

### 3. Network Medicine Analysis

#### 3.1 Disease Module Identification for Alzheimer's Disease (AD)

To localize the AD disease module within the human interactome, 101 AD-associated genes (CUI: **C0002395**) were extracted from *disease\_gene.tsv*. Of these, **91 genes** were successfully mapped onto the protein–protein interaction (PPI) network. The disease module was defined as the **Largest Connected Component (LCC)** of the subgraph induced by these genes.

**Final module characteristics:**

- **46 genes (nodes)**
- **132 raw interactions → 87 cleaned interactions** (after removal of 45 self-loops)

The Gephi visualization shows a cohesive topological structure with central hubs such as **APP, MAPT, and PSEN1**, forming a dense core surrounded by peripheral genes organized in short-path communities. These hubs align with well-established biological mechanisms underlying AD.

**Module significance.**

To evaluate statistical significance, the AD module was compared with **200 random modules** of equal size (46 genes). Random LCC sizes followed a distribution with **mean = 2.21** and **SD = 1.28**. The AD module's LCC size of **46** lies far beyond this range, producing a **z-score ≈ 34.19** and **empirical p-value = 0**, indicating an **extremely significant and non-random** molecular structure.

#### 3.2 Disease Separation Analysis

Molecular relationships between AD and two additional diseases were examined:

- **Presenile Dementia** (CUI: C0011265), expected to be biologically close
- **Liver Fibrosis** (CUI: C0239946), representative of a distant disease

**Gene-level overlap:**

- AD vs Presenile Dementia: **89 genes shared**
- AD vs Liver Fibrosis: **4 genes shared**

Using the separation metric proposed by Menche et al. (2015):

- **s(AD, Presenile Dementia) = 0.0** → complete overlap in interactome space
- **s(AD, Liver Fibrosis) = 0.0709** → small but positive separation

The overlap network highlights a dense common region between AD and Presenile Dementia, confirming that both diseases occupy an almost identical molecular neighborhood. Conversely, Liver Fibrosis genes appear sparse and peripherally located, reflecting their involvement in distinct biological pathways.

**Implication:** Presenile Dementia and AD share substantial mechanistic foundations, whereas Liver Fibrosis is molecularly distant and unlikely to display shared pathogenic processes.

#### 3.3 Disease–Drug Proximity and Repurposing Opportunities

Drug targets from *drug\_target.csv* were mapped to the interactome and compared with the AD module using the network proximity metric, defined as the average shortest-path distance between drug targets and AD genes. Drugs already indicated for Alzheimer's or dementia were excluded to produce a conservative repurposing set.

**Interpretation of proximity:**

- **Proximity = 0.0:** drug directly targets genes inside the disease module
- **Small positive proximity:** drug targets neighbors of the module → potential mechanistic relevance
- **Larger proximities:** targets progressively more distant from AD biology

**Top mechanistic repurposing candidates (proximity = 0):**

Drugs acting on module genes including **ENO1, GAPDHS, TPI1, ESR1, INS, CALM1, TF, CYP2D6, BCHE, and PPARG**. These genes are directly implicated in metabolic regulation, neurotransmission, hormonal signaling, and neuronal homeostasis—processes strongly associated with AD pathophysiology.

The proximity distribution exhibits three characteristic clusters:

1. **A small group at 0.0**, representing drugs that target AD genes directly
2. **A dominant peak near ≈1**, corresponding to first-neighbor interactions
3. **A broader distribution around ≈2**, reflecting increasingly distant network regions

**Conclusion:** Drugs with **proximity = 0 or very close to 0** represent the most compelling candidates for repositioning, as their targets lie within or immediately adjacent to the mechanistic core of AD.