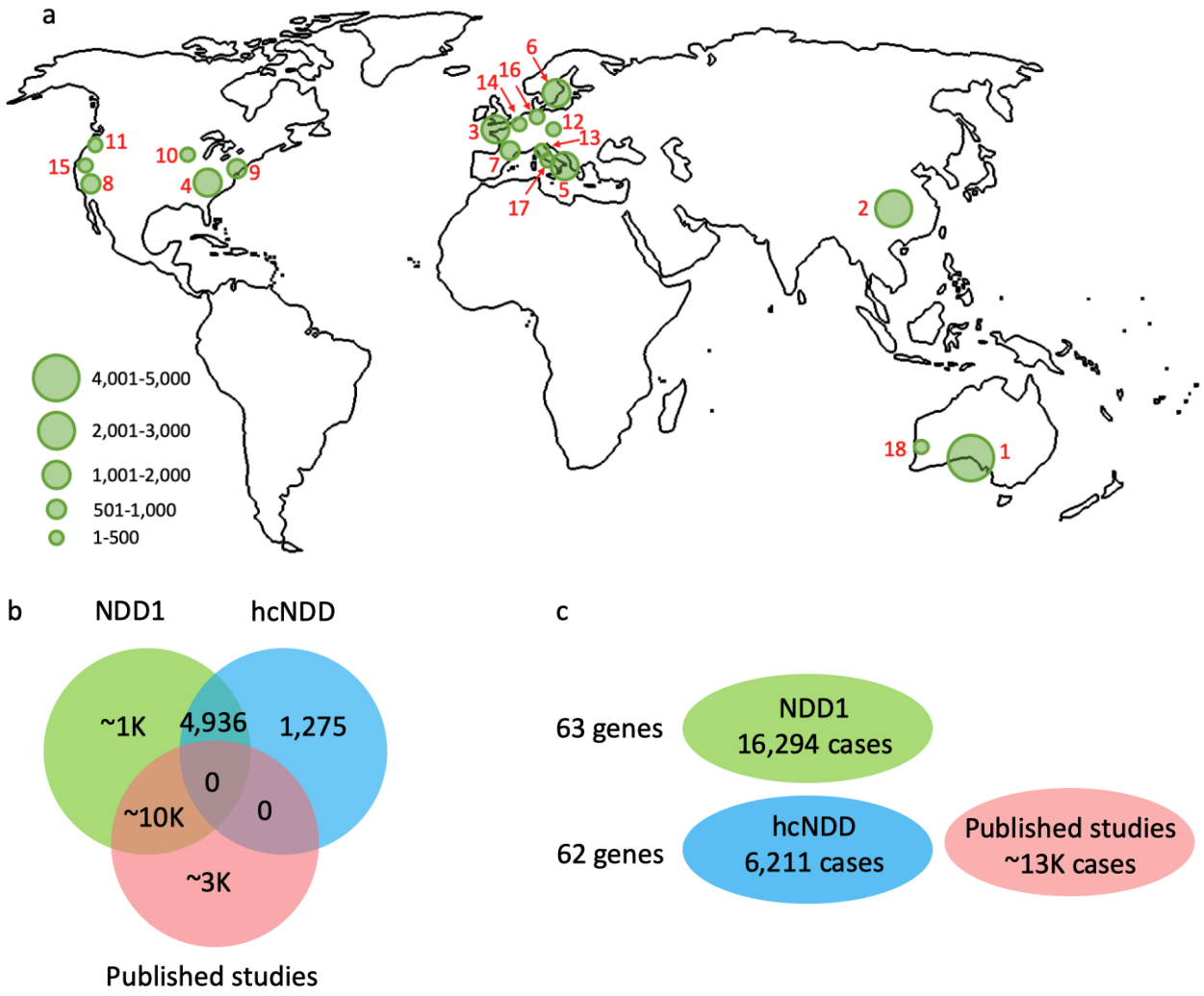
# Large-scale targeted sequencing identifies risk genes for neurodevelopmental disorders

**Wang et al.**

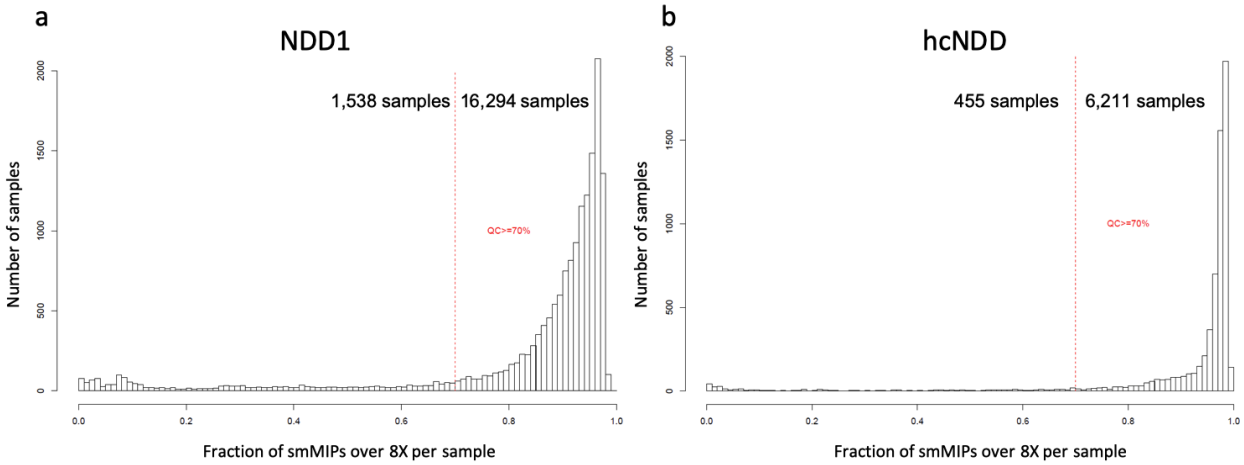
## Supplementary Information

Supplementary Information includes Supplementary Figures 1-4, Supplementary Table 1, and Supplementary Data 1-18.

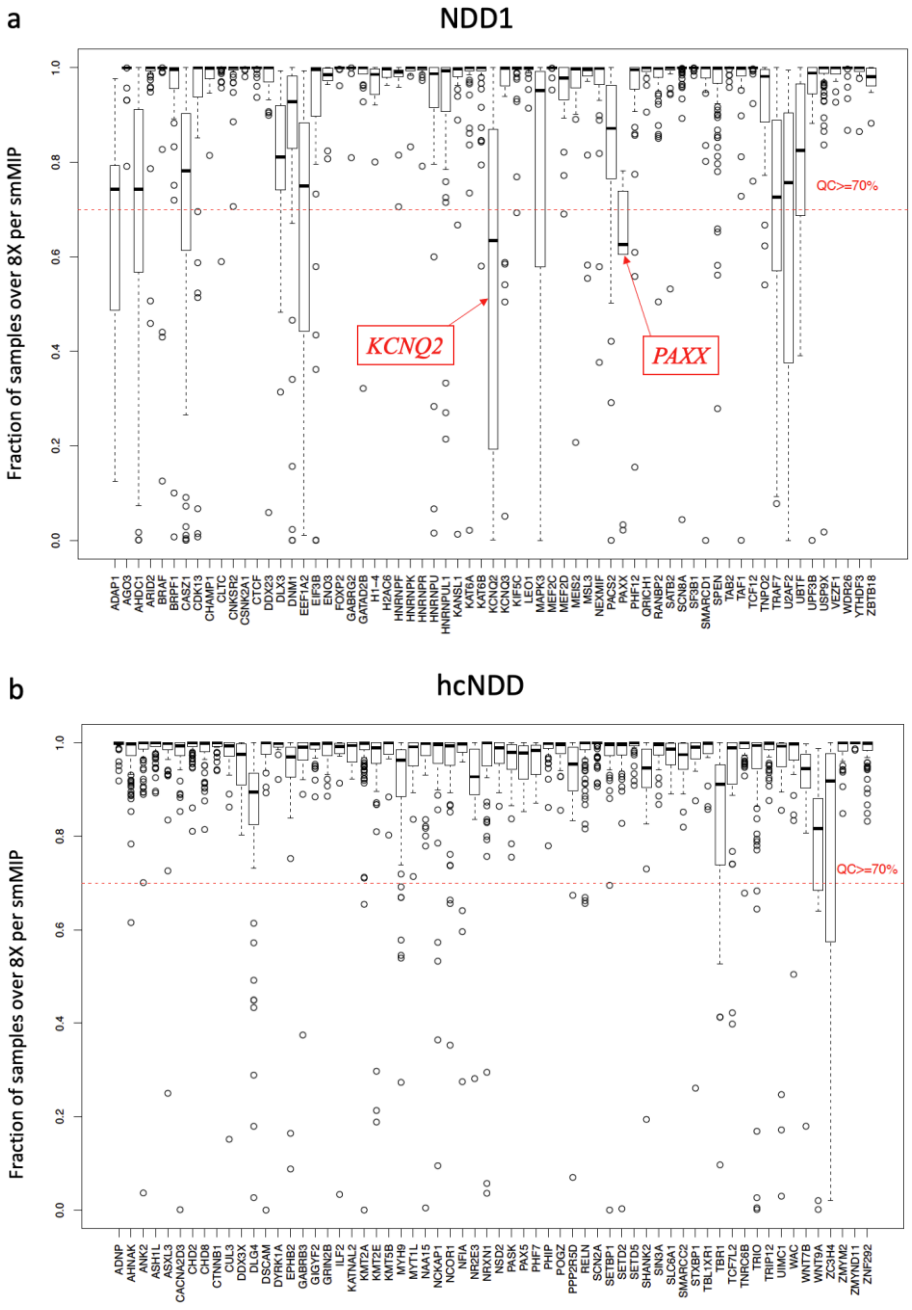
**Supplementary Figures**



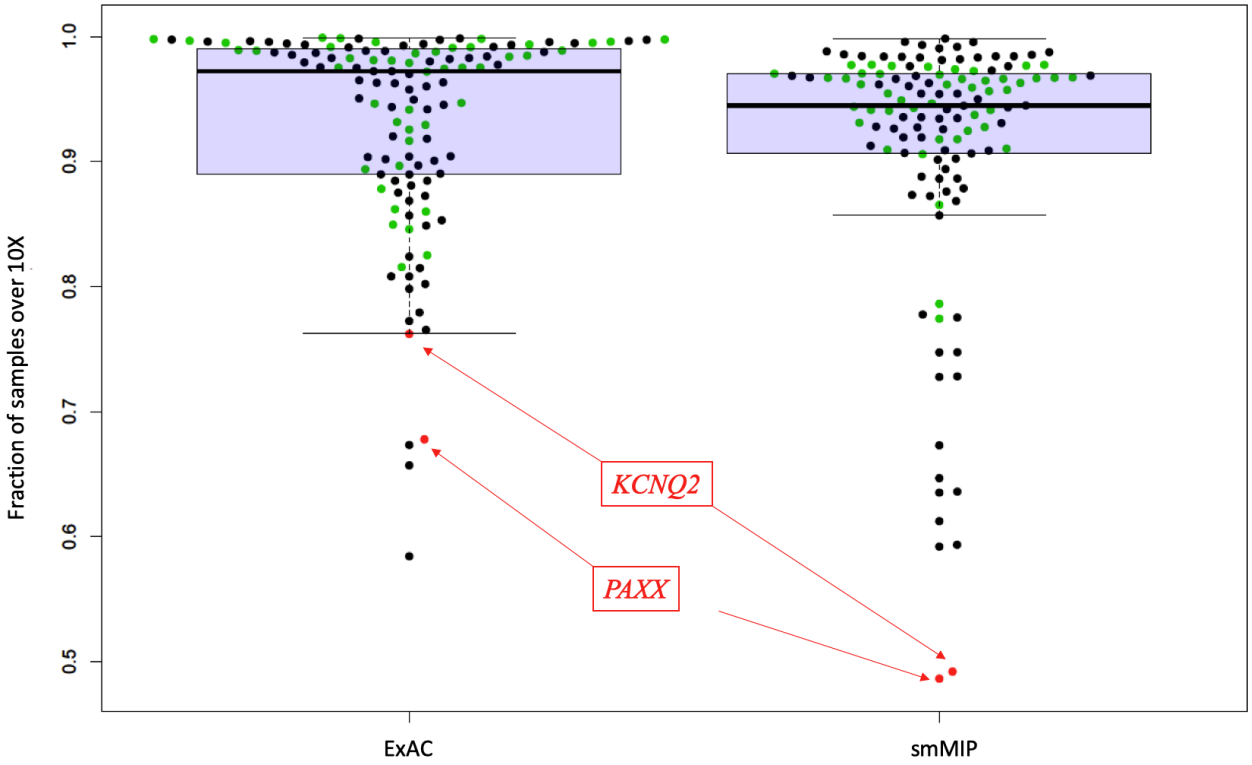

**Supplementary Figure 1. Samples and genes sequenced in this study.** All samples sequenced in this study using smMIPs are from the ASID network. a) Probands (n > 18K) with a primary diagnosis of ASD, DD, or ID were collected from 18 international cohorts. Circle size corresponds to the number of samples from each cohort; red numbers correspond to the cohort number in Supplementary Table 1. b) The numbers (after QC) differ slightly depending on the number of genes and therefore we indicate with an approximation sign (~). Sample overlap is indicated for three designs: NDD1 (63 genes) represents a design targeting 63 genes that were not yet established as high confidence; hcNDD (62 genes) represents a design targeting genes many of which were already known; the third portion of the Venn represents previous published smMIP studies, where variants from 62 genes in hcNDD were retrieved for a combined analysis. c) The 63 genes in panel NDD1 were screened in largest number of 16,294 NDD patients, while the 62 genes in hcNDD were screened only in 6,211 NDD cases where they had not been screened before, and the same category of variants were retrieved from ~13K NDD cases (precise number of cases may different for each gene) for the same 62 genes in hcNDD.




**Supplementary Figure 2. QC of samples in NDD1 and hcNDD.** The histogram shows the fraction of smMIPs with a read depth of over 8X per individual sample. There are 1,538 samples in NDD1 (a) and 455 samples in hcNDD (b). Less than 70% of smMIPs with a read depth over 8X failed QC and were removed from downstream analyses.




**Supplementary Figure 3. QC of genes in NDD1 and hcNDD.** Box and whisker plots show the fraction of samples with target bases at 8X or greater coverage for genes in NDD1 with 65 genes sequenced in 16,294 samples (after QC) (a) and hcNDD with 62 genes sequenced in 6,211 samples (after QC) (b). Two genes (*KCNQ2* and *PAXX*) in NDD1 failed QC and were removed from downstream analyses. For the box plots, the lower whisker indicates the lowest data point excluding outliers (minima), the upper whisker indicates the largest data point excluding outliers (maxima), the lower bound indicates the first quartile which is the median of the lower half of the dataset (25$^{th}$ percentile), the upper bound indicates the third quartile which is the median of the upper half of the dataset (75$^{th}$ percentile), and with the middle value of the dataset (50$^{th}$ percentile) indicates in the middle.




**Supplementary Figure 4. Fraction of samples over 10X in ExAC and smMIP data.** The fraction of samples with ≥10X read depth for ExAC (now available at https://gnomad.broadinstitute.org/) was retrieved for the same capture region as in smMIP sequencing. The average fraction was calculated per gene and plotted as the average fraction (by gene) of samples with ≥10X coverage in ExAC and smMIP data. Each dot represents a gene: green dots indicate the 48 genes at FDR significance in mutation burden analysis, and red dots indicate the two genes (*KCNQ2* and *PAXX*) that failed QC and were excluded from downstream analyses. For the box plots, the lower whisker indicates the lowest data point excluding outliers (minima), the upper whisker indicates the largest data point excluding outliers (maxima), the lower bound indicates the first quartile which is the median of the lower half of the dataset (25$^{th}$ percentile), the upper bound indicates the third quartile which is the median of the upper half of the dataset (75$^{th}$ percentile), and with the middle value of the dataset (50$^{th}$ percentile) indicates in the middle.

**Supplementary Table 1. ASID cohorts and smMIP panels sequenced in this study.**

| Cohort# on Figure 1a | Cohort | Location | PI | Cohort_ID | Primary diagnosis | NDD1 | | | hcNDD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | All | QC failed | Post QC | All | QC failed | Post QC |
| 1 | Adelaide | Adelaide, Australia | Jozef Gecz | Adelaide1, Adelaide2 | DD | 2,206 | 161 | 2,045 | - | - | - |
| | | | | Adelaide3 | DD | 1,440 | 74 | 1,366 | 1,440 | 105 | 1,335 |
| | | | | Adelaide4 | DD | 839 | 41 | 798 | 839 | 47 | 792 |
| 2 | ACGC | Changsha, China | Kun Xia | ACGC | ASD | 2,829 | 317 | 2,512 | - | - | - |
| 3 | Leuven | Leuven, Belgium | Hilde Peeters | Leuven1 | ASD | 904 | 24 | 880 | 864 | 11 | 853 |
| | | | | Leuven2 | ASD | 988 | 7 | 981 | 988 | 56 | 932 |
| 4 | AGRE | Seattle, USA | Raphael A Bernier | AGRE | ASD | 1,662 | 52 | 1,610 | - | - | - |
| 5 | Troina | Troina, Italy | Corrado Romano | Troina1, Troina2 | DD | 1,175 | 153 | 1,022 | - | - | - |
| | | | | Troina3, Troina4 | DD | 441 | 42 | 399 | 441 | 49 | 392 |
| 6 | Karolinska | Karolinska, Sweden | Magnus Nordenskjöld | Swedish | DD | 1,499 | 142 | 1,357 | - | - | - |
| 7 | Antwerp | Antwerp, Belgium | R Frank Kooy | Antwerp | DD | 900 | 38 | 862 | - | - | - |
| 8 | San Diego | San Diego, USA | Eric Courchesne | SanDiego1 | ASD | 488 | 6 | 482 | - | - | - |
| | | | | SanDiego2 | ASD | 404 | 0 | 404 | 404 | 0 | 404 |
| 9 | TASC | Seattle, USA | Raphael A Bernier | TASC | ASD | 737 | 203 | 534 | - | - | - |
| 10 | Iowa | Iowa, USA | Jacob J Michaelson | Iowa | ASD | 472 | 4 | 468 | 472 | 76 | 396 |
| 11 | SAGE | Seattle, USA | Raphael A Bernier | SAGE | ASD | - | - | - | 388 | 27 | 361 |
| 12 | Charles | Prague, Czech Republic | Zdenek Sedlacek | Czech | ID | 384 | 93 | 291 | 384 | 55 | 329 |
| 13 | ITAN | Verona, Italy | Elisabetta Trabetti | ITAN | ASD | - | - | - | 248 | 26 | 222 |
| 14 | Leiden | Leiden, Netherlands | Gijs W.E. Santen | Leiden | DD | 210 | 10 | 200 | - | - | - |
| 15 | Autism Phenome Project | Davis, USA | David G Amaral | APP | ASD | 198 | 132 | 66 | - | - | - |
| 16 | Radboudumc | Nijmegen, Netherlands | Nanda Rommelse | Radboud | ASD | - | - | - | 112 | 0 | 112 |
| 17 | Naples | Naples, Italy | Nicola Brunetti-Pierri | Naples | ASD | - | - | - | 86 | 3 | 83 |
| 18 | Melbourne | Melbourne, Australia | Ingrid E Scheffer | Melbourne2 | ASD | 56 | 39 | 17 | - | - | - |
| | | | | | **Total** | 17,832 | 1,538 | 16,294 | 6,666 | 455 | 6,211 |

**Supplementary References**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