benefits**: Personalized treatment selection
- **Risks**: Stigma, discrimination, deterministic thinking

7.2 Neurodiversity vs. Medical Model

Tension: Biological research vs. neurodiversity movement.

Neurodiversity perspective:

- ADHD/autism are differences, not deficits
- Society should accommodate, not "fix"
- Genetic research may pathologize natural variation

Medical model perspective:

- ADHD/autism cause significant impairment
- Individuals deserve treatment options
- Understanding biology enables better interventions

Integration:

- Research aims to reduce suffering, not eliminate diversity
- Treatments should be optional, not coercive
- Genetic insights can inform both medical and social interventions

7.3 Responsible Communication

Avoid:

- Oversimplifying: "ADHD is a dopamine disorder"
- Determinism: "Your genes determine your fate"
- Premature clinical claims: "Genetic test predicts treatment response"

Emphasize:

- Complexity: Thousands of genes, environment matters
- Probabilistic: Genes influence risk, not destiny
- Limitations: Current findings are exploratory, require replication

8 Limitations of Biological Interpretation

8.1 Inferential Gaps

Gap 1: GWAS signal → Causal gene

Problem: LD structure means multiple genes in same region.

Example: GABRB3 locus includes multiple genes; attributing signal to GABRB3 alone may be incorrect.

8.2 Ascertainment Bias

Gap 2: Well-studied genes → More literature

Problem: Publication bias favors known genes.

Example: GABRB2 vs. GABRB3 disparity may partially reflect historical research focus, not just biological importance.

8.3 Population Specificity

Gap 3: European ancestry GWAS → Universal biology

Problem: Genetic architecture may differ across populations.

Evidence: Some GWAS hits fail to replicate in non-European cohorts.

Implication: Findings may not generalize globally.

8.4 Simplification of Biology

Gap 4: Gene list → Biological pathway

Problem: Pathways are interconnected; clean categories are artificial.

Reality: Genes interact in complex networks transcending simple pathway boundaries.

9 Summary of Biological Insights

9.1 Core Findings

1. **Glutamatergic system**: Highest shared genetic enrichment; E/I imbalance hypothesis

2. **GABAergic system**: High shared enrichment; inhibitory dysfunction

3. **Serotonergic system**: Trans-diagnostic role (TPH2); aggression/mood

4. **Dopaminergic system**: ADHD-specific; lower shared enrichment
5. **Polygenetic background**: Mixed genes; likely general psychiatric risk

9.2 Novel Observations Requiring Follow-Up

1. **GABRB2 prominence**: Stronger signal than GABRB3 despite less prior literature
2. **E/I imbalance**: Quantitative relationship between glutamate and GABA enrichments
3. **Dopaminergic specificity**: Pattern suggests ADHD-specific vs. shared mechanisms
4. **TPH2 consistency**: Trans-diagnostic effects across all disorder comparisons

9.3 What This Does NOT Tell Us

- Patient heterogeneity or subtypes
- Treatment response predictors
- Causal mechanisms (vs. correlations)
- Clinical actionability
- Interaction with environment

9.4 Next Steps

1. **Replication**: Independent cohorts, diverse ancestries
2. **Functional validation**: Cell models, animal studies
3. **Clinical associations**: Genotype-phenotype correlations
4. **Multi-omic integration**: Expression, protein, imaging data
5. **Mechanistic studies**: How do variants affect protein function?