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# Connection between Genetic and Clinical Data in Bipolar Disorder

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## Abstract

Complex diseases may be associated with combinations of changes in DNA, where the single change has little impact alone. In a previous study of patients with bipolar disorder and controls combinations of SNP genotypes were analyzed, and four large clusters of combinations were found to be significantly associated with bipolar disorder. It has now been found that these clusters may be connected to clinical data.

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## Introduction

Modern analytical methods, particularly in the field of molecular genetics, produce large amounts of data that pose a challenge to statistical and data-mining methods for extracting useful information [1,2]. Thus, in polygenic diseases finding disease related genetic changes, among the vast number of changes (e.g., millions of SNPs), is a daunting task. In a recent study [3], 803 SNPs in samples from 607 bipolar patients and 1355 control subjects were analyzed. All the SNPs were from genes selected based on theoretical and experimental studies that suggested that signal transduction and particular ion channels were involved in bipolar disorder [4–6]. The number of combinations of 3 SNP genotypes was counted in the material [3]. The theoretical number of combinations of 3 SNP genotypes taken from 803 SNPs is 2,321,319,627 $(803!/3!(803-3)!\times 3^{3)}$, and as many as 1,985,613,130 combinations were found in the participants. 1,719,002,329 combinations were common between controls and patients, 208,699,590 combinations were found in controls only, and 57,911,211 combinations were found in patients only, of these 45,285,770 occurred only once, and not more than 1181 combinations were shared by 9 or more patients. None of the 803 single SNPs or the nearly two billion of SNP genotype combinations showed a statistically significant association with bipolar disorder. However, among the 1181 combinations shared by 9 or more patients and no controls, four clusters of combinations, were identified that were significantly associated with bipolar disorder. Within a cluster, each patient had a personal pattern of SNP genotypes that was somewhat similar to the patterns of other patients in the same cluster, but quite different from the patterns of patients in the other three clusters, hereby suggesting an extreme degree of genetic heterogeneity [3,7].

## Results

The four clusters, are shown in Table 1, 2, 3, 4. Of the 607 patients, 156 were members of the 4 clusters. The clusters contained 41, 48, 41, and 37 patients; 11 patients were members of two clusters, and no patient was a member of three clusters. The clusters contained 60, 60, 65, and 53 SNP genotypes; 29 SNP genotypes were located in two clusters, and one SNP genotype (rs1380452 positioned in the *ANK3* gene) was located in three clusters.

The 156 patients were subdivided into the 4 clusters and into three groups based on three geographic areas in Scandinavia (Oslo, Aarhus, and Copenhagen). The available clinical data were not the same in the three areas. The number of hypomanic, manic and depressive episodes was available from the Norwegian patients, the number of hospital admissions and the presence of alcohol dependence was available from the patients from Copenhagen (Tables 5, 6, 7).

Table 5 shows number of hypomanic and manic episodes and depressive episodes for the single patients. The median for the number of hypomanic and manic episodes is 3, and the median for the number of depressive episodes is 3.5. Patients having numbers of episodes above the median for both hypomanic and manic episodes and depressive episodes (more serious disease) were compared with patients having at least one type of episodes below or equal to the medians ($p = 0.0045$ for clusters 1+2+4 versus cluster 3).

**Table 1.** Cluster 1. defined by SNP1 = AVPR1B_rs33976516 = 1.

| SNP2 | SNP3 | GT[a] | Patients[b] |
|---|---|---|---|
| ANK3_rs2288358 | SCN2B_rs8192614 | 2 0 | 94 126 132 166 333 393 409 413 528 |
| ANK3_rs2288358 | CAMKK2_rs11065502 | 2 0 | 94 126 132 166 333 393 409 413 528 |
| ANK3_rs2288358 | PPP2R2C_rs17721365 | 2 0 | 94 126 132 166 333 393 409 413 528 |
| ANK3_rs2288358 | ANK3_rs10994322 | 2 0 | 94 126 132 166 333 393 409 413 528 |
| ANK3_rs2288358 | KCNC2_rs1880840 | 2 0 | 94 126 132 166 333 393 409 413 528 |
| ANK3_rs4948255 | KCNQ2_rs3787119 | 2 0 | 94 126 151 166 333 393 409 413 528 |
| ANK3_rs4948255 | KCNN3_rs12029542 | 2 0 | 94 126 151 166 333 393 409 413 528 |
| ANK3_rs4948255 | ATP1A3_rs4803520 | 2 0 | 94 126 132 151 166 333 393 409 528 |
| ANK3_rs4948255 | ATP1A3_rs2217342 | 2 0 | 94 126 132 151 166 333 393 409 528 |
| ANK3_rs4948255 | CACNG2_rs4821512 | 2 1 | 94 132 151 166 333 393 409 413 528 |
| CNTNAP2_rs6945513 | NFASC_rs17415523 | 0 0 | 105 149 197 200 210 231 278 333 390 |
| CNTNAP2_rs6945513 | ANK3_rs17805456 | 0 0 | 6 105 149 197 200 210 231 333 390 |
| CNTNAP2_rs6945513 | ANK3_rs7895653 | 0 0 | 6 105 149 200 210 231 278 333 390 |
| CNTNAP2_rs6945513 | ANK3_rs10994322 | 0 0 | 6 105 149 200 210 231 278 333 390 |
| CNTNAP2_rs6945513 | KCNN3_rs951241 | 0 0 | 6 105 149 200 210 231 278 333 390 |
| KCNN3_rs6699080 | SCN2B_rs8192614 | 2 0 | 6 94 100 126 151 278 285 366 409 593 |
| KCNN3_rs6699080 | TNR_rs2236885 | 2 0 | 6 94 100 126 151 278 285 366 409 593 |
| KCNN3_rs6699080 | ANK3_rs4359155 | 2 0 | 6 94 100 126 151 278 285 409 593 |
| KCNN3_rs6699080 | MCTP2_rs3784644 | 2 0 | 6 94 100 151 278 285 366 409 593 |
| P2RX7_rs6489794 | OLIG2_rs762178 | 1 1 | 42 151 166 210 248 330 393 421 511 |
| P2RX7_rs6489794 | CACNG2_rs2284016 | 1 0 | 57 151 248 330 356 366 393 421 511 |
| P2RX7_rs6489794 | ANK3_rs2393602 | 1 0 | 57 210 231 248 304 330 366 421 511 |
| KCNN3_rs1218575 | CREB1_rs2551921 | 1 1 | 57 126 151 200 210 231 393 436 511 593 |
| KCNN3_rs1218575 | CNTNAP2_rs2620460 | 1 1 | 126 151 200 231 248 393 409 436 545 |
| KCNN3_rs1218575 | ANK3_rs1010556 | 1 2 | 6 57 94 200 393 409 511 545 593 |
| ANK3_rs10821695 | TRPM2_rs1556314 | 0 1 | 42 94 114 231 285 333 393 421 575 |
| ANK3_rs10821695 | TRPM2_rs734336 | 0 1 | 42 94 114 166 231 285 333 393 575 |
| NFASC_rs16854930 | IMPA2_rs628419 | 1 1 | 51 94 132 248 390 393 394 511 575 593 |
| NFASC_rs16854930 | SCN1B_rs8100085 | 1 1 | 166 285 356 393 409 413 436 511 575 |
| NCAM1_rs12794326 | TNR_rs2239821 | 2 1 | 88 149 200 210 304 330 390 394 528 596 |
| NCAM1_rs12794326 | CNTN1_rs7315781 | 2 0 | 149 200 210 330 390 393 528 575 596 |
| CNTN2_rs16855045 | P2RX7_rs1718161 | 1 1 | 51 149 166 210 231 248 330 393 413 421 593 |
| CNTN2_rs16855045 | CACNG2_rs2283970 | 1 0 | 51 57 149 210 248 330 333 356 413 421 |
| ANK3_rs1010556 | ANK3_rs10994195 | 0 1 | 42 51 88 114 149 153 166 278 366 413 421 |
| ANK3_rs1010556 | CNTNAP2_rs1024676 | 2 0 | 6 57 94 132 304 409 511 545 593 |
| KCNN3_rs6426998 | TNR_rs1385541 | 2 0 | 6 94 126 278 285 393 409 421 593 |
| KCNQ2_rs6122454 | NRCAM_rs759548 | 0 1 | 57 149 200 285 304 356 421 511 545 |
| SCN4B_rs868344 | ANK3_rs7893313 | 2 1 | 35 42 88 94 100 149 333 356 409 |
| ANK3_rs1380452 | ANK3_rs10994171 | 2 0 | 6 57 100 105 132 304 409 436 511 593 |
| SCN4B_rs678262 | ANK3_rs2018783 | 0 2 | 42 94 100 114 248 304 393 409 436 |
| CACNG2_rs2283970 | CNTN2_rs3767298 | 0 1 | 51 57 149 210 248 333 356 413 421 |
| P2RX7_rs1718161 | CNTN2_rs3767298 | 1 1 | 51 114 149 166 210 231 248 393 413 421 593 |
| ATP1A3_rs4803520 | KCNN3_rs6426998 | 0 2 | 6 94 126 151 285 366 393 409 421 |
| ANK3_rs17805456 | CNTNAP2_rs10808044 | 0 2 | 6 105 149 153 197 210 231 366 390 413 |
| NCAM1_rs584427 | AQP4_rs3763043 | 2 0 | 35 151 200 366 390 409 413 421 436 545 |
| NFASC_rs2802853 | KCNQ3_rs869710 | 1 0 | 57 88 94 105 248 285 356 393 575 593 |

Cluster 1 contains 41 patients, 46 combinations of 3 SNP genotypes, and 58 SNP genotypes.
a) GT = genotype for SNP2 and SNP3 (0: Normal homozygote.1: Heterozygote. 2: Variant homozygote); b) Dummy ID.
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**Table 2.** Cluster 2. defined by SNP1 = KCNN3_rs884664 = 2.

| SNP2 | SNP3 | GT[a] | Patients[b] |
|---|---|---|---|
| ANK3_rs2018783 | KCNQ3_rs7002144 | 0 0 | 22 24 153 212 359 375 553 573 584 585 |
| ANK3_rs2018783 | KCNQ3_rs10217015 | 0 0 | 22 24 153 212 375 553 573 584 585 |
| ANK3_rs2018783 | CNTNAP2_rs1587048 | 0 0 | 22 24 305 351 359 421 500 553 573 |
| ANK3_rs2018783 | CNTNAP2_rs1524339 | 0 1 | 22 153 212 351 359 421 573 584 585 |
| ANK3_rs2018783 | CNTN1_rs11178111 | 0 0 | 22 24 153 305 351 359 573 584 585 |
| CAMKK2_rs2686343 | NCAM1_rs584427 | 0 0 | 22 50 154 188 201 351 359 500 524 |
| CAMKK2_rs2686343 | SCN2A_rs17184707 | 0 1 | 22 91 154 188 201 351 383 417 422 |
| CAMKK2_rs2686343 | NFASC_rs10900430 | 0 1 | 22 50 91 188 201 212 280 359 422 |
| CAMKK2_rs2686343 | NFASC_rs11240304 | 0 1 | 22 91 154 188 201 351 383 417 422 |
| CAMKK2_rs2686343 | NFASC_rs7535216 | 0 1 | 22 91 154 188 201 351 383 417 422 |
| CNTNAP2_rs10808044 | BACE1_rs522843 | 0 0 | 5 24 78 91 156 178 293 375 421 422 503 553 |
| CNTNAP2_rs10808044 | BACE1_rs473210 | 0 0 | 5 24 78 91 156 178 293 375 421 422 553 |
| CNTNAP2_rs10808044 | BACE1_rs525493 | 0 0 | 5 24 78 91 156 178 293 421 422 553 |
| CNTNAP2_rs10808044 | BACE1_rs477036 | 0 0 | 5 24 78 91 156 178 293 421 422 553 |
| BDNF_rs908867 | AQP4_rs3875089 | 1 0 | 5 24 50 123 156 176 201 359 378 500 |
| BDNF_rs908867 | NRG1_rs2975500 | 1 0 | 5 24 50 123 156 176 201 378 500 |
| BDNF_rs908867 | KCNQ3_rs17575754 | 1 0 | 5 24 50 123 156 176 359 378 500 |
| IMPA2_rs636173 | KCNC1_rs7110441 | 2 0 | 38 62 74 154 201 422 500 524 526 553 |
| IMPA2_rs636173 | KCNC1_rs16934680 | 2 0 | 38 62 74 154 201 422 500 524 526 553 |
| CNTNAP2_rs9640245 | TNR_rs859437 | 1 2 | 27 74 176 189 283 383 500 526 585 |
| CNTNAP2_rs9640245 | TNR_rs12119177 | 1 0 | 27 74 176 189 283 383 500 526 585 |
| ANK3_rs16914644 | KCNN3_rs11264248 | 1 2 | 38 62 74 123 147 178 201 305 422 |
| ANK3_rs16914644 | KCNN3_rs6695232 | 1 0 | 38 62 74 123 147 178 201 305 422 |
| CACNG2_rs2284010 | KCNQ3_rs713148 | 0 2 | 62 78 86 147 156 176 189 212 359 422 503 526 |
| CACNG2_rs2284010 | KCNQ3_rs17595945 | 0 2 | 62 78 86 156 176 189 212 359 422 526 |
| KCNC3_rs1559133 | PDE4B_rs4288570 | 1 0 | 22 62 147 212 280 375 422 500 538 |
| KCNC3_rs1559133 | ANK3_rs10821695 | 1 0 | 62 147 212 280 293 375 422 526 538 |
| KCNC2_rs2926150 | CNTN2_rs11240351 | 1 1 | 22 38 78 86 123 176 189 378 503 |
| KCNC2_rs2926150 | BACE1_rs522843 | 1 0 | 5 47 78 123 156 176 378 417 503 |
| NRCAM_rs11767318 | BACE1_rs525493 | 1 0 | 5 62 78 178 293 305 421 422 585 |
| NRCAM_rs11767318 | BACE1_rs477036 | 1 0 | 5 62 78 178 293 305 421 422 585 |
| CAMKK2_rs11065502 | SCN2A_rs17184707 | 1 1 | 27 91 176 188 201 212 305 359 375 422 |
| CAMKK2_rs11065502 | KCNN3_rs11264254 | 1 1 | 27 30 156 176 201 305 417 422 524 |
| KCNQ3_rs713148 | KCNC2_rs1379963 | 2 1 | 47 62 78 86 156 189 421 422 503 585 |
| CNTN1_rs11178111 | NRCAM_rs10953566 | 1 0 | 27 47 153 176 178 212 359 524 584 585 |
| MBP_rs12962017 | PPP2R2C_rs4689408 | 1 1 | 5 30 62 135 154 351 378 500 538 |
| KCNN3_rs11264254 | P2RX7_rs1718134 | 2 1 | 5 38 74 91 188 212 314 421 584 |
| KCNA1_rs1048500 | P2RX7_rs503720 | 0 0 | 22 47 50 62 135 293 359 417 526 |
| KCNA2_rs3887820 | KCNQ2_rs6062929 | 1 1 | 24 30 147 153 154 156 188 305 503 |
| IMPA2_rs3859296 | PPP2R2C_rs16838658 | 1 1 | 22 38 47 176 283 417 524 526 584 |
| NFASC_rs11240304 | KCNQ2_rs6090403 | 1 1 | 22 91 188 189 229 293 383 417 422 |
| BDNF_rs11030102 | SLC12A6_rs17236791 | 1 1 | 30 47 74 188 280 293 375 422 538 573 |
| NFASC_rs10900430 | KCNQ2_rs6090403 | 1 1 | 22 91 188 189 229 293 383 417 422 |
| SCN1B_rs8100085 | YWHAH_rs929036 | 0 2 | 22 24 74 86 153 154 383 417 538 |
| SPTBN4_rs4803342 | MAP2_rs17745941 | 1 1 | 30 50 62 78 283 422 500 524 553 |

Cluster 2 contains 48 patients, 45 combinations of 3 SNP genotypes, and 60 SNP genotypes.
a) GT = genotype for SNP2 and SNP3 (0: Normal homozygote.1: Heterozygote. 2: Variant homozygote); b) Dummy ID.
doi:10.1371/journal.pone.0044623.t002

**Table 3.** Cluster 3.defined by SNP1 = CACNG2_rs2179871 = 2.

| SNP2 | SNP3 | GT[a] | Patients[b] |
|---|---|---|---|
| KCNN3_rs6426998 | NCAM1_rs7130671 | 2 1 | 44 65 119 146 224 227 232 290 380 417 599 602 |
| KCNN3_rs6426998 | KCNC2_rs1458606 | 2 1 | 44 65 119 224 290 331 380 417 515 599 602 |
| KCNN3_rs6426998 | CAMKK2_rs1653594 | 2 1 | 44 119 146 224 227 290 331 380 417 599 602 |
| KCNN3_rs6426998 | CAMKK2_rs1140886 | 2 1 | 44 65 119 290 331 380 417 515 599 602 |
| KCNN3_rs6426998 | CAMKK2_rs1063843 | 2 1 | 44 65 119 290 331 380 417 515 599 602 |
| KCNN3_rs6426998 | SPTBN4_rs17656504 | 2 0 | 44 65 146 224 227 232 290 380 515 602 |
| KCNN3_rs6426998 | CNTNAP2_rs6962824 | 2 1 | 44 65 119 224 232 290 331 515 602 |
| KCNN3_rs6426998 | CNTNAP2_rs2972112 | 2 1 | 44 65 146 224 227 380 515 599 602 |
| KCNN3_rs6426998 | CNTNAP2_rs7803315 | 2 0 | 44 65 119 146 224 227 232 417 602 |
| KCNN3_rs6426998 | NFASC_rs6593917 | 2 1 | 44 65 146 224 232 290 417 515 599 |
| KCNN3_rs6426998 | SCN2A_rs17185905 | 2 0 | 44 65 119 146 224 227 232 331 515 |
| KCNN3_rs6426998 | ANK3_rs1380452 | 2 1 | 44 65 146 224 227 290 417 515 599 |
| KCNN3_rs6426998 | CREB1_rs10932201 | 2 1 | 44 65 224 232 290 331 417 515 602 |
| CNTNAP2_rs4493828 | IMPA2_rs3889500 | 2 0 | 65 380 388 417 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | SLC12A6_rs8028501 | 2 0 | 65 380 388 417 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | TNC_rs1330351 | 2 1 | 65 380 388 417 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | TNC_rs2071520 | 2 1 | 65 380 388 417 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | ANK3_rs16914571 | 2 0 | 380 388 417 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | AQP4_rs151245 | 2 1 | 65 380 388 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | MBP_rs2282557 | 2 0 | 65 380 388 417 449 469 484 570 574 599 |
| CNTNAP2_rs4493828 | MBP_rs470330 | 2 0 | 65 380 388 417 449 484 570 574 599 |
| CNTNAP2_rs4493828 | MBP_rs470131 | 2 0 | 65 380 388 417 449 484 570 574 599 |
| CNTNAP2_rs4493828 | DLG4_rs2586539 | 2 0 | 65 380 388 417 449 469 570 574 599 |
| CNTNAP2_rs4493828 | KCNC2_rs1880840 | 2 0 | 65 380 388 417 449 469 484 570 574 |
| CNTNAP2_rs4493828 | SLC12A6_rs16958875 | 2 0 | 65 380 388 417 449 484 570 574 599 |
| CNTNAP2_rs2972112 | SLC12A6_rs4577050 | 0 0 | 44 65 146 224 292 331 449 498 515 522 570 |
| CNTNAP2_rs2972112 | SLC12A6_rs436552 | 0 0 | 44 146 224 292 331 449 498 515 522 |
| CNTNAP2_rs2972112 | PDE4B_rs599381 | 0 1 | 44 83 146 227 232 292 420 498 522 |
| CNTNAP2_rs2972112 | KCNC1_rs757511 | 0 2 | 44 65 119 146 331 415 420 449 498 |
| CREB1_rs2551645 | P2RX7_rs6489794 | 1 1 | 44 145 227 290 292 330 420 449 484 533 |
| CREB1_rs2551645 | ANK3_rs9888033 | 1 0 | 44 145 227 265 307 330 380 415 436 |
| PPP2R2C_rs6814782 | NRG1_rs4236709 | 0 0 | 113 335 415 417 420 436 449 515 533 |
| PPP2R2C_rs6814782 | CAMKK2_rs2686343 | 0 1 | 44 113 119 290 380 415 515 522 533 |
| CNTNAP2_rs1024676 | SPTBN4_rs4803342 | 0 0 | 224 265 307 388 415 417 436 533 570 574 |
| CNTNAP2_rs1024676 | NRG1_rs2466051 | 0 1 | 44 224 330 388 469 504 522 533 599 |
| CACNG2_rs738974 | BACE1_rs525493 | 1 2 | 65 290 292 335 388 420 504 533 560 |
| CACNG2_rs738974 | BACE1_rs477036 | 1 2 | 65 290 292 335 388 420 504 533 560 |
| KCNC3_rs636567 | PPP2R2C_rs10937735 | 1 1 | 44 83 119 146 265 290 335 388 522 533 560 |
| KCNC3_rs636567 | CNTNAP2_rs2373289 | 1 1 | 83 119 146 335 388 516 522 533 560 |
| CNTNAP2_rs1730399 | KCNQ2_rs6090403 | 1 2 | 65 113 388 420 449 471 515 516 602 |
| PPP2R2C_rs10937735 | SCN2A_rs3769949 | 1 0 | 44 113 146 227 265 307 417 471 515 522 533 |
| BACE1_rs525493 | SCN2A_rs2060199 | 2 1 | 65 290 323 335 388 420 504 533 560 |
| CNTN1_rs1596509 | KCNC2_rs1458613 | 0 1 | 65 83 145 227 232 330 388 504 533 574 |
| MAP2_rs2663652 | KCNN3_rs906280 | 1 1 | 224 232 307 331 388 415 482 484 504 515 602 |
| NCAM1_rs2196456 | CACNG2_rs926543 | 0 1 | 65 145 290 292 330 498 515 516 574 |
| SCN5A_rs7430407 | MAP2_rs6733319 | 1 0 | 44 146 224 265 335 388 417 436 482 |
| PPP2R2C_rs4386675 | KCNN3_rs883319 | 1 1 | 83 292 331 388 482 484 497 516 533 574 |

**Table 3.** Cont.

| SNP2 | SNP3 | GT[a] | Patients[b] |
|---|---|---|---|
| NFASC_rs16854930 | SCN4B_rs678262 | 1 0 | 119 265 330 335 388 436 516 533 570 |
| KCNN3_rs7547552 | NCAM1_rs1807939 | 1 0 | 65 83 145 265 331 388 484 497 515 516 560 574 599 |

Cluster 3 contains 41 patients, 49 combinations of 3 SNP genotypes, and 65 SNP genotypes.
a) GT = genotype for SNP2 and SNP3 (0: Normal homozygote.1: Heterozygote. 2: Variant homozygote); b) Dummy ID.
doi:10.1371/journal.pone.0044623.t003

**Table 4.** Cluster 4. defined by SNP1 = KCNQ3_rs2469515 = 2.

| SNP2 | SNP3 | GT[a] | Patients[b] |
|---|---|---|---|
| ANK3_rs12049756 | SCN2A_rs3769949 | 1 1 | 111 268 294 358 360 385 399 444 491 521 538 |
| ANK3_rs12049756 | SCN2A_rs997508 | 1 1 | 111 294 354 358 360 385 399 444 491 521 538 |
| ANK3_rs12049756 | SCN2A_rs12469667 | 1 1 | 13 111 294 354 358 360 399 444 491 538 |
| ANK3_rs12049756 | ANK3_rs10821702 | 1 1 | 13 111 196 268 294 354 360 385 399 |
| ANK3_rs12049756 | AQP4_rs9951307 | 1 0 | 98 111 196 294 360 385 399 521 538 |
| SCN5A_rs7430407 | ANK3_rs1010556 | 1 1 | 10 13 20 56 72 196 268 328 336 567 |
| SCN5A_rs7430407 | CNTNAP2_rs4431524 | 1 1 | 10 20 56 59 72 268 328 336 567 |
| SCN5A_rs7430407 | KCNN3_rs11264250 | 1 1 | 13 20 56 59 72 268 328 336 567 |
| SPTBN4_rs8107961 | TBR1_rs7564766 | 2 0 | 0 10 13 18 196 328 343 358 492 527 599 |
| SPTBN4_rs8107961 | CAMKK2_rs1140886 | 2 1 | 0 18 196 343 358 360 492 527 538 599 |
| ANK3_rs10821677 | ANK3_rs12767186 | 1 1 | 56 62 98 111 268 294 360 385 399 431 596 |
| ANK3_rs10821677 | KCNC1_rs757511 | 1 2 | 62 98 111 294 399 444 521 527 596 |
| OLIG2_rs762178 | ANK3_rs2393602 | 2 1 | 0 20 72 323 328 491 527 538 567 596 |
| OLIG2_rs762178 | KCNC2_rs11180386 | 2 0 | 0 20 72 323 444 491 492 527 538 |
| CNTNAP2_rs2462603 | ANK3_rs10994200 | 2 0 | 20 62 72 189 268 354 358 538 567 596 |
| CNTNAP2_rs2462603 | ANK3_rs10761454 | 2 0 | 20 62 72 189 268 358 538 567 596 |
| PPP2R2C_rs2269920 | TRPM2_rs9974831 | 1 1 | 0 20 72 111 323 336 343 354 360 521 562 |
| PPP2R2C_rs2269920 | KCNC3_rs683856 | 1 1 | 196 268 294 354 360 369 492 527 562 |
| MAG_rs1034597 | CNTNAP2_rs2972112 | 1 0 | 10 13 18 111 294 358 360 385 444 483 538 |
| KCNQ2_rs6089908 | NFASC_rs6677763 | 0 1 | 294 323 336 343 354 358 492 527 567 596 |
| CNTN1_rs3794247 | NRG1_rs4535704 | 0 1 | 0 10 62 98 111 294 385 399 491 492 |
| ANK3_rs1380452 | TNC_rs1330351 | 0 1 | 0 294 328 343 399 444 521 527 567 |
| NRG1_rs2439311 | IMPA2_rs662383 | 1 1 | 56 111 328 354 360 431 483 567 599 |
| P2RX7_rs7958311 | BACE1_rs522843 | 1 1 | 59 98 111 189 196 294 360 399 431 |
| CNTNAP2_rs4431524 | MBP_rs470826 | 1 1 | 20 56 59 72 328 354 358 399 527 596 |
| DLG4_rs507506 | SPTBN4_rs814501 | 0 2 | 13 18 196 328 360 492 521 538 599 |
| IMPA2_rs628419 | KCNQ2_rs6062925 | 1 2 | 20 72 98 268 323 336 358 431 527 |
| PPP2R2C_rs3796403 | IMPA2_rs3786305 | 1 1 | 0 59 98 111 196 336 358 431 483 |
| CNTNAP1_rs2271029 | NRCAM_rs6958498 | 1 1 | 10 13 196 354 399 492 527 538 562 |
| KCNQ2_rs884851 | CNTNAP1_rs9897724 | 1 0 | 0 56 294 328 369 385 431 562 596 |
| NRG1_rs3924999 | ANK3_rs10761482 | 1 1 | 10 13 59 98 294 360 399 431 521 |
| CREB1_rs2551921 | MBP_rs9676113 | 1 1 | 18 20 72 369 399 483 527 567 599 |

Cluster 4 contains 37 patients, 32 combinations of 3 SNP genotypes, and 53 SNP genotypes.
a) GT = genotype for SNP2 and SNP3 (0: Normal homozygote.1: Heterozygote. 2: Variant homozygote); b) Dummy ID.
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**Table 5.** Number of hypomanic, manic and depressive episodes in patients from Oslo with bipolar disorder.

| Cluster 1 | | Cluster 2 | | Cluster 3 | | Cluster 4 | |
|---|---|---|---|---|---|---|---|
| **Hypo-manic and manic episodes** | **Depres-sive episodes** | **Hypo-manic and manic episodes** | **Depres-sive episodes** | **Hypo-manic and manic episodes** | **Depres-sive episodes** | **Hypo-manic and manic episodes** | **Depres-sive episodes** |
| **11** | **20** | **50** | **40** | **39** | **40** | **22** | **22** |
| **8** | **10** | 4 | 0 | **32** | **32** | 12 | 3 |
| 6 | 0 | 3 | 0 | **30** | **20** | **5** | **15** |
| 4 | 3 | 3 | 0 | **25** | **30** | 5 | 5 |
| 3 | 4 | 2 | 25 | **22** | **21** | 3 | 20 |
| 2 | 0 | 2 | 7 | **14** | **10** | 3 | 5 |
| 1 | 1 | 2 | 3 | **13** | **20** | 3 | 2 |
| 1 | 0 | 2 | 1 | **10** | **51** | 2 | 10 |
| | | 2 | 1 | **10** | **20** | 2 | 1 |
| | | 1 | 1 | **7** | **10** | 2 | 1 |
| | | 1 | 1 | **6** | **6** | 1 | 1 |
| | | | | 3 | 30 | 1 | 0 |
| | | | | 3 | 0 | 1 | 0 |
| | | | | 2 | 5 | | |
| | | | | 1 | 2 | | |
| | | | | 1 | 2 | | |
| | | | | 1 | 2 | | |
| | | | | 1 | 1 | | |
| 25% with more serious disease | | 9% with more serious disease | | 61% with more serious disease | | 15% with more serious disease | |

Each double cell shows the number of hypomanic and manic episodes and the number of depressive episodes for a single patient. The median for the number of hypomanic and manic episodes is 3, and the median for the number of depressive episodes is 3.5. Patients having numbers of episodes above the median for both hypomanic and manic episodes and depressive episodes (bold types) are compared with patients having at least one type of episodes below or equal to the medians. $p = 0.0045$ for clusters 1+2+4 versus cluster 3 (Fisher's exact test, two-tailed).

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**Table 6.** Number of hospital admissions for patients with bipolar disorder in Copenhagen.

| Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 |
|---|---|---|---|
| 38 | 38 | 14 | 70 |
| 25 | 32 | 12 | 41 |
| 13 | 22 | 8 | 40 |
| 7 | 17 | 4 | 20 |
| 7 | 12 | 3 | 19 |
| 6 | 9 | | 11 |
| 5 | 5 | | 7 |
| 5 | 5 | | 7 |
| 3 | 4 | | 6 |
| 3 | 4 | | |
| 2 | 3 | | |
| 1 | 3 | | |
| 1 | 3 | | |
| 0 | 2 | | |
| | 2 | | |
| | 2 | | |
| | 0 | | |
| | 0 | | |

Each box represents one patient.
doi:10.1371/journal.pone.0044623.t006

**Table 7.** Alcohol dependence (1) or non-dependence (0) in patients from Copenhagen with bipolar disorder.

| Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 |
| 0 | 0 | 1 | 0 |
| 0 | 0 | 1 | 1 |
| 0 | 0 | | 1 |
| 0 | 0 | | 1 |
| 0 | 0 | | 1 |
| 0 | 0 | | |
| 0 | 0 | | |
| 0 | 0 | | |
| 1 | 0 | | |
| 1 | 1 | | |
| 1 | 1 | | |
| | 1 | | |
| | 1 | | |

Each box represents one patient.
doi:10.1371/journal.pone.0044623.t007

## Discussion

The four clusters of combinations of SNP-genotypes were statistically significantly associated with bipolar disorder; whereas biological or clinical significance of the clusters was not apparent, apart from the original selection of genes related to signal transduction [3]. These genes are shown in Table 8. The relatively little overlap between the patients in the clusters led to an analysis of available clinical data from the psychiatric departments that had recruited the patients from three locations in Scandinavia. The division of patients according to locations, clusters and availability of clinical data led to the small groups of patients shown in Tables 5–7.

Using numbers of hypomanic, manic and depressive episodes higher than the median for these episodes as an indication of severity of disease, it was found that the number of patients with more severe disease was higher in one cluster compared with the three other clusters (Table 5). This result suggests that it may be possible to connect combinations of genetic data to clinical data. The figures in Table 6 and 7 may led to similar suggestions, but although significant differences may be found between the distributions in these tables, the statistical power is low and no significant results may remain after correction for multiple testing.

Due to the relatively low number of patients as well as of clinical data, no strong conclusions can be drawn from this study. However, the results in Table 5 indicated that some genetic subgroups may be more affected by their illness than other subgroups, hereby justifying further work with combinations of genetic data as a method to connect genetic and clinical data. Hopefully, other studies with more patients, more genetic data and more clinical data will try to look at combinations of their data.

## Materials and Methods

The patient sample, genes, SNP selection and genotyping, statistics and data processing regarding Table 1, 2, 3, 4 were described previously [3]. The Norwegian Scientific-Ethical Committees, the Norwegian Data Protection Agency, the Danish Scientific Committees, and the Danish Data Protection Agency approved the study. All patients gave written informed consent prior to inclusion in the project. The data in Table 5 were analyzed statistically with Fisher's exact test, two-tailed. In Tables 5, 6, 7 each box represents one patient. The numbers of hypomanic, manic, and depressive episodes in Norwegian patients were obtained by SCID [8]. The numbers of admissions in Copenhagen were obtained from patient records. Patients from Copenhagen were diagnosed with alcohol dependence when the patient was, or had been, treated in an alcohol clinic.

## Author Contributions

Conceived and designed the experiments: EM PK. Analyzed the data: EM PK GLM. Contributed reagents/materials/analysis tools: HD BB IM OAA SD OM TH. Wrote the paper: EM PK.

**Table 8.** Selected genes and function [3].

| Gene | Location | Name and/or Function |
|---|---|---|
| *ANK3* | 10q21 | Role for structure and function of nodes of Ranvier |
| *AQP4* | 18q11.2–q12.1 | Regulator of vasopression secretion |
| *ATP1A2* | 1q21–q23 | Na+/K+ ATPase alpha-2 subunit |
| *ATP1A3* | 19q13.31 | Na+/K+ ATPase alpha-3 subunit |
| *AVPR1B* | 1q32 | Arginine vasopressin receptor 1B |
| *BACE1* | 11q23.2–q23.3 | Regulation of the voltage dependent Na-channels. |
| *BDNF* | 11p13 | Involved in neuroplasticity and stress response |
| *CACNG2* | 22q13.1 | Neuronal calcium channel gamma subunit, stabilize the channel in an inactive state |
| *CAMKK2* | 12q24.2 | Involved in activation of CREB1 |
| *CLDN11* | 3q26.2–q26.3 | Role in myelinisation |
| *CNTN1* | 12q11–q12 | Cell adhesion molecule |
| *CNTN2* | 1q32.1 | Cell adhesion molecule |
| *CNTNAP1* | 17q21 | Contactin-associated protein, may be the signaling subunit of contactin |
| *CNTNAP2* | 7q35–q36 | Cluster voltage-gated potassium channels, localized at the juxtaparanodes |
| *CREB1* | 2q34 | Transcription factor |
| *DLG4* | 17p13.1 | Neuronal development, recruited into potassium channel clusters |
| *ERBB4* | 2q33.3–q34 | Neuregulin-1 receptor, involved in mitogenesis and differentiation |
| *GSK3B* | 3q13.3 | Neuronal cell development (Related to lithium respons) |
| *IMPA2* | 18p11.2 | Inositol monophosphatase (Related to lithium respons) |
| *KCNA1* | 12p13.32 | Voltage-gated delayed potassium channel |
| *KCNA2* | 1p13 | Voltage-gated delayed potassium channel, delayed rectifier class |
| *KCNC1* | 11p15 | Mediates the voltage-dependent potassium ion permeability of excitable membranes |
| *KCNC2* | 12q14.1 | Mediates the voltage-dependent potassium ion permeability of excitable membranes |
| *KCNC3* | 19q13.3–q13.4 | Mediates the voltage-dependent potassium ion permeability of excitable membranes |
| *KCNN3* | 1q21.3 | Potassium conductance Ca-activited channel, regulate neuronal excitability |
| *KCNQ2* | 20q13.3 | Voltage-gated potassium channel plays a role in the regulation of neuronal excitability |
| *KCNQ3* | 8q24 | Voltage-gated potassium channel plays a role in the regulation of neuronal excitability |
| *MAG* | 19q13.1 | Central role i myelinisation, involved in myelin-neuron cell-cell interactions |
| *MAP2* | 2q34–q35 | Microtubule-associated protein, involved in neurogenesis |
| *MBP* | 18q23 | Major constituent of the myelin sheath of oligodendrocytes in the nervous system |
| *MCHR1* | 22q13.2 | Inhibit cAMP accumulation stimulate intracellular Ca-flux |
| *MCTP2* | 15q26.2 | Intercellular signal transduction |
| *MOG* | 6p22.1 | Involved in completion and maintenance of the myelin sheath and in cell-cell communication |
| *NCAM1* | 11q23.1 | Neural cell adhesion molecule 1 |
| *NFASC* | 1q32.1 | Cell adhesion; organization of the axon initial segment (AIS) and nodes of Ranvier |
| *NRCAM* | 7q31.1–q31.2 | Ankyrin-binding protein is involved in neuron-neuron adhesion |
| *NRG1* | 8p12 | Associated with ERBB receptors |
| *NTRK1* | 1q21–q22 | Neurotrophic tyrosine kinase, receptor, type 1 |
| *OLIG2* | 21q22.11 | Oligodendrocyte lineage transcription factor 2 |
| *P2RX7* | 12q24 | Ligand-gated ion channel |
| *PDE4B* | 1p31 | Phosphodiesterase 4B, cAMP-specific |
| *PPP2R2C* | 4p16.1 | Protein phosphatase 2, regulatory subunit B, gamma isoform |
| *SCN1B* | 19q13.1 | Sodium channel beta subunit, propagation of nerveimpulses, binding to contactin |
| *SCN2A* | 2q23–q24 | Sodium channel alpha subunit, generation and propagation of action potentials in neurons |
| *SCN2B* | 11q23 | Sodium channel, voltage-gated, type II, beta |
| *SCN4B* | 11q23.3 | Sodium channel, voltage-gated, type IV, beta |
| *SCN5A* | 3p21 | Sodium channel, voltage-gated, type V, alpha subunit |
| *SCN8A* | 12q13 | Sodium channel, voltage gated, type VIII, alpha subunit, associated with ANK3 |
| *SLC12A6* | 15q13–q15 | Electroneutral potassium-chloride cotransporter 3 |

**Table 8.** Cont.

| Gene | Location | Name and/or Function |
|---|---|---|
| *SPTBN4* | 19q13.13 | Involved in location of specific membrane proteins in polarized regions of neurons |
| *TBR1* | 2q24 | Transcription factor, critical for early cortical development |
| *TNC* | 9q33 | Regulation of Na channels. Interaction with CNTN1 |
| *TNR* | 1q24 | Extracellular matix protein expressed primarily in the central nervous system |
| *TRPM2* | 21q22.3 | Transient receptor potential cation channel, subfamily M, member 2 |
| *YWHAH* | 22q12.3 | Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide |

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